Age-Dependent Seroprevalence of Toscana Virus in Central Italy and Correlation with the Clinical Profile

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In order to estimate the antibody prevalence rates for Toscana virus (TOSV) among children and adults, we evaluated the seroprevalence of TOSV in a population (n = 2,737) living in Tuscany during the period of 1999 to 2006. The seroprevalence rate was 19.8% in adults and 5.8% in children, showing an age-dependent increase in TOSV-specific immunity. Meningitis due to TOSV infection was more frequent in adults than in children.

Many viruses are transmitted to humans through arthropods, and many species of insects are found in numerous geographical areas. Among the types of arbovirus transmitted to humans, phleboviruses of the Bunyaviridae family are found in Europe, Africa, central Asia, and the Americas (13, 16). There are some viruses circulating in Europe which are responsible for acute, nonfatal, influenzalike symptomatology (10). One strain of these viruses, Toscana virus (TOSV), which can cause aseptic meningitis or meningoencephalitis, is present in Italy, Algeria, Spain, France, Portugal, Greece, and Cyprus, as noted in studies of the indigenous population and cases of infection in tourists visiting these areas (4, 5, 7–9, 11, 12, 14). Serological analyses have also shown the presence of asymptomatic TOSV infections (2, 3). A recent study of 360 subjects in a high-risk, professionally exposed population reported seropositivity in 70% of subjects, without neurological symptomatology (18). This confirmed that TOSV infection can occur with mild symptoms or none at all. Based on this information, we performed a serological analysis to estimate the antibody prevalence rates for TOSV infection among children and adults living in Tuscany, an area of endemicity, and we evaluated the frequency of meningeal symptomatology in seropositive subjects.

Serum samples were collected from 2,737 patients, residents of Tuscany, 1 to 60 years old, that had been hospitalized at the Clinic of Infectious Diseases of the University of Siena for pathologies not related to neurological forms during the 1999 to 2006 period. Among these, 2,097 were children 1 to 15 years old and 640 were adults 16 to 60 years old. Serum collection was grouped by age, as shown in Table 1. During the same time period, 70 patients (12 children and 58 adults) were hospitalized in the same unit with meningitis or meningoencephalitis, with the diagnosis of TOSV infection, as determined by viral molecular detection (reverse transcription-PCR) on the cerebrospinal fluid of these patients, as previously described (17). Briefly, cerebrospinal fluid was subjected to RNA extraction by using a Total RNA isolation kit (Promega, Mannheim, Germany) and amplified by reverse transcription-PCR. Sequences of the primers used to amplify a 465-bp fragment of the TOSV N gene were as follows: forward primer, 5’ GGTG AAGAATCGTCCACTCA 3’ (nucleotides [nt] 1184 to 1203); reverse primer, 5’ CCAAGGGCATGTGAAGAAGAT 3’ (nt 1593 to 1615). The amplified product was then subjected to nested PCR using the forward primer 5’ TTGTTCCTCAGAG ATGGATTATA 3’ (nt 1255 to 1277) and reverse primer 5’ AACCTGATTTCGTCCTACCAGTT 3’ (nt 1542 to 1564) to provide a 309-bp fragment. The products were then gel purified and sequenced for confirmation. No particular amino acid difference was revealed among the strains isolated from patients with meningitis or meningoencephalitis, as previously stated (19). Diagnosis was further confirmed in these patients by serological analysis. Anti-TOSV immunoglobulin G (IgG) was evaluated by enzyme-linked immunosorbent assay (Diesse S.p.A., Siena, Italy) (15). Briefly, microtiter plates (Labsystem, Helsinki, Finland) were coated with the purified TOSV n protein and tested with 100 μl of serum samples (diluted 1:100) per well. After 45 min of incubation at 37°C, the plates were washed and 100 μl of a peroxidase-conjugated anti-human IgG monoclonal antibody (Diesse, Monteriggioni, Italy) was added to each well. After incubation at 37°C for 45 min, the substrate (tetramethylbenzidine) was added and the enzymatic reaction was stopped with 1 N H2SO4. Samples giving an optical density at 450 nm of 0.360 were considered to be positive, as suggested by the manufacturer.

The statistical differences among groups were determined by Pearson’s chi-square test. A probability (P) value of less than 0.05 was considered statistically significant. The Armitage test was used for the linear trend in proportions. During the period of 1999 to 2006, the seroprevalence of TOSV was 19.8% in adults and 5.8% in children (P < 0.001) (Table 1). In particular, seroprevalence was significantly lower (P = 0.01) in children 1 to 10 years old than in older children, and it increased with age, reaching 12.3% in the 11-to-15-year age group (Table 1). However, there was no significant difference (P = 0.06) in seroprevalence between the 11-to-15-year age group and the adults, indicating that TOSV infection is more frequent in people over the age of 10 years. The mean percentage observed in adults was 19.8% (Table 1), and although a peak of seroprevalence was observed in the group of subjects 41 to 50 years old, no linear age-related exposure to
the virus was shown among adults (P = 0.22). It is worth noting that a retrospective study highlighted a TOSV seroprevalence of 20% in children in 1983 and 1986, indicating that at that time TOSV was circulating at a higher rate among children (data not shown). Unfortunately, we do not have serum samples collected from children in previous years, which would have provided more information regarding the virus circulation in this area and would have indirectly allowed for dating the circulation of TOSV isolated in Tuscany in 1971 (20).

In order to correlate the seroprevalence to the clinical profile, we evaluated the percentages of seropositive children and adults who showed neurological signs and we included the subjects hospitalized with TOSV meningitis in the age categories analyzed in this study. It was noted that, out of 185 seropositive adults, 58 (31.40%) had clinical signs, such as meningitis or meningoencephalitis. However, only 12 (9.02%) out of 133 seropositive children had meningitis (P < 0.01), indicating that TOSV infection is correlated with minor clinical symptoms at the pediatric age. In fact, only a few showed symptoms of the clinical disease (headache, vomiting, neck rigidity, myalgia), while most children were asymptomatic. Since TOSV infection is often asymptomatic or the clinical picture is not relevant, mainly characterized by a bad headache, diagnosis is frequently missed, as in sandfly fever Sicilian virus and sandfly fever Naples virus infections. A severe illness involving meningitis and, rarely, the brain (1, 6) develops in only small percentage of patients; thus, a large underestimate of the number of TOSV infections in children is possible. Although a lower TOSV seroprevalence in children than in adults could be ascribed to less exposure of children to the vector, less neurological involvement in the course of infection could be related to other factors, such as immunological or genetic factors.

Moreover, epidemiological data show that TOSV was circulating in Tuscany in the 1980s. Unfortunately, due to the lack of previously dated serum samples, it was not possible to evaluate the circulation of this virus in the previous decade, before TOSV isolation and identification. Thus, a retrospective serological study would be interesting.

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