

Surveillance reports

TOSCANA VIRUS MENINGITIS IN PORTUGAL, 2002-2005.

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Toscana virus infection is endemic in Italy, but has also been documented in other Mediterranean countries. Our aim was to investigate the occurrence of Toscana virus (TOSV) meningitis in children and young adults in a metropolitan area in the north of Portugal. Cerebrospinal fluid samples from 308 patients with the diagnosis of meningitis and with negative bacterial culture were tested for enteroviruses and herpesviruses by reverse transcription PCR. Those samples that proved negative for enterovirus and herpesvirus were tested for Toscana virus with a commercial reverse transcription nested PCR assay. In total, we investigated 106 samples, collected between May and September during the four-year period between 2002 and 2005 from patients younger than 30 years old. Toscana virus was the cause of meningitis in six (5.6%) of the cases, three children and three young adults. All had a benign course and self-limited disease. Since a first case report of TOSV infection 1985 and another in 1996, both in foreign tourists, these six cases of Toscana virus meningitis are, to our knowledge, the first diagnosed in Portuguese inhabitants, and they underline the need for more studies on the prevalence of this virus in Portugal.

Introduction

With the improvement of diagnostic techniques such as PCR, it is now possible to rapidly diagnose viral meningitis through identification of the pathogen [1]. In Portugal, enteroviruses are the most frequent cause of aseptic meningitis but, despite thorough testing, a significant number of patients are discharged from the hospital without an etiological diagnosis [2,3]. The inclusion of Toscana virus (TOSV) diagnosis in the laboratory tests for enterovirus- and herpesvirus-negative samples was an attempt to improve the knowledge about aseptic meningitis. Viral meningitides, including TOSV meningitis, are non-notifiable diseases in Portugal and are characterised by non-specific symptoms. Consequently, their seroprevalence is unknown.

TOSV belongs to a group of sandfly fever viruses are arboviruses, transmitted by the sandfly (genus *Phlebotomus*), and classified in the *Bunyaviridae* family, genus *Phlebovirus*. Three sandfly fever viruses have been identified in Mediterranean area: sandfly fever Naples virus, sandfly fever Sicilian virus, and Toscana virus. The latter is endemic in central Italy and described as the most frequent cause of aseptic meningitis in children in that region [4,5]. As a consequence of the life cycle of *Phlebotomus*, TOSV is more frequent during summer, with a peak in August. The most common presentation is an acute febrile illness or meningitis, and more rarely a meningoencephalitis [5-7]. Most of the studies on TOSV have been done in central Italy, but the occurrence of TOSV in other countries such as France, Spain, Slovenia, Greece, Cyprus, Turkey and Egypt, has also been reported recently [8]. TOSV can be identified by culture of cerebrospinal fluid (CSF), a time-consuming method with low sensitivity, but very

useful for virus characterisation and genetic studies [8]. Immunoenzymatic tests, (IgM by IFA or ELISA) are rapid and sensitive; however cross-reactivity can occur. Nowadays, reverse transcription (RT)-PCR is considered the method with the highest sensitivity and specificity for virus detection [8,9]. Many vectors implied in human diseases, including *Phlebotomus*, are present in Portugal [10]. The aim of this study was to investigate the occurrence of TOSV meningitis in children and young adults in an urban area in the north of Portugal.

Methods

During the period 2002 to 2005, we have investigated the occurrence of TOSV in CSF samples of aseptic meningitis patients. Aseptic meningitis was defined by a CSF cytosis greater than six leukocytes/ml and a negative bacterial culture. RNA was extracted from the samples with the Qiamp viral RNA mini kit (Qiagen). Samples that were PCR-negative for enterovirus and herpes virus and that had been taken from patients younger than 30 years and hospitalised between May and September each year were tested for TOSV with a nested RT-PCR assay purchased from Amplimedical SpA (Toscana, N prot Kit). Each run included a negative control (water) and a positive control (Toscana virus positive control, Amplimedical SpA). Some negative samples (n=26) were tested with an 'in-house' nested RT-PCR protocol with published outer and inner primers [11].

Results

Over the four-year period (2002-2005), 308 patients were admitted to the emergency department of our hospital with aseptic meningitis and hospitalised for observation and study. They were analysed by RT-PCR for the presence of a number of viruses known to cause meningitis (enterovirus, herpes simplex virus (HSV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), and West Nile virus) during the acute phase of the disease. The results are indicated in the Table.

RT-PCR for enterovirus and herpes simplex virus were done in an order determined by the patient's mental status at admission. CSF samples from patients with a normal level of consciousness or with somnolence (Glasgow Coma Scale ≥ 14) were analysed first for enterovirus, and if this was negative, subsequently for HSV. Samples from patients with further impaired mental status (Glasgow Coma Scale < 14) were first analysed for HSV, and then, if negative, for enterovirus. RT-PCR for CMV and EBV was performed only in cases with a negative result for HSV and enterovirus. RT-PCR for West Nile virus was done only in 15 patients aged over than 60 years without aetiological diagnosis. It was negative in all of them.

For this study, we chose 106 of those 308 samples to be tested for TOSV using the Amplimedical SpA nested RT-PCR

TABLE

Diagnosis of aseptic meningitis by reverse transcription PCR in cerebrospinal fluid samples, Portugal, May-Sept 2002-2005 (pool of samples = 308)

PCR	No. of positive samples	No. of negative samples
Enterovirus	110	178
Herpes simplex virus	20	178
Epstein-Barr virus	5	173
Cytomegalovirus	3	175
West Nile virus	0	15
Toscana virus	6	100
Total no. of viral meningitis cases*	144 (47%)	

* positive for one of the six viruses analysed

assay. This included only samples from patients who had been admitted between May and September in the years from 2002 to 2005 and had been negative for enterovirus, HSV, EBV and CMV when tested upon hospital admission. All patients were younger than 30 years of age (range 2.5 months to 30 years). TOSV was detected in six (5.6%) of those 106 samples. Three were children – one aged four years and two aged eight years – and three were young adults, aged 16, 23 and 30 years. Three of the patients were male. All six patients live in an urban area, and none of them had a recent history of travelling abroad.

All cases had occurred between May and July, two cases in 2002, three in 2003 and one in 2005. Admission symptoms were fever, headache and vomiting, and lasted between one and five days. On admission, meningeal symptoms were present in all patients and somnolence in three. Two of them had brain-computed tomography that was normal. CSF cytosis ranged from 70 to 1,090 cells/ μ l, with normal glucose and protein levels. The blood leukocyte count varied from 5.6 to 11.8x10⁹/L, and C-reactive protein was normal in all patients. All underwent only supportive treatment with antipyretics and intravenous fluids and had a benign and self-limited disease. They were discharged between four and seven days after admission. Twenty-six of the 106 samples were examined only by an 'in-house' RT-PCR assay. The sensitivity of this assay was comparable to the commercial RT-PCR when tested using TOSV-positive clinical samples. All 26 samples that were only tested by this method were negative.

Discussion

In the Mediterranean countries, especially Italy and Spain, the interest in TOSV has increased in recent years [12-14]. PCR is the most frequent method used for TOSV diagnosis. It can be complemented by immuno-enzymatic tests, which are rapid and sensitive [8,11], or culture, which in combination with PCR is useful for genetic characterisation of the virus. Most of the reported cases of TOSV infections occur throughout the summertime in the central region of Italy, particularly in the Siena province, in children [4,5,9,15]. A study by Valassina *et al.* [5] describes the analysis of 277 meningitis cases that occurred in Tuscany between 1995 and 1998. TOSV was identified as the cause for 58% of the cases admitted in the period between June and September, and for 10% of the cases admitted from May to October, reflecting the seasonality of the infection. Other studies investigate the seroprevalence of TOSV in humans in southern Europe [8]. More recent reports have demonstrated the occurrence

of TOSV in Spain, in the Granada and Madrid provinces [8,16-18]. A study by Navarro *et al.* shows that this virus is responsible for 7% of aseptic meningitis cases in Spain [16]. In another Spanish study using samples collected between 1988 and 1996, TOSV was the cause of 8% of aseptic meningitis cases; these authors analysed 1,268 serum samples from adults and children for the presence of antibodies against TOSV, and found a prevalence of 26.2% [17]. Echevarria *et al.* [18] identified TOSV as the cause of 8.6% of aseptic meningitis cases in the region of Madrid, where 5% of the healthy population were shown to have had a past infection. Another Spanish publication on the seroprevalence of TOSV in the community of Madrid, comparing two periods (1993 to 1994 and 1999 to 2000) found past infections in 7.2% and 5.7%, respectively. It further showed that seroprevalence is age dependent, with the antibody prevalence increasing with age [14]. Two TOSV cases have been identified in southern France, one of aseptic meningitis and the other of influenza-like illness [19,20]. In addition, several reports have been published on TOSV infection in travellers returning from Mediterranean countries [21-24].

The climate conditions in Portugal favour *Phlebotomus* survival. Two infections with TOSV, acquired in Portugal, have been reported in the past. Both were in male tourists, one Swedish and one German, returning from their holidays in Portugal in 1985 and in 1996, respectively [25, 26]. One of them had documented meningitis. Studies on the occurrence of TOSV in Portugal have so far not been done, and the six cases of TOSV meningitis reported here are the first cases diagnosed in Portuguese inhabitants. Thus, in our opinion, the investigation of TOSV in summer cases of meningitis in Portugal should be continued in the future, as it is done in other Mediterranean countries, even though the prevalence of TOSV appears to be lower in Portugal. Serological surveys are planned to document the prevalence of antibodies against TOSV in all age groups of the Portuguese population, in order to gain a clearer picture of the occurrence of this form of viral meningitis.

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