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Seroprevalence of and risk factors for Toscana and Sicilian virus infection in a sample population of Sicily (Italy)

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Abstract

Objective—The present study aimed to assess seroprevalence of and risk factors for Toscana (TOSV) and Sicilian (SFSV) virus infections in a sample of Sicilian subjects.

Methods—A cross-sectional seroepidemiological study was conducted on 271 individuals. Each participant completed a self-administrated questionnaire and provided a serum sample which was analyzed for the presence of IgG specific anti-TOSV and anti-SFSV viruses.

Results—Overall, 90 subjects (33.2%) were positive for TOSV IgG, 25 (9.2%) were positive for SFSV IgG and 11 (4%) were positive for both the viruses. A higher risk for TOSV seropositivity was found in participants who were older (adjOR=1.02 per year; 95% CI=1.01–1.03), having a pet living outdoors (adjOR=2.62; 95% CI=1.42–4.83) and being obese (adjOR=2.37; 95% CI=1.06–5.30).

Conclusions—TOSV seroprevalence appears to be relatively high in Sicilian general population, especially in older adults, representing a potential public health concern. The observations that seropositivity for TOSV was not significantly associated with SFSV seropositivity, and none of the risk factors associated with TOSV were associated with SFSV seem to suggest that these two phleboviruses may have different ecology and transmission pathways.

Keywords

Toscana virus; Sicilian virus; re-emergence; Italy

Introduction

Toscana virus (TOSV) and Sicilian virus (SFSV) are two arthropod-borne viruses (family Bunyaviridae, genus Phlebovirus) that are transmitted to humans by vectors, predominantly by sand flies of Phlebotomus species. In particular, *Phlebotomus perniciosus* and *Phlebotomus perfiliewi* have been documented to transmit TOSV whereas SFSV is known to be transmitted by *Phlebotomus papatasii*. Both viruses are considered to be endemic in

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several Mediterranean countries including Italy (1), Portugal (2), France (3), Cyprus (4) and Algeria (5). In such geographic areas, several domestic and wild animal species (e.g., rodents, insectivore and carnivore) have been hypothesized to be the primary reservoirs for these viruses (6,7), although vertical (transovarial) transmission seems to be also important for their survival (8). Humans are susceptible to infection, but they are not considered necessary for the maintenance cycle of the viruses, due to the short duration of viremia and the lack of evidence for a persistent infection (9).

TOSV and SFSV are the causative agents of self-limiting disease, known as sand-fly fever, which has an average duration of three to five days and manifests as high-grade fever, myalgia and severe headache (8,10). Many or most of the infections are asymptomatic (11), especially in children. Thus, laboratory studies are needed to evaluate the circulation of these viruses in the population (12). A small fraction of people with TOSV infection can have neurologic manifestations (13). During the summer, TOSV is one of the most important causes of aseptic meningitis and meningoencephalitis in endemic areas such as Italy, Portugal and Cyprus (14). Because of this, several authors have suggested implementing active surveillance systems to control the circulation of phleboviruses like TOSV, especially in areas with high vector density (9). Considering the high TOSV antibody prevalence recently reported in the general adult population of Sicily (15), the present study sought to confirm such findings among residents in a small city of western Sicily and to identify risk factors associated with TOSV and SFSV infection.

Materials and methods

The study was carried out from September to July 2009 in Calatafimi-Segesta, a small city located in the province of Trapani, western Sicily, Italy. Calatafimi-Segesta has an overall surface area of 154 km^2, at an altitude of 338 m above sea level. The economy of Calatafimi-Segesta is primarily agricultural, the most important crops being citrus, grapes and olives.

Stratified by age and sex, we sought to recruit a 10% random sample (n=614) of the 6,161 people registered at the National Health System Patients Roster for Calatafimi-Segesta who were at least 4 years of age. Subjects were asked to participate and, after giving informed consent (for themselves and, as appropriate, for their minor children), were enrolled by their own medical practitioners.

A total of 134 subjects were excluded because of severe cognitive dysfunction (N=34), death before recruitment (N=10), residence other than Calatafimi-Segesta (N=52) or inability of the general practitioner to reach them within three months (N=38). The study was approved by the Institutional Review Board of the AOUP “P. Giaccone” of Palermo, Italy.

Questionnaire and blood sample collection

Demographic and risk factor data were collected with a structured questionnaire. Most of the questions were derived from 2001–2002 NHANES questionnaire (16). Each interview was conducted face-to-face by well trained medical personnel in a mean time of 20 minutes. All participants, on a voluntary basis, were asked to provide a venous blood sample. Interviews were performed in healthcare settings provided by the Local Health Agency (Azienda Sanitaria Provinciale) of Trapani.

The following questionnaire data were collected and analyzed:

- Sociodemographics: age; sex; marital status (single, married or free union, or widow or widower); occupation (student or un-employed, housewife, farmer,

employed, retired, or other); highest education level [illiterate, low (primary school), intermediate (lower secondary school), high (higher secondary school), or university]; living arrangement; household size; family housing characteristics [size in square meters, year of construction, ownership (yes or no) and ownership of an extra-urban holiday residence (yes or no)].

- Anthropometry: weight [kilograms (kg)], height [meters (m)] and body mass index (BMI) categorized as <18.5, 18.5 to 24.9, 25 to 29.9 and >29.9 kg/M^2.
- Lifestyle habits and animal exposure: cigarette smoking (non-smoker, former smoker or current smoker) and pet ownership (no; yes, living indoors; or yes, living outdoors).

Serological analyses

Sera were stored at 4°C for a maximum of 24 hours until they were delivered to a central laboratory where they were aliquoted and frozen at −20 °C for future analyses. All assays were performed, using blinded methods, in specialized laboratories that met national and international quality-control standards.

Anti-TOSV-specific immunoglobulin G (IgG) was detected in serum using an enzyme immunoassay with recombinant N protein (IgG/IgM TOS virus detection kit; DIESSE, Siena, Italy), following the procedures described in the kit instructions. Sera with a borderline value were further analysed by immunofluorescence assay (IFA) to detect anti-TOSV IgG with a procedure described elsewhere (17) Anti-SFSV-specific IgG was detected using a commercial IIFT (SFV IgG mosaic I; Euroimmun, Germany), as indicated by the manufacturer.

Statistical analysis

Data were analyzed using the R statistical software package (18). Statistical significance was defined as $p \leq 0.05$, two-tailed. Absolute and relative frequencies were calculated for qualitative variables. Categorical variables were analyzed using chi-square (Mantel-Haenszel) or chi-square for linear trend tests, as indicated. Chi-square also was used to determine if seropositivity against both TOSV and SFSV was greater than could be expected by chance.

Odds ratio (OR) and 95% confidence interval (95% CI) were calculated. Variables that were statistically significantly associated with seropositivity for TOSV IgG were included in backward stepwise logistic-regression models. Goodness of fit was calculated for each model, and the model with the lowest Akaike Information Criterion (AIC) was considered to have the best fit. Age was included in the models as a continuous variable. Models with age categorized as <15 years, 15 to 34 years, 35 to 54 years, 55 to 74 years and >74 years yielded nearly identical results (not presented).

Results

From the initial sample (N=614), 134 (21.8%) subjects did not meet inclusion criteria of the study, and 209 (34%) refused to participate overall or for this serological research. Sex and age did not differ significantly between enrolled and non-enrolled subjects (p=0.49 and p=0.25, respectively).

The general characteristics of the 271 subjects who participated in the study are reported in Table 1. Overall, 90 subjects (33.2%) were positive for TOSV IgG, 25 (9.2%) were positive for SFSV IgG, and 11 (4%) were positive for both the viruses. The observed frequency (4%)

of simultaneous TOSV and SFSV seropositivity was not significantly different than expected (3%) by considering the probability of single TOSV or SFSV positivity (p=0.23).

Risk factors for TOSV and SFSV seropositivity are presented in Table 2. No variable was significantly associated with SFSV seropositivity. A higher risk for TOSV seropositivity was found in participants who were older (p_{trend}=0.005), married or free union (OR=2.59; 95% CI=1.36–4.91), overweight or obese (OR=1.94; 95% CI=1.08–3.46 and OR=2.73; 95% CI=1.28–5.84, respectively), farmers (OR=8.5; 95% CI=1.74–41.5), former smokers (OR=2.0; 95% CI=1.05–3.78), owners of an extra-urban holiday residence (OR=1.98; 95% CI=1.19–3.33) and owners of a pet living outdoors (OR=2.68; 95% CI=1.49–4.82).

In the multivariate analysis, age (OR_{adj} =1.02 per year; 95% CI=1.01–1.03), having a pet living outdoors (OR_{adj} =2.62; 95% CI=1.42–4.83) and being obese (OR_{adj} =2.37; 95% CI=1.06–5.30) were significantly associated with an increased risk of TOSV seropositivity (Table 3).

Discussion

Following World War II, the widespread application of dichlorodiphenyltrichloroethane (DDT) in much of southern Europe was associated with a considerable decrease of malaria and other arthropod-borne virus vectors (19). This included a decline in the circulation of phleboviruses and the incidence of sand-fly fever. The banning of DDT could be responsible for an inversion of this trend as observed in Brazil where the discontinuation of DDT has been associated with a marked increase in abundance of the Anopheles vector and increased malaria cases (20). Consistent with this re-emergence hypothesis, an increasing abundance of some phlebovirus vectors has been reported in Italy (19). Concurrently, relatively high seroprevalence against phleboviruses has been found in general populations of the Mediterranean region, ranging from 5% in Spain (21) to 51.7% in Corfu (4) for TOSV and from 1% in France (22) to 32% in Cyprus for SFSV (23). The TOSV seroprevalence observed in our study well fits within the previously reported ranges, although it is higher than the 3% observed in northern Italy (24) and the 22% observed in central Italy (25). Data for SFSV seroprevalence in northern and central Italy are lacking. The high TOSV seroprevalence that we found seems to suggest a higher density or longer time of circulation of vectors in areas with a mild climate such as Sicily and, consequently, a higher probability of exposure to viruses and infection.

As reported by others (12), TOSV seroprevalence in our study was significantly lower in childhood and showed a linear increase with age. This is clinically important, as adults have a higher risk of TOSV-associated neurological complications than do children (12). It also is noteworthy that TOSV seroprevalence was elevated in subjects who had a pet living outdoors and, marginally significant, in owners of an extra-urban holiday residence. Moreover, TOSV seroprevalence was quite high (67%) in the 9 farmers in our study, although this was not significant in multivariable analysis. These variables suggest a relationship with the extra-urban environment, and, possibly, with domestic animals that may be reservoirs contributing to the basic maintenance cycles of the viruses. The last variable independently associated with TOSV infection was obesity but further studies are needed to understand why obese persons can have this higher infection risk.

Some of our null results should be noted, as they suggest a dissimilar ecologic distribution of the vectors or hosts involved in maintenance of the basic life cycle and transmission of these two phleboviruses. Seropositivity for TOSV was not significantly associated with SFSV seropositivity, and none of the risk factors associated with TOSV were associated

with SFSV. A possible explanation for the lack of these associations is that the two viruses are transmitted by different vectors which have different habitats.

Although SFSV seroprevalence was 9.2% overall, it was similar or even somewhat reduced in subjects who were obese (9.8%), owners of an outdoor pet (7.8%) or extra-urban residence (6.4%), and farmers (11%). SFSV seroprevalence, unlike TOSV, did not show a linear age-related trend. SFSV seroprevalence was, however, about 2-fold higher after age 64 (15.9%) compared to the younger subjects (7%). Perhaps when our oldest subjects were children, they were heavily exposed of *P. papatasii*, the main SFSV vector, until it was markedly suppressed by the extensive use of DDT. Otherwise, SFSV seropositive young individuals could be exposed to SFSV vectored by other sand-fly species, where the virus has also been recently isolated (5,26). Further investigations in other areas and larger populations would be required to test this hypothesis and assess if *P. papatasii* repopulation with discontinuation of DDT could result in increasing SFSV seroprevalence in the future.

The major limitations of this study are its relatively small sample size and restriction to the general population of a small city of western Sicily. Nonetheless, the TOSV and SFSV rates that we found in subjects aged 55 years or more (40.2% and 11.6%, respectively) were similar to those reported in a previous study (37% and 9%, respectively) carried out on adult individuals representative of the broader Sicilian population (15). Thus, our findings for Calatafimi-Segesta may be generalizable to the entire island population.

Despite these limitations, to our knowledge, the present study is the first that has investigated TOSV and SFSV seroprevalence with respect to socio-demographic and lifestyle characteristics in a general population. The relatively high seroprevalence, especially against TOSV among older adults who are susceptible to neurological complications, is a concern. The possibility that an increasing density of vectors could intensify circulation of these and other arboviruses should encourage the implementation of active surveillance systems to control phlebovirus infections and their related human health burden.

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Biography

Biographical Sketch: Dr. Giuseppe Calamusa is a medical doctor, specialist in Hygiene and Preventive Medicine and researcher at the University of Palermo (Italy). His main research interests involve the epidemiology of infectious diseases and public health.

Table 1

Characteristics of the study population (n=271)

		No.* (%)
Total participants		271 (100)
Sex	- M	126 (46.5)
	- F	145 (53.5)
Age group (years)	- <15	23 (8.6)
	- 15 to 24	16 (6.0)
	- 25 to 34	28 (10.5)
	- 35 to 44	42 (15.8)
	- 45 to 54	45 (16.9)
	- 55 to 64	49 (18.4)
	- 65 to 74	40 (15.0)
	- 75 or more	23 (8.6)
Body mass index (kg/M^2)	- <18.5	14 (5.3)
	- 18.5 to 24.9	89 (33.5)
	- 25 to 29.9	121 (45.5)
	- >29.9	42 (15.8)
Marital status	- Single	74 (27.6)
	- Married/free union	179 (66.8)
	- Widow/er	15 (5.6)
Highest education level	- Illiterate	17 (6.3)
	-Low education	82 (30.5)
	- Intermediate education	90 (33.5)
	- High education	43 (16.0)
	- University degree	37 (13.7)
Job	- Student/unemployed	42 (15.6)
	- Housewife	73 (27.0)
	- Self-employment	39 (14.4)
	- Farmer	9 (3.3)
	- Employed	49 (18.1)
	- Retired	50 (18.5)
	- Other	8 (3.0)
TOSV IgG positive		90 (33.2)
SFSV IgG positive		25 (9.2)
TOSV and SFSV IgG positive		11 (4.1)

Table 2

Univariate analyses of risk factors for TOSV and SFSV IgG seropositivity

	SFSV positives No. (%)	Odds ratio (95% Confidence interval)	TOSV positives No. (%)	Odds ratio (95% Confidence interval)
Sex				
- F	14 (9.7)	1	42 (29)	1
- M	11 (8.7)	0.89 (0.38–2.07)	48 (38.1)	1.51 (0.91–2.51)
Age group (years)				
- <15	1 (4.3)	1	1 (4.3)	1
- 15 to 24	2 (12.5)	3.14 (0.26–37.99)	4 (25)	7.3 (0.73–73.2)
- 25 to 34	2 (7.1)	1.69 (0.14–19.9)	8 (28.6)	8.8 (1.01–76.6) *
- 35 to 44	2 (4.8)	1.1 (0.09–12.8)	14 (33.3)	11.0 (1.34–90.1) *
- 45 to 54	4 (8.9)	2.14 (0.23–20.4)	17 (37.8)	13.4 (1.64–108.1) *
- 55 to 64	3 (6.1)	1.43 (0.14–14.6)	19 (38.8)	13.9 (1.73–111.9) *
- 65 to 74	5 (12.5)	3.14 (0.34–28.7)	21 (52.5)	24.3 (2.99–197.9) **
- 75 or more	5 (21.7)	6.1 (0.65–57.1)	5 (21.7)	6.1 (0.65–57.1)
Body mass index (kg/M^2)				
- < 24.9	7 (6.7)	1	25 (24)	1
- 25 to 29.9	14 (11.6)	1.81 (0.70–4.68)	46 (38)	1.94 (1.08–3.46) *
- >29.9	4 (9.8)	1.5 (0.41–5.42)	19 (46.3)	2.73 (1.28–5.84) **
Marital status				
- Single	9 (12.2)	1	15 (20.3)	1
- Married/free union	14 (7.8)	0.61 (0.25–1.48)	71 (39.7)	2.59 (1.36–4.91) **
- Widow/er	2 (13.3)	1.11 (0.21–5.75)	4 (26.7)	1.43 (0.4–5.13)
Highest education level				
- Illiterate	2 (11.8)	1	8 (47.1)	1
- Low, intermediate	15 (8.7)	0.72 (0.15–3.43)	51 (29.7)	0.47 (0.17–1.29)
- High, university	8 (10)	0.83 (0.16–4.32)	31 (38.8)	0.71 (0.25–2.04)
Job				
- Student, unemployed	3 (7.1)	1	8 (19)	1
- Housewife	8 (11)	1.6 (0.4–6.39)	21 (28.8)	1.72 (0.68–4.31)
- Farmer	1 (11.1)	1.62 (0.15–17.7)	6 (66.7)	8.5 (1.74–41.5) **
- Employed	2 (4.1)	0.55 (0.09–3.48)	18 (36.7)	2.47 (0.94–6.47)
- Retired	4 (8)	1.13 (0.24–5.36)	20 (40)	2.83 (1.09–7.37)
- Other	1 (12.5)	1.86 (0.17–20.5)	4 (50)	4.25 (0.87–20.74)
Smoking habits				
- No smoker	16 (11)	1	43 (29.5)	1
- Former smoker	2 (3.6)	0.31 (0.07–1.38)	25 (45.5)	2.0 (1.05–3.78) *

	SFSV positives No. (%)	**Odds ratio (95% Confidence interval)**	**TOSV positives No. (%)**	**Odds ratio (95% Confidence interval)**
- Current smoker	7 (10)	0.9 (0.35–2.3)	22 (31.4)	1.1 (0.59–2.03)
Owner of extra-urban holiday residence				
- No	17 (11.8)	1	38 (26.4)	1
- Yes	8 (6.4)	0.51 (0.2–1.22)	52 (41.6)	1.98 (1.19–3.33) **
Having a pet				
- No	18 (9.8)	1	50 (27.2)	1
- Yes, living indoor	0 (0)	0.47 (0.02–2.78)	7 (35)	1.44 (0.54–3.82)
- Yes, living outdoor	5 (7.8)	0.78 (0.28–2.2)	32 (50)	2.68 (1.49–4.82) ***

* p-value<0.05

** p-value<0.01

*** p-value<0.001

Table 3

Best fitting logistic regression model, by Akaike's Information Criterion, for TOSV IgG seropositivity

	Adjusted OR (95% CI)	p-value
Age, in years	1.02 (1.01–1.03)	0.027
Owner of extra-urban holiday residence		
- No	1	
- Yes	1.75 (0.99–2.93)	0.054
Having a pet		
- No	1	
- Yes, living indoor	1.56 (0.56–4.32)	0.39
- Yes, living outdoor	2.64 (1.41–4.93)	0.002
BMI		
- < 24.9	1	
- 25 to 29.9	1.51 (0.81–2.82)	0.19
- >29.9	2.37 (1.06–5.30)	0.036