Occupational risk associated with Toscana virus infection in Tuscany, Italy

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**Background** Sandfly fever virus serotype Toscana is endemic in Mediterranean countries and is a widespread public health problem as it may be associated with acute neurological diseases such as aseptic meningitis.

**Aim** To assess whether Toscana virus infection is associated with occupational exposure.

**Methods** During the summer of 2001, a total of 678 blood samples were taken from healthy subjects residing in Tuscany (349 agricultural and forestry workers and 329 control subjects living in the same areas). Information on age, type of job and lifestyle was collected in a questionnaire, and sera were analysed using enzyme-linked immunosorbent assay for Toscana virus-specific antibodies.

**Results** Seropositivity to antibodies to Toscana virus was 30% in the control group and 23% in the agricultural and forestry workers ($P < 0.05$). Questionnaire responses suggested that, although the agricultural and forestry workers had a potentially greater continuous exposure to sandfly fever virus (outdoor activities, rural residence and at-risk lifestyle), these risk factors did not add significant information about greater susceptibility to disease.

**Conclusion** Our findings indicate a need for better understanding of the best preventive measures to avoid the risk related to sandfly bites, especially for people who live or work in areas at risk.

**Key words** Agricultural workers; forestry workers; occupational infection; outdoor activity; risk factors; sandfly fever; seroprevalence; Toscana virus.

**Introduction**

Sandfly fever virus serotype Toscana (TOSv) (family Bunyaviridae, genus Phlebovirus) was originally isolated from the sandfly Phlebotomus perniciosus, in central Italy in 1971 [1]. TOSv is endemic in Mediterranean countries, where the insect vectors ($P$ perniciosus and Phlebotomus perfilievi) are common [2]. It is a widespread public health problem since it may be associated with acute neurological diseases, mainly aseptic meningitis [3]. It can infect humans under favourable environmental and climatic conditions. Deaths and disease resulting from climate change [4] are a focal point in the human health sector today, and the geographical distribution and population dynamics of arthropod vectors causing infectious diseases are closely related to patterns of temperature, rainfall and humidity. Current mitigation measures (e.g. reduction of exposure, preventive behavioural measures etc.) are therefore important. TOSv diseases in humans occur particularly during the summer, with a peak in August, correlating with the life cycle of the Phlebotomus vectors [5–8].

Most disease cases have been reported in residents or travellers in central Italy or Spain and sporadically from other Mediterranean countries such as Cyprus, southern France, Greece and also Portugal [9]. Studies in Italy have documented the circulation of the virus not only in Tuscany but also in other regions of the country such as Marche, Umbria, Emilia-Romagna, Campania, Piedmont and Sardinia [8,10–14].

The clinical manifestation of meningitis from TOSv is similar to that caused by other viral agents. The clinical course is generally benign, and asymptomatic infection or infections without central nervous system involvement have also been described [15].

A high prevalence of antibodies against TOSv has been found in the healthy population [2], but meningitis is less frequent among individuals from endemic zones, probably because of anti-TOSv antibodies in resident adults [16].
Populations living in rural areas and with high levels of outdoor activity are at the greatest risk of TOSv infection. Similarly, outdoor workers may be exposed to the sandfly fever-borne virus as well as other vector-borne microorganisms, if not adequately protected.

This study gathers the results of a serological survey, performed in August 2001 in agricultural, forestry workers and others living in the same areas in Tuscany. Specific antibodies to TOSv were investigated by serological techniques and screening of anti-sandfly fever Naples virus (SFNv) antibodies was also performed in people >55 years of age. The aim of the study was to evaluate whether this infection was associated with occupational exposure and to identify if any group was particularly vulnerable.

**Methods**

The study was conducted in Tuscany (Italy), and the study population comprised a total of 678 healthy adults; there were 349 agricultural and forestry workers, assumed to be at risk of exposure to sandfly bites (exposed subjects), and 329 controls. A total of 94 agricultural and forestry workers and 136 controls came from Florence province and 255 and 193 from Arezzo province.

The study was conducted in the context of periodical health surveillance of agricultural and forestry workers. In accordance with Italian ethical guidelines, an interview questionnaire was administered to all subjects and written informed consent was obtained from all participants who agreed to participate in the study. All data were treated confidentially and analysed anonymously.

The exposed group, referred to as outdoor workers, was in principle considered at greater risk of exposure to sandfly bites. The control group was selected as coming from the same areas as exposed workers and were all blood donors. Subjects in the control group were not occupationally exposed. Each person's main medical history and personal details were recorded, and none had any history of neurological symptoms. The seroprevalence to the infection under study was analysed in two cities: Florence and Arezzo.

Information collected in questionnaires included patient medical history data, occupation, past duties, leisure activities, time spent outdoors, hobbies (frequency and duration), history of recent and past insect bites, place of residence (rural or urban), diseases in the past or still present and use of medicinal drugs. Exposed workers also gave information about personal protective equipment (PPE), its use at work and the duration of the current employment. The use of insect repellents was not included in the questionnaire as it was felt that they were reasonably used by both groups.

This information was used to identify risk factors, which were then analysed to obtain a general description of the two groups and to see whether these risk factors might influence exposure to the virus.

Sera were collected from all exposed workers and controls and stored at −80°C until laboratory analysis. Serologic tests were done at the Arbovirus Unit of the Istituto Superiore di Sanità, Rome, Italy. Anti-TOSv IgG in sera were tested by an enzyme-linked immunosorbent assay (ELISA), previously described [3,7,17–19]. TOSv and SFNv neutralization tests were done with TOSv IgG-positive sera. Virus used for neutralization tests was Sandfly fever Naples used as a 10% brain suspension in the 52nd mouse passage. Infectivity titrations were performed by plaque assay using VERO cells. The assay was carried out as previously described [20].

About half of the samples were also tested using a commercial kit ELISA (Disesse Kit, Siena, Italy) to compare the results and to verify if the in-house-developed test gave comparable results.

The statistical package SPSS 13.0 for Windows was used for data entry and analysis. Data analysis was divided into three phases: first, the exposed workers and the controls were examined for differences in demographic characteristics, seropositivity distribution and differences in risk factors; even if the data come from a serological survey, it is not uncommon to apply retrospectively case-control techniques [21]. Then, only the seropositive subjects were considered from both groups, and differences in the presence of risk factors between seropositive controls and seropositive exposed workers were checked. In both analyses, Pearson chi-square tests on two-way contingency tables were computed, with statistical significance value set at the standard threshold of ≤0.05. Finally, a multivariate logistic regression model was estimated on the whole data sample to evaluate the joint effect of risk factors on seropositivity.

**Results**

Controls and exposed subjects had different sex distributions: there were 26.5% of women in the control group and 73.5% men. Among exposed workers, the figures were 5.5 and 94.5%, respectively (chi square \( P < 0.001 \)). This difference was not considered a problem in the retrospective analysis since sex was not significantly related to seropositivity (chi square \( P > 0.05 \)).

The mean age ± standard deviation was similar in the two groups: 43 ± 8 years for the controls and 44 ± 11 years for the exposed group. When age was subdivided into <35, 35–45, 46–55 and >55 years, the 35–45 years category predominated in the control group, while the exposed workers were more equally age distributed. However, here again, this difference was not relevant in the retrospective analysis since age classes were not significantly related to seropositivity (chi square \( P > 0.05 \)) (Figure 1a).

As regards the place of origin, the exposed group came mainly from Arezzo and the control group was more evenly distributed between the two cities (Figure 1b).

In analysing the questionnaires, the risk factors were grouped under environmental factors, work-related
outdoor activities and extra-occupational activities. For the exposed workers, we also considered the use of PPE. In the whole dataset recreational trekking and camping were significantly more frequent among controls, and all the other risk factors were predominant among exposed workers (data not shown).

The exposed subjects from Arezzo and Florence were divided into three occupational categories: forestry, farmer and agricultural labourers. The seropositivity of samples for these different categories showed no significant difference (data not shown).

Exposed and control subjects. 55 years old, from Florence and Arezzo, who had a positive anti-TOSv IgG ELISA result, were also screened for cross-reactions due to anti-SFNv antibodies by the SFNv neutralization test. All 36 subjects analysed tested negative for anti-SFNv neutralizing antibodies.

Of 678 human sera collected, just over a quarter were positive and nearly three quarters negative (Table 1). In the control group, 30% of samples were positive compared to 23% in the agricultural and forestry workers group; this difference was significant \( P < 0.05 \). Therefore, the factor ‘work at risk’ did not play a major role in the transmission of TOSv infection to people. We also confirmed about half of our IgG ELISA results, obtained with an in-house-developed test, using a commercial kit, and the results were comparable (data not shown).

The results regarding the place of origin indicated that there were 88 positive samples from Arezzo (20% of the total of Arezzo samples) and 90 from Florence (39% of the total of Florence samples). Table 2 shows that the distribution of seropositive samples among controls and exposed workers differed depending on their origin; in Florence 63% of the positive subjects were controls, while the percentage of positive samples in Arezzo was more evenly distributed between controls and exposed workers (48 and 52%).

Several environmental and lifestyle risk factors differed significantly between the exposed and control seropositive subjects. In the seropositive exposed group, rural residence, presence of insects, garden and rivulet close to home, recreational activities like gardening, livestock breeding, agriculture, hunting and fishing were more frequent. Trekking and camping were in contrast significantly more frequent in seropositive controls (Table 3).

Finally, results of the stepwise multivariable logistic regression model on the whole dataset analysing the joint effects of risk factors on positive results indicated that the controls were at a 1.4 times higher risk than exposed subjects [odds ratio (OR) = 1.41, 95% confidence interval (CI): 1.1–2.07] and people from Arezzo were less vulnerable to TOSv infection than those from Florence (OR = 0.4, 95% CI: 0.28–0.59).

From the questionnaires, it was possible to analyse the habits of exposed workers about using PPE in the workplace (i.e. gloves, clothes, masks, caps, safety shoes and boots and personal hygiene—shower at the end of shift). The lower seroprevalence of antibodies to TOSv in the exposed workers might reflect their regular good utilization of PPE. The results also suggested that these subjects probably also adopted preventive strategies in their extra-occupational and recreational activities.

**Discussion**

This study found that 23% of agricultural and forestry workers potentially exposed to the Toscana virus were
positive to anti-TOSv antibodies compared to 30% of the subjects in the control group. Seroprevalence results were in agreement with the statistics for the circulating virus in Tuscany [2]. Data from the questionnaires showed that environmental and lifestyle risk factors differed significantly between the occupationally exposed and the control group. Details of the demographic and behaviour characteristics of all subjects represent a strong point of our study since we believe that only data arising from a well-characterized population can be reliably compared.

Little is known about the role of arthropod-borne viruses in occupationally acquired infections. Regarding TOSv infection, Valassina et al. [22] reported a seroprevalence of Toscana virus in 77.2% of forestry workers, higher than in a non-exposed population, in the Tuscany region. Our study, however, did not show a higher seroprevalence in exposed workers when compared to non-exposed controls—rather the opposite. Differences in laboratory techniques in these two studies cannot account for the results since we also confirmed our IgG ELISA data, obtained with an in-house-developed test, using a commercial kit.

The possibility that in people >55 years of age the positive anti-TOSv IgG ELISA results might be due to cross-reactive anti-SFNv antibodies was investigated, but they were all negative. Screening of anti-SFNv antibodies was not performed in younger subjects since the circulation of SFNv, another member of the Phlebovirus group, was interrupted in Italy after malaria control programmes, which resulted in a dramatic reduction of the vector, Phlebotomus papatasi, to levels not permitting viral transmission.

The exposed workers selected for this study were expected to have greater susceptibility to sandfly bites since they had more risk factors, including a lot of hours spent outdoors. However, they were less likely to have an exposure to TOSv than the control group because the sandflies bite during the first hours of the night [23].

Moreover, the exposed workers did seem to apply preventive measures confirming a significant role of PPE in the field of occupational health and safety, probably because they had a sharper perception of the risk. Current information about risk assessment in the workplace suggests that when the risk cannot be eliminated, strict protective and preventive measures must be adopted.

Our study indicated that the circulation of TOSv in Toscana requires active and sensitive surveillance in different settings: educating people about self-protection, instituting public health programmes directed against sandflies and specifically training professionals such as veterinarians, physicians and biologists in wildlife health management.

Moreover, since a limitation of this research is that it describes the seroprevalence to TOSv only in Tuscany, further similar studies will be necessary in other areas to better evaluate the spread of TOSv infection in Italy.

Table 2. Distribution of the place of origin of positive results in controls and exposed subjects

<table>
<thead>
<tr>
<th>Place of origin</th>
<th>Positive results</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls n (%)</td>
<td>Exposed subjects n (%)</td>
</tr>
<tr>
<td>Arezzo</td>
<td>42 (48)</td>
<td>46 (52)</td>
</tr>
<tr>
<td>Florence</td>
<td>57 (63)</td>
<td>33 (37)</td>
</tr>
<tr>
<td>Total</td>
<td>99 (56)</td>
<td>79 (44)</td>
</tr>
</tbody>
</table>

Chi square P < 0.05.

Table 3. Main demographic characteristics and risk factors in seropositive control subjects and exposed workers from Tuscany, Italy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Seropositive controls n (%)</th>
<th>Seropositive exposed n (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>78 (81)</td>
<td>70 (97)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Female</td>
<td>18 (19)</td>
<td>2 (3)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>13 (13)</td>
<td>17 (24)</td>
<td>n.s.</td>
</tr>
<tr>
<td>35–45 years</td>
<td>30 (31)</td>
<td>23 (32)</td>
<td></td>
</tr>
<tr>
<td>46–55 years</td>
<td>41 (42)</td>
<td>18 (25)</td>
<td></td>
</tr>
<tr>
<td>&gt;55 years</td>
<td>14 (14)</td>
<td>4 (19)</td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>74 (75)</td>
<td>37 (47)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rural</td>
<td>25 (25)</td>
<td>42 (53)</td>
<td></td>
</tr>
<tr>
<td>Presence of insects</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Garden at home</td>
<td>48 (48)</td>
<td>51 (65)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cesspool</td>
<td>12 (12)</td>
<td>9 (11)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Rivulet close to home</td>
<td>14 (14)</td>
<td>26 (33)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Domestic animals in house</td>
<td>52 (52)</td>
<td>48 (61)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Recreational activities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hunting</td>
<td>12 (12)</td>
<td>30 (38)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fishing</td>
<td>7 (7)</td>
<td>13 (16)</td>
<td>0.05</td>
</tr>
<tr>
<td>Gardening</td>
<td>35 (35)</td>
<td>51 (65)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Animal breeding</td>
<td>21 (21)</td>
<td>27 (34)</td>
<td>0.05</td>
</tr>
<tr>
<td>Trekking</td>
<td>58 (59)</td>
<td>31 (39)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Agriculture</td>
<td>21 (21)</td>
<td>32 (40)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Camping</td>
<td>16 (16)</td>
<td>5 (6)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Data may be limited because of missing values for some variables. n.s., not significant.

Key points
- Of the 678 human blood samples (agricultural and forestry workers and control subjects), 26% reported positivity to anti-Toscana virus antibodies.
- Highest prevalence of Toscana virus antibodies was reported in the control group.
- Agricultural and forestry workers did seem to apply effective preventive measures, confirming a significant role of PPE in the field of occupational health and safety.
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Conflicts of interest
None declared.

References