Serosurvey Study of Toscana Virus in Domestic Animals, Granada, Spain

José María Navarro-Marí,1 Begoña Palop-Borrás,2,* Mercedes Pérez-Ruiz,1,* and Sara Sanbonmatsu-Gámez1

Abstract

Toscana virus (TOSV) is transmitted by infected sandflies. In Mediterranean countries, TOSV is one of the major viral pathogens involved in aseptic meningitis and meningoencephalitis in humans. It remains unclear if there are animal reservoirs able to maintain the virus through the cold months of the year, when the vector is not circulating. From May to October of 2006 and 2007, we conducted a serosurvey study on domestic animals from Granada province (south of Spain). TOSV was investigated in 1186 serum samples from horses, goats, pigs, cats, dogs, sheep, and cows by serology (indirect fluorescence assay), viral culture, and RT-polymerase chain reaction. Specific anti-TOSV antibodies were detected in 429 (36.2%) serum samples. The highest seropositivity rates were observed in cats (59.6%) and dogs (48.3%). These results suggest that an important percentage of the domestic animals have been infected by TOSV. Significantly different seroprevalence rates were detected in goats among distinct geographical areas. All viral cultures were negative. TOSV was detected by RT-polymerase chain reaction in only one serum sample from a goat. Thus, the studied animals do not seem to act as reservoirs for TOSV; otherwise, they could be amplifying hosts for the virus.

Key Words: ecology—seroprevalence—Toscana virus.

Introduction

TOSCAN A VIRUS (TOSV; genus Phlebovirus, family Bunyaviridae) is an arbovirus (Arthropod-borne virus) transmitted by the bite of a phlebotomine fly. TOSV was first isolated from the sandfly Phlebotomus perniciosus (Verani et al. 1980) and later from P. perfiliewi. TOSV has been isolated from Phlebotomus spp. in Italy (Verani et al. 1984) and Spain (Sanbonmatsu-Gámez et al. 2005), and recently from Sergentomyia minuta in France (Charrel et al. 2006).

TOSV is one of the major pathogens involved in aseptic meningitis and meningoencephalitis in Mediterranean countries (Charrel et al. 2005) during summer, coinciding with the maximum activity of the vector. Serosurvey studies in humans from endemic areas have demonstrated high TOSV seroprevalence rates, ranging from 16% to 26% (Eitrem et al. 1991, Braito et al. 1998, Echevarría et al. 2003, Sanbonmatsu-Gámez et al. 2005, de Ory-Manchoñ et al. 2007). These data suggest that many TOSV infections might be asymptomatic or paucisymptomatic. Mild febrile illness due to TOSV-related phleboviruses such as sandfly Naples and Sicilian viruses has been reported (Depaquit et al. 2010). Similarly, TOSV could be responsible of mild diseases, which are underdiagnosed because TOSV is not usually investigated in outpatients.

Survival of TOSV during the cold months of the year, when the vector is not circulating, could be explained by vertical and sexual transmission of TOSV among sandflies (Tesh and Modi 1987, Verani et al. 1988, Tesh et al. 1992). It remains unknown if animal reservoirs could also be involved in TOSV survival throughout the year. Up to date, no evidence of TOSV infection in domestic and wild vertebrate animals has been documented, except for one isolate of TOSV recovered from the brain of a bat (Verani et al. 1988).

To identify possible animal reservoirs of TOSV, we conducted a study to analyze the seroprevalence rates as well as the presence of TOSV by viral culture and RT-polymerase chain reaction (PCR) in domestic animals in Granada province (south of Spain), where TOSV had been previously detected in humans (Mendoza-Montero et al. 1998) or in sandflies (Sanbonmatsu-Gámez et al. 2005).
Materials and Methods

Samples

Serum samples from the following domestic animals were used: dogs, cats, cows, sheep, goats, horses, and pigs. Sera from cats, dogs, and horses were provided by a veterinary laboratory (ANLAVE) in Granada, and sera from the remaining animals were provided by the Health Animal Laboratory (Junta de Andalucía, Santa Fé, Granada, Spain). The latter lived in livestock farms and villages from areas where TOSV had been detected in humans or in sandflies.

To determine the role of these animals in the maintenance of TOSV during the cold months of the year, when the vector is not circulating, the study period was comprehended between October and May of 2006 and 2007.

Seroprevalence study

Anti-TOSV seroprevalence study was carried out by indirect fluorescent assay (IFA) (Hodinka 1999). TOSV antigen was prepared from isolates obtained in Vero cell cultures. For this purpose, a TOSV strain, Spanish genotype (Sanbonmatsu-Gámez et al. 2005), was inoculated in tubes with Vero cell monolayers following standard protocols (Karabatos 1994, Hsiung 1994), and incubated at 35°C until the appearance of cytopathic effect (CPE). Cell cultures containing infected and uninfected cells were harvested and fixed on 18-well slides with cold acetone and subsequently frozen at −80°C until use. IFA was carried out by adding 10 µL of serum sample to each well and incubated at 35°C for 30 min in humidity chamber. After incubation, species-specific fluorescein isothiocyanate (FITC)-labelled antiglobulins were added to the wells and incubated at 35°C for 30 min as described above. Observation of the specific fluorescent foci by 40× field examination was recorded. Positive controls obtained with anti-TOSV-positive human serum samples were used to determine the pattern of fluorescence.

Specificity of the assay was indicated by the absence of fluorescence when animal sera were tested with uninfected Vero cells. For this purpose, up to 20 serum samples from each animal species were randomly selected. An initial 10-fold dilution and subsequent twofold dilutions of each sample were subjected to IFA using slides with uninfected Vero cells. The working dilution of each animal serum sample was the lowest titre that did not show nonspecific fluorescence in uninfected cells (Table 1).

Detection of TOSV by viral culture and real-time RT-PCR

Aliquots of the serum samples were used each for viral culture and TOSV-specific real-time RT-PCR.

Tubes with Vero cell monolayers were inoculated with 100 µL of serum sample, incubated at 35°C, and examined daily for the appearance of CPE. After 14 days of incubation or when CPE was observed, 1 mL of cell culture supernatant from each tube was frozen at −80°C for further investigation of TOSV by RT-PCR.

Qiamp RNA viral kit (Qiagen, Hilden, Germany) was used for nucleic acids extraction. For this purpose, a 100-µL aliquot from each serum sample was mixed with 400 µL of lysis buffer AVL (provided with the kit), and subsequent RNA extraction was carried out according to the manufacturer’s instructions.

For molecular detection of TOSV in viral culture, a 100-µL aliquot of cell culture supernatants was pooled in order of three and subjected to automatic nucleic acids extraction by using the Nuclisens easyMAG system (BioMérieux, Lyon, France) following the manufacturer’s instructions.

TOSV real-time RT-PCR was carried out using a previously described protocol (Pérez-Ruiz et al. 2007), with minor modifications. Briefly, cDNA was synthesized from 10 µL of extracted RNA using iScriptTM cDNA synthesis (Bio-Rad Laboratories Headquarters, Hercules, CA). Real-time PCR was performed with 6 µL of cDNA, 4 µL of LightCycler FastStart DNA MasterPLUS Hybridization Probes kit (Roche Diagnostics, Mannheim, Germany), 0.5 µM of each sense and antisense primer, and 0.2 µM of 6FAM-labelled Taqman® probe. The amplification was performed in a LightCycler II Instrument (Roche Diagnostics). Reaction conditions were denaturation at 95°C for 10 min followed by 45 cycles of denaturation for 5 s at 95°C (ramp rate, 20°C/s) and annealing of primers, and extension for 20 s at 60°C (ramp rate, 20°C/s). A single fluorescence reading in channel 530 was taken in each cycle at the extension step. Semiquantitative measures were expressed by determination of the threshold cycle of detection (Ct).

Positive (TOSV strain) and negative (water) controls were included from the beginning of the procedure in each run.

Statistical analysis

Data were statistically analyzed with the SPSS 15.0 software (SPSS, Chicago, IL). Along with descriptive statistics, bivariate analysis was performed to compare demographic

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Starting dilution</th>
<th>Antiglobulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow</td>
<td>1:20</td>
<td>Anti-bovine IgG1,2 polyclonal antiserum FITC (goat)a</td>
</tr>
<tr>
<td>Goat</td>
<td>1:80</td>
<td>Anti-goat IgG polyclonal antiserum FITC (rabbit)a</td>
</tr>
<tr>
<td>Pig</td>
<td>1:20</td>
<td>Anti-pig IgG polyclonal antiserum FITC (rabbit)a</td>
</tr>
<tr>
<td>Sheep</td>
<td>1:80</td>
<td>Anti-sheep IgG antiserum FITC (rabbit)b</td>
</tr>
<tr>
<td>Cat</td>
<td>1:20</td>
<td>Anti-feline IgG(H+L)-FITC conjugatec</td>
</tr>
<tr>
<td>Horse</td>
<td>1:40</td>
<td>Anti-equine IgG(Fe)-FITC conjugatec</td>
</tr>
<tr>
<td>Dog</td>
<td>1:20</td>
<td>Dog IgG(H+L)-FITC conjugatec</td>
</tr>
</tbody>
</table>

*aVMRD (Fullman, WA).

bBETHYL Lab (Fullerton, CA).

Fuller Lab (Fullerton, CA).
and epidemiological data with laboratory results by chi-square test, and subsequent analysis of corrected residues to compare variables two-by-two from statistically significant analysis by the chi-square test. A p-value < 0.05 was considered statistically significant.

Results

A total of 1186 serum samples were analyzed from the following animals: 286 dogs, 243 goats, 229 sheep, 213 cats, 151 cows, 50 pigs, and 14 horses. Anti-TOSV antibodies were detected in 429 (36.2%) specimens from 138 dogs, 43 goats, 74 sheep, 127 cats, 27 cows, 11 pigs, and 9 horses. The observed distribution of seroprevalence rates was 48.3% in dogs, 17.7% in goats, 32.3% in sheep, 59.6% in cats, 17.9% in cows, 22% in pigs, and 64.3% in horses (p < 0.001).

To determine differences in seroprevalence rates within the same animal among distinct areas, cats, dogs, and horses were subgrouped by five geographical areas in Granada province, as reported previously (Sanbonmatsu-Gámez et al. 2005): urban, metropolitan, south, west/southwest, and north/northeast. Goats, sheep, pigs, and cats were subgrouped by the village of origin, all of which were within the metropolitan area.

Anti-TOSV seroprevalence rates in each animal species were analyzed by geographical areas, and no statistical differences were observed except for goats (Tables 2 and 3). Chi-square test showed different seroprevalence rates among goats compared by geographical origin (p < 0.001). Analysis of corrected residues demonstrated that significantly higher rates were found in goats from village no. 1 (Table 3).

A TOSV-positive serum sample from a goat was detected by RT-PCR, belonging to a herd from village no. 1, collected in December 2006. TOSV was not detected in any of the 1186 serum samples by cell culture and subsequent RT-PCR.

Discussion

Life cycle of many arboviruses includes arthropods as vectors and vertebrates as reservoirs or amplifying hosts (Gould et al. 2006). Rift Valley fever virus, a member of the genus Phlebovirus, has been isolated from humans, cattle, and rodents during epidemic outbreaks (Beaty and Calisher 1991). Although the reservoir for Rift Valley fever virus during interepizootic periods remains unknown, bats have been proposed as potential reservoirs (Boiro et al. 1987, Oelofsen and Van der Ryst 1999). Previous data from TOSV seroprevalence studies in domestic and wild animals showed no evidence of infection. TOSV could only be isolated from the brain of a bat (Pipistrellus kuhlii) (Verani et al. 1988).

The most probable vector for TOSV in Spain is P. perniciosus (Sanbonmatsu-Gámez et al. 2005). P. perniciosus feeds on almost all animals when they are accessible. However, previous studies have demonstrated that this species has some preferences and rarely feeds on chickens, but frequently feeds on dogs and sheep (Colmenares et al. 1995, Bongiorno et al. 2003).

The aim of this study was to identify a possible vertebrate host as reservoir for TOSV that could explain the persistence of the virus during the cold months of the year, when the vector is not circulating. From October to May, we conducted a study of TOSV infection in domestic animals that might be potential hosts for P. perniciosus from areas where TOSV had been previously detected in humans (Mendoza-Montero et al. 1998) or in sandflies (Sanbonmatsu-Gámez et al. 2005).

Unlike Verani’s study (Verani et al. 1988), we found a high seroprevalence rate for TOSV antibodies (36.2%) among domestic animals. These data agree with the feeding habits of P. perniciosus, since high seroprevalence rates were observed in cats (59.6%), dogs (48.3%), and sheep (32.3%) (Colmenares et al. 1995, Bongiorno et al. 2003). Although we ruled out unspecific fluorescent reading with the serum working dilution, we did not carry out neutralization assays to confirm positive results. Cross-reactions might have contributed to overestimate the seroprevalence rate. Serological cross-reactions have been reported within the sandfly fever Naples virus complex (Depaquit et al. 2010), which TOSV is member of. However, the use of serum dilutions from each animal would yield a deleterious effect on sensitivity but would increase specificity of the IFA.

Serological data indicate that an important percentage of domestic animals have been infected by TOSV; however, the virus could not be isolated from any of the 1186 serum samples. Only one sample from a goat was positive for TOSV by RT-PCR. This goat was from the location where the highest seroprevalence rate was detected. The test was repeated to rule out cross-contamination and the same result was obtained. Low-level viremia was observed since a cycle threshold value in the real-time PCR above 35 was obtained in the two assays. Further, Sanbonmatsu-Gámez et al. (2005) reported TOSV detection in 3 out of 103 pools of phlebotomines collected in different areas of Granada province. One of these pools was obtained from sandflies within the same village (village no.1) where the highest seroprevalence rate was observed in goats and where the positive TOSV RT-PCR was obtained in this study. The other two positive pools were collected from a village neighbouring village 1, both

Table 2. Anti-Toscana Virus Antibodies in Cats and Dogs by Geographical Area

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Anti-Toscana virus antibodies by geographical area</th>
<th>Total</th>
<th>Urban</th>
<th>Metropolitan</th>
<th>South</th>
<th>West/southwest</th>
<th>North/northeast</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>Pos. (%)‡</td>
<td>213</td>
<td>55</td>
<td>127</td>
<td>23</td>
<td>2</td>
<td>6</td>
<td>0.673</td>
</tr>
<tr>
<td>Pos. (%)‡</td>
<td></td>
<td>127</td>
<td>34</td>
<td>72</td>
<td>15</td>
<td>1</td>
<td>5</td>
<td>0.673</td>
</tr>
<tr>
<td>Dogs</td>
<td></td>
<td>286</td>
<td>57</td>
<td>183</td>
<td>35</td>
<td>5</td>
<td>1</td>
<td>0.502</td>
</tr>
<tr>
<td>n</td>
<td>Pos. (%)‡</td>
<td>138</td>
<td>22</td>
<td>95</td>
<td>16</td>
<td>3</td>
<td>2</td>
<td>0.502</td>
</tr>
<tr>
<td>Pos. (%)‡</td>
<td></td>
<td>138</td>
<td>22</td>
<td>95</td>
<td>16</td>
<td>3</td>
<td>2</td>
<td>0.502</td>
</tr>
</tbody>
</table>

‡Nine out of 14 horses were positive for anti-Toscana virus antibodies.

Table 2. Anti-Toscana Virus Antibodies in Cats and Dogs by Geographical Area

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Anti-Toscana virus antibodies by geographical area</th>
<th>Total</th>
<th>Urban</th>
<th>Metropolitan</th>
<th>South</th>
<th>West/southwest</th>
<th>North/northeast</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>Pos. (%)‡</td>
<td>213</td>
<td>55</td>
<td>127</td>
<td>23</td>
<td>2</td>
<td>6</td>
<td>0.673</td>
</tr>
<tr>
<td>Pos. (%)‡</td>
<td></td>
<td>127</td>
<td>34</td>
<td>72</td>
<td>15</td>
<td>1</td>
<td>5</td>
<td>0.673</td>
</tr>
<tr>
<td>Dogs</td>
<td></td>
<td>286</td>
<td>57</td>
<td>183</td>
<td>35</td>
<td>5</td>
<td>1</td>
<td>0.502</td>
</tr>
<tr>
<td>n</td>
<td>Pos. (%)‡</td>
<td>138</td>
<td>22</td>
<td>95</td>
<td>16</td>
<td>3</td>
<td>2</td>
<td>0.502</td>
</tr>
<tr>
<td>Pos. (%)‡</td>
<td></td>
<td>138</td>
<td>22</td>
<td>95</td>
<td>16</td>
<td>3</td>
<td>2</td>
<td>0.502</td>
</tr>
</tbody>
</table>
separated by approximately 1 km (S. Sanbonmatu-Ga´ mez, pers. comm.). Indeed, this area was selected in this work and Sanbonmatu-Ga´ mez et al. (2005) study because cases of TOSV meningitis had taken place in past few years. Thus, this seems to be a TOSV hyperendemic area.

Vertical and sexual transmission of TOSV among sandflies has been demonstrated (Ciufolini et al. 1985), which could explain the survival of TOSV during the cold seasons of the year, when the vector does not circulate (Tesh et al. 1992). However, the rate of vertically infected sandflies decreases in each generation, suggesting that alternative mechanisms for virus maintenance should exist (Tesh and Modi 1987). Transient and low-level viremia has been described in humans and animals after natural or experimental Phlebovirus infection (Bartelloni et al. 1976, Cusi et al. 2001). Even though humans may play a role in the life cycle of TOSV by infecting naïve sandflies, the vector must ingest large quantity of virus to become infected. Thus, only vertebrate hosts with high-level viremia could be capable of infecting sandflies (Ciufolini et al. 1985).

We were not able to detect enough amounts of TOSV RNA in serum sample from the studied animals. Otherwise, Leishmania spp., also transmitted by phlebotomines, have a recognized canine reservoir, and high-level parasitemia has been demonstrated by PCR in dogs (Aoun et al. 2009). Similarly, we could speculate that TOSV RNA should have been detected in serum samples in this study to argue that an animal reservoir is involved in TOSV life cycle. Indeed, previous reports have suggested that TOSV reservoir can be the sandfly itself, and vertebrate animals would serve as amplifying hosts during warm season when the activity of sandflies is maximum (Tesh et al. 1992).

Further TOSV investigation conducted on wild and/or other domestic animals should be carried out to acknowledge if other reservoirs for the virus exist.

**Acknowledgments**

We are grateful to the veterinary laboratory ANLAVE (Granada, Spain) and Laboratory of Animal Health (Santa Fe, Granada, Spain) for kindly providing us the serum samples used in this work. We also thank the staff from the Virology Unit, Microbiology Service, from the Hospital Universitario Virgen de las Nieves (Granada, Spain), for their excellent technical assistance. This study was supported in part by founds from a research project, 05/305 (Junta de Andalucı´ a, Spain).

**Disclosure Statement**

No competing financial interests exist.

**References**


---

**Table 3. Anti-Toscana Virus Antibodies in Goats, Sheep, and Cows by Geographical Area**

<table>
<thead>
<tr>
<th>Village no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>n</td>
<td>243</td>
<td>45</td>
<td>31</td>
<td>25</td>
<td>22</td>
<td>19</td>
<td>15</td>
<td>12</td>
<td>62</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Pos. (%)</td>
<td>n</td>
<td>43</td>
<td>7</td>
<td>12</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>18</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Goats</strong></td>
<td>n</td>
<td>50</td>
<td>30</td>
<td>25</td>
<td>20</td>
<td>15</td>
<td>10</td>
<td>8</td>
<td>5</td>
<td>25</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Pos. (%)</td>
<td>n</td>
<td>11</td>
<td>7</td>
<td>10</td>
<td>6</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>9</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Sheep</strong></td>
<td>n</td>
<td>31</td>
<td>12</td>
<td>15</td>
<td>10</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>6</td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Pos. (%)</td>
<td>n</td>
<td>7</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Cows</strong></td>
<td>n</td>
<td>19</td>
<td>10</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Pos. (%)</td>
<td>n</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Pigs</strong></td>
<td>n</td>
<td>50</td>
<td>25</td>
<td>12</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Pos. (%)</td>
<td>n</td>
<td>11</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Percentage by animal species and geographical area. Pigs belonged to the same herd and were collected in different periods.*

---

Ciufolini, P, Nascetti, A, Genoni, M, et al. Vertical and sexual transmission of TOSV among sandflies has been demonstrated (Ciufolini et al. 1985), which could explain the survival of TOSV during the cold seasons of the year, when the vector does not circulate (Tesh et al. 1992). However, the rate of vertically infected sandflies decreases in each generation, suggesting that alternative mechanisms for virus maintenance should exist (Tesh and Modi 1987). Transient and low-level viremia has been described in humans and animals after natural or experimental Phlebovirus infection (Bartelloni et al. 1976, Cusi et al. 2001). Even though humans may play a role in the life cycle of TOSV by infecting naïve sandflies, the vector must ingest large quantity of virus to become infected. Thus, only vertebrate hosts with high-level viremia could be capable of infecting sandflies (Ciufolini et al. 1985).

We were not able to detect enough amounts of TOSV RNA in serum sample from the studied animals. Otherwise, Leishmania spp., also transmitted by phlebotomines, have a recognized canine reservoir, and high-level parasitemia has been demonstrated by PCR in dogs (Aoun et al. 2009). Similarly, we could speculate that TOSV RNA should have been detected in serum samples in this study to argue that an animal reservoir is involved in TOSV life cycle. Indeed, previous reports have suggested that TOSV reservoir can be the sandfly itself, and vertebrate animals would serve as amplifying hosts during warm season when the activity of sandflies is maximum (Tesh et al. 1992).

Further TOSV investigation conducted on wild and/or other domestic animals should be carried out to acknowledge if other reservoirs for the virus exist.

**Acknowledgments**

We are grateful to the veterinary laboratory ANLAVE (Granada, Spain) and Laboratory of Animal Health (Santa Fe, Granada, Spain) for kindly providing us the serum samples used in this work. We also thank the staff from the Virology Unit, Microbiology Service, from the Hospital Universitario Virgen de las Nieves (Granada, Spain), for their excellent technical assistance. This study was supported in part by founds from a research project, 05/305 (Junta de Andalucı´ a, Spain).

**Disclosure Statement**

No competing financial interests exist.

**References**


Address correspondence to: Mercedes Pérez-Ruiz
Servicio de Microbiología
Hospital Universitario Virgen de las Nieves
Avda. Fuerzas Armadas, 2
Granada 18014
Spain
E-mail: mercedes.perez.ruiz.sspa@juntadeandalucia.es
This article has been cited by: