Testing insecticide susceptibility of *Phlebotomus perniciosus* and *P. papatasi* (Diptera: Psychodidae) in Italy

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Summary. - A study was undertaken to establish the susceptibility of two recently colonized sand fly species, *Phlebotomus perniciosus* and *P. papatasi* to some insecticides. A laboratory colony of *P. papatasi*, unexposed to insecticides for a period of ten years, was used as reference strain. Bioassay tests were carried out according to the WHO standard protocols based on the mortality rates recorded at different exposure times. Serial (from 1 to 60 min) exposures to DDT 2%, lambda-cyhalothrin 0.06% and permethrin 0.2% were tested on groups of 30 adult sugar-fed female sand flies, for a total of 7470 specimens. Data were subjected to probit analysis and the susceptibility was calculated at LT50 and LT95 by comparison with the laboratory reference strain. The LT50 values for *P. perniciosus* were 19.9, 3.2 and 6.9 min, and for *P. papatasi* 18.0, 7.4 and 11.0 min, using DDT, lambda-cyhalothrin and permethrin, respectively. The results showed that two Italian populations of *P. perniciosus* and *P. papatasi* from Campania region and from Rome, respectively, are susceptible to the insecticides tested as compared with the reference strain used.

**Key words:** sand flies, insecticide susceptibility, DDT, lambda-cyhalothrin, permethrin, *Phlebotomus perniciosus*, *Phlebotomus papatasi*, Italy.

Riassunto (Sensibilità agli insetticidi di *Phlebotomus perniciosus* e *P. papatasi* (Diptera: Psychodidae) in Italia). - E’ stato compiuto uno studio per stabilire la sensibilità ad alcuni insetticidi di due colonie di *Phlebotomus perniciosus*, il principale vettore di leishmaniosi e *P. papatasi*. Una colonia di *P. papatasi*, mai esposta agli insetticidi negli ultimi dieci anni, è stata utilizzata come ceppo di riferimento. I saggi biologici sono stati compiuti secondo i protocolli standard del WHO. Sono stati saggiati gruppi di 30 femmine adulte nutriti con glucosio (7 470 esemplari) al DDT 2%, alla lambda-cyhalotrina 0,06% ed alla permetrina 0,2%, a diversi tempi di esposizione (da 1 a 60 min). I dati ottenuti sono stati valutati mediante analisi probit e la sensibilità è stata espressa in TL50 e TL95. I valori TL50 per *P. perniciosus* sono stati 19,9-3,2 e 6,9 min e per *P. papatasi* 18,0-7,4 e 11,0 min rispettivamente per DDT, lambda-cyhalotrina e permetrina. I risultati ottenuti mostrano che una popolazione di *P. perniciosus* e una di *P. papatasi* che originano rispettivamente dalla Campania e da Roma, comparate con il ceppo di riferimento, sono sensibili agli insetticidi testati.

**Parole chiave:** flebotomi, sensibilità, DDT, lambda-cyhalotrina, permetrina, *Phlebotomus perniciosus*, *Phlebotomus papatasi*, Italia.

Introduction

Among the sand fly species involved in the transmission of leishmaniasis in Italy, *Phlebotomus perniciosus* is regarded as the main vector of human and canine leishmaniasis caused by *Leishmania infantum* [1-3]. The species exhibits remarkable anthropophilic behaviour in peri-domestic and domestic habitats and it is widespread throughout the country in both rural and urban [4, 5]. Although the vectorial role of *P. papatasi* in Italy is limited to Phleboviruses transmission, this species is of medical importance because of its endophilic and anthropophilic behaviour and the abundance in domestic resting sites of urban areas, which result in bite-associated dermatological pathologies [6]. The above considerations justify investigations on the impact of domestic and peridomestic sand fly control based on the use of chemical insecticides.

In domestic and peridomestic situations, insecticides such as DDT, malathion, fenitrothion, propoxur and, more recently, synthetic pyrethroids such as deltamethrin and lambda-cyhalothrin, have been successfully used to control sand flies in many countries [7-22]. However, the spectrum of susceptibility of sand flies to a range of insecticides has been only partially...
studied and exiting information has not yet been fully collated and critically evaluated. The pressure of insecticides employed by departments of public health, as well as in agricultural activities, may contribute to develop a resistance in sand fly vector populations. Kaul et al. [23], working in the kala-azar epidemic area of Bihar State, India, first reported the existence of a DDT-resistant population of *P. papatasi*.

In Italy, very limited observations are available on levels and distribution of susceptibility to insecticides for sand fly vector populations and thus there is concern about the readiness to implement a response plan in the event of local recrudescence episodes of leishmaniasis [24]. Only one study was carried out on the susceptibility levels to DDT, malathion and permethrin of Sicilian populations of *P. perniciosus* and *P. perfiliewi* [25], which indicated the susceptibility of these species to the insecticides tested.

Thus, before planning any control measure against *Leishmania* vectors, a study has been undertaken in order to establish the base line of susceptibility to representative insecticides. The present paper reports the results of time-mortality bioassay to DDT, lambda-cyhalothrin and permethrin carried out in 1999 to evaluate the susceptibility levels in recently-colonized *P. perniciosus* and *P. papatasi* populations.

### Table 1. - Insecticide susceptibility of adults females of *Phlebotomus perniciosus*, *P. papatasi* and reference strain

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Sand fly species</th>
<th>Reference strain</th>
</tr>
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<tbody>
<tr>
<td></td>
<td><em>P. perniciosus</em></td>
<td><em>P. papatasi</em></td>
</tr>
<tr>
<td>DDT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposure times</td>
<td>10 9 10</td>
<td>900 900 990</td>
</tr>
<tr>
<td>Females tested</td>
<td>19.9</td>
<td>18.0</td>
</tr>
<tr>
<td>LT&lt;sub&gt;50&lt;/sub&gt;</td>
<td>17.4-22.5</td>
<td>14.8-21.6</td>
</tr>
<tr>
<td>FL</td>
<td>1.19</td>
<td>1.08</td>
</tr>
<tr>
<td>RR</td>
<td>160.7</td>
<td>127.7</td>
</tr>
<tr>
<td>LT&lt;sub&gt;95&lt;/sub&gt;</td>
<td>117.7-248.5</td>
<td>82.3-207.5</td>
</tr>
<tr>
<td>FL</td>
<td>0.12</td>
<td>0.89</td>
</tr>
<tr>
<td>RR</td>
<td>1812 ± 0.163</td>
<td>1931 ± 0.237</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lambda-cyhalothrin</th>
<th></th>
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<tbody>
<tr>
<td>Exposure times</td>
<td>6 7 6</td>
</tr>
<tr>
<td>Females tested</td>
<td>630 720 630</td>
</tr>
<tr>
<td>LT&lt;sub&gt;50&lt;/sub&gt;</td>
<td>3.2</td>
</tr>
<tr>
<td>FL</td>
<td>1.7-5.7</td>
</tr>
<tr>
<td>RR</td>
<td>0.65</td>
</tr>
<tr>
<td>LT&lt;sub&gt;95&lt;/sub&gt;</td>
<td>62.4</td>
</tr>
<tr>
<td>FL</td>
<td>23.9-171.2</td>
</tr>
<tr>
<td>RR</td>
<td>1.43</td>
</tr>
<tr>
<td>Slope ± SE</td>
<td>1275 ± 0.273</td>
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<table>
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<tr>
<th>Permethrin</th>
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<tr>
<td>Exposure times</td>
<td>8 9 9</td>
</tr>
<tr>
<td>Females tested</td>
<td>810 900 900</td>
</tr>
<tr>
<td>LT&lt;sub&gt;50&lt;/sub&gt;</td>
<td>6.9</td>
</tr>
<tr>
<td>FL</td>
<td>5.5-8.3</td>
</tr>
<tr>
<td>RR</td>
<td>0.88</td>
</tr>
<tr>
<td>LT&lt;sub&gt;95&lt;/sub&gt;</td>
<td>126.5</td>
</tr>
<tr>
<td>FL</td>
<td>88.4-202.1</td>
</tr>
<tr>
<td>RR</td>
<td>0.48</td>
</tr>
<tr>
<td>Slope ± SE</td>
<td>1300 ± 0.099</td>
</tr>
</tbody>
</table>

(a) Letal time in minutes. FL: fiducial limits at the LT<sub>50</sub> and LT<sub>95</sub>. RR: resistance ratio = LT<sub>50</sub>, LT<sub>95</sub> of tested species/ LT<sub>50</sub>, LT<sub>95</sub> of reference strain.
Materials and methods

Sand flies

Due to the difficulty of obtaining large numbers of wild *P. perniciosus* and *P. papatasi* needed for the bioassay tests in a definite period of time, laboratory colonies of the two species were established during the sand fly season of 1999. The rearing methods are described by Maroli *et al.* [26] and Killick-Kendrick *et al.* [27]. In brief, each gravid female collected in the field was retained individually in a glass tube (20 ml) lined with a triangle of filter paper for oviposition. From the second generation the mass-rearing technique of Modi and Tesh [28], was used. Time-mortality tests were performed starting with females of the third laboratory generation.

*P. perniciosus population.* - The colony of *P. perniciosus* (PERNI-VE) originated from fed females collected from a leishmaniasis focus of the Campania region, where a recrudescence of visceral disease caused about 300 clinical cases in the period 1993-1999 [24, 29, 30]. No insecticides in public health control programmes have been reported to be used in the area. However all classes of insecticides have been employed for agricultural purposes in this area.

*P. papatasi population.* - The colony of *P. papatasi* (PAPA-RM) originated from fed females collected in a bedroom of a flat located in the historical centre of Rome, where they are commonly found in bedrooms during the early sand fly season (i.e., June-July) [6].

Reference strain. - As there are no international standard susceptible strains of sand flies to insecticides, a *P. papatasi* colony originated from Rocca Priora, Central Italy, was used as reference strain. This colony has been maintained since 1990 for more than 80 laboratory consecutive generations.

Bioassay tests for susceptibility

Bioassay tests were carried out according to the WHO [31] protocols for testing the susceptibility of sand flies to insecticides based on the mortality rates observed at different exposure times. Tests were performed by using WHO test tubes for adult mosquitoes. The insecticides tested were one organochlorine and two pirethroids. Tested concentrations of DDT, lambda-cyhalothrin, and permethrin were 2%, 0.06% and 0.2%, respectively. A serial time exposure (between 1 and 60 min) was used. Six to ten exposure times of each insecticide were tested in at least three replicates by using different rearing batches. For each exposure time 30 adult, 3-4 days old, sugar-fed females were used. Batches of 30 females were also exposed into the test tubes filled with control papers for the higher time of exposure required for the insecticide to be tested. After the exposure, the sand flies were transferred to holding tubes and the mortality rates were recorded after 24 hrs. The bioassay tests were carried out at standard conditions (T = 23 ± 2°C; RH = 60-70%). The insecticide papers were furnished by Vector Control Division, Ministry of Health, Venezuela. Papers were impregnated according to the protocols of WHO [32].

Analysis

Data were subjected to probit analysis (Finney 1971) [33] using a Windows program (Praxeme R & D, Version 2.0, 1997). Resistance ratios (RR) were calculated at LT<sub>50</sub> and LT<sub>95</sub> by comparison with the laboratory reference strain.

Results

A total of 7 470 female sand flies were used to evaluate the susceptibility of *P. perniciosus* and *P. papatasi* to the selected insecticides. To obtain mortality rates between 2% and 98%, it was necessary to test 9-10 exposure times for DDT, while for lambda-cyhalothrin and permethrin the number was lower, being 6-7 and 8-9, respectively (Table 1). The values of LT<sub>50</sub>, LT<sub>95</sub> with their fiducial limits as well as the calculated RR and regression line slopes for each insecticide tested are given in Table 1.

The data observed reveal a consistent and uniform response of the Italian populations of *P. perniciosus* and *P. papatasi* towards the compounds tested when compared to the reference strain used in this study. The time-mortality regression lines also reveal the two species to be homogenous in their response to the insecticides assayed (Fig. 1). No evidence of pronounced heterogeneity, indicating a potential shift in tolerance levels, was observed. Nevertheless, it should be mentioned that the LT<sub>50</sub>, LT<sub>95</sub> values for DDT were higher when compared with that of the other two insecticides tested.

Considering the LT<sub>50</sub>, LT<sub>95</sub> values observed, the results clearly revealed that the sand fly population tested were more susceptible to lambda-cyhalothrin and permethrin compared with DDT. The insecticide efficacy of the three insecticides was in the following descending order: lambda-cyhalothrin, permethrin, DDT.

Discussion

Following the first record on the existence of DDT-resistant population of *P. papatasi* [23], laboratory and field studies have documented the resistance for DDT and other insecticides in *P. papatasi*, *P. argentipes* and...
more recently in Sergentomyia shortii [34-39]. Among the sand fly species of medical importance tested for their susceptibility levels to insecticides, *P. papatasi* and *P. argentipes* are the most common species tested, followed by *Lutzomyia longipalpis*, *L. youngi*, *P. brevis*, *P. transcaucasicus*, *S. shortii*, *S. punjaubensis*, *P. bergeroti*, *P. langeroni*, *P. sergenti* [40-44].

However, a comprehensive review of existing information on sand fly susceptibility to insecticides is particularly difficult since published data are widely dispersed in the literature. The majority of the studies have been performed by using dose-mortality bioassays or discriminating concentration only, and thus it is difficult to compare the resulting data with our results based on time-mortality bioassay.

As for the previous study carried out in Italy by Lavagnino and Ansaldi [25] on the susceptibility levels to DDT (0.5%, 1.0%, 2.0% and 4.0%), malathion (0.5%) and permethrin (0.2%) of Sicilian populations of *P. perniciosus* and *P. perfiliewi*, only the mortality rates observed at a limited number of exposure times (three) were reported and no LT50 and LT95 values were calculated. By reference to the limited amount of comparable data available in the literature [40] and to our laboratory reference strain, the population of the two species tested in the present study can be regarded as susceptible to DDT, lambda-cyhalothrin and permethrin.

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**REFERENCES**


INSECTICIDE SUSCEPTIBILITY OF P. PERNICIOSUS AND P. PAPATASI IN ITALY


