INVESTIGATION OF SANDFLIES (DIPTERA, PHLEBOTOMIDAE) IN AN ENDEMIC FOCUS OF VISCERAL LEISHMANIASIS IN YUGOSLAVIA

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Abstract. The paper presents the results of faunistic, ecological and viral investigations concerning phlebotomine sandflies in an endemic focus of visceral leishmaniasis in Yugoslavia. These investigations were carried out in the period from 1969 to 1981.

Sandflies belong to the medically important species of hematophagous insects, attracting great attention in Yugoslavia. They are vectors of the causative agents of sandfly fever and also of cutaneous and visceral leishmaniasis. The Diptera roused special interest in the period immediately following World War II, because at that time the mentioned diseases (with the exception of cutaneous leishmaniasis) acquired alarming proportions in some parts of the country. In Serbia, sandfly fever occurred in the form of epidemics. The first autochthonous cases of visceral leishmaniasis in the Republic were noted in the period from 1944 to 1945 after which the disease spread not only to new areas but also to many localities affected earlier. Sandflies have been studied all over Yugoslavia but mostly in South-East Serbia, especially in the area of Dobrić. Since this area is still the focus of visceral leishmaniasis, it is the object of special interest to parasitologists, pediatricians and entomologists, and of late to virologists, as well.

Detailed faunistic investigations of sandflies in South-East Serbia were begun in 1947 and subsequently, when new cases of visceral leishmaniasis were detected in children in 1968, an extensive ecological study was started in 1969. Within the viral investigation, the first of its kind with these Diptera in our country, three strains of highly contagious viruses were isolated.

MATERIAL AND METHODS

The collecting of sandflies, the method of processing them, and the determination of the origin of their blood meals have been described in earlier papers (Mišović 1979, 1980, 1982; Živković and Mišović 1979). The number of studied specimens of sandflies in settlements for the period 1969—1981 was 31,126, and in natural biotopes 40,850. Moreover, the collection and processing of sandflies, of serologic reactions in the viral investigations, may also be found in previous papers (Gligorić et al. 1981, 1982, 1983).

RESULTS AND DISCUSSION

Both faunistic and ecological investigations of sandflies were carried out in South-East Serbia (the area of Dobrić) in the period from 1969 to 1981, as well as in the area Aleksinac in 1976 to 1981. The field study took place from the second half of July to the end of August, coinciding with the greatest population density of these Diptera.
Sandflies were investigated in settlements, in some biotopes, as well as in the various types of natural microhabitats. A special attention was given to the study of sandflies in the artificial light in settlements and out doors. The study of the origin of blood meals in these insects is important for the determination of the degree of affinity of the various species of sandflies (as vectors of disease) in relation to man and some kinds of animals as hosts and reservoirs of causative agents which is of special interest to medicine. During our continuous field investigations, the climate was also an object of our study, especially the temperature and relative humidity of the air. Viral research comprised the isolation of several strains of viruses and their identification.

In Yugoslavia the fauna of the Phlebotominae family consists of ten species: Ph. papatasi (Scopoli, 1786), Ph. sergenti Parrot, 1917, Ph. major Annandale, 1910, Ph. perfiliewi Parrot, 1910, Ph. perniciosus Newstead, 1911, Ph. tobbi Adler et Theodor, 1930, Ph. simici Nitulescu, 1931, Ph. chimenos balcanicus Theodor, 1938, Sergentomyia minuta Rondani, 1853 and S. dentata Sinton, 1933. In the area of Dobrić six species are represented: Ph. papatasi, Ph. sergenti, Ph. major, Ph. perfiliewi, Ph. tobbi and Ph. simici (1969—1981) and in the area of Aleksinac (1976—1981) four species: Ph. papatasi, Ph. major, Ph. perfiliewi and Ph. tobbi.

Investigation of sandflies in settlements

These investigations were of two types and they covered both human dwellings and animal houses also in the artificial light at night. In the area of Dobrić 14 villages were studied: Azbrenica, Krajkovac, Dešilovo, Lepaja, Oblačina, Jug Bogdanovac, Rožina, Balševac, Morokina, Brest, Aleksandrovo, Mramorski Potok, Kruše and Lainske Pojate. In 1968 kala-azar was detected in children aged 4 months to 7 years in the following five villages out of the 14 investigated: Mrorokina, Brest, Azbrenica, Kruše and Mramorski Potok.

The study of sandflies in the area of Aleksinac comprised eight villages: Žitkovac, Trnjane, Gornja and Donja Pešanica, Gredetin, Donji Androvc, Ošćina, Boboviste and Bovin. In 1968 the disease was detected only at Gredetin. In the area of Dobrić, detailed ecologic investigations were carried out in the following villages: Morokina, Brest, Jug Bogdanovac (primary school), Kruše and Aleksandrovo and in the Aleksinac area at Gredetin, Trnjane and Gornja Pešanica (Fig. 1).

The faunistic composition of sandflies (area of Dobrić) was most abundant at Jug Bogdanovac and Azbrenica, whereas all the species in the investigated part of South-

Table 1. Detection of various species of sandflies in the period from 1969 to 1981 in the 14 investigated villages situated in the area of Dobrić

<table>
<thead>
<tr>
<th>Villages</th>
<th>Species</th>
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<tbody>
<tr>
<td>Azbrenica</td>
<td>Phlebotomus papatasi, Ph. sergenti, Ph. major, Ph. perfiliewi, Ph. tobbi, Ph. simici</td>
</tr>
<tr>
<td>Krajkovac</td>
<td>Ph. papatasi, Ph. major, Ph. perfiliewi, Ph. tobbi, Ph. simici</td>
</tr>
<tr>
<td>Dešilovo</td>
<td>Ph. papatasi, Ph. major, Ph. perfiliewi, Ph. tobbi, Ph. simici</td>
</tr>
<tr>
<td>Jug Bogdanovac</td>
<td>Ph. papatasi, Ph. sergenti, Ph. major, Ph. perfiliewi, Ph. tobbi, Ph. simici</td>
</tr>
<tr>
<td>Oblačina</td>
<td>Ph. papatasi, Ph. major, Ph. perfiliewi, Ph. tobbi, Ph. simici</td>
</tr>
<tr>
<td>Lepaja</td>
<td>Ph. papatasi, Ph. major, Ph. perfiliewi, Ph. tobbi, Ph. simici</td>
</tr>
<tr>
<td>Rožina</td>
<td>Ph. papatasi, Ph. major, Ph. perfiliewi, Ph. simici</td>
</tr>
<tr>
<td>Balševac</td>
<td>Ph. papatasi, Ph. perfiliewi, Ph. tobbi</td>
</tr>
<tr>
<td>Morokina and Brest</td>
<td>Ph. papatasi, Ph. major, Ph. perfiliewi, Ph. tobbi, Ph. simici</td>
</tr>
<tr>
<td>Aleksandrovo</td>
<td>Ph. papatasi, Ph. major, Ph. perfiliewi</td>
</tr>
<tr>
<td>Mramorski Potok</td>
<td>Ph. papatasi, Ph. sergenti, Ph. perfiliewi, Ph. tobbi, Ph. simici</td>
</tr>
<tr>
<td>Kruše</td>
<td>Ph. papatasi, Ph. major, Ph. perfiliewi</td>
</tr>
<tr>
<td>Lainske Pojate</td>
<td>Ph. papatasi, Ph. perfiliewi</td>
</tr>
</tbody>
</table>
East Serbia was found. The smallest number was found at Baličevac, Aleksandrovto, Kručke and Lalinske Pojate. In these localities only two (at Lalinske Pojate), or three species of sandflies were found (Table 1).

From the data presented one may conclude that in all the villages Ph. popatasi and Ph. perfilievi were regularly detected, followed by Ph. major, Ph. simici and Ph. tobi, while Ph. sergenti was found in a negligible number only at Jug Bogdanovac (primary school), Mramorski Potok and Azbrecište. The most dense populations were those of Ph. popatasi and Ph. perfilievi (Zivković and Mišićnović 1972, 1973, Mišićnović 1978, Mišićnović and Marković 1983).

In order to study a number of ecologic characteristics such as: the density of the mixed populations of sandflies, the faunistic composition, the relative abundance of the various species, the fluctuations of their populations, the sex ratio, the age of female specimens, the origin of blood meals and changes of temperature and relative humidity of the air, detailed investigations were performed in six of the 14 localities studied: Merošina, Brest, Azbrecište, Aleksandrovto, Jug Bogdanovac and Kručke. In five localities sandflies were investigated in human dwellings and stables, while at Jug Bogdanovac the study concentrated in primary school at the entrance to the village along the Niš-Prokuplje road.

In the eight localities studied around Aleksinac, three of them were systematically investigated on sandflies: Trnjane, Gornja Pećanica and Gredetin. This investigation concerned both human dwellings and animal houses and the following four species were found: Ph. popatasi, Ph. major, Ph. perfilievi and Ph. tobi. Ph. popatasi and Ph. perfilievi were observed to be most abundant, the latter being the dominant species in the seasons investigated, and sometimes the only one found (Mišićnović 1983).

Special attention was devoted to the study of sandflies collected in the settlements in the artificial light. Sandflies leave their diurnal resting sites at sunset and during the night they attack man and animals. This is the time at which infection occurs due to the causative agents they transmit. It is for these reasons that the study of their nocturnal activities is undoubtedly of great importance in the epidemiology of diseases in which they act as vectors. Sandflies were collected in the artificial light in three villages situated in the Dobrić area: Merdišina, Kručke and Aleksandrovto. In the last village, systematic studies of the nocturnal activities of sandflies covered several years. At Merdišina and Kručke such study took place only in a few of these years. In the samples collected at night at Aleksandrovto and Kručke the species were found in each locality (Ph. major, Ph. perfilievi, Ph. simici), at Merdišina four (the same species plus Ph. major, Ph. tobi) (Zivković and Mišićnović 1979, Mišićnović et al. 1983).

Investigation of sandflies in natural biotopes

A detailed investigation of sandflies outside settlements was carried out in natural biotopes, especially on the expanses under crops, but also in various types of microhabitats (stone walls along roads, rodents' burrows, cracks in the ground).

It is known that various microhabitats are used as diurnal resting sites by sandflies, and some of them are the sites of their pre-imago development. Of all the microhabitats examined greatest attention was paid to the supporting stone walls and rodents' burrow. The first such investigations in our country were initiated by Zivković and Adamović (1973, 1974). They investigated these Diptera in some microhabitats located in the area of Baličevac, as well as in one artificial shelter along one asphalt road. It is interesting to note that these authors indicated the absolute domination of the species Ph. perfilievi.

The damaged supporting stone walls run along the asphalt road between Merdišina and Jug Bogdanovac on the Niš-Prokuplje highway. The investigations were carried out in the period from 1975 to 1977. Four species were found in this place: Ph. major, Ph. perfilievi, Ph. tobi and Ph. simici. The species Ph. perfilievi prevailed in all the three years, while the remaining three species were found in a negligible number.

In the rodents burrows, probably the habitat of field mice (Apodemus syvaticus), as well as in the ground full of cracks round the holes between the villages Kručke and Lalinske Pojate, Ph. perfilievi was the only species found. Systematic investigations were undertaken in the period from 1974 to 1975, while in 1976–1981 only the faunistic composition was studied. The results obtained from the mentioned types of microhabitats indicated that they had been only diurnal resting sites but also possible breeding sites of sandflies (Mišićnović 1982, Mišićnović and Miltinović 1983).

Sandflies were collected in the artificial light in some natural biotopes: in a small forest, in a cornfield, wheat-field, on a melon patch, on a tobacco patch and in a vineyard. While a 100W bulb was used as the source of light in the villages, torches of 2.5W were used in natural biotopes, and in some of them even car head lights served the purpose. After processing the material collected in light traps, differences were found between the individual villages and natural biotopes as far as the density of the mixed populations was concerned. There were also some differences with regard to their faunistic composition. While at Merdišina four species were found, at Kručke and Aleksandrovto only three were encountered. In all the samples, regardless of the village covered by our study, Ph. perfilievi prevailed numerically and sometimes was the only species found. The rest were rarely detected. In three out of the six biotopes (cornfield, wheat-field, melon patch) four species were found at Merdišina, in the wood only two (Ph. perfilievi, Ph. simici) and in the tobacco patch and vineyard exclusively Ph. perfilievi species was encountered (Zivković and Mišićnović 1975).
Temperature and relative air humidity

The influence of temperature and relative air humidity on the flight of sandflies in the artificial light was traced in the settlements within a five-year period (1973 to 1977). Of the four species found in the examined localities (the village Aleksinac), Ph. papatasi was the only species not attracted by artificial light. Ph. perfiliewi held the dominant place, while Ph. major and Ph. simici were considerably less often found. The nocturnal activity of sandflies depended particularly on the meteorological conditions, first of all, on the temperature, the air currents and rainfall. Relative humidity did not play a decisive role in the flight of these insects.

The optimal temperature favouring the adults was between 20 and 25°C in the presence of relative humidity of 72-78 %, while most of the species found preferred temperatures ranging from 21 to 26°C. The number of sandflies decreased above and below these temperatures regardless to the remaining possible favourable factors.

The most suitable conditions for Ph. perfiliewi are temperatures ranging from 22 to 24 °C and relative humidity from 65 to 70 %; for Ph. major — 23-26 °C and 60 to 66 % RH respectively and for Ph. simici — 20—24 °C and 70-75 % RH respectively (Miščević 1981).

Dynamics of mixed sandy populations, relative abundance and sex ratio

Analysis of the population dynamics of sandflies in the period from 1969 to 1981 in the area of Dobrič indicated the following: the highest percentage of these Diptera was found in 1974 and the lowest in 1977. In the localities around Aleksinac, they were most abundant in 1979 and slightly less so in 1977 (Fig. 2).

The relative abundance of individual species in the area of Dobrič during the period investigated was as follows: Ph. papatasi — 6.10%, Ph. sergentii — 0.7 %, Ph. major — 0.65 %, Ph. perfiliewi — 9.26 %, Ph. tobbi — 0.16 % and Ph. simici — 1.18 %. In the villages around Aleksinac, where the investigations were concentrated exclusively in the settlements, the following percentage was found: Ph. papatasi — 30.15 %, Ph. major — 1.94 %, Ph. perfiliewi — 67.81 % and Ph. tobbi — 0.10 %.

Further examination of the dynamics of the populatons of individual species in the area of Dobrič showed that the dominant are two species: Ph. papatasi and Ph. perfiliewi. The first found only in the settlements, could be noted: the period between the third ten-day period of July and the middle of the second ten-day period of August. The second species also found in the settlements, was most abundant from the first ten-day period to the middle of the second ten-day period of August. In some seasons, towards the end of August, a growth of population density was noted which would favour the appearance of a second generation of the species. This especially applied to the period from 1972 to 1975. The remaining four species would appear in a larger number at the beginning of the season or in the first half of August, as was the case with Ph. major and Ph. simici. The species Ph. tobbi and Ph. sergentii were very seldom found, usually in the second half of each season investigated (Zivković 1972, 1973, Miščević 1979). However, in the area of Aleksinac Ph. papatasi reached its maximum abundance in the middle of the second ten-day period of August and afterwards it rapidly decreased, so that in the middle of the third ten-day period of August this species was not found at all. Ph. perfiliewi was also most abundant in the middle of the second ten-day period of August, but it decreased gradually so that its specimens were only sporadically found in the first ten-day period of September. The other two species were encountered rarely (Miščević 1983).

In the examined natural microhabitats supporting stone walls, Ph. perfiliewi was most numerous at the beginning of the second and third ten-day periods of August. However, in two seasons Ph. perfiliewi was the only species present in rodents’ burrows and in the first year attained two separate maxima: in the middle of the first ten-day period and at the end of the second ten-day period of August. In the second year there was only one maximum in the first ten-day period of September (Miščević 1982, Miščević and Milićutnović 1983). As for the sex ratio of sandflies collected in the settlements, one may conclude that, as a rule, females predominate in human dwellings and males in animal houses. This was the case in both examined areas. Taking into account the length of our investigations, it may be concluded that engorged female sandflies were found quite often and that in some samples they were not only the most numerous form but also they outnumbered the males, as is found in other species (Miščević and Milićutnović 1983). For the females was found with eggs in their abdomens, while unfed females were much less abundant. This primarily applies to the species Ph. papatasi and Ph. perfiliewi. The results obtained confirm that the first species is exclusively found in settlements and sites at which complete development takes place. This also applies to Ph. perfiliewi, the only difference being that this sandfly is quite regularly found outside settlements where it represents the dominant species, unlike the situation in the settlements where it is found with Ph. papatasi with which it takes turns. As for the species Ph. major and Ph. simici, their sex ratio is almost identical in both of them, and the detection of unfed, fed and gravid females was in favour of fed forms while unfed and gravid specimens were only rarely found (Miščević 1979, Miščević and Marković 1983).

In the area of Aleksinac the sex ratio was almost the same as that in the Dobrič area, especially regarding the Ph. perfiliewi species. Ph. papatasi was found in one village (Gredetić) there were 61.80 % of females in the rooms, while there were none in the domestic animal quarters. The species Ph. major and Ph. tobbi were encountered in a small number. As for the unfed, fed and gravid forms, the fed ones prevailed in the Ph. papatasi species, while the unfed ones prevailed in the Ph. perfiliewi and Ph. major species. The highest percentage of females with eggs was found in the Ph. perfiliewi species (Miščević 1983).

While considering the sex ratio of sandflies collected in the artificial light in settlements, it should be noted in what follows: in open fields, during flight at dusk (between 7.30 and 8 pm), and their number considerably increases after 8 pm. In the first hours it is usually males that appear in a greater number and then their number declines so that towards midnight it is very low. The number of females also rapidly increases between 8 and 10.30 pm and then gradually falls, although individual specimens also appear after midnight, but their number is negligible. Without saying that in some seasons as well as on some nights there are deviations, depending to a great extent on the weather conditions, primarily on temperature, air currents and rainfall (Miščević 1981). In the material collected in the period from 1973 to 1977, as well as in that from 1978 to 1981, there were more females than males. As for females the most abundant were those unfed (80%), about 19 % being fed and 0.50 % gravid. The largest number of unfed females was found between 9 and 11 pm in all investigation seasons and it amounted to 80.50 %. Engorged females were most frequently found in the first collecting hours (between 7.30 and 9 pm), accounting for about 23 %. Their number slightly decreased subsequently. Gravid females were very seldom found (Miščević 1979, Miščević et al. 1983). First of the examined microhabitats males were predominant. As for the age structure of the females the most numerous were unfed specimens, followed by fed and gravid
forms (Miščević 1982, Miščević and Milutinović 1983). The most abundant species was Ph. perfiliewi. With some exceptions, when the number of males and females was identical, the males prevailed in the artificial light in natural biotopes, whereas females absolutely prevailed in the settlements. Among the females gravid forms were most abundant together with fed specimens in comparison to the settlements where unfed forms were dominant (Živković and Miščević 1975).

The origin of blood meals in sandflies

Since 1970 immunochemical studies have been performed in order to examine the origin of blood meals in sandflies, so as to determine the susceptibility of the individual species to various hosts. By the method of immunoprecipitation in 1% agar gel the authors studied the sera of some pro-microfilaria of the hosts to see if sandflies of the hosts to see if sandflies of the hosts to see if sandflies of the hosts to see if sandflies of the hosts to see if sandflies of the hosts to see if sandflies of the hosts to see if sandflies of the hosts to see if sandflies of the hosts to see if sandflies of the hosts to see if sandflies of the hosts. The studies comprised three species: Ph. papatasi, Ph. major, and Ph. perfiliewi. Out of the total number of sandflies analyzed (285 specimens), immunoglobulins were detected in 92.24% i.e. from man, some domestic animals and the rat. Only a small number was negative (7.76%) in the process of immunodiffusion. As a positive finding we considered any immunoreaction in agar gel irrespective of the kind or number of hosts, i.e. regardless of the type of blood meal. Out of the total number of positive sandflies collected in the human dwellings, 59.53% pertained to Ph. papatasi, 33.93% to Ph. perfiliewi and only 6.54% to P. major.

After investigating the abdominal contents of all sandflies by means of eight specific antisera to IgG namely from man, swine, sheep, cow, horse, dog, chicken and rabbit, the investigations confirmed that the immunoglobulins of one to five hosts might be found in the abdominal contents of individual sandflies.

The results of our studies indicated that the two most abundant species Ph. papatasi and Ph. perfiliewi collected in the human dwellings contained the largest percentage of serum proteins from man, sheep and dog in their gut. Where the Ph. papatasi species was concerned, human serum protein was predominant (79.06%), which confirms the already known fact that this sandfly is a typically anthropophilic species.

The Ph. major species, found in a negligible number, contained the serum proteins of man, swine and the rat. The species Ph. perfiliewi was positive in the largest percentage with the antiserum of human IgG (51.72%), then with that of the dog (27.58%) and sheep (10.34%). For this species the presence of canine serum proteins is important (in human dwellings — 27.58%, and in the artificial light — 15.03%) whereas the incidence of serum proteins from rats ranged from 6.80 to 8.27%. These two kinds of animals are known to be reservoirs of the causative agent of visceral leishmaniasis (Šimić et al. 1985, Petrović et al. 1975).

On the basis of our investigations it might be concluded that the abdominal contents of females depend on the locality where they are collected, as well as on the host accessible to them, which primarily is true of the Ph. perfiliewi species. This phenomenon has been pointed out by Tesh et al. (1972), Lewis (1974) and Miščević (1977, 1980).

Viral investigations

Since 1976 detailed viral investigations have been undertaken in relation to these Diptera in South-East Serbia — Dobroć.

From the species Ph. perfiliewi three viral strains of high contagiousness to laboratory animals have been isolated. One of these three strains was identified as the Naples sandfly fever virus (Bunyaviridae, Phlebovirus) (Gligić et al. 1982). The identification of the virus is under way and the third virus was identified on the basis of the morphology observed by electron microscopy, as well as serologic findings. It has been confirmed as a new member of the Vesicular Stomatitis virus serogroup of Rhabdoviridae: Vesiculovirus, named Jug Bogdanovac virus (Gligić et al. 1981, 1983, Tesh et al. 1985). It is worth mentioning that the conducted investigations were the first of a kind in Yugoslavia.

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REFERENCES


Simč C., Petrović Z., Boroški A., Contribution to the knowledge of the epidé-
The economic losses caused every year by horse flies larvae amount to more than 600 million rubles in the USSR. The problems of the control of these parasites are therefore very important. One of the possible ways how to regulate the number of horse flies populations is the utilization of their pathogens and parasites. The adults of this family have already been widely studied, whereas the knowledge of their larvae is still insufficient. The present publication, in which the author summarized her own results, as well as the available literary data on this topic, is therefore very useful.

The first part entitled "Morphology and ecology of the larvae" deals with the structure of body and its segmentation, nutrition, development, ecological morphotypes of larvae, their associations, and zonal distribution. The second part entitled "Organisms attacking larvae and pupae of horse flies" concerns besides the infections caused by viruses, bacteria and fungi also parasitic protozoans, helminthes and insects. It contains also chapters discussing the peculiarities of pathogenesis and diagnostics of diseases of horse flies larvae and methods used in ecological studies of these larvae. The list of literature comprises 285 citations. It is a valuable publication which can instigate further research in this still little known field.

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