

ECOLOGY OF ANOPHELES MOSQUITOES IN BELGRADE AREA. ESTIMATING VECTOR POTENTIAL FOR MALARIA RETRANSMISSION

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Belgrade is situated in the area that is potentially at risk from malaria outbreaks. Until eradication, the main vector of malaria in this area was Anopheles maculipennis s. s. (previous name An. typicus) and secondary vectors were An. messeae and An. atroparvus. In this study we examined the distribution and ecology of Anopheles mosquitoes (Diptera, Culicidae) in Belgrade. Females of Anopheles mosquitoes were collected from animal shelters in Belgrade at eight locations during 2003. Egg morphology was used to identify the specimens. A total of 3704 females deposited eggs ready for identification. Three species of An. maculipennis complex were identified: An. messeae, An. atroparvus and An. maculipennis s. s.. The most abundant species were An. messeae (64%). The relative frequency of three species varied depending on the site of collection. Seasonal fluctuations of mosquitoes' species varied. Each develops in a distinct type of water, too. The three species of the An. maculipennis complex, particularly An. messeae and An. atroparvus, are considered as potential vectors of malaria in Belgrade. With the possible reintroduction of Plasmodium species due to climatic changes and increased travel to and from the countries where malaria is endemic, a more efficient vector control is necessary.

Key words: potential vectors, An. maculipennis complex, receptivity, imported malaria, gametocyte carriers, Belgrade

INTRODUCTION

Reintroduction of malaria is more frequent in the regions where the parasite was eradicated or its incidence was significantly reduced (Baldari *et al.*, 1998; Kruger *et al.*, 2001; WHO, 2002). The reasons are numerous and involve global climatic changes that cause the change of life conditions in the habitat (water, number and kind of hosts, ecological factors); the increase of tourist trips and commercial contacts with the consequent increase of malaria imported into non-

endemic regions, as well as the ecological changes under the influence of man that are favoured by local mosquito populations (back to nature) and infectious diseases spread by them (Marchant *et al.*, 1998; Coluzzi, 2000; Romi *et al.*, 2001). Infectious potential of *Anopheles* mosquitoes is not only obvious in their previous and future transmission of malaria vectors, canine filariasis, but in their transmission of other less-known infectious agents, as well. Only 70 species out of a great number of *Anopheles* mosquitoes in the world are able to complete the sporogonic development of *Plasmodium* and introduce malaria transmission (Service, 2000).

The Belgrade area is rich in localities which are ideal habitats for the development and survival of mosquitoes. Before eradication, the main vector of malaria in Belgrade and its surroundings was, thanks to its spread and density, *An. maculipennis* s.s. (*An. typicus*) and secondary vectors were *An. messeae* and *An. atroparvus* (Simic, 1948; Vukasovic, 1950; Sitar, 1977). In the last decades they have, however, relocated their habitats, which is the consequence of not only a reduced vector control (Sitar, 1977; Coluzzi, 2000) but global warming as well.

The lack of entomological surveillance in our country, especially in the last decade, with global warming and numerous ecological changes caused by global warming, in our extremely dynamic and changeable ambience, as well as sporadic cases of autochthonous malaria in the neighbouring countries Greece, Bulgaria, Italy, Germany (Baldari *et al.*, 1998; Kruger *et al.*, 2001; WHO, 2002; Kampen *et al.*, 2003) made us take a better look at the ecology and distribution of *Anopheles* mosquitoes, and analyze the current prevalence of potential malaria vectors in Belgrade.

MATERIAL AND METHODS

Study area

The study was carried in eight different localities in the vicinity of Belgrade (Pancevacki Rit, Crvenka, Batajnica, Surcin, Galovica, Stara Bezanija, Grocka and Mislodjin). These localities are mainly in areas of the Danube and Sava river basin, rich in still waters, especially in spring time, and this is a favorable condition for the development and survival of mosquitoes. These localities have different geographical features, vegetation, social infrastructure and agricultural development. Belgrade represents a combination of urban, semi-urban and rural environments and is characterized by non-hygienic settlements and numerous animal shelters in suburban and rural areas. Pigs, cattle, sheep and goats are the most common domestic animals in Belgrade surrounding area.

Pancevacki Rit is suburban area situated 5 km from the city of Belgrade, between the rivers Danube and Tamis. Its 400 km² large wetland was constantly flooded, but since World War II it has been drained area by area and almost half of it is turned into a very fertile patch of land, suitable especially for cultivating grains and vegetables. Many meandering canals and bogs remained in the marsh: slow streams and large bogs. In the south, the area ends with a river island.

Crvenka is a weekend-settlement in Palilula, located in Banat section of this municipality, half kilometer away from the left bank of the Danube, on the Batin

canal stream. The canal, with colonies of wild ducks and white herons, flows through the settlement.

Batajnica is an urban neighborhood located in the northern part of Zemun. It is both the northernmost and the westernmost part of the Belgrade's urban area. It is close to the Danube's right bank, but not on the river itself, due to flooding. A small, 114 meter-high hillock separates the settlement from the river.

Surcin is a suburban settlement located 20 kilometers west of downtown Belgrade. The most important feature of Surcin is the Belgrade Nikola Tesla Airport. This area with numerous smaller streams is flat and marshy as the entire southern section belongs to the floodplain of the Sava river. The majority of population is into agriculture ("suburban agriculture" – fruits, early vegetables, etc). Extensive farms for pig breeding are located in Surcin.

Galovica is a suburban area, near to the international airport in Surcin. It stretches from New Belgrade to Ostruznica (10 km in length). The Galovica canal drains a catchment area in south-east Srem that stretches on 74100 ha and is itself 51 km long. As a primary channel of the upper area Galovica is an effluent stream for numerous sloughs and other smaller channels.

Stara Bezanija is an urban neighborhood situated in the central part of the Novi Beograd municipality. Bezanija is located west of downtown Belgrade, across the Sava river. Once a suburb of Belgrade, separated from it by the vast marshlands on the Sava's left bank, Bezanija today forms one completely urbanized area of Belgrade city.

Grocka is a suburban settlement, in the northern part of Sumadija region, located on the right bank of the Danube, where small river of the Grocica empties into the Danube, 30 km east of Belgrade. Grocka is center of one of the best known fruit growing areas in Serbia. Conditions are especially good for growing peaches, apricots, plums, cherries and grapes.

Mislodjin is a suburban village situated in the vicinity of the rivers Kolubara and Sava, in the Belgrade's municipality of Obrenovac. In the area there are numerous animal shelters.

Mosquito collections

Between June and October of 2003, adult mosquitoes were caught inside selected animal shelters: cow barns, sheep and goat pens, pigsties, henhouses (none was found inside a house), once a month.

The females of *Anopheles* mosquitoes were kept individually in test tubes with wet filter paper. In the laboratory, the female *Anopheles* mosquitoes were stored at +24° to +26°C and 60% of humidity until laying eggs or dying. The identification of species was based on the morphology of laid eggs. The species of *Anopheles maculipennis* complex were identified on the basis of shape, surface-pattern, and float-structure of the eggs according to identification keys (Russell *et al.*, 1943; Smart, 1956; Vukasovic, 1976; Sinerge *et al.*, 1979; White, 1979). Breeding sites which did not match any species were marked as atypical ones. Breeding sites where eggs with patterns typical of two species were found, were marked as hybrids according to Vukasovic (1976), who explains this phenomenon, previously noticed in this area, as fertilization between individuals

of different species within the *An. maculipennis* complex. In a certain number of breeding sites immature eggs were prevailing.

Weather conditions, amount of precipitation (L/m²), relative humidity (%) and temperature (°C) were analysed according to meteorological records from the Republic's Hydro Meteorological Institute.

Water specimens were taken in all localities with the habitats (larval breeding sites) of *Anopheles* mosquitoes and physical and chemical analyses (water color, turbidity, pH, ammonium, chloride and sodium hydrogen carbonate) were made at the Institute of Public Health "Milan Jovanovic-Batut".

The statistical analysis was based on the application of a linear trend.

RESULTS

Climatic factors in Belgrade

During 2003 the amount of rainfall in Belgrade was 551.9 L/m². The greatest amount of rainfall was recorded in October and June and the least during August. The average humidity was 65.5% and the average temperature 12.8°C (Table 1).

Table 1. Climatic factors in Belgrade in 2003

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Total monthly amount of precipitation (l/m ²) in Belgrade, 2003												
62.9	26.5	11.4	23.1	39.5	37.0	111.8	6.4	58.0	115.2	23.4	36.7	551.9
Mean relative humidity (%) in Belgrade, 2003												
82.2	74.7	58.5	55.8	55.7	53.3	63.0	49.9	64.2	74.4	76.7	77.6	65.5
Mean temperatures (°C) in Belgrade, 2003												
0.4	-2.1	7.1	12.1	21.5	25.0	23.1	25.6	17.8	10.8	9.2	2.8	12.8

The highest average temperature in 2003 was recorded in August 25.6°C (minimum temperature 20.2°C, maximum temperature 29.2°C) and in June 25.0°C (minimum temperature 19.9°C, maximum temperature 28.8°C). In comparison to June and August, the average temperature in July was 23.1°C (minimum temperature 17.0°C, maximum temperature 30.3°C) (Table 2).

Table 2. Summer temperatures in Belgrade in 2003

°C	Jun	Jul	Aug	Sep
min	19.9	17.0	20.2	13.2
max	28.8	30.3	29.2	23.7
mean	25.0	23.1	25.6	17.8

Anopheles mosquitoes in Belgrade

A total of 3704 (52%) out of 7074 collected females of *Anopheles* mosquitoes laid eggs. By the determination of the laid eggs only three *Anopheles*

mosquitoes were found, all belonging to the *An. maculipennis* group. The most prevalent was *An. messeae* 64% (n=2397) then *An. atroparvus* 21% (n=778) and *An. maculipennis* s. s. 8% (n=288). The rest were atypical breeding sites 3% (n=95), hybrids 2% (n=65) and breeding sites with immature eggs 2% (n=81) (Figure 1).

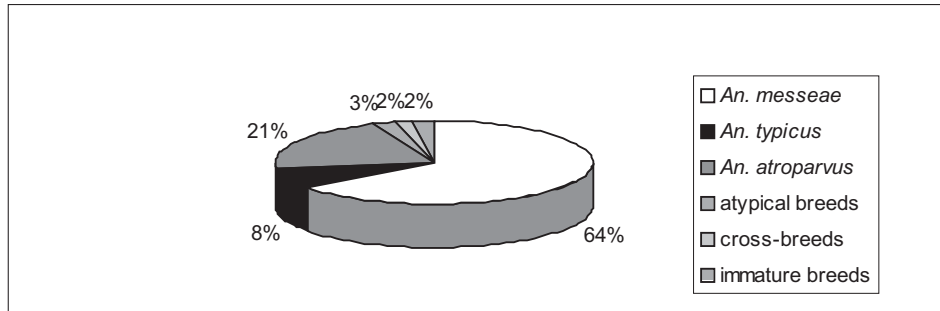


Figure 1. The prevalence of species within the *An. maculipennis* complex in Belgrade and its surroundings during 2003

Three *Anopheles* species had different levels of prevalence depending on the locality, month, choice of place populated by different animal hosts and choice of water habitat.

In six out of eight localities *An. messeae* was prevailing. Its presence in the total population of *Anopheles* mosquitoes in the examined localities was between 48% and 86%. The presence above 80% was determined in three localities: Grocka, Crvenka and Pancevacki Rit. In two localities, Surcin and Galovica the prevalence of *An. atroparvus* was determined (50% and 55%, respectively). In these localities *An. messeae* ranged between 36% and 42%. *An. maculipennis* had significant prevalence only in Mislodjin, Batajnica and Stara Bezanija (Figure 2).

In the examined localities population density of *Anopheles* mosquitoes in animal shelters was unequal: Pancevacki rit (100-800 females per animal shelter), Crvenka (100-1000 females per animal shelter), Galovica (50-500 females per animal shelter), Mislodjin (20-350 females per animal shelter) and Batajnica (0-400 females per animal shelter). In the inhabited parts of town: Grocka (5-180 females per animal shelter), Surcin (10-250 females per animal shelter), Stara Bezanija (0-150 females per animal shelter), residual populations of *Anopheles* mosquitoes are present (Table 3).

Analyzing a relative number (%) of *An. messeae* using a linear trend and according to the examined months (June-October) a decline of 6% was noticed regarding its presence in the total population of *Anopheles* mosquitoes; on the other hand the presence of *An. maculipennis* s.s. decreased by 1.9% when its maximum presence of 11% was noticed in August. Unlike them, a monthly increase of 8.2% was noticed regarding the presence of *An. atroparvus* in percentages in the total population of *Anopheles* mosquitoes (Figure 3).

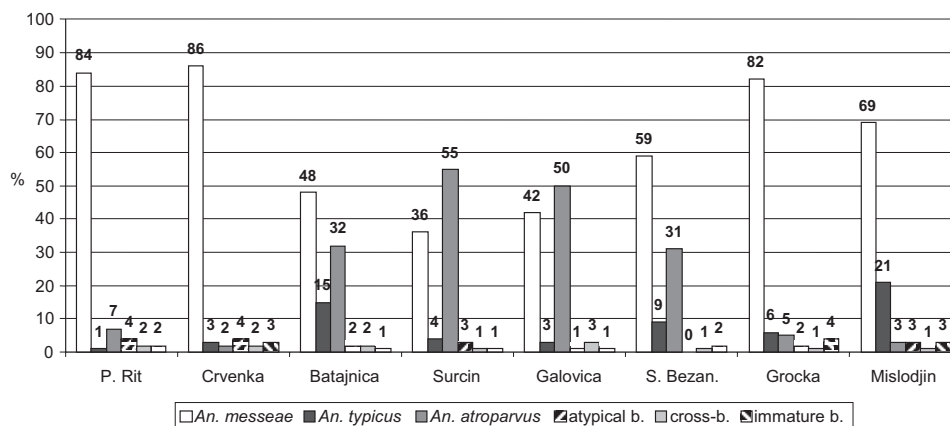


Figure 2. The distribution of three mosquito species within the *An. maculipennis* complex according to localities in Belgrade during 2003

Table 3. Density *An. maculipennis* s. l. in examined localities in Belgrade in 2003

Locality	Features of the locality	Density (number of females per animal shelter)
Pancevacki Rit	Swamps, irrigation canals	100 - 800
Crvenka	Swamps, irrigation canals	100 - 1000
Batajnica	The outskirts of Pustara, lake	0 - 400
Surcin	Canals, swamps	10 - 250
Galovica	Canal	50 - 500
Stara Bezanija	Urban area on the bank of the Sava	0 - 150
Grocka	On the bank of the Danube	5 - 180
Mislodjin	Canal, vicinity of the rivers Kolubara and Sava	20 - 350

The species *An. messeae* was equally prevalent in animal shelters with different animal hosts (cows, pigs, sheeps, goats, turkeys) whereas the two other species were selective. *An. atroparvus* was extremely rare in animal shelters with different kinds of domestic animals especially turkeys and goats while *An. maculipennis* s. s. mainly chose animal shelters with cows and pigs, but was also found in others (Figure 4).

An. messeae was mostly found in localities where the habitats with clean, alkaline waters (pH ↑9) were prevalent, as well as fresh waters with a lower quantity of chloride (↓40-50 mg/L) and a minimum quantity of bicarbonate of about 1 mg/L (Pancevacki rit, Sibnica). *An. atroparvus* was prevalent in localities with clean, alkaline and salty waters with a greater quantity of chloride (60-90 mg/L) and bicarbonate (↑500 mg/L) (Galovica, Batajnica, Stara Bezanija). *An.*

maculipennis s. s. was most prevalent in Mislodjin where the greatest quantity of ammonia 47.44 mg/L and muddiness were recorded (Table 4).

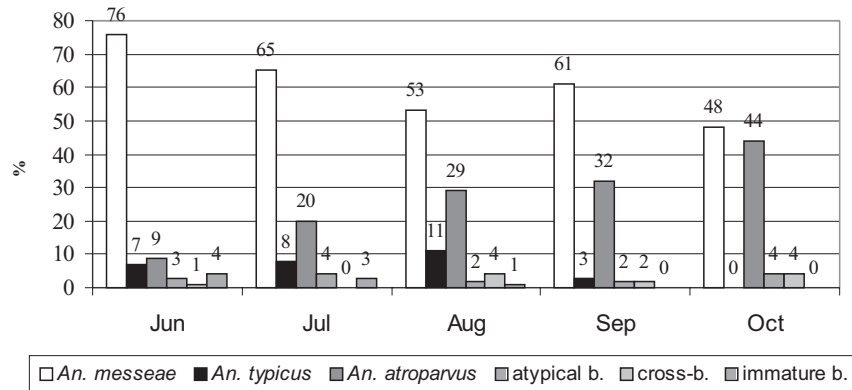


Figure 3. A monthly distribution of three mosquito species within the *An. maculipennis* complex in Belgrade in 2003

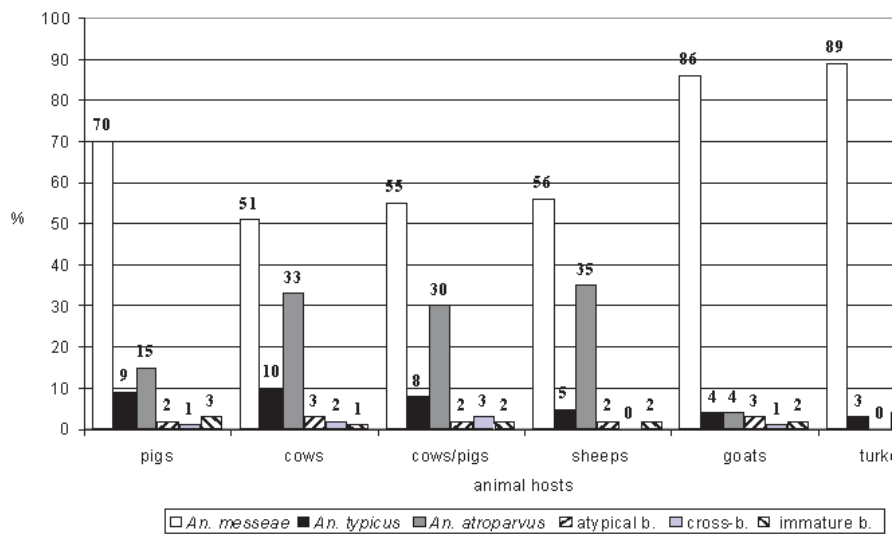


Figure 4. A distribution of three mosquito species within the *An. maculipennis* complex in comparison to the kind of the host in Belgrade in 2003

Table 4. Physical and chemical parameters of water habitats of *An. maculipennis* s. l. in Belgrade during 2003

Parameter	Unit	P. Rit	Crvenka	Batajnica	Surcin	Stara B.	Grocka	Mislodjin
Water color	Co-Pt	25	22	25	80	90	25	30
Turbidity	NTU	4.0	1.8	4.1	2.3	2.7	3.2	16
pH	-	9.50	9.39	7.64	7.92	8.62	7.40	7.59
NH ₄	mg/l	3.26	0.42	1.06	3.94	0.44	2.04	47.44
Cl ⁻	mg/l	39.80	47.69	59.05	65.99	92.70	70.24	58.10
NaHCO ₃	mg/l	1.16	0.98	497.76	695	464	756.4	561.2

DISCUSSION

Belgrade and its surroundings have a moderate continental climate and the hottest month is July with an average temperature of 22.5°C (Unkasevic, 1994). In the urban area the number and prevalence of some mosquito species change from year to year, depending on climatic conditions, type of a habitat and measures taken in the previous year. The temperature and humidity are basic abiotic factors that influence the development and survival of mosquito populations. Year 1999 was taken as an example when elements of a tropical climate were recorded in Belgrade which resulted, besides the inability of systematic air-treatment of mosquitoes (because of bombing), in a significant increase in their number.

Raising domestic animals in suburban areas is typical of Belgrade. In these conditions *Anopheles* habitats survive. At the same time an efficient zoo-barrier is provided protecting people from the *Anopheles* sting.

The identification of species within the *An. maculipennis* complex is still made by using an old method of morphological identification of laid eggs that we also applied, but molecular techniques for more reliable identification of species within this and other *Anopheles* complexes are making an advanced progress (Proft *et al.*, 1999; Romi *et al.*, 2000; Linton *et al.*, 2002). Financial troubles in the last decade have resulted in the lack of vector control and thus in the increase of the *Anopheles* density in localities in the alluvial plain of the rivers Sava and Danube.

In some localities in Belgrade, hydro-geological characteristics of the area are suitable for the development of *Anopheles* mosquitoes and cause their denser population (Pancevacki Rit, Crvenka, Galovica, Mislodjin and the outskirts of Batajnica). In the inhabited parts of town where intensive zoo-hygienic measures are taken (Grocka, Surcin, Stara Bezanija), residual populations of *Anopheles* mosquitoes are present. Our research has confirmed changed relations between species within the *An. maculipennis* complex in comparison to the period before eradication (Simic, 1948; Vukasovic, 1950; Sitar, 1977; Stajkovic *et al.*, 1991). As a result of ecological changes and urbanization, in the first place, the previous main malaria vector *An. maculipennis* s. s. is very rare now. *An. messeae* is prevalent

and although zoophilic, it used to have a vector potential for malaria in Serbia together with *An. maculipennis* s. s. (Simic, 1948).

An. atroparvus, which was seldom found in comparison to the other two species, is in the second place regarding its prevalence, and previous entomological research (Vukasovic, 1950; Sitar, 1977; Stajkovic *et al.*, 1991) shows a continual increase of its number in the general population of *Anopheles* mosquitoes in the Belgrade area. This species is considered to be the secondary malaria vector in Europe (Service, 2000). *An. atroparvus* is most prevalent in localities close to the international airport Surcin. According to the International Sanitary Code the airport area should be malaria vector-free 400 m in diameter (Guillet *et al.*, 1998). The length of development of *Plasmodium* in a mosquito is different with different species and is conditioned by air temperature, and if it is below 15°C the cycle cannot be completed (Service, 2000). Although there are favourable weather conditions in Belgrade from June until mid September for potential completion of the sporogonic cycle of *Plasmodium*, there is a question whether previous vectors are susceptible to infection of imported *Plasmodium* species, in the first place with the prevalent imported *P. falciparum* of African origin (Dakic, 2005). No experimental research has been done in our country on the ability of domestic *Anopheles* mosquitoes to complete a sporogonic cycle of certain *Plasmodium* species. *An. atroparvus*, according to the experiments made in Italy, England and Portugal, is not receptive to the African *P. falciparum* species (De Zulueta *et al.*, 1975; Marchant *et al.*, 1998). However, its vector potential for *P. vivax* of an exotic origin has been experimentally confirmed (De Zulueta *et al.*, 1975; Daskova and Rasnicyn, 1982). In Russia *An. messeae* has become as prevalent as it used to be before eradication in the last decade and the erosion of health care and an increase of imported malaria have caused *P. vivax* malaria transmission (WHO, 2002).

In Serbia no research has been done regarding antropophilia of *Anopheles* mosquitoes. In the Ulcinj area Vitanovic (1980) found out that man's role in *Anopheles* feeding is insignificant because of man's changed living conditions comparing to the past. In the localities where we entomologically examined a greater number of animal shelters with different animal hosts, local *Anopheles* populations adapted themselves to the existing animal hosts, which confirms that they are ready to chose an alternative host in the absence of the original one (Service, 2000).

CONCLUSION

In the examined localities in the Belgrade area we have identified three different mosquito species all of them belonging to the *An. maculipennis* complex: *An. messeae*, *An. atroparvus* and *An. maculipennis* s. s. A great density of *An. messeae* in the first place, as well as *An. atroparvus* does not necessarily mean a high risk of malaria reintroduction. Their obvious tendency to live in animal shelters and sting mostly animals, as well as the unreceptiveness of European *An. atroparvus* and *An. messeae* to *P. falciparum* regarding a scarce number of gametocyte bearers in our country during summer, in the first place *P. falciparum*

of the African origin, is quite relieving. However, the possibility of a sporadic outbreak of autochthonous urban malaria in some unusual circumstances like in 1999 with the presence of *Anopheles* mosquitoes, cannot be totally excluded. The expectations that easier communication with the world will cause an increasing import of tropical parasitic diseases as well as *P. vivax* which these European species of *Anopheles* mosquitoes are probably receptive to, obligate us to a continual epidemiological observation and observation of species distribution (introducing new techniques), density, change in the behaviour of *Anopheles* mosquitoes, as well as the level of their resistance to the pesticides in use.

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Ekologija komaraca roda *ANOPHELES* na području Beograda u proceni vektorskog potencijala za ponovno uspostavljanje transmisije malarije

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SADRŽAJ

Beograd je smešten u području koje je potencijalno rizično za ponovno uspostavljanje transmisije malarije. Do eradikacije malarije, glavni vektor malarije na području Beograda bio je *An. maculipennis* s. s. (raniji naziv *An. typicus*) a sekundarni vektori bili su *An. messeae* i *An. atroparvus*. Mi smo analizirali distribuciju i ekologiju komaraca roda *Anopheles* (*Diptera, Culicidae*) na području Beograda. Ženke komaraca roda *Anopheles* sakupljali smo tokom 2003. godine na 8

lokaliteta šireg područja Beograda. Identifikaciju vrsta vršili smo na osnovu morfologije položenih jaja.

Od ukupnog broja izlovljenih ženki komaraca roda *Anopheles*, njih 3704 je položilo jaja, a njihovom identifikacijom nađene su tri vrste komaraca roda *Anopheles*, svi pripadnici *Anopheles maculipennis* kompleksa: *An. messeae*, *An. atroparvus* i *An. maculipennis* s. s.. U ukupnoj populaciji najzastupljeniji je bio *An. messeae* 64%, zatim *An. atroparvus* 21%, a najmanje *Anopheles maculipennis* s. s. 8%. Postojala je razlika u procentualnoj zastupljenosti ovih vrsta u ukupnoj *Anopheles* populaciji prema lokalitetima, po mesecima, prema izboru vodenih staništa. Prisutne vrste *Anopheles* komaraca, posebno *An. messeae* i *An. atroparvus* ukazuju da je Beograd receptivan za transmisiju malarije, a klimatski uslovi tokom leta pogodni su za kompletiranje sporogoničnog razvoja pripadnika roda *Plasmodium*.