

The Species Composition of Malaria Mosquitoes in the Kharkov Region (Ukraine): Natural Factors of Malaria Spread

Ukrayna'daki Kharkov Bölgesi'ndeki Sıtma Sivrisineklerinin Tür Bileşimi: Sıtma Yayılmasının Doğal Faktörleri

Lyudmila Gazzavi-Rogozina¹, Olga Filiptsova¹, Olga Naboka¹, Alexandr Ochkur²

¹ Biology Department, National University of Pharmacy, Kharkov, Ukraine

² Pharmacognosy Department, National University of Pharmacy, Kharkov, Ukraine

ABSTRACT

Objective: Article describes the species composition of malaria mosquitoes dominating in the Kharkov region, Ukraine, season of their possible effective infection, as well as antimalarial precautions taken.

Methods: When collecting the material, the conventional methods of evaluation of abundance of mosquitoes were used. Collection of larvae and pupae was carried out with standard butterfly net or photo tray with subsequent recalculation per m².

Results: On the territory of the region were studied 30 species of bloodsucking mosquitoes of such three genera as *Anopheles*, *Culex*, *Aedes* were found.

Conclusion: Facts demonstrate the favorable environmental conditions for malaria spread such as; increase in the number of vectors, increase in precipitation, long temperature period of transmission of infection.

Key Words: Malaria, *Anopheles* genus, epidemiology, evaluation of abundance, hydrotechnical measures

Received: 10.09.2016

Accepted: 11.28.2016

ÖZET

Amaç: Bu makale, Ukrayna'nın, Kharkov bölgesinde egemen olan sıtma sivrisineklerinin, muhtemel etkili enfeksiyon mevsimindeki tür bileşiminin yanı sıra alınan antimalaryal önlemlerini açıklamaktadır.

Yöntem: Materyali toplarken, sivrisineklerin miktarının değerlendirilmesi için geleneksel yöntemler kullanılmıştır. Larvaların ve pupaların toplanması standart kelebek ağı veya fotoğraf tepsisi ile yapıldı ve m² başına sonraki yeniden hesaplamalar yapıldı.

Bulgular: Bölgenin topraklarında *Anofel*, *Kuleks*, *Aedes* gibi 30 tür içerisinde üç cins kan emen sivrisinek bulundu.

Sonuç: Durum; sıtma yayılımı için olumlu çevresel koşulları göstermektedir. Bunlar arasında, vektör sayısındaki artış, yağış artışı, enfeksiyonun aktarımında uzun sıcaklık periyodu sayılabilir.

Anahtar Sözcükler: Sıtma, *Anofel* türü, epidemiyoloji, miktarın değerlendirilmesi, hidroteknik önlemler

Geliş Tarihi: 09.10.2016

Kabul Tarihi: 28.11.2016

INTRODUCTION

Bloodsucking mosquitoes (*Diptera, Culicidae*) are numerous annoying bloodsuckers and vectors of pathogens of dangerous diseases of humans and animals-parasitic invasions, viral and bacterial infections. Change in abundance and the species composition of bloodsucking mosquitoes have a significant impact on the course of the epizootic process, and therefore, on the epidemiological safety of the area, the health status of the population and domestic animals. Many authors have investigated the fauna of blood-sucking mosquitoes, but today the issues of ecology and the epidemiological significance remain relevant all over the world.

In the longitudinal study conducted in Sekong Province in the southern region of Laos was studied the prevalence of malaria mosquitoes in three malaria endemic villages. Of the 16 species of mosquitoes of *Anopheles* genus being under study *An. dirus* A, *An. maculatus* sl. and *An. jeyporiensis* appeared to be infected with sporozoites was observed high correlation between the sporozoite index and humidity of the malaria epidemiological season. It was also noted the ambivalence of mosquitoes in the choice of food since about 50% of insects with equal probability attack humans both indoors and outdoors (1). According to the studies conducted in the malaria endemic areas of such villages as Lenia, Kuala Lipis, Pahang in Malaysia the population of *Anopheles maculatus Theobald* mosquitoes was 31.0%. High frequency level of attacks of *Anopheles maculatus Theobald* on humans was recorded in December, and in January the activity of attacks decreased. Larval stage of mosquitoes was taken by a rapid flow of the river, thereby causing a reduction in their number. Of the five dominant species of *Culicidae* family only *An. annulitarsis* had a significant positive correlation of abundance with monthly precipitation. Activity of attacks of *An. maculatus* was observed from 10:00 to 11:00 p.m. (2). According to the studies conducted by the Center for Control of Diseases in Papua New Guinea when catching mosquitoes of *Anopheles* genus using baits and insect light traps *Anopheles koliensis* Owen was caught most often, then *An. punctulatus* Dönitz, *An. karwari* (James), *An. farsauti* Laveran (*sensu lato*), *An. longirostris* Brug and *An. bancroftii* Giles followed. Percentage of mosquitoes affected by sporozoites of *Plasmodium falciparum* Welch and *P. vivax* in light traps was much higher than in mosquitoes caught by the "on oneself" method. It implies the attractiveness of light traps for older mosquitoes. *An. punctulatus* and *An. Farsauti* were more often affected by sporozoites (3). In Western and East Georgia three related species of malarial mosquitoes of the *An. maculipennis* complex – *An. maculipennis* Meigen, 1818; *An. melanoon* Hackett, 1934; *An. sacharovi* Favre, 1903 were identified (East Georgia) (4). Entomological observations of malaria-transmitting mosquitoes were conducted in the area of the Korean demilitarized zone (Paju, Gyeonggi Province) from April to October 1999 where there were cases of malaria. Trapping of mosquitoes of the adult stage was performed using the dark field and light traps with ultraviolet radiation in five and two stations, respectively. Weekly capture of the larval stages was also conducted in five rice fields located close to the stations of trapping of adult species. Malarial index appeared to be higher in mosquitoes of 11 species selected throughout the study period in 1999, and it was 47-48% of the total number of species caught in dwelling houses and cattle barns. In all five observation stations mosquitoes of *Anopheles* genus were caught in ultraviolet light traps from May, and they were the most numerous in the stations located near ponds and rice fields. Percentage of the population density of the larval and adult stage constantly grew since June and reached its maximum value in the second decade of July (112 females/trap/night). Cross correlation showed a significant relationship between the number of adult females of *Anopheles* genus and the number of larvae collected on the same day, previous day, as well as 3 and 7 days ago (5).

According to the data of the European Center for Control and Prevention of Diseases *Anopheles labranchiae* species is of special interest of researchers. It is the endophilic species and a harmful bloodsucker involved in transmission of malaria in Spain, Portugal, France, Corsica in recent years, and in 2011 – in Italy. *Anopheles labranchiae* was and is the most important link in spreading malaria (Becker et al., 2010). It was suggested that *An. labranchiae* was involved in transmission of malaria in Spain, Portugal, France and Italy (6). Historically, there is the evidence of natural infection with *An. labranchiae* by exotic strains of *malaria plasmodium*, and recently the studies have shown that these data are confirmed in the laboratory. In general, *An. labranchiae* is considered to be non-effective for transmission of exotic strains (7). Recently, it was reported that *An. labranchiae* was a vector of an imported case of tertian malaria (*pl. vivax*) in Corsica in 1970-ies (8). Difficulties of the population control and the prevalence of *An. labranchiae* are explained by a high fertility of this species (9).

The European Center for Control and Prevention of Diseases also reports that *Anopheles sacharovi* was distributed in the coastal areas of Italy, Sardinia, Corsica, Croatia, the former Yugoslav Republic of Macedonia, Albania, Bulgaria, Romania, in the southern regions of the former USSR, Turkey, Lebanon, Israel, Jordan, Syria, Iraq, Iran (10, 11, 12, 13). Preliminary results of the recent research in Moldova suggest that there is a significant part of the population of this species on the south of the country. For the first time this species was registered in Greece in 1928, and later a numerous population was found in all coastal areas (14). Previously *Anopheles sacharovi* was registered in abundance in Armenia, but disappeared in 1965. The recent studies indicate re-colonization of some regions of Armenia (15). There are also records of occurrence of this species in Cyprus since 2009. *Anopheles sacharovi* is an important factor in spreading malaria (10). Historically, this is a known vector of malaria in Armenia (15), and it was confirmed not only in Turkey, Syria, Northern Iraq and Iran (12), but in Greece as well (14). In Turkey, it remains the main vector of malaria (16). It was also suggested that *Anopheles sacharovi* was involved in transmission of malaria in Corsica, France, the Balkans, Italy, Romania and Greece (6). It was the main vector in the North-Eastern coast of Italy (15). In 2011 *Anopheles sacharovi* was the vector of 42 cases of tertian malaria in Greece (17, 18, 19). Presumably, malaria transmission was by *An. sacharovi* because of migrants from countries endemic for malaria. This is the most common species of malarial mosquitoes in Greece (17, 20, 21). Taking into consideration the high epidemiological importance, as well as frequent complaints of people on the bites of troublesome bloodsuckers we considered it appropriate to analyze the modern status of the population of *Anopheles* genus mosquitoes within the territory of the Kharkov region (Ukraine).

MATERIALS and METHODS

Analysis of the entomological and meteorological situation in Ukraine was conducted in the Kharkov region according to the data of the Ukrainian Center of Control and Monitoring of Diseases at the Ministry of Health of Ukraine, as well as the Kharkov Regional Laboratory Center.

Collection of the material (adult and larval) was carried out on the territory of natural and artificial reservoirs the Kharkov region in the period of 2013 – 2014.

When collecting the material the conventional methods of evaluation of abundance of mosquitoes were used. Collection of larvae and pupae was carried out with standard butterfly net (net diameter 20 cm, bag depth 25 cm, handle length 1 m) or photo tray (area 13 × 18) with subsequent recalculation per m². Unit of account is carrying out a net in the water for one meter. During examination net was submerged in the water to half of the rim and quickly carried out on the surface of one meter. Content of the net was rinsed in the tub and out of it were caught larvae and pupae of different stages, counting the number of both. Accounting of winged mosquito population was carried out 1 hour before sunset, at sunset or 1 hour after sunset. Considering that various species attack man at different times, were carried out three accounting for one hour, and the average number of mosquitoes of all species on one account. Mosquitoes were caught by the method "on the body" for 15 minutes on a naked forearm with a test tube-killing bottle (22).

RESULTS

On the territory of the region were studied 30 species of bloodsucking mosquitoes of such three genera as *Anopheles*, *Culex*, *Aedes* were found. Determination of species belonging of mosquitoes was carried out at the larval and imaginal stages using determinants (22, 23). Epidemiological role of each species is determined by a number of conditions. Dangerous vectors can be only the species occurring in large amount with a significant percentage of the population feeding on the human blood, having a rather long season of activity and a sufficient number of females surviving to the age of the possible maturation of sporozoites in their bodies (24).

Depending on the situation each species of *Anopheles* meets these conditions to varying degree. The same *Anopheles* species can be a dangerous vector in one area and be for nothing in another locality. There are species that are dangerous vectors under a wide range of conditions, while others transmit malaria only in exceptional cases or do not have the epidemiological value at all (24).

In Ukraine the most important vectors are *Anopheles maculipennis*, *An. m. messeae*, *An. m. atroparvus*, *An. claviger* (25) (Table 1).

List of the dominant species of mosquitoes (Diptera, Culicidae) of the Kharkov region includes: *Anopheles* genus – *An. maculipennis*, *An. messeae*; *Aedes* Meigen genus – *Ae. cataphylla*, *Ae. leucomelas*, *Ae. dorsalis*, *Ae. excrucians*, *Ae. vexans*; *Culex* genus – *C. pip. pipiens*, *C. pip. molestus*.

Table 1. Relative abundance of *Anopheles* mosquitoes Kharkov region

List of species		*Relative abundance
Anopheles mosquitoes	1 <i>Anopheles maculipennis</i>	10.2 on a sq.m.
	2 <i>An.m. messeae</i>	10.8 on a sq.m.
	3 <i>An.claviger</i>	7.4 on a sq.m.
	4 <i>An.atroparvus</i>	6.8 on a sq.m.

All mosquito species registered on the territory of the Kharkov region are susceptible to the species of human malaria parasites currently known. Moreover, dominant species in urban landscapes are *An. maculipennis* and *An. messeae*. These species possess all the qualities that are necessary to be considered a dangerous vector of malaria. They are well infected with three main species of human malaria parasites (24).

On the territory studied under the conditions of urban landscapes the gonotrophic females occurred within 3.5–4 months, and the larval stages in ponds – approximately within 4.5 months. Maximum number of species was observed in mid-July. Due to the high number and activity of the attack in the summer, as well as proximity of breeding places to human settlements such species as *An. maculipennis*, *An. messeae* are of the greatest epidemiological risk (25).

DISCUSSION

As previously noted, in order to correctly identify malaria control the knowledge concerning the species composition of *Anopheles* that are prevalent in the given area, epidemiological values of each species, subspecies and biology of major vectors is required. Control measures against a vector must be based on the knowledge of its biology and the seasonal changes. Rational duration of antimalarial precautions is determined primarily by seasonal phenomena in the life of malaria mosquitoes. When planning and implementing the measures for prevention of malaria in Ukraine the scientifically proven terms for conducting such measures based on the long-term study of phenology of malaria mosquitoes are used. Best evaluation criterion of anti-mosquito measures is the age composition of female malaria vectors. Malaria transmission occurs by *Anopheles* mosquitoes in the presence of favorable temperatures for the maturation of malarial parasites in the body of a mosquito (25).

According to the data of the Kharkov regional hydrometeorological center the end of spring in 2013 was hot. Temperature reached +30°C. Summer of 2013 was moderate with sufficient rainfall. Daytime temperatures, as a rule, did not exceed the level of +35°C. July was the hottest month, the average temperature of the month was +25 °C. Maximum temperature observed in August 7 (+37 °C). Autumn was warm and rainy, maximum temperature reached up to +25°C. All facts mentioned above demonstrate improvement of environmental conditions in the possible transmission of imported cases of malaria (26).

In order to monitor the possible occurrence of indigenous cases of malaria and transmission of imported, it is critical to track the beginning and end of the season of effective infection of mosquitoes with malaria plasmodium not only in the Kharkov region, but throughout Ukraine. Season of effective infection of malaria mosquitoes by a causative agent of tertian malaria – *P. vivax* in 2013 began in 18 regions of Ukraine before May 10, earlier (before March 18) – in the Chernivtsi region; later – in the Ivano-Frankivsk region (May 31, 2013). End of the season of effective infection of mosquitoes in 13 regions of the country was in the I-II decade of August; in 6 regions – in the III decade of August, in 4 regions – in September; and in the Sumy region, the Chernihiv region and Kyiv – before August (August 7, 2013); later – in the Odesa region (October 7, 2013). The longest season of effective infection of mosquitoes by a causative agent of tertian malaria was observed in the Odesa region (163 days) (25).

Season of a possible infection of persons with a causative agent of vivax malaria began in the II-III decades of May in 15 regions and in the I-II decades of June in 7 regions; prior the specified period this season began in the Chernivtsi region – before April 15; later – in the Ivano-Frankivsk region – before July 28. End of the season was in October in 19 regions and in September in 3 regions; in the Cherkasy region – in September 10, 2013; in the Odesa region – in November 25, 2013.

The longest season of the possible transmission of malaria was recorded in the Odesa region – 191 days (25).

Epidemiological situation of malaria is complicated by deterioration of the entomological monitoring of vectors – bloodsucking mosquitoes. In 2012 specialists of entomological groups of sanitary-epidemiological institutions conducted supervision over the sanitary condition of water reservoirs, fish breeding ponds, ornamental ponds, basements, and other areas. Antimalarial hydrotechnical measures, monitoring in determining the species and age composition of malaria mosquitoes were conducted. After reforming the sanitary and epidemiological service in December 2012 the Parasitological and Entomological Sections were significantly reduced.

In the Kharkov region during 2012 compared to 2011 the average number of the larval stages of *Anopheles* genus mosquitoes increased by 3.9% (25).

In 2013 on the territory of the Kharkov region 5082.6 ha of anophelogenous areas of water reservoirs with the average seasonal index of abundance of larvae of *Anopheles* genus mosquitoes – 8.7 were registered. In 2013, as well as in previous years, to control the number of bloodsucking mosquitoes environmental (hydrotechnical) measures and chemical (insecticidal) agents were used, but in much smaller amounts (25). But it should be remembered that resistance to insecticides can be a challenge all over the world. For instance, the study on resistance to insecticides to reduce the number of mosquitoes of *Anopheles* genus was conducted in southern Turkey in Adana, Adiyaman, Antalya, Aydin and Mugla. These areas where malaria is endemic are diverse with relation to the use of insecticides, geographical features, social infrastructure, development of agriculture and peculiarities of tourism. All these factors can influence on the mosquito resistance to insecticides. Dominant species *An. sacharovi*, *An. superpictus* were exposed to different insecticides – DDT, dieldrin, malathion, pirimiphos methyl. Resistance to dieldrin was detected in *An. sacharovi* in 1970; resistance to karbofos was first registered in 1974. Cross-resistance was observed for a wide range of organophosphorous and carbamate insecticides although previously they had never been used for treating dwelling houses and premises. Aim of the study was to compare the baseline information on susceptibility of *Anopheles* genus mosquitoes under experimental conditions with the results obtained on the samples in nature in order to assess the current trends in resistance of mosquitoes to insecticides on the southern coast of Turkey (27).

Operational area of treatment against adult malaria mosquitoes in Ukraine in the premises was reduced by 20% of the amount of work of 2012 and was the lowest in the last 5 years, and against the larvae of malaria mosquitoes – by 2.5 times (25).

Antimalarial hydrotechnical measures were conducted in all sectors to the fullest extent from the work leading to elimination of the mosquito breeding to the work creating unfavorable conditions for larval development (25).

CONCLUSION

Abovementioned facts demonstrate the favorable environmental conditions for malaria spread: increase in the number of vectors, increase in precipitation, long temperature period of transmission of infection.

Conflict of interest

No conflict of interest was declared by the authors.

REFERENCES

1. Vythilingam I, Phetsouvanh R, Keokenchanh K, Yengmala V, Vanisaveth V, Phompida S et al. The prevalence of *Anopheles* (Diptera: Culicidae) mosquitoes in Sekong Province, Lao PDR in relation to malaria transmission. *Trop Med Int Health*. 2003; 8:525-35.
2. Ali WN, Ahmad R, Nor ZM, Ismail Z, Lim LH. Population dynamics of adult mosquitoes (Diptera: Culicidae) in malaria endemic villages of Kuala Lipis, Pahang, Malaysia. *Southeast Asian J Trop Med Public Health*. 2011; 42:259-67.
3. Hii JL, Smith T, Mai A, Ibam E, Alpers MP. Comparison between anopheline mosquitoes (Diptera: Culicidae) caught using different methods in a malaria endemic area of Papua New Guinea. *Bull Entomol Res*. 2000; 90:211-9.
4. Bezzhonova OV, Babuadze GA, Gordeev MI, Zvantsov AB, Ezhov MN, Imnadze P et al. Malaria mosquitoes of the *Anopheles maculipennis* (Diptera, Culicidae) complex in Georgia *Med Parazitol* (Mosk). 2008; 3:32-6.

5. Shin EH, Lee WJ, Lee HI, Lee DK, Klein TA. Seasonal population density and daily survival of anopheline mosquitoes (Diptera: Culicidae) in a malaria endemic area, Republic of Korea. *J Vector Ecol.* 2005; 30:33-40.
6. Alten B, Kempen H, Fontenille D. Malaria in southern Europe: resurgence from the past. In Takken W, Knols BGJ, editors. *Emerging pests and vector-borne diseases in Europe*. Wageningen (the Netherlands): Wageningen Academic Publishers; 2007. p. 35-58.
7. Toty C, Barre H, Le Goff G, Larget-Thiery I, Rahola N, Couret D et al. Malaria risk in Corsica, former hot spot of malaria in France. *Malar J.* 2010; 9:231.
8. Baldari M, Tamburro A, Sabatinelli G, Romi R, Severini C, Cuccagna G et al. Malaria in Maremma, Italy. *Lancet.* 1998; 351:1246-7.
9. Boccolini D, Toma L, Di Luca M, Severini F, Cocchi M, Bella A et al. Impact of environmental changes and human-related factors on the potential malaria vector, *Anopheles labranchiae* (Diptera: Culicidae), in Maremma, Central Italy. *J. Med Entomol.* 2012; 49:833-42.
10. Becker N, Petric D, Zgomba M, Boase C, Madon M, Dahl C et al. *Mosquitoes and their control*. Second ed. Berlin: Springer-Verlag Berlin Heidelberg; 2010; 577.
11. Schaffner F, Angel G, Geoffroy B, Hervy JP, Rhaïem A, J. B. The mosquitoes of Europe (CD ROM). Montpellier, France: IRD Edition and EID Méditerranée; 2001.
12. Yurttas H, Alten B. Geographic differentiation of life table attributes among *Anopheles sacharovi* (Diptera: Culicidae) populations in Turkey. *J Vector Ecol.* 2006; 31:275-84.
13. Merdic E. Checklist of mosquitoes (Diptera: Culicidae) of Croatia. *European Mosquito Bulletin.* 2004; 17:8-13.
14. Patsoula E, Samanidou-Voyadjoglou A, Spanakos G, Kremastinou J, Nasioulas G, Vakalis NC. Molecular characterization of the *Anopheles maculipennis complex* during surveillance for the 2004 Olympic Games in Athens. *Med Vet Entomol.* 2007; 21:36-43.
15. Romi R, Boccolini D, Hovanesyan I, Grigoryan G, Di Luca M, Sabatinelli G. *Anopheles sacharovi* (Diptera: Culicidae): a reemerging malaria vector in the Ararat Valley of Armenia. *J Med Entomol.* 2002; 39:446-50.
16. Alten B, Caglar SS, Simsek FM, Kaynas S. Effect of insecticide-treated bednets for malaria control in Southeast Anatolia. Turkey. *J Vector Ecol.* 2003; 28:97-107.
17. Danis K, Baka A, Lenglet A, Van Bortel W, Terzaki I, Tseroni M et al. Autochthonous Plasmodium vivax malaria in Greece, 2011. *Euro Surveill.* 2011; 16:19993.
18. European Centre for Disease Prevention and Control. Meeting Report: Consultation on Plasmodium vivax transmission risk in Europe, Stockholm 17-18 January 2012. Stockholm: ECDC, 2012.
19. Hellenic Center for Disease Prevention and Control. Epidemiological Surveillance Report, Malaria in Greece, 2013. Athens; 2013.
20. Vakali A, Patsoula E, Spanakos G, Danis K, Vassalou E, Tegos N, et al. Malaria in Greece, 1975 to 2010. *Euro Surveill.* 2012; 17:20322.
21. Kousoulis AA, Chatzigeorgiou KS, Danis K, Tsoucalas G, Vakalis N, Bonovas S et al. Malaria in Laconia, Greece, then and now: a 2500-year-old pattern. *Int J Infect Dis.* 2013; 17:e8-e11.
22. Prudkina NS. Krovososuschie dvukrylyie nasekomyie. Fauna, biologiya, ekologiya, mediko-veterinarnoe znachenie. Uchebnoe posobie: H. Kollegium; 2011 (in Russian).
23. Sheremet VP. Krovosisni komarl' Ukrayini. Navchalniy posibnik dlya studentiv biologichnogo fakultetu. K.: "KiYivskiy univrsitet"; 1998 (in Russian).
24. Zvantsov AB, Ezhov MN, Artemev MM. Perenoschiki malyarii (Diptera, Culicidae, Anopheles) sodruzhestva nezavisimiyh gosudarstv (SNG). Kopenhagen: Vsemirnaya organizatsiya zdorovoohraneniya. Evropeyskoe regionalnoe byuro. Programma «Obratim vspyat malyariyu»; 2003 (in Russian).
25. Statistical data of the Ukrainian Center of Control and Monitoring of Diseases at the Ministry of Health of Ukraine (<http://ucdc.gov.ua/en/>).
26. <https://www.timeanddate.com/weather/ukraine/kharkiv/historic>.
27. Kasap H, Kasap M, Alptekin D, Herath PRJ. Insecticide resistance in *Anopheles sacharovi* Favre in southern Turkey. *Bull World Health Organ.* 2000; 78:687-92 (in Russian).