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THE USE OF MATHEMATICAL MODELS IN MONITORING THE RISK OF RE-EMERGING MALARIA IN ROMANIA

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Malaria, considered to be the oldest disease on earth, has a high annual mortality rate and represents a global threat. Being transmitted by mosquitoes, this disease is expanding its spatial range, gradually manifesting itself in previously unaffected areas and reappearing in areas where it was eradicated in the past. With mosquitoes – poikilothermic arthropods – as its definitive host, the development of the *Plasmodium* protozoan depends entirely on the environmental temperature. Thus, predictions of mosquito population development and pathogen evolution within vectors, in contrast to climate change, are becoming a necessity, especially for European countries, which are currently free of many of the targeted diseases, especially malaria, with only imported cases remaining. The climatologic analysis highlighted that in 2023 in the Danube Delta area cumulative temperature-humidity conditions for malaria infection have been met. From the 13^{th} of May to the 30^{th} of September infection with *Plasmodium vivax* was possible and from the 9^{th} of June to the 30^{th} of September infection with *Plasmodium falciparum* was likely. We estimate that the periods in which mosquitoes can multiply in the Danube Delta will increase by 2 weeks in next 50 years, in the context of climate change.

Keywords: Plasmodium protozoan; malaria; mosquitoes.

INTRODUCTION

The transmission of diseases through mosquito bites depends on a series of very complex interactions between the environment and susceptibility, the adaptive capacity of populations, and exposure. Variation in climatic factors, including rainfall and temperature, modulates the spatiotemporal distribution of vector mosquitoes, their hosts and the pathogens they transmit. Thus, climate change increases the risk of disease transmission, through increased vector survival time as well as blood feeding rates, increased replication of vector pathogens, shorter breeding periods and longer transmission seasons[1].

Malaria and dengue are the most important mosquito-borne diseases threatening the entire world, diseases that are expanding their spatial range, gradually manifesting themselves in previously unaffected areas and reappearing in areas where they were previously eradicated[2–4]. Studies show that the range of malaria transmission has also expanded in the African highlands, with the climate conducive to transmission increasing by 30% between 2012 and 2017[2].

Malaria is a highly fatal disease caused by protozoa of the Plasmodium genus. Five species are involved in causing the disease in humans, and two of these species – *Plasmodium falciparum* and *Plasmodium vivax* – are of greater importance, with Pl. falciparum producing about 97% of the cases recorded globally (230 million cases), 93% being recorded in sub-Saharan Africa[5–7].

Malaria is a vector-borne disease, transmitted by mosquitoes of the genus *Anopheles*; in Africa the species *Anopheles gambiae* is responsible for transmission, as it is a strictly anthropophilic species that feeds both indoors and outdoors, especially at night[8]; and for Europe species belonging to the *Anopheles maculipennis* complex are involved, especially *Anopheles labranchiae*[9].

Physiological characteristics of vectors (such as growth and blood-feeding rates) and pathogens (*e.g.* extrinsic incubation period) increase almost exponentially with increasing temperatures until a thermal optimum is reached[10,11]. Increased rainfall also favours the creation of mosquito breeding sites, but floods could destroy these breeding sites[12]. Nevertheless, at the same time droughts leading to water shortages can favour the creation of breeding sites by increasing water storage, especially near dwellings. Research suggests that high humidity shortens mosquito incubation and blood-feeding intervals[13].

Anopheles mosquitoes transmitting *Plasmodium vivax* are still present in Europe, with the most widespread being *Anopheles atroparvus*, but other species belonging to the maculipennis complex have been reported, such as *An. labranchiae*, *An. messeae*, *An. sacharovi* and *An. melanoon*, and the current environmental conditions would be suitable for mosquito development at high densities[14–19].

For *Pl. vivax* a minimum of 14.5–15°C is required to develop inside the mosquito, while *Pl. falciparum* requires a temperature of 16–19°C[20–22]. A modelling study conducted by Mordecai *et al*. in 2013[23] suggests optimal malaria transmission at 25°C, which makes several areas in Europe vulnerable to malaria transmission, and the starting point of transmission decrease at 28°C. This is consistent with the result of studies in Portugal, which state that *Anopheles* mosquito abundance is favoured by temperatures between 19 and 25°C18. *Plasmodium* development and transmission occur within a certain temperature range, *i.e*. between 15°C and 35°C, and at 25°C the extrinsic life cycle occurs between 9 and 21 days. The extrinsic life cycle cannot be completed for *Pl. falciparum* species at temperatures below 20°C; and for *Pl. vivax* the life cycle ceases at temperatures below 15°C. However, temperatures above 25°C will shorten the extrinsic development period.

Vectors capable of transmitting *Pl. vivax* are also present in Europe. Potential malaria vectors in Europe belong to the *An. maculipennis* complex (*e.g*. An. messeae)[24]. Understanding the vector population will lead to a clearer understanding of the malaria transmission process and enhance our ability to predict what may happen to disease intensity in the future. At 16°C, larval development can take more than 45 days (leading to a reduction in the number of mosquito generations per year by subjecting larvae to increased risk to predators), compared to only 10 days at 30°C. By affecting the duration of the aquatic phase on the life cycle of mosquitoes, temperature and precipitation determine the timing and abundance of mosquitoes.

Recent study by Shapiro *et al*. (2017) states, based on field and laboratory data and empirical mathematical models, that the optimal temperature for malaria transmission is between 25 and 26°C, with the minimum temperature required for transmission being 16–17°C and the maximum 34–35°C25. Mosquito mortality rates increase at temperatures above 25°C, while vector competence begins to decline. At 22°C the parasite life cycle lasts 19 days and at 30°C only 8 days[26]. The upper limit of mosquito longevity can be 56 days, as used in previous studies[27,28], depending on environmental conditions[29]. For malaria transmission, there are different values of temperatures and number of days in which transmission is possible: for *Pl. falciparum* – at 28°C, 9–10 days, and at 20°C, 22 days. *Pl. vivax* – at 28°C, 8–10 days, and at 20°C, 16 days. *Pl. malariae* – at 28°C, 14 days, and at 20°C, 30–35 days. *Pl. ovale* – at 28°C, 12–14 days.

MATERIAL AND METHOD

To demonstrate the risk of transmission of a vector-borne disease, we must follow three steps: the existence of the pathogen in nature, the presence of the mosquito vector in nature, and the existence of favourable climatic conditions.

Thus, we followed the cases of malaria reported in Romania in the last 25 years, proving the presence of the pathogen in the human reservoir host. Data were provided by the National Centre for Surveillance and Control of Communicable Diseases (NCSCCD) within the Institute of Public Health[31].

To demonstrate the presence of the Anopheles vector in nature, we identified mosquitoes from Tulcea area because in the past, when malaria was endemic in Romania, over 50% of the cases were registered in this area[31]. Our study on the existence of the vector in nature was conducted over a period of 6 months, April–September 2023, in the regions of Tulcea and Braila, where in the past, when malaria was endemic in Romania, most cases of Plasmodium spp. infestation were recorded.

During May, June, July, August and September, at the beginning and end of the month, in Mărașu Township, Plopi Village, located in the Great Braila Island, on the bank of one of the two arms of the Danube, the Old Danube, at a distance of 50 kilometres from the city of Braila, CDC Light Traps with dry ice as an attractant, and Laika traps also adapted for trapping mosquitoes (Figure 1, Figure 2), as well as a hand vacuum cleaner for collecting females directly from the human host were set. One of the nearby tourist attractions is the nature park on the Small Island of Braila.

Figure 1. CDC light traps placed in areas favourable to the development of mosquito populations

The traps were placed in areas considered favourable for the development of the vector (trees with bark, accumulations of water, abundant vegetation). The traps were set during the night, between 8 p.m. and 8 a.m. for the *Anopheles and Culex* species, and from morning till dusk for the *Aedes albopictus* species. The female *Aedes albopictus* has an anthropophilic behaviour and bites at dawn and dusk, between 3 p.m. and 7 p.m. Mosquitoes were preserved in ethyl alcohol for identification based on morphological characters, using identification keys presented by Becker et al. (2010)[32] and interactive keys from the MosKeyTool software (Pasteur Institute, France).

Making predictions on the development of mosquito populations and the evolution of pathogens within vectors, in contrast to climate change, is becoming a necessity, especially for European countries, which are currently free of many of the diseases of concern, especially malaria, with only imported cases remaining. Mathematical modelling of malaria transmission began in the 20th century when in 1911, Ronald Ross provided the first mathematical model. Ross proposed a mechanistic model of transmission that included the human host and the mosquito, and in the early 1950s, Georges Macdonald improved Ross' model and introduced an expression for the number of base replications[33]. The Intercomparison Project (ISI-MIP) is a community-led initiative that provides a consistent framework for cross-sectoral modelling of climate change effects. Studies show that rainfall accumulation greater than 80 mm, an average temperature of 18–32°C, and relative humidity greater than 60% combined represent the lower limit for Pl. falciparum transmission[34].

In order for a vector-borne disease to manifest itself in certain territories, the coexistence of three factors is necessary: the presence of the mosquito vector in nature, the presence of the pathogen in nature and, most importantly, the existence of favourable environmental conditions. The climatologic data were collected at the Tulcea weather station (Latitude: 45.183056, Longitude: 28.816667, Elevation: 4 meters) which measures the weather elements from the Danube Delta. The Tulcea station is identified with STAID 967 in the European Climate Assessment & Dataset (ECA&D). The precipitation, as well as minimum, maximum and average of air temperatures at 2 meters above ground were recorded daily from the 1st of January 2023 to the 30th of September 2023[35].

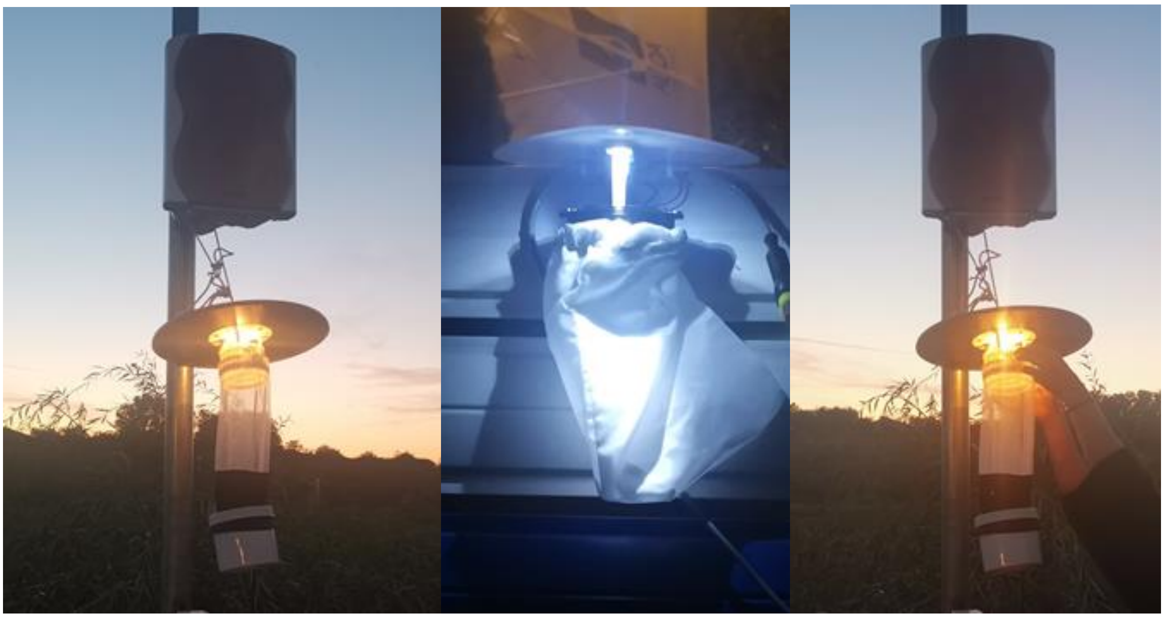

Figure 2. CDC light traps placed in areas favourable to the development of mosquito populations

RESULTS AND DISCUSSION

MALARIA CASES IN ROMANIA

Between 1995 and 2020, 667 cases of malaria were reported in Romania, all of them imported, but it is noted that cases were reported every year, with a decrease during the pandemic period (Figure 3). Considering that in Romania there is no malaria monitoring and control program, if the conditions are met (existence of mosquito vector in nature, existence of favourable environmental conditions, as well as the presence of malaria cases in humans), then the risk of re-emergence of malaria in Romania is present.

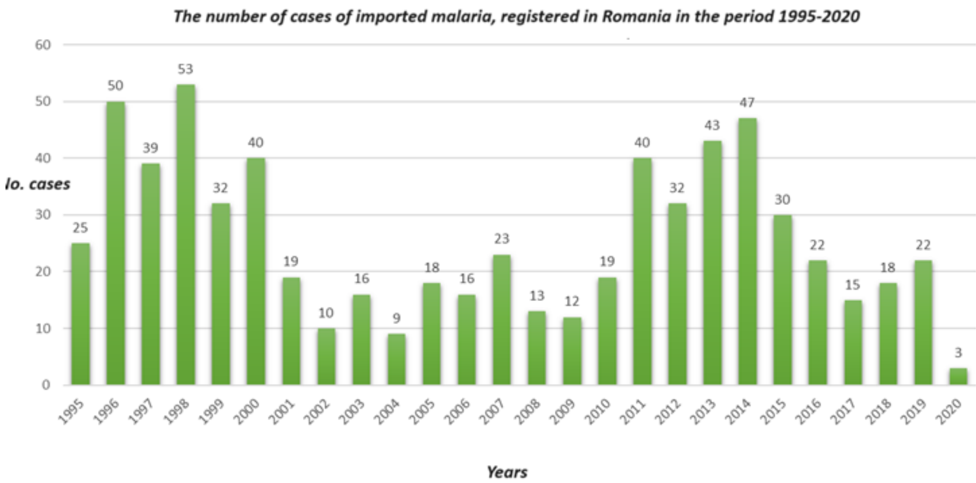

Figure 3. The number of malaria cases registered in Romania in the period 1995-2020 (National Centre for Surveillance and Control of Communicable Diseases (NCSCCD) within the Institute of Public Health (IPH))31

ANOPHELES VECTOR

In the area of the Great Braila Island 9 species have been identified: *Aedes albopictus, Culex pipiens*, *Aedes caspius*, *Culex modestus*, *Aedes vexans*, *Anopheles maculipennis*, *Anopheles hyrcanus*, *Coquillettidia richiardii*, *Culiseta anulata* (Table 1). The *Anopheles maculipennis* complex was identified on the basis of morphological characters provided by Becker N.[32].

Ten cryptic species belong to this complex, with *An. messeae* being the most widespread. Seven of these cryptic species are distributed throughout Europe, including Romania: *An. atroparvus, An. daciae, An. labranchiae, An. maculipennis s.s., An. melanoon, An. messeae and An. sacharovi*. The identification of these species in the adult stage is done only by PCR molecular biology techniques.

Table 1

Mosquito species identified in the Great Braila Island area

Location	**Date of collection**	**Exposure time**	**Identified species (no. specimens)**
Mărașu Township, Plopi Village, located in the Great Braila Island	15–18/05 /2023	The traps were placed during the night, between 8 p.m. and 8 a.m., for the species of the genus Anopheles and Culex, and from morning till dusk, for the species Aedes albopictus.	Culex pipiens **117** Aedes caspius **98** Culex modestus **148** Aedes vexans **215** Anopheles maculipennis **73** Coquillettidia richiardii **85** Culiseta anulata **138**
	16–19/06 /2023		Aedes albopictus **279** Culex pipiens **109** Aedes caspius **110** Culex modestus **99** Aedes vexans **93** An. maculipennis **55** Anopheles hyrcanus **17** Coquillettidia richiardii **38** Culiseta anulata **91**
	15–18/07 /2023		Aedes albopictus **212** Culex pipiens **109** Culex modestus **39** Aedes vexans **89** Anopheles hyrcanus **17** Coquillettidia richiardii **31** Culiseta anulata **22**
	12–15/08 /2023		Aedes albopictus **142** Culex pipiens **109** Aedes caspius **75** Culex modestus **99** Aedes vexans **153** Coquillettidia richiardii **38** Culiseta anulata **89**
	14–18/09 /2023		Aedes albopictus **123** Culex pipiens **109** Aedes caspius **205** Culex modestus **74** Aedes vexans **175** Anopheles maculipennis **68** Anopheles hyrcanus **17** Coquillettidia richiardii **38** Culiseta anulata **65**

The presence of species of the *Anopheles maculipennis* complex fulfils one of the three conditions for the spread of a vector-borne disease, namely the presence of vectors in nature, which together with the presence of the *Plasmodium* pathogen in nature and the existence of favourable environmental conditions, increase the risk of re-emergence of malaria in nature.

Traps were also placed in Tulcea area: Murighiol township, Victoria village and Mineri village, in the same period as in the Great Braila Island, respecting the types of traps and time period.

Eight species of mosquitoes have been identified in the Tulcea area: *Culex pipiens, Aedes caspius, Culex modestus, Aedes vexans, An. maculipennis, Anopheles hyrcanus, Coquillettidia richiardii and Culiseta anulata* (Table 2).

Table 2

Mosquito species identified in Tulcea area

Location	**Date of collection**	**Exposure time**	**Identified species (no. specimens)**
Murighiol township Victoria village Miners village	15–18/05 /2023	The traps were placed during the night, between 8 p.m. and 8 a.m., for the species of the genus Anopheles and Culex, and from morning till dusk, for the species Aedes albopictus.	Culex pipiens **173** Culex modestus **183** Anopheles maculipennis **84** Coquillettidia richiardii **91** Aedes vexans **198**
	16–19/06 /2023		Culex pipiens **109** Aedes caspius **103** Culex modestus **99** Aedes vexans **93** An. maculipennis **55** Anopheles hyrcanus **17** Coquillettidia richiardii **38** Culiseta anulata **91**
	15–18/07 /2023		Culex pipiens **109** Culex modestus **39** Aedes vexans **89** Anopheles maculipennis **17** Culiseta anulata **22**
	12–15/08 /2023		Culex pipiens **109** Culex modestus **99** Aedes vexans **153** Culiseta anulata **89** Anopheles maculipennis **72**
	14–18/09 /2023		Culex pipiens **109** Culex modestus **74** Aedes vexans **175** Anopheles maculipennis **68** Anopheles hyrcanus **17** Culiseta anulata **65**

Mosquitoes were also included in the Anopheles maculipennis complex using the identification keys provided by Becker N.[32], they were not identified in July and August, probably due to the high temperatures averaging 30°C at which mortality among the species begins to occur.

BIOCLIMATIC CONDITIONS

To study the influence of climatologic factors on both mosquito population and *Plasmodium* incubation, we used bioclimatic indexes that evaluate the potential conditions for malaria infections.

In entomology, one of the most frequently used thermal indexes is Growing Degree Days (GDD), which calculates cumulative heats units required by mosquitoes to pass from eggs to adults. GDD is useful to estimate the moments when the Anopheles mosquito transits from one development stage to another. This index calculates the temperature from the lowest threshold (T_b) to the ceiling (T_c) at which mosquito biological activities take place, for each day:

$$GDD = \sum_{i=1}^{n} \frac{(T_{\text{maximum}} + T_{\text{minimum}})}{2} - T_{\text{b}} \quad (°C)$$

For a certain period, it is calculated as the sum of the degrees between the two thresholds from all days.

For *Anopheles* mosquito, the base temperature T_b = 12 °C and the ceiling threshold T_c = 35 °C.

Anopheles mosquitos need only 40 °C to pass from egg to larva and another 185 °C to transit from larva to adult. In Tulcea, starting with May conditions necessary to accumulate enough GDD for change the development stages are met (Table 3).

Table 3

Monthly climatological conditions in 2023 - Tulcea.

Month	**Maximum air temperature (ºC)**	**Minimum air temperature (ºC)**	**Average air temperature (ºC)**	**Precipitation (mm)**	**Growing degree days (ºC)**
January	8.5	1.8	5.1	31.8	3.1
February	8.0	0.2	4.1	1.2	2.8
March	13.5	3.0	8.2	18.1	8.4
April	15.5	6.9	11.2	92.8	10.0
May	22.7	10.3	16.5	34.0	141.9
June	27.8	15.5	21.6	63.4	293.4
July	30.8	19.1	24.9	136.0	413.8
August	31.4	19.1	25.2	13.2	426.5
September	27.3	14.6	20.9	0	265.4

To evaluate all thermal intervals in which the female mosquitoes can develop, we used potential mosquito development period index (PMI)[36]. This index establishes the windows in which thermal conditions are met. It is obvious that the development of the mosquito population depends on many other factors. Therefore, this bioclimatic index does not show that there are mosquitoes, but indicates that from the point of view of thermal abiotic factors mosquitoes could develop. The potential mosquito development period index (PMI) calculates, based on the gaussian function f(T), the numbers of windows in which thermal conditions allow mosquitos to develop:

$$\text{PMI} = \int_{T_b}^{T_c} f(T)\,dT$$

The function f(T) describes the developing rate of mosquitoes. The Anopheles mosquito develops from 12°C to 35°C, with an optimal interval from 25–27ºC.

Moreover, to identify the transmission of infection with malaria, it is necessary to know the periods favourable for the virus to develop, which can be determined based on potential virus development index (PVI). PVI counts the thermal windows appropriate for the development of the *Plasmodium falciparum* and *Plasmodium vivax*, respectively:

$$\text{PVI} = \int_{T_{bv}}^{T_{cv}} g(T)\,dT$$

where g(T) is the function of optimal conditions for developing of virus. *Plasmodium falciparum* can develop from base temperature T_{bv} = 16°C to T_{cv} = 38°C with an optimal range from 28°C to 33°C. Instead, Plasmodium vivax requires temperatures between T_{bv} = 16°C and T_{cv} = 37°C, with an optimal interval between 27°C to 31°C. The second mathematical formula calculates the possibility of having thermal conditions favorable to the development of mosquitoes and protozoa, taking into account the minimum and maximum thresholds, using the number of days as a unit of measure.

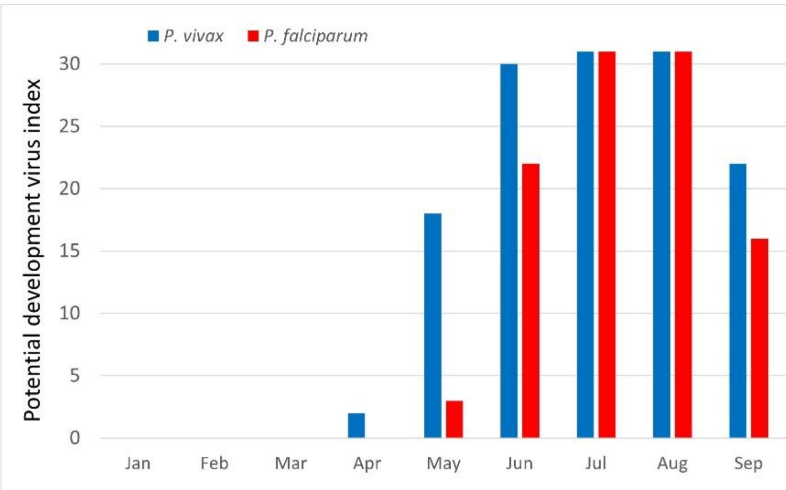

Figure 4. The monthly number of days with temperatures favourable for the development of the malaria spp. in 2023 – Tulcea.

The Danube Delta ensures humidity conditions that favour a suitable environment for the development of mosquitoes during the whole year. The many standing water holes along with the abundant rainfall from April to September (Table 3) are a natural incubator for mosquito eggs.

The two potential indexes are important to understand if there is a correlation between infection and environmental conditions. The climatologic analysis highlighted that in 2023 in the Danube Delta area cumulative temperature-humidity conditions for malaria infection were met. From the 13^{th} of May to the 30^{th} of September infection with *Plasmodium vivax* was possible and from the 9^{th} of June to the 30^{th} of September infection with *Plasmodium falciparum* was likely. We estimate that the periods in which mosquitoes can multiply in the Danube Delta will increase by 2 weeks in next 50 years, in the context of climate change (Figure 4).

In the Danube Delta, the maximum number of days with temperatures favourable for the development of the malaria virus in 2023 is calculated based on all above parameters superimposed (Figure 4). We considered only the most common species found in the Danube Delta – *Plasmodium vivax* and *Plasmodium falciparum*. The potential development indexes of both mosquitoes and viruses indicate that malaria can occur from spring to autumn, in the Danube Delta. The incidence of *Plasmodium vivax* is higher than P.F.

CONCLUSIONS

This study shows the necessity of introducing in Romania monitoring and control programs for mosquito vectors, because of the risk of disease transmission, in the context of favourable climatic factors.

Thus, in Romania, cases of malaria are diagnosed every year and, although they are all imported, it shows that the *Plasmodium protozoan* is present in nature in the human host.

Mosquito vectors belonging to the *Anophles maculipennis* complex, responsible for the transmission of malaria in Europe, were also identified.

The study has the disadvantage of lacking molecular biology testing of the species of mosquitoes included in this complex, although the entire complex is involved to some extent in the transmission of malaria.

From the point of view of the climatic conditions, they proved favourable for the transmission of malaria, from May to September 2023. Thus, during this period, actions to control the mosquito population should be enhanced, in order to avoid blood feeding on human hosts.

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