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## Research Article

# Mapping Malaria Transmission Risk in Northern Morocco Using Entomological and Environmental Data

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Malaria resurgence risk in Morocco depends, among other factors, on environmental changes as well as the introduction of parasite carriers. The aim of this paper is to analyze the receptivity of the Loukkos area, large wetlands in Northern Morocco, to quantify and to map malaria transmission risk in this region using biological and environmental data. This risk was assessed on entomological risk basis and was mapped using environmental markers derived from satellite imagery. Maps showing spatial and temporal variations of entomological risk for *Plasmodium vivax* and *P. falciparum* were produced. Results showed this risk to be highly seasonal and much higher in rice fields than in swamps. This risk is lower for Afrotropical *P. falciparum* strains because of the low infectivity of *Anopheles labranchiae*, principal malaria vector in Morocco. However, it is very high for *P. vivax* mainly during summer corresponding to the rice cultivation period. Although the entomological risk is high in Loukkos region, malaria resurgence risk remains very low, because of the low vulnerability of the area.

## 1. Background

Until recently, malaria was endemic in Morocco. Three plasmodial species were present: *Plasmodium falciparum*, *P. vivax*, and *P. malariae*. *Plasmodium falciparum* was the dominant species until the beginning of the 1950s, when *P. vivax* became more important [1]. The most affected areas were those, which were most inundated, mainly the plains comprising swamps, which are the main breeding sites of the major vector in Morocco: *Anopheles labranchiae*.

Drainage and irrigation projects, undertaken at the beginning of the twentieth century have contributed to the reduction of malaria transmission, but disease was still common in the early 1960s. In 1965, a National Malaria Control Program was launched. It was based on vector control using DDT indoor residual spraying and parasite reservoir control by treatment and chemoprophylaxis. The

effect on *P. falciparum* transmission was rapid, and the last autochthonous case was notified in 1973. Thirty years later, it was possible to interrupt *P. vivax* transmission. In 2008, Morocco undertook the process for certification of malaria-free status according to WHO criteria.

However, Morocco still reports, every year, about one hundred of imported malaria cases. These are mainly detected in large urban areas, mainly Casablanca and Rabat, but also Fes and Agadir. Most of imported cases are *P. falciparum* originating from sub-Saharan Africa [2, 3].

Thus, malaria resurgence in Morocco remains a risk, because vectors are present in formerly malarious areas and because parasites are regularly imported. This risk may change with climatic and environmental modifications as well as increasing numbers of malaria parasite carriers coming from sub-Saharan Africa.

The prevention of the reappearance of malaria transmission in Morocco relies on surveillance which should be based on a spatial and temporal stratification with respect to malariogenic risk factors, that is, vulnerability, receptivity, and infectivity [4]. The receptivity takes into account all parameters of the vectorial capacity of anopheline population (density, trophic behaviour, longevity, and duration of sporogonic cycle of *Plasmodium*). The infectivity (closely related to vectorial competence) is the ability of a particular parasite species to replicate in a given vector. Vulnerability corresponds to the risk of importing parasite carriers and of these becoming infective. National Malaria Control Program has a considerable inventory of entomological and parasitological information, and several areas of high risk are regularly studied and monitored [5, 6]. Unfortunately, most of this information is only available in descriptive formats. Analysis of these data in association with environmental factors could reduce the need for very detailed field studies and allow generating maps of vector distribution and transmission risk. These would be useful for decision makers, by rapidly elucidating spatial patterns and permitting a better orientation and targeting of control measures.

In this study, we analyze the receptivity of the Loukkos area, one of the largest wetlands in Northern Morocco, to quantify and map malaria transmission risk in this region by integrating biological and environmental factors derived from high spatial resolution imagery.

## 2. Methods

**2.1. Study Area.** The study was carried out in the Loukkos area located in Northwestern Morocco on the Atlantic coast. It is situated between northwestern coordinate 35°09'47"N, 06°09'16"E and southeastern coordinate 34°56'55"N, 05°59'58"E (Figure 1). This region is characterized by a typically Mediterranean climate, with marked oceanic influences (low annual thermic amplitude and frequent dew). The total annual rainfall ranges from 600 and 800 mm concentrated mainly between October and April. The monthly mean low and high temperatures are ranging from 6 to 32°C, respectively, during winter and summer. The landscape is composed of wetlands (coastal lagoons, swamps and rice fields), forests, and agricultural zones. Two study sites, in two different biotopes, were chosen to carry out this study. (1) Boucharen site located in an artificial wetland with controlled hydrology and characterized by the presence of large rice cultivation surfaces in continuous extension. The altitude is 1 to 15 m above sea level. (2) Beggara site located in a shallow swampy zone fed by river water and subject to the Atlantic Ocean tidal influence.

### 2.2. Entomological Data

**2.2.1. Larval Sampling.** Fifteen potential breeding sites, in each study area, located in the main biotopes potentially suitable to *An. labranthiae* larvae were visited every month from February to November 2008. Mosquito larvae, when

present, were collected using plankton nets, counted and preserved in ethanol before being identified morphologically [7].

**2.2.2. Sampling of Adult Mosquitoes.** Adult mosquitoes were caught in the two sites, using three sampling techniques.

- (i) Indoor resting mosquitoes were collected monthly from February to November in 4 stations (animal shelters) for two consecutive nights in each site. Mosquitoes were collected during 10 minutes per station using a sucking tube.
- (ii) Human bait collection was conducted in two stations in each site. Landing catches were made, in each station, on two volunteers, one indoor and one outdoor from 08:00 pm to 05:00 am during two consecutive nights. These catches were made monthly from April to November in Boucharen and only in June and July in Beggara. Human biting rate was estimated as the average number of bites per person per night.
- (iii) Five CDC light traps were deposited in each site during two consecutive nights in five different stations (animal shelters).

All collected adult mosquitoes were identified morphologically [7] and 3% of adults of *An. maculipennis* complex was identified by multiplex polymerase chain reaction (PCR) [8].

All larvae and adults collection stations were georeferenced using a Global positioning system receiver (GPS).

**2.2.3. Vectorial Capacity.** Vectorial capacity (VC) was calculated using the formula of Garrett-Jones [9] derived from the works of MacDonald [10]:

$$VC = \frac{ma^2 \cdot p^n}{-\ln p}, \quad (1)$$

where  $m$  is vector-host ratio, that is, the anopheline density in relation to man,  $a$  is vector biting rate, the average number of people bitten by one mosquito in one day,  $p$  is daily survival rate, calculated as  $p = \sqrt[n]{P}$  (where  $P$  is the parous rate and  $u$  is the trophogonic cycle duration in days), and  $n$  is length in days of the sporogonic development.

The following parameters used in calculation of VC were estimated.

- (i) Parity rate: was calculated during every session of capture according to the method of Detinova [11].
- (ii) Human blood index: was measured by bloodmeal analysis of fed females using ELISA [12].
- (iii) Trophogonic cycle: the duration of this cycle was calculated using the formula:  $u = f_1 / (T - g_1)$  where  $f_1$  and  $g_1$  are parameters depending on humidity and  $T$  is the temperature [11].  $f_1$  and  $g_1$  correspond respectively to 36.5°C-days and 9.9°C, for a relative humidity of 70–80% which characterizes the study region.

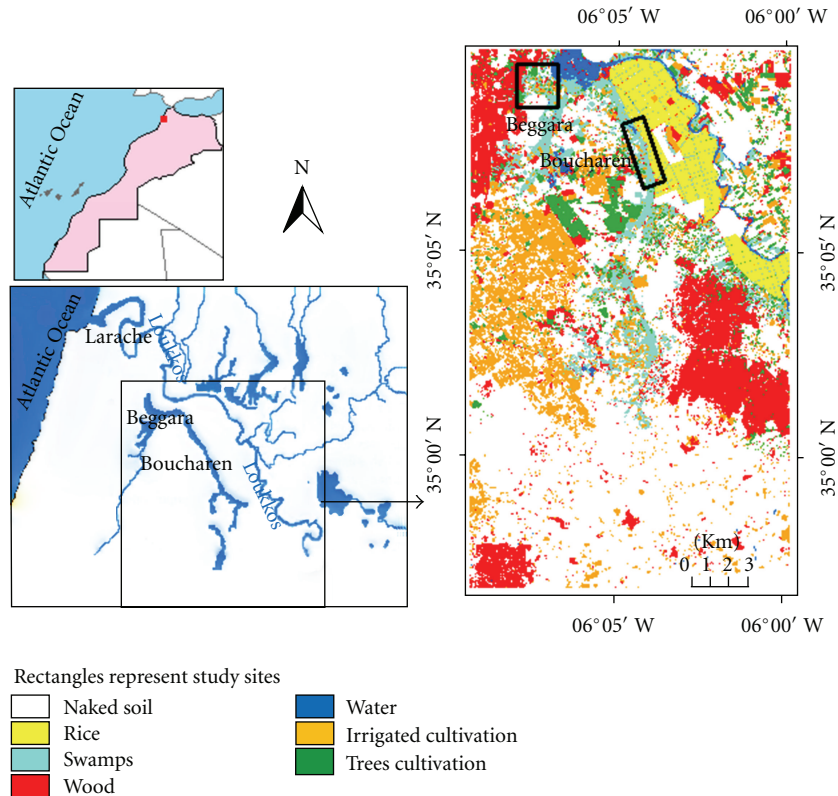


FIGURE 1: Maps showing location of study sites and land use in study area.

(iv) Sporogonic cycle: its duration was calculated as follows:  $n = f_2 / (T - g_2)$  where  $f_2$  and  $g_2$  are parameters depending on *Plasmodium* species [11] and are experimentally evaluated, respectively, at 111°C-days and 16°C for *P. falciparum* and at the 105°C-days and 14.5°C for *P. vivax*. Temperature above which the sporogony is impossible is estimated at 18°C and 16°C for *P. falciparum* and *P. vivax*, respectively [11].

(v) Infectivity: *An. labranchiae* infectivity to *P. vivax* and *P. falciparum* was estimated based on literature, respectively, to 0.5 [1, 13, 14] and 0.05 [15, 16].

### 2.3. Environmental Data

**2.3.1. Landscape Description.** To identify and map the landscape units within the study area, a spot satellite image taken on July 22, 2007 was used. This image allows the study area (14 × 24 Km) to be covered in one scene with a spatial resolution of 10 × 10 meters. The classification of this image resulted in a land use map depicting the ecological units likely to impact *Anopheles* abundance (Figure 1).

**2.3.2. Meteorological Data.** Meteorological data recorded by the meteorological station of Loukkos were used. Mean monthly temperatures were used for the estimation of sporogonic and trophogonic cycle durations. Relative humidity

average is considered in the calculation of the duration of the trophogonic cycle.

**2.4. Risk Mapping Approach.** To spatially characterize the risk, we used the approach developed by Tran et al. [17] based on a mosquito abundance regression logistic model which consists in using the results of the followup of larvae in different ecological units to identify specific larvae breeding sites (rice fields, different wetlands) and quantify the relationship between land cover, larvae presence, and adult abundance. Analysis of larval data led to the definition of a larval index that was defined as the probability of observing larvae in a point at least once during the mosquito season and was estimated by applying the logistic transformation to each pixel, within the image of the study area, according to its biotope. An adult abundance index was generated from the larval index and was also calculated for each pixel in the study area. This adult index was defined for each trap location as the mean value of the larval index within a buffer radius of 300 m around the trap location. This model showed a good overall accuracy (80%) [17].

Hence, the model developed by Tran et al. [17] was applied to the land cover map of the Loukkos region. It resulted in a map of a larval index, corresponding to the probability of observing *An. labranchiae* larvae in a given breeding site. Then, an adult abundance index was produced from the larval index. Comparison of the adult abundance index and the number of indoor resting anophelines really

captured in the same pixel showed a highly significant linear regression ( $r^2 = 0.71$ ,  $P < .01$ ), allowing us to infer adult abundance index for each pixel in the Loukkos.

From this, the human biting rate (ma) was inferred from a comparison between mean of indoor resting anopheline catches and mean of human landing catches. Captures conducted the same night, in the same area and in pixels of the same adult abundance index class, were compared. Comparison showed a significant linear relationship between indoor resting anopheline and human landing catches ( $r^2 = 0.61$ ,  $P < .01$ ). Subsequently, monthly predictive maps of human biting rate (ma) were produced.

Integration of the other vectorial capacity parameters (see Section 2.2.3), which are supposed to vary in time but not in space, as well as *An. labranchiae* susceptibility to *Plasmodium* species, *P. falciparum*, and *P. vivax* has allowed the spatiotemporal mapping of the entomological risk for each *Plasmodium* species.

### 3. Results

**3.1. Collected Fauna.** During this study, a total of 10168 adult anophelines among which 1239 in Beggara and 8928 in Boucharen were caught by the three collection methods. Catches by CDC light traps were very irregular with low production: only 405 mosquitoes were collected by this method while 7574 were caught on man and 2188 by indoor resting catches. Hence, catches by CDC light traps were not considered in this study.

The morphological identification of collected anophelines showed that they all belong to Maculipennis group. Molecular identification of 325 mosquitoes (3% of the total), collected in the two study sites by the three collection methods, showed that they all belong to *An. labranchiae* species. Results of indoor resting catches and human landing catches are presented in Table 1. Total density is higher in Boucharen, even development seems to begin earlier in Beggara. Peak densities are observed in May and June, respectively, in Beggara and Boucharen sites.

The larval investigations have permitted to collect 1137 larvae belonging all to *An. labranchiae* species and 861 *Culicinae* larvae belonging to *Culex* and *Aedes* genera.

*Anopheles. labranchiae* larvae were collected in the two study sites (Boucharen and Beggara) mostly in rice fields and swamps with, respectively, 80% and 56% of positive sites. About three quarters (73%) of *An. labranchiae* larvae were collected in Boucharen. Collected larvae per type of larval habitats are represented in Table 2.

#### 3.2. Parameters Used in the Calculation of Vectorial Capacity.

- (i) The human blood index was measured on 354 fed females: only eleven mosquitoes were engorged on man. The HBI was, then, estimated to 0.03.
- (ii) Parity rate: a total of 621 unfed females collected throughout the study period were dissected to estimate this parameter. It varies between 0.6 and 0.92.

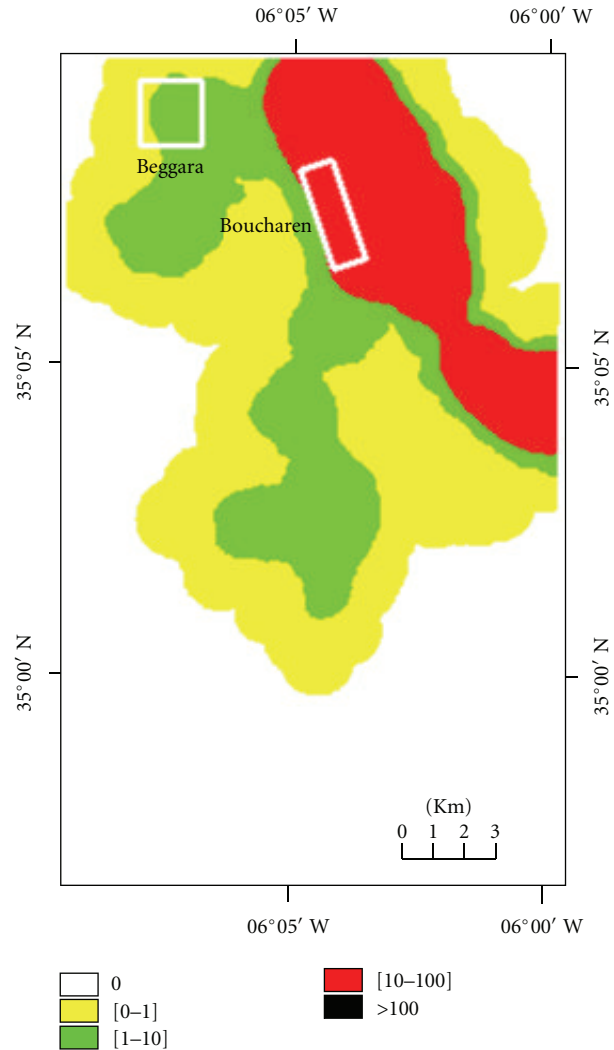


FIGURE 2: Spatial distribution of *Plasmodium vivax* transmission risk estimate in July in Loukkos. Classes were arbitrary chosen with a logarithmic scale.

Table 3 recapitulates the results of different parameters used in the calculation of the vectorial capacity during the study period in the two study sites. VC is higher in Boucharen than in Beggara for both Plasmodial species.

**3.3. Entomological Risk.** Entomological risk for *P. vivax* estimated during July and August are represented in Figures 2 and 3.

This risk is unimportant during the whole year for *P. falciparum*, presenting a maximum in August (Figure 4) while it is significant for *P. vivax* from May to October peaking in August.

### 4. Discussion

In the study area, *An. labranchiae*, the only representative of Maculipennis group in Morocco [5, 6, 18, 19], was the only anopheline species collected. Indeed, *An. labranchiae* finds its

TABLE 1: *Anopheles labranchiae* in indoor resting catches and human landing catches, Loukkos 2008.

Collection method	Locality	Coordinates	Feb.	March	April	May	June	July	August	Sept.	Oct.	Nov.	Total	
Indoor resting mosquitoes	Boucharen	35°07'49"N 06°04'34"W	6	14	22	86	131	52	70	23	21	—	425	
		35°07'40"N 06°04'34"W	4	17	15	82	75	53	34	19	70	—	369	
		35°07'37"N 06°04'34"W	2	8	34	17	43	20	18	41	37	—	220	
		35°07'38"N 06°04'34"W	3	7	20	20	119	43	57	22	44	—	335	
		Total	15	46	91	205	368	168	179	105	172	—	1349	
	Beggara	35°09'49"N 06°06'56"W	25	33	56	39	37	25	16	19	25	7	—	282
		35°09'53"N 06°06'51"W	6	4	8	25	8	15	4	1	2	12	—	85
		35°09'47"N 06°06'57"W	7	10	9	31	3	11	7	2	4	11	—	95
		35°10'31"N 06°06'47"W	17	30	49	98	86	1	27	38	19	13	—	378
		Total	55	77	122	193	134	52	54	60	50	43	—	840
Human landing catches	Boucharen	35°07'09"N 06°04'48"W	—	—	7	65	634	861	1373	300	113	—	3353	
		35°07'55"N 06°04'23"W	—	—	11	82	694	1027	1565	372	121	—	3872	
		Total	—	—	18	147	1328	1888	2938	672	234	—	7225	
	Beggara	35°09'53"N 06°06'57"W	—	—	—	—	62	94	—	—	—	—	—	156
		35°09'45"N 06°06'50"W	—	—	—	—	79	114	—	—	—	—	—	193
		Total	—	—	—	—	141	208	—	—	—	—	—	349

TABLE 2: *Anopheles labranchiae* larvae collected per larval habitat in study sites.

		Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.
Boucharen	Rice field	0	0	0	87	171	157	119	92	69	2
	Swamp	3	8	29	37	33	9	7	5	2	0
	Irrigated cultivation	0	0	0	0	0	0	0	0	0	0
	Total	3	8	29	124	204	166	126	97	71	2
Beggara	Swamp	9	46	86	79	59	11	8	5	4	0
	Irrigated cultivation	0	0	0	0	0	0	0	0	0	0
	River	0	0	0	0	0	0	0	0	0	0
	Total	9	46	86	79	59	11	8	5	4	0

preference area in the Atlantic plains in the Northwestern of the country where it constitutes more than 95% of the anopheline populations [20]. The study site, a large wetland situated in the plain of Loukkos, contains various types of larval habitats favourable to the development of mosquitoes particularly the swamps and rice fields, considered as preferred breeding sites of *An. labranchiae*. The proximity of human and animal (particularly bovine) agglomerations to the breeding sites makes this zone a very favourable biotope for the development of this species. In fact, the most dramatic

malaria epidemics in the history of Morocco were registered in this area [1] when *An. labranchiae* was involved in the transmission of *P. vivax*, as well as of *P. falciparum* and *P. malariae* [21].

Larvae and adults of *An. labranchiae* were collected, all over the study period from February to November. These results corroborate those of Sicault et al. [22] as well as those of Guy and Holstein [23] who demonstrated that *An. labranchiae* has no spontaneous ovarian diapauses in Morocco; it may lay eggs even in winter in moderate

TABLE 3: Vectorial capacity in the study sites, Loukkos 2008.

		April	May	June	July	August	Sept.	Oct.	Nov.
Average temperature		15.71	18.31	21.36	23.58	23.48	22.00	20.37	16.58
Trophogonic duration in days ( $u$ )		6.28	4.34	3.18	2.67	2.69	3.02	3.49	5.46
Pf sporogonic cycle duration in days ( $n$ )		—	48.05	20.71	14.64	14.84	18.50	25.40	—
Pv sporogonic cycle duration in days ( $n$ )		—	27.56	15.31	11.56	11.69	14.00	17.89	50.48
Boucharen	Human biting rate (ma)	2.25	18.38	166	236	367	84	57.25	1.25
	Parity rate (females dissected)	0.92 (13)	0.91 (68)	0.64 (25)	0.78 (82)	0.81 (112)	0.89 (120)	0.83 (72)	0.60 (10)
	Pf. vectorial capacity	—	2.06	0.61	7.29	16.32	10.58	3.23	—
	Pv. vectorial capacity	—	3.21	1.31	9.71	20.89	12.59	4.59	0
Beggara	Human biting rate (ma)			32.25	52				
	Parity rate (females dissected)			0.63 (78)	0.85 (41)				
	Pf. vectorial capacity			0.10	3.93				
	Pv. vectorial capacity	—			0.23	4.75			

Pf: *Plasmodium falciparum*.

Pv: *Plasmodium vivax*.

temperature. The increase of temperatures in resting shelters increases the activity of females after the winter [22, 24]. In this study, larval development began in February in the swampy zones (Beggara). The adults became abundant in the neighbouring villages from March. The rice fields (Boucharen area) became favourable to the development of larvae from their inundation in May, and larvae densities reached their maximum in June/July during the rice growing phase. In this period, wide areas of the swampy zones dried out or became unsuitable to the development of larvae because of their exploitation by man or standing about by cattle. Adult densities in the neighbouring villages are then appreciably lower. So, the complex swamp/rice field assures *An. labranchiae* development in the region of Loukkos all over the year.

Rice fields offer, by their biotic and abiotic characteristics, a much more favourable biotope to *An. labranchiae* larvae development in Morocco [5, 25, 26]. The results of this study confirm these earlier observations; in June, the receptivity of Boucharen village located near rice fields is 6 times higher than receptivity of Beggara situated near swamps.

Malaria transmission risk assessment or entomological risk is based on the evaluation of the vectorial capacity and vectorial competence of local vectors as defined by Garrett-Jones and Shidrawi [27]. The vectorial capacity is the best measure to quantify receptivity to malaria in a given region. Thus, the entomological risk corresponds to the product of the vectorial capacity and the infectivity (vectorial competence for a particular plasmodial species or strain). It is in the same area time, space and *Plasmodium* species dependant [28]. To study its variations in Loukkos, we characterized it spatially according to environmental markers generated from satellite images. Examination of the risk maps so produced shows that transmission risk varies strongly by environmental determinants which control the

presence and extension of breeding sites and consequently the presence of vector and its density. This density is much higher around rice fields during the favourable transmission season. Indeed, rice fields are known for their role in maintaining a high entomological risk in the Mediterranean basin as this was demonstrated by several authors [5, 29–31].

The risk variations in time are mainly influenced by climatic determinants, particularly the temperature which modulates the vectorial capacity of the vector populations by increasing their densities (by shortening their development cycle) and by decreasing the duration of trophogonic and sporogonic cycles.

Results obtained in this study clearly indicate that transmission risk is strongly higher for *P. vivax* than for *P. falciparum* in the various biotopes and during all the transmission period, which is extending from May to October. This is due to differences in the infectivity and the length of sporogonic cycle. Results of the current study suggest a possible transmission of *P. falciparum*, although low in Loukkos. The findings are at odds, with earlier reports of the apparent refractoriness of European *An. labranchiae* to tropical *P. falciparum* strains [32]. Such a different vectorial capacity between allopatric populations of the same species has been found in a number of other anopheline species. For example, *An. stephensi mysorensis* and *An. fluviatilis* T are known to act as vectors of malaria in Iran and Pakistan but seem not to be vectors in India [33–35].

Even though entomological risk is very high in Loukkos region, the malaria vulnerability (risk of presence of infective *Plasmodium* carriers) is very low. Although Morocco notifies every year about one hundred imported malaria cases, these are notified particularly in big cities where transmission conditions are absent. The probability that these carriers contact the vector in the potential transmission zones during

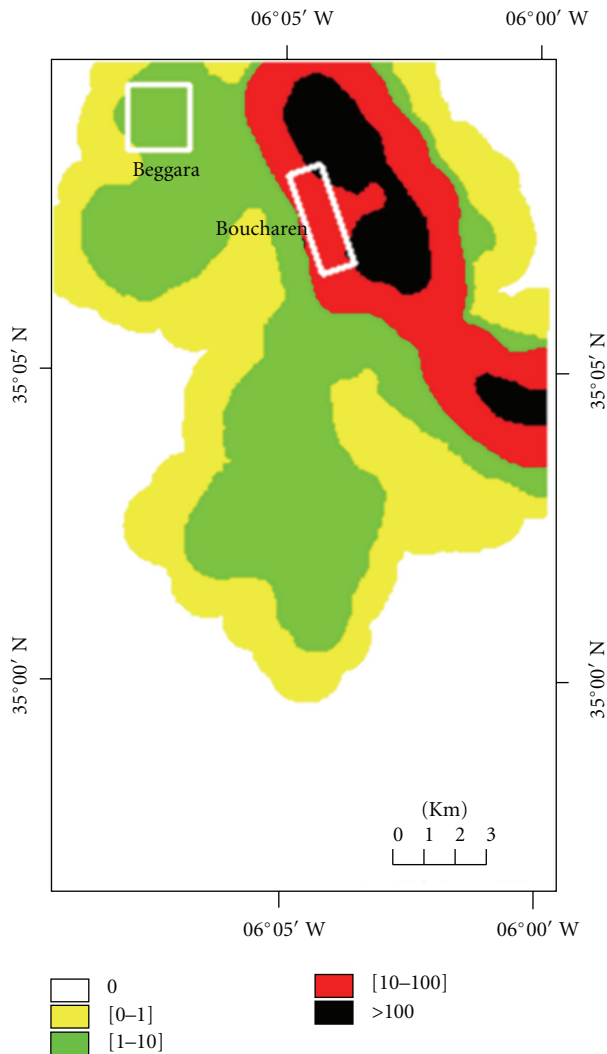


FIGURE 3: Spatial distribution of *Plasmodium vivax* transmission risk estimate in August in Loukkos. Classes were arbitrary chosen with a logarithmic scale.

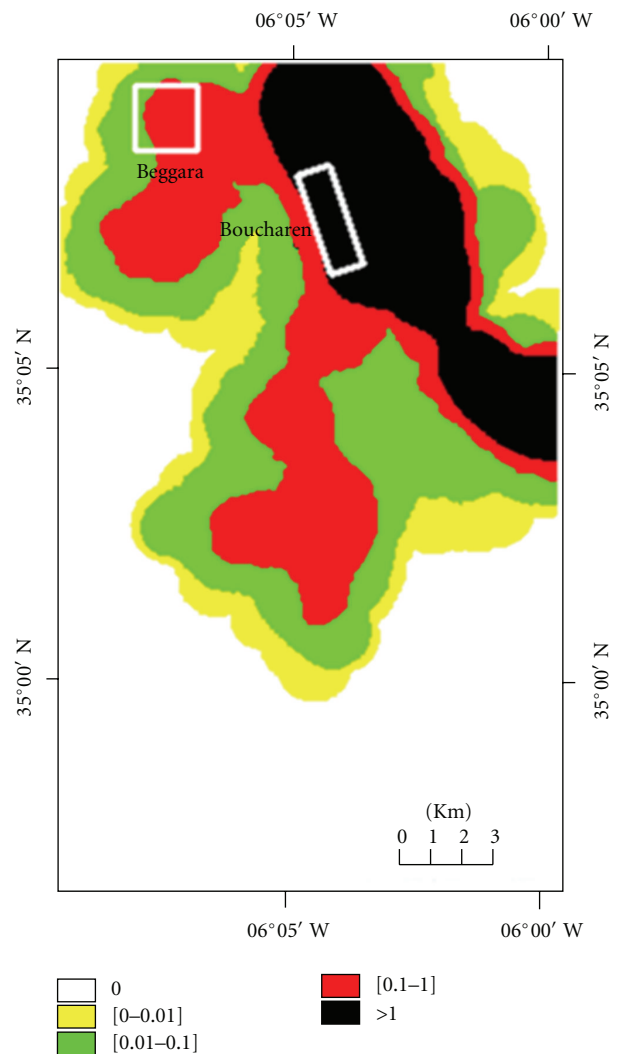


FIGURE 4: Spatial distribution of *Plasmodium falciparum* transmission risk estimate in August in Loukkos. Classes were arbitrary chosen with a logarithmic scale.

the transmission season is very low. Since 1996, only 5 imported malaria cases were notified in Larache city, about 10 km from the study area (Ministry of Health, unpublished data).

The most worrying situation would result from the exposure of local vectors to imported *P. vivax*. Indeed the number of cases of *P. vivax* imported malaria is in net growth; it increased from 1.5% (1/69) in 2003 to 9% (13/142) in 2008. These cases come particularly from Mauritania. It is therefore important to maintain strict surveillance, particularly for importation of *P. vivax* to areas with environmental characteristics similar to Loukkos.

In synthesis, the malaria resumption risk in Loukkos is low but growing. The increase of African populations arriving clandestinely from malaria endemic zones and the perpetual change of land cover factors can lead to increased malaria risk. Continuous contact with exotic strains of *Plasmodium* could in the long term lead to the selection

and/or to the adaptation of strains able to develop better in local vectors.

This indicates the need for focused, but flexible, epidemiological surveillance to prevent any possibility of encounters between potential vectors and gametocyte carriers. The present work can be considered as a public health tool designated to help manage malaria risk and to better use available resources by focusing actions to areas most at risk.

**Conflict of Interests**

The authors declare that they have no Conflict of interests.

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