Acta Tropica 109 (2009) 70–73

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Entomological investigations in the region of the last malaria focus in Morocco

C. Faraj[a,*], E. Adlaoui[a], S. Ouahabi[a], M. Rhajaoui[b], D. Fontenille[c], M. Lyagoubi[a]

[a] Laboratoire d'Entomologie Médicale Institut National d'Hygiène, 27 Avenue Ibn Batouta, Agdal 11400 Rabat, Morocco
[b] Laboratoire d'Immunologie Parasitaire, Institut National d'Hygiène, Rabat, Morocco
[c] Institut de Recherche pour le Développement (IRD), 911 avenue Agropolis, BP 64501, 34394 Montpellier Cedex 5, France

ARTICLE INFO

Article history:
Received 30 April 2008
Received in revised form
23 September 2008
Accepted 30 September 2008
Available online 17 October 2008

Keywords:
Malaria
Resumption risk
Anopheles labranchiae
Anopheles sergenti
Chefchaouen
Morocco

ABSTRACT

To evaluate the risk of malaria transmission resumption in Morocco, we have studied the current level of receptivity of the region of the last malaria focus in the country. *Anopheles (Anopheles) maculipennis labranchiae* and *Anopheles (Cellia) sergentii*, the major vectors of malaria in Morocco, are still presents but their anthropothic index was low and no parasite positive samples were detected. *An. labranchiae* was very rare; only 34 females were caught over all the study period. The human biting rate was nil and none of its blood meal was human. *An. sergenti* was more abundant but its low human aggressiveness and its zoophilic behaviour would not attribute to this species an important vectorial capacity. Thus, the receptivity of Chefchaouen province, the region of the last malaria focus in Morocco, under the current vector control measures undertaken by Public Health services, is low and despite the likely presence of *Plasmodium vivax* gametocyte carriers, the malariogenic potential appears to be low and the risk of malaria resumption is, at this time, unimportant.

1. Introduction

Malaria exists in Morocco for centuries as an endemic disease with frequent summer and autumn outbreaks. *Anopheles labranchiae* was considered, on epidemiological bases, as the major vector in the north of the country and *Anopheles sergenti* in the south (Gaud, 1948; Senevet and Andarelli, 1956; Guy, 1963). In 1965, a national programme for malaria control was launched. This programme, based on indoor treatment with DDT and a large-scale chemoprophylaxis has led to the interruption of the transmission of *Plasmodium falciparum* from through Morocco since 1974 and the progressive reduction of *Plasmodium vivax* incidence (Ministry of Health, unpublished data). The last outbreak was reported in 2002 (18 cases) in Chefchaouen province in the north of the country. To control this outbreak, DDT insecticidal residual spraying was undertaken for the last time in Morocco. Since then, Public health services attempt to avoid the resumption of a local transmission through surveillance and systematic anophelines larvae control by release of larvivorous fishes in water-bodies and chemical control of breeding sites by organophosphates mainly temephos. However, *Anopheles* vectors are still present, the "malariogenic potential" of the area has never been evaluated and very few data exist on the *Anopheles* potential vectors. This entomological study was carried out in the Chefchaouen province with the aim to provide a good knowledge on the distribution and bionomics of *Anopheles* vectors to estimate their vectorial capacity and so, to consider, the risk of recurrence of malaria transmission in this region.

2. Materials and methods

2.1. Study area

Chefchaouen province is located in the Northwest of Morocco in the Rif Mountains (Fig. 1). This zone presents a variety of climates; a typical Mediterranean climate in the mountainous zone and a semi-arid climate in the coastal zone. So, two study areas about 44 km apart were chosen.

1) **Assoul locality** (35°18′N; 004°59′W) situated in the Northeast of Chefchaouen city on the Mediterranean coast, at an altitude of 60 m. This station is characterised by a semi-arid climate with moderate winters. Precipitations divide up generally over a long period of the year, from October till May with peaks in December, January and February. The total annual rainfall ranges between 400 and 500 mm. The monthly mean high and low temperatures are ranging from 30.6 °C to 4.8 °C, respectively during summer and winter. Reproduction sites for mosquitoes

* Corresponding author. Tel.: +212 37771902/65; fax: +212 37772067.
E-mail addresses: c.faraj@menara.ma, chafikaf@gmail.com (C. Faraj).

0001-706X/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.actatropica.2008.09.021

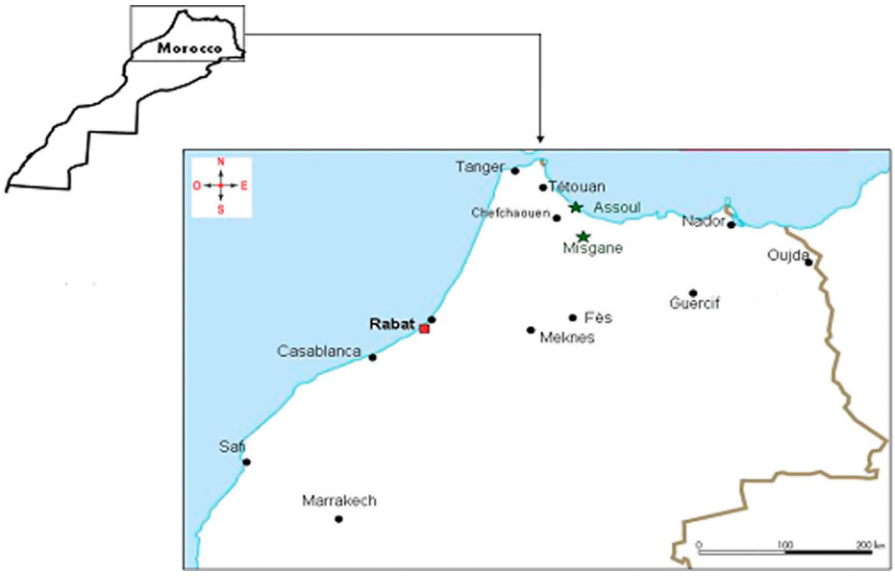

Fig. 1. Location of the study sites.

are mainly constituted by collections of water along the river.

2) **Mizgane locality** (34°55′N, 004°53′W) situated in the southeast of Chefchaouen city at a height of 500 m. Mizgane is characterised by a sub-humid bioclimate with moderate winters. This site receives more precipitations; about 700–800 mm from October to May. The minimal and maximal temperatures are respectively 4.5 °C and 32 °C. The main mosquito breeding sites are small collections of water on the stagnant edges of rivers, natural sources and ponds for storing water.

2.2. Mosquito collections

Adult mosquitoes were collected monthly, from April to October 2005 using several collection techniques:

- Five CDC-light traps were placed monthly, in each locality, for three consecutive nights in five locations (2 in animal shelters, 2 in human habitations and 1 outdoor) from 07:00 p.m. to 05:00 a.m.
- Collections on human volunteers were made by four persons, two indoor and two outdoor from 07:00 p.m. to 05:00 a.m., three times in June, July and October in each locality.
- Resting fauna was investigated monthly from April to October in two animal shelters, two human habitations and one natural shelter in each locality for three consecutive nights. Mosquitoes were collected using mouth aspirators.

2.3. Mosquitoes' analysis

All collected mosquitoes belonging to *Anopheles* genera were preserved individually in a numbered tube with desiccant for laboratory processing. They were all identified morphologically (Brunhes et al., 2000).

All *Anopheles* females belonging to the *Anopheles maculipennis* complex were identified up to species using the PCR technique (Proft et al., 1999). All head–thorax of *An. labranchiae* and *An. sergenti* females were tested by specific ELISAs (Wirtz et al., 1992) to detect the presence of CS antigens for two *P. vivax* phenotypes (PVK210 and PVK 247). Blood meal sources of fed females were identified by ELISA (Beier et al., 1988) using five antibodies: anti-human, anti-dog, anti-sheep, anti-bovine and anti-horse.

Table 1
Proportion of *Anopheles* species collected per locality in 2005.

	Assoul (n = 850)	Mizgane (n = 433)	Total (n = 1283)
An. sergenti	57.6%	40.0%	51.6%
An. cinereus	37.2%	51.5%	42.0%
An. d'thali	4.8%	0.9%	3.5%
An. labranchiae	0.4%	7.1%	2.7%
An. claviger	0.0%	0.5%	0.2%

3. Results

3.1. Species inventory

During the study, 1283 Anopheles adult, belonging to five species, were collected in the two studied localities: *An. sergenti*, *Anopheles* (*cellia*) *cinereus*, *Anopheles* (*cellia*) *d'thali*, *Anopheles* (*Anopheles*) *maculipennis* s.l. and *Anopheles* (*Anopheles*) *claviger*. The proportions of *Anopheles* species collected per locality are presented in Table 1.

All *An. maculipennis s.l.* adults (n = 34) collected in the two sites and analyzed by PCR belong to *An. labranchiae* species.

3.2. Population dynamics

Figs. 2 and 3 based on collections from CDC traps show the trend of Anopheles densities from April to October in each locality. Results

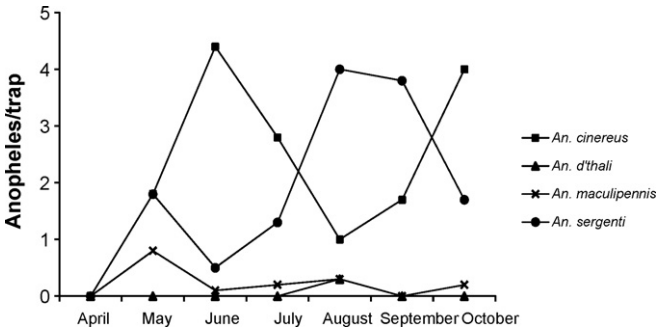

Fig. 2. Trend in *Anopheles* species density collected by CDC trap at Mizgane locality, Chefchaouen, 2005.

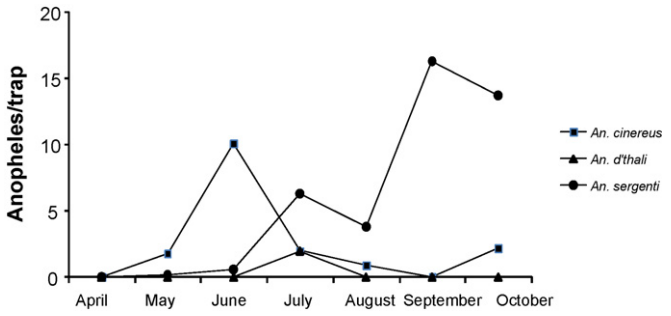

Fig. 3. Trend in *Anopheles* species density collected by CDC trap at Assoul locality, Chefchaouen, 2005.

Table 2
Anopheline mosquitoes captured using an aspirator in either human, animal or natural shelters per locality in 2005.

		Assoul	Mizgane	Total
An. sergenti	Houses	0	2	2
	Animal shelters	69	24	93
	Natural shelters	4	0	4
An. cinereus	Houses	0	2	2
	Animal shelters	129	20	149
	Natural shelters	4	0	4
An. d'thali	Houses	0	0	0
	Animal shelters	1	1	2
	Natural shelters	0	0	0
An. labranchiae	Houses	0	1	1
	Animal shelters	1	11	12
	Natural shelters	1	0	1

show the mean number of mosquitoes collected per locality by five traps during three nights. The total density was very low and has slightly varied during the study period in Mizgane. It was more important in Assoul where it increased progressively to reach a peak in September.

3.3. Resting fauna

The number of *Anopheles* collected in resting fauna is shown in Table 2. *An. claviger* was never collected in these shelters. *An. d'thali* and *An. labranchiae* were rare, *An. sergenti* and *An. cinereus* were captured at more relevant density and at different abdominal conditions (Table 3). All species showed a distinct preference for animals' shelters.

3.4. Infection rates

A total of 697 of *An. sergenti* and *An. labranchiae* were tested by CSP ELISA and all were found negative for PVK210 and PVK 247 CS antigens.

Table 3
Proportion of *Anopheles* females classified according to their abdominal conditions upon capture in human, animal or natural shelter in 2005.

	Unfed	Fully fed	Fed	Semi-gravid	Gravid
An. sergenti (n = 99)	2%	10%	75%	8%	5%
An. cinereus (n = 155)	1%	24%	66%	9%	0%
An. labranchiae (n = 14)	0%	7%	86%	7%	0%

Table 4
Blood meal analysis of fed mosquitoes.

	Blood meal sources						Other
	Human	Cow	Horse	Dog	Sheep	Mixed	
An. sergenti (n = 150)	9	63	14	3	4	30	27
An. cinereus (n = 131)	6	69	22	0	3	17	14
An. labranchiae (n = 18)	0	10	3	0	3	0	2

3.5. Human biting rate

Only 11 specimens were captured on human volunteers, demonstrating a very low human biting rate in the study area.

3.6. Feeding preferences

A total of 299 blood meals were tested by ELISA. Results show a marked preference for zoophily across all Anopheles species analyzed (Table 4).

4. Discussion

The five species collected in Chefchaouen are part of the inventory of the Moroccan anophelines fauna (Himmi et al., 1995). However, *An. d'thali* had just been found in this humid zone of Rif in the far north of Morocco (Faraj et al., 2008). The extension of the distribution area of this species, considered to be common in the desert areas, could be related to environmental change noted in this region. The decrease of the water courses flow during the summer season, as a consequence of the drop in rainfall and the increase in the water mobilization for the needs of irrigation, resulted in the creation of small collections of water on the beds of rivers. These, colonized by seaweeds, constitute breeding sites favourable to the development of *An. d'thali.* This species was not frequent in the studied area. It was captured only in the middle of the summer in both localities.

Anopheles sergenti was the most abundant species in Chefchaouen province particularly at Assoul locality where it represents 58% of collected mosquitoes. This species has been considered, a long time ago, as a southern species. It constitutes more than half of the *Anopheles* population in the Moroccan Sahara (Gaud et al., 1950; Gaud, 1953; Guy, 1959, 1963). It was signalled in the Rif, for the first time in 1967 by Bailly-Choumara (unpublished data). Although *An. sergenti* is known to be vector in other countries, it has never been found naturally infected in Morocco. It has been always considered, on the epidemiological basis, as a potential vector which play, in the south, the role of *An. labranchiae* in the north (Gaud, 1948; Senevet and Andarelli, 1956; Guy, 1963; Guy and Holstein, 1968).

During this study *An. sergenti* was mainly collected in summer and autumn particularly in animal shelters. The summer-autumnal development of this species has already been reported in Morocco (Gaud et al., 1950; Guy, 1963).

Anopheles cinereus was less abundant but it prevailed at Mizgane. This species is widespread in Morocco but is more common in mountainous areas (Trari et al., 2004). Its presence on the coastal strip is exceptional (Ristorcelli, 1946; Gaud, 1953; Gaud et al., 1950; Guy, 1959, 1963). During this study *An. cinereus* was collected in abundance in spring. It was progressively replaced, later, by *An. sergenti*. This species has never been considered as a malaria vector in Morocco in view of its weak longevity as well as its exophilic and zoophilic behaviour (Guy and Holstein, 1968).

Anopheles labranchiae, the unique representing of the *maculipennis* complex (Faraj et al., 2004) and the principal vector of malaria in Morocco was relatively rare (3%) in the two study areas, particu-

larly in Assoul locality. These results confirm what is reported in the literature; *An. labranchiae* is a ubiquitous mosquito that present in a very large part of the country but it predominates in the Atlantic plains (Gaud, 1953; Guy, 1959, 1963) where it constitutes 95% of *Anopheles* population (Gaud et al., 1950).

During 2005, *An. claviger* was very rare in Chefchaouen; only three females were captured. The same abundance was reported in 2001 (Faraj et al., 2003). According to Gaud (1953), Senevet and Andarelli (1956) and Guy (1959, 1963) this species exists in low densities in Morocco. Its zone of preference is the northern slopes of the Middle-Atlas and the interior plains (plain of Saïs, mainly between Meknes and Fes). Its presence in the Rif in very low density was mentioned in 1967 by Bailly-Choumara (unpublished data).

Among the five *Anopheles* species collected in Chefchaouen, only *An. labranchiae* and *An. sergenti* could be responsible for a resumption of malaria transmission. However, the results presented here show that the role that *An. labranchiae* could play is unimportant because of its weak density (only 34 specimens were collected during the study) and its zoophilic behaviour (none blood meal processed was human). This behaviour was already been reported in other areas of Morocco (Houel, 1955; Guy, 1963).

As for *An. sergenti*, although it has an endophilic behaviour, its low human biting rate, its short seasonality and its zoophilic behaviour, confirmed by blood meal analyses, do not confer it a high vectorial capacity. However it remains the only species which can be responsible for a possible emergence of autochthonous cases.

The study of the infectivity showed that neither *An. labranchiae* nor *An. sergenti* was found infected during 2005. This permits to advance the absence of sporozoites circulation in *Anopheles* without guaranteeing for all the absence of dormant human reservoir which could be at the origin of a new resurgence. *P. vivax* is known by its very long period incubation (Izri et al., 1994).

So, the overall receptivity of Chefchaouen province seems to be low. Although the vulnerability of the region could be considered high because of the possible presence of gametocytes carriers of *P. vivax*, the risk of reappearance of autochthonous malaria transmission appears to be low in the current conditions of surveillance and vector control.

The risk of malaria reintroduction via travellers coming from endemic tropical countries is unimportant because of the reduced number of gametocyte carriers of *P. falciparum* in this area. Since 1995, only one imported case was reported in Chefchaouen province (Ministry of Public Health, unpublished data). Furthermore this region is not a common passage for the illegal African immigrants. Besides, it is not certain that Moroccan *Anopheles* species are susceptible to infection with the tropical Africans strains of *Plasmodium*.

Acknowledgements

We gratefully thank C. Brengues, IRD Montpellier, for PCR processing and the staff of the Laboratoire d'Entomologie Médicale: M. Elkohli, L. Laqraa, M. Elrhazi, M. Sbika and A. Arous for their technical assistance in field and laboratory. We also thank staff from Chefchaouen province; Dr. A. Mountassir, A. Lyalli, B. Dahbi, S. Nouhassi and K. Oufkir for their information and help in collecting specimens.

This work was funded by EU grant GOCE-2003-010284 EDEN (the paper is catalogued by the EDEN Steering Committee as EDEN0114). The contents of this publication are the sole responsibility of the authors and can in no way be taken to reflect the views of the European Union.

References

Beier, J.C., Perkins, P.V., Wirtz, R.A., Koros, J., Diggs, D., Gargan, T.P., Koech, D.K., 1988. Blood meal identification by direct enzyme-linked immunosorbent assay (ELISA), tested on *Anopheles* (Diptera: Culicidae) in Kenya. J. Med. Entomol. 25, 9–16.

Brunhes, J., Rhaim, A., Geoffroy, B., Angel, G., Hervy, J P., 2000. Les moustiques de l'Afrique méditerranéenne. Logiciel d'identification et d'enseignement. Montpellier, France, IRD & IPT, CD-Rom collection didactique, Éditions IRD.

Faraj, C., Adlaoui, E., Rhajaoui, M., Lyagoubi, M., 2003. Malaria transmission estimation in high-risk provinces of Morocco. East Mediterr. Health. J. 9, 542–547.

Faraj, C., Adlaoui, E., Saaf, N., Romi, R., Boccolini, D., Di Luca, M., Lyagoubi, M., 2004. Note sur le complexe Anopheles maculipennis au Maroc. Bull. Soc. Pathol. Exot. 97, 293–294.

Faraj, C., Adlaoui, E., Ouahabi, S., Lakraa, L., Elkohli, M., El Aouad, R., 2008. Extension vers le nord de l'aire de distribution de *Anopheles (Cellia) d'thali* Patton 1905. Bull. Soc. Pathol. Exot. 101, 62–64.

Gaud, J., 1948. Fréquence au Maroc et rôle vecteur possible d'*Anopheles sergenti* Theobald. Bull. Soc. Pathol. Exot. 12, 498–501.

Gaud, J., 1953. Notes biogéographiques sur les Culicidés du Maroc. Arch. Inst. Pasteur. Maroc. 4, 443–490.

Gaud, J., Faure, F., Maurice, A., 1950. Répartition et fréquence relative des espèces anophéliennes au Maroc. Ann. Parasitol. Hum. Comp. 25, 53–60.

Guy, Y., 1959. Les Anophèles du Maroc. Mémoires de la Société des Sciences Naturelles et Physiques, Maroc, Zoologie (N S). Rabat 7, 1–235.

Guy, Y., 1963. Bilan épidémiologique du paludisme au Maroc (données recueillies entre 1960, 1961 et 1962). Ann. Parasitol. Hum. Comp. 38, 823–857.

Guy, Y., Holstein, M., 1968. Données récentes sur les Anophèles du Maghreb. Arch. Inst. Pasteur. Alger. 46, 142–150.

Himmi, O., Dakki, M., Trari, B., El Agbani, M.A., 1995. Les *Culicidae* du Maroc: clés d'identification, avec données biologiques et écologiques. Travaux de l'Institut Scientifique. Série Zool. Rabat 44, 1–51.

Houel, G., 1955. Note sur l'orientation trophique de *Anopheles labranchiae* au Maroc. Bull.de l'Inst. d'Hyg. Maroc. 15, 387.

Izri, M.A., Lortholary, O., Guillevin, L., Rousset, J.J., 1994. Accès palustre à *Plasmodium vivax* plus de cinq ans après un séjour à Meknes. Maroc. Bull. Soc. Pathol. Exot. 87, 189.

Proft, J., Maier, W., Kampen, H., 1999. Identification of six sibling species of the *Anopheles maculipennis* complex (Diptera: Culicidae) by polymerase chain reaction assay. Parasitol. Res. 85, 837–843.

Ristorcelli, A., 1946. Sur la présence à Marrakech d'*Anopheles hispaniola*. Ann. Parasitol. Hum. Comp. 21, 1–4.

Senevet, G., Andarelli, L., 1956. Les Anophèles de l'Afrique du Nord et du bassin méditerranéen. Encyclopédie Entomologique, Paris XXXIII, 280.

Trari, B., Harbach, R.E., Himmi, O., Dakki, M.A., Agoumi, A., 2004. An inventory of the mosquitoes of Morocco. I. Genus *Anopheles* (Diptera: Culicidae). Eur. Mosq. Bull. 18, 1–19.

Wirtz, R.A., Sattabongkot, J., Hall, T., Burkot, T.R., Rosenberg, R., 1992. Development and evaluation of an enzyme-linked immunosorbent assay for *Plasmodium vivax* VK247 sporozoites. J. Med. Entomol. 29, 854–857.