ORIGINAL ARTICLE

Epidemiological Patterns of Malaria Infection diagnosed at La Rabta Teaching Hospital in Tunisia

Caractéristiques épidémiologiques des cas de paludisme diagnostiqués au CHU La Rabta de Tunis

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Abstract
Introduction :
Since the last indigenous case in 1979, there has been no active transmission of malaria in Tunisia. However, imported malaria cases are increasing. Our study aimed to describe malaria cases detected in la Rabta Hospital from 2015 to 2018 and to assess adherence of Tunisian travelers to malaria prophylaxis.
Methods: We conducted a descriptive and retrospective study of cases of malaria diagnosed in the laboratory of Parasitology and Mycology of La Rabta Hospital in Tunis during four years (2015 to 2018). Patients were addressed from several deparments of the same hospital as well as other public and private health facilities. Data were collected from patients' information forms at the Laboratory which included demographic data, travel history, preventive measures taken and symptoms. Patients were tested by direct microscopic examination of thick and thin blood smears and rapid diagnostic tests.
Results :
A total of 339 patients consulted at the laboratory. Blood smears were positive among124patients (36.6% [31.9- 41.6]). Most of the cases were positive to *Plasmodium falciparum* (81.5%). The highest positivity rate was recorded in 2017 (p=0.003). Most positive cases were males (83%; p=0.001) and aged from 15 to 45 years (76.4%). More than half of positive cases were Tunisians (54%). All the cases travelled or were originated from sub-saharian countries except one transfusion-transmitted malaria Tunisian patient. Among Tunisian positive cases, pre-travel consultation, chemprophylaxis and personal protection were used in 25.7%, 22.7% and 42.4% of the cases respectively.
Conclusions :
In the absence of national data, our study provided an overview of the current malaria situation in Tunisia. Imported malaria cases are increasing and transfusion-transmitted malaria is still observed.With competent anopheles species still existing in the country, a strict surveillance is highly needed as well as regulations on blood donor screening and educationof travelers regarding malaria prophylaxis.
Keywords: Malaria, *Plasmodium,* prevention, transfusion, travel,Tunisia

Résumé
Introduction :
Depuis le dernier cas autochtone en 1979, il n'a pas été rapporté de transmission active de paludisme en Tunisie. Cependant, les cas importés de paludisme semblent augmenter ces dernières années. Notre objectif était de décrire les cas de paludisme importé diagnostiqués à l'Hôpital La Rabta durant une période de 4 ans (2015 à 2018) et d'évaluer l'adhérence des voyageurs tunisiens à la prophylaxie antipaludique.
Méthodes :
Il s'agit d'une étude rétrospective descriptive des cas de paludisme diagnostiqués au Laboratoire de Parasitologie-Mycologie de l'Hôpital La Rabta durant 4 ans (2015-2018). La collecte de données était basée sur la fiche d'information des patients. En plus des données démographiques, nous avons collecté les variables concernant les antécédents de voyage, les mesures de prophylaxie et les symptômes cliniques. Tous les patients ont été testés par goutte épaisse, frottis sanguin et test de diagnostic rapide.
Résultats :
Au total, 339 patients ont consulté au laboratoire pour suspicion de paludisme, dont 124 cas étaient positifs (36.6% [31.9-41.6]). *Plasmodium falciparum* était l'espèce la plus fréquemment identifiée (81,5%). Les taux de positivité les plus élevés ont été notés en 2017 (p=0.003). La majorité des cas positifs étaient de sexe masculin (83% ; p=0,001) et âgés de 15 à 45 ans (76.4%). Les cas positifs étaient tunisiens dans 54% des cas. Tous les cas diagnostiqués étaient originaires ou ont voyagé en Afrique sub-saharienne à l'exception d'un cas de paludisme post-transfusionnel. Chez les Tunisiens ayant eu un paludisme d'importation, la consultation pré-voyage, la chimioprophylaxie et la prophylaxie antivectorielle ont été notées dans 25.7%, 22.7% et 42.4% des cas respectivement.
Conclusion :
Notre étude a montré que les cas de paludisme importés en Tunisie n'étaient pas négligeables et même en augmentation. Nos résultats ont aussi permis d'avoir une meilleure compréhension et une idée globale sur la situation actuelle. Cette étape est essentielle pour cibler les actions de prévention et de lutte dans le but d'éviter la réintroduction du paludisme en Tunisie.
Mots-clés : Malaria, *Plasmodium,* prévention, transfusion, voyage, Tunisie

INTRODUCTION

Malaria is still a significant cause of mortality worldwide and a cause of morbidity in returned travellers and migrants in non-endemic areas (1). According to the World Health Organization (WHO) World Malaria Report 2017, most of malaria cases remain in the WHO African Region with 90% in 2016, followed by the WHO South-East Asia Region (7%) and the WHO Eastern Mediterranean Region (2%) (2). It is estimated that 44% of the population of the Eastern Mediterranean Region are living in areas at risk of local malaria transmission in 2015(3).

In Tunisia, malaria was for a long-time endemo-epidemic (4) but thanks to the efforts of the national program of malaria eradication established since 1968, the country became malaria-free in 1979 (4, 5). Besides four cases of airport malaria notified in 2013, no local case has been registred since the elimination (6, 7).

However, the specific geographic location of Tunisia increases the risk of reintroduction of this disease, especially with the continual population movement from and to malaria-endemic countries. In fact, international travel, growing rapidly worldwide, has made distant endemic regions easy to reach and hosting an increasing number of travellers. In Europe, it is estimated that over 10 million people travel annually to endemic areas leading to nearly 15 thousands imported malaria cases per year (8).

Likewise, imported malaria cases seem to increase in Tunisia. With the persistance of a local anophelism (9), these cases may lead to the reappearance of autochthonous malaria such as seen in the neighboring country Algeria (10) and other countries of the Mediterranean region; France (11) and Grece (8) where malaria was previously eliminated. The main challenge is therefore to prevent the reintroduction of malaria transmission mainly through strengthening malaria surveillance, preventive measures for travellers and collaboration in border areas (12). Better understanding of epidemiological profile of imported malaria cases is essential to adapt these action plans.

The current study aimed to describe malaria cases detected in la Rabta Hospital from 2015 to 2018 and to assess adherence of Tunisian travelers to malaria prophylaxis.

METHODS

It was a descriptive study of the diagnosed imported malaria cases among all cases consulting for malaria testing in the Laboratory of Parasitology of the Teaching Hospital “La Rabta” in Tunis from january 2015 to december 2018.

The Laboratory of Parasitology of la Rabta Hospital, one of the four main laboratories responsible for diagnosing malaria in Tunis capital, is characterised by its linkage to the only Infectious Diseases Department of the capital, located in the same hospital. Patients were addressed from this department as well as other medical deparments of la Rabta hospital and other public and private health facilities.

Data were collected from the patient information forms at the Laboratory. These forms included demographic *data* such as age, gender, nationality, profession, travel history (date of arrival in Tunisia or date of return from travel, travel duration, country of destination or origin, reasons of travel), clinical and biological *data*, laboratory results (identified species) as well as information on chemoprophylaxis and personal protection for Tunisian travelers.

Malaria diagnosis was based on direct microscopic examination of thick and thin blood smears (BS) (Giemsa and MGG colorations respectively) ; these techniques are the reference methods recommended by the WHO. Rapid diagnostic test (RDTs) (OptiMAL-IT, Biorad) was also performed. This test represents a diagnostic aid due to its simplicity, speed and good sensitivity. Besides, an estimation of the percentage parasitaemia of *Plasmodium* in Giemsa-stained thin blood films was carried out.

We used a computerized database (Access; Microsoft) for data entry and storage and SPSS-20 software for data analysis. A descriptive analysis was conducted to assess gender distribution, age, profession, reasons for travel for all consulting patients in addition to *Plasmodium* species for positive malaria cases. Quantitative data were presented by mean values and standard deviation. Qualitative variables were expressed as relative and absolute frequencies and were compared using Chi-square test or logistic regression. The significance level was set at $p < 0.05$. All the participants were assigned an unique ID to ensure anonymity and confidentiality.

RESULTS

Description of all patients tested for malaria

During the study period of 4 years (2015-2018), a total of 339 subjects consulted at the laboratory for malaria testing with an average of 84.75 cases per year. Male to female sex ratio was equal to 2.6. Age ranged from 1 to 71 years with an average of 34.6 13.2 years. More than two thirds of the cases were aged between 30 and 45 years (n=137). Most cases consulted during autumn and summer (58.4%) with two peaks in october (12.4%) and august (11.2%). More than half of the cases were Tunisians (58.7%) (Table 1).

Almost all of Tunisian cases had travel history (92.9%). The remaining tested patients (n=14) were mainly weakened (after transplantation, leukemia) or had prolonged unexplained fever. Among the 185 Tunisians with travel history, only five cases traveled to Asia (India, n=2 and Saudi Arabia, n=3). All the others traveled to African countries (Figure 1).

Table 1: Demographic characteristics and time of consultation of cases tested for malaria, La Rabta Hospital, 2015-2018

	n	%
Gender		
Female	93	27.4
Male	246	72.6
Age (n=335)		
[0-15[	15	
4.5		
[15-30[	109	32.5
[30-45[	137	40.9
≥45	74	21.1
Nationality		
Tunisian	199	58.7
Foreigner	140	41.3
Year		
2015	71	20.9
2016	89	26.3
2017	90	26.5
2018	89	26.3
Season		
Winter	56	16.5
Spring	85	25.1
Summer	97	28.6
Autumn	101	29.8

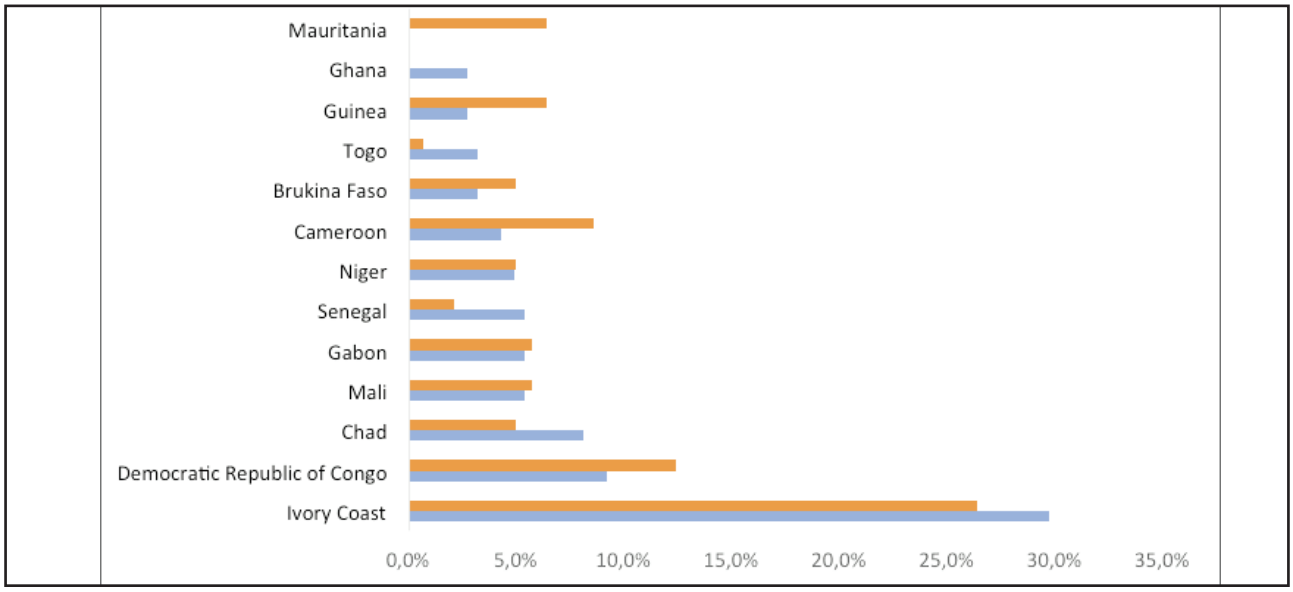

Figure 2: Postivity rates by year, La Rabta Hospital, 2015-2018
BS: Blood Smear; RDT: Rapid Diagnostic Tests

Pre-travel medical consultation was reported among 36.8% of Tunisian travelers while 9.7% were informed of malaria risk through Internet (Table 2). Tunisian travellers used chemoprophylaxis in 39.5% of the cases mainly Mefloquine (52%). The molecule was not precised in 35.6% of the cases. Anti-vector prophylaxis was used in 47% of the cases; long sleeves (31.4%), mosquito nets (27%), repellent (27%), not going out at night (9.2%).

Table 2 : Malaria prophylaxis among Tunisian travelers

	Consulting patients (n=185)		Positive cases (n=66)	
	n	%	n	%
Pre-travel medical consultation	68	36.8	17	25.7
Information of malaria risk through Internet	18	9.7	6	9.1
Chemoprophylaxis	73	39.5	15	22.7
Personal protection	87	47	28	42.4

Description of positive malaria cases

All the 339 patients consulting from 2015 to 2018 were tested by thick smear and blood smear (BS) and 324 were tested by RDTs (95.6%). Thick and blood smears were positive for 124 cases (36.6% [31.9- 41.6]) and RDTs were positive for 113 cases (34.9% [29.6- 40.4]).

The highest positivity rates were recorded in 2017 (p=0.003 and p=0.001 resepectively for BS and RDTs) (Figure 2). Positivity rates were also significantly associated with season of diagnosis time (p=0.001 and p=0.002 resepectively for BS and RDTs). The highest rates were recorded during autumn (48.5% for both tests).

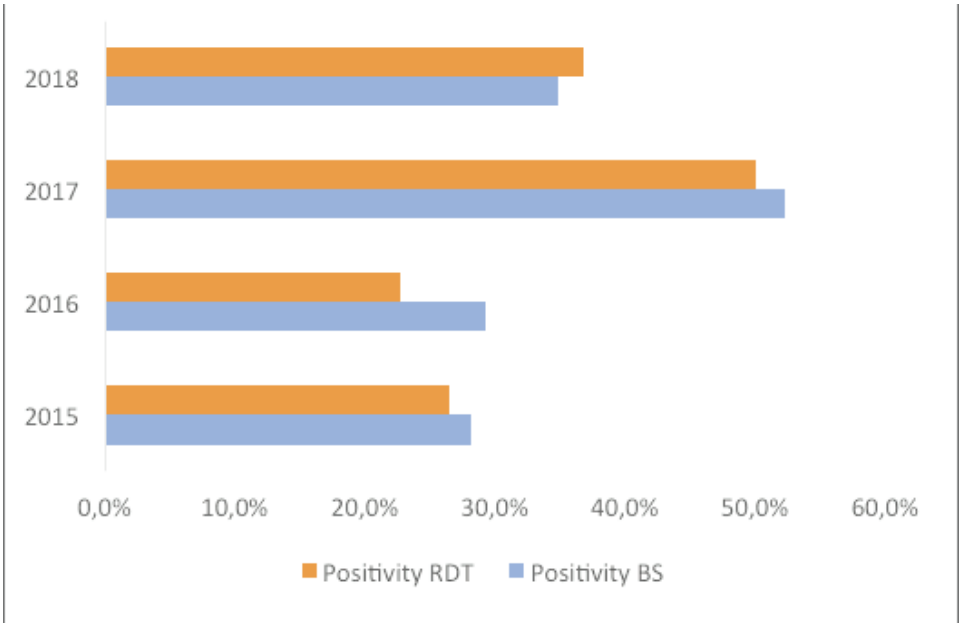

Figure 2: Postivity rates by year, La Rabta Hospital, 2015-2018
BS: Blood Smear; RDT: Rapid Diagnostic Tests

Most positive cases were males (83%; p=0.001). Positive cases were aged between 10 and 64 years with an average of 34.2 ± 12 years (p=0.65). The age groups 15 to 30 and 30 to 45 years were the most affected (38.2 % each) (p=0.12) (Table 3). More than half of positive cases were Tunisians (54%). Positivity rates did not differ significantly with the origin (40.7% among foreigners *versus* 33.7% for Tunisians, p=0.81).

Among the 14 Tunisian cases without travel history, only one was positive. It was a 26-year- old Tunisian man, allografted and having received globular pellets and platelet units during his hospitalization. With the occurrence of a fever resistant to antibiotics by 15 days post-graft, a blood smear was carried out and showed trophoizoites of *P. falciparum* with a 20% parasitemia.

Table 3: Demographic and clinic characteristics among cases with positive Blood Smears

	N	%	p
Gender (N=124)			**0.001**
Female	21	16.9	
Male	103	83.1	
Age (N=123)			0.12
[0-15[	2	1.6	
[15-30[	47	38.2	
[30-45[	47	38.2	
≥45	27	22.0	
Nationality (N=124)			0.18
Tunisian	67	54.0	
Foreigner	57	46.0	
Countries of Origin /Destination			0.17
Ivory Coast	40	32.5	
Congo	18	14.6	
Gabon	10	8.1	
Cameroun	7	5.7	
Chad	7	5.7	
Mali	6	4.8	
Burkina Faso	4	3.3	
Mauritania	4	3.3	
Others	27	22.0	
Profession (N=109)			**0.03**
Unemployed	3	2.7	
Higher professional occupations	29	26.6	
Intermediate occupations	27	24.8	
Low technical occupations	16	14.7	
Students	30	27.5	
High-level athlete	4	3.7	
Reasons of Travel (N=111)			0.51
Studies	29	26.1	
Work	56	50.4	
Tourism	14	12.6	
VFR	8	7.2	
Medical care	2	1.8	
Others	2	1.8	
Clinical Signs			
Fever (N=114)	91	79.8	**0.004**
Chills (N=92)	25	27.2	**0.015**
Headache (N=98)	34	34.7	**<10-3**
Altered consciousness (N=94)	18	19.1	**<10-3**
Nausea and/or vomiting (N=99)	41	41.4	**0.001**
Gastric pain (N=93)	17	18.3	0.72
Anemiae (N=93)	10	10.8	0.43
Thrombopenia (N=94)	11	11.7	0.16
Leucopenia (N=90)	5	5.6	0.25

The epidemiological investigation found a foreigner originating from Ivory Coast among the blood donors who had a positive indirect immunofluorescence (1/20) and the real-time PCR was positive for *P. falciparum*. Diagnosis of transfusion-transmitted malaria was thus retained.

The remaining Tunisian positive cases travelled mainly to Ivory Coast (39.4%) and Democratic Republic of Congo (15.2%). Those who travelled to Asian countries had negative BS. Positive foreigners were also mainly from Ivory Coast (24.6%) and Republic Democratic of Congo (14%) .Less than one third of Tunisian positive cases had pre-travel consultation (25.7%) and used chemprophylaxis (22.7%) (Table 2). Proportion of pre-travel consultation was significantly lower among positive cases, 25% *vs* 41.9% among negative cases (p=0.021) as well as chemoprophylaxis (20.5% *vs* 45.5%; p=0.001). However, there was no significant difference in the use of personal protection (p=0.44).

Fever was the most common clinical sign, present in 79.8% of positive cases. Other clinical signs included nausea and/or vomiting, headache, chills, and gastric pain (Table 3). The mean time from arrival to Tunisia to diagnosis was significantly higher among positive cases (p=0.01) (11.8 *vs* 8.8 days). There was no significant difference in mean times from arrival to onset of clinical signs (p=0.7) (7.1 among positive *vs* 7.5 days among negative patients).

Positive laboratory results

Plasmodium falciparum was the most frequent species (82.2%) identified in 101 imported cases and the post-transfusion malaria case. Parasitemia was mainly between 1 and 4 (85%) and >4 in 8.4% of the cases.

The remaining cases were positive to *P. ovale* (n=7), *P. vivax* (n=4) and *P. malariae* (n=3). The species was not identified in 7.3% of the cases (n=9). *P. falciparum* positive cases were mainly originating or travelling to Ivory Coast (31.7%) and Democratic Republic of Congo (15.8%). Ivory Coast was also the main country of infection by *P. ovale* (5/7) while cases of *P. vivax* were originating or travelling to Mauritania (3/4) and Djibouti (1/4) (Table 4).

Table 4 : Parasites types by country of origin or travel (N=123)

	P. falciparum	***P. ovale***	***P. vivax***	***P. malariae***	**Not specified**
Ivory Coast	32	5		1	2
Congo	16				2
Gabon	9			1	
Chad	6	1			
Guinea	6				1
Cameroun	5			1	1
Mali	5	1			
Togo	4				
Burkina Faso	3				1
Benin	3				
Mauritania	1		3		
Djibouti			1		
Soudan					1
Others	11				
Total	**101**	**7**	**4**	**3**	**8**

Evolutive stage of *Plasmodium* was reported in 91.1% of positive cases. The majority were trophozoites (90.3%). Trophoizoites were associated with gametocytes in 6.2%. Isolated gametocytes were found in 1.8%. Most of the cases had at least one laboratory results control (73.4%). The majority had only one control (51.7%) on Day 3 after starting treatment and only five patients had 4 controls (on day 3,7, 14 and 28). First control of BS was positive in 9.9% (9/91) and RDTs in 17.6% of the cases (16/91). The third and fourth controls of both BS and RDTs were negative for all the cases who had control.

DISCUSSION

Since the last autochtonous malaria case in 1979, imported malaria has been on the rise in Tunisia. However, estimations of the total number of these cases are limited, and there is considerable underreporting in the mandatory disease reporting at the national level. Despite the retrospective nature of the study and its restriction to one department of the capital, our study provided important results on the increasing number of imported malaria cases in our laboratory. In fact, during the 4-year study period, we registered 124 cases with the highest numbers in 2017

and 2018, while previous studies conducted in the same department showed 130 cases in 10 years (1980 – 1989) and 291 cases in 15 years (1991 – 2006) (13, 14).

This increase can be explained by a growing number of sub-saharian african students coming to Tunisia for university and higher education (especially West Africa and other francophone countries). On the other hand, commercial and professional exchanges with subsaharian countries are extending rapidly these last years, and this can be partly attested by the establishment of new direct flights between Tunis and some african capitals such as Abidjan, Bamako, Dakar, Nouakchott, and Ouagadougou. Indeed, the vast majority of our cases were originating or travelling to sub-Saharian countries. This was also reported in other series in Tunisia (15), in Morocco (16) as well as in Europe (17, 18). Ivory Coast and the Democratic Republic of Congo were the most frequently implicated countries in malaria contamination, serving as both the origin and destination of travel. Additionally, a Moroccan review between 1997 and 2007 found that the majority of foreigners also came from these countries (19).

Besides, one transfusion-transmitted malaria (TTM) case was observed. In Tunisia, the first TTM case was reported in the 90s and so far, 11 cases have been registered (20).This route of transmission, although accidental and negligeable, continues to be a threat in non-endemic countries (21). In fact, the possibility of carriers of Plasmodium among blood donors is quite high, considering the parasite's persistence for at least one year (depending on the species) and the increasing travel and immigration from sub-Saharan Africa. Blood donor screening is the most suitable strategy and should be adapted to each country according to its national guidelines (22).

Imported malaria cases were mostly detected during summer and autumn. Similar findings were reported in other Tunisian series as well as in the mediterranean region (4, 8). These months correspond with the return of Tunisians travellers and the arrival of students from malaria endemic countries. It also corresponds with the season of mosquitos activity, including Anopheles vectors. Several entomological studies reported the presence of anopheles in the country with some species keeping the anthropophilic property such as *A. sergenti, A. labranchiae* especially in the north region (4,5). Simultaneous presence of these anopheles with infected persons increases the risk of local transmission. This risk is becoming higher these last years with the changing ecological and climatic factors which may facilitate the resurgence of the transmission (9).

The majority of imported malaria cases were men, young adults. Most of the reviews report similar results not only at the national but also the international level (19, 23). This predominance expresses travel characteristics rather than specific risk factors. In fact, in Tunisia and Morocco, this is mostly due to movement of foreign students and Tunisian professionals whereas in Arabian Gulf countries men were mainly expatriates working in construction sector and originating from Asian countries (23).

Regarding preventive measures, chemoprophylaxis was used by less than a quarter of Tunisian travellers. These rates remain low like seen among European travellers (8). Similarly to other series, Mefloquine was the most used drug, partly due to the standard protocol of chemoprophylaxis for Tunisian travellers which is based on Chloroquine for zone 1 and Mefloquine for zone 2 and 3, and since it is provided for free for all travellers to endemic regions by the National Program of Malaria Control and Prevention. Pretravel consultation and personal protection rates remain also low. This is mainly explained by a lack of health education as well as lack of awareness of malaria risk by travellers, which was found as a risk factor for malaria infection in a french series (24).

Plasmodium falciparum was the most predominant species, as reported in many studies in North Africa as well in Europe (18, 19, 25). This species often results from an african contamination and responsible of most severe malaria forms in addition to a growing concern of Chloroquine resistance. In about 8% of the cases, the species was not identified, probably due to a low level of parasitemia and rare trophozoites in the blood smears. This is often seen among asymptomatic foreigners students. Besides, some blood smears showed gametocytes, whether isolated or not which could be another risk factor for malaria resurgence. In our study, RDTs were less sensitive (positive cases=113) than microscopic examination of a thin blood smear and thick blood smear (positive cases = 124) which remains the method of reference in term of sensitivity and specificity. These tests detecting *Plasmodium* antigens are simple and fast. But their sensitivity depends on parasitemia of the infected patients, thus they are used as complementary tests and are not recommended in the screening (26).

Duration of monitoring depends on the drugs taken and their pharmacological properties. Usually, a monitoring of 28 days is recommended. In our study, 95.3% of the cases had at least one control. This rate was higher than seen in previous series (4). Close monitoring is useful to evaluate the treatment efficacy and to detect a possible drug resistance.

CONCLUSION

During the 4-year study period, 339 patients consulted for malaria testing with a positivity rate of 36.6% and the highest numbers registered in 2017 and 2018. Except the transfusion-transmitted malaria case, all the positive cases had traveled or were originated from sub-Saharan African countries. Adult males were the most affected,

mainly Tunisians travelling for work or students coming to Tunisia for university and higher education.

In the absence of national *data*, our study provided an overview of the current malaria situation in Tunisia. Imported malaria cases are increasing and transfusion-transmitted malaria is still observed. Since anthropophilic anopheles species still exist in the country, a strict surveillance is highly needed to avoid any potential risk of reintroduction of this parasitosis in the country. Screening and early treatment of positive cases originating or travelling to endemic regions are highly needed. Regulations on blood donor screening should also be updated and adapted to the national context.

Malaria prophylaxis remain low among Tunisian travellers. Targeted education and awareness especially on chemoprophylaxis and personal protection need to be improved with expanding travel medicine counseling, more training of health professionals and better communication to the public.

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