Master of Public Health

Master international de Santé Publique

“ Characteristics and risk factors of severe imported malaria in adults over 15 years between 2000 and 2009 in metropolitan France ”

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Master of Public Health 2

Location of practicum: Centre National de Référence du Paludisme, Pitié-Salpêtrière Hospital

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List of acronyms :

CNR Palu:	Centre National de Référence du Paludisme
InVS:	Institut national de Veille Sanitaire
AIDS:	Acquired immunodeficiency syndrome
PCR:	Polymerase Chain Reaction
WHO:	World Health Organization
ARDS:	Adult respiratory distress syndrome
ALAT:	Aspartate amino transferase
ASAT:	Alanine amino transferase
IQR:	Interquartile Range
Hb:	Hemoglobin
RBC:	Red Blood Cells
OR:	Odd Ratio
CI:	Confidence Interval
IU:	International Unit

Summary:

Imported malaria became an important public health issue in many developed countries, because of severe cases of malaria that can progress quickly to complications and death. To explore characteristics and risk factors for severe malaria in travelers, we conducted a retrospective study of imported *P. falciparum* malaria among adult travelers returning to France from malaria-endemic areas from 2000 through 2009. The study also intends to explain the evolution of the severe cases during the study period.

During the study period, epidemiological, clinical and biological data were collected by a network of 120 laboratories in metropolitan France and were reported to the “CNR Paludisme”. WHO definition (2000) was used to define the patients with severe malaria. Factors associated with severe malaria were identified by logistic regression analysis.

The study evaluated 20,921 patients who presented *P. falciparum* malaria, including 1,103 patients with severe malaria. In multivariate analysis, risk factors independently associated with severe malaria were: absence of chemoprophylaxis, older age, caucasian origin, onset of symptoms before returning to France, time between diagnosis and symptoms between 4 and 12 days, and male gender. The study period has also been marked by an increased proportion of severe cases which represented 3.1% in 2000 and 10.7% in 2009.

Vigilance should be increased for adults above 60 year and for patients with caucasian origin. Regarding delay in diagnosis, greater physician awareness of the disease is needed. Increasing compliance to chemoprophylaxis remains necessary for all travelers, including African patients, which represent an increased proportion of the severe cases.

Keywords: *Plasmodium falciparum,* severe malaria, risk factors, evolution of severity

<u>Sujet :</u> « Caractéristiques et facteurs de risque du paludisme grave d'importation chez l'adulte de plus de 15 ans en France métropolitaine entre 2000 et 2009 »

<u>Résumé:</u>

Le paludisme d'importation est devenu un enjeu de santé publique dans de nombreux pays développés, par la fréquence et la létalité de ses formes graves liées à *Plasmodium falciparum.* Nous avons conduit au CNR Paludisme une étude rétrospective sur les cas de paludisme d'importation à *P. falciparum* en France métropolitaine chez l'adulte entre 2000 et 2009.

Les objectifs étaient de décrire les principales caractéristiques des patients présentant des formes graves de paludisme et de mettre en évidence les facteurs de risques associés au développement de formes graves. Une analyse de l'évolution des formes graves pendant la période d'étude a également été réalisée.

Les données épidémiologiques et cliniques ont été recueillies par un réseau de 120 correspondants en France métropolitaine et transmises au CNR Paludisme. La définition OMS 2000 a été utilisée pour déterminer les cas graves. Les facteurs associés à la gravité ont été mis en évidence par une régression logistique.

L'étude a porté sur l'analyse de 20 921 patients infectés par *P. falciparum*, dont 1103 présentaient une forme grave de paludisme. Après l'analyse multivariée, les facteurs indépendamment associés à la sévérité étaient : l'absence de prophylaxie, un âge élevé, l'origine caucasienne, le fait d'être un homme, l'apparition des symptômes avant le retour en France et un délai entre les symptômes et le diagnostic de 4 à 12 jours. La période d'étude a également été marquée par une augmentation significative de la proportion des formes graves, qui sont passées de 3,1% en 2000 à 10,7% des patients en 2009.

La vigilance doit être accrue chez deux groupes particulièrement à risque : les patients âgés de plus de 60 ans ainsi que les patients d'origine caucasienne. Une meilleure détection des signes de paludisme par les médecins est nécessaire afin d'éviter un retard diagnostic. L'utilisation de chimioprophylaxie doit être amplifiée chez tous les voyageurs, notamment les patients d'origine africaine qui représentent une proportion croissante des patients présentant une forme grave.

Mots-clés : *Plasmodium falciparum,* paludisme grave, facteurs de risque, évolution de le sévérité

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I) The host institution

The host institution of my internship was the "**Centre National de Référence du Paludisme (CNR Paludisme)"**.

The French national reference centers "Centres Nationaux de Référence (CNR)" are laboratories located within public or private health establishments, education or research institutions. They are appointed for 5 years by "l'Institut de Veille Sanitaire (InVS)", under the direction of the Ministry of Health. There are 45 CNR in France with the main mission the surveillance of infectious disease (expertise, monitoring, warning and advice). Each CNR sends to the InVS an annual progress report.

The specific missions of the "CNR Paludisme" are:

- to ensure the epidemiological surveillance of imported malaria in France
- to support the investigation of autochthon cases in metropolitan France
- to evaluate the chemosensitivity of *Plasmodium falciparum* isolates
- to provide a warning role in case of emergency situations in the epidemiological or biological plan
- to advise the authorities on the prevention and treatment of imported malaria
- to design and implement surveys to study the causes of morbidity and mortality in imported malaria.

Malaria in metropolitan France is not a mandatory notifiable disease, unlike tuberculosis, typhoid fever, or Acquired immunodeficiency syndrome (AIDS). This explains why a CNR, which has established a network of correspondents, was created.

The CNR Paludisme is organised in three main groups initially with 120 correspondents. Two are located in Paris: Bichat Hospital mostly studies the chemoresistance, with Pitié-Salpêtrière hospital, for north of France cases and total epidemiological surveillance is done in the P.& M. Curie faculty of Medicine, Pitié-Salpétrière University Hospital, where I am based. Another one is located in Marseille and works on south of France cases. Each center collects samples from specific collector sites (correspondents able to send quickly a blood sample from malaria infected individuals diagnosed in their hospitals).

II) **Introduction**

Malaria is still one of the most prevalent infectious diseases. In 2008, there were an estimated 243 million cases of malaria worldwide and malaria accounted for an estimated 863,000 deaths in 2008. Half of the world's population is at risk of malaria (1).

Malaria is a vector-borne infectious disease caused by a eukaryotic protozoan of the genus *Plasmodium.* Malaria is naturally transmitted by the bite of a female *Anopheles* mosquito (Annex 1, Figure 1). Human malaria is caused by four main principal *Plasmodium* species: *Plasmodium falciparum (P. falciparum), Plasmodium. vivax, Plasmodium. ovale* and *Plasmodium. malariae*. *Plasmodium falciparum* is the predominant and most dangerous of the malaria species. It causes multi-organ involvement and, in the absence of prompt and appropriate treatment, is associated with high mortality rates. At least two-third of all imported malaria cases into the developed countries are due to *P. falciparum* (2)**.**

Imported malaria is a symptomatic or asymptomatic *Plasmodial* infection with laboratory confirmed malaria parasitemia (microscopy or Polymerase Chain Reaction (PCR)) that occurs in a subject living in metropolitan France after travel in endemic area (Malaria acquired outside metropolitan France). Imported malaria has been an important public health problem in developed countries in the last few decades. The reason is multifactorial; increasing international travel for non-immune population, tourism, immigration and the spread of drug-resistant *P. falciparum* strains (3)**.** Among the Western countries, France is the most affected by imported malaria due to the large influx of travelers to Africa (4).

Studies have already been undertaken in order to identify persons running the highest risk of severe disease. However, most of these studies are case reports or included a limited number of patients (5-14)**.** And few studies have examined the full spectrum of malaria in travelers who had no exposure during childhood. Our study can provide valuable informations on a large population group with no immunity and which is not complicated by other confounding factors (immunosuppression, multiple pathologies…).

Recently, an analysis of factors associated with mortality among 21,888 patients diagnosed with imported *P. falciparum* malaria between 1996 and 2003 in France was published. Major risk factors revealed were older age, being native from a non-endemic area, infection in East Africa and absence of effective chemoprophylaxis (15)**.**

At the “Centre national de Reference du Paludisme”, we handle a large database regarding imported malaria into metropolitan France, which provides a unique opportunity to analyze the risk factors for severe imported malaria.

Here, we describe and retrospectively analyze the main features of severe imported malaria observed in France during 2000-2009, and compared them with those for uncomplicated cases in order to determine risk factors for severe cases. The objective was to have a better knowledge of the characteristics and risk factors for severe malaria, which might be helpful for malaria cases management, the establishment of prevention measures or control programs. The study also tend to describe the evolution of the different factors associated with malaria and to explain the evolution of the proportion of severe cases in metropolitan France .

One interest of our study, compared to previous studies, was the large number of patients included, which gives power to our results. As the definition of severe malaria was the same throughout the study period (16)(WHO definition, 2000), we were also able to use uniform and relevant criteria to characterize patients with severe malaria. We also increased the accuracy of our study, by defining precise classes to define each of our criteria.

III) **Materials and methods**

a) Data sources

All the malaria cases, confirmed by blood film or Polymerase Chain Reaction (PCR), are reported to the “CNR Paludisme” by health care providers or laboratory staff members based exclusively in metropolitan France and called the “correspondents”. Case investigations are conducted by local clinician correspondents. Data from exhaustive studies suggested that the collecting network data accounts for 50%-55% of total malaria cases imported to France (17, 18).

Reports were transmitted through the “CNR Paludisme ” using a uniform case-report form that contains clinical, laboratory, demographical and epidemiological information’s. The “CNR Paludisme” first used a hard copies before the year 2006 then electronically (secure Internet site) from 2007 on.

b) Study population

The study was limited to reported *P. falciparum* infection, which is the most predominant species and is responsible for almost all severe and fatal cases. All cases of mixed infections containing *P. falciparum* and another subspecies were removed from the analysis.
We excluded the children under fifteen years to reduce the heterogeneity in the study population. Clinical presentation of severe malaria is indeed different in young children (19, 20) and they likely have specific risk factors. The pediatric population will be analyzed separately in a future study.
The study period was from 2000 through 2009; the WHO definition of severe malaria was revised in 2000 and we therefore had a consistent definition during the entire period.
The study population consisted of all *P. falciparum*-infected patients reported in metropolitan France to “CNR Paludisme” during the years 2000 through 2009.
During this period, 1,103 cases of severe and 18,772 of uncomplicated *P. falciparum* malaria cases have been reported (Figure 1).

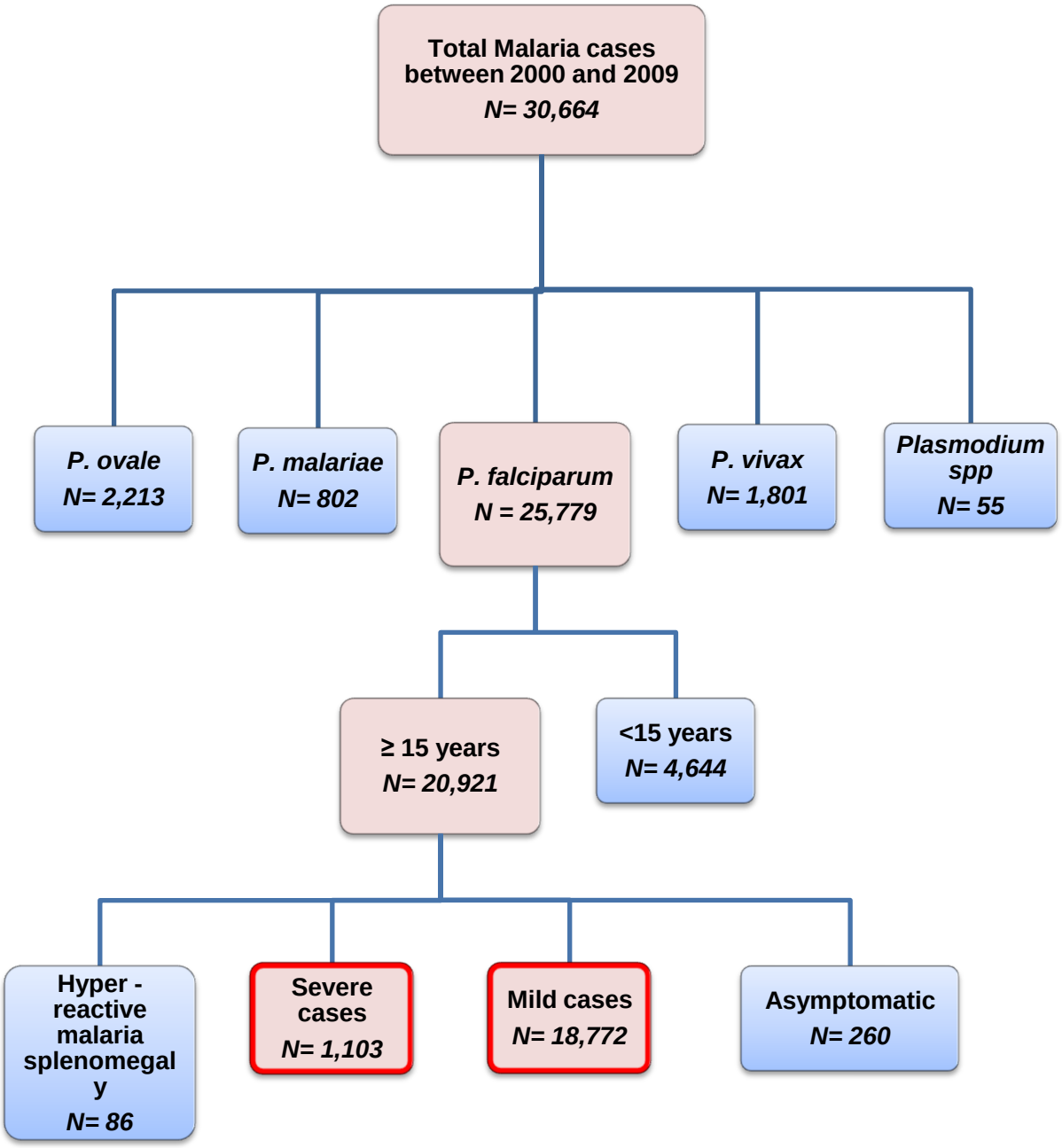


Figure 1: Patient selection and exclusions in the study

c) Variables

i) Outcome

The primary outcome was a binary variable: severe Malaria versus uncomplicated Malaria. Severe malaria was defined using the World Health Organization (WHO) criteria (2000 definition) (16) (see below): In a patient with *P. falciparum* asexual parasitaemia and no other confirmed cause for their symptoms, the presence of one or more of the clinical or laboratory features classifies that patient as suffering from severe malaria.

World Health Organization (WHO) criteria (2000 definition) used were:
(1) Cerebral malaria: Unrousable coma not attributable to any other cause, with a Glasgow Coma Scale score < 9. Coma should persist for at least 30 min after a generalized convulsion;
(2) Prostration or weakness;
(3) Impaired consciousness with rousable mental condition;
(4) Adult respiratory distress syndrome (ARDS);
(5) Repeated generalized convulsions (more than two episodes in 24 hours);
(6) Circulatory collapse with systolic blood pressure of less than 80 mmHg despite adequate volume repletion;
(7) Pulmonary oedema ;
(8) Spontaneous bleeding and/or disseminated intravascular coagulation;
(9) Jaundice with total bilirubin of more than 50 µmol/L;
(10) Macroscopic haemoglobinuria;
(11) Severe anemia with haemoglobin < 5g/L;
(12) Hypoglycaemia with blood glucose of less than 2.2 mM;
(13) Acute renal failure with urine output < 400 mL/24 hours in adults and serum creatinine of more than 265 µmol/L;
(14) Acidaemia (pH of less than 7.25) or acidosis (serum bicarbonate of less than 15mM);
(15) Hyperlactatemia with plasma lactates > 5mmol/L;
(16) Hyperparasitaemia with ≥ 4% of parasitized red blood cells.

ii) Explanatory variables

We selected the variables, which will be treated in our analysis:
- Qualitative variables:
  - **Gender** in two modalities : Male / Female
  - **Birth country** in two modalities : Endemic countries / Non endemic countries

The classification was based on the world distribution of malaria from WHO (Annex 2, Figure 2)

- **Ethnic group** in four categories: African / Caucasian / Asian / Others
  Captured by attending physician based on the color of the skin (i.e., not devoid of subjectivity).
- **Region of malaria acquisition** in seven categories: West Africa / East Africa / North Africa / Austral Africa and Indian Ocean Islands / Asia / East Europe / Others
- **Use of Chemoprophylaxis** in two categories: Yes/No. Based on patient declaration i.e., not confirmed by presence of the drug in patients blood.
- **Objectives of travel** in six categories : Migrants visiting their origin country/ Residents or expatriates ≥ six months/ Military/ Professionals/ Tourism/ Others
- **Frequency of travel** in 3 categories : >1/ 1/ resident. It refers to the number of travels in an endemic country during the last 12 months.
- **Place of stay in 3 categories :** Urban area / Rural area/ Mixed or itinerant
- **Previous consultation**: Yes/No. It refers to a medical consultation before the actual consultation when the case is reported.

- Quantitative variables:
  - **Age** in years
  - **Duration** of stay in days
  - **Time between return from endemic country and symptoms** in days
  - **Time between onset of symptoms and medical consultation** in days
  - **Time between onset of symptoms and diagnosis** in days
  - **Hemoglobin count** in g/L**,**
  - **White blood cell level** in Giga/L**,**
  - **Platelet count** in G/L
  - **Parasitemia** in % of parasitized red blood cells

Others variables, collected from hospitalization reports for the severe cases, were shown to describe precisely the severe cases. The biological and clinical information were collected at D0, defined as the hospitalization date of the patient. We did not take into account the clinical beyond D0 (i.e., health status upon admission).

- **Type of chemoprophylaxis**
- **Duration of hospitalization** in days
- **Duration of hospitalization in intensive care** in days
- **Temperature** in °C

- **Clinical criteria :** Impaired consciousness/ Coma/ Repeated convulsions/ Circulatory collapse/ Abnormal bleeding/ Respiratory distress/ Acidosis/ Haemoglobinuria/Jaundice; in two categories :Yes/No
- **Biological criteria:** Renal failure / Hyperglycemia/ Hyperbilirubinemia/ Hyperlactatemia in two categories: Yes/No
- **Others biological values:** ALAT and ASAT level (markers of hepatic, liver failures), PH and HCO3- count (parameters of acidosis), lactates

d) Data analysis:

The case-report form changed from 2007 on, and we therefore handled two types of databases with different codes and criteria between the two periods. An important part of my work was based on data management and consisted in merging and homogenizing the two bases into a single database.
In addition, from 2000 and 2005, a lot of information was missing. I collected the missing data (essentially biological and clinical data) from the hospitalization charts and reports in order to limit bias and increase the power of the study.

We first described the general study population and then focused on patients with severe disease. In the descriptive analysis, categorical variables were summarized by frequencies and percentages, and numerical variables were summarized by means and standard deviation if normally distributed and by medians and interquartiles ranges if non-normally distributed. The comparisons between groups were done using Chi-square test or Fisher exact test for proportions when appropriated. For quantitative variables, we used t-test or Wilcoxon test and Anova or Krushkal-Wallis test. The correlation between two quantitative variables was assessed by correlation test. To test the trend on the evolution on certain variables, a test of trend was used.

A univariate analysis was performed to determine the factors associated with the severe malaria. The association between dependant variables and explanatory variables was assessed using odds ratio and the 95% confidence interval. We decided to reject the null hypothesis when the P-value was less than 0.25. Dummy variables were used for variables with more than one category. Chi square test is applicable only for qualitative variables, we used subgroups for the quantitative variables.

Then, a multivariate logistic regression approach was used with the preselected variables. Only variables with $p<0.05$ were retained in the final model. Interactions were sought by introducing interaction terms in the logistic regression model and testing for their significance at the 0.05 level.

Statistical analysis was performed using Stata 10 (Stata Corporation, College Station, TX).

IV) **Results**

a) Description of the study population

The total number of monoinfection *P. falciparum* malaria cases (≥ 15 year olds) from 2000 through 2009 was 20,921. Among the study population, 18,772(92.8%) and 1,103(5.5%) patients had uncomplicated and severe malaria respectively; 260(1.3%) were asymptomatic and 86(0.4%) were hyper-reactive malarial splenomegaly. Globally, 106 deaths were related to malaria.

- ***Evolution of the number of malaria infected patients***

The annual number of *P.falciparum* malaria infected patients reported by the network decreased between 2000 and 2009 with 2,510 cases in 2000 and 1,541 cases in 2009. However, the proportion of severe cases increased steadily from 3.1% in 2000 to 10.7% in 2009 (Figure 2).The proportion of deaths was stable over the period with a mean fatality rate of 0,5% (Annex 3, Table 1).

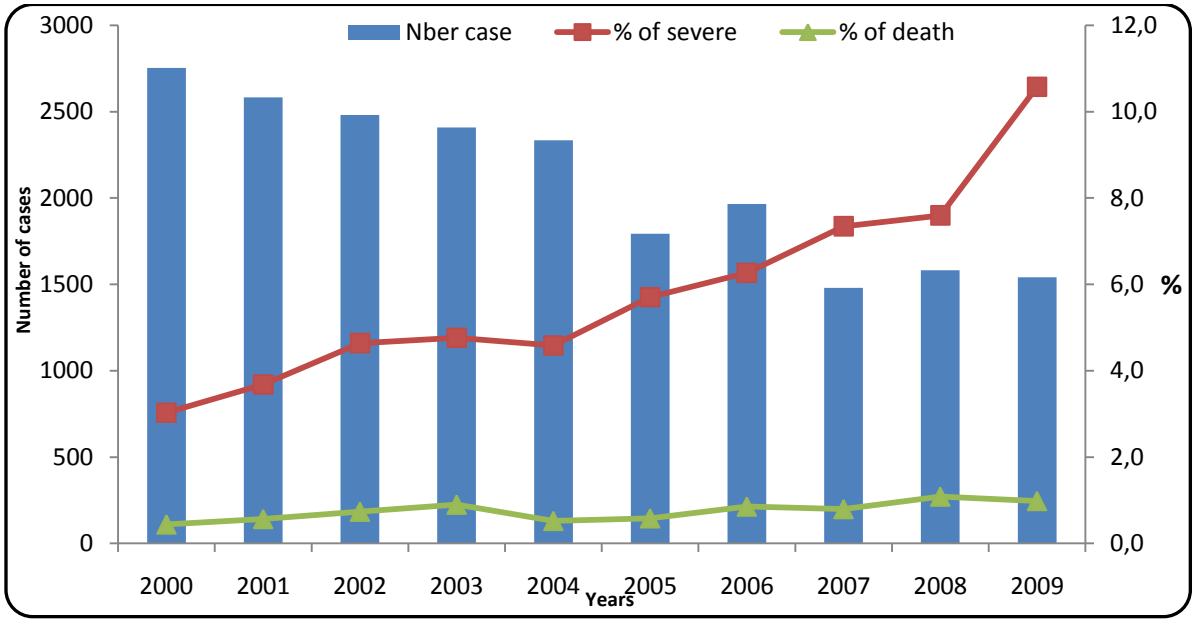


Figure 2: Evolution of the number of malaria cases, of the severity rate (%) and the fatality rate (%) (N=19,875)

- ***Characteristics of the patients***

-Gender: The majority of patients were male (64,7%). The global sex ratio (M/F) of 1.8 was constant over the years. (p=0.9)**.**

-Age**:** The patients were between 15 and 94 years old with a median age of 36 years old

(interquartile range, [27-46]). The proportion of adults of 15-59 years was 94% with a median age of 34 years and the proportion of people over 60 years was 6 % with a median age of 64 years. The proportion of the 15-60 years old slowly decreased while the proportion of the people over 60 years old increased during the period (p<0.001).

- *Ethnic origin*: Africans and Caucasians accounted for 67%, and 31,1% of the population respectively, followed by Asians (0.7%)(N=18,736). The proportion of Caucasians decreased significanlty over the years, accompanied by an increase in the proportion of Africans, which reached respectively 23.1% and 74.1% of cases in 2009 (p<0.001)(Figure 3).

-*Birth country*: Moreover, 10,052 (58%) of the patients were born in endemic countries and 7265(42%) in non-endemic countries. (N=17,317).

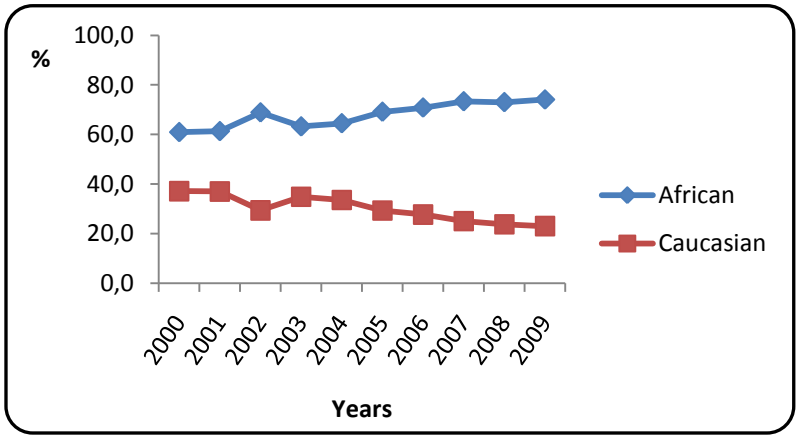


Figure 3: Evolution of the proportion of Caucasians and Africans between 2000 and 2009

- ***Characteristics of the travel***

-*Purpose of travel*: More than half of the patients were Africans travelers visiting their countries (53.7%), followed by resident (14.4%) and tourists (13.6%) (Figure 4).

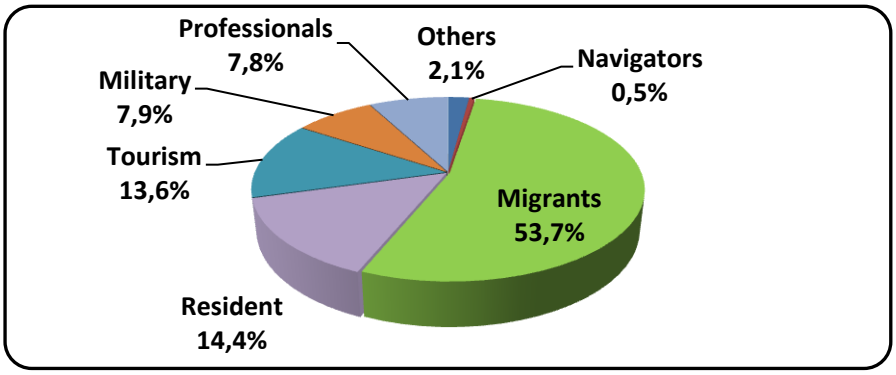


Figure 4: Distribution of the objective of travel in %(N=18,046)

-*Endemic country visited*: Most patients had acquired malaria in Africa: 60.4% in West Africa, 26.6% in Central Africa, 9.7% in Austral Africa, 0.8% in East Africa (Figure 5). The

other area of endemicity were minority and were South America (0.9%), Asia (0.6%), and North Africa (0.6%) (N=20,909) (Figure 5).

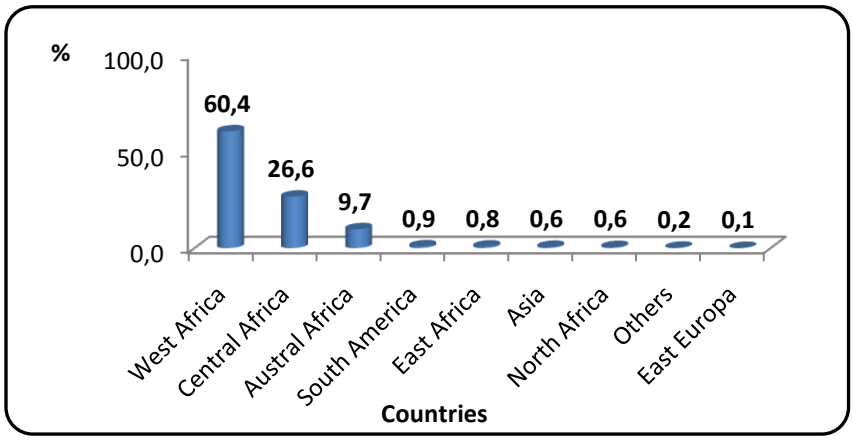


Figure 5: Repartition of cases relative to endemic area (N=20,909)

The five countries counting for the larger number of malaria cases were: Ivory Cost, Cameroun, Mali, Senegal and Comoros islands (Annex 4, table 2).

-Place of residence: Fifty-six percent patients were itinerant. Around 25% stayed exclusively in urban area and 20% in rural area. (N=9612). Africans are more likely to stay in a strictly urban area (30.8%) compared to Caucasians (14.4%) (p<0.001).

-Duration of stay: The median duration of stay was 31 days (IQR [20,61]).The duration was not different between the ethnic groups (Kruskal-Wallis test, p=0.07) and was constant over the years(p=0.09).

-Period of diagnosis: We observed a peak of frequency in the period after summer vacations, between July and September (Figure 6). The tendency was the same in all the years of observations (p=0.11 for test of trend).

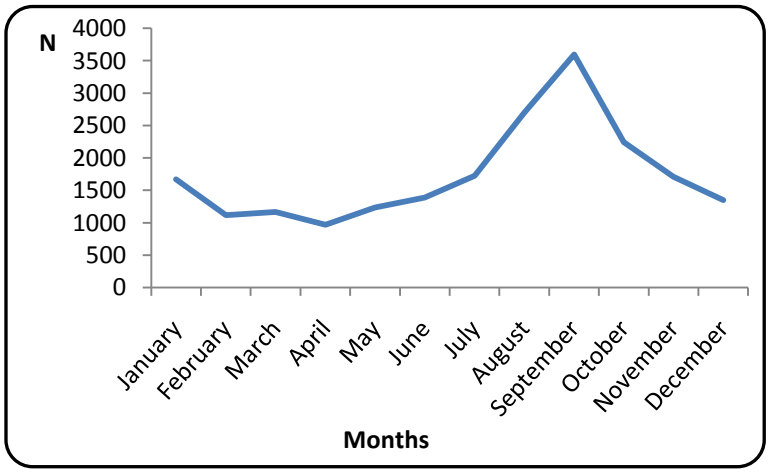


Figure 6: Period of diagnosis of P. falciparum malaria cases in France (N=19,823)

- ***Delay***

-Return/ symptoms: The median time from return to onset of symptoms was 5 days (IQR [0,11]). And 12.2 % (2099) had their first symptoms before return (N=16,973).
-Symptoms/consultation: The median time from onset of symptoms to consultation (general practitioner or hospital) was 2 days (IQR [1,5]) (N=12,403).
-Symptoms/diagnosis: The median time from onset of symptoms to diagnosis of malaria was 4 days (IQR [2,6]) (N=16,025).

- ***Prophylaxis***

Globally, 39.9% of the patients reported taking chemoprophylaxis and 60.1% didn't take any (N=19,339). Besides, the proportion of patients reported taking prophylaxis decreased during the study period (from 44.1% in 2000 to 28.7% in 2009) (p<0.001) (Figure 7).
Differences in the use of chemoprophylaxis between Africans and Caucasians were observed. Indeed, 54.1% of the Caucasians declared taking prophylaxis whereas only 32.6% of the Africans did it (p<0.001).

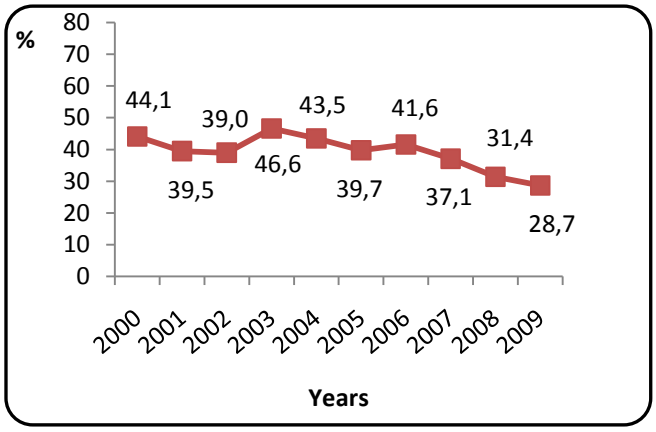


Figure 7: Proportion of patients who reported taking chemoprophylaxis in % (N=19,339)

- ***Biological data***

- Parasitemia: Concerning biological data, 1,737 (11.4%) had a high-level parasitemia (>=4% parasitized red blood cells). The level of parasitemia was significantly higher in older patients (p<0,001). Around 20% of the patients above 60 years olds had high level parasitemia, whereas 10% in patients between 15 and 60 years old.
- Hemoglobin: 36.6% patients were anemic (Hb<120g/L) and 0.2% had severe anemia (Hb<50g/L) (N=15,481).

-White blood cells: 3.9% of the patients had a level of white blood cells above 10 Giga/L (N=15,188).

-Platelets: Two third of the patients had thrombocytopenia (platelet< 150 G/L) and 16% had severe thrombocytopenia(platelets<=50G/L)(N=15,640).

b) Description of severe cases

The number of severe *P.falciparum* cases analyzed in our study was 1,103.

- ***Characteristics of the patients***

-Gender: Male were in majority (70.3%) with a sex ratio M/F = 2.4.This ratio didn't change during the study period (p=0.06).

-Age: The median age was 44 (IQR, [32-55]). And in overall 166 patients (15.1%) were aged more than 60 years old. During the study period, the proportion of older patients (>60 years old) increased gradually (p<0.001), reaching 19% of the patients in 2009.

-Ethnic group: Among all severe cases, Caucasians were most numerous (56.3%), followed by Africans (39.2%), and Asians (2.1%). During the study period, the proportion of Africans increased steadily, while the proportion of Caucasians decreased. And since 2008, Africans were more numerous than Caucasians (Figure 8).

-Birth country: 64.8 % of the patients were born in non-endemic countries and 35.2% in endemic countries (N=960).

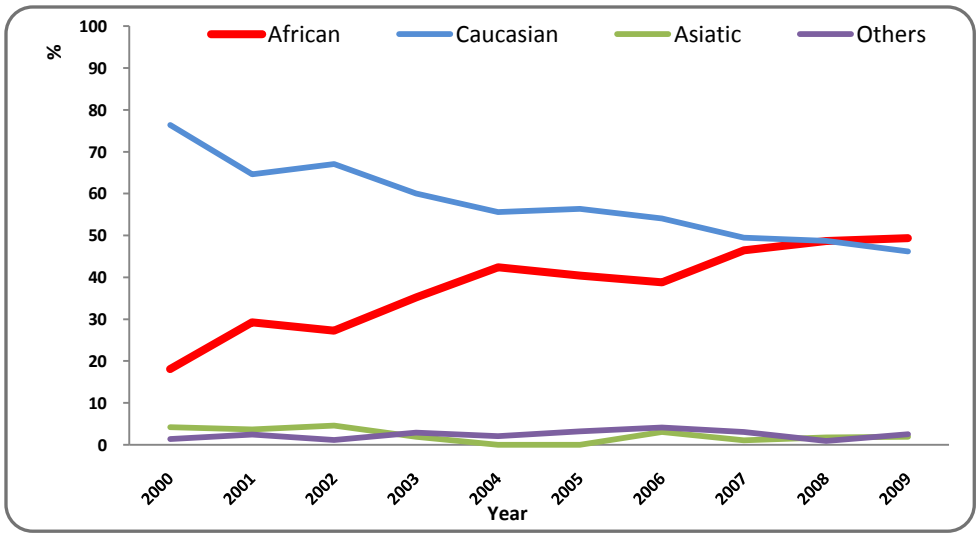


Figure 8: Repartition of Ethnicity between 2000 and 2009 in % (N=1,010)

- ***Characteristics of the travel***

-Purpose of travel: Of those who presented severe malaria, 284 (29.6%) were migrants visiting their origin country, 261 (27.2%) were tourists, 132 (13.8%) were resident or expatriates more than 6 months, 131 (13.7%) were professionals, 95 (9.9%) military (Figure 9).

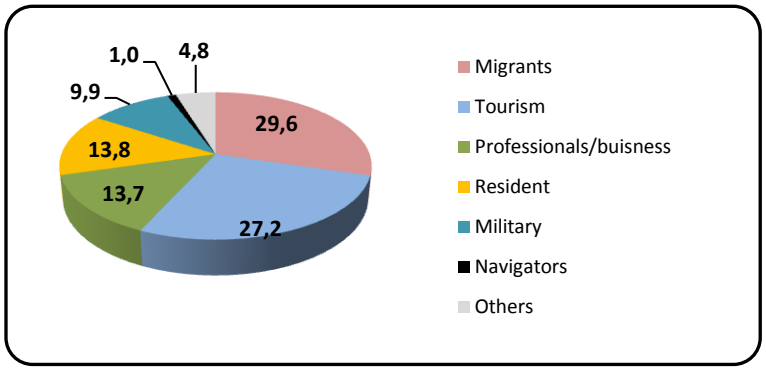


Figure 9: Distribution of the objective of travel in % (N= 959)

-Endemic country: Most patients acquired severe malaria in Africa: 62.6% in West Africa, 22.5% in Central Africa, 9.8 % in Austral Africa, 2.09% in East Africa. Very few patients acquired it in Asia or in South America. The repartition of endemic countries didn't change significantly between 2000 and 2009.

East Africa had the most important severity rate of malaria with a rate of 12.3% (N=1103). (Figure 10).

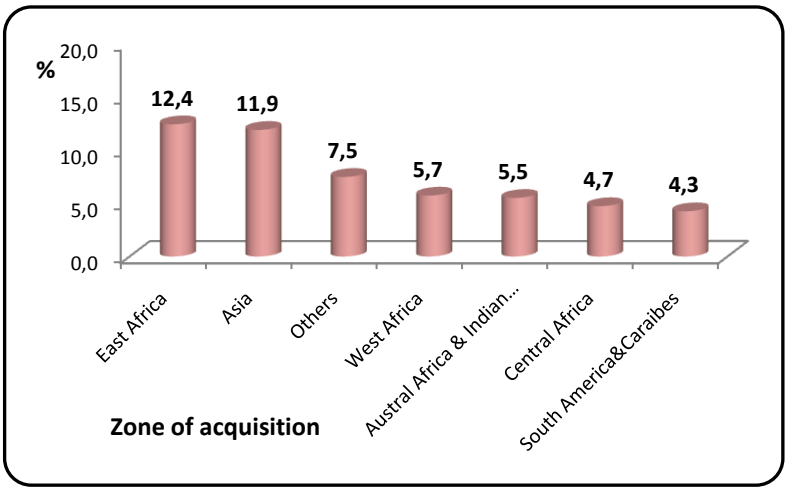


Figure 10: Severity rate of malaria in % relative to the zone of acquisition (N=1,103)

-Duration of stay: The median time of stay in the endemic country was 29 days (IQR [16-61]) and didn't change during the period (p=0.8).

- ***Delay***

-Return/ symptoms: The median time between return to onset of symptom was 8 days (IQR [4-12]) (N=715). And 167 patients (23.4%) had their first symptoms before return.
-Symptoms/consultation: The median time between onset of symptoms to consultation was 3 days (IQR [1-5]) (N=932).
-Symptoms/diagnosis: The median time from onset of symptoms to diagnosis was 4 days (IQR [3-7]) (N=934).
These three variables were constant over the years in the study period (p respectively=0.06, p=0.2 and 0.5 with trend test).
-Hospital stay: The median hospitalization length was 7 days with a range between 0 to 180 days (N=734). Among those, 75% (N=550) were in intensive care units..

- ***Prophylaxis***

More than half of the patients (66.4%) declared not having taken chemoprophylaxis (N=1049) (Figure 11a). Among patients reported taking chemoprophylaxis, only 38.6% took it regularly, 35.4% of the patients took it occasionally and 26% stopped taking chemoprophylaxis before their return to France (N=246) (Figure 11b).

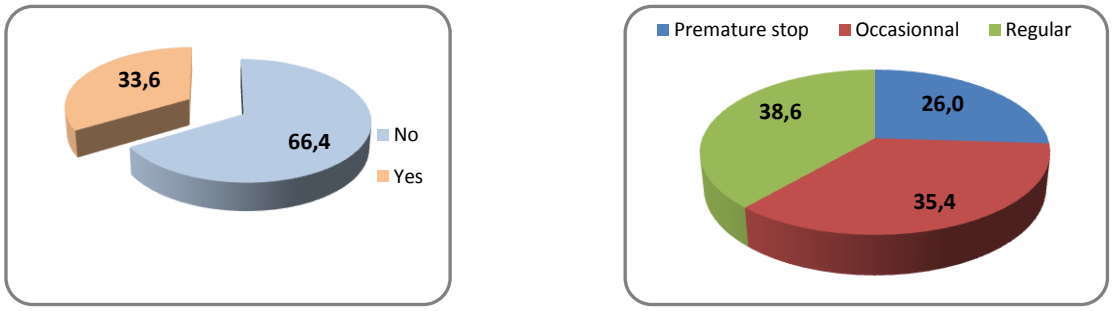


Figure 11: (a) Use of chemoprophylaxis in % (N=1049) and (b) chemoprophylaxis state of use in % (N=246)

The chemoprophylaxis state of use decreases significantly, from 36.8 % in 2000 to 25.7% in 2009. Globally, the prophylaxis treatment used was mostly the association Chloroquine-Proguanil (37.5%), followed by Chloroquine (25.3%), Doxycycline (20.5%) and to a lesser extent, Mefloquine (7.6%), Proguanil (7.2%) and the association Atovaquone-Proguanil (3.6%). (N=249).

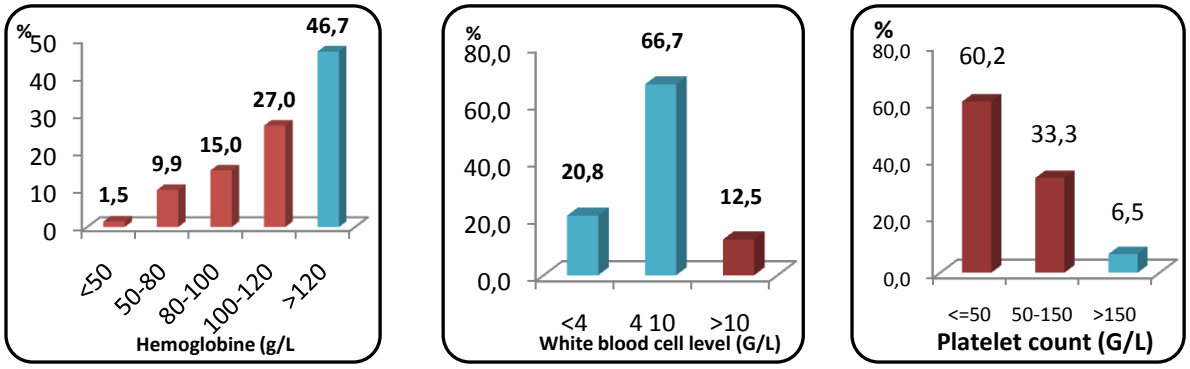
- ***Biological data***

Six hundred and twenty six patients (61.2%) had high level of parasitemia (>=4% of parasitized red blood cells)(N=1023). Caucasians had a significantly higher level of parasitemia compared to Africans (p<0.001). Among the severe cases, 69,2% of the Caucasians and only 50.5 % of the Africans had a level above 4% of parasitemia. Age was also positively correlated with the rate of parasitemia (p<0.001),

-Hemoglobin: Regarding laboratory features, 53.3% of the patients were in anemia (<120g/L) (Figure 12a). 11.20% presented a level of hemoglobin < 80g/L and 1.45% were in severe anemia (<50g/L) (N=964).

-White blood cells: 12.5% had a level of white blood cell more than 10 G/L (N=912) (Figure 13b).

-Platelets: 95% of the patients were in thrombocytopenia (<150 G/L), and 60.2% presented very low platelet counts (<50 G/L) (N=979) (Figure 13c).



<u>Figure 12:</u> (a) Repartition relative to level of Hemoglobin (g/L) in % (N=964), (b) Repartition relative to White blood cells level (G/L) in % (N=912), (c) Repartition relative to platelet counts (N=979)

For the severe forms, we collected additional data concerning clinical criteria(Annex 5,Table 3) and biological criteria (Annex 5, Table 4), which permit to draw precisely the profile of severe cases. The most frequent criteria found were: A creatinine level > 265µmol/l or an acute renal failure (33.2%), clinical jaundice (33.8%), Bilirubin >50µmol/L (37.2%), impaired consciousness (42.2%) and parasitemiae ≥4%(61.2%)

c) Univariate Analysis

Univariate analysis was performed to determine the factors associated with severity and the results obtained were as follows (Annex 6, Table 4):

- The **age** was significantly associated with severe malaria (p<0.001). The odd of developing severe malaria increased with the increase of the age.

- The **sex** was significantly associated with severe malaria (p= 0.001). Men were more likely to develop severe malaria than women (OR=1.30 [1.14-1.49]).

- Patients who were **born in non-endemic** countries were significantly (p<0.001) more likely to develop severe malaria (OR=2.40 [2.75-3.16]) compared to those who were born in endemic-countries.

- **Caucasians** were significantly (p<0.001) more likely to develop severe malaria (OR=2.75 [3.02-3.95]) compared to **Africans**.

- The **duration of stay** was significantly associated with severe malaria (p<0.001). Patients who stayed less than 20 days are more likely to present severe malaria than those who stayed more than 20 days.

- The **type of travel** was significantly associated with severe malaria (p<0.001). The expatriates (OR=1.84 [1.49-2.28]), the military (OR=2.36 [1.86-3.00]), the professionals (OR=3.37 [2.72-4.19]) and then the tourists (OR=3.90 [3.27-4.66]) were more likely to develop severe malaria than the migrants visiting their countries.

- The **frequency of travel** was significantly associated with severe malaria (p<0.001). Patients who declared having travelled more than one time in an endemic country during the last 12 months were more likely to develop severe malaria (OR=1.63 [1.34-1.97]) comparing to the residents or those who had travelled one time or less.

- The time **between return and symptoms** was associated with severe malaria (p<0.001). Patients who had their first symptoms before return had a higher proportion of severe cases than those who develop their first symptoms after return.
Among those who had their symptoms after return, patients who had them between 5 and 13 days developed more severe malaria than patients who had them just after return (0 to 4 days) or a long time after (> 13days).

-An increase in the **time between onset of symptoms and consultation** was significantly associated with an increased risk of severe malaria.

- The **time between onset of symptoms and diagnosis** was associated with severe malaria ($p<0.001$). A time to diagnosis from 3 to 12 days was associated to an increased risk of severe malaria. And there were no significant differences between patients who were diagnosed in the first 48 hours and those who were diagnosed above 12 days.

- The odd of having severe malaria increased gradually when **the level of hemoglobin** decreased between 120g/L to 50g/L.($p<0.001$)

- The **platelet count** was associated with the proportion of severe malaria ($p<0.001$). Comparing to patients who had normal platelet count > 150 G/L, patients who had a count between 51 and 150 had a higher risk (OR=2.08; [1.59-2.73]). And a level ≤50 G/L was associated with a very high odd ratio of developing severe malaria (OR=17.57 [13.26-23.29]).

- The **white blood cell** count was associated with the severity of malaria ($p<0.0001$). Comparing to patients who have a count < 4G/L, patients with a count between 4 and 10 G/L (OR=1.16 [0.95-1.33]) and with a count >10G/L (OR=4.58 [3.54-5.92]) had a higher risk of developing severe malaria.

- Patients who states they don't take any **chemoprophylaxis** had a significant higher risk to develop severe malaria (OR, 1.35 [1.19-1.55) ($p<0.001$).

- A high-level **parasitemia** (≥4% of parasitized red blood cells) was significantly ($p<0.001$) associated with a higher risk of severe malaria (OR=16.94 [14.59-19.67]).

- **Season** of diagnosis in France was significantly associated with severe malaria ($P<0.001$). Diagnosis in spring or in summer was associated with a lower risk of severe malaria compared to a diagnosis in winter. No significant difference was observed between winter and fall (OR=1.03 [0.87-1.22]).

- Patients who were itinerant were the most likely to develop severe forms of malaria. However, the place of residence was not significantly associated with the severe malaria ($p= 0.85$).

- Having a previous medical consultation was not associated with severity neither ($p=0.36$).

In conclusion, the variables “place of residence” and “previous consultation” won’t be kept in the multivariate analysis.

As the variables “Frequency of travel” and “Time between symptom and consultation” had more than 50% of missing values, we didn’t keep them in the multivariate analysis neither.

Parasitemia and platelet counts (via the criteria “disseminated intravascular coagulation”) were variables, which were part of the definition of severe malaria. We therefore decided not to include them in the multivariate analysis to limit bias.

The others factors, associated with an increased risk of severe malaria in univariate analysis, were tested in the multivariate analysis. For the variable ”Season”, we also created only two group by grouping “Winter and Fall together”, and “Spring and Summer”, as the odds ratio were closer.

d) Multivariate analysis

After controlling for all the variables in a multivariate model (Annex 7, Table 5), the parameters “Region of malaria acquisition”, “Duration of stay” and “Season of diagnosis” were no longer predictive of severity. The variable “Place of birth”, correlated with others parameters was excluded from the model.

Many factors were still associated with an increase risk of severe malaria:

There is a weak association between **sex** and severity (p=0.004). Men seem more likely to develop severe malaria (OR=1.18 [1.06-1.33]).

Increasing **age** was associated with an increase risk of severe malaria (p<0.001). The odds ratio of having severe malaria compared to the 15-30 year olds were: 1.33 [1.09-1.63] for the 31-45 year olds, 1.86 [1.51-2.29] for the 46-60 year olds. The most risky class was the >60 years with an odd ratio of 2.77 [2.11-3.64].

Caucasians are significantly more likely to develop severe malaria comparing to Africans. (OR=1.93 [1.22-3.04]).

The **objective purpose** of travel was significantly associated with severe malaria (p=0.005). Comparing to the migrants visiting their countries, the military are more likely to develop

severe malaria (OR=1.97 [1.33-2.92]). The other groups have an odd ratio above one but not significantly different from the migrants.

The odd of severe malaria was significantly (p=0.04) higher when **chemoprophylaxis** was absent (OR=1.20 [1.01-1.43]) compared to those who declared taking chemoprophylaxis.

Patients presenting **onset** before **return** was associated with an increased risk of severe malaria (OR=1.60 [1.24-2.06]). The risk was not significantly different between categories when the symptom onsets were after return.

Time to diagnosis of 3 to 12 days after symptoms was associated with an increased risk of severe malaria (p<0.001) compared to a diagnosis in the first 48hours. However, after 12 days, the risk of having severe malaria decreased with an odd ratio only of 1.55 [1.04-2.31].

The odd of having severe malaria increased gradually when the level of **hemoglobin** decreased (p<0.001). And patients presented severe anemia (<80g/L) had a very high odd (OR=10.53 [7.59-14.63]) of developing severe malaria.

A **white blood cell** count above 10G/L was significantly (p<0.001) associated with an increased risk of severe malaria (OR=4.42 [3.13-6.24]).

V) **Discussion**

The aim of this study was to identify risk factors for severe imported *P. falciparum* malaria in metropolitan France. The objective was also to describe the evolution of severe imported malaria between 2000 and 2009. To our knowledge, the present study, which included 20,921 patients was one of the largest study conducted in Europe about imported malaria.

France has a large number of migrants of African origin, who are at risk of acquiring malaria when visiting friends and relatives. Transmission of malaria is more efficient in sub Saharan Africa than in other endemic areas. This factor explains why France, in comparison with many other European countries or the United States has so many malaria cases and why the country received high numbers of malaria-infected returning travelers from Africa, rather than from Asia or South America (15, 21)**.**

Despite the fact that the number of travelers to endemic areas has gradually increased, the number of imported cases has decreased since 2000 (Annex 8, Figure 3). This discordance can be due to better prevention among travelers or because of a change in travelers habits who are now going more frequently to areas where the transmission risk is lower *(CNR Paludisme Report 2009).*

However, the proportion of severe imported *P. falciparum* malaria has increased. Indeed between 2000 and 2009, the percent of severe cases has increased from 3.1% to 10.7%. Among severe cases, the distribution of endemic countries didn't change significantly and the delay between onset of symptoms and diagnosis stayed stable between 7 and 12 days. These variables can not therefore explain the increase of severe cases. However, we observed an increase in the proportion of African patients among severe cases, which becomes higher than the proportion of Caucasians in 2008. The proportion increased from 18% in 2000 to 49% in 2009. In addition, the proportion of patients declaring taking antimalarial prophylaxis decreased from 36.8% to 25.8% during the study period.
One explanation of these findings is that Africans are less likely to take antimalarial prophylaxis, first because they are less likely to have pretravel encounters with a health care provider and because they are less able to pay for prophylaxis drug (22)**.** A more efficient therapy atovaquone-proguanil (Malarone®) was recently available for prophylaxis (since 2001). Its main advantage is that it can be stopped 7 days only after the return, compared to 30 days for concurrent drugs. It seems more acceptable for travelers and may explain why there is a better compliance to treatment. At the opposite, its main disadvantage is its

expensive cost, 80 to 100 € for 2 weeks of stay in endemic area for a single person. So we can suppose that Caucasians had more access to this expensive drugs than Africans, That may probably explain, for a part, the changes we recently observe in the metropolitan France imported malaria population.
We also noticed an increase in the proportion of patients over 60 years in the severe cases. A global increase of age in travelers may also partly explain the progressive increase of the proportion of severe cases we observed during the study period.

According to our multivariate analysis, the following characteristics were independently associated with severe malaria: Caucasian origin, older age, chemoprophylaxis, sex, time between onset of symptoms and diagnosis, low level of hemoglobin, and hyperleukocytosis.

Severe malaria was particularly frequent among Caucasian patients, as previously reported in smaller series (5, 8-10).These results are consistent with the hypothesis of persistent acquired immunity, even after several years in a non-endemic area, which may be partly protect African immigrants from severe malaria (5, 8). A recent study has suggested that this immunity to disease may be acquired after relative short-time exposure(5). Our results showed that Africans, supposed immune or semi-immune have significantly lower parasite densities than Caucasians, supposed to be naïve individuals. However, there is no marker that has reliably been shown to correlate with immunity to malaria, and precisely what contributes immunity to malaria is still much debated. It's also difficult to obtain complete information about the duration and number of stays in endemic countries. Differences in genetic background may also explain the difference in malaria disease expression between Africans and Caucasians. Genetic factors, selected at the population level over centuries of exposure to the parasite, may partly explain the relative protection of Africans from severe illness(23, 24). Lewis et al (9) suggested that there may be genetic advantages in this ethnic group, by reporting that Africans not taking chemoprophylaxis had significantly less risk of severe malaria than Europeans, despite being resident in the United Kingdom for several years and thus having lost some of their acquired immunity to malaria.

The results of the present study confirm that the risks of severe disease due to *P. falciparum* increase with age, as has already been reported (10-14).We found a gradual increase in risk over the entire age spectrum and a particularly high risk among elderly patients above 60 years old (12, 15). This gradual increase with age, especially in non-immune populations (12, 13), reflects age-dependant innate protective mechanisms, which are independent of acquired immunity. This innate protection has an "inverted" profile; naïve younger patients

have a smaller risk of severe disease than naïve adults. One hypothesis is that older patients may have less efficient parasite clearance mechanisms in the spleen and less efficient clearance of non-infected RBC than do younger patients.
As discussed by Dondorp et al.(2005) (25), the total parasite biomass was associated with disease severity and with other markers of severity. In our study, we also detected a positive association between age and parasitemia; elderly patients have significantly higher rate of parasitemia compared to youngest patients. These findings suggest that the level of parasitemia could be a mediator in the relation between age and severity of the disease.
In addition to the innate protection, which decreases with age, another factor may explain the huge increase of severe cases in patients over 60 years old. Indeed, elderly patients have a more-fragile health state and have in general an increased susceptibility to many infectious diseases, mostly due to comorbidity (diabete, renal failure…) (26, 27).

The risk for severe malaria was higher when chemoprophylaxis was absent. These results are consistent with previous studies reported that patients who had taken prophylaxis are less likely to develop severe forms than those who had not taken any prophylaxis (9,11). However, our study relied on the patients account of the use of chemoprophylaxis and thus may have underestimated the full effects antimalarials could have in a fully compliant population. These results underline the importance of recommending antimalarial prophylaxis for travelers to malaria endemic areas.

In our study, male gender was associated with an increased risk of severe malaria. However, this result must be interpreted with caution. Indeed, the odd ratio was low (1.18 [1.08-1.33]) and didn't reflect an important difference of risk between male and female. In literature, gender as a risk factor has conflicting results, Swenson et al (28) reported that the risk for disease severity was higher in female patient, and Sabatinelli et al (29) reported that female patients were at higher risk for mortality; however, many other studies (5, 13, 14) were unable to show that gender was a risk factor.

The military seems to be a population at particular risk of severe malaria. Although we have no data concerning their conditions of stay, we can hypothesize that they may work or live in places where the contamination is high. In addition, although most of the military had access to chemoprophylaxis (70% declared taking it), only 40% of them declared taking it regularly, so we can assume that the compliance to chemoprophylaxis for this population group should be increased.

Having the first symptoms before returning to France was associated with an increased risk of severe malaria. This result can easily be explained by the fact that many patients presenting symptoms before the return to France were patients repatriated in France because of the severity of their symptoms, and we therefore had a population with an important number of severe cases in this group.

Decreased levels of hemoglobin were associated with increased risks of severe malaria. Anemia is caused by the destruction of red blood cells parasitized. As described previously, the parasite load is associated with malaria severity(25). And anemia is an intermediate marker, which reflects the intensity of the parasite load. Therefore, a severe anemia (<80g/L) reflects an important parasite density and is associated with higher risks of severe malaria.

High white blood cell counts are associated with higher risk of severe malaria and the effect was particularly marked at counts >10G/L. Hyperleukocytosis is a sign of bacterial infection, which were probably more frequent in our patients with severe malaria and partly could be explain this association between leukocytosis and severity.

Time to diagnosis of 4 to 12 days was associated with an increased risk of severe malaria, which is consistent with what has previously been reported (11, 15).These observations are probably linked to the dynamic of parasite densities in blood(25). There is an first fast-activating but short-active innate response, which controls parasite density in the first days. There is then a slower-activating but long acting antibody like response, which explains that above 12 days parasitemia is controlled (30, 31). A long duration to diagnosis reflects benign and controlled malaria.

Our study presents some limitations:
First, our statistics did not capture cases and deaths declared out of hospital, and the network for collecting data accounted for only 50%-55% of total malaria cases imported to France. (Annexe8, Figure 3) However, exhaustive studies (18) suggest that the representativeness of the correspondent sites was correct. Thus it appears unlikely that risk factors associated with severity differ for cases not seen in our study.
Concerns about the quality of data from this analysis remain. Although quality has improved greatly in recent years, the data need careful interpretation. Indeed, two data tables were used to design the study population and the difference in the format of certain variables can lead to misclassification.
A further problem is that our statistics cannot distinguish the simple cases, that turn into severe form later. This may lead to misclassification and induced underestimation of severe cases. In addition, the statistics didn't record all comorbidities and underlying health

conditions of the patients, which may be confounding factors of the study. Some factors were also not introduced in the multivariate analysis because of a large amount of missing data. Other possible biases may include: response bias for the variable “Use of chemoprophylaxis” because patients may not have declared their real use of drugs; interviewer bias, for variables such as “Ethnic group” which depend on the judgement of the person who collects the data; or memory bias, particularly for some variables such as “Duration of stay”, “Delay for symptoms”, or “Delay for diagnosis”.

VI)**Conclusion:**

As international travel continues to expand, malaria will gain more and more attention in the developed countries. And whereas the acquisition of *P. falciparum* malaria among travelers from endemic countries could never be completely avoided, our data suggest that severe complications can be prevented by a combination of factors. Given the large number of patients included in the study, our results can be interpreted with confidence.

It is first important to increase the compliance of antimalarial chemoprophylaxis; preventive measures remain necessary for all travelers, including African patients, for whom adherence is often poor and which represent an increased proportion of the patients with severe malaria.

These findings should also lead clinicians to increase vigilance on two populations particularly at risk for severe malaria: travelers above 60 years and patients with caucasians origins. Determining how these findings of variation in susceptibility could be translated into management guidelines, will probably require further research.

The finding that male sex was associated with a higher risk of severe malaria wasn’t shown before and trigger further research too.

Post travel care should also be reinforced to reduce the interval between onset of symptoms and diagnosis and greater physician awareness of the disease is needed in order to treat the patients when they show the first clinical and biological signs of severe malaria . Despite emerging drug resistance, imported drug malaria remains a treatable disease and the importance of early diagnosis and prompt therapy cannot be overemphasized.

VII) **References**

1. **WHO**. Malaria Report 2009.
2. **Freedman DO**. Clinical practice. Malaria prevention in short-term travelers. *N Engl J Med* 2008; 359:603-612.
3. **Kain KC, Shanks GD, Keystone JS**. Malaria chemoprophylaxis in the age of drug resistance. I. Currently recommended drug regimens. *Clin Infect Dis* 2001; 33:226-234.
4. **WHO ROfE**. Centralized Information System for Infectious Disease (CISID)/Malaria. 2007.
5. **Phillips A, Bassett P, Zeki S, et al.** Risk factors for severe disease in adults with falciparum malaria. *Clin Infect Dis* 2009; 48:871-878.
6. **Ben-Ami R, Siegman-Igra Y, Anis E, et al.** Malaria in travelers returning from short organized tours to holiday resorts in Mombassa, Kenya. *Isr Med Assoc J* 2005; 7:364-367.
7. **Kain KC, Harrington MA, Tennyson S, et al.** Imported malaria: prospective analysis of problems in diagnosis and management. *Clin Infect Dis* 1998; 27:142-149.
8. **Bouchaud O, Cot M, Kony S, et al.** Do African immigrants living in France have long-term malarial immunity? *Am J Trop Med Hyg* 2005; 72:21-25.
9. **Lewis SJ, Davidson RN, Ross EJ, et al.** Severity of imported falciparum malaria: effect of taking antimalarial prophylaxis. *Bmj* 1992; 305:741-743.
10. **Matteelli A, Colombini P, Gulletta M, et al.** Epidemiological features and case management practices of imported malaria in northern Italy 1991-1995. *Trop Med Int Health* 1999; 4:653-657.
11. **Jensenius M, Ronning EJ, Blystad H, et al.** Low frequency of complications in imported falciparum malaria: a review of 222 cases in south-eastern Norway. *Scand J Infect Dis* 1999; 31:73-78.
12. **Muhlberger N, Jelinek T, Behrens RH, et al.** Age as a risk factor for severe manifestations and fatal outcome of falciparum malaria in European patients: observations from TropNetEurop and SIMPID Surveillance Data. *Clin Infect Dis* 2003; 36:990-995.
13. **Schwartz E, Sadetzki S, Murad H, et al.** Age as a risk factor for severe Plasmodium falciparum malaria in nonimmune patients. *Clin Infect Dis* 2001; 33:1774-1777.
14. **Calleri G, Lipani F, Macor A, et al.** Severe and complicated Falciparum malaria in Italian travelers. *J Travel Med* 1998; 5:39-41.

15. **Legros F, Bouchaud O, Ancelle T, et al.** Risk factors for imported fatal Plasmodium falciparum malaria, France, 1996-2003. *Emerg Infect Dis* 2007; 13:883-888.
16. Severe *falciparum* malaria. World Health Organization, Communicable Diseases Cluster. *Trans R Soc Trop Med Hyg* 2000; 94 Suppl 1:S1-90.
17. **Legros F, Danis M**. Surveillance of malaria in European Union countries. *Euro Surveill* 1998; 3:45-47.
18. **Legros F**. Enquete national de recensement des cas de paludisme d'importation en France métropolitaine pour l'année 1997. *BEH* 1999.
19. **Dondorp AM, Lee SJ, Faiz MA, et al.** The relationship between age and the manifestations of and mortality associated with severe malaria. *Clin Infect Dis* 2008; 47:151-157.
20. **Reyburn H, Mbatia R, Drakeley C, et al.** Association of transmission intensity and age with clinical manifestations and case fatality of severe Plasmodium falciparum malaria. *Jama* 2005; 293:1461-1470.
21. **Freedman DO, Weld LH, Kozarsky PE, et al.** Spectrum of disease and relation to place of exposure among ill returned travelers. *N Engl J Med* 2006; 354:119-130.
22. **Leder K, Black J, O'Brien D, et al.** Malaria in travelers: a review of the GeoSentinel surveillance network. *Clin Infect Dis* 2004; 39:1104-1112.
23. **Modiano D, Luoni G, Sirima BS, et al.** Haemoglobin C protects against clinical Plasmodium falciparum malaria. *Nature* 2001; 414:305-308.
24. **Williams TN, Mwangi TW, Wambua S, et al.** Sickle cell trait and the risk of Plasmodium falciparum malaria and other childhood diseases. *J Infect Dis* 2005; 192:178-186.
25. **Dondorp AM, Desakorn V, Pongtavornpinyo W, et al.** Estimation of the total parasite biomass in acute falciparum malaria from plasma PfHRP2. *PLoS Med* 2005; 2:e204.
26. **Yoshikawa TT**. Epidemiology and unique aspects of aging and infectious diseases. *Clin Infect Dis* 2000; 30:931-933.
27. **Castle SC**. Clinical relevance of age-related immune dysfunction. *Clin Infect Dis* 2000; 31:578-585.
28. **Svenson JE, MacLean JD, Gyorkos TW, et al.** Imported malaria. Clinical presentation and examination of symptomatic travelers. *Arch Intern Med* 1995; 155:861-868.
29. **Sabatinelli G, Majori G, D'Ancona F, et al.** Malaria epidemiological trends in Italy. *Eur J Epidemiol* 1994; 10:399-403.

30. **Kochin BF, Yates AJ, de Roode JC, et al.** On the control of acute rodent malaria infections by innate immunity. *PLoS One*; 5:e10444.
31. **McQueen PG, McKenzie FE**. Host control of malaria infections: constraints on immune and erythropoeitic response kinetics. *PLoS Comput Biol* 2008; 4:e1000149.

VIII) **Annexes**

ANNEX 1

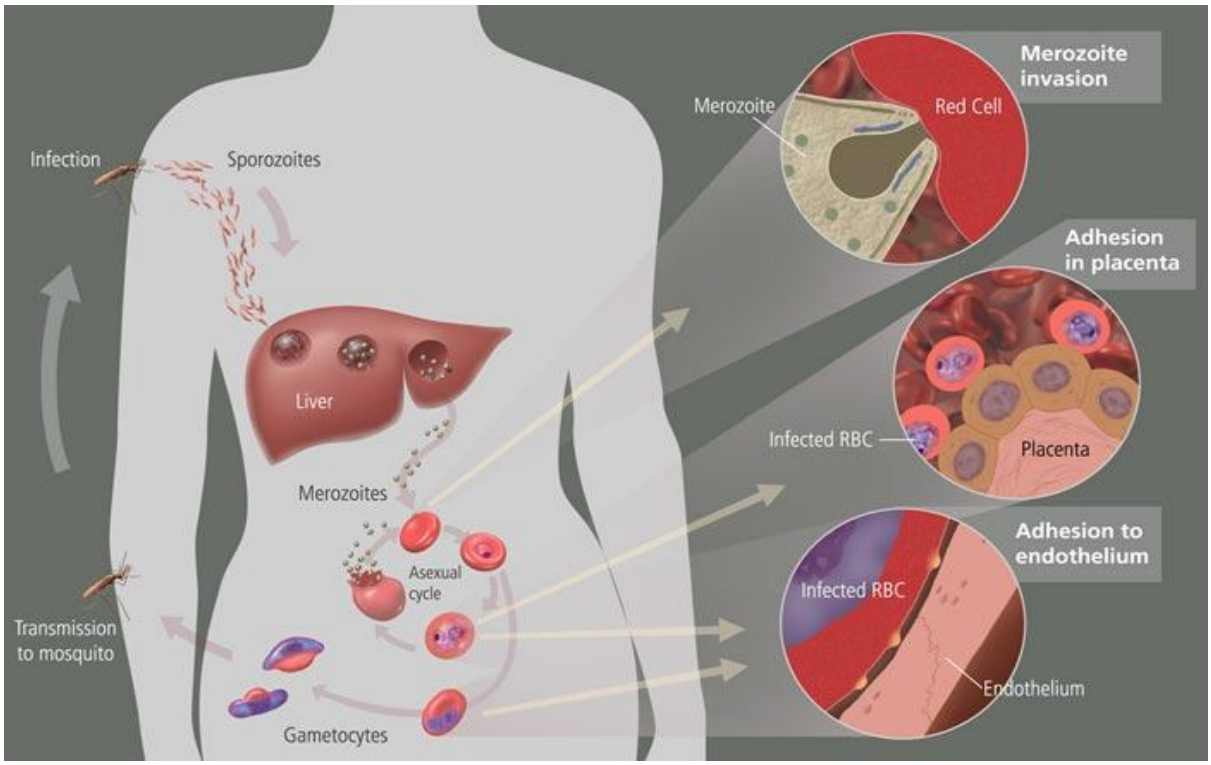


***Figure 1*: The life cycle of malaria parasites in the human body.**

(from http://history.nih.gov/exhibits/bowman/SSmalaria.htm)

A mosquito infects a person by taking a blood meal. First, sporozoites enter the bloodstream, and migrate to the liver. They infect liver cells (hepatocytes), where they multiply into merozoites, rupture the liver cells, and escape back into the bloodstream. Then, the merozoites infect red blood cells, where they develop into ring forms, then trophozoites (a feeding stage), then schizonts (a reproduction stage), then back into merozoites. Sexual forms called gametocytes are also produced, which, if taken up by a mosquito, will infect the insect and continue the life cycle.

ANNEX 2:

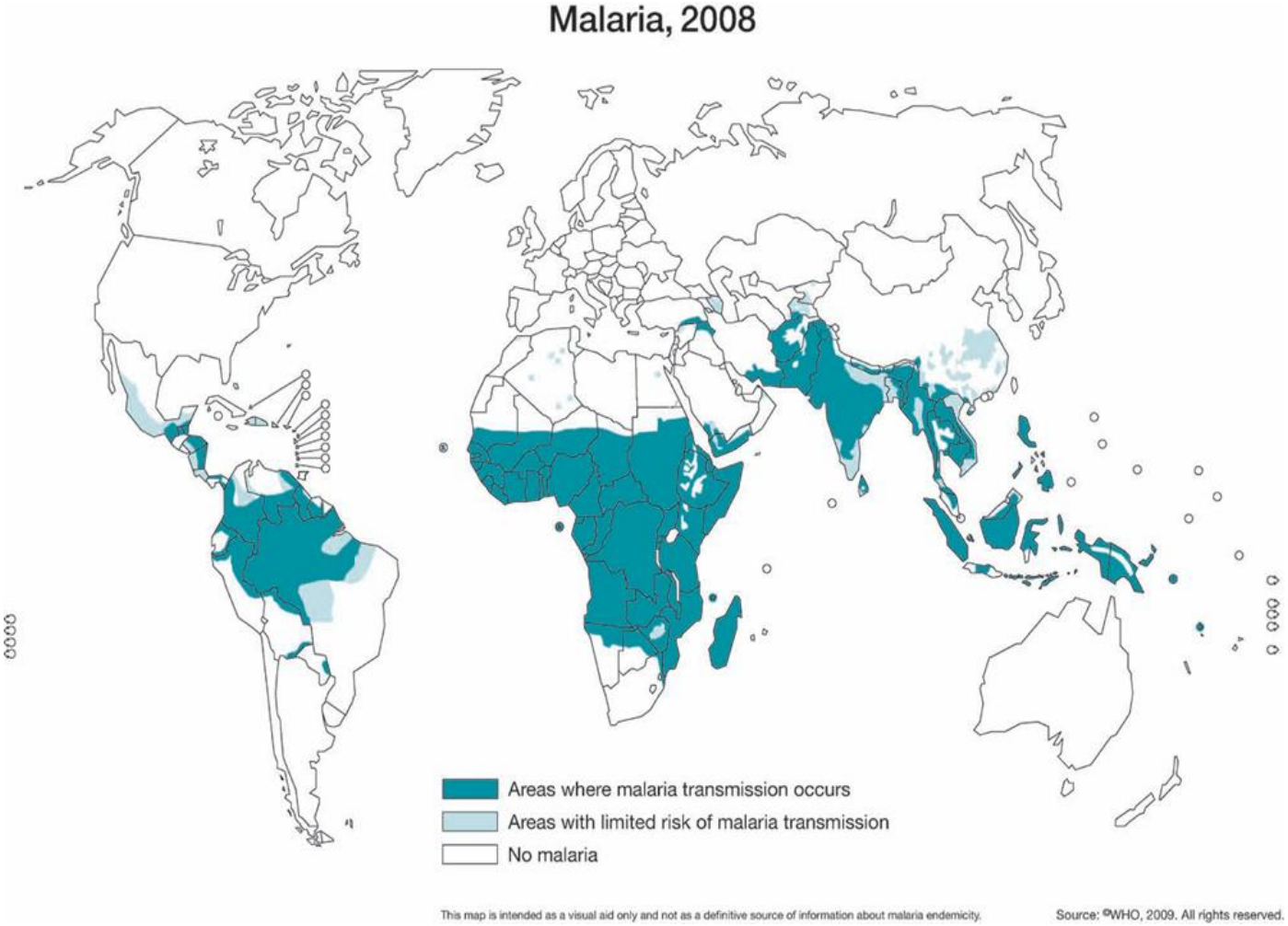


***Figure 2*: Malaria endemic area in 2008** *(from WHO malaria report 2009)*

ANNEX 3:

Table 1: Distribution of mild and severe P. falciparum malaria cases by calendar year,2000-2009, France (N=19,875)

Year	Number of cases	Number of Mild cases	% Mild cases	Number of Severe cases	Case severity rate (%)	Number of deaths	Case fatality rate (%)
2000	2464	2388	96.9	76	3.1	11	0.4
2001	2289	2203	96.2	86	3.8	13	0.6
2002	2420	2306	95.3	114	4.7	15	0.6
2003	2358	2244	95.2	114	4.8	19	0.8
2004	2254	2147	95.3	107	4.7	11	0.5
2005	1737	1636	94.2	101	5.8	7	0.4
2006	1869	1751	93.7	118	6.3	9	0.5
2007	1431	1325	92.6	106	7.4	6	0.4
2008	1533	1415	92.3	118	7.7	8	0.5
2009	1520	1357	89.3	163	10.7	7	0.5
Total	19875	18772	94.1	1103	5.9	106	0.5

ANNEX 4:

<u>Table 2:</u> Distribution of P. falciparum malaria cases by country of acquisition, 2000-2009

	Number of cases	Number of mild cases	No of severe cases	Number Death	Severity rate (%)
Ivory Coast	4501	4301	200	15	4.4
Cameroon	2998	2856	142	12	4.7
Mali	1989	1890	99	11	5.0
Senegal	1857	1736	121	18	6.5
Comoros	1486	1444	42	1	2.8
Burkina-Faso	1079	1001	78	5	7.2
Congo	747	712	35	3	4.7
Guinea	727	688	39	4	5.4
Benin	705	655	50	4	7.1
Central African Republic	557	538	19	2	3.4
Togo	512	467	45	6	8.8
Gabon	473	450	23	5	4.9
Madagascar	353	298	55	6	15.6
Democratic Republic of C	271	259	12	1	4.4
Ghana	193	183	10	2	5.2
Nigeria	154	137	17	1	11.0
Niger	145	132	13	1	9.0
Tchad	134	122	12	1	9.0
French-Guyana	125	120	5		4.0
Mauritania	80	72	8	1	10.0
Kenya	63	53	10	1	15.9
Others	722	654	68	6	9.4

ANNEX 5:

***Table 3*: Clinical criteria**

Criteria	Total Number	Positive	%
Impaired consciousness	1002	423	**42.2**
Coma	1000	53	**5.3**
Multiple convulsions	999	24	**2.4**
Circulatory collapse	998	67	**6.7**
Abnormal bleeding	998	67	**6.7**
Respiratory distress	1000	118	**11.8**
Acidemia or Acidosis	996	74	**7.4**
Haemoglobinuria	999	68	**6.8**
Jaundice	1000	338	**33.8**
Hyperlactatemia	231	36	**15.6**

***Table 4:* Biological criteria**

Criteria	Total Number	positive	%
Parasitemiae >= 4%	1023	626	**61.2%**
Creatinemiae >265 µmol/L	769	255	**33.2%**
Glycemia >2.2 mmol/L	769	20	**2.6%**
Bilirubin >50 µmol/L	769	286	**37.2%**
Hyperlactatemia	231	36	**15,6**

The median value of temperature at the admission was 38.8 °C (IQR [37.8-39.55]).

	N	P25	P50	P75	Normal values
ALAT(IU/L)	266	34	64.5	106	8-35
ASAT(IU/L)	267	51	79	143	8-30
PH	137	7.37	7.43	7.46	7
Hco3-(mmol/L)	90	16.6	20.6	24.2	20-30
Blood pressure(cmHg)	431	9.5	11	12.5	9-14

ANNEX 6 :

<u>Table 5:</u> Univariate analysis. Factors associated with severe malaria among patients treated for P. falciparum malaria in French hospitals, 2000-2009 (N=20,921)

Variables		No. Of cases	No of severe cases(%)	No of mild cases(%)	OR	95% CI	p
Total			1,103	18,772			
No of death		109	102	7			
Age (in years)							
	15-30	7,040	256 (23.21%)	6,784 (36.14%)	1		
	31-45	7,488	327 (29.65%)	7,161 (38.15%)	1.2	1.02-1.43	
	46-60	4,298	371 (33.64%)	3,927 (20.92%)	2.50	2.12-2.95	**<0.001**
	>60	1,049	149 (13.51 %)	900 (4.79%)	4.39	3.53-5.44	
Gender							
	Female	6,980	327 (29.7%)	6653 (35.5%)	1		
	Male	12,854	774 (70.3%)	12,080 (64.5%)	1.30	1.14-1.49	**0,001**
Birth country							
	Endemic-country	9,883	338 (35.2%)	9,545(59.9%)	1		
	Non-endemic country	7,004	622(64.8%)	6,382 (40.1%)	2.75	2.40-3.16	**<0,001**
Ethnic group							
	Africans	12,194	396 (39.2%)	11,798 (69.3%)	1		
	Caucasians	5,480	569 (56.3%)	4,911 (28.9%)	3.45	3.02-3.95	**<0,001**
	Others	359	45 (4.5%)	314 (1.84%)	4.27	3.07-5.93	
Region of malaria acquisition							
	West Africa	12,038	69 (62.6%)	11,348 (60.5%)	1		
	East Africa	185	23 (2.09%)	162 (0.9%)	2.33	1.50-3.64	**<0.001**
	Austral Africa and Indian Ocean Islands	1,961	108 (9.8%)	1,853 (9.9%)	0.96	0.79-1.18	
	Central Africa	5,239	248 (22.5)	4,991 (26.6)	0.82	0.70-0.95	
	Asia	126	15 (1.4)%)	111 (0.6%)	2.22	1.29-3.83	
	Others	321	19 (1.72%)	302 (1.61%)	1.03	0.65-1.66	
Duration of stay (in days)							
	0-20	3,406	264 (34.7%)	3,142 (26.1%)	1		
	21-31	3,504	181 (23.78%)	3,323 (27.6%)	0.65	0.53-0.79	**<0.001**
	32-61	2,930	136 (17.87%)	2,794 (23.2%)	0.60	0.47-0.72	
	>61	2,948	180 (23.65%)	2,768 (23.0%)	0.77	0.64-0.94	

Objectives of travel							
	Migrants back to the country of origin	9,467	284 (29.6%)	9,183 (55.2%)	1		
	Residents or expatriates ≥ 6 months	2,449	132(13.8%)	2,317 (13.9%)	1.84	1.49-2.28	
	Military	1,395	95 (9.91%)	1,300 (7.82%)	2.36	1.86-3.00	**<0.001**
	Professionals/ business	1,387	131 (13.7%)	1,256 (7.6%)	3.37	2.72-4.19	
	Tourists	2,423	261(27.2%)	2,162 (13.0%)	3.90	3.27-4.66	
	Others	90	56 (5.84%)	396 (2.38%)	4.57	3.37-6.20	
Frequency of travel							
	1 per year	4,982	268 (43.5%)	4,714 (50.4%)	1		
	>1 per year	2,316	196 (31.8%)	2,120 (22.7%)	1.63	1.34-1.97	**<0.001**
	resident	2,674	152 (24.7%)	2,522 (27%)	1.06	0.86-1.30	
Place of residence							
	Rural	1,831	117(19.5%)	1,714 (19.5%)	1		
	Urban	2,341	144(24%)	2,197 (24.9%)	0.96	0.75-1.23	0.85
	Mixed	5,236	340 (56.7%)	4,896 (55.6%)	1.02	0.82-1.26	
Time between return and onset (in days)							
	<0	2,065	167 (17.1%)	1,898 (12%)	1		
	0-4	5,796	293(30.0%)	5,503(34.9%)	0.61	0.50-0.73	**<0.001**
	5- 8	3,118	191(19.6%)	2,927 (18.6%)	0.74	0.60-0.92	
	9-13	2,823	179(18.3%)	2,644 (16.76%)	0.77	0.62-0.96	
	>13	2,950	146 (15%)	2,804 (17.8%)	0.59	0.47-0.74	
Time between onset and diagnosis (in days)							
	0-2	7,116	268 (26,5%)	6,848 (41,4%)	1		
	3-4	4,217	278(27,5%)	4,217 (25,5%)	1.68	1.42-2.0	**<0.001**
	4-6	1,998	207(20.47%)	1,998 (12,08%)	2.65	2.19-3.20	
	6-12	1,904	180 (17.80%)	1,904 (11,51%)	2.42	1.98-2.94	
	>12	1,569	78 (7,72%)	1,569 (9,49%)	1.27	0.98-1.64	
Hemoglobin (g/L)							
	<50	31	14 (1.45%)	17 (0,1%)	16.83	8.21-34.50	
	50-79	412	95 (9.9%)	317 (2.24%)	6.12	4.76-7.87	**<0.001**
	80-100	45	8 (0.8%)	37 (0,3%)	4.42	2.04-9.55	

	101-120	4,987	397 (41.2%)	4,590 (32,42%)	1.77	1.54-2.03	
	>120	9,645	450 (46.7%)	9,195 (64,95%)	1		
Platelet count (G/L)							
	>150	3,667	589 (60.2%)	1,887 (13,2%)	1		
	51-150	9,141	326 (33.3%)	8,815 (61,6%)	2.08	1.59-2.73	**<0.001**
	<= 50	2,476	64 (6.5%)	3,603 (25,2%)	17.57	13.26-23.29	
White blood cell count							
	<4	3,693	190 (20.8%)	3,503 (25.2%)	1		
	4 10	10,569	608 (66.7%)	9,961 (71.5%)	1.16	0.95-1.33	**<0.001**
	>10	573	114 (12.5%)	459 (3.3%)	4.58	3.54-5.92	
Chemoprophylaxis							
	Yes	7,437	352 (33,6%)	7,085 (40,7%)	1		
	No	11,025	697 (66,4%)	10,328 (59,3%)	1.35	1.19-1.55	**<0.001**
Parasitemia (percent)							
	< 4	14,913	397(38.8%)	14,516 (91.4%)	1		
	>=4	1,977	626 (61.2%)	1,351 (8,5%)	16.94	14.59-19.67	**<0.001**
Season of diagnosis in France							
	Winter	3,741	245 (22.3%)	3,496 (18.5%)	1		
	Spring	3,413	176 (16%)	3,237 (17.3%)	0.78	0.64-0.95	**<0.001**
	Summer	7,624	340 (30.9%)	7,284 (38.9%)	0.67	0.56-0.79	
	Fall	5,045	340 (30.9%)	4,705 (25.1%)	1.03	0.87-1.22	
Previous consultation							
	Yes	10,407	614(61.5%)	9,793(60.51%)	1		
	No	6,776	385(38.5%)	6,391(39.5%)	0.96	0.84-1.1	0.36
Time between onset and consultation							
	0-2	6,185	414(45.5%)	5,771(51.5%)	1		
	3-4	2,813	228(25.1%)	2,585(23.1%)	1.23	1.04-1.45	**<0.001**
	5-7	1,675	166(18.2%)	1,509(13.5%)	1.53	1.27-1.85	

ANNEX 7

Table 6: Multivariate analysis. Factors independently associated with severe malaria among patients treated for P. falciparum malaria in French hospitals, 2000-2009 (N=20,921)

Variables	Odds Ratio	[95% IC]	P>z
Sex			
Female	1.00		**0.004**
Male	1.18	1.06 1.33	
Age			
15-30	1.00		**<0.001**
31-45	1.33	1.09 1.63	
46-60	1.86	1.51 2.29	
>60	2.77	2.11 3.64	
Ethnie			
African	1.00		**<0.001**
Caucasian	1.93	1.22 3.04	
Others	1.74	1.33 2.29	
Region of malaria acquisition			
West Africa	1.00		0.57
Central Africa	0.91	0.76 1.09	
East Africa	1.33	0.74 2.40	
Austral Africa and Indian Ocean Islands	1.08	0.83 1.41	
Asia	0.90	0.44 1.84	
Others	0.71	0.39 1.29	
Duration of stay			
0-20	1.00		0.72
21-31	0.87	0.68 1.11	
32-61	0.91	0.69 1.19	
>61	0.93	0.71 1.21	
Objectives of travel			
Migrants back to the country of origin	1.00		**0.005**
Residents or expatriates >= 6 months	1.23	0.90 1.68	
Military	1.97	1.33 2.92	
Professionnals/ business	1.37	0.97 1.94	
Tourism	1.36	0.98 1.88	
Others	2.08	1.34 3.24	

Chemoprophylaxis				
Yes	1.00			**0.04**
No	1.20	1.01	1.43	
Season of diagnosis in France				
Winter/fall	1.00			0.79
Spring/summer	0.98	0.84	1.14	
Time between return and onset (in days)				
<0	1.60	1.24	2.06	
0-4	1.00			
5 8	1.08	0.86	1.36	**0.002**
9 13	1.15	0.91	1.46	
>13	0.91	0.70	1.17	
Time between onset and diagnosis (in days)				
0-2	1.00			
3 4	1.44	1.10	1.88	
4 6	1.86	1.39	2.50	**<0.001**
6 12	2.40	1.77	3.27	
>12	1.55	1.04	2.31	
Platelet count (G/L)				
>150	1.00			
51-150	2.20	1.62	2.98	**<0.001**
<= 50	8.27	6.04	11.33	
White blood cell count (G/L)				
<4	1.00			
4 10	1.54	1.25	1.89	**<0.001**
>10	4.42	3.13	6.24	

ANNEX 8:

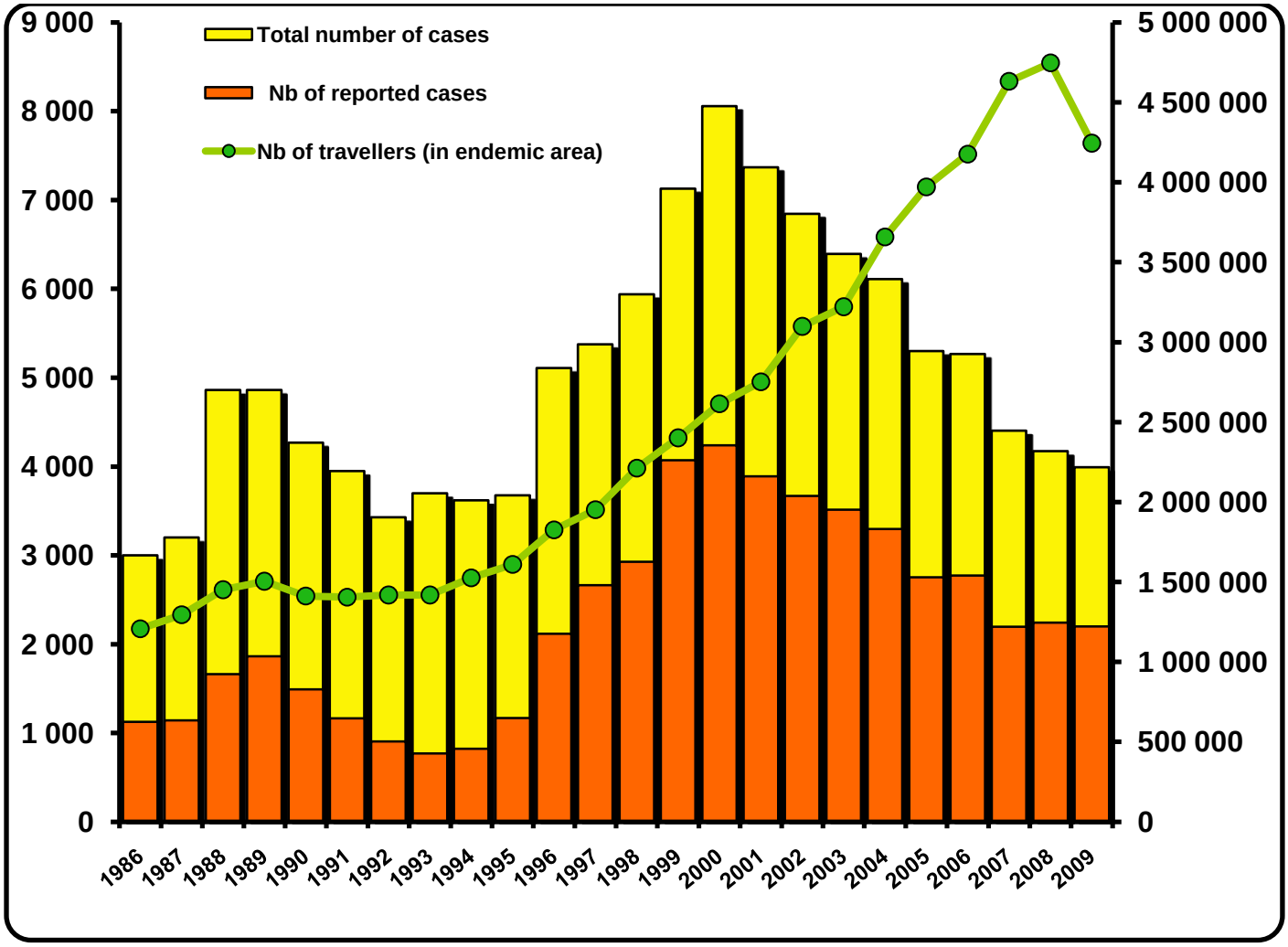


Figure 3: ***Evolution of the number of travelers , the total number of cases, and the number of reported cases in metropolitan France (from CNR Paludisme report 2009)***