First Detection of Malaria in Dry Season in Dakar Senegal

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Introduction: With 225 million people ill and 781,000 deaths in 2009, malaria remains the most important parasitic disease in the world.80% of cases occur in sub-Saharan Africa. Senegal, which is located in the high-risk area, devotes a lot of resources in the fight against this disease. This is in such context that we have carried out this retrospective study of malaria cases at ABASS NDAO Hospital (CHAN).
Materials and Methods: This study, involving 486 patients, was conducted from January 2011 to June2011. Data on the sex of the patients were recorded. Screening methods used are thick film, blood smear and Rapid Diagnostic Test (RDT).
Results: A total of 486 subjects were tested for malaria diagnosis and 33 patients were identified, only one case is due to the *Plasmodium malariae*, others are caused by the *Plasmodium falciparum*. The hospital prevalence of malaria according to this study is 6.79% (33/486). A significant monthly variation was observed for the number of cases: 17 cases in January, 4 cases in March, 1 case in April,1 in May and none in June. Both men and women are affected by malaria, but with a number of cases slightly higher among men: 19 malaria cases against 14 cases for

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women.

Conclusion: During period of low transmission (January-June) the prevalence of malaria in Dakar is high, caused by the *Plasmodium falciparum*. More effort should be made in the fight against this epidemic in urban and suburban areas.

1. INTRODUCTION

Malaria is a disease caused by a parasite of the *Plasmodium* kind (*Phylum apicomplexa*, class of Coccidia, *Haemosporida* order, *Plasmodidae* family). The parasite is transmitted through the bite of a female Anopheles mosquito, the latter is infected after biting a warm-blooded animal [1]. The life cycle of Plasmodium is quite complex and requires two hosts: a final host (a man) in whom the parasite multiplies by schizogony, and a primary host (the female anopheles hematophagous) in which the parasite sexually reproduces. Five species of Plasmodium are involved in human pathology: *P. falciparum* responsible for the most severe form of malaria with the majority of deaths, *P. ovale, P. vivax, P. malariae* and *P. knowlesi* that cause typical "benign" forms. Each year, malaria causes 400 to 900 million cases of fevers, and between one and three million deaths [2], on average one death every 30 seconds. The vast majority of victims are children under 5 years [3], pregnant women are also particularly vulnerable because the placenta is a target where the *P. falciparum* parasites can accumulate [3].

Efforts have been undertaken to reduce the transmission of the disease and improve treatment. There has been little change in the 1990s [4]. But in recent years a decline in malaria cases was observed with 225 million patients and 781,000 deaths in 2009 [1]. Malaria remains despite this reduction the most important parasitic disease in the world. Indeed, it is endemic in the tropics, in the America, in many parts of Asia, and Africa. However, it is in sub-Saharan Africa that 85 to 90% of deaths [1] are recorded. Accurate statistics are difficult to obtain, due to the prevalence of the disease in rural areas, where people do not always have access to a hospital, or do not have the means to get a treatment. Most cases are not documented [2].

Senegal, which is located in the sub-Saharan zone (high risk) dedicates most resources in the fight against the disease through its National Programme for the Fight against Malaria (PNLP), among others with the distribution of 6 million treated mosquito nets between 2005 and 2010, making available free intermittent preventive treatment during pregnancy in 14 regions, the distribution of one million Rapid Diagnostic Tests (RDT) and 1.5 million ACT (Artemisinin -based combination therapy) since 2007 [5]. This high coverage has reduced the burden of disease and precisely saved lives. Indeed, the number of confirmed malaria cases was reduced by 41% in one year, from nearly 300,000 in 2008, to 175,000 in 2009. Dramatically, the number of malaria cases in children under 5 decreased from 400,000 of suspicious cases in 2006 to 78,000 confirmed in 2008, then 30,000 confirmed cases in 2009.As a result, the mortality of children under 5, all causes were reduced by 30% between 2005 and 2008/2009. (It went from 121 deaths per 1,000 births in 2005 to 85 deaths per 1,000 births in 2008/2009). The mortality of children aged 1 to 4 years, went down by 48% [5].

Epidemiological variations are important from North (Sahelian and hypo-endemic) to South (Guinean Sudanese and hyper-endemic). These geographical variations explain in part the differences in prevalence of parasitaemia recorded in 2008 (Table 1) [5].

It is in this context that we proposed to perform a retrospective study to assess malaria cases detected in Medical Biology Laboratory of ABASS NDAO Hospital of Dakar during the period of low transmission, which extends from January to June 2011.Indeed; malaria in Senegal is defined as a steady endemic with seasonal peaks. The rainy season lasts from July to October. The peak of malaria, which is the consequence, is between July and December [5].

The aim is to determine the epidemiological data such as prevalence and incidence in this period of low transmission. Then the data will be compared with results provided by the WHO or reported by other studies. This will allow us to assess the prevalence and incidence of malaria during low transmission in Dakar.

2. MATERIALS AND METHODS

Sample: This retrospective study was about 486 patients who came to take malaria diagnosis at the Dakar CHAN from 1 January 2011 to 30 June 2011. The sample consisted of 227 males and 259 female subjects, 46.7% and 53.3% respectively.

Diagnostic methods: The methods used to detect the parasite are thick, blood smear and Rapid Diagnostic Test (RDT).

Thick and thin blood smear: For the thick film making is to deposit a large drop of capillary blood in the middle of a leaf or several small drops of venous blood slightly apart from each other , and with the tip of another blade , it is spread circularly while not exceeding 1 cm in diameter, this corresponds to the defibrination ; then allowed to dry the slide object in the open air by placing flat protected from dust, flies and cockroaches .Coloring occurs when the slide object are dried coating of water and the left in contact for 15 minutes corresponding to the deshemoglobinisation; then water is poured, then the plate dilution of Giemsa solution is coated (3 drops Giemsa for 2 ml water) and left in contact for 15 to 20 minutes following the slow or fast nature of the Giemsa solution. Finally rinsed with water until the water being drained is colorless and dried slide object vertically.

The dried leaf is given to the objective optical microscope at 100 x immersing a drop of paraffin oil and it will be observed granulocytes, platelets reddish mass and the presence or absence of trophozoites or gametocytes of Plasmodium as a ring set with a reddish core or as a banana with or without reddish core inside. And at this level identification will be based on morphological characters when you know that five (5) species are responsible for malaria that are: *Plasmodium falciparum, vivax, ovale, malariae and P. knowlesi*.

For Blood Smear (Thin Film): A small drop of blood is collected at 1 cm from the slide object and another slide object with softened edges dry and grease, we advance starting to the left of the slide object until the blood is in contacting therewith; it will fuser by capillarity and the slide object is tilted 45° and push slightly to the left not too strongly supported so as to spread the blood thin and uniform layer with a single stroke while

avoiding arriving at the opposite end. Then the slide object was dried by stirring in the open air.
Once dry smear, coated alcohol at 95° for 10 minutes: the fixation; then washed with water and stained with Giemsa (fast or slow), and finally rinsed with water and dried vertically. The dried leaves are recorded in the optical microscope at 100 x objective immersing a drop of paraffin oil. Note the presence or absence of trophozoites, gametocyte and schizonts inside RBCs Calculating parasite density in the case of a thick film was done by counting the number of parasite in 200 leukocytes multiplied by the total number of leukocytes in the blood. For our work, the interpretation is done on the basis of an average of 8,000 WBC per ul of blood leukocytes. In the case of blood smears, the number of parasitized erythrocytes is multiplied by the number of red blood cells given by the Complete Blood Count (CBC) and divided by 1000. It is expressed as the number of parasitized erythrocytes /mm3, and allows diagnosis.

Rapid Diagnostic Test (RDT): We also used the rapid tests for antigens. These are immune chromatographic tests (or Rapid Diagnostic Test (RDT)) which significantly contributes in the detection of malaria in hospital because of their high sensitivity and specificity [6]. The process is simple and has the advantage to overcome the microscope. Furthermore, a single drop of blood is sufficient and only lasts about ten minutes to confirm the diagnosis. Yet, the detection limit with these tests is in the range of 100 parasites per microlitre (µl) of blood against 5 by screening microscope. There are several TDR versions, but rely almost on the same principle [7]. We have used for this study the ICT Malaria® test or the ParaHIT® focused on HRP2 antigen specific for P. falciparum. The HRP2 test cassette contains round and square windows, a surface where lines appear C (control) and T Pf (test), a dryer, 25 handles collection parameterized 5 µl, 25 lancets, 25 alcohol swabs and a reagent bottle (buffer solution with dropper). The test is used at room temperature and opened just before use. So we proceed successively to the following operations: Mention code, the patient number and date. Then take 5 µl of capillary blood in the finger using the sampling loop, remove the 5 microliters of blood in the square window, vertically deposit 4 drops of the buffer solution in the round window, leave the test on the flat surface and then wait up to 15 minutes for the purpose of reading. It is negative if only one band appears on Line C. It is positive if there are two (2) bands appearing on lines C and T

(*Pf*). However, the test is invalid if no band appears at the end of 15 minutes and in this case we use the same technique with a new test.

Ethical Clearance: The principal investigator were made brief explanation for all study population about the study protocol and after that written informed consents were obtained from all study population. By using codes confidentiality was maintained. The study proposal ethically approved by Ethical clearance committee from Biology   Laboratories of Cheikh Anta Diop University of Dakar.

3. RESULTS

3.1 Monthly Variation of Malaria

The number of malaria patients, 17 cases in January (3,50 %) , decreases from 10 cases in February (2,06%), 4 in March (8,23%), one in April (0,21%), one in May (0,21%) and none in June (Table 2, Fig. 1). There is thus a clear and rapid decline of malaria cases from 17 cases in January, 1 case in April, and zero cases in June despite the high number of patients gradually

who have been tested for malaria: 84 patients in January, 97 in May and 107 patients in June (Table 2).

3.2 Cases of Malaria by Plasmodium Species

Out of 486 patients as a whole who were submitted to a diagnostic test in our study, 33 positive cases(6,79%) were detected, 32 cases are cases of *P. falciparum* (6,58%) and only one *P. malariae (0,21*%) was identified.

3.3 Malaria Cases by Sex

The number of women who have undergone the test were higher than that of men: 259 versus 227 (Table 3 and Figs. 1, 2 and 3). However, we observed more cases of malaria among men than among women: 19 versus 14. For example, we observed 11 cases of malaria in men against 6 cases among women in January 2011 (Fig. 3). Calculations give overall percentage. The malaria cases are 57.57% for men and 42.43% for women, the sex ratio is 1.35 per cent for men, while the latter represent 53.3% of the patients tested.
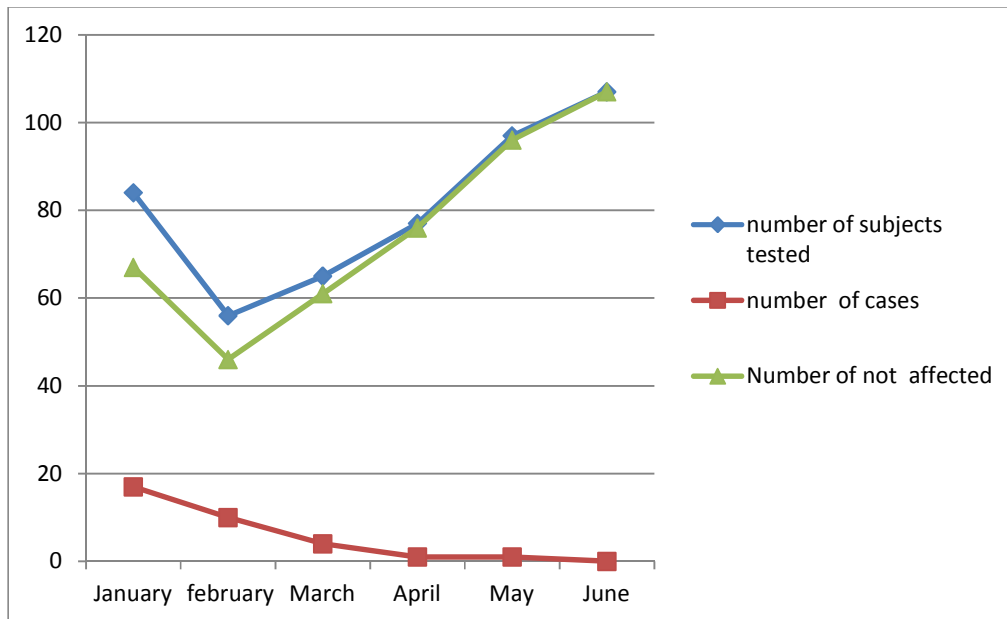
Fig. 1. Monthly variation of malaria cases between January and June 2011at Abass Ndao's hospital

Table 1. Prevalence of parasitaemia in Senegal

| Region	Saint-Louis	Louga	Diourbel	Matam	Kaolack	Fatick	Ziguinchor	Thiès	Dakar	Kolda	Tambacounda
Parasitemia	0	0,01	0,03	0,04	0,07	0,08	0,05	0,08	0,08	0,19	0,23

Source: National Survey on Malaria in Senegal (NPHS-II), 2008-2009

Table 2. Monthly variation of malaria cases between January and June 2011at Abass Ndao's hospital

Month	Number of patients tested	Number of malaria cases	Number of non-malaria patients
January	84	17	67
February	56	10	46
March	65	4	61
April	77	1	76
May	97	1	96
Jun	107	0	107

Table 3. Monthly variation of malaria cases by sex at Abass NDAO'S hospital from January to Jun 2011

Month	Males						Females						Total
	January	February	March	April	May	Jun	January	February	March	April	May	Jun	
Examined	39	19	33	39	48	49	45	37	32	38	49	58	486
Positive	11	4	2	1	1	0	6	6	2	0	0	0	33
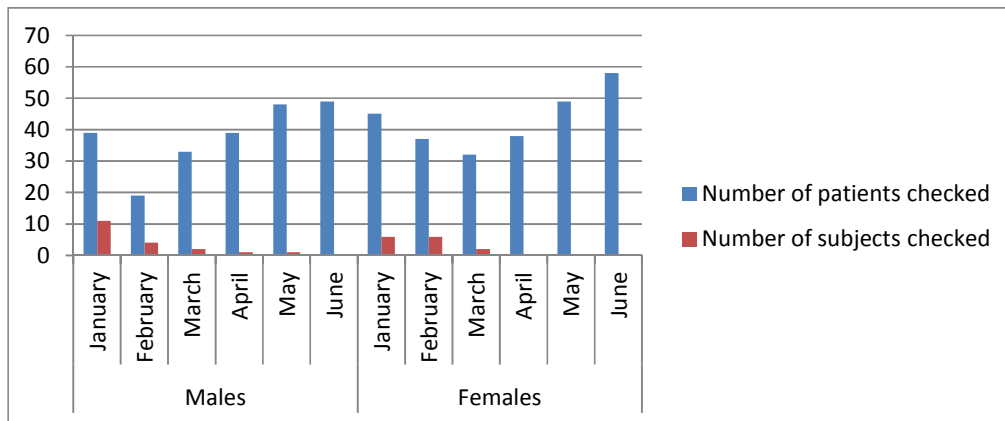
Fig. 2. Monthly variation of malaria cases by sex between January and June 2011at Abass Ndao's hospital
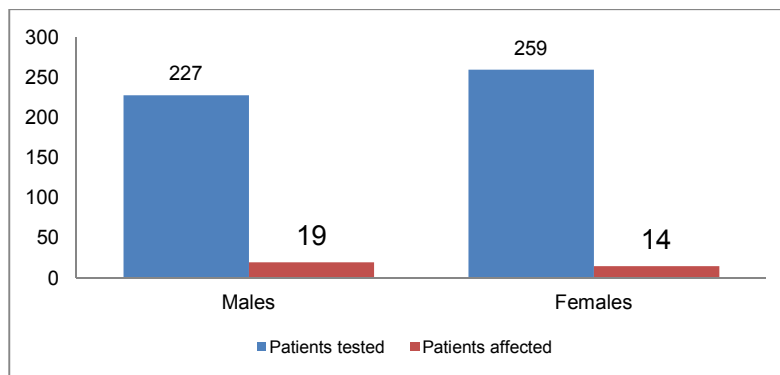
Fig. 3. Variation of malaria cases by sex between January and June 2011at Abass Ndao's hospital

3.4 Calculation of Prevalence and Incidence of Malaria

In the 486 patients tested, we have 33 malaria (Table 3), which corresponds to a prevalence of 6, 79%.

4. DISCUSSION

This study was carried out randomly over the period: 1 January to 30 June 2011 on patients for the diagnosis of malaria at ABASS NDAO Hospital. The diagnostic tests are successively TDR, thin and thick blood smear. Bench-top records have been developed for the collection of patient information and results.

All detected cases of carriage are due to *P. falciparum* except one case caused by *P malariae*. This shows that almost all cases of malaria in Dakar encountered are due to *P.*

falciparum, which is responsible for severe forms according to the WHO's World Report on malaria published in 2010 (World Malaria Report 2010) [1]. According to Mendis K et al., *P falciparum* is the most common cause of infections and accounts for approximately 80% of all malaria cases and 90% of deaths [8]. Malaria should therefore draw attention to Dakar, for the cases encountered are "potentially serious". Our results also showed a significant monthly variation of malaria. Thus, we observed a maximum of malaria cases in January followed by a decline which goes down over the months (Fig. 1). It goes from 17 malaria cases in January to zero cases in June. The monthly variation of malaria cases is well illustrated by (Fig. 1). It could be due to the gradual onset of the dry season characterized by the lack of points of stagnant water, spawning and larval mosquito vectors of disease. This variation is observed at the national level by the PNLP in N° 4 issue of *Roll Back Malaria* published in 2010 [5].

We are also interested in fluctuations observed by gender (Figs. 2, 3 and 4). The results show that men are more affected by the disease than women with 19 cases of malaria among men and 14 cases in women for all cases registered, the respective percentages of patients are 57.57% and 42 43%. This variation is quite sensitive to the extent of 53.3% of patients undergoing the test are women (Fig. 4). These fluctuations could be due to the fact that men, especially young people, are outside at night until late before entering the house to sleep. The risk of malaria is high for them because it is at the night that the mosquito vector of malaria becomes very active as it is in search of blood meals. This assumption can be based on the results of a team of Dutch and Gambian researchers in the "*mosquito trapped in a dirty sock*" published by the journal "Science et Vie" [9]. In addition, this team showed at the end of experiments in a small village in Gambia in West Africa, the attraction of mosquitoes to humans is indeed a good mix of odor detection and carbonic gas expelled from breathing [9]. This variation of malaria cases by sex could also be linked to the provision of free preventive treatment during pregnancy in 14 regions of Senegal according to Roll Back Malaria in the 4th issue, November 2010 [5]. The overall prevalence obtained from 1 January to30 June 2011is6.79%.This value is slightly less than that of the prevalence of parasitaemia in Dakar which is 8% in 2008-2009 as per ENPS-II, *Roll Back Malaria* No. 4, November 2010 issue [5] (Table 1). However, this 6.76% prevalence cannot reflect a significant decrease in the transmission or annual prevalence of malaria to

the extent that such period coincides with the dry season characterized by a decrease in transmission. Indeed malaria in Senegal is a steady endemic with seasonal peaks. The rainy season lasts from July to October. The peak of malaria, which is the consequence, is between July and December. The difference we have noticed compared to the work of *Roll Back Malaria* [5] could be related to a question of scale. Our study focused on 486 patients at the Laboratory during a period from January to June, while the data obtained by *Roll Back Malaria* [5]. are the result of observations made in 2008-2009 over the region of Dakar, therefore a much higher number of patients. Moreover, in this same article by *Roll Back Malaria* [5], we note that the Dakar region, although having a prevalence of 6.79% in our study, less than that published by 8%, is still in a worrying position as it still has a higher prevalence than those recorded in Saint-Louis (0%), Louga (1%), Diourbel (3%), Matam (4%) and Ziguincho r(5%) in 2008-2009 according to results published in the National Survey on Malaria in Senegal, 2008-2009 (NPHS-II), results reported by *Roll Back Malaria* [5]. However, we could not exclude that the decrease in prevalence could be related to Senegal's efforts in the fight against malaria. Indeed, many actions have been taken in the fight against malaria, including: the distribution of ITNs, the distribution of a million TDR and 1.5 million of ACT according to *Roll Back Malaria* [5]. Efforts are also in line with prevention methods recommended by the World Health Organization (WHO).
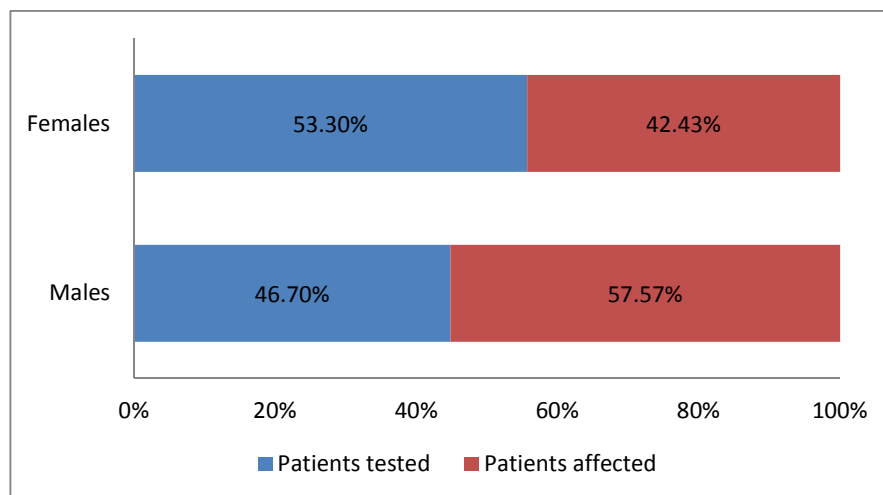
Fig. 4. Percentage of patients tested and malaria patients in relation to sex between January and June 2011at Abass Ndao's hospital

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5. CONCLUSION

With 225 million patients and 781,000 deaths in 2009, malaria remains the most important parasitic disease in the world, 80% of cases in sub-Saharan Africa [1], a high-risk area, occur in Senegal. This retrospective study of cases of malaria at the Medical Biology Center (MBC) of the ABASS NDAO Hospital (CHAN) that we conducted from January 2011to June2011 (period of low transmission) allowed us to demonstrate that the case malaria met in Dakar are potentially serious forms as caused by *P. falciparum* [8] responsible for the malignant tertian fever. A very marked monthly variation was observed between January and June with a frequency of 20.24% in January and 0% in June. We also found in our study that the disease affects both women and men but with a higher number of men affected. The study also allowed us to assess the hospital prevalence of 6.79%. Despite the slight decline compared to the prevalence in 2008-2009(6.79% vs. 8%), efforts must intensify the fight against malaria in Dakar, since the value of the prevalence remains high even if the period is characterized by a low rate of transmission.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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