



## Search for Plasmodium Trophozoites in Volunteer Blood Donors at the Djougou Blood Transfusion Station

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### Authors' contributions

*This work was carried out in collaboration among all authors. All the authors have contributed positively to this study. Each one of us played his or her score up to the writing of the manuscript. After the first manuscript, they all worked for; the completion of the final version. They even remain ready to work towards the publication of our original research article in case of any corrections. All authors read and approved the final manuscript.*

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### ABSTRACT

**Aims:** Malaria is a real public health problem in sub-Saharan Africa; more than 300 million new cases and about two million deaths occur each year. Blood transfusion is a potential route of *Plasmodium* transmission, but there is still no consensus on measures to prevent post-transfusion malaria in endemic areas. In order to determine the prevalence rate of asymptomatic Plasmodium carriers among voluntary blood donors and to prove the risk of transfusion malaria.

**Patients and Methods:** we conducted a study from 29 March 2018 to 31 May 2018 in the laboratory of the Centre de Santé de la Commune (CSC) in Djougou, which houses a Blood Transfusion Station (BTS) and handled a total of 337 blood samples collected. Thick drop (TG),

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blood smear (FS) and microscopy were the methods used to enhance our results.

**Results:** At the end of the work, out of 337 cases studied, we obtained 58 positive cases of Plasmodium trophozoites, i.e. 17.21% and 279 negative cases, i.e. 82.79%. The positivity is higher in subjects with a body temperature between 36° C and 37.4°C, i.e. 56.89%, and therefore considered to be asymptomatic carriers.

**Conclusion:** Based on these facts, it is advisable to establish a system for the prevalence of asymptomatic Plasmodium carriers among blood donors and to eliminate malaria after a blood transfusion.

*Keywords: Malaria; parasitic prevalence; voluntary blood donor; blood transfusion; Djougou commune (Benin).*

## 1. INTRODUCTION

Blood transfusion is a very delicate medical act that must be followed from the moment the blood is donated until it is actually transfused to the recipient, which is the patient in need [1-5]. Transfused blood must be tested to ensure that it does not contain any viral, bacterial, fungal or parasitic diseases for the safety and survival of the patient. Among the parasitic diseases, the case of malaria is a sobering one as malaria alone accounts for almost all cases of tropical parasitic diseases transmitted by blood transfusion [6-9]. Malaria is in fact an erythrocytopathy caused by Plasmodium and transmitted by the bite of the female Anopheles. This parasite in the circulating blood multiplies when conditions are favourable and the inoculation of a patient with contaminated blood can cause malaria [10-13].

Our work aims to search for Plasmodium trophozoites in the blood of voluntary blood donors in the commune of Djougou.

## 2. PATIENTS AND METHODS

The search for Plasmodium trophozoites was carried out on 337 volunteer blood donors aged between 18 and 58 years old. Subjects are submitted to an information sheet (appendix) and their constants (weight, temperature and blood pressure) are taken. After their blood has been collected in tubes containing Ethylene Diamine Tetra Acetic (EDTA) bearing a registration number, a thick drop and a thin blood smear are taken, which is then dried and fixed (the smear alone is fixed) to the MAYGRUWAL, the slide is stained with GIEMSA 10% for 15 minutes. Once the slide is dried, it is read under the microscope with the 100 objective with the immersion oil. The ad hoc sheets used by the National Blood Transfusion Programme and the laboratory

registers were reviewed in order to collect the variables of interest, in this case, age, sex, the results of the thick drop, parasite density and plasmodium species. These data were supplemented by an interview to provide information on the antimalarial prevention measures used by the subjects. Each blood sample was used to make a thin smear and a thick drop stained with Giemsa on the same slide. L'espèce plasmodial was identified on the smear, the parasite density on the thick drop, according to the formula used by l'Organisation Mondiale de la Santé. Parasitaemia was assessed by counting asexual parasites for 200 leukocytes, i.e. an evaluation for one microlitre of blood (based on an average of 8,000 leukocytes per microlitre). When the number of parasites counted was less than 10 per 200 leukocytes, the count was made against 500 leukocytes. Parasitaemia was expressed per microlitre.

## 3. RESULTS

Fig. 1 shows the distribution of blood donors by gender.

Fig. 2 shows the distribution of Plasmodium prevalence according to age.

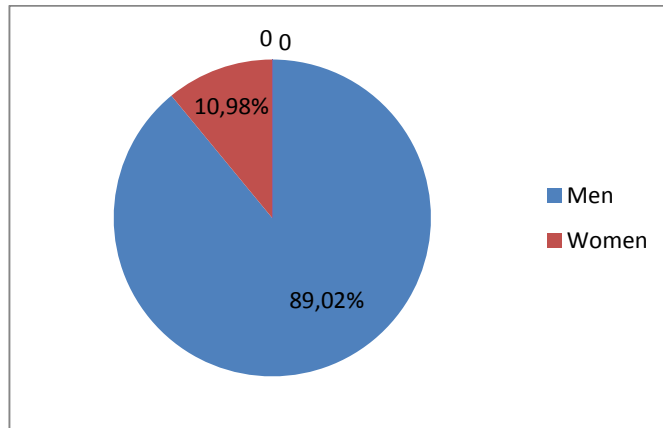
Fig. 3 shows the search results for Plasmodium trophozoites in blood donors of all ages and sexes.

Fig. 4 shows the distribution of parasitized donors by gender.

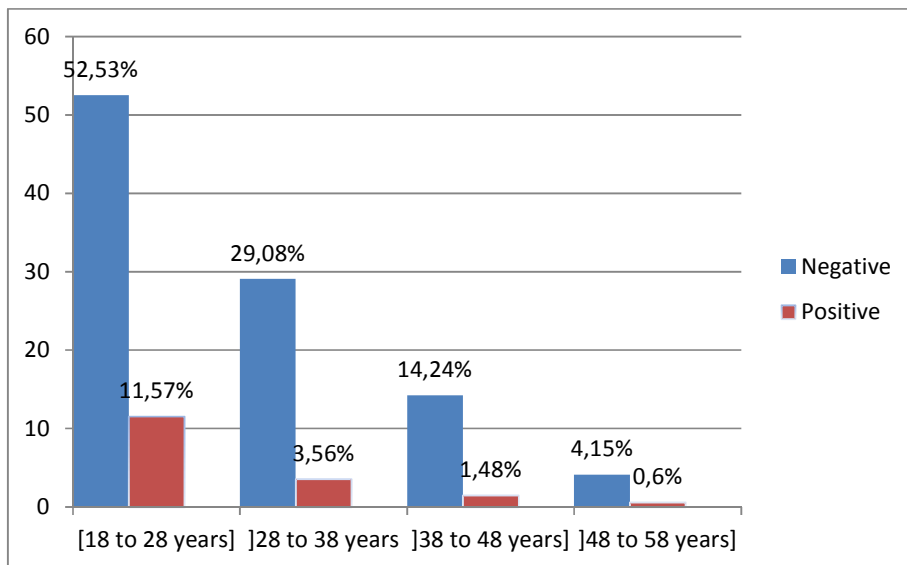
Fig. 5 shows the distribution of parasitaemia in parasitized subjects.

Fig. 6 shows the distribution of parasitized subjects as a function of body temperature.

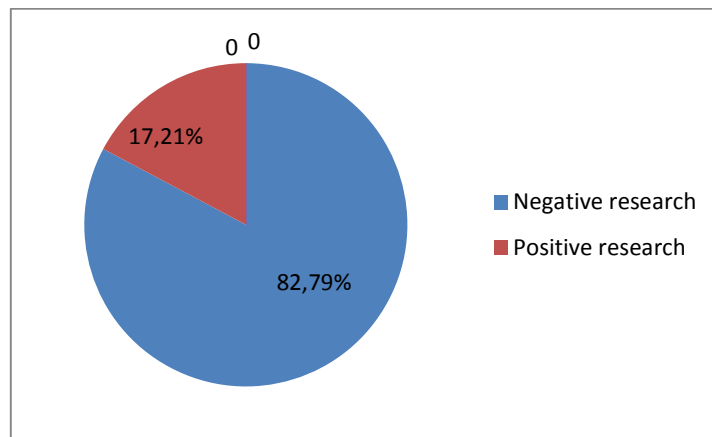
Fig. 7 shows the distribution of parasitized subjects in relation to the use of insecticide-treated nets.



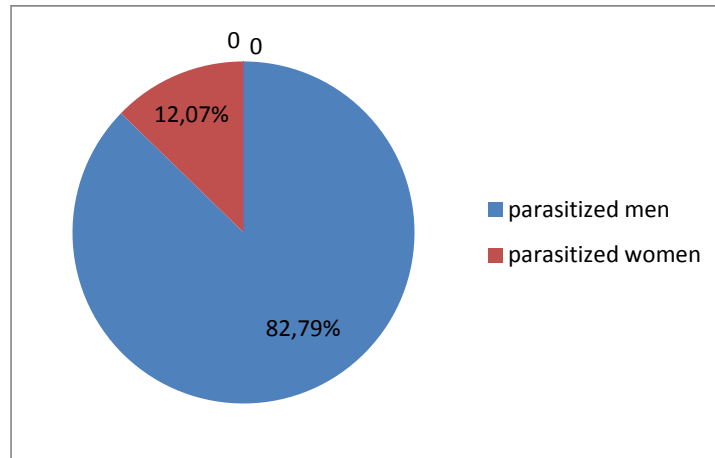
**Fig. 1. Distribution of blood donors by gender**



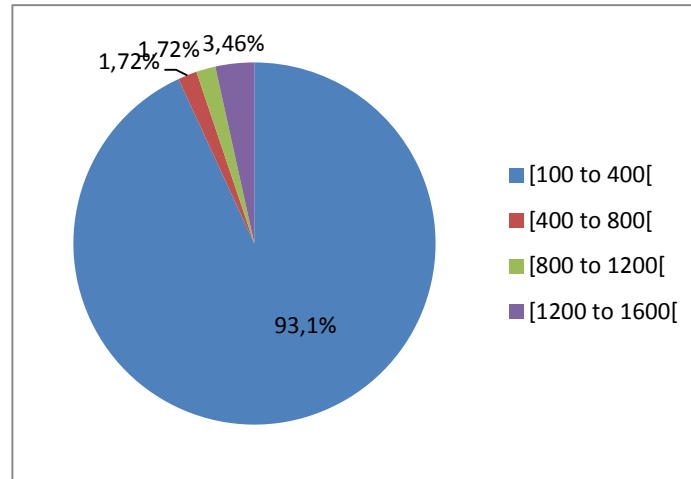
**Fig. 2. Distribution of Plasmodium prevalence according to age**



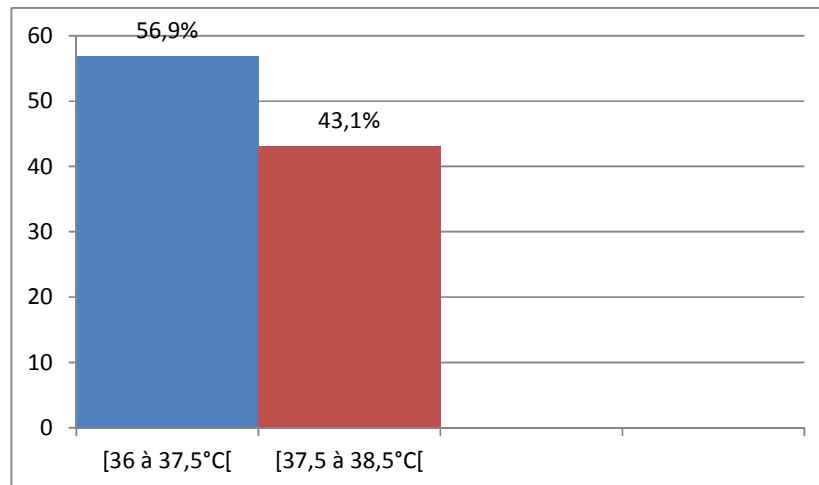
**Fig. 3. Search results for Plasmodium trophozoites in blood donors**



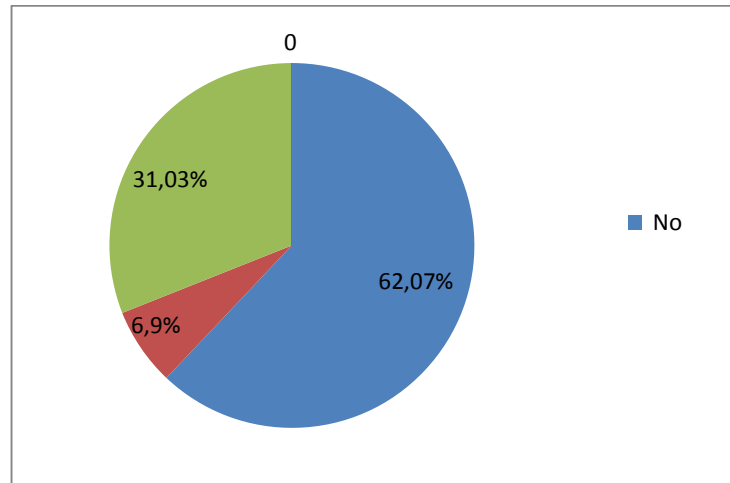
**Fig. 4. Distribution of parasitized donors by gender**



**Fig. 5. Distribution of parasitaemia in parasitized subjects**



**Fig. 6. Distribution of parasitized subjects as a function of body temperature**



**Fig. 7. Distribution of parasitized subjects in relation to the use of ITNs**

#### 4. DISCUSSION

Our study, based on the search for Plasmodium trophozoites in volunteer blood donors at the Djougou Blood Transfusion Station, was carried out on 337 subjects, 17.21% of whom were declared positive for Plasmodium trophozoites; this explains why we have asymptomatic donors with malaria.

A review of the literature on the risk of malaria transmission through blood transfusion in sub-Saharan Africa over a period from 1980 to 2009 had shown that parasite prevalence among blood donors varied considerably from 0.1% in Kenya to 55% in Nigeria. The highest prevalences were reported in West Africa. We found a predominance of male donations over female donations which is probably explained by the fact that men make four (04) donations and women make three (03) donations per year.

The age group that makes more donations is between eighteen (18) and twenty-eight (28) years old. It constitutes the active population and is the most infested age group.

The study revealed that 17.21% of the subjects sampled were carriers of Plasmodium trophozoites and the vast majority of these carriers were male, i.e. 87.93%.

Moreover, 93.1% of subjects declared positive for Plasmodium trophozoites have a low parasitaemia. Parasitemia ranges from one hundred (100) parasites per microlitre to four hundred (400) parasites per microlitre.

We also found that 56.9% of subjects positive for Plasmodium trophozoites have a body temperature between thirty-six (36) and thirty-seven point four degrees Celsius (37.4) and are apparently considered to be "normal" donors.

Our study also revealed that 62.07% of parasitized donors do not sleep under insecticide-treated nets.

The method of detection of the different types of Plasmodium on thin smears is not superior to that of Polymerase Chain Reaction PCR. The PCR technique allows for targeted in vitro replications. It allows obtaining from a complex and scarce sample, large quantities of a specific DNA fragment of defined length. The order of magnitude is that of a million copies in a few hours of replication cycles. Each reaction uses two oligonucleotide primers with 3' 5' 5' 3' ends pointing towards each other. It is therefore necessary to use original and specific matrices for each type of Plasmodium. In the framework of this research work we have five types of Plasmodium, namely:

- Plasmodium falciparum
- Plasmodium vivax
- Plasmodium oval
- Plasmodium knowlesi
- Plasmodium malariae

So we have to buy the original and specific matrices for each type of parasite, which would make this research work more expensive, if we should use PCR.

Apart from the cost, there is also an important factor is the choice of one of the important elements of the parasite that can highlight the parasite, namely:

- Sporozoites
- Hypnozoites
- Schizontes
- Trophozoites
- Gametocytes

All of this may add to the time spent on research work. Even in Real Time PCR (RT-PCR) the time factor always plays against us if we should use this method.

Even if the thin smear method is not superior to PCR, at least it is used in all blood transfusion centers. However far away the country is, this staining method can still be applied to May Grünwald Geimsa (MGG) in fifteen minutes to get results for a blood transfusion. Moreover, demand for blood transfusion in BENIN exceeds supply. As an example, for one bag of blood to be transfused, there are about ten recipients waiting. In these conditions, it is a real race against death.

As for the alternative method, there is the ELISA chain, which can highlight the IgM immunoglobulins of the parasite. But it is also an expensive and long method, with the difference that it allows to get out after four hours, ninety-six tests.

Still as an alternative method, there is that of immunofluorescence, which is a microscopic method, but which uses fluorescent substances specific to the parasites involved. It is similar to the thin smear method, but more expensive.

The various methods that can detect the presence of plasmodium in the blood of blood donors are as follows:

- Thin smear
- ELISA
- RT-PCR
- Immunofluorescence
- QCB

All other methods are more expensive than smear tests and take longer.

Our point of view on the thin and colored smear method used is that it is the one that allows us to realize the originality of the research work done. If we use PCR in this research work when rural blood transfusion centers do not use it, there

would be a bias. We would have more positive cases compared to what they do routinely. We have worked under the same conditions as they do to be able to identify errors and take corrective action.

## 5. CONCLUSION

The results of this study show that the prevalence of asymptomatic Plasmodium carriage among voluntary blood donors in the commune of Djougou was high, thus constituting a significant risk of transmission of the parasite to recipients who are often in poor general condition. However, the use of insecticide-impregnated mosquito nets and the retention of volunteer donors appear to be useful means of reducing the risk of asymptomatic Plasmodium carriage. A way should also be found to screen donor blood bags. But until this is achieved, all transfused patients should be routinely given presumptive anti-malarial treatment and voluntary blood donors, especially younger ones, should be sensitized to the use of insecticide-treated nets. This could significantly reduce the carriage of Plasmodium among blood donors and thus reduce the risk of transfusion malaria.

## DISCLAIMER REGARDING CONSENT/ETHICAL APPROVAL

As per university standard guideline participant consent and ethical approval has been collected and preserved by the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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