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Frequency and Identification of Plasmodial Species in the NZABA Health Zone, Mbujimayi City (Democratic Republic of the Congo)

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Abstract

Method: We used the prospective method supported by the techniques of analysis of Medical Biology (malaria microscopy which consisted in taking blood samples to make thick drops and thin smears), and the data were collected via Kobo software—collect V.2021.2.4 and analyzed by using SPSS.20 software. This study is carried out in the NZABA Health Zone. It is limited to determining the frequency and identifying plasmodial species. **Results:** After collecting and analyzing the data, we arrived at the results according to the following: out of 201 subjects subjected to our study, 140 people had a positive thick drop, or 69.7% had a thick drop positivity rate; the women come first with 83 cases or 59.70%; the age group from 0 to 15 years is much more affected by malaria, with 44.3%, followed by the age group of 41 years and over; the age groups between 16 to 30 and 31 to 40 occupy the bottom of the scale with 10 and 10.7 respectively. There is a predominance of *Plasmodium falciparum* at 97.4% followed by *Plasmodium ovale* at 2.14%. *Plasmodium malariae* is the last species with 0.7% and the bottom of the scale is occupied by *Plasmodium vivax* which has not been found. **Conclusion:** The frequency of malaria is 69.7%; *Plasmodium falciparum* is predominant with 97.4%. 3% of malaria cases escape the rapid diagnostic test and consist of *Plasmodium ovale* at 2.14% and *Plasmodium malariae* at 0.7%. *Plasmodium vivax* was not found; women come first with 83 cases or 59.70% followed by men with 57 cases or 40.30%; the age group from 0 to 15 years is the most affected.

Subject Areas

Public Health

Keywords

Frequency, Identification, Plasmodial Species

1. Introduction

1.1. Problem

Malaria is a febrile and haemolytic erythrocytopathy caused by the presence and development, multiplication in the liver, then in the red blood cells of a haematozoa of the genus Plasmodium transmitted by the infecting bite of an infected female Anopheles mosquito of the Culicidae family [1]. Currently, five specifically human Plasmodium species have been identified: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi*. *Plasmodium knowlesi*, genetically close to *Plasmodium vivax*, and microscopically close to *Plasmodium malariae*, was recently discovered in humans in Malaysia (but was previously known in monkeys in Southeast Asia) [2].

Plasmodium falciparum and *Plasmodium malariae* are ubiquitous species, the first largely dominated by its workforce. *Plasmodium vivax*, also very widespread in the world, does not exist in Central Africa and West Africa, where it is

replaced by *Plasmodium ovale*, a specifically African and Malagasy species [3].

According to the World Health Organization (WHO), malaria affected approximately 229 million people worldwide in 2019 and caused 409,000 deaths [4]. The situation is more worrying for several years since parasites have been developing resistance to antimalarial molecules and mosquitoes are less and less afraid of insecticides [5]. In France, approximately 5500 cases are reported of importation each year, among which 94% of the cases are from Malaria, in the tropical zones of Africa [6].

Malaria particularly affects underprivileged tropical areas of Africa, Asia and Latin America. The African region is by far the most affected with 94% of malaria cases recorded in this region. Epidemics can occur when populations exposed to malaria move to highly endemic areas [4]; children and pregnant women are the most vulnerable groups [7].

Malaria is responsible for 36.5% of the reasons for consultation in health services [6]. It is the leading cause of death for children under 5 after the neonatal period [8].

6 countries (Nigeria, DRC, Burkina Faso, Mozambique, Côte d'Ivoire, and Mali) account for 60% or 390,000 deaths due to malaria. About 40% of mortality attributable to malaria in the world is concentrated in two countries, including Nigeria and the DRC, and mainly affects children under five years of age [9].

In the DRC, *Plasmodium falciparum* remains the most predominant Plasmodiale species in the country, and it is responsible for serious forms of malaria; *Plasmodium malariae* and *Plasmodium ovale* are found either separately or in co-infection with *Plasmodium falciparum*. *Plasmodium vivax* was found as much in Tshimbulu in Kasaï Central at a low prevalence as in a faithful Kimbanguist from Tshiopo who came on pilgrimage to Nkamba in Kongo-Central [10]. The three DPS of Kasaï, namely Kasaï, Kasaï—Central and Kasaï Oriental, have experienced the highest prevalence rates in the country, oscillating between 32% and 38% [11].

In Kasaï Oriental, the species that remains dominant is *Plasmodium falciparum*, which is why the rapid diagnostic tests that are available (Care stare or SD Bioline) are HRP2-based tests [12].

Indeed, all four species of Plasmodium have a first enzyme called Aldolase, this protein has a short persistence after successful treatment. The second is the Parasite Lactate Dehydrogenase (pLDH) produced by the asexual and sexual stages of the parasite with a distinction between isoenzymes of *Plasmodium falciparum*, *Plasmodium vivax* and others with short persistence after successful treatment. The third is specific to the *Plasmodium falciparum* species (Histidine Rich Protein-2 (HRP-2): it is produced by the trophozoites and gametocytes of *Plasmodium falciparum* with persistence of ±43 days after successful treatment [12].

This creates difficulty in distinguishing species and determining the positivity rate for malaria rapid diagnostic tests in places where there are only HRP 2 tests such as in Eastern Kasaï, from where, conventional microscopic techniques, thin smear and thick smear remain the reference [2].

In Eastern Kasaï, it is accepted that malaria is due to *Plasmodium falciparum*,

Plasmodium ovale, more rarely found and *Plasmodium malariae*, the populations of this region are not infected by *Plasmodium vivax*, because this Plasmodium requires the presence of Duffy subgroup on red blood cells, the Duffy antigen being the *Plasmodium vivax* receptor [2]. However, in this region, Africans are mostly Duffy negative [13].

Analysis of data made by the Provincial Service of the National Program for the Fight against Malaria in Eastern Kasaï reveals in its report a positivity rate for Rapid Diagnostic Tests higher than 79% in the province and the interpreter of a counter-performance with regard to the indicator in 2021 in the second quarter, among the 19 HZs that make it up, there is a low rate of positivity for RDTs in the Health Areas of the Nzaba, 59.2%, qualified as performing by the PNLP, which motivates the choice of this NZABA health entity in this study.

Knowing that the Rapid Diagnostic Test kits available on the market are specific to a single species of *Plasmodium falciparum* parasite and in cases where a malaria infection can be caused by other Plasmodium species and does not react to test positive of Rapid Diagnosis at HRP 2, a question arises on this particularity between the Health Zones of the same province as follows: are there no other species of plasmodia which would reveal negative the test at HRP 2 while the patient suffers from malaria? What is the true frequency of malaria in this health zone?

1.2. Goal

1.2.1. Main Objective

Determine the frequency and identify plasmodial species in the Health Areas of the Nzaba in order to improve the management of malaria.

1.2.2. Specific Objectives

- Identify suspected malaria patients;
- Prepare a thick drop coupled with the thin smear and analyze;
- Identify the different species of plasmodia;
- Calculate the frequency of all parasite species in patient samples;
- Make suggestions based on the results obtained.

2. Methodology

2.1. Materials

To carry out this work, we used the following materials:

- Stirrer
- Plastic staining tray and Metal staining bridges
- Absorbent wadding
- Funnel
- Registration form
- Withers
- Glass Slides (Object Holder)
- Honed blades
- The Olympus CX23 microscope; Timer
- Paper towels
- Filter paper
- Put ph paper
- Pasteur pipette
- Metal tray
- Racks
- Rack
- Sterile syringes

Reagents

- Buffered Water
- Immersion Oil
- Methanol
- Giemsa's Stock Solution

Annex

- Ordinary Water
- Denatured Alcohol

2.2. Method

To carry out our work and achieve the objectives assigned above, we used the prospective method supported by the techniques of analysis of Medical Biology (malaria microscopy which consists in taking blood samples to make thick drops and thin smears), then observation under the microscope and the data were collected using Kobo-collect V2021.2.4 software, analyzed using SPSS.20 software.

This study is carried out in the 18 Health Areas of the NZABA. It is limited to determining the frequency and identifying plasmodial species for the patient samples that we have taken. It covered a period from May to July for theory and from August to September 2021 for laboratory analyses.

The laboratory of the General Reference Hospital of DIPUMBA served as a framework for the realization of our work; we chose this laboratory because of the good working conditions it offers us in terms of size, workspace and materials available for our research.

2.3. Population and Sample

We carried out our study among individuals in the Health Areas of the Nzabawho attended curative services during our study; they amount to 201 people thanks to the non-probability sampling technique by convenience.

Indeed, the Health Areas of the Nzaba is located in the Provincial Division of Kasaï Oriental in the Democratic Republic of the Congo (DRC). Administratively, Nzaba is in the commune of Bipemba. It is bordered to the east by the Bonzola zone, to the west by the Mukumbi health zone, to the north by the Health Areas of the Mpokolo, to the south by the Tshishimbi health zone and to the northeast by Bipemba health zone.

It has a relief that is made up of plains and valleys with a sandy-clayey soil; the vegetation is dominated by grassy savannah as well as small gallery forests. It has a humid tropical type climate with alternating two seasons: a long rainy season from mid-August to mid-May (9 months) period during which there is intense multiplication of Anopheles mosquitoes. It is connected by road 14 kilometers from the city center of Mbujimayi, the capital of the province [14].

2.4. Selection Criteria

2.4.1. Inclusion Criteria

1) Having developed clinical symptoms of malaria (fever, headache, chills, digestive disorders, body aches, physical asthenia, bone and joint pain and having consulted one of the health units in the health areas of the Nzaba during the period of our study;

2) Have consented and adhered to the study;

3) Not having resorted to self-medication with antimalarial before consulting and before microscopic analyses.

2.4.2. Exclusion Criteria

Anyone who does not meet the inclusion criteria listed above is excluded from

this study.

2.5. Type of Study

This analytical study is of a transversal and descriptive type on the frequency and identification of plasmodial species in the NZABA health zone.

2.6. Data Collection Plan

To succeed in collecting the data of this treaty, we went through the following steps:

- Implementation of data collection software;
- Obtaining the visa of the MCZ of NZABA;
- Briefing of nurses in charge of health areas in this area;
- Blood sampling from patients who have attended the care services;
- Confections of thick drops coupled with thin smears and their colorations;
- Readings of the slides under the microscope with the 100× objective;
- Actual data collection by encoding in the Kobo collection software.

2.7. Data Analysis Plan

Kobo Collection Box central server was encoded in software deployed via an Android brand phone and the statistical calculations were performed using SPSS software, the data is presented in tables and expressed as a percentage.

2.8. Study Parameters

We have retained the following parameters or variables for our work:

2.8.1. Independent Variables

- Age;
- Gender;
- Origin;
- Civil state;
- Occupation;
- Religion.

2.8.2. Dependent Variables

- Positivity of the GE to malaria;
- Plasmodium species.

2.9. Ethical Considerations

This study obtained the authorization of the ethics committee of the Higher Institute of Medical Techniques of Mbujimayi and the approval of the Provincial Health Division of Kasaï Oriental. The oral consent of the patients was obtained after having clearly provided explanations of the objectives and the interest of the study. The patient's medical confidentiality was not disclosed. In addition, patient anonymity was guaranteed by the use of codes.

2.10. Description of Analysis Techniques (Blood Films)

2.10.1. Thick Gout

Interest:

The thick drop is used to make the biological diagnosis and to quantify the parasitaemia of malaria [10].

Principle:

A drop of blood is deposited, spread by circular movements starting from the center (defibrination), dried on a microscope slide and then stained with Giemsa. Red blood cells are lysed, and hemoglobin is dissolved during staining. Only parasites and white blood cells remain visible under the microscope. Giemsa's solution stains the cytoplasm of plasmodia in blue while the component chromatin of the nucleus is stained in intense red. During the staining of the dried blood spot, the hemoglobin in the red blood cells is dissolved and removed by the stained water [15].

2.10.2. Thin Smear

Interest: the Thin Smear is a technique that allows the identification of species through observation of the morphology of the parasite and the parasitized erythrocyte. It requires methanol fixation, followed by Giemsa staining.

Principle: a drop of blood (2 µl) is deposited, then spread on a microscope slide and stained after fixing with methanol.

2.10.3. Procedure

1) Making the GE

- Start by degreasing the blades.
- Note the patient's coordinates (number) on the slide.
- Disinfect the place to be pricked (volar side of the tip of the 3rd left finger) with a cotton swab slightly soaked in alcohol. Then let it dry for a few seconds.
- With the left hand firmly press the proximal part of the cleaned finger to stimulate circulation and with a sterile vaccinostyle, prick the fingertip with a sharp and strong blow.
- Squeeze gently, and wipe off the first drop of blood with a dry cotton ball (make sure there is no cotton fiber left on the finger).
- Gently squeeze again and collect 2 - 3 drops of blood in the middle of the slide.
- Dab the stung area with a dry cotton pad (for haemostasis).
- With the corner of a second slide, quickly gather the drops of blood and spread them in a circular motion in spirals (1 cm in diameter) starting from the middle of the drop to form an even layer.

2) Preparation of thin smear

- A drop of blood (2 µl) is placed on one edge of a slide.
- Then use a second clean slide to touch the drop and allow the blood to spread along the edge of the slide.
- Tilt the blade at a 45˚ angle with respect to the first and push it towards the

free edge with a rapid and firm movement (without interruption until the blood runs out along the blade containing the drop).

3) Slide drying

The slides should be placed flat horizontally in a WHO-type box to allow uniform drying away from dust, flies and heat.

2.11. Quick Staining Techniques

2.11.1. Working Solution

- Prepare a 10% Giemsa solution (10 ml of Giemsa stock solution in 90 ml of water buffered at pH 7.2).

2.11.2. Fast Coloring

- Place the slides back to back in the staining tray, pour the solution gently until the slides are totally immersed and leave to rest for 10 to 15 minutes away from sunlight.
- Gently remove the solution by slowly adding clean water; evacuate the stain scum deposit on the tray then rinse the slides (valid in the case where the staining is done on small quantities of slides).
- Remove the slides one by one, let them drain and dry them on a rack; the sides bearing the samples facing down.

NB: After making the thick film and the smear, the smear must first be fixed before drying the slide, followed by staining.

A few remarks regarding the quality of Giemsa staining according to the PNLP:

- Use the working solution extemporaneously.
- Maintain the pH of buffered water at 7.2 (if possible, measure with pH paper).
- If the thick drop is too blue, add a few drops of 2% KH_2PO_4 to the buffered water (too alkaline).
- If the preparation is too pink, add a few drops of 2% Na_2HPO_4 to the buffered water (too acidic) [10].

2.12. Reading under an Optical Microscope

A thick drop is read using an optical microscope with a 100 objective (immersion objective). It consists in putting the blade on the plate and putting a drop of oil on the spot. First place the 10× objective, then the 100× objective and bring it into contact with the oil; Lamp dimmer fully open, Capacitor lifted; fully open iris diaphragm. Make sure that the chosen area has the required quality and examine the slide on at least 100 microscopic fields using the Rampart method. (**Figure 1**)

2.13. Expression of Results

The "plus" system is already outdated; there is currently an emphasis on the use of parasite density if the thick film is positive for Malaria [10].

Figure 1. Reading a thick drop and Thin Smear.

Determination of Parasite Density

It consists of counting the parasites per µl of blood on a thick drop, compared to a predetermined number of white blood cells. In each field, the parasites are counted at the same time as the leukocytes. The number of leukocytes counted varies between 200 and 500 according to the following diagram: If after having counted 200 leukocytes, the number of parasites counted is greater than or equal to 100, in this case the reading stops and the density is calculated according to the formula downstairs [16]. However, if on the other hand, at 200 leukocytes, the number of parasites counted is less than 100, it is then necessary to continue up to 500 leukocytes, and calculate the density according to the formula below [16].

$$\text{Parasite density} = \frac{\text{Number of trophozoites counted}}{\text{Number of white blood cells counted}} \times 8000$$

NB: 8000 is the average leukocyte count per µl in humans.

3. Results

In the light of Table 1, it can be seen that women were much more questioned than men with 59.70% against 40; 30%, Analysis of this data shows that out of 201 subjects in our study, 140 people had a thick smear positive, which is 69.7% as a thick smear positivity rate, And let's see that women come out on top with 83 cases or 59.70% followed by men with 57 cases or 40.30%.

In view of these results (Table 2), the analysis shows a predominance of *Plasmodium falciparum* with 136 cases or 97.4% followed by *Plasmodium ovale* with 3 cases, or 2.14% and the bottom of the scale is occupied by *Plasmodium malariae* with 1 case. Out of the total of 140 subjects 0.7%, *Plasmodium vivax* was not found.

By analyzing the data in Table 3, we notice that the age group from 0 to 15 years is affected much more by malaria with 44.3%, followed by the age group of 41 years and over.

In the light of Table 4, we have noticed that single people are much more affected with 50% of cases, or a number of 70 subjects suffering from malaria, followed by married people with 45.7% of cases, or a number of 64 subjects with

malaria and widowers occupy the last position with 4.3% or 6 cases.

We note that individuals without work are much more affected than others who have a known exercise, this category of unemployed comes at the top of our analysis with 93 cases, or 66.5% of our sample positive for malaria, followed by farmers with 21 cases or 15% the bottom of the scale is occupied by health personnel with one case or 0.7%. (Table 5)

In view of these results (Table 6), we find that individuals who did not have a known religion were much more affected with 83 cases or 59.3% of our sample followed by the Catholic Church with 21 cases or 15% of cases, the bottom of the scale is occupied by Protestants and Jehovah's Witnesses who have 11.4% and 7.9% respectively.

For all the subjects received in our study and suffering from malaria, we find that the health areas of Gérôme, Kadima Diba and Luaba; Lutulu, Mayiba and Mbikayi were much more affected than the others, followed by the Airport, Dinanga and Market health areas. (Table 7)

Table 1. Distribution of results by sex and positivity of thick gout in malaria.

	Workforce			Percentage		
	Positive	Negative	Total	Positive	Negative	Total
Feminine	83	37	120	41.29	18.41	59.70
Male	57	24	81	28.36	11.94	40.30
Total	140	61	201	69.65	30.34	100.00

Table 2. Presentation of the results according to the identification of the Plasmodium species.

Identification of Plasmodial Species	Workforce	Percentage
Falciparum	136	97.14%
Malariae	1	0.7%
Vivax	00	00
Ovale	3	2.14
Total	140	100.0%

Table 3. Presentation of results by age group.

Age Range	Workforce	Percentage
[0 - 15]	62	44.3%
[16 - 30]	14	10.0%
[31 - 40]	15	10.7%
[41 years and over]	49	35.0%
Total	140	100.0%

Table 4. Presentation of the results according to the marital status of the subjects.

Marital Status	Workforce	Percentage
Singles	70	50%
Bride	64	45.7%
Widowed	6	4.3%
Total	140	100.0%

Table 5. Presentation of results by profession.

Occupation	Workforce	Percentage
Unemployed person	93	66.5%
digger	9	6.4%
Farmer	21	15.0%
Teacher	16	11.4%
Personal health	1	0.7%
Total	140	100.0%

Table 6. Presentation of results by religion.

Religion	Workforce	Percentage
Others	83	59.3%
Catholic	21	15.0%
Muslim	9	6.4%
Protestant	16	11.4%
Witnesses	11	7.9%
Total	140	100.0%

Table 7. Presentation of results by origin.

Origin	Workforce	Percentage
Airport, Dinanga and Market	20	14.3%
Gerome, Kadima Diba and Luaba	45	32.1%
Health Areas of the Nzaba	18	12.9%
Lutulu, Mayiba and Mbikayi	46	32.9%
Mercy, Mukangala and Mutombo Kaci	1	0.7%
Tatu Muya, Tudikolela and Nzibabua	10	7.1%
Total	140	100.0%

4. Discussion

During this study on the frequency and identification of plasmodial species in

the Health Areas of the Nzaba, we found that women were much more investigated than men. The analysis shows that out of 201 subjects subjected to our study, 140 people had a positive thick drop, or 69.7% as the thick drop positivity rate, among women come first with 59.70% and men with 40.30%. This shows that the female sex is exposed and makes much more malaria than the male sex. Our results differ from those presented by the provincial coordination of the national malaria control program in Eastern Kasaï, which shows in a quarterly and epidemiological bulletin that the positivity rate is always less than 60% for RDTs [17], this would be said in fact that the thick film gives the possibility of diagnosing in addition to *Plasmodium falciparum* other species such as *Plasmodium ovale*, malariae and vivax [2] [18].

The age group from 0 to 15 years is much more affected by malaria, with 44.3% and comes first, followed by the age group from 41 years and over, the two age groups between 16 to 30; 31 to 40 years old are not much more affected by malaria, occupying the bottom of the scale with respectively 10% and 10.7%.

Single people are much more affected with 50% of cases, or a total of 70 subjects with malaria followed by married people with 45.7% of cases, or a total of 64 subjects with malaria and widowers. Occupy the last position with 4.3% or 6 cases. Individuals without work are much more affected than others who have a known exercise, this category of unemployed comes at the top of our analysis with 93 cases, or 66.5% of the sample positive for malaria, followed by farmers with 21 cases or 15% the bottom of the scale is occupied by health personnel with one case or 0.7%. This would be said to the fact that the other categories are not briefed on the prevention and transmission of malaria while the health personnel are indeed well informed in the matter [19]. these results corroborate with those found in 2013-2014 during the Demographic and Health Survey which shows in its results that this same age group from 0 to 15 years was much more affected by malaria than those whose age group age is greater than 15 years [20], this would be due to the fact that adults in general are already immunized and that children under 15 have not yet developed an effective means of defense against this pathology, especially since children under 5 are included in this age group. Stefani Aurélia's study in 2021 supports our results because, it shows that during her study, the category of unemployed individuals was much more affected by malaria than any other category, this would be said to the fact that civil servants are somehow informed about malaria prevention and behave responsibly than those without business [13] [21]. With regard to religion, individuals who did not have a known religion were much more affected with 83 cases or 59.3% of our sample followed by the Catholic Church with 21 cases or 15% of cases, the bottom of the scale is occupied by Protestants and Jehovah's Witnesses who have 11.4% and 7.9% respectively.

For all the subjects with malaria, we found that the health airs of Gérôme, Kadima Diba and Luaba; Lutulu, Mayiba and Mbikayi were much more affected than the others. This would be to the position of each health area because the first two were crossed by the KANSHI River and inhabited by diamond diggers

in the mine next door.

With regard to the frequency of plasmodial species, the analysis shows a predominance of *Plasmodium falciparum* at 97.4% followed by *Plasmodium ovale* with 2.14% and the bottom of the scale is occupied by *Plasmodium vivax* which has not been found. Our results are supported by the national strategic plan for the fight against malaria in the DRC, which in its introduction shows that *Plasmodium falciparum* remains the most predominant plasmodial species in the country and is responsible for severe forms of malaria, especially in infants and children. Under 5, pregnant women; *Plasmodium malariae* and *Plasmodium ovale* are found either separately or in co-infection with *Plasmodium falciparum*. *Plasmodium vivax* was found in TSHIMBULU in central Kasaï with a low prevalence [10].

Our results are opposed on the one hand to this same National Strategic Plan because during our investigations, we did not have to detect a case of co-infection of *Plasmodium falciparum* in co-infection with other species.

5. Conclusions

This study on the frequency and identification of plasmodial species in the Health Areas of the NZABA is prospectively supported by the techniques of medical biology analysis, during which we investigated 201 subjects and many more women than men. The analysis shows that out of 140 people had a positive thick smear, or 69.7% as the frequency of malaria in the Health Areas of the Nzabaand shows that the female sex came first with 83 cases followed by men with 57 cases. *Plasmodium falciparum* predominates with 136 cases or 97.4% followed by *Plasmodium ovale* with 3 cases out of the total of 140 subjects or 2.14% and the bottom of the scale is occupied by *Plasmodium malariae* with 1 case or 0.7%. *Plasmodium vivax* was not found. The age group from 0 to 15 years is much more affected by malaria, with 44.3% followed by that of 41 years and over. The two age groups from 16 to 30, 31 to 40 years are not much more affected by malaria, occupying the bottom of the scale with respectively 10% and 10.7%.

These results will help health decision-makers, especially those involved in the fight against malaria, to consider the use of a brand of Rapid Diagnostic Test with several antigens (aldolase, parasite lactate dehydrogenase) instead of only Histidine Rich Protein 2, for better biological than therapeutic management of malaria.

In view of the above, we suggest:

- To the PNLP, please intensify and favor the thick drop coupled with thin smears in all the favorable and viable structures of the Health Zone; consider using Pan Antigen RDTs (Aldolase, PLDH and HRP 2);
- To field service providers, to search for the malaria parasite in the thick film in cases where the results of RDTs are negative in front of a clinic that invokes malaria;
- To future researchers, continue with in-depth investigations and expand the

sample.

To the population of the Health Areas of the Nzaba, please protect themselves with all families by applying preventive measures to fight against malaria (mosquito net impregnated with long-acting insecticide, Sulfadoxine Pyrimethamine and Environmental Sanitation).

Conflicts of Interest

The authors declare no conflicts of interest.

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