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"Joint Approach malaria and toxoplasmosis about 2727 cases at the Center for Biological and Medical Analysis of Hospital Abass Ndao Dakar (CHAN) from 1 January 2011 to 30 September 2012."

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Abstract

Parasitology includes a wide variety of extracellular parasites, some of which are among the most serious, have a development in the blood visible after intracellular staining (Plasmodium, Babesia, Leishmania and Toxoplasma) or mobile (trypanosomes and microfilariae). Their detection and diagnosis is the first goal which is necessary for a diagnostic and prognostic biological technique. It is in this context that we assess parasitological examinations of blood. The diagnostic methods used are rapid diagnostic test (RDT), the thick blood, blood smears and serology for toxoplasmosis. The study was performed on 2727 patients in 2548 to 179 for malaria and toxoplasmosis. Indeed 105 cases of malaria are recorded including 103 due to P. falciparum is the most dangerous species and two cases caused by P. malariae. Hospital prevalence found for malaria is 4, 12% with a significant monthly variation confirming seasonal resurgence of the disease. The number of cases of toxoplasmosis is 82 also found a prevalence of 45.81%. While efforts are being made, but they must be stepped up to significantly reduce the prevalence of these parasites.

Keywords: Balance, parasitological examinations, Malaria, Blood, Toxoplasmosis.

Introduction

Parasitism, reactivity association in which one partner (host) environment and serves food to another (parasite), is one of the most important biological relationships in the animal world. Characterized by high species diversity and a variety of modes (both by the location of the parasite in the host for the duration of the parasitic relationship), parasitism for almost all animal species (air, land and water) including man. This parasite can affect the health of the host, ranging from simple problems to the most severe illnesses and even death. It is therefore easy to understand, besides the obvious consequences in human health of this parasitic relationship, its impact in the global economy. Parasitology studying a variety of parasites, some of which are among the most pathogens thrive in the blood. Species and parasitic forms are numerous and can interfere, in the form intra-or extracellular different organs. Major parasites in temperate and tropical zones are caused by parasites spend part or all of their lives in the blood of man. Therefore, their detection and diagnosis is the first goal which is necessary for a diagnostic and prognostic biological technique. Direct microscopic diagnosis should identify the parasite species, its stage of development and the number of parasites (parasitemia), essential figure in the prognostic assessment and therapeutic monitoring. [26] The overall objective of this study is to take stock of parasitological examinations of blood made the Analysis of Medical Biology Centre (VBM) of the Hospital Abass Ndao (CHAN). The specific objectives are the determination of various blood flukes found in the laboratory, the calculation of the prevalence of each of bloodstream parasites found and the comparison of these data with the results reported by previous studies.

Technical equipment

-**For the rapid diagnostic test** (RDT) test cassette, thinner capillary pipette (5µl) or loop sampling single-use, lancet (lancet) and alcohol swab.

-**For thick and thin blood** smears gloves, alcohol 70 ° and 95 °, cotton, sterile lancet, blades object holders, test tube pipette bottle, mother Giemsa 10% tap water, distilled water, rack, dryer, paraffin oil and microscope.

-**For toxoplasmosis serology**: Precision pipettes (Pasteur pipettes) with disposable tip for dispensing 10 mu.l, 25µl and 100µl, refrigerator, Scissors, Laboratory timer or watch, Centrifuge, Garrote, common markers (sets) , dry tubes for sample dilution, Oven, Bath - bath at 37 °, Cotton wool, paper adsorbent deal to cover the mattress, sterile gloves, needles, alcohol pad at 70 ° racks (medium - tubes), Kit ® Immunocombs Toxo IgG, code: 50440002 Version: L3 Format: 3 x 12 tests consisting of 3 combs: composed of 12 teeth in each unit which is sensitized by two points or spots reaction (upper spot: human immunoglobulins (control internal), lower spot: inactivated antigens Toxoplasma gondii RH) 3 trays development each of which contains six compartments (AF) of 12 wells each Compartment A: Sample Diluent, Compartment B: washing solution, Compartment C: anti goat antibodies - human IgG conjugated to alkaline phosphatase, Compartment D: washing solution, Compartment E: washing solution of F: chromogenic substrate containing 5 - bromo - 4 - chloro -3 - indolyl phosphate (BCIP) and nitro-blue tetrazolium (NBT) Positive Control: 1 tube (red top) of 0.20 ml of human plasma, inactivated by heat treatment containing anti-IgG antibodies to Toxoplasma diluted to 10 IU / ml level. Negative Control: 1 tube (green cap) containing 0.20 ml of diluted human plasma inactivated by heat treatment and negative for anti-Toxoplasma antibodies. Punch: for perforating the aluminum foil covering the wells of trays development. CombScals TM: for reading results (calibration range of colors).

Kit Immunocombs ® Toxo IgM: 50441002 Code Version: L3 Format: 3 x 12 tests with the same components as the kit above with an IgM specificity without CombScals TM because it is a qualitative test and a capacity of 0 , 15 ml for controls. It also has an adsorption solution (1 vial of 4 ml transparent cap) containing goat antibodies directed against human IgG.
Human sera from patients fasted for at least 8 hours.

Biological material

The biological material is composed of blood samples taken from patients to diagnose a parasitic blood flukes in the Analysis Centre of Medical Biology (VBM) of the Hospital Abass NDAO (CHAN).

Methods of study

Malaria¬

Rapid diagnostic test (RDT)

The procedure: All kit components and samples must be brought to room temperature before starting. First remove the test from the pouch and place it on a flat, dry surface of tape. Disinfect the finger and prick with a lancet. With a capillary pipette (5µl), draw blood to black and then transfer it to the round window of the test line. Or, with a loop sampling disposable (5µl), dip the end into the circular drop of blood and place it carefully into the round window of the test. Then add four drops of diluent in the square window. And finally interpret the results after fifteen minutes.
Results and Interpretation: The presence of a single color in the control window C-band indicates a negative result. By cons if there are two colored bands in the results window, the result is positive, regardless of the order of the bands. If the control band does not appear in the results window, the result is considered invalid. In this case it is recommended that a new test on the sample.

Thick film

Levy: It occurs at the finger or the earlobe, the heel or the big toe to the newborn. With a sterile lancet, prick the bottom edge of the nail, the 3rd or 4th finger of a jerk in the first with a disinfectant alcohol swab. Then press the finger, remove the first drop and collect a drop of blood the size of the head of a pin in the middle of a clean and degreased blade held by the edges. To collect venous blood, the patient's arm should be attached with a tourniquet to highlight the veins of the forearm cleansed with alcohol swab. And then select a vein with a needle prick in and the blood is collected in a tube or a syringe needle adapted.
Making: After removal, place a large drop of capillary blood in the middle of a blade or three small drops of venous blood slightly apart from each other. Then spread with the corner of another slide in the form of a circle of one centimeter in diameter rotating in a circular motion, it is the defibrination and then dry the blade with a dryer.
Colouring: Once dry leaf, pour tap water or distilled water on the blade and let it sit for 5 to 10 minutes for déshémoglobiniser. Then pour the water, then cover the slide with Giemsa solution (3 drops of Giemsa in 2 ml of water), let stand for 15 to 20 minutes depending on the method used. Finally rinsing the blade moderately until dripping water was colorless and dried with a dryer blade.
Reading: A drop of immersion oil (paraffin oil) is deposited on the thick film. It is the focus with the x100 objective. We must examine 100 microscopic fields before declaring a slide negative. Once a pest is identified, start counting parasites and leukocytes. If no noise is seen in a field, it simply counts the field.
Results and Interpretation: The presence of parasites certify that the result is positive. In this case it is necessary to calculate the parasite density. DP = number of parasites counted x 8000 / Total leukocyte count (200). Example: 25 200 parasites

found on leukocytes then Dp = 25 x 8000/200 = 1000 parasites/mm3 blood. The assessment of the parasite density is made by evaluating the number of parasites per field reading: If n / field (n = number of parasites) <1 → Dp low, n / field <19 → average Dp, n / field ≥ 20 → Dp high.

Blood smear

Sampling: it is the same as for the thick film, but here, the drop is thin and is filed 1cm on the blade.

Making: we must stabilize the blade where the samples, then with a clean slide, touch the small drop and let the blood spread along the edge. Then the second blade is inclined at 45 ° and pushes it firmly along the other blade, stopping a little short of the edge. Ensure that the two plates are in contact during spreading. When the thin smear is dry, identify the sample with a pencil or felt (not ballpoint pen) in its thickest part.

Colouring: Once dry smear, it is fixed with methanol at 95 ° for 10 minutes, then washed with water. Then it is stained with Giemsa, rinsed with water and finally dried with a blow dryer.

Reading and recognizing the dried blades are brought under an optical microscope with 100x objective. Note the presence or absence of trophozoites, gametocytes of merozoites inside red blood cells when looking for Plasmodium. The identification will be done according to the morphological characteristics of the species of Plasmodium.

Results and Interpretation: the interpretation is in the light of the parasite density. DP = number of parasitized erythrocytes x number of red blood cells given by the NFS / number of red blood cells per field x 10 (= 1000).

Toxoplasmosis:¬

For toxoplasmosis we used solid-phase enzyme immunoassay (EIA). It is identical for the determination of IgM antibodies and IgG with a difference ie pretreatment of samples to capture the IgG to IgM.

Procedure: The procedure summarized below is available to users of the package Immunocomb Toxo IgM or IgG.

First, balance reagents and samples to be tested at room temperature and perform the test at room temperature. Then pre-dilute 10 ml of each sample and control in 100 mu.l of sample diluent and incubate for 10 minutes (IgM only). Then distribute 25 ml of each pre-diluted sample for IgM or 10 ml of each pure sample for IgG and two controls specific amount as the case in the well compartment A and homogenize (incubate for 10 minutes for IgM). And finally insert the comb into compartment A, mix, incubate 20 minutes for IgM (10 minutes for IgG), absorb.

Reading and interpreting

Reading: Reading is done visually, for qualitative and quantitative IgM to IgG. It consists in comparing the intensity of each spot of the lower tooth with the intensity of the spot of the lower tooth of the positive control.

Thus, a spot having an intensity greater than or equal to the intensity of the positive control spot indicates the presence of IgM or IgG antibodies toxoplasmosis in the test sample. The absence of a spot or a spot having an intensity lower than the intensity of the positive control spot indicates no detectable levels toxoplasmosis IgM or IgG in the test sample (negative) antibodies.

The quantification is done using the CombScals TM calibrating the lower spot of the positive control in the corresponding color in the color scale by adjusting the rule so that the '10 'or' C + 'appears in the window at above the intensity of the selected color and finally reads the results of different samples without moving the calibrated position of the rule.

Interpretation: Because the presence of IgM antibodies do not persist beyond one year or observable reactivation, the qualitative detection of IgM antibodies provides a quick confirmation of acute or recent infection only. The quantitative detection of IgG antibodies can provide a determination of immune status in pregnant women and newborns, because of the persistence of anti-Toxoplasma IgG throughout life. In case of an increase of the title beyond four times the previous level, we can diagnose an active infection.

Results

Our study involved the participation of 2,727 patients. The 2548 patients have come to a diagnosis for malaria and 179 are pregnant women who came to the serology of toxoplasmosis. The study was conducted over a period of 21 months (1 January 2011-30 September 2012), but for toxoplasmosis we did a retrospective study because of the unavailability of reagent serology. The period chosen is from January to November 2011.

Malaria¬

Variation depending on the species of malaria parasite in CHAN

Of the 105 patients diagnosed in 2548 had malaria. The 103 cases are caused by Plasmodium falciparum and two other cases are caused by Plasmodium malariae.

Annual variation of malaria in CHAN

From Figure 3, we see that the number of malaria cases is decreasing from 91 in 2011 to 14 in 2012. Note that for 2012 we stopped in September.

Monthly variation of malaria CHAN

From Figure 4, we find that the number of malaria cases was 24 in January 2011 to rapidly decreases to become zero in June From July it rises back up to 24 in October. Then there is decline in malaria cases which become zero between February and May. In June the number of cases increases up to 9 cases in September.

Variation of malaria cases by sex: The results (Appendix 1) show us that there is more malaria men (54) than women (51) despite the higher number of women screened (1484 women against 1064 men).

Calculation of prevalence and incidence of malaria in CHAN: Table 1 shows that the 2548 patients tested, 105 were malaria is a prevalence of 4.12%.

Toxoplasmosis¬

Monthly change toxoplasmosis CHAN: From Table 2 we see that the number of women with toxoplasmosis who is 14 in January decreases sharply to 5 in March. In April it goes up to 12 and can be canceled by May He still believes up to 25 in June, gradually decreases and vanishes again in October and November. Incidence and prevalence of toxoplasmosis CHAN: Table 3 shows that out of 179 pregnant women diagnosed, 82 were IgG and / or IgM anti-toxoplasma a prevalence of 45.81%.

Discussion

The test results show that for almost all malaria positive cases is due to Plasmodium falciparum with 103 cases against two cases for P. malariae. P. falciparum is the most dangerous species responsible for severe and fatal cases according AHOUANGAN B. J. O. in its steady in 2005 [2] thesis. According Ndiaye S. Mr. AYAD and malaria remains, Senegal, the major endemic disease and the leading cause of morbidity and mortality in the most vulnerable groups such as children under five years and pregnant women. [24] Despite efforts, malaria remains a public health problem. Between the years 2011 and 2012 we noted a significant decrease in malaria cases. These results are confirmed by those found in the 2011 report on malaria in the world, published on December 13, 2011 by the World Health

Organization (WHO). According to the report, there were, in 2010, 216 million cases of malaria (with a margin of uncertainty range between 149 million and 274 million), which caused 655,000 deaths (with a margin of uncertainty between 537,000 and 907,000), a decrease in mortality of 25% globally since 2000 and 33% in the African Region of the WHO [26]. This decrease can be explained by a considerable extension of the measures to combat and prevent malaria, including through the extension of nets, the awareness and better availability of antimalarial drugs. This study also showed a monthly variation quite remarkable. Thus we found two peaks in January and October, with 24 cases of malaria each two months. Between January and June, we noted a significant decrease of malaria. 24 cases in January we go to 0 cases in June The number increases from July to return to 24 cases in October before falling sharply to become zero in February. The number remains zero until May to increase again. The monthly variation (Figure 4) is consistent with that found by Sagna in 2011. [20] It can be explained by the seasonal nature of the vector of Plasmodium. In fact, with the arrival of the rainy season, the breeding sites increase, thus leading to a proliferation of mosquitoes of the parasite. However, the peak in January 2011 can be explained by the persistence of standing water caused by the 2010 floods. If we look at the variation of malaria cases by sex (Appendix 1), we realize that there are more men suffering from malaria (54) than women (51). These results do not correllent with those of the study Ndiaye S. and Mr. AYAD 2009 "National Survey on Malaria in Senegal from 2008 to 2009" [24]. According to this study, Senegal, as in most countries south of the Sahara, malaria represent 35% of the reasons for consultation and remains endemic and major cause of morbidity and mortality in the most vulnerable groups, namely children under five and pregnant women. This may be caused by the fact that those at risk are more protected than others. This hypothesis is supported by the 2011 report of Roll Back Malaria "Collection, progress and impact of the RBM Partnership." According to the report, the 14 regions of the country have benefited from the free provision of intermittent preventive treatment during pregnancy. 52% of pregnant women received two doses of sulfadoxine - pyrimethamine (SP) during prenatal consultations in 2008-2009 against 13% in 2005. [25] The calculation of the hospital prevalence gives us 4.12% (Table 1). This prevalence is lower than that found by Sagna in 2011 in the very center, which was 6.79%. This difference can be explained by the fact that our sample is representative. In addition to our study period is larger. This prevalence reflects a decrease in the annual prevalence of malaria since our study period covers two seasons (the dry season and the rainy season). The decrease in prevalence may be related to many efforts by Senegal in the fight against malaria.
For toxoplasmosis: we are interested in the monthly variation and hospital prevalence. If we look at the monthly change in toxoplasmosis, we see that the number of women with toxoplasmosis which is 14 decreases sharply in January to 5 March. In April it goes up to 12 and is canceled in May. He still believes up to 25 in June, gradually decreases and vanishes again in October and November. The Toxoplasma prevalence in our study population is 45.81% (Table 3). It is slightly higher than that found by Ndiaye in 2010 (44.4%) [15]. This difference may be related to a problem of scale. Our study was performed on 179 patients between January and November 2011 while that Ndiaye is performed on 231 patients during the whole year 2010. However it is included within the range established by Faye [8] January to November 1993, 40.2% (CI: 30.6% to 49.8%; ELISA, 353 women). This prevalence is comparable to that found in France in 2003 was nearly 44%. Studies in recent years have shown a gradual increase in Toxoplasma prevalence in Senegal (18% in 1997

and 36% in 2004). [15] This increase can be explained by the level of health of populations, systematic serology in pregnant women and eating habits but also by contact more permanant with cats especially in cities. This assumption can be based on the modes of transmission of the disease described in the development cycle of the parasite ingestion of infected raw meat containing cystic forms of T. gondii ingestion of oocysts from cat feces (dirty hands), meat or contaminated vegetables.

Conclusion

This assessment of parasitological examinations of blood made the VBM CHAN between January 2011 and September 2012, we found that for malaria, the majority of cases are caused by Plasmodium falciparum. Hospital prevalence of malaria is 4, is 12% lower than those found in previous studies. This reflects the efforts of the national program against malaria (NMCP). Despite this decline, much remains to be done to significantly reduce mortality and morbidity due to malaria in the country. This study also confirms the seasonal outbreaks of malaria from July to December. Hospital prevalence of toxoplasmosis found between January and November is 45.81%. This means that 54.19% of women are not immunized against toxoplasmosis. Indeed it is important to strengthen the serological monitoring of toxoplasmosis in pregnant women to reduce the risk of maternal-fetal transmission of the protozoan Toxoplasma gondii after maternal primary infection. Given the high percentage of non-immune pregnant women, it is imperative to undertake persuasive efforts towards health authorities in Senegal for the serological tests are available to the public, also to raise awareness among women of childbearing age about the risks of seroconversion during pregnancy and diet and lifestyle measures to observe.

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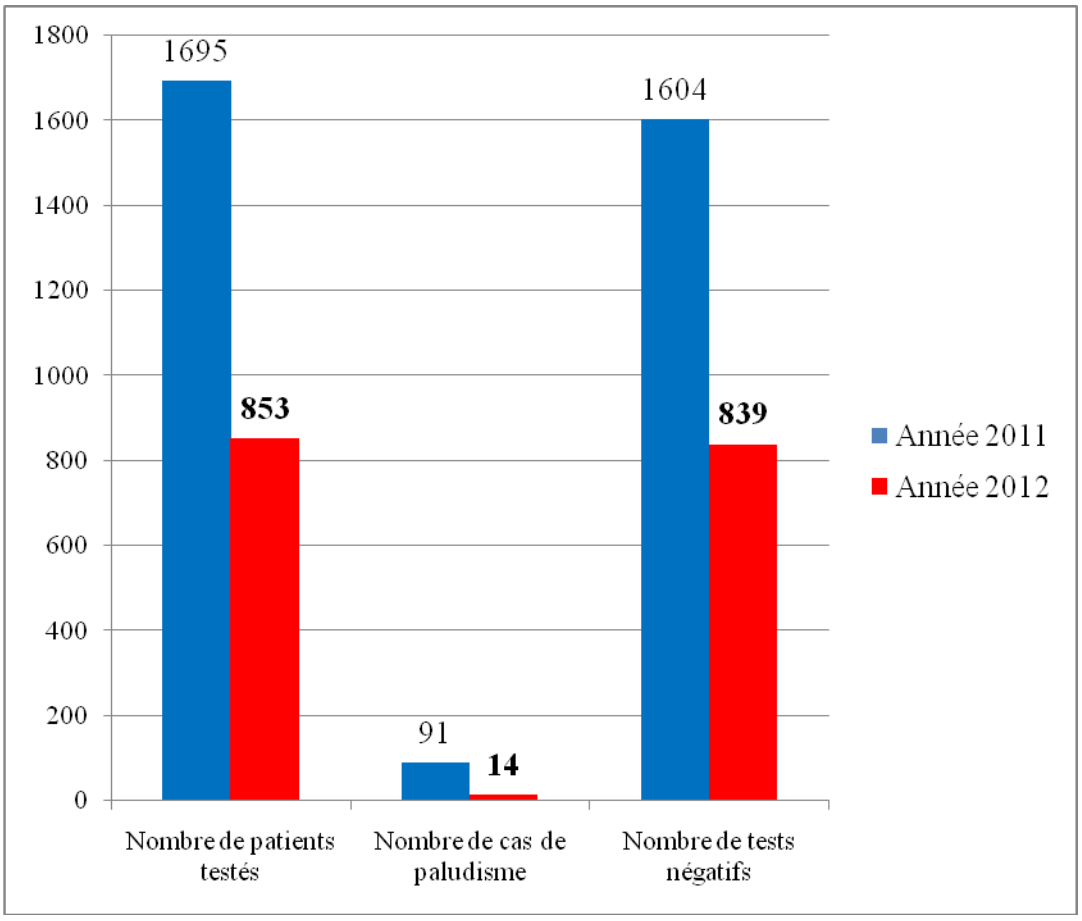


Figure 3 : Variation of malaria between January 2011 and September 2012 at CHAN.

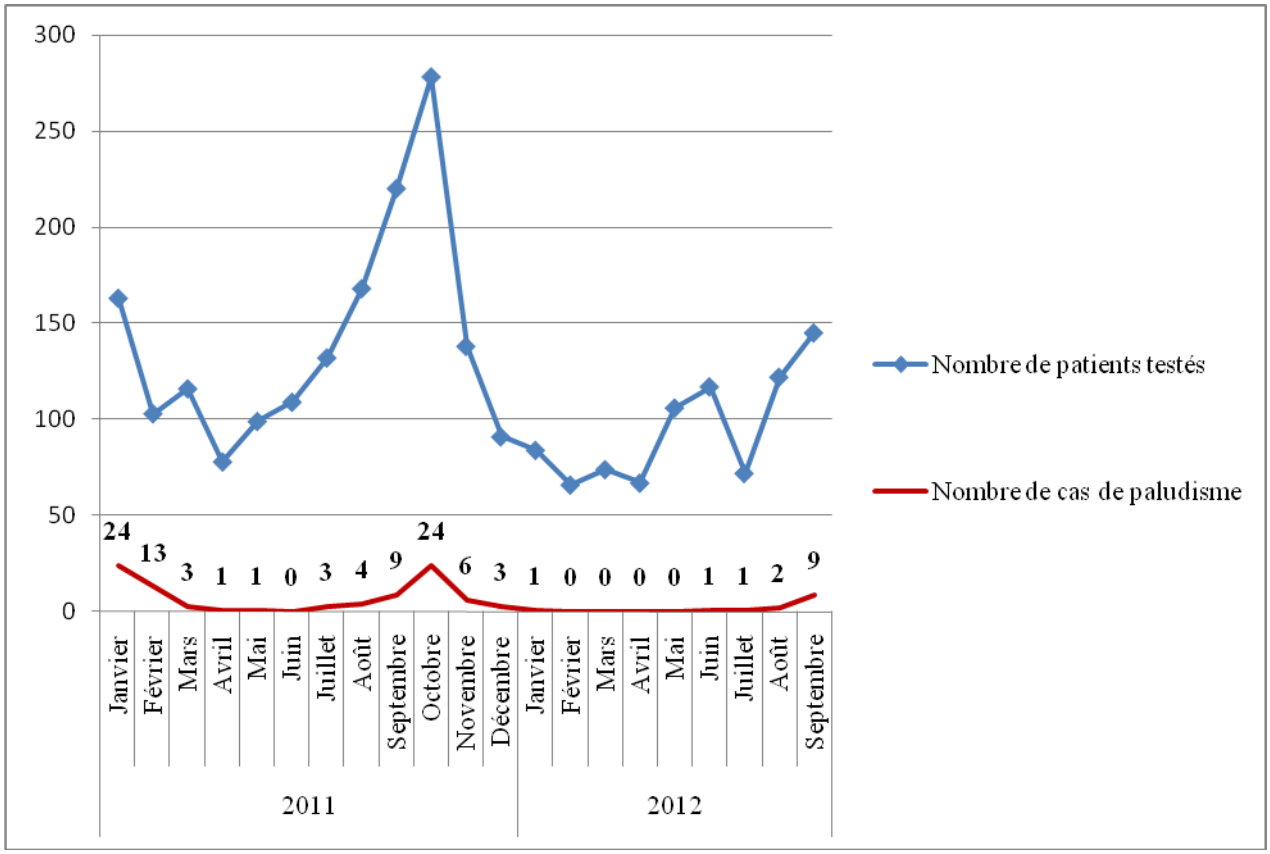


Figure 4 : Monthly variation of malaria cases at CHAN

	Number of patients tested	Number of malaria cases	Frequencies
Total	**2548**	**105**	
average frequency *****			**3,26%**
Prevalence of 1 January 2011 to 30 September 2012			**4,12%**

Table 1 : Incidence and prevalence of malaria at CHAN.

2011			
	Number of patients tested	Number of positive cases	Number of negative cases
January	28	14	14
February	8	3	5
March	20	5	15
April	26	12	14
May	2	0	2
Jun	33	25	8
July	42	20	22
Auguste	8	2	6
September	4	1	3
October	5	0	5
November	3	0	3
Total	**179**	**82**	**97**

Tableau 2 : Monthly change toxoplasmosis at CHAN.

	Nombre de patients testés	Nombre de cas positifs	Fréquences
Total	**179**	**82**	
Fréquence moyenne *			**30,18**
Prévalence du 1er Janvier au 30 Novembre 2011			**45,81%**

Tableau 3 : Fréquence et prévalence de la toxoplasmose au CHAN.

ANNEX

Annex 1: Monthly variation of malaria cases by sex.

Years	Month	Women		Men	
		Examined	Positive	Examined	Positive
2011	January	98	11	65	13
	February	69	8	34	5
	March	63	1	53	2
	April	37	0	41	1
	May	53	1	46	0
	Jun	60	0	49	0
	July	69	1	63	2
	Auguste	91	3	77	1
	September	137	8	83	1
	October	156	7	122	17
	November	78	1	60	5
	Décember	61	2	30	1
2012	January	54	0	30	1
	February	45	0	21	0
	March	45	0	29	0
	April	39	0	28	0
	May	65	0	41	0
	Jun	68	0	49	1
	July	46	0	26	1
	Auguste	75	2	47	0
	September	75	6	70	3
Total		**1 484**	**51**	**1 064**	**54**

Annex 2: Example of thick blood smears and properly made

Source :http://www.amazon.com/Techniques-base-diagnostic-microscopique-paludisme/dp/9242544302

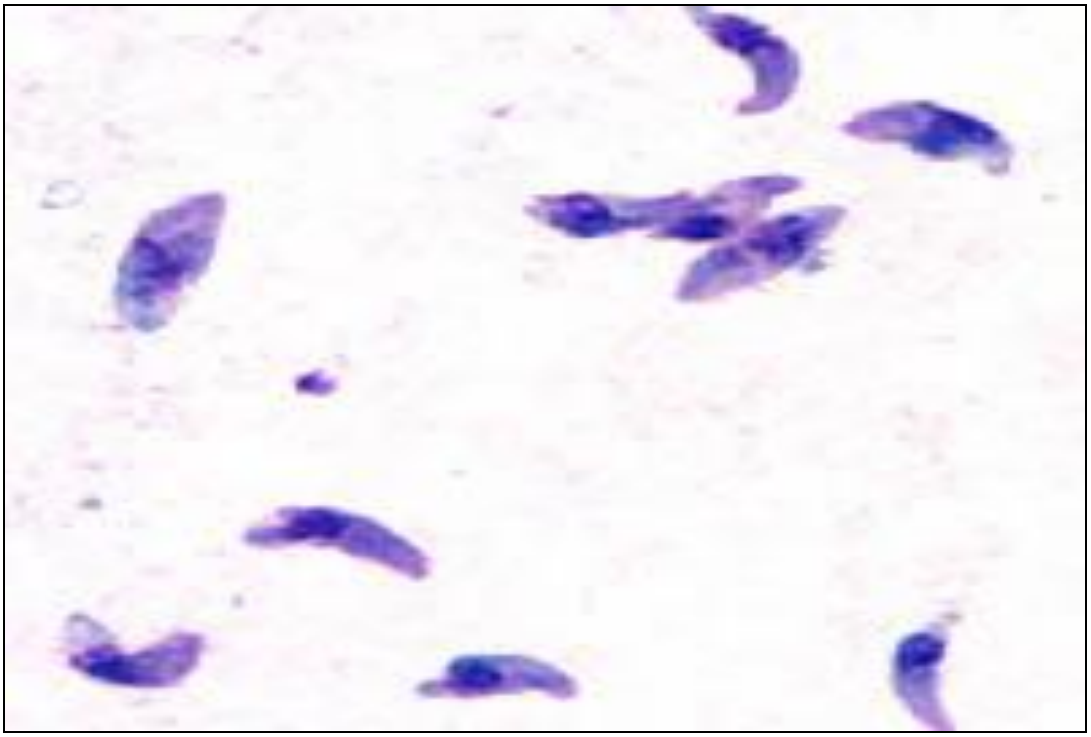
Annex 3: Averaging and true prevalence of malaria CHAN.

Years	Month	Number of patients tested	Number of malaria cases	Prevalence
2011	January	163	24	14,72%
	February	103	13	12,62%
	March	116	3	2,59%
	April	78	1	1,28%
	May	99	1	1,01%
	Jun	109	0	0%
	July	132	3	2,27%
	Auguste	168	4	2,38%
	September	220	9	4,09%
	October	278	24	8,63%
	November	138	6	4,35%
	Décember	91	3	3,30%
2012	January	84	1	1,19%
	February	66	0	0%
	March	74	0	0%
	April	67	0	0%
	May	106	0	0%
	Jun	117	1	0,85%
	July	72	1	1,39%
	Auguste	122	2	1,64%
	September	145	9	6,21%
Total		**2548**	**105**	
average prevalence				**3,26%**
Prevalence of 1 January 2011 to 30 September 2012				**4,12%**

Annex 4: Average and actual prevalence of toxoplasmosis at CHAN

2011			
Month	Number of patients tested	Number of positive cases	Prevalence
Janvier	28	14	50%
Février	8	3	37,5%
Mars	20	5	25%
Avril	26	12	46,15%
Mai	2	0	0%
Juin	33	25	75,76%
Juillet	42	20	47,62%
Août	8	2	25%
Septembre	4	1	25%
Octobre	5	0	0%
Novembre	3	0	0%
Total	179	82	
average prevalence			**30,18**
True prevalence of 1st January to 30 November 2011			**45,81%**

Annex 5 : Tachyzoites of Toxoplasma gondii



Source : http://fr.wikipedia.org/wiki/Toxoplasmose

Annex 6: Table Identification of Plasmodium sp.

	P. falciparum	*P. vivax*	*P. ovale*	*P. malariae*
Geographical distribution	Tropical climates: Africa, Southeast Asia, South America, Oceania	Southeast Asia, South America, Oceania	Especially African tropics	Tropical climates: Africa, Southeast Asia, South America, Oceania
Clinic	Malignant tertian fever	Benign tertian fever		Quartan
Revival	Absence	May occur more than 5 years after infection	can occur up to three years after infection	10 to 20 years reactivation after infection
Severe	Cerebral malaria or cerebral malaria	No mortal form		
Parasitemia	Can be massive (> 10%)	Rarely exceeds 2%	Rarely exceeds 2%	Generally <2%
Parasitized erythrocytes	Same size non-parasitized erythrocytes	Larger than non-parasitized erythrocytes Distorted irregularly	Larger than non-parasitized erythrocytes oval shape or fringed	Smaller than uninfected erythrocytes
Multi-parasitism	Common	Absent	Absent	Absent
Trophozoïte (young form: form into a ring)	Thin cytoplasmic ring. Small core often divided	Cytoplasmic thick ring Large nucleus	Thin cytoplasmic ring	Cytoplasmic thick ring Large nucleus
Trophozoïte (old shape)	Shaped ring wider or distorted	Amoeboid body: fingered cytoplasm or nucleus fragmented + big + / - + deformed thin black pigment	Amoeboid body: fragmented nucleus and cytoplasm	Very thick ring shape or form flag (rectangular) Large black pigment
Pigments	Spot Maurer (dark red spot in his nail to the erythrocyte surface)	Grits Schüffner the erythrocyte surface (fine granules)	Grits Schüffner the erythrocyte surface (large granules)	
Gametocytes	Banana shape characteristic (10µm), central mass of nuclear speckles (red) and pigment (B)	Round (10-12µm), pale purple or blue cytoplasm, fine black pigment dispersed	Round (7-8µm), pale purple or blue cytoplasm, fine pigment black scarce	Rounded (5-6µm), pale purple or blue cytoplasm, black pigment and abundant large grains
Schizonts (body rosette)	Absent (their presence is a sign of seriousness)	Large (10-14µm), 12-24 cores for black pigment end + / - dispersed	Average size (10µm), 6-11 large nuclei, large black pigment + / - dispersed	Small (5-6µm), 6-8 cores arranged in rosette. Big black pigment in the center

Source : http://www.memobio.fr/html/para/pa_pa_cr.html

Ndiaye, A. (2014). Joint approach malaria and toxoplasmosis about 2727 cases at the Center for Biological and Medical Analysis of Hospital Abass Ndao Dakar (CHAN) from 1 January 2011 to 30 September 2012. Open Science Repository Medicine, Online(open-access), e23050479.