1) There is no pathognomonic symptoms of malaria "simple crisis" and the lack of reliability of clinical diagnosis (even if and when a clinical examination is done) has been long ago underlined (Baudon et al., 1988; Baudon et al., 1986);
2) There is a lack of technical equipment such as microscope which is critical to diagnose malaria (Baudon et al., 1988; Besnard et al., 2006; Rogier et al., 2001) and in countries where 50% or more children are asymptomatic carriers, parasite density could be of great help even with its limitations (Bouvier et al., 1997; Delley et al., 2000). To overcome this issue, WHO recommended a large scale use of Rapid Detection Tests (RDT) and actually "The WHO African Region has had the largest increase in levels of malaria diagnostic testing, from 36% of suspected malaria cases tested in 2005, to 41% in 2010 and 65% in 2014" (World Health Organization, 2015). But RDT has some well-known drawbacks and limitations such as false negative, no detection of low densities, no estimation of parasite densities, etc. Nevertheless they can be considered as interesting tools for malaria diagnosis at Peripheral Health Centre (PHC). Immunological method based on PCR are scientifically the best to detect parasite (Fancony et al., 2013) even at a very low density but they required some skills and equipment which can be implemented only at the Central level and not at the Peripheral one.
3) There is a usual behavior in several PHC to consider any fever as malaria and to implement Artemisinin-based combination therapy (ACT) without going any further. This attitude has 3 main consequences: (1) lack of reliability of malaria diagnosis ("overdiagnosis") and therefore of data dealing with actual malaria; (2) lack of diagnosis of other diseases that do not receive needed appropriate treatment and are not quoted in official statistics such as diseases that could blow up (recent dengue epidemics); (3) increase of selective pressure due to over scale use of ACT.

Although the Angolan Ministry of Health has put efforts these past 12 years to rebuild the health-care infrastructure, child (167/1,000) and maternal (460/100,000) mortality rates remain among the highest in the world (Luckett et al., 2016). Recent reports have shown that in Angola the available statistics for the burden of malaria are not reliable because of the poor case reporting system and the scarcity of nationally representative malaria surveys (Gosoniu et al., 2010; Luckett et al., 2016). For 2002, official numbers reported 1,4 million malaria cases with 11,344 deaths; in 2004, 3,2 million cases and two-thirds occurred in children > 5 years old (UNICEF, 2008). Then, in 2006–2007, a large scale Angola Malaria Indicator Survey was implemented at the national level to assess the prevalence of malaria and anemia among children > 5 years old. For 2013, a total of 2,3 million cases were officially reported with 5,714 deaths. According to recent surveys in Angola, malaria is by far the highest cause of morbidity and mortality and has an epidemic potential in five provinces (Gosoniu et al., 2010).

In the framework of the National Malaria Control Programme, which implemented several integrated measures, including case management based upon ACT and large scale distribution of long lasting impregnated nets (LLIN), a cross sectional study was done to monitor three public health centers and to compare their malaria diagnosis with crosscheck microscopy done in the laboratory of the Medical Department (MD) of the private SONAMET® Company.

2. Materials and methods

2.1. Study setting and design

Between July 2010 and March 2011, at the request of the National Malaria Control Programme (NMCP), the Sonamet MCP team implemented a classical case-control study during the dry and rainy seasons in three health centers, one in Alto Liro (district of Lobito town) and two in neighboring villages, Cambambi and Asseque, located in the Benguela Province. Carriers of *Plasmodium*, among 724 patients of newly-born to 14 years old, with or without fever, were analyzed (Table 1). Information on age, gender and temperature (front) were taken. Thick blood smears were systematically processed in order to estimate their parasite load by classical microscopy. Parasitological data were compared to the malaria diagnosis done in the health center according to clinical symptoms, and eventually parasitological observations, which was considered as "presumptive malaria". In these cases, antimalarial drugs were systematically given, free of charge, by the nurse of these health centers. Initially, the aim of the study was to evaluate the proportion of malaria cases among feverish patients, then to use this information for other health centers and improve the statistical data on malaria procured by Peripheral Health Centre. However, it quickly appeared that even the notion of "fever" was not reliable and could not be used as good discriminant factor of "cases" and "control" because we noticed records of "fever" with body temperature of 35 °C–36 °C (Carnevale, unpublished observation). Therefore, we changed and considered clinically diagnosed malaria (or "presumptive malaria") as cases and "other pathologies" as controls.

Table 1
Number of patients, presumptive and confirmed malaria cases with their proportions (%), and diagnosis error rate per health center.

Health Centers	Alto Liro	Cambambi	Asseque	Total
Patients	227	152	345	724
Presumptive cases	156	78	339	573
%	68.7%	51.3%	98.3%	79.1%
Confirmed cases[a]	3	13	74	90
% per Presumptive cases	1.9%	16.7%	21.8%	15.7%
% per Patients	1.3%	8.6%	21.4%	12.4%
Rate of error (%)	98.1%	83.3%	78.2%	**84.3%**

[a] Thick blood smears.

2.2. Location

Three health centers were chosen for our study. One is in Alto Liro (12°21′S; 13°32′E) located on the upper part of Lobito, a town of 200,000 inhabitants. Vegetation is scarce and the landscape is dry. Until 2007, domestic water was kept permanently in tanks close to habitations, which were suitable breeding sites for *Anopheles gambiae* (Carnevale et al., 2015; Toto et al., 2011). The Alto Liro health center was selected by the NMCP for monitoring data, as it is well equipped with biological service and microscopes. Asseque (12°39′S; 13°27E) is a village of 8,000 inhabitants, close to Benguela town, in an agricultural area with numerous irrigation canals. The health center is well equipped with microscopes and solar panels given by Sonamet. Cambambi village (12°33′S; 13°32′E) is close to Asseque although located in a dryer area with fewer plantations and the health center has no microscope. In addition, some classical cross-sectional malaria surveys were done by MCP team among asymptomatic children of the same three locations to evaluate their *P. falciparum* prevalence for a comparison with the plasmodial prevalence and parasite load among patients of the health centers.

2.3. Ethics statement

This study was conducted in accordance with the Edinburgh revision of the Helsinki Declaration and was approved by the National Malaria Control Program of the Ministry of Health of Angola, the ethical authority in charge of approving studies on malaria research in Angola.

3. Results

Presumptive malaria was diagnosed for 573 cases of the 724 children selected for the study (79.1%), while only 90 (15.7%) of them were confirmed malaria cases with positive thick blood smears giving thus an overall error rate of 84.3% (Table 1). Presumptive malaria cases were diagnosed 6 times more than the confirmed cases with positive thick blood smears done on the same children. Therefore, the official percentage of presumptive malaria cases constituted nearly 80% of the patients, while reliable parasitological diagnosis showed that 12.4% of the patients were actually parasite carriers and 15.7% of clinically diagnosed malaria were *Plasmodium* positive (Table 1).

3.1. Variations according to season

The comparison of "presumptive" and "confirmed" malaria cases according to dry and rainy seasons and health centers is shown in Table 2. It is worth noticing that the number of presumptive malaria cases diagnosed during the dry and the rainy seasons was not significantly different (respectively 81.9% and 77.7%) ($\chi^2 = 1.79$; $p = 0.091$) and the rate of diagnosis error was around 80% for both seasons (Table 2).

For the confirmed cases of *Plasmodium,* with positive thick smears, the percentage was significantly higher during the dry season than the rainy one, respectively 20.2% and 13.2% ($\chi^2 = 6.05$; $p = 0.008$).

3.2. Variations according to health center

The Alto Liro health center had a similarly elevated rate of diagnosis errors with 97.6% and 98.2% for the dry and rainy seasons respectively (Table 2). The overall of 68.4% of patients (n = 227) were clinically diagnosed as malaria cases, while only three (2%) were confirmed cases with positive blood smear. All 98% of presumptive malaria cases received an ACT treatment in spite of the presence of a microscope and a laboratory unit. The percentage of "presumptive clinical malaria cases" among patients was similar during the dry (67.7%) and the rainy (69.1%) seasons ($\chi^2 = 0.038$; $p = 0.42$). In random cross-sectional surveys done by the MCP team, among 428 symptomless children of the Alto Liro community, the result showed that during the dry season, 12.9% (n = 18) of the 140 children tested had positive blood smears, while during the rainy season, 13.5% (n = 39) were positive among 288 children. Thus, the plasmodial prevalence (PP) was significantly higher among symptomless children (PP = 13.3%; n = 428) than in patients of the Alto Liro health center (PP = 1.3%; n = 227), even if the official declaration of presumptive malaria cases in this health center was very high with two thirds of the patients diagnosed with malaria. The reliability of data from such health center is matter of great concern.

In the Cambambi health center, a total of 78 out of 152 patients were clinically diagnosed with malaria, while only 13 of them (16.7%) were confirmed positive, representing 8.6% of the total patients instead of 51.3% of officially reported cases by the health center, with an overdiagnosis rate of malaria cases reaching 83.3% (Table 2).

The percentage of presumptive malaria cases among patients was similar during the dry (59.2%; n = 49) and the rainy (47.6%; n = 103) seasons ($\chi^2 = 1.79$; $p = 0.09$). The percentage of positive thick smears confirmed as malaria cases was much higher in the dry compared to the rainy season (37.9% and 4.1% respectively) and the overdiagnosis of malaria is therefore significantly higher in the rainy season, reaching nearly 96%, than in the dry season (62.1%) ($\chi^2 = 12.69$; $p = 0.0001$) (Table 2). When compared to Alto Liro, it is interesting to notice that the percentage of positive thick smears among presumptive malaria cases is significantly higher in Cambambi (16.7%; n = 78) without microscope, than in Alto Liro with microscope (1.9%; n = 156) ($\chi^2 = 15.5$; $p < 0.05$) where clearly this tool is not correctly used.

In the Asseque health center, close to Cambambi and well equipped in microscopes and solar panels, presumptive malaria was diagnosed in 339 out of 345 patients (98.3%), while *Plasmodium* confirmed cases were actually reported in 74 thick blood smears (21.8%) giving a diagnosis error rate of 78.2% (Table 2). When Asseque health center reported 98.3% malaria cases among all patients, there were actually 21.4% of confirmed cases. There is an obvious high rate of overdiagnosis of malaria in patients (100%) during the rainy season, while 22% only had confirmed *Plasmodium* parasites on their thick blood smears (Table 2). The percentage of positive blood smears among presumptive malaria cases were similar during the dry and rainy seasons (21.9% and 21.8%; ($\chi^2 = 0.0006$; $p = 0.49$). The prevalence of *Plasmodium* confirmed cases in Asseque was similar to Cambambi, respectively 21.8% (n = 339) with microscopes available and 16.7% (n = 78) ($\chi^2 = 1.02$; $p = 0.31$), in the latter case where no microscope (Table 1). Cross-sectional surveys done by MCP team among the Asseque community covered by this health center showed an overall plasmodial prevalence of 31.1% among the 363 asymptomatic children less than 14 years examined. This plasmodic index appeared significantly higher than the prevalence of *Plasmodium* among the 345 patients of Asseque in our study (21.4%) ($\chi^2 = 7.76$; $p < 0.05$). Plasmodial prevalence was also significantly higher during the dry season (43.2%; n = 132) than the rainy season (24.2%; n = 231) ($\chi^2 = 14.05$; $p < 0.05$), while this prevalence during both seasons was the same (21.8%) in our study (Table 2).

The densities of parasites in thick blood smears of patients and asymptomatic children were analyzed with arithmetic mean of respectively 93,535 [± 148,343] and 1,665 [± 3,176] parasites/µl during the dry season and 47,878 [± 50,353] and 2,695 [± 5,825] parasites/µl during the rainy season (Table 3). Parasitaemia of malaria patients was 56 times higher than in asymptomatic children during the dry season and 18 times during the rainy season. However, the standard deviationaisdu7nts37(p)-420.79(o8)-259.275icdeviationade,(inde-8.1children)]TJ

Table 3
Parasitaemia, arithmetic mean and median (parasites/μl) for patients and symptomless children according to season.

Season	Patients (parasites/μl)			Asymptomatic children (parasites/μl)		
	Parasitemia (mini-maxi)	Mean (sd)[a]	Median	Parasitemia (mini-maxi)	Mean (sd)[a]	Median
Dry	185–480,000	93,535 [± 148,343]	14,169	32–16,320	1,665 [± 3,176]	533
Rainy	31–192,471	47,878 [± 50,353]	34,376	32–34,933	2,695 [± 5,825]	889

[a] sd, standard deviation.

Table 4
Antimalarial drugs misused for the treatment of presumptive malaria patients according to the respective health center and number of patients.

Drug		Presumptive malaria cases treated/total presumptive malaria cases			
		Alto Liro (n = 156)	Cambambi (n = 78)	Asseque (n = 339)	TOTAL (n = 573)
Coartem®	N (%)	142/145 (97.9%)	52/64 (81.3%)	235/309 (76.1%)	429/518 (82.8%)
Quinine	N	1/1	1/1	24/24	26/26
Amodiaquine	N	1/1	0	5/5	6/6
Arsucam®	N	0	2/2	0	2/2
Arinate®	N	0	5/5	0	5/5
Arthemeter®	N	3/3	1/1	0	4/4
Unspecified drug	N	0	1/1	0	1/1
No antimalarial drug	N (%)	4/4	3/4 (75%)	0	7/8 (87.5%)
Unknown	N	2/2	0	1/1	2/2

3.3. Misusage of antimalarial drugs

Among the 573 presumptive malaria cases that received antimalarial drugs, 90 only were parasitological confirmed cases, meaning that 84.3% of the patients (n = 483) received useless antimalarial drugs that were inadequately recommended (Tables 1 and 4). Such misdiagnosis induced not only a large misusage of antimalarial drug, but also 83% of patients who received Coartem® had an inappropriate treatment, such as, to a lesser extent, those who had other antimalarial drugs including Quinine, Amodiaquine, Arsucam®, Arinate®, or Arthemeter® (Table 4).

4. Discussion

A health facility-based survey dealing with 30 centers in Luanda (Angola), gathering 864 patients with fever (≥37.5 °C) or history of fever in the last 24 h, showed that 31 patients (3.6%) only had positive malaria blood smears (Thwing et al., 2009), and among patients with fever, 4.6% had positive blood smears, and 2.7% were positive even without fever. According to these authors, a very small minority of patients with fever at health facilities in Luanda actually had laboratory-confirmed *Plasmodium* infection, despite the large proportion of patients being diagnosed and treated for malaria (Thwing et al., 2009). They rightly underlined that "this low prevalence even among fever has two major implications: massive overuse of Artemisinin-based combination therapies (ACTs) or other antimalarials in patients who do not need it, and likely under-diagnosis of other causes of fever, some of which may be potentially life-threatening" (Thwing et al., 2009). A cross-sectional cluster survey was done in October-November 2007 in 33 health facilities of Huambo Province of Angola to assess the quality of malaria diagnosis and treatment (Rowe et al., 2009). Suspected malaria was defined as either fever (by history or measured axillary temperature ≥37.5 °C) or at least three of the following symptoms: headache, joint pain, chills, sweating, anemia, anorexia, fatigue, vomiting, or diarrhea. Rowe et al. (Rowe et al., 2009) imagined scenarios of hypothetical patients and noticed that in case of adults with fever and negative test (RDT or microscopy), most health workers (72.0–81.7%) seemed to ignore the result of the test and gave an incorrect diagnosis of malaria, and nearly all patients (96–100%), such as malaria diagnosed or suspected ones, were treated with an antimalarial drug. In this survey, it is worth underlining that even if fever seemed to be the main cause of consultation, temperature was measured in only 26% of consultations and assessment quality was poor for all other symptoms needed to identify suspected malaria. Combining the well-known lack of reliability of clinical symptoms (Baudon et al., 1988), the lack of pathognomonic symptoms and the poor quality of both clinical examinations (if any) and microscopy (if any), some concerns need to be addressed about the statistics, the actual burden of malaria, and its evolution which is of paramount importance for the evaluation of the efficacy of the different measures implemented to control the disease.

In a more recent survey done in the same Province of Huambo (Angola), laboratory supervision, including increase in number and level of training of laboratory technicians, allowed an improvement of malaria diagnostic capacity with a significant reduction in false-positive microscopy slide reading (Luckett et al., 2016).

The current monitoring implemented in the three health centers, one in the Lobito urban part and two in suburban and rural areas of the Benguela Province, showed that among outpatients, parasitological observations confirmed only 15% of malaria cases which were diagnosed according to clinical examination or "presumptive" malaria cases. Our data showed that with or without microscope, the rate of misdiagnosis was similar. In the case of Asseque center, plasmodial prevalence was significantly higher during the dry season than during the rainy season, which could be due to vegetables and other crops cultivated during the dry season that induce large scale use of water for irrigation which favored suitable breeding sites for vectors. This interesting seasonal variation is not indicated in the statistics of the health center where 98.3% of patients were supposed to have malaria (and received ACT treatment) when, in fact, nearly 22% only were confirmed with positive blood smears (Table 1).

Therefore, according to official reports, malaria is considered as

Delley, V., Bouvier, P., Breslow, N., Doumbo, O., Sagara, I., Diakite, M., Mauris, A., Dolo, A., Rougemont, A., 2000. What does a single determination of malaria parasite density mean? A longitudinal survey in Mali. Trop. Med. Int. Health 5, 404–412.

Fancony, C., Sebastiao, Y.V., Pires, J.E., Gamboa, D., Nery, S.V., 2013. Performance of microscopy and RDTs in the context of a malaria prevalence survey in Angola: a comparison using PCR as the gold standard. Malar. J. 12, 284.

Gosoniu, L., Veta, A.M., Vounatsou, P., 2010. Bayesian geostatistical modeling of malaria indicator survey data in Angola. PLoS One 5, e9322.

Hume, J.C., Barnish, G., Mangal, T., Armazio, L., Streat, E., Bates, I., 2008. Household cost of malaria overdiagnosis in rural Mozambique. Malar. J. 7, 33.

Luckett, R., Mugizi, R., Lopes, S., Etossi, R.C., Allan, R., 2016. The role of laboratory supervision in improving the quality of malaria diagnosis: a pilot study in Huambo, Angola. Am. J. Trop. Med. Hyg. 94, 659–662.

Ministry of Health, WHO, 2005. Ministerio da Saude consolida mecanismos para travar a epidemia de Marburg, Luanda, Angola. Ministry of Health, WHOpp. 1–2.

Ministry of Health, 2005. Controlo de doenças infecciosas – Situaçao epidemiologica na cidade do Uige., Luanda, Angola. Ministry of Healthpp. 1–3.

Mudhune, S.A., Okiro, E.A., Noor, A.M., Zurovac, D., Juma, E., Ochola, S.A., Snow, R.W., 2011. The clinical burden of malaria in Nairobi: a historical review and contemporary audit. Malar. J. 10, 138.

Mwanziva, C., Shekalaghe, S., Ndaro, A., Mengerink, B., Megiroo, S., Mosha, F., Sauerwein, R., Drakeley, C., Gosling, R., Bousema, T., 2008. Overuse of artemisinin-combination therapy in Mto wa Mbu (river of mosquitoes), an area misinterpreted as high endemic for malaria. Malar. J. 7, 232.

Njuguna, J., Menge, D., Nzou, J., Chege, C., 2015. Impact of an intervention to minimize overdiagnosis of malaria cases in a low risk kenyan sub-county. J. Health Care Poor Underserved 26, 802–810.

Olivar, M., Develoux, M., Chegou Abari, A., Loutan, L., 1991. Presumptive diagnosis of malaria results in a significant risk of mistreatment of children in urban Sahel. Trans. R. Soc. Trop. Med. Hyg. 85, 729–730.

Orish, V.N., Ansong, J.Y., Onyeabor, O.S., Sanyaolu, A.O., Oyibo, W.A., Iriemenam, N.C., 2016. Overdiagnosis and overtreatment of malaria in children in a secondary healthcare centre in Sekondi-Takoradi, Ghana. Trop. Dr. 46, 191–198.

Reyburn, H., Mbatia, R., Drakeley, C., Carneiro, I., Mwakasungula, E., Mwerinde, O., Saganda, K., Shao, J., Kitua, A., Olomi, R., Greenwood, B.M., Whitty, C.J., 2004. Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study. BMJ 329, 1212.

Rogier, C., Henry, M.C., Spiegel, A., 2001. Diagnosis of malaria attacks in endemic areas: theoretical aspects and practical implications. Med. Trop. (Mars) 61, 27–46.

Rowe, A.K., de Leon, G.F., Mihigo, J., Santelli, A.C., Miller, N.P., Van-Dunem, P., 2009. Quality of malaria case management at outpatient health facilities in Angola. Malar. J. 8, 275.

Thwing, J.I., Mihigo, J., Fernandes, A.P., Saute, F., Ferreira, C., Fortes, F., de Oliveira, A.M., Newman, R.D., 2009. How much malaria occurs in urban Luanda, Angola? A health facility-based assessment. Am. J. Trop. Med. Hyg. 80, 487–491.

Toto, J.C., Besnard, P., Le Mire, J., Almeida, D.S., Dos Santos, M.A., Fortes, F., Foumane, V., Simard, F., Awono-Ambene, H.P., Carnevale, P., 2011. Preliminary evaluation of the insecticide susceptibility in *Anopheles gambiae* and *Culex quinquefasciatus* from Lobito (Angola), using WHO standard assay. Bull. Soc. Pathol. Exot. 104, 307–312.

UNICEF, 2008. Progress report for malaria no more. Angola 2007. UNICEF.

Wang, S.J., Lengeler, C., Smith, T.A., Vounatsou, P., Diadie, D.A., Pritroipa, X., Convelbo, N., Kientga, M., Tanner, M., 2005. Rapid urban malaria appraisal (RUMA) I: epidemiology of urban malaria in Ouagadougou. Malar. J. 4, 43.

Wang, S.J., Lengeler, C., Mtasiwa, D., Mshana, T., Manane, L., Maro, G., Tanner, M., 2006a. Rapid urban malaria appraisal (RUMA) II: epidemiology of urban malaria in Dar es Salaam (Tanzania). Malar. J. 5, 28.

Wang, S.J., Lengeler, C., Smith, T.A., Vounatsou, P., Akogbeto, M., Tanner, M., 2006b. Rapid urban malaria appraisal (RUMA) IV: epidemiology of urban malaria in cotonou (Benin). Malar. J. 5, 45.

Wang, S.J., Lengeler, C., Smith, T.A., Vounatsou, P., Cisse, G., Tanner, M., 2006c. Rapid urban malaria appraisal (RUMA) III: epidemiology of urban malaria in the municipality of Yopougon (Abidjan). Malar. J. 5, 29.

World Health Organization, 2015. World Malaria Report, WHO Global Malaria Program. WHO, Geneva, pp. 1–280.