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Rapid test for the detection of Malaria (Pan/Pv/Pf)

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INDEX

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OTHER EVALUATIONS

INDEX	
S. No.	Name of the Evaluation Body
4.	Malaria Research Centre (ICMR, Goa, 2004)

A comparison of microscopic examination and rapid diagnostic tests used in Guyana to diagnose malaria

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Abstract

The aim of this study was to compare rapid diagnostic tests (RDTs) for malaria with routine microscopy for a prompt and accurate diagnosis of malaria and to provide an effective disease management in Guyana. Blood samples were collected randomly from 624 patients with clinical suspicion of malaria from four private hospitals in Georgetown, Guyana. The five different test methods [Paramax-3, Optimal-IT, VISITECT Malaria Combo PAN/Pf, Standard Diagnostic (SD) Bioline and conventional Giemsa stain microscopy] were performed independently by well trained and competent laboratory staff to assess the presence of malaria parasites. Results from the rapid diagnostic kits were analyzed and compared to those obtained by general microscopy. Of the 624 patients involved in the study, 197 (31.6%) tested positive and 427 (68.4%) tested negative to RDT whereas 190 (30.4%) tested positive and 434 (69.6%) tested negative to microscopy. The positive agreement index between RDT and microscopy was 89%. A comparison of microscopy with the RDTs, Paramax, Opitmal-IT, Omega, SD, showed a positive agreement index of 93%, 86%, 80% and 86%, respectively. The study, therefore, highlights the importance of both methods in diagnosis of malaria in endemic areas. Microscopy is the more reliable method in rural areas where malaria is most prevalent. RDT offers a good alternative, being an easy and rapid method that does not require an experienced laboratory technician.

Introduction

Malaria is an extremely complex disease that has been responsible for deaths and social disruption since the beginning of recorded human history. A large number of suspected malaria cases are still not suitably identified, resulting in the overuse of anti-malarial drugs and poor disease monitoring.[1-3] The World Health Organization has recommended that management of all malaria cases should be confirmed by quality-assured, parasite-based diagnosis before treatment is started.[3-4] Parasite densities of around 200 parasites per microliter (parasites/ L) should be detected to ensure high field sensitivity for clinically significant malaria infection in many malaria endemic populations.[5] High sensitivity of malaria diagnosis is important in all settings, and is essential for the most vulnerable population groups. This is particularly important for infants and pregnant women who are put at greater risk by a wrong diagnosis. In these subjects, malaria infection produces an acute illness that can rapidly progress to death.[6-7]

Malaria remains a very serious health problem in Guyana, South America. It affects mainly the indigenous population living in rural/hinterland communities and miners working in those areas. Malaria is not considered to be the major cause of death overall in Guyana but becomes a great threat when combined with malnutrition or if repeated episodes are experienced. According to available data, cases of malaria are on the increase, with the majority occurring in inland regions.[8]

Currently, microscopic examination of thin or thick blood smears is widely used for diagnosis of malaria. Giemsa microscopy is regarded as the most suitable diagnostic instrument for malaria control because it is inexpensive to perform, and it is able to differentiate malaria species and quantify parasites.[9] At low level parasitemia, the sensitivity of microscopy is limited and the method is time consuming, labor intensive, and requires an experienced microscopist.[10] The sensitivity of various diagnostic methods also depends on the malaria species, the parasite density, previous treatment, gametocytemia and quality of the diagnostic method.[11]

The national malaria control programs of several countries have been using rapid diagnostic tests (RDTs) as a definitive diagnostic tool where microscopy is not readily available to confirm suspected malaria cases.[12] However, in Guyana, most of these RDTs are used in the private sector.

This study, therefore, explores the importance of microcopy and different diagnostic methods for the detection of *Plasmodium* species in Guyana.

Materials and Methods

This prospective study was conducted in Georgetown (Region n. 4), an urban region of Guyana, between July and October 2011. Samples were obtained from one private laboratory (Laboratory A), one private clinic (Laboratory B), and two private hospitals (Laboratory C and Laboratory D) in the city of Georgetown, Guyana. These private institutions use RDTs as their main diagnostic tool for malaria. However, Laboratory B would follow up with microscopy only if the RDT result is positive.

All tests were carried out immediately and examined by well trained and competent laboratory staff. For microscopy, the test was examined independently by expert microscopists in different settings who did not know the results of RDT. Written results were communicated immediately to the clinicians.

Eligibility for participation

All participants were patients who presented with signs and symptoms of a malaria infection and for whom a malaria test was requested by a physician. Those who had recently returned from an endemic rural region of Guyana with a high prevalence of malaria were also suitable candidates for this investigation. A total of 624 samples were obtained.

Rapid diagnostic tests

Four different brands/types of RDT were used by the different laboratories: Paramax-3, OptiMal-IT, VISITECT Malaria Combo PAN/Pf, and Standard Diagnostic Bioline, Malaria Ag Pf/Pv (SD). Laboratory A used SD (batch n. 018110); Laboratory B used VISITECT Malaria Combo PAN/Pf (batch ns. 126013, 126041 and

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Contributions: RK, conception and design, analysis and interpretation of data as well as revising and final approval of the version to be published; RM, collecting samples for diagnosing and drafting the article.

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126043) and OptiMal-IT (batch n. 0E0015M); Laboratory C used Paramax-3 (batch ns. 9117 and 9121), and Laboratory D used OptiMal-IT (batch ns. 0K0027M and 0E0015M) and Paramax-3 (batch ns. 9111 and 9114).

Paramax-3 (rapid test for malaria pan/*P. vivax*/*P. falciparum*)

This is a rapid, self-testing, qualitative, 2-site sandwich immunoassay utilizing whole blood to detect *Plasmodium falciparum* specific histidine rich protein-2 (Pf HRP-2), *Plasmodium vivax* specific parasite lactase dehydrogenase (pLDH) and pan malaria specific pLDH. This test can be used for the specific detection of *P. falciparum* and *P. vivax* malaria, and to differentiate other malarial species.

OptiMal-IT

DiaMed OptiMal-IT is an immune-chromatography test using monoclonal antibodies against the metabolic enzyme pLDH of *Plasmodium spp*. These monoclonal antibodies are classified in two groups: i. specific for *P. falciparum;* ii. using pan-specific monoclonal antibodies reacting with all four species of *Plasmodium spp*. that can occur in human beings: *P. falciparum*, *P. vivax, P. ovale, and P. malariae.*

It is specific for the detection of pLDH, an enzyme produced by both sexual and asexual forms of the parasites and sensitive for the detection of peripheral parasitemia levels of 0.001-0.002% (50-100 parasites/ L of blood).

VISITECT Malaria Combo PAN/ *P. falciparum*

VISITECT Malaria Combo Pan/Pf is a rapid test for the detection of *P. falciparum,* non-*P. falciparum* or mixed infections that utilizes the principle of immune-chromatography. This kit determines malarial infection by the detection of pan malaria specific pLDH released from parasitized red blood cells. Additionally, it determines the specific infection of *P. falciparum* by the detection of *P. falciparum* specific Pf HRP-2, a water soluble protein that is released from parasitized erythrocytes of infected individuals and is species specific.

SD Bioline Malaria Ag *P. vivax*/*P. falciparum*

The SD Bioline Malaria Pf/Pv test is a rapid immunochromatographic test for qualitative detection of antibodies of all isotypes (IgG, IgM, IgA) specific to *P. falciparum* and *P. vivax* simultaneously in human whole blood. Whole blood can be used for testing immediately or stored at 2-8°C for up to three days. This test is intended for initial screening and all positive specimens should be confirmed by microscopic examination.

Microscopy diagnosis

All of the samples obtained were first tested by RDTs, then the same samples were used to make thick and thin smears. A small number of smears were made from fresh capillary blood. The remaining smears were made from venous blood obtained from the patient added to an EDTA collection tube. These were stored at 2-8°C for between 24 to 48 h after being tested by RDT and prior to microscopic examination. The smears were processed by fixing the thin film in absolute methanol (methyl alcohol), heat fixed and stained with 10% Giemsa solution in buffered water, pH 7.2 for 10-12 min. After staining, the smears were rinsed with normal water, drained and air dried. They were then examined by light microscopy under 1000x magnification for malaria parasites, *Plasmodium species* and quantization. A malaria blood film was considered negative after 100 high power fields had been examined and no parasite observed. The parasite count per microliter of blood was obtained by using the formula: [parasite count/200 white blood cell (WBC)] × absolute WBC count.[13] If the parasite density was found to be more than 100 parasites/field in a thick film, the thin film was used for the count. Upon the observation of asexual malaria parasites, parasitized red blood cells (RBCs) were counted against 1000 RBCs. The parasite count per microliter of blood was obtained using the formula: (parasite count/1000 RBC) × absolute RBC count.[14] The thin film was used for species identification of detected malaria parasites.

Ethical approval

Ethical approval for this research was granted by the Ethical Review Committee of the Ministry of Health, Georgetown, Guyana, South America.

Table 1. Performance of different rapid diagnostic tests methods compared to Giemsa stain microscopy.

RTDs	Microscopy Positive	Negative	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%)	NPV (%)	Accuracy
Positive	172	25	90.5 (86.6-93.5)	94.2 (92.5-95.6)	87.3	95.8	93.1
Negative	18	409					

Mc Nemar test=0.36; P<0.05; Accuracy=93.3%; kappa=83.9. RTDs, rapid diagnostic tests; PPV, positive predictive value; NPV, negative predictive value.

Table 2. Diagnostic performances of Paramax, OptiMAL-IT, Omega and SD tests compared to that of microscopy.

RDTs	Microscopy Positive	Negative	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%)	NPV (%)	Accuracy (%)	χ^2	P value
PARAMAX									
Positive	84	11	98.8 (93.8-99.9)	94.9 (92.9-95.3)	88.4	99.5	96.0	247.19	<0.05
Negative	1	204							
OPTIMAL-IT									
Positive	57	4	80.3 (73.2-84.0)	97.9 (95.3-99.3)	93.4	93.1	93.2	177.9	<0.05
Negative	14	148							
OMEGA									
Positive	18	6	85.7 (67.4-95.8)	88.7 (81.4-92.7)	75.0	94.0	87.8	38.0	<0.05
Negative	3	47							
SD									
Positive	13	4	100 (78.7-100)	71.4 (51.6-71.4)	76.5	100	85.2	14.75	<0.05
Negative	0	10							

RTDs, rapid diagnostic tests; PPV, positive predictive value; NPV, negative predictive value.

Statistical analysis

Since none of the diagnostic methods tested in this study is considered as gold standard, statistical analysis was carried out according to Erhart *et al.,* Bhattarai *et al*. and Speybroeck *et al.*[15-17] The agreement between the two tests was evaluated by calculating positive and negative agreement indices according to the theory proposed by Graham and Bull.[18] Considering the readings of the two tests reported as either positive or negative, the values a, b, c and d denote the observed frequencies for each possible combination of ratings by tests 1 and 2.

a. the number of samples positive with both tests;
b. the number of samples negative with test 1 and positive with test 2;
c. the number of samples positive with test 1 and negative with test 2; and
d. the number of samples negative with both tests.

The proportion of specific agreement for the overall agreement (P_o), the positive ratings (P_{pos}) (the positive agreement index), and for the negative ratings (P_{neg}) (the negative agreement index) were calculated as follows:

$$Po = (a + d) / total$$
$$P_{pos} = 2a / 2a + b + c$$
$$P_{neg} = 2d / 2d + b + c$$

This method excludes the limitation of the kappa statistics. Mean, standard deviation, prevalence and confidence intervals were calculated using SPSS 11 software. The calculations of positive and negative agreement indices and overall agreement indices were made according to Erhart *et al.,* Bhattarai *et al.* and Speybroeck *et al*.[15-17]

Results

A total of 624 patients, aged 1-83 years (mean 33.6 years ± 14.4 years) suspected of malaria or presenting a history suggestive of malaria were included in the study. The study involved more males 443 (71%) than females 181 (29%).

Giemsa stain microscopy

The results of parasite detection by microscopy are shown in Tables 1 and 2. Diagnostic performances of the tests with their batch numbers are shown in Table 3. The Giemsa stain microscopy test reported 190 slide positive cases of malaria (30.5%). Positivity with RDTs was 31.6%. Out of these positive films, 163 slides (85.8%) were positive with mono-infection. Species determination identified 99 (52%) slides with mono-infection of *P. falciparum* (Pf), 63 (33%) slides with mono-infection of *P. vivax*, and one (0.5%) slide with mono-infection *P. malariae* (Pm). Mixed infections were recorded in 27 (14.2%) slides (Table 4).

Microscopy recorded parasite count with *P. falciparum* ranging from 24 to 105,600 parasites/µL (mean count 11,002.4 parasites/µL), *P. vivax* ranging from 96 to 163,800 parasites/µL (mean count 12,736.3 parasites/µL), *P. malariae* with mean count 1469 parasites/µL and *P. falciparum* gametocyte recorded 24 to 12,330 parasites/µL (mean count 2325.1 parasites/µL).

Rapid diagnostic tests

Paramax

Of the total 300 patients tested for malaria with Paramax against microscopy, 95 (31.6%) were Paramax positive compared to 85 (28.3%) positive by microscopy: 59 cases were *P. falciparum* positive, 28 cases were *P. vivax* positive, and 8 cases were both *P. falciparum* and *P. vivax* positive.

OptiMAL-IT

Of the 223 patients tested for malaria with OptiMal-IT against microscopy, according to OptiMAL-IT testing, 35 cases were *P. falciparum* positive, 23 cases were *P. vivax* and 3 cases both *P. falciparum* and *P. vivax* positive (Table 4).

VisiTect

Overall, 74 patients were tested for malaria with VisiTect against microscopy: 7 were *P. falciparum* positive, 7 *P. vivax* positive, and 10 cases both *P. falciparum* and *P. vivax* positive.

Standard Diagnostic

Of the 27 patients tested for malaria with SD against microscopy, 6 cases were *P. falciparum* positive, 5 cases were *P. vivax* positive and 6 cases both *P. falciparum* and *P. vivax* positive.

Concurrence between microscopy and rapid diagnostic tests

Comparison between microscopy and RDTs were assessed by calculating positive and negative agreement indices. Table 2 shows positive and negative agreement index among different RDT kits. A high positive agreement index was observed in all RDTs except SD.

Discussion

Malaria can be a life-threatening disease in a vulnerable population if not treated. Therefore, a quick and accurate diagnosis is very important. To prevent unnecessary antimalarial treatments, it is important to confirm clinical suspicions with a good laboratory test. RDTs for malaria are being increasingly adopted across endemic countries to strengthen parasitological diagnosis and appropriate management of all cases of fever. They are particularly valuable in areas which do not have good resources for microscopy.

Most RDTs only record the presence or absence of antigens but cannot measure the parasite density. These should, therefore, only be considered as an extended means of parasite based diagnosis where microscopy is absent due to its varied diagnostic applications and importance of supportive patient management.[4,19-21] The national malaria control programs of several countries have been using these RDTs as a definitive diagnostic tool where microscopy is not readily available to confirm suspected malaria cases.[12] However, in Guyana most of these RDTs are used in the private sector.

In this study, we assessed the field performance of the RDT among different hospital laboratory and private laboratories using conventional Giemsa stain thick and thin blood films. In the current study, more infections were detected by RDT than by blood slide microscopy.

Among all the four RDTs used in this study, OptiMAL had the lowest positive results compared to microscopy. This could be because some malaria infections detected by blood films were not detected by the OptiMAL test because the latter detects pLDH which is produced only by living parasites. It is possible that some of the patients infected with malaria medicated themselves when malaria symptoms appeared during this outbreak and did not report this to the attending clinician. Self treatment of malaria is widespread in Guyana (Kurup and Kumar; unpublished results, 2011). There are other possible explanations for discrepancies in test results obtained by blood film examination and by the OptiMAL test, including: i) insufficient detection of low parasitemia levels by OptiMAL; ii) the sequestration of parasites; and iii) false-positive reactions.

In areas where there is a high incidence of malaria, the lack of facilities undermines the benefits of RDTs. RDTs, however, are sensitive diagnostic tools for malaria. They are also simple to use and provide quick results without the need for good microscopic equipment and electricity, making them a good alternative to microscopy in endemic areas.[22]

Marx and others,[23] in their systematic review on the accuracy of RDT for malaria in returning travelers, indicates that, despite a low sensitivity, RDT will lead to the detection of most clinically relevant *P. falciparum* cases with considerably better accuracy than that to be expected from routine microscopy. The current study confirms that RDT in conjunction

with microscopy should improve the diagnosis of malaria. However, RDT use should be considered as more cost-effective in the areas characterized by high-moderate intensity malaria transmission and in situations where health services are inadequate or absent.[23]

On other hand, RDTs only record the presence or absence of antigens but cannot measure the parasite density. They should, therefore, only be considered to be an extended means of parasite based diagnosis where microscopy is absent due to its varied diagnostic applications and the importance of supportive patient management.[4,19-21]

Reports from elsewhere indicate that RDTs have shown a comparable level of accuracy to microscopy in clinical settings.[24,25] Even though the clinical history of the participants was not recorded in our study, evidence from other studies showed that RDT positive cases missed by microscopy might be individuals who had been treated but in whom antigenemia persists.[25,26] A potential alternative explanation for this level of false positives is sequestration: erythrocytes containing mature parasites clump together in the microvasculature and are, therefore, not seen in the peripheral circulation and blood films, while antigen continues to be released.[27] It may also be possible that the parasite density was too low to be

Table 3. Diagnostic performances of individual rapid diagnostic tests and their Batch number compared to that of microscopy.

RDTs	Microscopy Positive	Microscopy Negative	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%)	NPV (%)	Accuracy (%)	χ^2	P value
PARAMAX									
Batch 91111									
Positive	16	8	96.9(86.4-99.8)	97.0 (91.9-98.4)	93.9	98.5	96.9	85.0	<0.05
Negative	0	66							
Batch 91114									
Positive	31	2	100 (79.3-100)	89.2 (84.7-89.2)	66.7	100	91.9	53.5	<0.05
Negative	1	64							
Batch 91117									
Positive	27	1	100 (89.2-100)	98.1 (92.6-98.1)	96.4	100	98.8	75.7	<0.05
Negative	0	52							
Batch 91121									
Positive	10	0	100 (75.2-100)	100 (88.8-100)	100	100	100	32.0	<0.05
Negative	0	22							
OPTIMAL - IT									
Batch 0E0015M									
Positive	42	4	84.0 (74.9-89.1)	96.0 (91.5-98.6)	91.3	92.3	92	100.3	<0.05
Negative	8	96							
Batch 0K0027M									
Positive	15	0	71.4 (56.2-71.4)	100 (93.8-100)	100	100	91.8	46.7	<0.05
Negative	6	52							
OMEGA									
Batch 126013									
Positive	4	4	80 (33.3-98.9)	78.9 (66.6-83.9)	50.0	93.8	79.2	6.2	<0.05
Negative	1	15							
Batch 206041									
Positive	8	1	80 (51.8-89.5)	95.7 (83.4-99.8)	88.9	91.7	90.9	20.1	<0.05
Negative	2	21							
Batch 206043									
Positive	6	1	100 (60.7-100)	91.7 (72.0-91.7)	85.7	100	94.4	14.1	<0.05
Negative	0	11							
SD									
Batch 018110									
Positive	13	4	100 (78.7-100)	71.4 (51.6-71.4)	76.5	100	85.2	14.7	<0.05
Negative	0	10							

RTDs, rapid diagnostic tests; PPV, positive predictive value; NPV, negative predictive value.

Table 4. Comparison of slide microscopy and rapid diagnostic tests by species detected.

RDTs	Microscopy Neg	Pf	Pfm	Pfv	Pfvm	Pm	Pv	Pvm	Total
Neg	409 (65.5)	14 (2.2)	0.0	0.0	0.0	0.0	4 (0.6)	0.0	427 (68.4)
Pf	19 (3.0)	78 (12.5)	0.0	4 (0.6)	2 (0.3)	0.0	4 (0.6)	0.0	107 (17.1)
Pfv	1 (0.2)	6 (1.0)	3 (0.5)	14 (2.2)	0.0	0.0	3 (0.5)	0.0	27 (4.3)
Pv	5 (0.8)	1 (0.2)	0.0	3 (0.5)	0.0	1 (0.2)	52 (8.3)	1 (0.2)	63 (10.1)
Total	434 (69.6)	99 (15.9)	3 (0.5)	21 (3.4)	2 (0.3)	1 (0.2)	63 (10.1)	1 (0.2)	624

RTDs, rapid diagnostic tests. Pf, *P. falciparum*; Pfm, *P. falciparum* & *P. malariae*; Pfv, *P. falciparum* & *P. vivax*; Pfvm, *P. falciparum*, *P. vivax* & *P. malariae*; Pm, *P. malariae*; Pv, *P. vivax*; Pvm, P. vivax & *P. malariae*.

seen by microscopy but that there was sufficient parasite antigen to result in a positive RDT.[28]

Although molecular tests such as RDT should be preferred to microscopy, RDT testing for confirmation of malaria can not be used in countries like Guyana since the protocols are too cumbersome, too expensive, and are not simple or rapid, or even not available at all, because of limited resources such as a lack of electricity and inadequate laboratory infrastructure.[29]

In this study, both RDT and microscopy provided comparable results. Therefore, RDT in conjunction with microscopy should be used to improve the diagnosis of malaria. RDT use should be considered more cost-effective in the areas characterized by high-moderate intensity malaria transmission and in situations where health services are inadequate or absent.[30]

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A Study of Evaluation of Rapid Diagnostic Techniques of Malaria in Urban Slums of Vijayawada, Krishna District, Andhra Pradesh, India.

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ABSTRACT

200 clinically suspected malaria patients from Urban Slums of Vijayawada from June to November, 2009 were chosen for the study ranging ,from infants to 80 years old. Maximum number of cases were seen in the age group of 20-30 years. The mean age was 25 and SD of 12.82.The male to female ratio was 2.1: 1, showing a male predominance. 62 cases were positive by peripheral smear.Out of which 50 (81%) were positive for *Plasmodium vivax* and 12 (19%) cases were positive for *Plasmodium falciparum*. One case was positive for both. The cases which were not detected by peripheral smear and SD-Bioline were detected by Paramax-3 kit which showed a sensitivity and specificity of 88% and 100% respectively for pLDH (*Plasmodium vivax*), sensitivity and specificity of 83.3% and 98.9% for HRP-2 (*Plasmodium falciparum*). The SD-Bioline test results indicated that 22% (44 of 200) of the patient samples were positive for malaria parasites,showing a sensitivity and specificity of 74% and 100% for *Plasmodium vivax* ,Sensitivity of 58.33% and specificity of 100% for *Plasmodium falciparum*. The sensitivity of SD-Bioline for *Plasmodium vivax* was as low as 74% while that of Paramax-3 was 88%.The sensitivity of SD-Bioline for *Plasmodium falciparum* was as low as 58.33% while that of Paramax-3 was 88.3%.

Keywords: *Plasmodium*, Specificity, Sensitivity, Peripheral smear.

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INTRODUCTION

Malaria is a major global health problem. It continues to afflict the poor nations and is among the top ten killer diseases of the world. More than 3 million people worldwide live in malaria in endemic areas. Every year 300-500 million people are infected with malaria and 1.5-2 million of these die from disease, 90% of which occur in tropical Sahara. Outside Africa, 70% of the remaining cases occur in just 3 countries, India, Srilanka and Brazil. The global dream of eradicating malaria has remained elusive because of several complex issues that pose a challenge to the health system. In 1992, the global malaria control strategy was introduced with 4 basic elements- early diagnosis, prompt treatment, selective and sustainable vector control and early forecasting of epidemics; yet the desired results could not be achieved. Roll back malaria initiative launched by WHO in 1998 as a global project brought together the biggest players in health system but again with no satisfactory outcome. Clinical diagnosis is imprecise but remains the basis of therapeutic care for the majority of febrile patients in malaria endemic areas, where laboratory support is often out of reach. Scientific quantification or interpretation of the effects of malaria misdiagnosis on the treatment decision, epidemiologic records, or clinical studies has not been adequately investigated. Despite an obvious need for improvement, malaria diagnosis is the most neglected area of research, accounting for an expenditure less than 0.25% ($ 700,000) out of U.S. $323 million which was invested for research and development in 2004 (Chansuda Wongsrichanalai, Trop. Med. Hyg., 2007).

In India malaria has been a serious problem in north eastern states mainly due to topography and climatic conditions which are congenital for perennial malaria transmission. About 70% of infections are reported to be due to *Plasmodium vivax*, 25-30% due to *Plasmodium falciparum* and 4-8% due to mixed infection. *Plasmodium* malariae has a restricted distribution and is responsible for <1% of infections in India.Even with the implementation of modified plan of operation (MPO) which was launched in 1984, the epidemiological situation has not shown any great improvement in our country. During the year 2001, the Annual parasite incidence is 2.04.The identified malaria priority areas are forests, forested foot hills, forest fringe areas and developmental project sites.

One main element of global malaria control strategy for effective management is prompt and accurate diagnosis. In many endemic countries, the current approach to malarial diagnosis, especially, in peripheral health canters, is entirely based on clinical diagnosis, which is of limited accuracy due to the poor specificity of symptoms and signs of malaria. Consequently, presumptive antimalarial treatment is widely administered for any fever with no obvious alternative cause, leading to significant overuse of antimalarial drugs. In addition, over- diagnosis of malaria in the formal health care sector often co-exists with under diagnosis of malaria in the community.With chloroquine and sulfadoxine-pyrimethamine resistance becoming widespread and more effective but expensive antimalarial medicines, including artemisinin-based combination therapies, being used in most of countries, there is a need to improve the diagnosis of acute febrile illness at various levels of health care system; this is now a public health priority, as it would ensure that antimalarial drugs can be targeted to patients who need them.

In general view is that the “gold standard” method for malaria diagnosis is the detection of *Plasmodium* species by microscopic examination of blood films. This method is

relatively simple and has low direct costs, but it is labour-intensive, time-consuming, and requires well- trained personnel who can differentiate between the different *Plasmodium* species. Alternative diagnostic tests for malaria and in particular rapid diagnostic tests (RDTs) have been developed over the past 20 years. These tests are fast easy to perform, and do not require electricity or specific equipment. They include those based on histidine-rich protein 2(HRP2) alone or a modified test format of HRP and parasite-specific aldolase enzyme (pan-malarial antigen), parasite lactate dehydrogenase (pLDH), and qualitative detection of antibodies of all isotypes (IgG, IgM, IgA). The technical performances of these alternative techniques have already been assessed in various populations and epidemiologic settings. Stipulations for these rapid tests include the capability to detect 100 parasites/µl from all *Plasmodium* species and the ability to perform semi quantitative measurements for monitoring drug treatment results. However, the main limitation of the majority of these studies was that microscopy was used as the "gold standard". The majority of malaria cases are found in countries, where cost-effectiveness is an important factor and ease of diagnostic test performance and training of personnel are also major considerations. Most new technology for malaria diagnosis incorporates immunochromatographic capture procedures, with conjugated monoclonal antibodies providing the indicator of infection. Preferred targeted antigens are those which are abundant in all asexual and sexual stages of the parasite.

The main aim of this study was to assess the accuracy of available RTDs for the diagnosis of malaria, as a first step to improve malarial management at different levels of the health care system in Vijayawada, where malaria is endemic. So I made this study to compare 2RDTs (PARAMAX-3 Pan/Pv/Pf and SD BIOLINE Malaria P.f/P.v tests) with the conventional microscopy, using blood samples of patients with clinical suspicion of uncomplicated malaria at urban slums of Vijayawada.

MATERIALS AND METHODS

It is a prospective case study consisting of 200 clinically suspected malaria patients from Urban Slums of Vijayawada . The processing of samples was done in the Department of Microbiology, Siddhartha medical college, Vijayawada. The **inclusion criteria** in this study group consist of fever with chills and rigors followed by sweating. After obtaining brief clinical history about the duration of symptoms, age, sex, occupation, socio-economic status and past medication, the samples were collected. The **exclusion criteria**: Patients who have already taken anti-malarial drugs are not included. Fever with defined cause is excluded.**Collection of Samples:** (Monica Cheesbrough, 2002) 2ml of intravenous blood was collected with a sterile syringe and is transferred into sterile bottle containing EDTA i.e., anticoagulant.**Processing of Blood Samples:** (G.K. Sharma, 1998).Thin and Thick Smears were prepared from the collected blood sample. After smear preparation, smears were stained with Leishman's stain. The blood sample was subjected to serological tests (Rapid Diagnostic Tests):

To detect plasmodial antigen: A third generation Rapid Diagnostic test for malaria (PARAMAX-3Pan/Pv/pf. Manufactured by Zephyr Biomedicals. M.L.No: 558. Lot No: 91061. Mfg.Dt: 10-2008. Exp.Dt: 09-2010) was used. Paramax-3 malaria Pf/Pv/Pan test is an

immunochromatographic test. Procedures were followed strictly as contained in the manufacturer's standard operating manual inserted in the kit.

To detect plasmodial antibody: A rapid one step malaria anti-*Plasmodium falciparum* and *Plasmodium vivax* test (SD-Bioline One Step Malaria Anti-P.f/ P.v test. Manufactured by SD BIOLINE STANDARD DIAGNOSTICS PVT.LTD. Lot No: 18043. Mfg.Dt: 21-10-2008. Exp.Dt: 20-04-2010), was used alongside the gold standard (microscopy). The SD Bioline malaria Pf/Pv test is an immunochromatographic test for the qualitative detection of the antibodies of all isotypes (IgG, IgM and IgA) specific to P.*falciparum* and P.*vivax* simultaneously in human serum, plasma or whole blood. The test cassette contains a membrane strip, which is precoated with recombinant malaria P.f capture antigen on test b and 1 region and with recombinant P.v capture antigen on test b and region 2. Procedures were followed strictly as contained in the manufacturer's standard operating manual inserted in the kit. The RDTs for malaria (Paramax-3 and SD-Bioline) are compared with microscopy.

RESULTS

Statistical software: The statistical software namely SPSS11.0 and Systat8.0 were used for the analysis of the data and Microsoft and Excel have been used to generate graphs, tables etc.

A total of 200 blood samples were tested for malaria parasites by both Paramax-3, SD-Bioline methods, and the results were compared to results obtained from reading thin and thick- smear blood films. The blood film results indicated that 31% (62 of 200) of the patients were infected with malaria based on the morphologies of the parasite stages.Table 1: Among them, P. *vivax* was present in 50(25%) samples while P.*falciparum* was present in 12(6%). [The one mixed infection has been tabulated with the P.*falciparum* infection numbers for ease of analysis].Correspondingly, the Paramax-3 test results indicated that 28% (56 of 200) of the patient samples were positive for malaria parasites. Infections with P. *vivax* accounted for 44(22%) samples, while infections with P.*falciparum* accounted for 12(6%)of the total malaria cases. Both methods identified one patient with a mixed infection of P. *falciparum* and P. *vivax*. [The one mixed infection has been tabulated with the P.*falciparum* infection numbers for ease of analysis].

The SD-Bioline test results indicated that 22% (44 of 200) of the patient samples were positive for malaria parasites. Infections with P. *Vivax* accounted for 37(18.5%) samples, while infections with P.*falciparum* accounted for 7(3.5%) of the total malaria cases. Both methods identified one patient with a mixed infection of P. *falciparum* and P. *vivax*. (The one mixed infection has been tabulated with the P.*falciparum* infection numbers for ease of analysis) . In the present study the patient's age group ranged from infants to elderly. According to (Table-2), the maximum number of cases is seen in the age group of 20-30 years .The mean age was 25 years.

Table-3 shows sex-wise distribution of malaria. Out of 102 samples tested in males, 42 were positive for malaria with a percentage of 67.74%. Among 98 samples tested in females 20 were positive for malaria with a percentage of 32.25%. It showed male predominance with male to female ratio of 2.1:1.

Table-4 & 5: Paramax-3 compared to traditional blood films for detection of P. *vivax* (Table-4) and P.*falciparum* (Table-5) infections .Sensitivity= 4400/ 50=88%, Specificity= 15000/ 150= 100%. Positive Predictive Value=4400/ 44= 100%, Negative Predictive Value=150 00/156= 96%.The blood films identified 6 P.*vivax*- positive samples that were not identified by the Paramax-3 test; however, there was 100% agreement between blood film results and Paramax-3 results for the other 44 samples containing P. *vivax*.Paramax-3 had sensitivity of 88% (95% confidence interval, 85.2 to 97.6%) and specificity of 100% (95% CI, 96.2 to 100.0%), when compared to traditional blood films for the detection of P. *vivax* (Table 4). Positive and negative predictive values were 100% (95% CI, 93.9 to 100.0%) and 96% (95% CI, 95.5 to 99.8%), respectively, for P. *vivax*.Sensitivity=1000/12=83.3%, Specificity=18600/188= 98.9%, Positive Predictive Value=1000/12= 83.3%, Negative Predictive Value= 18600/188=98.9%.Although both methods detected 12 cases of P. *falciparum* infection, there were 2 cases detected by Paramax-3 that were not detected by the blood films and 2 cases detected by blood film that were not detected by the Paramax-3 method. Paramax-3 had sensitivity of 85.7% (95% CI, 62.3 to 97.9%) and specificity of 98.9% (95% CI, 95.5 to 99.8%), when compared to traditional blood films for the detection of P.*falciparum* infections (Table 5). Positive and negative predictive values were 85.7% (95%CI, 62.3 to 97.9%) and 98.9% (95% CI, 95.7 to 99.8%), respectively, for P. *falciparum*.

Table 6 & 7: SD-Bioline test compared to traditional blood films for detection of P. *vivax* (Table-6) and P.*falciparum* (Table-7) infections:Sensitivity= 3700/50= 74%, Specificity=15000/150= 100%, Positive Predictive Value= 3700/37=100%, Negative Predictive Value= 15000/180= 83.33%.The blood films identified 13 P.*vivax* positive samples that were not identified by the SD-Bioline test; however, there was 100% agreement between blood film results and SD-Bioline results for the other 37 samples containing P. *vivax*.SD-Bioline had sensitivity of 74% [95% confidence interval [CI], 85.2 to 97.6%] and specificity of 100% (95% CI, 96.2 to 100.0%) when compared to traditional blood films for the detection of P. *vivax* infections (Table 6). Positive and negative predictive values were 100% (95% CI, 93.9 to 100.0%) and 83.33% (95% CI, 95.5 to 99.8%), respectively, for P. *vivax*.Sensitivity= 700/ 12= 58.33%, Specificity=18800/188= 100%, Positive Predictive Value= 700/12= 100%, Negative Predictive Value= 18800/19= 97.4%.The blood films identified 5 P. *falciparum* positive samples that were not identified by the SD-Bioline test; however, there was 100% agreement between blood film results and SD-Bioline results for the other 7 samples contains P. *falciparum*. SD-Bioline had sensitivity of 58.33% (95% CI, 62.3 to 97.9%) and specificity of 100% (95% CI, 95.5 to 99.8%), when compared to traditional blood films for the detection of P.*falciparum* infections (Table 7). Positive and negative predictive values were 100% (95%CI, 62.3 to 97.9%) and 97.4% (95% CI, 95.7 to 99.8%), respectively, for P. *falciparum*.

Table No.8 shows comparison of various methods of malaria diagnosis. The SD-Bioline gave the worst comparative results with a percentage of 22% of total positivity, while Paramax-3 gave a percentage of 28% of positivity when compared with peripheral smear which got a percentage of 31% of positivity.

Table No.9 shows comparison of various methods of malaria diagnosis for P.*vivax*. The SD-Bioline gave the low comparative results with a percentage of 18.5%, while aramax-

3 gave a percentage of 22% for P.*vivax*, when compared with peripheral smear which got a percentage of 25%.

Table No.10 shows comparison of various methods of malaria diagnosis for P.*falciparum*. The SD-Bioline gave the low comparative results with a percentage of 3.5%, when compared with Paramax-3 and peripheral smear which got a percentage of 6% for P.*falciparum*.

Table-1: Results of the Present Study: (n= 200)

Total no. of Samples tested	Number positive for Pv/Pf				Negative for malaria
	Pv alone	Pf alone	Pv+Pf	Total +ves	
200	50(25%)	11(5.5%%)	01(0.5%)	62(31%)	138(69%)

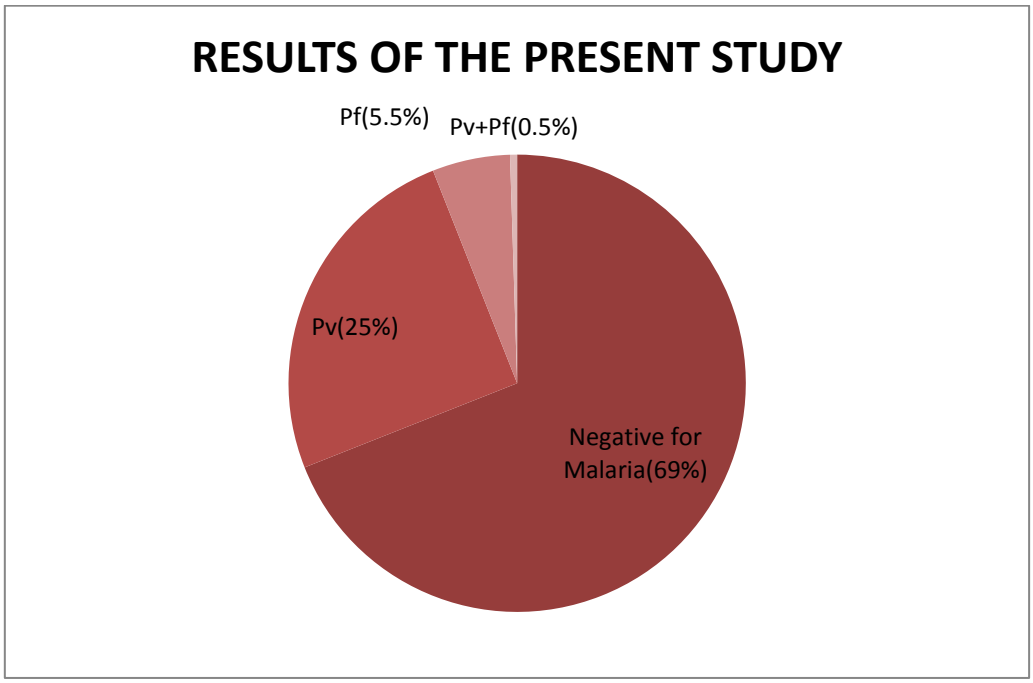


Table-2: Age-wise distribution of malaria in the present study: (n=200)

Age in Years	Total No. Of samples tested (%)	Out of total, No. Of samples positives (%)
00-10	14 (7%)	01 (1.61%)
11-20	20 (10%)	02 (3.22%)
21-30	42 (21%)	23 (37.09%)
31-40	33 (16.5%)	16 (25.80%)
41-50	31 (15.5%)	10 (16.12%)
51-60	30 (15%)	06 (9.67%)
61-70	29 (14.5%)	04 (6.45%)
71-80	01 (0.5%)	00 (0.0%)
Total	200 (100%)	62 (100%)

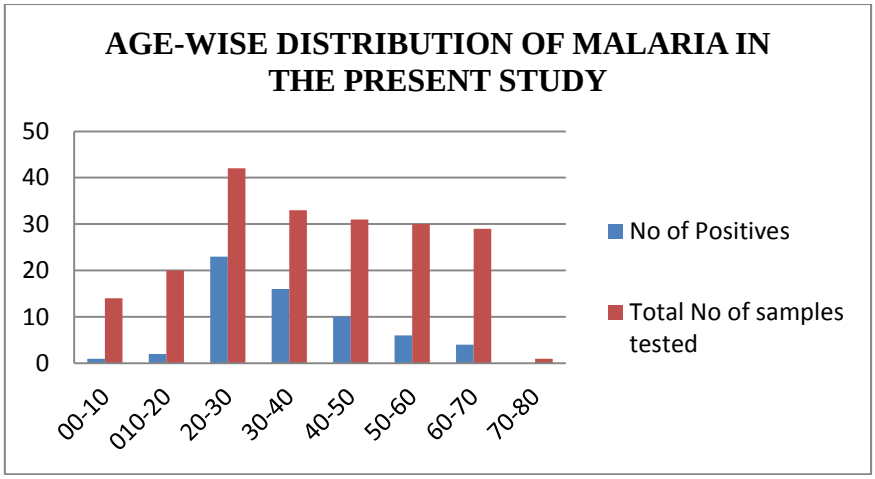


Table-3: Sex-wise distribution of malaria: (n=200)

Sex	Total No. of cases (%)	No. of positives (%)
Males	102 (51%)	42 (67.74%)
Females	98 (49%)	20 (32.25%)
Total	200 (100%)	62 (99.99%)

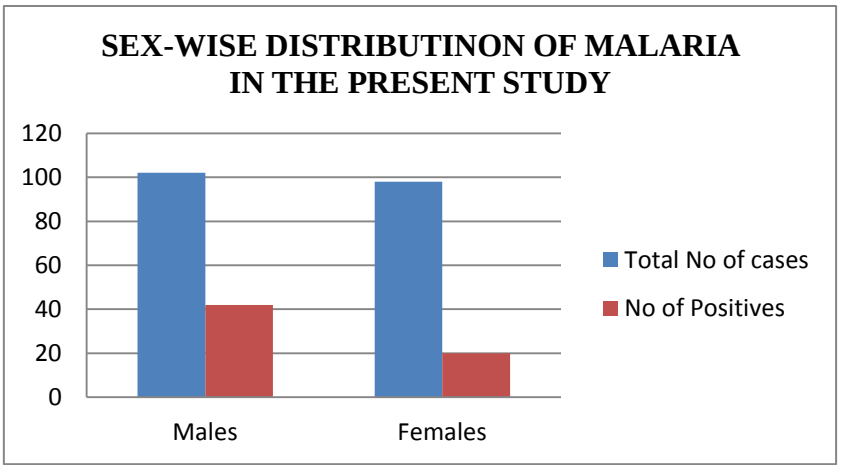


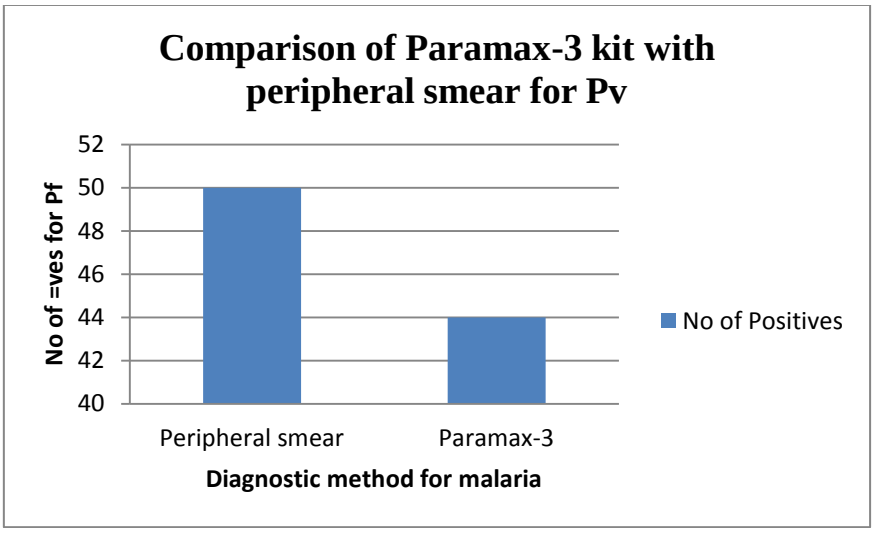
Table-4 & 5: Paramax-3 compared to traditional blood films for detection of P. vivax (Table-4) and P.falciparum (Table-5) infections:

Table-4: For P. vivax: (n=200)

Data	+ve for Pv by Smear Examination	-ve for Pv by Smear Examination	Total
+ve for Pv by Paramax-3	44	00	44
-ve for Pv by Paramax-3	06	150	156
Total	50	150	200

Table-5: For P. falciparum: (n=200)

Data	+ve for Pf by Smear Examination	-ve for Pf by Smear Examination	Total
+ve for Pf by Paramax-3	10	02	12
-ve for Pf by Paramax-3	02	186	188
Total	12	188	200



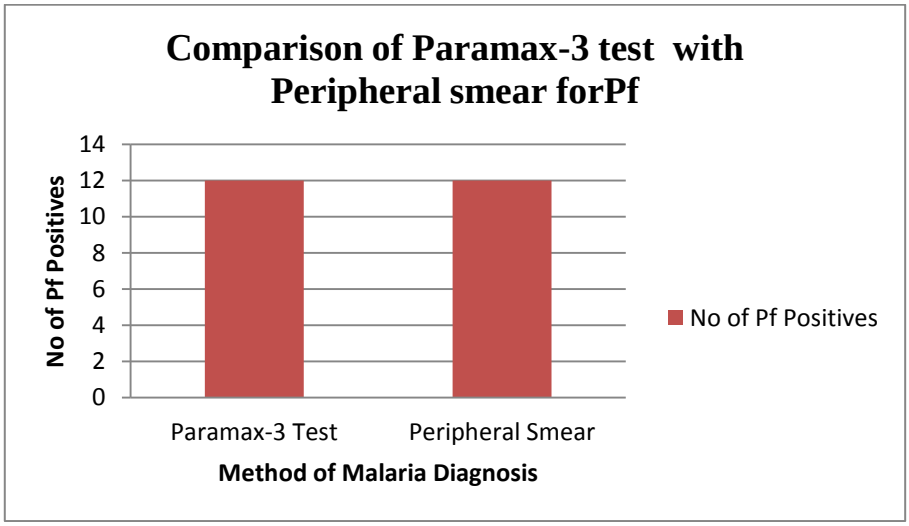
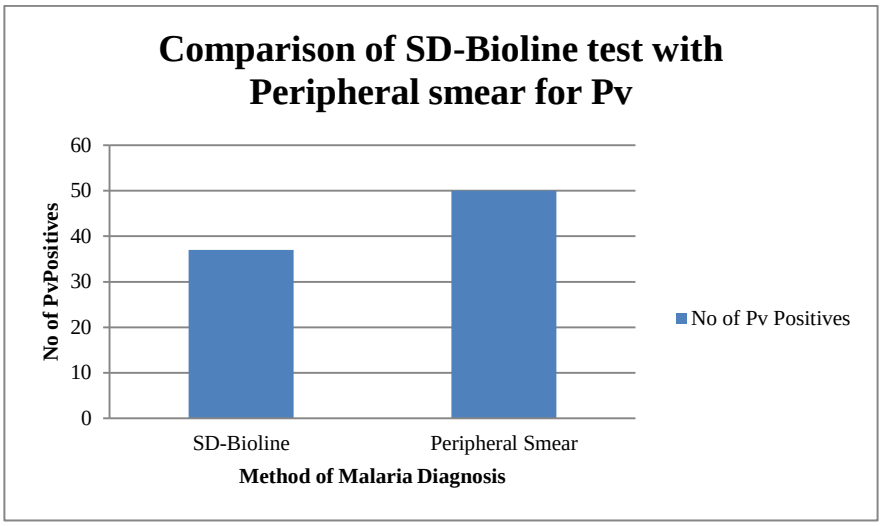


Table 6 & 7: SD-Bioline test compared to traditional blood films for detection of P. vivax (Table-6) and P.falciparum (Table-7) infections:
Table: For P. vivax: (n=200)

Data	+ve for Pv by smear examination	-ve for Pv by smear examination	Total
+ve for Pv by SD-Bioline	37	00	37
-ve for Pv by SD-Bioline	13	150	180
Total	50	150	200

Table 7: For P.falciparum: (n=200)

Data	+ve for Pf by smear examination	-ve for Pf by smear examination	Total
+ve for Pf by SD-Bioline	07	00	07
-ve for Pf by SD-Bioline	05	188	193
Total	12	188	200



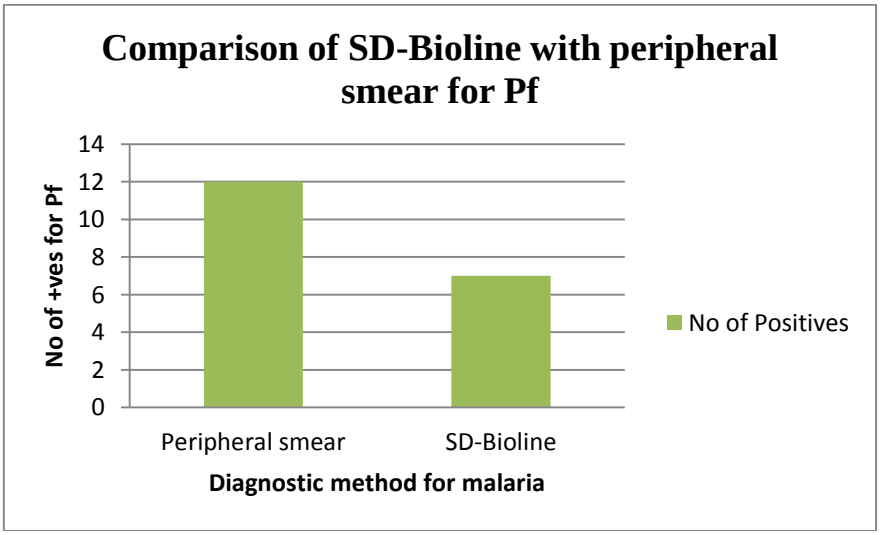


Table-8: Comparison of various methods of malaria diagnosis employed in the present study: (n=200)

Total No. of samples tested	+ve by smear	-ve by smear	+ve by Paramax-3	-ve by Paramax-3	+ve by SD-Bioline	-ve by SD-Bioline
200	62(31%)	168(69%)	56(28%)	144(62%)	44(22%)	156(78%)

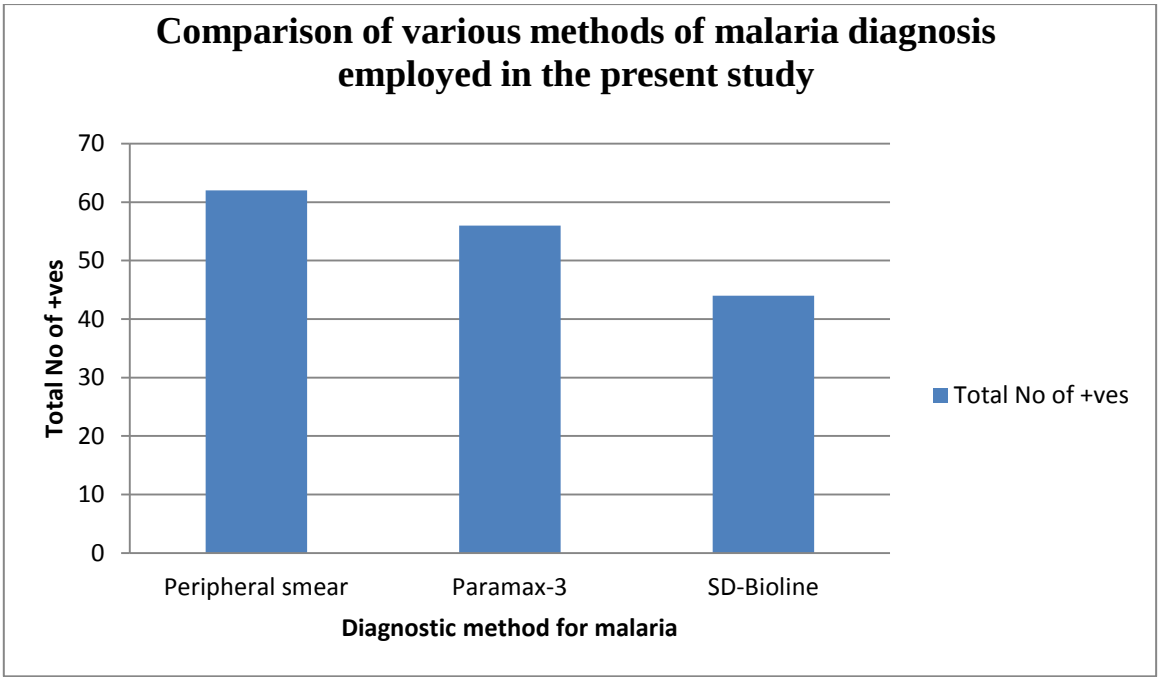


Table-9 Comparison of various methods of malaria in diagnosis of P.vivax in the present study: (n=200)

No. of samples tested	+ve for Pv by smear	-ve for Pv by smear	+ve for Pv by Paramax-3	-ve for Pv by Paramax-3	+ve for Pv by SD-Bioline	+ve for Pv by SD-Bioline
200	50(25%)	150(75%)	44(22%)	156(88%)	37(18.5%)	163(81.5%)

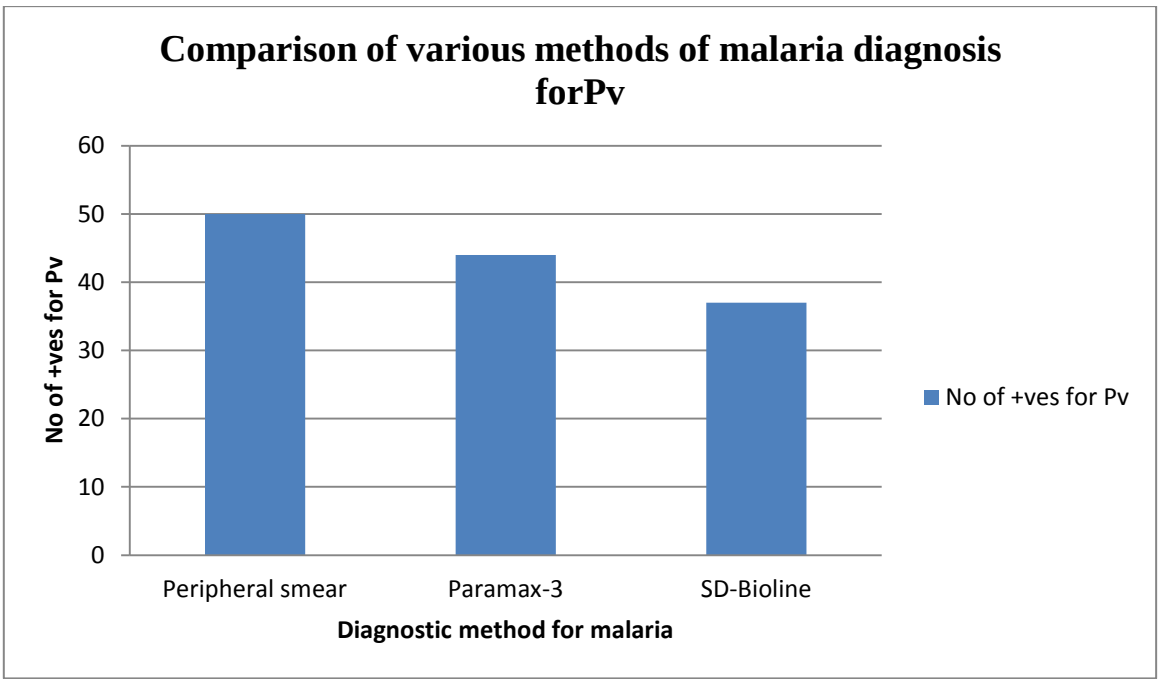


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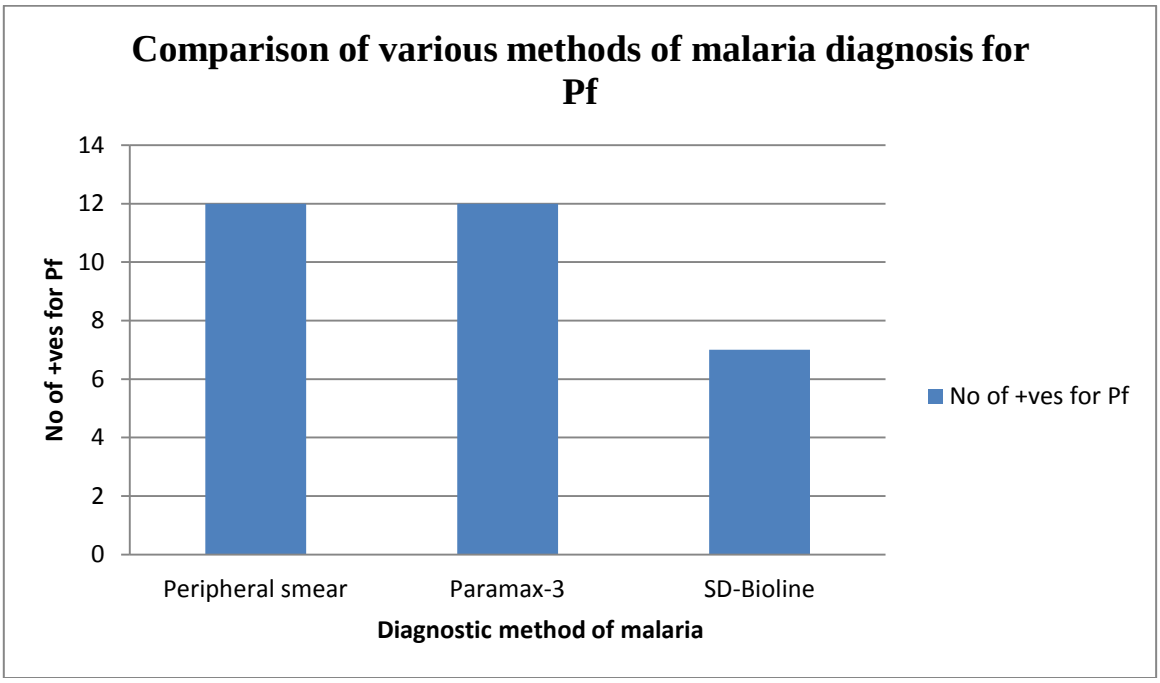
Table-10) Comparison of various methods of malaria diagnosis for P.falciparum in the present study: (n=200)

No. of samples tested	+ve for Pf by smear	-ve for Pf by smear	+ve for Pf by Paramax-3	-ve for Pf by Paramax-3	+ve for Pf by SD-Bioline	-ve for Pf by SD-Bioline
200	12(6%)	188(94%)	12(6%)	188(94%)	7(3.5%)	193(96.5%)



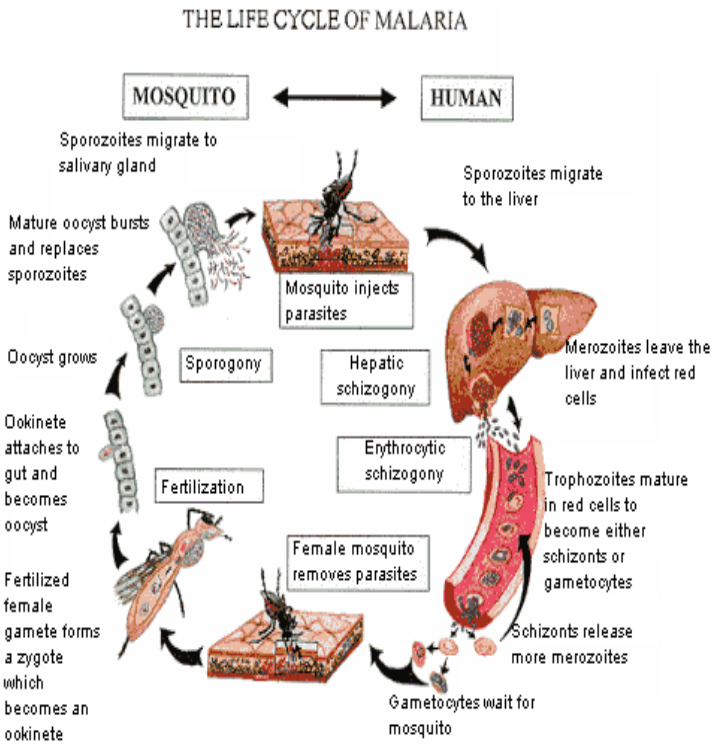


Fig-2, Plasmodium vivax schizonts (Leishman's stain 1000x)

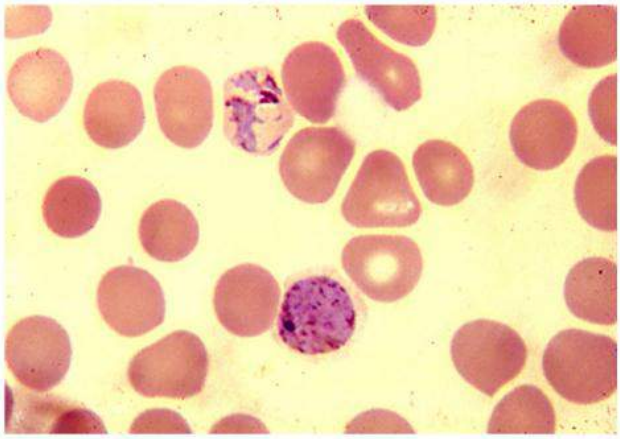


Fig-3, Plasmodium vivax rings and schizont
(Leishman's stain 1000x)
Fig-4, Plasmodium falciparum rings (Leishman's stain,1000x)

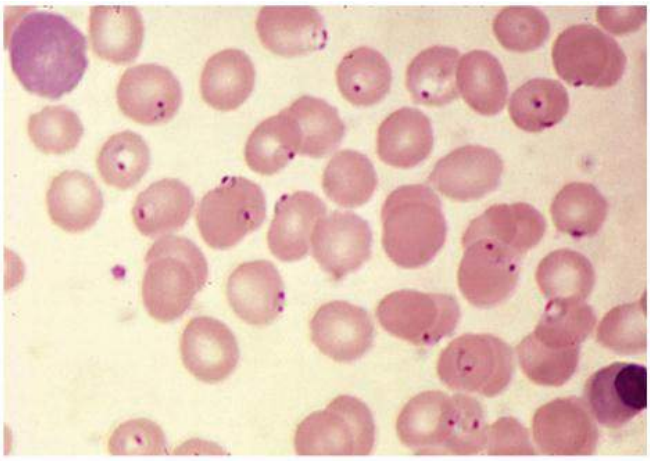
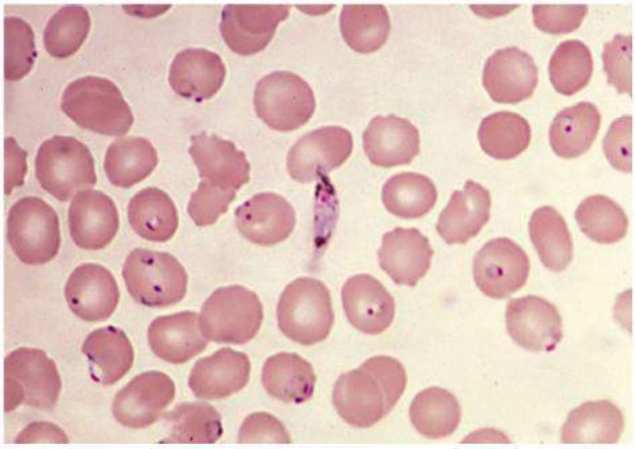


Fig-5, Plasmodium falciparum gametocytes and rings
(Leishman's stain, 1000x)



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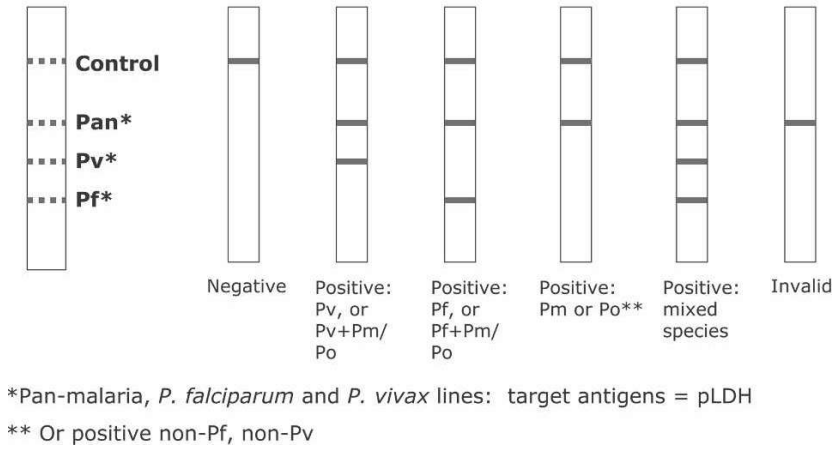

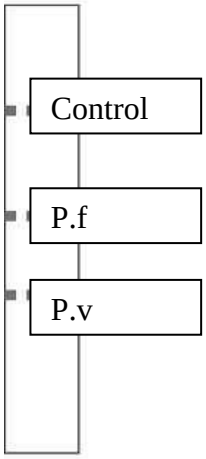

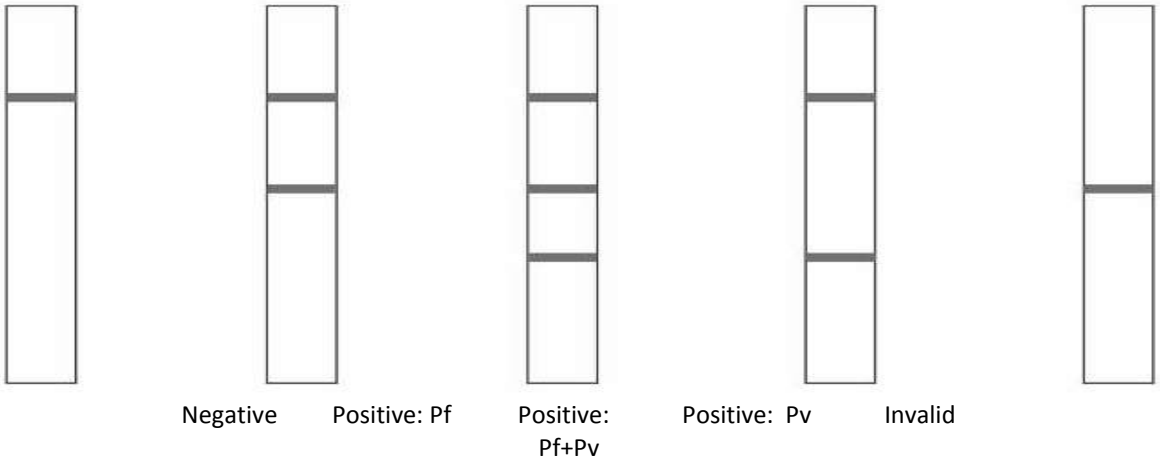

2. Plasmodium vivax schizont and rings

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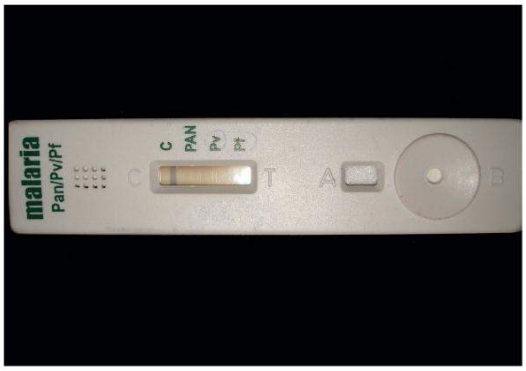
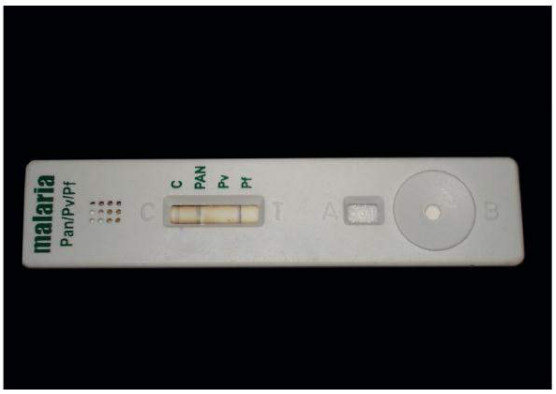
Paramax-3 Kit

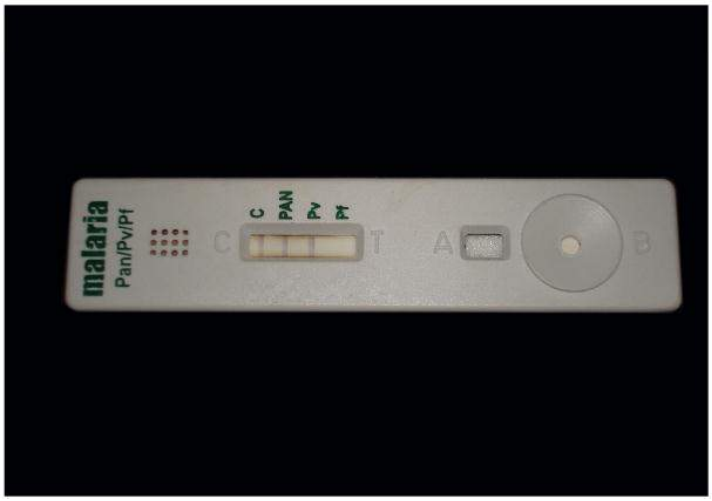
SD-Bioline Kit



Negative (Paramax-3 kit)



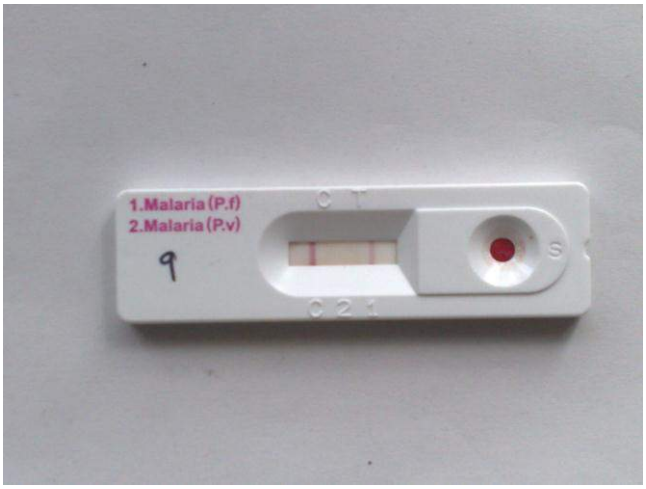
Plasmodium falciparum positive (Paramax-3 kit)



Plasmodium vivax positive (Paramax-3 kit)

Mixed infection (Paramax-3 kit)

Negative (SD-Bioline kit)



Plasmodium falciparum positive (SD-Bioline kit)

Mixed infection (SD-Bioline kit)

DISCUSSION

Malaria is a life threatening infection with a global impact extending from the most developed countries to regions of the world with only the most basic of health care infrastructure and the regions where malaria is highly endemic are increasing the need for rapid, prompt and accurate diagnosis. Endemic malaria, population movements and foreign travel – all contribute to malaria diagnostic problems in the Laboratory. Changing patterns of accepted morphological appearances of parasites, possibly due to drug pressure, strain variation, or approach to blood collection, have created diagnostic problems that cannot easily be resolved merely by reference to an Atlas. Even today microscopy still remains the most widely used method and the gold standard in malaria diagnosis in India.

In remote urban slums, control of malaria is a great challenge. Strengthening national capabilities to provide early diagnosis and treatment both within and outside the health services is of highest priority in WHO's action plan for malaria control 1995 – 2000 (WHO 1996).Currently, management of malaria by the Indian National Anti-Malarial programme (NAMP) is based on presumptive treatment of fever cases. Because symptoms lack specificity, moat diagnoses are inaccurate, resulting in both overtreatment with

antimalarial agents and undertreatment of those with other illnesses (Taylor & Mtambu, 1986). This places the population at undue risk of side-effects with no resulting benefit from the treatment and wastes drug supplies.

In view of this I have taken the recently developed Immunochromatographic tests i.e Paramax-3 (P.f/P.v/Pan malaria test) and the SD-Bioline(P.f/P.v malaria test) that was launched into the market and used in urban slums of Vijayawada. The RDTs results are compared with traditional blood film examination. I was able to carry out the test without any difficulty and achieved excellent results.Blood film examination still remains the gold standard in the diagnosis of malaria (Crooke et. al., 1999). Blood obtained by a finger or ear lobe is ideal because the density of parasites is greater in blood from this capillary rich area. The thick and thin smears can be utilized for diagnosis alone (or) in combination by employing malaria stains available (Giemsa, Leishman's Wright's, Field's stain, JSB stain). Peripheral smear is the standard, cost effective diagnostic technique for detection and differential of *Plasmodium* species.

It has several limitations like time consuming, labour intensive and requires the service of skilled technician. Further diagnosis of malaria can be missed if the parasite count is less than 60/µL of blood. This could be the possible reason for the failure to detect malaria in two samples which were smear negative and Paramax-3 positive in the present Study (Table-5). More over as P.*falciparum* may sequester in the deep capillaries the infection may easily be missed because there are insufficient number of parasites for detection in blood films (Moody, 2002).

In the present study out of 200 samples, the blood film results indicated that 31% (62 of 200) of the patients were infected with malaria based on the morphologies of the parasitic stages. Among them, P. *vivax* was present in 50(25%) samples while P.*falciparum* was present in 12(6%). [The one mixed infection has been tabulated with the P.*falciparum* infection numbers for ease of analysis].

The prevalence rate of P. *falciparum*, in this study found to be 6%, while the overall malaria prevalence was 31%. This prevalence rate was found to be lower than the earlier studies. Since the subjects were selected randomly from clinically suspected patients, it is possible that this could have influenced the prevalence rate of malaria infection in this study. The malaria prevalence rate in this study is however in consonance with previous studies.

The percentage of positivity by peripheral smear compared with other studies.

Study Series	Percentage (%)
Shiff et al., (1993)	50.80%
Kodsingh et al., (1997)	46.90%
Tarimo et al.,(1999)	52.00%
Pawan et al., (2000)	26%
P.U.Agomo et al., (2003)	35.83%
Das et al., (2003)	18%
Jeremaih et al., (2005)	40.98%
Zaccheaus AJ et al., (2007)	27.5%
Present Study	**31.00%**

Malaria effects all ages. In the present study, it is commonly seen in the age group 20-30 years with mean age of 25 and SD of 12.82.

Mean and Standard Deviation

Study Series	Mean	S.D (Years)
Rickman et al.,(1989)	30.6	15.9
Kodsingh et al.,(1997)	26	15.8
Mills et al., (1999)	39	-
Present Study	**25**	**12.82**

Males are more frequently exposed to the risk of acquiring malaria than females. Present study showed male predominance with ratio 2.1:1

Study Series	Ratio
Ugen et al., (1995)	1.3:1
Kodsinghe et al., (1997)	2:1
Mishra et al., (1999)	3:1
Singh et al.,(2001)	1.9:1
Present Study	**2.1:1**

Paramax-3 Kit

In the current scenario, Paramax-3 test is more accurate diagnostic technique as it is based on presence or absence of antigen of the parasite, HRP-2 & pLDH. In areas where microscopy is not readily accessible and it can take 4-6 weeks before slide results are available (Singh et al, 1996) which causes delay in the diagnosis and treatment of cases contribute to the continuing transmission. Whereas this RDT method requires a small amount of (5-6oμl) blood for the test to be performed, the results are obtained within 3-5 minutes and interpretation is easy depending on the presence or absence of a line on the test strip.

Further its need is increased in case of cerebral malaria, intravascular haemolysis where immediate and reliable diagnosis is very important. It can also be used for post treatment evaluation of P.*vivax* cases as pLDH antigen becomes negative immediately after effective treatment because antigen can be detected only in live parasites. The disadvantages include cost factor (the cost of the ICT malaria P.f/P.v (US$ 1.2 per test) is too high (Tjitra et al, 1999), and the test remains positive even after one week of Antimalarial treatment. This persistence can be due to persistent viable asexual stage of parasite below the detection limit of microscopy and delayed clearance of circulating antigen.

Sensitivity and Specificity of pLDH compared with other studies

pLDH	Sensitivity	Specificity
Robert Piper et al., (1994)	100%	100%
Carol J Palmer et al.,(1998)	94%	100%
Makler MT et al., (1998)	95%	100%
Anthony Moody et al., (2002)	100%	100%
Huong N M et al., (2002)	100%	100%
Moody,Anthonyet al., (2002)	96%	100%
Present Study	**88%**	**100%**

The sensitivity and specificity of HRP-2 antigen

Study Series	Sensitivity	Specificity
Premji et al., (1994)	89%	84%
Dietz et al., (1995)	89%	97%
Carballic and Acheet al., (1996)	86%	99%
Humar et al., (1997)	88%	77%
Singh et al (1997)	93%	92%
Anthony Moody et al.,(2002)	100%	100%
Huong NM et al.,(2002)	95%	97.2%
Present Study	**83.3%**	**98.9%**

SD- Bioline Kit

The SD Bioline malaria Pf/Pv test is an immunochromatographic test for the qualitative detection of the antibodies of all isotypes (IgG, IgM and IgA) specific to P.*falciparum* and P.*vivax* simultaneously in human serum, plasma or whole blood.

SD-BIOLINE was not able to detect some positive cases which Paramax-3 and microscopy could detect. However one interesting aspect of SD-Bioline is that the manufacturers used two surface proteins, namely, merozoite surface protein (MSP-1) and circumsporozoite protein (CSP) which has previously been documented to induce potent antibodies. They have been put forward as strong candidates for malaria vaccine development.

Several studies have been done on some of the rapid strips like Optimal (Jelinek et al., 1999; Cooke et al., 1999), SD-Bioline (Cavanagh et al., 1998), ICT (Singh et al., 2000) and others. Irrespective of the type of rapid malaria screening strip used, it was found that the only measurement for evaluating the diagnostic value of the strip to determine the sensitivity and specificity using the gold standard as a basis of comparison (Tarazon et al., 2004; WHO, 1995). The sensitivity of most of the reagent srips were found to reduce with decrease in parasite density, thus suggesting that they are most useful in areas where malarial endemicity prevails (Tarazon et al., 2004).

In this study, the SD-Bioline P.f/P.v RDT sensitivity was found to be low (58.33%) while the specificity was high (100%) for the diagnosis of P.*falciparum*.

This finding is similar to the observation of Agomo P.V et al.,(2003) in whose report, the SD-Bioline's sensitivty was reported to be 54.84%. The PPV and NPV of 58.0% and 68.0% respectevely, are however at variance with the PPV (100%), NPV (97.4%) which is obtained in this study. The specificity value of 42.9% in his report is also at varience with the specificity of 100% obtained in this study.

My study shows more or less similar results to that of Zaccheaus Awortu Teremiah et al., (2007) in Port Hercourt, who has reported a sensitivity of 47%, specificitity of 100%.a positive predictive value of 100% and a negative predictive value of 83.2% for P.*falciparum*

CONCLUSION

The major advantages of peripheral smear study are , it is least expensive, species differentiation is clear and quantisation of parasitaemia is possible. Through the technique of staining is simple, it is time consuming, labour intensive and requires the service of a skilled technician.

Paramax-3 test meets many of the criteria for an ideal diagnostic test, it is simple, rapid, sensitive, specific easy to perform and does not require any equipment.

This test can be used as an epidemiological tool because, in areas where both P.*vivax* and P.*falciparum* are prevalent, it can be used to identify the *Plasmodium* species infecting the patients in epidemics quickly and it allows public health workers to deliver the appropriate chemotherapy rather than just give chloroquine to each and everyone with malarial symptoms. This is particularly important where the preferred therapies for the two infections are different.

Further implementation of this test will result in time saving and reduce the use of expensive drugs unnecessarily by the clinically suspected malaria patients. This test can be used in urgent or epidemic situations for spot diagnosis and treatment of both P.*vivax* and P.*falciparum* infection by nonmedical staff. Hence this test method could help to attain the goals of the Roll Back malaria initiative.

The SD-Bioline RDT has an advantage of differenting P.*falciparum* from P.*vivax* malaria. Despite the few limitations(low sensitivity), the test can be used as an epidemiological tool in areas where P.*vivax* and P.*falciparum* are prevalent. It can also be used to identify the *plasmodium* species infecting the patients in the slums rapidly by nonmedical staff. The cost of the SD-Bioline P.f/P.v (Rs.33/- per test) at the time of study was quite affordable and would favour its widespread use in malaria endemic areas of developing countries where most of the patients need a fever screen.

Finally I want to conclude that the peripheral smear is gold standard, Paramax-3(antigen detection) kit is more sensitive and specific, and SD-Bioline (antibody detection) kit is specific and cost effective.

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Rapid decrease of malaria morbidity following the introduction of community-based monitoring in a rural area of central Vietnam

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Abstract

Background: Despite a successful control programme, malaria has not completely disappeared in Vietnam; it remains endemic in remote areas of central Vietnam, where standard control activities seem to be less effective. The evolution of malaria prevalence and incidence over two and half years in a rural area of central Vietnam, after the introduction of community-based monitoring of malaria cases, is presented.

Methods: After a complete census, six cross-sectional surveys and passive detection of malaria cases (by village and commune health workers using rapid diagnostic tests) were carried out between March 2004 and December 2006 in Ninh-Thuan province, in a population of about 10,000 individuals. The prevalence of malaria infection and the incidence of clinical cases were estimated.

Results: Malaria prevalence significantly decreased from 13.6% (281/2,068) in December 2004 to 4.0% (80/2,019) in December 2006. *Plasmodium falciparum* and *Plasmodium vivax* were the most common infections with few *Plasmodium malariae* mono-infections and some mixed infections. During the study period, malaria incidence decreased by more than 50%, from 25.7/1,000 population at risk in the second half of 2004 to 12.3/1,000 in the second half of 2006. The incidence showed seasonal variations, with a yearly peak between June and December, except in 2006 when the peak observed in the previous years did not occur.

Conclusion: Over a 2.5-year follow-up period, malaria prevalence and incidence decreased by more than 70% and 50%, respectively. Possibly, this could be attributed to the setting up of a passive case detection system based on village health workers, indicating that a major impact on the malaria burden can be obtained whenever prompt diagnosis and adequate treatment are available.

Background

In 1991, Vietnam experienced a devastating malaria epidemic that caused more than one million cases and 4,646 deaths. The same year the National Malaria Control Programme (NMCP) was launched and since then, thanks to the political commitment, the fast economic growth and strong international support, the burden of malaria has reduced dramatically [1,2]. Indeed, in 2006 only 91,635 malaria cases and 43 malaria deaths were reported nationwide, a 95% and 99% decrease respectively compared to the 1991 figures [3]. The national insecticide-treated bed net (ITN) campaign, supported by an intensive media campaign on the importance of malaria and ITN use, and the widespread use of artemisin derivatives for treatment, were at the basis of such a success. In addition, indoor-residual spraying (IRS) was employed in epidemic prone-area or where ITN coverage was low [1]. The recent development of Long-Lasting Insecticide Nets (LLINs) should overcome the problems of low re-treatment rates, washing and variation in insecticide dosing, possibly improving their effectiveness [4]; however this new tool is not yet available in Vietnam. Despite past successes, malaria has not completely disappeared in Vietnam; it is confined to some remote areas, mainly inhabited by ethnic minorities. Besides the burden on the local populations, malaria affects also migrant workers from non-endemic areas, with the potential of spreading where transmission has virtually stopped. Therefore, though geographically limited, the control of malaria in these areas is extremely important for the whole Vietnam and possibly for its neighbouring countries. Currently, about half of all malaria cases and 80% of severe cases and malaria-related deaths occur in the central highlands [5-7], where the main vector is *Anophele dirus s.s.*, a highly anthropophilic sylvatic species, whose exophagy and exophily as well as early biting habits limit the impact of interventions such as IRS or ITN [8,9] Indeed, in central Vietnam, forest activity has been identified as a strong risk factor for malaria infection [10-12]. It is, therefore, important to know the epidemiology of malaria in these areas to understand its dynamics. A longitudinal malaria surveillance system consisting of bi-annual cross-sectional surveys and passive case detection of malaria cases at community level, through an extensive network of trained hamlet health workers, was set up in Ninh Thuan Province (centre-south Vietnam) as a component of a larger study aiming at evaluating the effectiveness of long-lasting insecticidal hammocks. This paper reports the evolution of malaria prevalence and incidence in this population during a 2.5-year surveillance period by community-based monitoring of malaria cases.

Methods

Study site

The study was carried out in two neighbouring districts, Bac Ai (eight communes and 25 villages) and Ninh Son (two communes and five villages), situated in the hilly and forested part of Ninh Thuan province (southern coast of central Vietnam). The population is mainly represented by the Raglai ethnic group, whose members practise subsistence farming (maize, cashew, rice, beans and manioc), cultivate cash crops such as coffee and cotton, and exploit forest products (bamboo, resin, hunting).

The climate is a combination of tropical monsoon and dry and windy weather. The dry season occurs between January and April, with the coldest period in January and February, and the rainy season between May and December. The mean rainfall is 725 mm/year, with the mean temperature ranging between 25°C and 30°C and the humidity between 70% and 80% (Ninh Thuan provincial climatic data, from 2000 to 2006). Malaria transmission is perennial with two peaks, one in June and the other in October. Twenty two different Anopheles species have been identified: the two main malaria vectors are *An. dirus s.s.* and *Anopheles minimus A*, while secondary vectors such as *Anopheles maculatus* or *Anopheles jeyporiensis* probably play a non-negligible role in the local malaria transmission (Van Bortel & Coosemans, personal communication).

Census

A full census of the study population was done in March 2004: information on age, sex, socio-economic status, forest activity, bed net availability and previous vector control measures was collected. [12]. Each individual living in the study area was attributed a unique identifier code (including village, house and family member codes) which was used in all study activities (census update, surveys, PCD). The census file was routinely updated as births, deaths and migrations were collected by village health workers (VHW) and reported monthly to the malaria provincial station where the electronic census file was managed. The study area was divided into clusters of 1 to 3 neighbouring villages in order to reach a total of about 1,000 people per cluster. Size and number of clusters were determined according to the cluster design requirements and the expected effect of the intervention [12].

Cross-sectional surveys

Each year, between April 2004 and November 2006, two cross-sectional surveys, one at the beginning (April, May) and one at the end of the transmission season (November, December), were carried out. A random sample of 160 individuals aged 10–60 years per cluster was selected for the first survey in April 2004 [12]; and was subsequently increased from the 2nd survey onwards by 60 addi-

tional and randomly selected children aged 2–9 years, lately identified as a high risk age group. An individual, pre-coded standardised questionnaire was administered on previous malaria symptoms and anti-malarial treatment taken, and a clinical examination, body temperature and spleen size, was carried out. A blood sample (finger prick) for thick and thin blood film was collected. Suspected malaria cases were presumptively treated either with chloroquine (25 mg/kg in 3 days) or artesunate (16 mg/kg in 7 days) according to the national guidelines.

Passive case detection

Passive case detection of malaria was started in July 2004, and continued until the end of the study. Patients could attend either the Commune Health Centres (CHC) or consult the village health workers (VHW). The latter and the CHC health staff were trained to use rapid diagnostic tests, to take blood slides and administer the treatment to malaria patients according to the test results.*Plasmodium falciparum* cases (including mixed infections) were treated with a full course of artesunate (7 days), while *P. vivax* patients received a full course of of chloroquine (3 days); primaquine was not used. Patients attending the CHC or consulting the VHW were identified onto the census file, asked about fever in the past 48 hours, had their body (axillary) temperature registered, and a blood sample taken to detect malaria infection, i.e. a rapid diagnostic test and a thick and thin blood film for later microscopic examination. Rapid tests results were used only to decide if antimalarial treatment had to be immediately administered while microscopy on all blood films for species identification and parasite density determination was carried out later. Quality of case management, blood sampling, and reporting was assured by monthly supervision meetings with the staff of the Provincial Centre for Malariology, Parasitology and Entomology (PCMPE) and the District Health Centres (DHC), and retraining was provided whenever needed.

Laboratory tests

Rapid diagnostic test

Paramax-3™ (Zephyr Biomedicals, India) rapid tests for detecting *P. falciparum*-specific histidine rich protein-2 (Pf HRP-2), *P. vivax* specific lactacte dehydrogenase (pLDH) and a pan malaria-specific pLDH were used. Four drops of buffer solution were added to the blood drop onto the sample pad on the test device and results were read after 15 minutes. A control band served to validate the test performance.

Microscopic examination

Thin films were fixed with methanol, then stained, together with thick films, with a 3% Giemsa solution for 45 minutes, and kept in slide boxes at room temperature. The number of asexual parasites per 200 white blood cells (WBCs) was counted and parasite densities were computed assuming a mean WBC count of 8,000/μL. A slide was defined as negative if no asexual form was found after counting 1,000 WBCs. Microscopic examination was blinded to patients' identity and location: reading and quality control was performed at the National Institute of Malariology, Parasitology and Entomology in Hanoi. Discrepant results were re-read and agreed upon by a third senior technician.

Case definition

Patients with malaria symptoms consulting the VHW or attending the CHC were considered as suspected malaria cases. A malaria infection was defined as a positive blood slide with Plasmodium asexual forms, regardless of symptoms and parasite density. Clinical malaria was defined as a patient with fever (body temperature ≥ 37.5°C), and/or history of fever in the past 48 hours, and a positive blood slide for Plasmodium asexual forms. Recrudescence was defined as clinical malaria occurring within 28 days following the first episode. Splenomegaly was defined as any palpable spleen, independently of the Hackett classification.

Data management and statistical analysis

Data were double entered, checked and cleaned using Epi-Info v6.04d. The data set was analysed with STATA 9.0 software (Stata Corp., College Station, TX). Descriptive statistics were used to compute malariometric indices and a survey chi-square test ("svytab" command in STATA, taking into account the cluster effect) was used to test for significant differences in proportions ($p < 0.05$).

The population follow up was divided in 6-month periods. Malaria incidence rates were computed by dividing the number of new cases in a given semester by the corresponding total person-semester at risk (from the census file). The latter was obtained by computing for each individual the number of days spent in the study area in relation to the actual length in days of that specific semester. Incidence rates were compared and incidence rate ratios calculated using a Poisson survey regression model ("svypoisson" in STATA, allowing for the survey design).

Tests for trend, taking into account the survey design, were performed using the "lincom" command (test for linear combination of estimates) in the survey logistic and in the survey Poisson regression models.

Ethical considerations

The study was approved by the ethical committees of the Institute of Tropical Medicine, Antwerp, in Belgium, the National Institute of Malariology, Parasitology, and Entomology, Hanoi, as well as by the Ministry of Health in Vietnam. The fundamental principles of ethics in research

on human participants were maintained throughout the study period. The research procedures were disclosed to the participants and oral informed consent was sought from them or their legal representatives. Nobody was coerced into the study and if individuals wished to withdraw, they were allowed to do so without prejudice.

Results

Census

Nine thousands eight hundred and seventy five individuals were registered during the census carried out in March 2004 (Table 1). The Ra-glai ethnic group represented the majority (83%) of the population, which was young (median age: 19 years), and generally with a low education level. More than half of the people were forest workers, mainly working in the forest fields, and almost all households had forest fields (96.5%, 1,801/1,867). In total, one third (33%) of the population had only daily activities in the forest, while another 25% was working and sleeping there overnight, with a substantial number of days (median 23) and nights (median 16) per month spent in the forest. ITN use in the villages was high (88.2%; a few additional people were sleeping under an untreated bed net), with a median of 2.5 people per bed net, regardless of insecticide treatment. Hammocks (locally bought, either in cotton material or knotted cord) were also popular: half of households had hammocks (48.4%). Among people staying overnight in the forest, ITN use was lower (70.4%) than in those just sleeping in the villages, hammocks were used by 16.4% of the people (with or without ITN), and 13.2% of the forest workers were sleeping without hammocks or ITNs. Socio-economic status was generally low with half (951/1,867) of the houses made of thatched bamboo, only 15% (282/1,867) made of bricks, and 46% (857/1,867) of them having no radio, TV nor motorbike.

Cross sectional surveys

Malaria prevalence steadily decreased from 11.7% (178/1,518) in the first survey carried out at the end of the dry season to 4.0% (80/2,019) in the last survey carried out in December 2006, at the end of the transmission season (Table 2). This decrease was even stronger when comparing the first and last end of transmission seasons (survey 2 (S2) and survey 6 (S6)): from 13.6% to 4.0%, over 70% reduction ($p < 0.001$). Plasmodium falciparum and P. vivax were the most common species and decreased in similar proportions, by 66% and 70%, respectively, between S2 and S6. A few P. malariae mono-infections were identified during the first four surveys, and the prevalence of mixed infections decreased by almost 89% between S2 and S6. The parasite density was consistently higher in P. falciparum than in P. vivax infections. The percentage of gametocyte carriers also significantly decreased from 8.0% (166/2068) in survey 2 to 1.0% (21/2,019) in the last survey ($p = 0.003$). Most infections (86%) were detected in individuals without any sign or symptom of malaria (asymptomatic carriers) and this proportion remained stable across surveys (Table 2). Splenomegaly was uncommon, with the highest number found in the first survey (1.9%, 29/1,518), while in the other surveys the prevalence was well below 0.5%. Overall, malaria prevalence decreased with increasing age group (test for trend, $p < 0.05$), children less than 10 years old having the highest values across all surveys (in survey 1, children had not been included).

Passive case detection

Between July 2004 and December 2006, 4,862 suspected malaria cases were identified through passive case detection (Table 3). Most of them (94.0%, 4,570/4,862) presented with fever and/or had a history of fever in the past 48 h (79.6%, 3871/4862). Malaria infection was detected in 18.4% (893/4,862) of suspected cases and P. falciparum was the dominant species (75.8%, 677/893). Plasmodium vivax represented the large majority of the remaining infections, while several mixed infections were also detected. The number of malaria cases decreased progressively with age with the highest number detected in the youngest age groups (≤ 9 years and 10–19 years old). Mean parasite density was higher for P. falciparum than for P. vivax, both in fever and non-fever cases, with higher densities among fever cases compared with non-fever cases. The 893 malaria infections were detected in 687 individuals. The large majority of patients (80.2%, 551/687) had only one malaria episode during the whole study period (Table 4). However, a few individuals had two (14.1%, 97/687) or three (4.1%, 28/687) episodes, with three individuals having experienced six malaria episodes. Twenty five recrudescences (recurrent parasitaemia within 28 days of the original episode), 21 P. falciparum, and four P. vivax patients, were detected, almost all among children under 10 (88%, 22/25).

In the rainy season (July-December), the malaria incidence rate per semester was estimated at 25.7/1,000 person-semesters at risk in 2004, 22.4/1,000 in 2005 but only 12.7/1,000 in 2006 (Table 5): a 52% reduction within 2 years (crude IRR = 0.48; 95%CI [0.27; 0.85]; $p = 0.02$). Conversely, the incidence rate did not show any variation between the 2 consecutive dry seasons (Jan-Jun 2005 and 2006), though the incidence was 60% lower than that in the first rainy season (July-December 2004) (Table 5). Seasonal variations in monthly malaria incidence were loosely related with monthly rainfall (Figure 1). Malaria incidence increased with decreasing age: in each semester, children less than 10 years old experienced the highest incidence rate compared to older age groups (test for trend, $p < 0.001$).

Table 1: Baseline characteristics of the study population

Study population, N = 9,875	**n**	**%**
Sex (ratio = 0.98)		
- Male	4,900	49.62
Age groups:		
- ≤ 9 years	2,684	27.18
- 10–19 years	2,379	24.09
- 20–29 years	1,733	17.55
- 30–39 years	1,110	11.24
- 40–49 years	954	9.66
- = 50 years	1,015	10.28
Ethnic groups:		
- Ra-glai	8,205	83.09
- K'ho	1,339	13.56
- Kinh	314	3.18
- Others (Chu, Cham, Ede)	17	0.17
Education level (age ≥ 20, n = 4,812):		
- None	2,197	45.66
- Primary school	2,258	46.92
- Secondary school or higher	341	7.09
- Missing	16	0.33
Occupation:		
- None (children, students, retired people)	4,376	44.31
- Forest work (farming & other)	5,242	53.08
- Other (teacher, health staff.)	240	2.43
- Missing	17	0.17
Bed net use in the village:		
- Sleep under ITN	8,707	88.17
- Sleep under an untreated bed net	494	5
- Sleep without bed net	656	6.64
- Missing	18	0.18
Forest activities:		
- Never	4,176	42.29
- Only during day	3,244	32.85
- Work and sleep in the forest	2,438	24.69
- Missing	17	0.17
Days/month spent in forest, median [range] (n = 5,682)		23 [1;30]
Nights/month spent in forest, median [range] (n = 2,438)		16 [1;30]
Bednet/hammock use in the forest (n = 2,438):		
- Sleep under ITN	1,717	70.43
- Sleep in a hammock	319	13
- Sleep under ITN and hammock	80	3.28
- Sleep without bed-net and hammock	322	13.21
Households N = 1,867		
House structure:		
- Thatched bamboo	951	50.94
- Wooden boards	374	20.03
- Dried mud	260	13.93
- Bricks	282	15.1
Socio economic level:		
- No radio, TV, motorbike	857	45.9
- Only a radio	517	27.69
- Only TV	99	5.3
- TV + radio (no moto)	94	5.03
- At least a motorbike (+/-radio, TV)	300	16.07

Table 2: Evolution of malariometric indices across 6 consecutive cross sectional surveys

Cross sectional surveys: Date **Participants, N**	**S1** **04/2004** **1,518**	**S2** **12/2004** **2,068**	**S3** **04/2005** **2,081**	**S4** **12/2005** **2,089**	**S5** **04/2006** **2,102**	**S6** **12/2006** **2,018**
Malaria prevalence: n (%) [95%CI]	178 (11.7) [6.8; 19.5]	281 (13.6) [8.8 ; 20.5]	126 (6.1) [3.5; 10.4]	173 (8.3) [4.6; 14.4]	116 (5.5) [2.6; 11.5]	80 (4.0) [2.3; 6.8]
By species:						
- *P. falciparum*	85 (5.6)	148 (7.1)	57 (2.7)	103 (4.9)	61(2.9)	49(2.4)
- *P. vivax*	77 (5.1)	93 (4.5)	52 (2.5)	55 (2.6)	43 (2.0)	27 (1.3)
- *P. malariae*	3 (0.2)	2 (0.1)	2 (0.1)	7 (0.3)	0 (0.0)	0 (0.0)
- *Mixed infections*	13 (0.9)	38 (1.8)	15 (0.7)	8 (0.4)	12 (0.6)	4 (0.2)
Asymptomatic infections*	153 (86.0) [66.4; 95.0]	230 (81.9) [67.7; 90.7]	97 (77.0) [40.6; 92.4]	139 (80.3) [70.2; 87.7]	99 (85.3) [68.0; 94.1]	68 (85.0) [75.6; 91.21]
Parasite density/μl (GM)° [95%CI]						
P. falciparum	187.9 [129.0–273.7]	169.0 [124.1–230.1]	172.6 [112.7–264.4]	225.0 [149.1–339.7]	245.0 [154.0–389.8]	370.5 [205.8–667.0]
P. vivax	76.3 [59.7–97.5]	79.5 [59.8–105.9]	68.6 [47.9–98.2]	81.2 [52.1–126.5]	82.1 [52.7–127.8]	145.1 [83.5–252.4]
P. malariae	140.7 [54.1–365.5]	81.1 [12.7–515.8]	148.1) [39.4–557.1]	93.5 [87.8–99.7]	-	-
Gametocyte prevalence, n (%) [95%CI]	65 (4.3) [2.4; 7.4]	166 (8.0) [3.0; 19.8]	33 (1.6) [0.7 ; 3.4]	55 (2.6) [1.5 ; 4.6]	44 (2.1) [0.9 ; 4.7]	21 (1.0) [0.4 ; 2.5]
Malaria prevalence by age group						
- <9 years	NA	101 (17.5) [11.3; 26.0]	54 (10.4) [5.3; 19.6]	59 (11.5) [6.8; 18.8]	42 (9.1) [4.2; 18.7]	28 (6.3) [3.6; 10.8]
- 10–19 years	70 (13.6) [7.3; 23.8]	78 (15.7) [9.3; 25.3]	35 (6.6) [3.3; 12.6]	41 (7.9) [3.5; 16.6]	37 (6.9) [3.1; 14.9]	28 (5.4) [2.9; 9.8]
- 20–29 years	49 (11.8) [6.3; 20.2]	42 (10.2) [5.9; 17.1]	10 (2.4) [0.8; 7.0]	27 (6.1) [3.5; 10.5]	15 (3.3) [1.2; 9.1]	9 (2.2) [0.9 ; 4.9]
- 30–39 years	30 (11.0) [7.1; 16.7]	19 (7.5) [3.6; 15.0]	15 (5.3) [2.7; 10.0]	23 (7.7) [3.5; 16.3]	7 (2.5) [0.6; 8.9]	5 (1.8) [0.7 ; 4.9]
- 40–49 years	20 (11.1) [5.8; 20.2]	27 (13.8) [7.5; 24.2]	8 (3.9) [1.0; 14.1]	17 (9.6) [4.4; 19.8]	10 (4.4) [1.6; 11.9]	7 (3.2) [1.3; 7.8]
- ≥ 50 years	9 (6.8) [2.8; 15.6]	14 (10.5) [5.2; 20.1]	4 (3.1) [1.3; 7.5]	6 (4.3) [1.8; 10.2]	5 (3.5) [1.2; 9.8]	3 (2.0) [0.6; 6.7]
Spleen rate	29 (1.9) [6.8; 19.3]	7 (0.3) [8.8; 20.5]	4 (0.2) [3.5; 10.4]	5 (0.2) [4.6; 14.4]	4 (0.2) [2]	4 (0.2) [2.3; 6.8]

* % asymptomatic infections/all infections; ° GM: geometric mean

Discussion

Ninh Thuan has always been considered one of the most endemic malaria provinces in Vietnam [6,7]. Indeed, the first survey, carried out in April 2004, at the end of the low transmission season, showed a relatively (for Vietnam) high value for malaria prevalence (11.7%). The large proportion of asymptomatic infections seems to indicate that transmission is sufficiently intense to stimulate some partial immunity able to control malaria parasitaemia and prevent its evolution towards clinical disease. Indeed, the highest incidence of clinical attacks observed among children <9 years of age and its decreasing value as age increases supports this hypothesis, i.e. people living in the study area are repeatedly exposed since infancy to malaria infection and acquire some partial immunity. This is different from a previous study carried out in the neighbouring province of Binh Thuan, where the malaria prevalence was 5% or less and where children were not particularly at

Table 3: Characteristics of malaria patients identified by passive case detection.

Suspected malaria cases		**4,862**	
	***n*,**	***%***	***(95%CI)***
History of fever previous 48 hours	3,871	79.6	(66.2 ; 93)
Fever	4,570	94.0	(88.9 ; 99.1)
Malaria infection	893	18.4	(10.4 ; 26.4)
P. falciparum	677	13.9	(20.5; 34.3)
P. vivax	164	3.4	(2.4 ; 4.4)
P. malariae	1	0.02	(0 ;0.06)
Mixed	51	1.0	(0.7; 1.6)
Parasite density/μl (GM), without fever			
P. falciparum	21	422.0	(974.0–1183.0)
P. vivax	8	174.0	(50.0–598.0)
Parasite density/μl (GM°), fever cases			
P. falciparum	696	1880.0	(1563.0–2262.0)
P. vivax	202	641.0	(477.0–863.0)
Gametocytes carriers	192	3.9	(2.7 ; 5.2)
Malaria cases by age group			
≤ 9 years	435	22.6	(13.7; 35.1)
10–19 years	202	22.2	(14.2; 32.9)
20–29 years	106	16.6	(9.5 ; 27.4)
30–39 years	74	14.1	(8.6 ; 22.5)
40–49 years	49	11.2	(7.0 ; 17.3)
≥ 50 years	27	6.3	(3.0 ; 12.5)

GM: geometric mean

risk [11]. The high malaria prevalence in small children in Ninh Thuan seems to indicate that transmission occurs in the villages themselves. Indeed, in Ninh Thuan the villages in the study area are located close or within the forest, increasing the probability of contact with the main vector *Anopheles dirus s.s.*, asylvatic species that is highly anthropophilic. In contrast, in Binh Thuan the study village was located along the district road and relatively far from the deep forest so that the malaria risk was particularly high in individuals with forest activities but not in small children [11]. The more intense transmission in Ninh Thuan as compared to Bin Thuan is supported also by the large proportion of asymptomatic cases detected by cross-sectional surveys in the former. A similar situation has been described in neighbouring countries, such as Cambodia [13], Indonesia [14] or Burma [15], where the malaria prevalence was comparable to that found in Ninh Thuan and with children having a higher risk of malaria infection. Obviously, the large proportion of asymptomatic malaria carriers in Ninh Thuan maintains malaria transmission in this area and represents an obstacle to malaria control. It is unclear what might be the impact of these asymptomatic infections on the carrier's health; the haematological status of the survey participants was not determined, mainly because in the study carried out in Binh Thuan no case of anaemia was detected [11]. However, asymptomatic infections were much less common. In Africa, asymptomatic infections have been associated with a higher risk of anaemia and lower school performances [16-18].

Table 4: Number of malaria episodes per person (N = 687) over the follow-up period

Total clinical episodes/person	**Patients n (%)**
1	551 (80.2)
2	97 (14.1)
3	28 (4.1)
4	8 (1.2)
6	3 (0.4)

Within a relatively short period, both the prevalence of malaria infection and the incidence of clinical cases decreased substantially, more than 70% and 50%, respectively. Prevalence decreased steadily over the 2.5-year follow up, while for the incidence such a decrease occurred only during the last 6-month period as the incidence peak usually observed during the second part of the year did not occur. It is difficult to attribute the observed trend to

Table 5: Malaria incidence rate (per 1,000 person-semesters at risk) per semester and age group

Age group (years)	Jul-Dec.04 Incidence [95%CI]	Jan-Jun.05 Incidence [95%CI]	Jul-Dec.05 Incidence [95%CI]	Jan-Jun.06 Incidence [95%CI]	Jul-Dec.06 Incidence [95%CI]
≤ 9	38.4 [18.2; 58.6]	16.9 [3.7; 30.0]	38.0 [4.7; 71.3]	20.3 [0.0; 43.4]	21.6 [0.0; 44.8]
10–19	20.7 [0.0; 42.1]	9.6 [2.2; 17.1]	22.4 [7.0; 37.7]	10.9 [0.0; 22.7]	12.9 [2.5; 23.3]
20–29	23.3 [2.6;44.0]	7.0 [1.0; 13.0]	13.7 [3.0; 24.3]	4.6 [0.0; 9.5]	9.0 [0.0; 20.1]
30–39	20.9 [2.7; 38.9]	5.9 [1.6;10.1]	20.9 [5.6; 36.3]	5.7 [0.5; 10.8]	8.1 [0.0; 16.9]
40–49	24.7 [0.0; 51.2]	5.0 [0.7;9.3]	13.9 [2.9; 25.0]	3.9 [0.0; 9.4]	8.8 [2.3; 15.2]
≥ 50	12.5 [0.0; 25.3]	2.8 [0.0; 6.2]	6.4 [0.0; 13.4]	0.9 [0.0; 3.0]	2.6 [0.0; 7.2]
Total	**25.7 [7.9;43.6]**	**9.5 [3.9; 15.1]**	**22.4 [7.1; 37.8]**	**9.9 [0.0; 19.7]**	**12.3 [0.4; 24.2]**

a specific cause; however this is unlikely to be a natural trend in the study area since the malaria incidence in the two neighbouring provinces of the study area, i.e. Lam Dong and Khan Hoa province, experienced much smaller reductions in malaria incidence, i.e. 13% and 22%, respectively. These provinces had low incidence rates in 2004 (between 0.6 and 1.3/1,000 population). In other provinces from Central Highlands such as Dak Lak, with an annual incidence rate of 4.5/1,000 in 2004, the reduction by 2006 was only by 5% [6,7] Therefore, considering that the environment did not change dramatically and that the yearly rainfalls did not vary considerably during the whole study period, the observed decrease may reflect the impact of the malaria surveillance system set up for the study, i.e. the prompt availability of diagnosis and treatment at village level. In this area, trained VHWs could use rapid diagnostic tests and treat the confirmed cases of *P. falciparum* or *P. vivax* (as well as other species suspected cases) with either artesunate or chloroquine, respectively. VHW are often employed by health programmes in rural and remote areas where public health services are not always or easily accessible and available. In Vietnam, 84% of villages (61,664/73,462) have VHWs who have participated to malaria control activities, usually health promotion and in some areas also rapid diagnosis and prompt treatment [3]. In the present study, about half of the confirmed malaria cases by PCD were detected by the VHWs in their respective villages. Therefore, the most important contributing factor on the observed decreasing trend in the study area may be the activity of the VHWs. Our results confirm previous reports about VHWs having played an important role in malaria diagnosis and treatment in many different settings and for more than 45 years [19]. Malaria morbidity and mortality were significantly reduced when the rural population at risk for malaria were treated at village level [20]. In Cambodia, after the scaling up of the VHW project (rapid tests + pre-packaged combination therapy for malaria) in Rattanakiri province, the annual malaria incidence decreased by more than 60%, from 165/1,000 in 2006 to 64/1,000 in 2007 [21]. Similarly, in a remote area of Mindanao, the Philippines, the parasite prevalence was significantly lower among individuals living in villages with a resident VHW [22].

In conclusion, despite a successful control program, malaria transmission in Vietnam is still ongoing at a relatively high intensity in some remote areas. Nevertheless, in Ninh Thuan province, a significant decreasing trend in malaria prevalence and incidence has been observed in a short period (2.5 years). This is probably due to the set up of a passive case detection system based on VHW, indicating that a major impact on the malaria burden can be obtained whenever prompt diagnosis and adequate treatment are available.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

TND contributed to the study design, study coordination and supervision, field work, data entry, cleaning and analysis, and paper writing. AE contributed to the study design, study coordination and supervision, field work, statistical analysis and reviewed the manuscript. HLX contributed to the study design, study coordination and supervision, and reviewed the manuscript. TLK contributed to the study design and reviewed the manuscript. TNN contributed to the field work, data entry & cleaning, the study coordination and supervision. XNX contributed to the field work, data entry & cleaning, the study coordination and supervision. KPV contributed to the field work, data entry & cleaning, the study coordination and

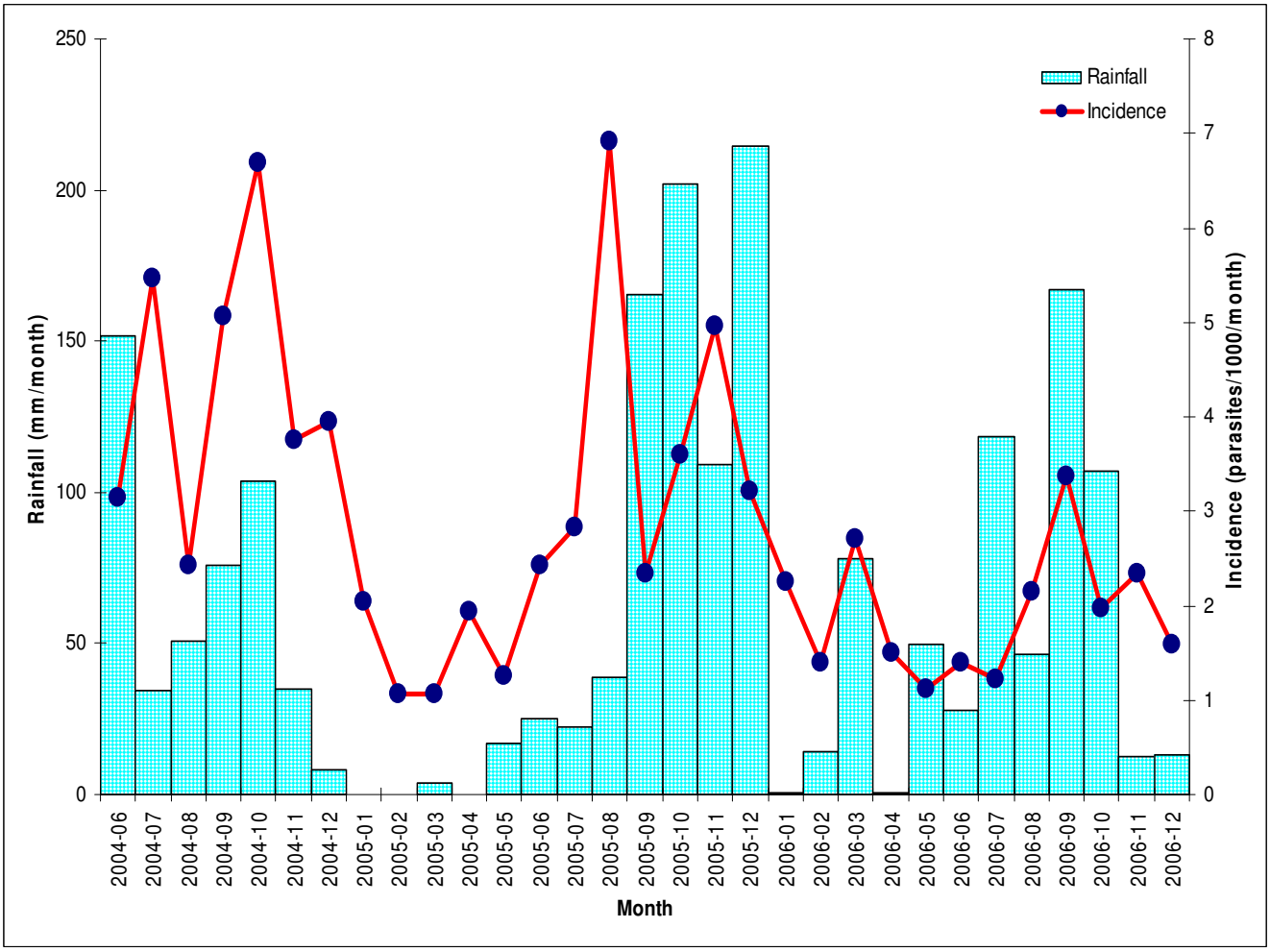

Figure 1
Malaria incidence (all species) and rainfall by month during the study period.

supervision. MC reviewed the paper. UD contributed to the study design, study coordination and supervision, data analysis and reviewed the manuscript.

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Performance Evaluations

ISO 9001: 2008
EN ISO 13485: 2012

OTHER EVALUATIONS

PARAMAX®

Rapid test for the detection of Malaria (Pan/Pv/Pf)

Tulip Group

Evaluation of Parscreen and Paramax malaria diagnostic tests Manufactured by M/S Zephyr Bio Medicals, Verna, Goa.

Testing Laboratory:
Malaria Research Centre (ICMR),
DHS Building, Campal, Panaji, Pin-403 001,
Goa, India.

Name of the Product: Parascreen and Paramax

Type of Product: Immunochromatic Rapid Diagnostic kit for malaria parasites *Plasmodium vivax* and *Plasmodium falciparum*

Principle Of diagnostic tests:
1. Parascreen: Immunochromatographic test capable of detecting pan malaria specific pLDH and Pf specific PfHRP-2.

A *P. falciparum* positive test will show both PfHRP-2 and pLDH detection lines.

P. vivax, P. malariae and *P. ovale* will show pLDH positive reaction only. In this case, the judgment of species could be made on the basis of local epidemiological situation and known Plasmodium species in the area. The limitation of the test is that the cases of mix infection of *Pv+Pf* can not be identified.

2. Paramax: Immunochromatographic test capable of detecting PfHRP-2, pan specific pLDH and *P. vivax* specific *Pv*LDH

A *P. falciparum* positive test would show both PfHRP-2 and pLDH detection lines.

A *P. vivax* positive case will show pLDH specific and *P. vivax* specific pLDH lines.

In case only pan specific LDH (pLDH) line appears and test is negative for PfHRP-2 and *P. vivax* specific LDH, then there could be either *P. malariae* or *P. ovale* but again the judgment of the third species could be made based on local distribution of Plasmodia.

The Paramax test is capable of detecting mix infection of *Pf* + *Pv* and

Pf + Po/Pm

Period of study: 8th March, 2004 to 29th March, 2004

Patients enrolled: 197 routine fever cases visiting for malaria test.

Type of blood sample used: Fresh whole blood directly from finger prick of fever cases in passive collection and detection facility. Thick and thin blood smears were simultaneously prepared for microscopy.

Time of reading of test: 15 minutes after test was applied as prescribed by the manufacturer.

Gold Standard Used for Comparison: Blood smear stained with Giemsa stain. Blood slides blinded and read by 3 qualified Laboratory Technicians independently.

Results: Results of the testing of the kit have been summarized in Table 1 and 2 given below.

Table. 1. Comparison of malaria diagnosis with microscopy with Parascreen and Paramax diagnostic tests

Nos.	Microscopy	Parascreen	Paramax
Total Tested (%+ve)	197 (68.0)	197 (68.0)	197(68.0)
Pv (% +ve)	68 (34.5)	68 (34.5)	68(34.5)
Pf (% +ve)	7 (3.55)	7 (3.55)	7(3.55)
Pv+Pf (% +ve)	0 (-)	0 (-)	0 (-)

Table: 2. Shows results of evaluation of Parascreen and Paramax malaria diagnostic tests

	P. falciparum (N=7)	*P. vivax* (N=68)	Overall
Sensitivity (%)	100	100	100
Specificity (%)	100	100	100
PPV (%)	100	100	100
NPV (%)	100	100	100
Efficacy (%)	100	100	100

PPV = Positive Predictive Value

NPV = Negative Predictive Value

Parasitaemia: In thick blood film parasites counted against 200 WBCs to work out parasitaemia / micro litre of blood taking 8000 WBCs per micro litre as standard

Parasitaemia Range :

1. *P. falciparum*: 400 - 22720 parasites/μl of blood
2. *P. vivax* : 520 - 33600 parasites /μl of blood

Inference: As table 2 reveals both Paramax and Parascreen diagnostic tests are of standard quality for the diagnosis of malaria showing absolute sensitivity, specificity and efficacy.

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