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Comparison of Rapid Malaria Test and Laboratory Microscopy Investigation for Community-Based Active Surveillance of Asymptomatic Falciparum Malaria in Yenagoa, Niger Delta, Nigeria

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ABSTRACT: Blood samples collected from 272 volunteers in two communities of Bayelsa State in the Niger Delta area were investigated for falciparum malaria parasite using the rapid test based on the detection of soluble antigen and laboratory microscopy test. The data showed that out of the 272 samples collected, 40(14.7%) falciparum malaria cases were identified by microscopic examination. 53(19.5%) were detected by rapid test. The Global (p.f) malaria test kit yielded 22 false negative and 35 false positive results when compared with microscopic findings. Only 18 cases shows true positive results. The specificity and sensitivity of the kit was 80% and 45% respectively when the parasite density is below 100 parasite/ μl. These results shows that rapid test would not give justifiable results as most of the low parasite density cases could escape detection, therefore, it cannot replace laboratory microscopic diagnosis in an active malaria surveillance programme in the study area. Therefore, regulatory agencies should insist on regular laboratory microscopic diagnosis in the surveillance of malaria. @ JASEM

Malaria is a debilitating disease that affects the physical and economic wellbeing of people living in endemic areas of Africa; (Nebe *et al* 2000). Malaria still remains the most important disease when compared with other tropical parasitic diseases. Malaria is a disease of the poor and has caused heavy toll of morbidity and mortality especially in children below five years of age and pregnant women, (Daniel, 1999) and about 300 – 500 million malaria cases are recorded globally, (WHO, 1997). Human malaria is caused by a protozoan parasite of the genus, *Plasmodium*. The four species of *Plasmodium* are *P. vivax, P. ovale, P. malariae* and *P. falciparum.* In Nigeria, *P. falciparum* is the most virulent specie and accounts for over 80% of human malaria infections leading to about 200,000 deaths annually, (Eneaya *et al*, 2003, Fabiyi, 1996, Okoyeh *et al,* 1994). The diagnosis of malaria is based on microscopic examination of a stained blood samples (thin and thick blood films) and this remains the primary diagnostic method in the study of malaria infection. The major drawback of routine microscopy in malaria studies is expertize in the diagnosis of the parasites. As a result of this, the use of a more rapid diagnostic test to ensure prompt and early diagnosis as a first step to the control of malaria has been advocated as a necessity for active surveillance (WHO, 1990, Wongsrichanlai 2001). Multiple studies have been advocated by other researchers, John *et. al*, (1995) who used optimal dipstick test for the diagnosis of malaria, while Oliver *et. al*, (2000) utilized para-sight RF in the diagnosis of imported malaria in travelers clinic. All these techniques were based on detecting the circulating antigens in individuals living in malaria endemic areas. In this study, we compared the antigenemia, and parasitaemia in individuals with asymptomatic malaria, using malaria Global RF rapid device and laboratory microscopy as indices of active surveillance of community - based malaria, with a view of mounting control measures in the reduction of malaria burden in the human population.

MATERIALS AND METHODS

Study area: Yenagoa is located between latitude $4^0$51'N and $6^0$10'E. and has a total population of about 1, 121693, spreading over a land area of 12,000 square kilometers, (Alagoa, 1999). Yenagoa is situated within the tropical rain forest region of the Niger Delta of Nigeria. The annual rainfall ranges from 2000mm to 4,000mm extending between 8 - 10months in each year. The topography of the study area is made up of effluent creeks and swamps which often retain high body of water after heavy rainfall. This ecosystem supports all season mosquitoes breeding activities. The occupation of the people is predominantly fishing and farming at subsistent level. The settlement pattern is a clustered type, where people are confined to their families and compounds. In most of the communities that make up Yenagoa, houses are built with mud walls and thatch roofs, and this has kept the residence of the area unprotected from mosquito bites – vector of malaria parasite. There are few maternities and clinics, dispersed over a distance of 5km, between each of the communities of Yenagoa. These health centres are without personnels with adequate laboratory diagnosis of falciparum malaria.

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*Consent from community heads and blood collection in Yenagoa***:** All necessary ethical clearance were obtained from the State Ministry of Health, Bayelsa State and the state epidemiologist for the investigation. Consent was also obtained from the community heads and from those who showed willingness to participate in the study. The study was conducted between May 2006 and May 2007. The purpose and benefit of the study were explained to the communities. Prior to the arrival of the research team on the agreed date, the volunteers from the communities – Azikoro and Agudama-Epie were mobilized to the health centres through their town crier. The other communities in Yenagoa were superstitious about blood collection for the investigation. This affected the sample size of the research. A total of 272, (143 females and 129 males), volunteered for the study, 2mls of intravenous blood samples were collected from each volunteer. At the time of blood collection, each volunteer was questioned about illness such as fever, headache, muscle aches and pains and the medication taken during previous two weeks. A thick and thin blood film of each volunteer's blood was made on a grease free slide. At the same time, 10ul of blood samples were collected and tested for circulating antigen using malaria Global device assay (serial No R0115864-02). The result of the test was interpreted on the appearance of lines at region T and C. The intensity of the antigen level was graded as O (absent), faint (low) and thick (high).

Microscopy: The thick and thin films prepared were stained with 10% giesma solution and examined microscopically using oil immersion. The parasites were counted on thick blood smears until 500 trophozoites were recovered and the parasite density was expressed in micro-litres (WHO, 1998).

Analysis of data: Considering the result of the microscopy as a good standard, various variables were measured, which include, True positive (Tp), True negative (T_N), False positive (Fp) and False negative (FN). The sensitivity, specificity and accuracy of the test kit were calculated based on Ruselle, (2002).

Sensitivity (ST) =

$$\frac{TP}{(TP+FN)} \quad \ldots\ldots\ldots\ldots (1)$$

Accuracy (AC) =

$$\frac{TP+TN}{No\ of\ test\ (N)} \quad \ldots\ldots\ldots\ldots (2)$$

Specificity (SP) =

$$\frac{TN}{(TN+FP)} \quad \ldots\ldots\ldots\ldots (3)$$

Reliability (RB) =

$$\frac{(TP \times TN) - (FP \times FN)}{(TP+FN)\,(TN+FP)} \quad \ldots\ldots\ldots\ldots (4)$$

*Condition for acceptance, if ST, AC, SP, RB. ≥1

RESULTS AND DISCUSSION

A total of 272 volunteers were involved in the study, 40(14.7%) were diagnosed malaria positive on the basis of parasite detection in thick blood films. 20 (7.4%) had parasite density ranging from 1 – 99 µl, 13 (4.8%) has parasite density between 100 and 499 µl while 7(2.6%) had parasite density above 500 parasite/µl. The average parasite density was 98.9 parasite / µl. (Table 1). The malaria (pf) Global device was positive in 53 (19.5%) cases. 23 cases, were weakly positive with a fainted positive line; while 30 cases was strongly positive with thick positive line. The comparative performance of the rapid test and standard microscopy showed 18 positive cases and 197 negative cases that corresponded with microscopy result: 22 false negative results were obtained, in which microscopic examination showed parasite density/ul ranging between (≤ 0 and ≥1). Also 35 false positive results were obtained when parasite density/ul was ≤ 0 (Tables 2 and 3). The positive negative predictive index values of the Global device kit for (pf) malaria compared with thin/ thick blood films were 85% and 92% respectively. The rapid test showed 18 true positive results, which corresponded with the specificity value of 80% when compared with microscopy result. The sensitivity and reliability values (45% and 30%) were below expected level (table 4). The performance characteristic of malaria (pf) Global device has been compared with laboratory microscopy result. Positive slides of falciparum malaria parasites were higher 52 (19.5%) with rapid test than in routine microscopic results, 40 (14.17%). The higher percentage of malaria with rapid test may be attributed to unknown factors, (Oliver et al, 2000). It is possible that most of the volunteers in the investigation have recently treated themselves of malaria. Such individuals may have residual malarial antigens, which can test positive with rapid test kit.

Table 1: Prevalence of *P. falciparum* by microscopy

Parasite density (ul)	No counted	% counted
< 1	232	85.2
≥ 1	20	7.4
≥ 100	13	4.8
≥ 500	7	2.6
Total	**272**	**100**

Table 2: Prevalence of *P. falciparum* by rapid test analysis

	Antigen intensity			Total	%
	No line	Faint	Thick	53	19.5
No (%) +ve	-	23	30		
No (%) -ve	219	-	-	219	80.5
	Grand Total			272	100

Table 3 Comparative performance characteristics of rapid rest and microscopy

		Microscopy		
Rapid test kit (RTK)		- ve	+ve	**Total**
	+ve	35	18	53
	-ve	197	22	219
	Total	232	40	272

In this study, 35 of 53 positive slides, showed false positivity while 22 of the 40 slides showed false negativity. Only 18 of the 272 blood samples had true positive result for falciparum malaria. The false positive results according to Russell *et. al,* (2002) may reflect the non-specific binding of the antibody to the capture lines. Further, the false positive results in this study could be due to higher percentage of people who have just treated themselves for malaria and other malaria related fever, therefore, more people still have circulating antigen in their blood, although it seems reasonable to assume that the true parasite density has been cleared off. When some of the false positive results were retested for other infections, it was observed that four of them had hepatitis. This observation agrees with Peyron, (1994) and Laferi *et. al,* (1997) who reported the hypothesis of antigenic variation in parasite immunity. *P. falciparum* malaria parasitaemia of 22, of the 272 individuals were lower in volunteers with false negative antigenemia using rapid test. These volunteers had parasites density below 100 parasite/ul. Therefore, the rapid test technique was not reliable enough since there is the possibility of low parasite density cases which will escape detection. This observation is consistent with Tidi and Akogun, (2005). The combination of poor assay specificity and low sensitivity, particularly in individuals with parasite density ≤ 500/ul, who also had no symptoms of malaria suggests that majority of the malaria cases in similar surveillance programme will not be accurately detected with rapid test. In addition, it is impossible using the rapid test to distinguish between true and false positive malaria results (Table 4).

Table 4: Recommendatory Performance characteristics index (RPCI) of the rapid malaria test

Parasite density	T_P	T_N	F_P	F_N	Sensitivity	Specificity	Test Accuracy	Kits Reliability
≤ 0	0	18.9.0	35	0	0.45	0.80	1.005	0.30
≥ 1	2	5.0	0	12.0				
≥ 100	6	3.0	0	10.0				
≥ 500	10	0	0	0				
%(RPCI)				%	45	80	10	30

It is suggested that under the condition of low parasite density and prevalence, conducting active malaria surveillance using rapid malaria test is not justifiable. The low sensitivity of the kits indicates that the rapid test techniques only identified individuals who have relatively high parasite density. These are individuals who would normally seek treatment at the road-side patent medicine dealers. The goal of active surveillance is to identify those individuals who normally would not be detected in a passive (clinic based) surveillance programme; i.e, asymptomatic individuals. It is believed that asymptomatic individuals with low malaria parasite density make parasite detection very much difficult. In conclusion, if active surveillance is to be conducted, it should be done with high quality equipment. However, in a community based surveillance, where superstitious belief is high and compliance to malaria diagnosis is low, the rapid test with 15 minutes result output could be used to sensitized the people for blood collection followed by reexamining the individuals with microscopy.

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