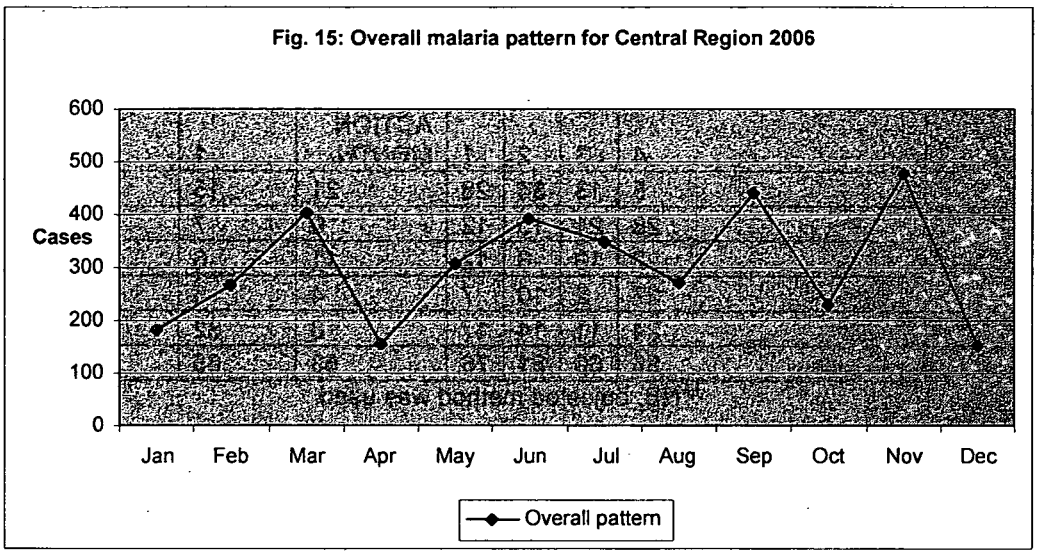


Fig. 15: Overall malaria pattern for Central Region 2006

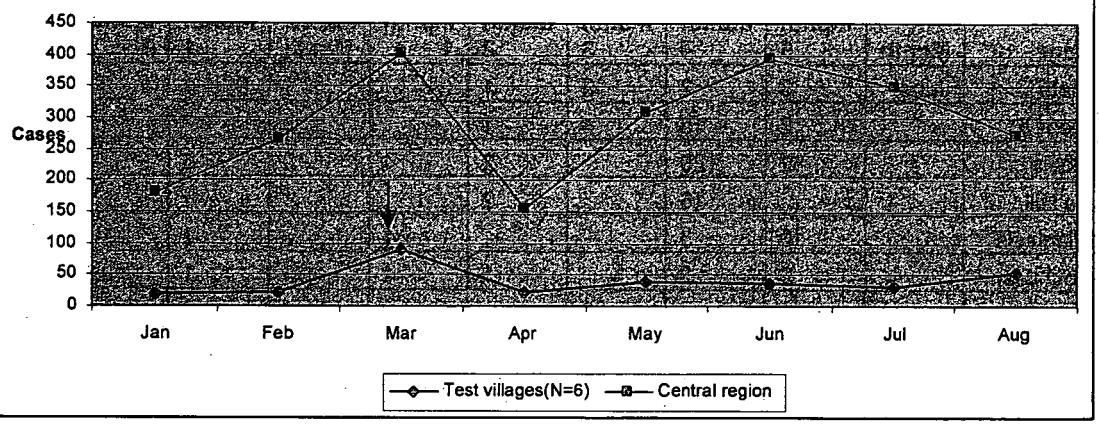
3.3.2.1.The impact of a double control measure (MBS and Bednet distribution/replacement) applied at the first malaria peak period of 2006.

Six villages were attended in March and malaria control measures used were MBS and Bednet distribution/Replacement (BR). Monthly cases of villages were monitored and compared with that of Central Region (overall) and are shown in Table 19 and Fig. 16.

Table: 19: Overall Malaria cases of Central region and total malaria cases of villages with control measures done in March 2006

			MONTHLY MALARIA CASES							
VILLAGE	DATE OF ACTIVITY	TYPE OF ACTIVITY	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG
6 test villages	March	MB,BR	21	24	91	24	39	37	30	54
126 villages			180	267	402	156	308	394	349	271

Fig. 16: Overall malaria pattern of Central region and malaria pattern of villages with control measures done in March 2006



The results indicated that at the height of the malaria peak period MBS and BR does not show any immediate suppression of malaria cases. The impact of the control measures were observed soon after the first malaria peak, two months later. When the second malaria peak started to ascend in May the malaria in the test villages continued to be suppressed.

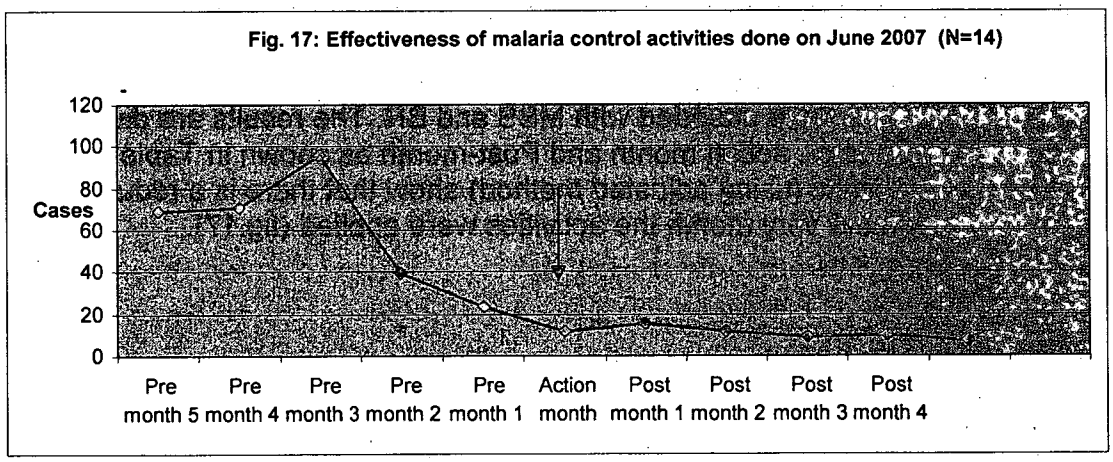
3.3.2.2.The impact of a double control measure (MBS and Bednet distribution/replacement) applied at the second malaria peak period of 2007.

The second malaria peak in 2007 occurred in May-July. In this month a total of 14 villages were attended with MBS and BR. The results are divided into pre-month data , action month and Post-month as shown in Table 20 and Fig. 17. The results (using adjusted method) show that there is a reduction of malaria on the very month the activities were applied (fig.17).

Table 20: Effectiveness of malaria control activities done in June 2007

Village	Month	Pre mpnth 5	Pre month 4	Pre month 3	Pre month 2	Pre month 1	Action Month	Post month 1	Post month 2	Post month 3	Post month 4
Aimela	June	3	2	3	1		1	1			
Aligegeo		2	15	4	1		3	2	3		1
Arabala		12	25		2	4	2	3	1		
Bina Hill			16		2				1		
Burianiasis		10	5			1		1		3	
Faibubulia		2	2		1	1		1	1	1	
Gegema		1		3							
Harasita		3	1		1		1			1	
Karara		3	1	1				1	1		1
Kwailabusu		1	2	4				1		1	
Kwareasi		6	3	3			2				4
Namorako		8	10				3	1	2	1	
Oibola		19	10	18	14	5				3	
Zion		1	4	3	1	1	3	1			1
TOTAL		**71**	**96**	**39**	**23**	**12**	**15**	**12**	**9**	**10**	**7**

** NB: The adjusted method is used

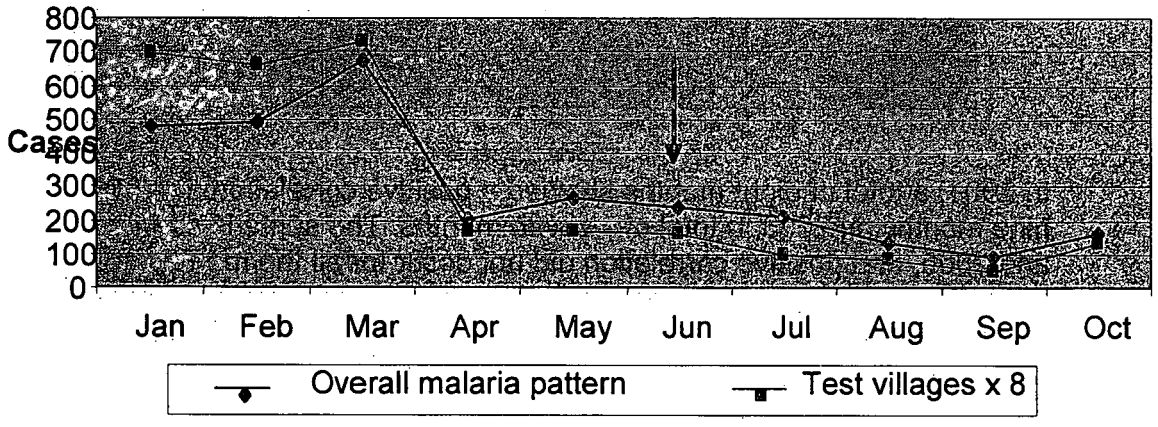


Using the routine method and comparing the malaria pattern of the test villages with that of Central region (overall) table 21 and figure 18 shows that the impact of the activities occurred on the second month. However this reduction was not sustained on the third month.

Table 21: Overall malaria cases for Central region and malaria pattern of test villages - June 2007

			MONTHLY MALARIA CASES									
VILLAGE	DATE OF ACTIVITY	TYPE OF ACTIVITY	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT
213 VILLAGES			478	490	674	202	269	236	212	134	91	163
14 test villages	June uncorrected	MB.BR	86	81	90	21	21	20	12	10	6	16
test x 8			698	657.4	730.4	170.4	170.4	162.3	97.4	81.2	48.7	129.9

Fig. 18: Overall malaria pattern for Central region and malaria pattern for villages (n=14) control measures done in June 2007



NB: Figures for test villages were small hence they were multiplied by 8 to increase the figures for better correlation.

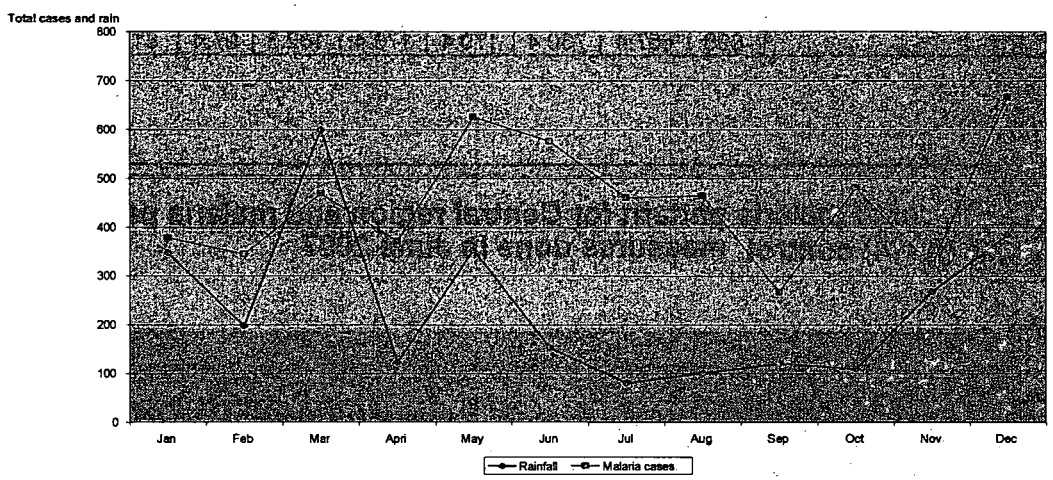
3.4. ANALYZING BOTH METEOROLOGY AND MALARIA DATA TO DEVELOP A SYSTEM THAT COULD ASSIST MALARIA OFFICERS IN PREDICTING EPIDEMICS AND FORCASTING MALARIA CASES. HENCE RESPONDING IN TIME, WHILE WAITING FOR MALARIA DATA TO ARRIVE FROM THE CLINICS - CENTRAL REGION RESULTS

3.4.1. Collecting climate and malaria data
See section 4.3.1.

3.4.2. Rainfall and Malaria cases correlation

Monthly rainfall and malaria data since 2000 were correlated. Results show that when monthly rainfall data is correlated with its corresponding malaria cases there is a positive correlation shown for the first eight months of the year (Jan-Aug). When rainfall increases in a particular month cases will increase simultaneously, though at different rates. A total of eight months showed positive correlation (fig. 19).

Fig. 19: Central region of Malaita-Rainfall and Malaria cases -2000



In 2001, a total of eight months showed a positive correlation. In 2002, a total of nine months, in 2003, a total of seven months. The same is seen also for 2004 and 2005, that positive correlation did not occur for all the months. Probably in the other months in which there was no correlation other factors that may be stronger determining factors than rain fall might have affected malaria endemicity.

3.4.3. Rainfall and Malaria Incidence correlation

When correlating monthly rainfall and its corresponding malaria incidence there was a positive correlation in the first eight months of the year (Jan-Aug) just as seen for rainfall and cases correlation in Fig.19.

The correlation of incidence and rainfall in 2001, 2003, 2004 and 2005, also show that the correlation pattern is not the same in each year.

Rainfall does correlates with malaria cases and malaria incidence. However where there were negative correlations other determining factors stronger than

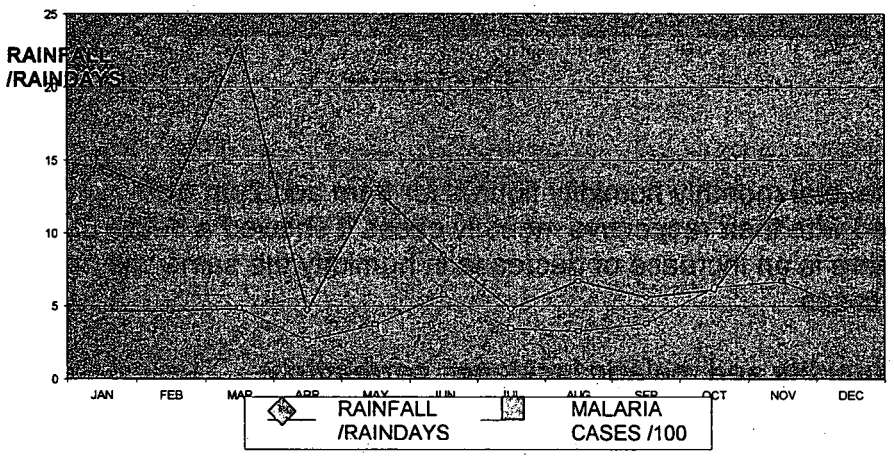
rainfall might have had their impact. These other factors do not have a consistant pattern.

3.4.4. Rainfall intensity and malaria cases correlation

Much of the favourable breeding sites for mosquitoes are created by rainfall. However tomuch rain causes flushing, destroying breeding sites resulting in low mosquito density thus less transmission. The total volume of rainfall falling (Rain intensity = Fainfall/raindays) may determine the creation of favourable breeding sites or flushing, thus may correlate with transmission and malaria cases.

When monthly rainfall intensities are correlated with their respective malaria cases (see Fig 20) only five months showed a positive correlation (March,April,May, Jul and November). In 2001, only five months and in 2002 only six months. All the other years (2003-2005) also showed that the relationship between rainfall intensity and malaria cases is not consistent each month.

Fig.20: CENTRAL RELIGION OF MALAITA -RAINFALL /RAINDAYS AND MALARIA CASES /100 FOR 2000



The impact of rainfall in a certain month is assumed to have an impact in the following month. Hence, when malaria cases for 2000 are dragged forward by one month, only five months had positive correlations (May, Jun,Jul,Aug,Oct). The same could be seen for the years 2001-2005, thus rainfall intensity do have an impact on malaria cases by a lag period of one month. But where there were no correlations there may be some other factors that had stronger impacts on malaria cases that had played a role.

3.4.5. Rainfall intensity and malaria incidence correlation

The same picture that is seen when comparing rain intensity and cases (fig. 20) is seen also for rain intensity and incidence. There is only five months that showed a positive correlation between rain intensity and malaria cases.

3.4.6. Humidity and malaria cases correlation

One of the parameters measured by the Meteorology division is humidity. Humidity is measured at 8 am and 2pm each day. Each month, total humidity is calculated. In 2000, total monthly humidity at 8 am compared with their respective malaria cases showed a consistent correlation (Fig.21). When there is an increase in humidity the same will happen for malaria cases. Humidity at 2pm also showed a similar picture, except for the month of July.

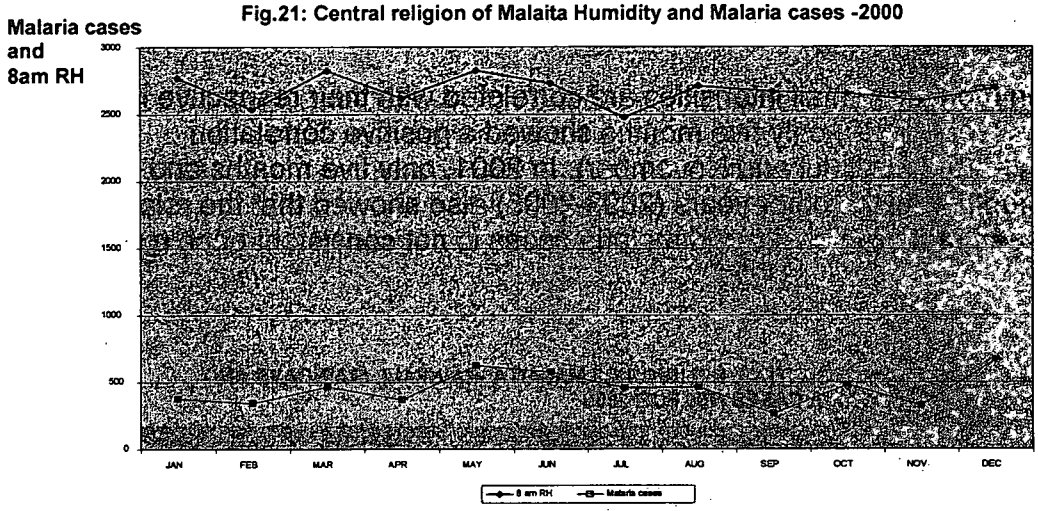


Fig.21: Central religion of Malaita Humidity and Malaria cases -2000

When the total monthly humidity figures for 8am and 2pm were averaged and correlated with their respective monthly cases it showed a better correlation . When there is an increase or decrease in humidity the same will happen for malaria cases

3.4.7. Humidity and malaria incidence correlation

When the 2000 humidity data is correlated with malaria incidence a similar picture as for humidity and malaria cases (fig.21) is shown.

Data for 2001 showed the same picture as that for 2000, a positive correlation between average humidity and cases/incidence.

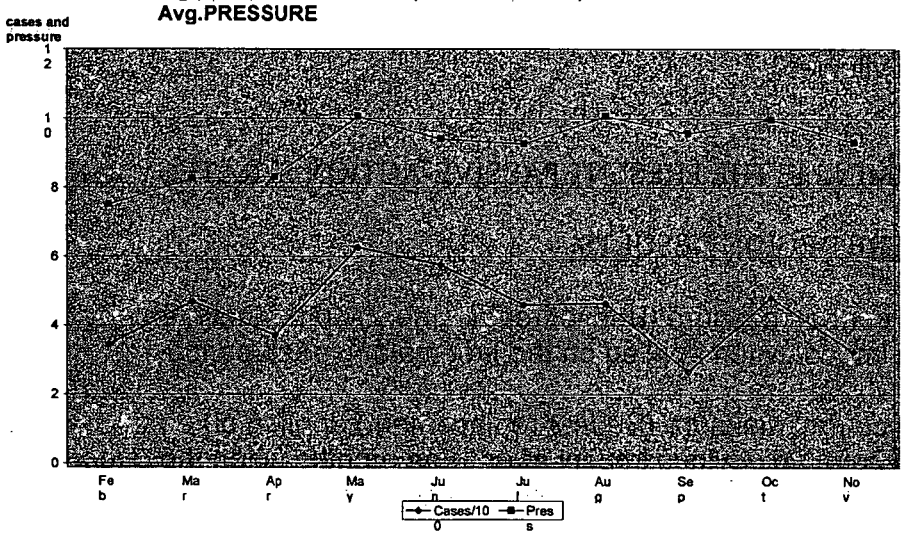
Humidity is a good indicator to use to determine whether malaria cases/incidence will increase or decrease. But it does not tell us by how much is the increase/decrease.

3.4.8. High pressure and malaria cases correlation

Air pressure is one of the parameters that the Meteorology Office measure daily. Each day the highest and lowest pressures are recorded. In 2000 the highest pressures each month are averaged and correlated with malaria cases for that respective month. The same is done for the low pressures. Results showed that there is no correlation.

However there is a good correlation between air pressure(averaged) and malaria cases when cases are shifted forward by one month (Fig.22). When pressure drops or increase malaria cases will drop or increase respectively, but by a lag of one month.

Fig.22: MALARIA CASES (Shifted forward) AND ITS CORESSPONDING Avg.PRESSURE



4. CONCLUSION

4.1. . DETERMINING THE COST –EFFECTIVE STRATEGY, PRO-ACTION or PASSIVE ACTION

4.1.2. TETERE REGION RESULTS

a) Identifying problem priority villages and controlling malaria in them is a cost effective strategy because these priority villages are only 13-15.7% of all the problem villages and yet contributes 76-77.4% of all cases per year.

b) 67.9% of all priority villages in the last malaria peak in 2005 appeared again in

the first malaria peak of 2006.

c) Using the top priority selection method, the villages that appeared >5 x as a priority village within one year is 91% likely to appear as a problem priority villages in the first malaria peak period of the following year. These villages are likely to contribute 32.6% of all cases of the first peak period of the year.

d) If the months in the first peak period are combined and the lowest cut-off point of priority villages in any of the months is selected as a common cut-off point the proportion of the villages in the first peak that will appear again in the second peak will be 63%.

e) Only a small proportion of priority villages (3.2-12.7%) appeared as priority villages in the same months within a two years period. 83% of these villages are top priority villages.

4.2.DETERMINE IF THE PRESENT PASSIVE-ACTION STRATEGY IS EFFECTIVE

4.2.1. TETERE REGION RESULTS

a) a single control measure (Bednet replacement) do not have an obvious immediate impact when applied on the first malaria peak period.

b) a single control measure (Bednet replacement) applied on a malaria peak period will have an obvious impact after three months when the overall trend was beginning to ascend for the second malaria peak.

c) a single control measure (Bednet replacement) applied on the lowest malaria period of the year will have an obvious impact for two months when the malaria trend starts to ascend for another malaria peak.

d) a single control measure (Bednet replacement) applied on the lowest malaria period do not suppress malaria during the height of the second malaria peak period.

e) a single control measure applied on the lowest malaria period could have an obvious impact when the second malaria peak of the year starts to descend.

f) a single control measure (Bednet replacement) applied on the height of the second malaria peak show no reduction in malaria. However the reduction of malaria becomes more obvious two months later, when the malaria peak begins to descend.

g) two control measures (Residual Spraying and Bednet replacement), do have an immediate impact when applied at the malaria peak period. Malaria was reduced significantly on the very month of application, but could not sustain it for another month.

4.2.2. CENTRAL REGION RESULTS

a) a double control measure (MBS with BR) do have an immediate impact on the month of action. The impact of the activity lasted for a total of three months - **Using the routine method of data calculation**

b) a double control measure (MBS and BR) using the adjusted method of calculation (Fig:12) show a significant immediate impact on the month of action. The reduction of malaria was not sustained the following month but on the third month there was a reduction again, cases started to increase again on the fourth month.

c) a double control measure (MBS and BR) applied in priority villages show no immediate impact but on the following month. The reduction of malaria could not be sustained to the second month -. using the routine method

d) a double control measure (MBS and BR) applied in priority villages show a significant impact on the very month they were applied. However the impact could not be sustained. Malaria increased again on the following month – using adjusted method.

e) a double control measure (MBS and BR) applied at the height of the first malaria peak period do not show any immediate suppression of malaria cases. The impact of the control measures were observed soon after the first malaria peak, two months later, when the second malaria peak started ascending.
f) a double control measure (MBS and BR) applied on the second malaria peak period show that there is a reduction of malaria on the very month the activities were applied – adjusted method.

g) a double control measure (MBS and BR) applied on the second malaria peak shows an impact on the second month. However this reduction was not sustained on the third month – routine method.

4.2.3. Comparing Tetere and Central region results

Results from both Tetere and Central regions supported that all malaria control measures applied did have an impact. Using the adjusted method of calculating data results showed that all activities applied did reduces malaria within 30 days.

Using the routine method of calculating data results (table 22) showed that a single control measure(BR) do not have any impact in reducing the malaria peaks. Their impact were obvious prior to the height of the peak or when the peak is descending.

Table 22: Comparing Tetere and Central region results

SINGLE CONTROL MEASURE IMPACT ON PEAK PERIODS(routine)				
Region	Type of measure	Applied at first peak	Applied at lowest malaria period	Applied at second peak
Tetere	Bednet Replacement	- No reduction. - Reduction observed three months later when the second peak starts ascending	- No reduction - Reduction observed when the second peak ascends	- No reduction - Reduction observed two months late when the peak descends.
DOUBLE CONTROL MEASURES IMPACT ON PEAK PERIODS(routine)				
Region	Type of measure	Applied at first peak	Applied at lowest malaria period	Applied at second peak
Tetere	Residual spraying + Bednet replacement			- reduction
Central	MBS + Bednet replacement	- No reduction - Reduction observed two months later when the second peak starts ascending.		- No reduction - Reduction observed a month later

The double control measures have their impacts a month earlier than single control measures. When applying control measures on the first peak period a single control measure took three months in Tetere region before its impact was observed. On the same period a double control measure in Central region took only two months. Similarly a single control measure applied on the last peak period in Tetere region took two months before the impact was observed. On the same period a double control measure in Central region took one month.

To significantly reduce malaria during the height of any malaria peak a strong double control measure has to be applied. In Tetere region it took bothe Residual spraying and bednet replacement before an impact was observed at the very height of the peak period.

4.3. ANALYZING BOTH METEOROLOGY AND MALARIA DATA TO DEVELOP A SYSTEM THAT COULD ASSIST MALARIA OFFICERS IN PREDICTING EPIDEMICS AND FORCASTING MALARIA CASES. HENCE RESPONDING IN TIME, WHILE WAITING FOR MALARIA DATA TO ARRIVE FROM THE CLINICS

a) The best meteorology parameters that could be used to predict an increase or decrease of malaria are Humidity and Air pressure.

b) Monthly Humidity is correlated with its respective monthly malaria cases/incidence. If field officer could measure their own humidity and monitor its trend they could predict within that month whether malaria will increase or decrease.

c) Air pressure would help field managers predict whether malaria cases/incidence would increase/decrease the coming month. Hence giving them enough time to act.

d) Air pressure is a more accurate parameter to predict malaria peak months. It could be used to predict a malaria peak period one month prior to its occurance.

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分担研究報告書

「アジアで流行している感染症の我が国への侵入監視の強化に関する研究」

PCR 法に使用する陽性対照の開発

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研究要旨

PCR 法は高感度かつ容易な手法として実験のみならず、検査診断に広く用いられるようになったが、実際の利用に当たっては高感度ゆえに PCR 産物の汚染による偽陽性がしばしば問題となる。特に陽性対照として用いる鋳型は十分な量があり、多量な反応産物を確実に作りつづけることから、陽性対照の混入を原因とした偽陽性の確率は必然的に高くなる。DNA 操作の訓練や実験設備の充実により問題発生は低減されるが、依然として操作ミスによる汚染が生じることに変わりは無い。そこで、万一陽性対照の汚染による偽陽性の反応が生じても、偽陽性であることが直ちに判別できるよう陽性対照の配列を改変し利用する方法を提案し、関係国への普及に努めた。

A. 研究目的

現在、各方面で遺伝子検査法が採用されている。感度や迅速性に優れ、共通の基本操作および装置により多様な病原体検査が可能である。一方、本試験方法ではかならず陽性対照が必要となり、その供給は必ずしも容易ではない。アジア地域での検査法普及に際してはわが国からの強力な支援が必須で、特に陽性対照(鋳型 DNA 等)の提供は経済面のみならず、精度管理の観点からも不可欠である。ところが、陽性対照による不用意な汚染は検査の信頼性を損なう最大の原因ともなる。そこで、国際的に提供することを前提とした腸管寄生性原虫類の遺伝子診断用鋳型 DNA(陽性対照)の開発を行なった。

遺伝子検査においては、陽性対照の汚染による事故(偽陽性)は往々防ぎ得ないものとし、反応産物の真偽判定を容易に行なえるよう予め陽性対照の鋳型 DNA の設計(配列を改変)を行なった。そのため、陽性対照(鋳型 DNA)によって得られる増幅産物は真の増幅産物のサイズと異なること、制限酵素切断部位が導入されていること、改変部位に署名となる配列を組み込むことを設計の基本とした。これにより、増幅産物の電気泳動での真偽の区別を容易にし、制限酵素切断の有無による確認が可能となり、最終的には遺伝子配列を読むことで改変部位の署名となる配列が確認できる。また、将来この挿入配列を用いて PCR やプローブによる検出へ拡張する余地が確保出来るものと考える。

B.研究方法

陽性対照となる鋳型 DNA は目的の PCR 産物とは異なる大きさで、プライマーダイマーよりも大きい必要がある。そこで、100bp 前後とした。任意の配列は市販のオリゴ合成により用意し(グライナージャパン)、PCR 法に

より適宜配列を接続した。用いる配列は機能が既知であり(rDNA 等)、特異配列の一部であることを条件に化学合成した。

ACGTの4つ塩基を利用し、A を00、Cを01、G を10、T を11と規定すると、2進数で2ビットの情報となる(表1)。署名に使う文字は ASCII コードに変換し、4 塩基の配列(8bit)で1文字を表現した(表2)。署名配列には国立感染症研究所の略称である"NIID, Japan"を用いることとした。これを 16 進数のASCIIコードで表現すると"4E 49 49 44 2C 20 4A 61 70 61 6E"となり、上述の術の規定に従って塩基配列に置き換えると "catg cagc cagc caca agta agaa cagg cgac ctaa cgac cgtg"の 44bp で表現される。Blastn サーチでこの配列と完全一致の登録配列が無いことを確認した。なお、部分的にはヒトの配列と20/22(90%)の一致が確認された。

制限酵素部位は 5'側に EcoRI、3'側に PstI を導入し、56bp とした"GAATTC catg cagc cagc caca agta agaa cagg cgac ctaa cgac cgtg CTGCAG"。一般に、6塩基認識の制限酵素認識部位がこの程度に近接して2 つ存在する頻度は低く、確認のための切断に用いるだけでなく、人工物であることを強調する意味も含めた。この配列を基本として、これに PCR プライマーを両端に接続すれば 100bp 前後の陽性対照用鋳型 DNA が完成する。PCR 用酵素には Ex Taq hotstart version(TakaraBio)を用いた。電気泳動による産物の確認にキャピラリー電気泳動装置 HDA-12(eGene)を使用した。なお、泳動に際して 15bp と 5kbp のアライメントマーカーを使用したので、泳動像には全てのレーンにこの 2 本のバンドが含まれている。

C. 研究結果

赤痢アメーバ

赤痢アメーバは病原性の *Entamoeba histolytica* と非病原性の *E. dispar* の鑑別が必要で、鑑別結果に従って治療の必要性を判断する。SSU rDNA の Nested-PCR では、*Entamoeba* 属共通の一次反応に、E1、E2 プライマーが用いられる。二次反応では *E. histolytica* 特異的プライマーに EH1、EH2 プライマー、*E. dispar* 特異的プライマーに ED1、ED2プライマーが用いられる。これらのプライマーを接続した配列すると 168bp となる(E1- EH1- ED1- Sign- ED2comp- EH2comp- E2comp(comp は complement 相補配列の略))。本当の赤痢アメーバからの増幅産物は 900bp 程度となり、区別は容易である。

SSU rDNA とは別に、29-kD システインリッチタンパク質遺伝子による鑑別法もあり、このプライマーを同じ陽性対照に組み込み、共通の陽性対照として構築した。すなわち、*E. histolytica* 特異的プライマーの P11 と P12、*E. dispar* 特異的プライマーの P13 と P14 である。全て接続語の配列は 252bp となった(P11- P13- (E1- EH1- ED1- Sign- ED2comp- EH2comp- E2comp) -P14comp- P12comp)。本来の *E. histolytica* および *E. dispar* の PCR 産物は 118bp、120bp であり、陽性対照はそれぞれ 252bp、211bp となることから、区別は容易である。

該陽性対照は経済性を考慮し、20bp 程度ずつ共通の配列を持たせた 70bp 弱の合成オリゴ5本を3回のPCRで接続した(図1)。完成した PCR 産物を希釈して鋳型として用いて P11/P12 プライマーで PCR を行い、反応することを確認した(図 2)。

クリプトスポリジウム

クリプトスポリジウムは 18S rDNA 遺伝子により種別(Xiao et al., 1999)、cpgp 遺伝子により亜型の鑑別が可能である(Leav et al., 2002)。当該研究ではこれら2遺伝子の8反応 16 本のプライマーを接続した 327bp のクリプトスポリジウム用陽性対照を作成した(cxof-cxif-cpbdiagf-cl2f-cpgpnewf1-cl1f-

cpgpwf-cpgpnewf2-sign-cpgpwr2-cpgpwr-cpgpwr1-cl1r-cl2r-cxir-cpbdiagr-cxor)。プライマーは18S rDNAの一部領域を増幅するNested-PCR(Xiao et al., 1999)の2セット4本(cxof, cxif, cxir, cxor)、18S rDNAの変異領域を増幅するPCR(Johnson et al., 1995)の2本(cpbdiafg, cpbdiagr)、cpgp遺伝子の一部領域を増幅するNested-PCR(Leav et al., 2002)の4本(cl2f, cl2r, cl1f, cl1r)、cpgp遺伝子の一部領域を増幅するPCR(Wu et al., 2003)の2本(cpgpwf, cpgpwr)、cpgp遺伝子の一部領域を増幅するNested-PCR(泉山ら、平成18年度当該研究報告書)の4本(cpgpnewf1, cpgpnewf2, cpgpnewr1, cpgpnewr2)を接続した。本当のクリプトスポリジウムの産物は400bp～1.3kb程度となるが、陽性対照では327bp未満となり判別が容易となる(表3)。

ジアルジア

ジアルジアはgdh遺伝子の型別により人獣に感染するAssemblageのAとB、イヌに感染するCとD、その他E、ネコに感染するFを分けることが可能である(Monis et al., 1999)。当該研究ではgdh遺伝子のNested-PCR用プライマー4本を接続した119bpのジアルジア用陽性対照を作成した(gdh1-gdh1seq-EcoRI-Signature-PstI-gdh4seq-gdh4)。本来のジアルジアのPCR産物は1st PCRが約770bp、2nd PCRが約750bpとなり、一方陽性対照の1st PCRでは119bp、2nd PCRでは96bpとなることから、判別は容易である。

D.考察

わが国では多分野において遺伝子検査法が採用されている。感度や迅速性に優れ、基本操作や装置の共有により多様な病原体検査が可能である。さらに、培養法や鏡検など従来の検査方法と比べ、結果に担当者の主観の入る余地が少ないこと(均質なデータ取得)、機材や設備あるいは人材といった導入に際しての障壁が少ないことも特徴として挙げられる。そのため、東南アジア地域との検査ネットワークの構築に際しては積極的に導入を図るべき手法と判断し、普及に努めてきた。一方、本試験方法では陽性対照(鋳型DNA等)が必要となり、その安定供給は必ずしも容易ではない。そのため、アジア地域での検査法普及に際してはわが国からの強力な支援が必須で、陽性対照(鋳型DNA等)の提供が不可欠の要因となる。そこで、全くの合成により各種腸管寄生性原虫類の鋳型DNAを用意した。その際、遺伝子検査法では陽性対照の汚染による事故(偽陽性)は防ぎ得ないものとし、反応産物の真偽が容易に判定できるよう予めの準備が必要と考えた。具体的には、以下のように鋳型DNAの設計(配列を改変)を行なった。まず、真の増幅産物とサイズを変え、電気泳動により違いを可視化することとした。また、制限酵素切断部位を導入し、確認試験を容易にした。さらに、改変部位に署名となる配列を導入し、遺伝子配列を読むことで改変部位の署名となる配列が確認できるようにした。署名とは、ACGTの4つ塩基を利用し、Aを00、Cを01、Gを10、Tを11と規定すると、2進数で2ビットの情報と読み替えることができる(表1)。一方、署名文字《NIID, Japan》をASCIIコードに変換し、4塩基の組み合わせ配列で1文字(8bit)を表現した(表2)。また、将来この挿入配列を用いてPCRやプローブによる検出へ発展的に拡張する余地が確保できるものと考える。さらに、この研究を足掛かりとして陽性対照の安全使用が周知・徹底されることを期待する。

E.結論

PCR法は高感度ゆえにPCR産物の汚染による偽陽性がしばしば問題となる。当該研究では陽性対照の汚染による人為ミス(偽陽性)は避けがたいものと認識し、反応産物の

真偽判定を容易にする陽性対照(鋳型DNA)を設計した。この陽性対照はアジアネットのタイ、フィリピン両国に常時供給を保証した。また、国内にあっても要望に応じ配布する予定である。

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F. 健康危機情報

なし

G. 研究発表

なし

表 1. 塩基の意味付け

nucleotide	binary
a	00
c	01
g	10
t	11

表 2　配列、アスキーコード、文字の変換テーブル

sequence	binary	hexadecimal	character	sequence	binary	hexadecimal	character	sequence	binary	hexadecimal	character
agaa	010 0000	20	(space)	caaa	100 0000	40	@	cgaa	110 0000	60	`
agac	010 0001	21	!	caac	100 0001	41	A	cgac	110 0001	61	a
agag	010 0010	22	"	caag	100 0010	42	B	cgag	110 0010	62	b
agat	010 0011	23	#	caat	100 0011	43	C	cgat	110 0011	63	c
agca	010 0100	24	$	caca	100 0100	44	D	cgca	110 0100	64	d
agcc	010 0101	25	%	cacc	100 0101	45	E	cgcc	110 0101	65	e
agcg	010 0110	26	&	cacg	100 0110	46	F	cgcg	110 0110	66	f
agct	010 0111	27	'	cact	100 0111	47	G	cgct	110 0111	67	g
agga	010 1000	28	(	caga	100 1000	48	H	cgga	110 1000	68	h
aggc	010 1001	29	)	cagc	100 1001	49	I	cggc	110 1001	69	i
aggg	010 1010	2A	*	cagg	100 1010	4A	J	cggg	110 1010	6A	j
aggt	010 1011	2B	+	cagt	100 1011	4B	K	cggt	110 1011	6B	k
agta	010 1100	2C	,	cata	100 1100	4C	L	cgta	110 1100	6C	l
agtc	010 1101	2D	-	catc	100 1101	4D	M	cgtc	110 1101	6D	m
agtg	010 1110	2E	.	catg	100 1110	4E	N	cgtg	110 1110	6E	n
agtt	010 1111	2F	/	catt	100 1111	4F	O	cgtt	110 1111	6F	o
ataa	011 0000	30	0	ccaa	101 0000	50	P	ctaa	111 0000	70	p
atac	011 0001	31	1	ccac	101 0001	51	Q	ctac	111 0001	71	q
atag	011 0010	32	2	ccag	101 0010	52	R	ctag	111 0010	72	r
atat	011 0011	33	3	ccat	101 0011	53	S	ctat	111 0011	73	s
atca	011 0100	34	4	ccca	101 0100	54	T	ctca	111 0100	74	t
atcc	011 0101	35	5	cccc	101 0101	55	U	ctcc	111 0101	75	u
atcg	011 0110	36	6	cccg	101 0110	56	V	ctcg	111 0110	76	v
atct	011 0111	37	7	ccct	101 0111	57	W	ctct	111 0111	77	w
atga	011 1000	38	8	ccga	101 1000	58	X	ctga	111 1000	78	x
atgc	011 1001	39	9	ccgc	101 1001	59	Y	ctgc	111 1001	79	y
atgg	011 1010	3A	:	ccgg	101 1010	5A	Z	ctgg	111 1010	7A	z
atgt	011 1011	3B	;	ccgt	101 1011	5B	[	ctgt	111 1011	7B	{
atta	011 1100	3C	<	ccta	101 1100	5C	\	ctta	111 1100	7C	|
attc	011 1101	3D	=	cctc	101 1101	5D	]	cttc	111 1101	7D	}
attg	011 1110	3E	>	cctg	101 1110	5E	^	cttg	111 1110	7E	~
attt	011 1111	3F	?	cctt	101 1111	5F	_				

表3　クリプトスポリジウム用陽性対照のサイズ一覧

標的遺伝子	プライマーセット	予定産物サイズ	
		本当の鋳型	陽性対照
18S rDNA	cxof/cxor	1.3kb	327bp
18S rDNA	cxif/cxir	840bp	276bp
18S rDNA	cpbdiagf/cpbdiagr	440bp	260bp
cpgp	cl2f/cl2r	981bp	207bp
cpgp	cl1f/cl1r	949bp	175bp
cpgp	cpgpwf/cpgpwr	889bp	115bp
cpgp	cpgpnew1f/cpgpnew1r	923bp	149bp
cpgp	cpgpnew2f/cpgpnew2r	883bp	109bp

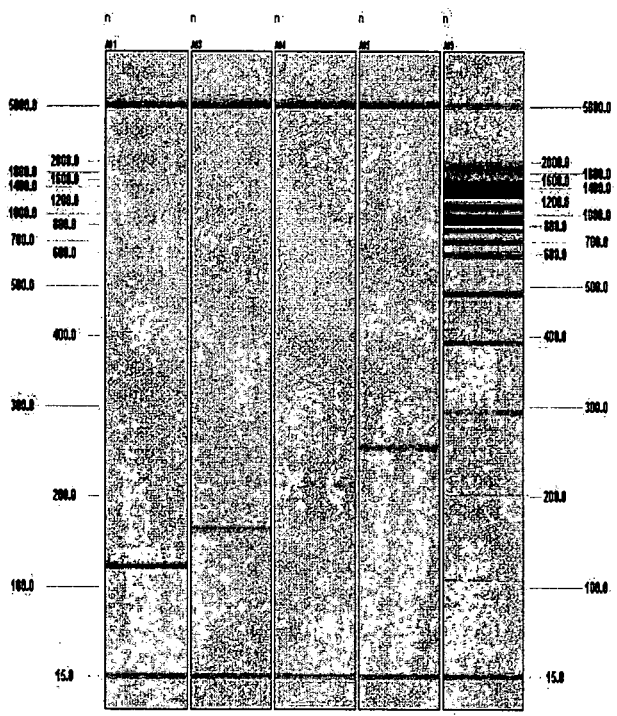

図2　赤痢アメーバ陽性コントロールの合成

各種の鋳型DNAをPCR反応で連結して、一本の鋳型DNAとした。これを用い、それぞれのprimerセットを用いることで、大きさの異なる特異バンドが合成される。

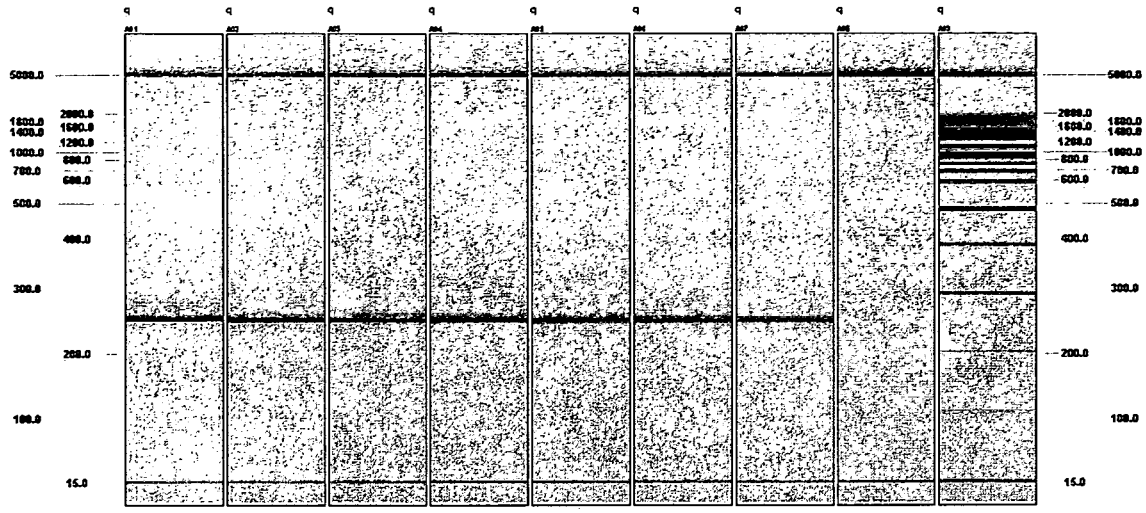

図 3　陽性コントロールの希釈と PCR による確認

ここでは鋳型 DNA 保存液を 10^{-1} から 10^{-7} まで希釈し、反応性を確認した。その結果、全ての希釈段階で鋳型として機能し、反応産物を得た。

Molecular Characterization of Isospora belli Oocysts from Patients in Thailand

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ABSTRACT

To investigate the extent of genetic heterogeneity in the genus *Isospora* infecting patients in Thailand, a total of 38 fecal samples containing *Isospora* oocysts from human immunodeficiency virus/acquired immunodeficiency syndrome patients (n=30), corticosteroid-treated patients (n=3) and immunocompetent individuals (n=5) were recruited for analysis. Though remarkable variation in the maximum width and length of oocysts both within and between isolates was observed, the small subunit ribosomal RNA (rRNA), 5.8S rRNA, internal transcribed spacer 1 (ITS-1),and ITS-2 were highly conserved, indicating that there were no cryptic species or extensive strain variation.

INTRODUCTION

Isospora belli is a coccidian protozoon in phylum Apicomplexa that parasitizes epithelium of upper small intestine of humans and causes diarrheal disease. The entire life cycle of Isospora consists of asexual development and sexual reproduction that take place in the same host. Transmission of *I. belli* oocysts seems to be confined to the anthroponotic cycle because humans are the only known natural host. Although both immunocompetent individuals and immunosuppressed patients are susceptible to infections, the prevalence of isosporiasis seems to occur more frequently in the latter. After the pandemic of human immunodeficiency virus-1 (HIV-l) infection, human isosporiasis has been more commonly identified as an opportunistic infection of the gastrointestinal tract of those who have lowCD4+1ymphocyte counts (usually <200cells/μl).

Human isosporiasis seems to be cosmopolitan in distribution, especially in tropical and subtropical regions such as Haiti, Mexico, Brazil, EI Salvador, Venezuela, and Southeast Asia. However, the prevalence of this infection is occasionally under estimated because oocysts are usually excreted in small numbers or may not be found in spite of actual infection. Moreover, the transparent appearance of *I. belli* oocysts could be overlooked in direct fecal smears.

It is of note that infection with *I. belli* usually produces more aggressive and