世代シーケンサーを利用したサルマラリア原虫*Plasmodium cynomolgi*の新規ゲノム構築と比較ゲノム解析, 第９回分子寄生虫・マラリア研究フォーラム, (長崎) 2010.10.9

4. **彦坂健児**・中井裕・渡邊洋一・橘真一郎・有末伸子・Palacpac Nirianne・遠山知子・本間一・堀井俊宏・北潔・田邉和裕, Eimeria tenella のミトコンドリアゲノムはコンカテマー構造をとる, 第９回分子寄生虫・マラリア研究フォーラム, (長崎) 2010.10.8.
5. 有末伸子・Nirianne M. Q. Palacpac・川合覚・平井 誠・田邉和裕・堀井俊宏, マラリア原虫 SERA 遺伝子ファミリーの系統特異的な進化, 第９回分子寄生虫・マラリア研究フォーラム, (長崎) 2010.10.8.
6. K. Tanabe and T. Mita. Intimate association of evolutionary history of the human malaria parasite, *Plasmodium falciparum*, with ancient demography of modern humans. The 10th Awaji International Forum on Infection and Immunity. 2010.9.10.
7. N. Arisue, N. M. Q. Palacpac, S. Kawai, M. Hirai, K. Tanabe, and T. Horii. The SERA gene family from several *Plasmodium* species: Picking important clues for the evolutionary puzzle of this gene family. The 10th Awaji International Forum on Infection and Immunity. 2010.9.9.
8. K. Hikosaka, Y. Nakai, Y. Watanabe, S. Tachibana, N. Arisue, N. Marie Q. Palacpac, T. Toyama, H. Honma, T. Horii, K. Kita, K Tanabe. Concatemer mitochondrial genome of the coccidian parasite *Eimeria tenella*. The 10th Awaji International Forum on Infection and Immunity. 2010.9.9.
9. S. Tachibana, S. Kawai, N. Goto, S. Nakamura, N. Arisue, Y. Katakai, H. Honma, N. Palacpac, H. Sawai, T. Tougan, K. Kita, Y. Yasutomi, T. Horii, T. Yasunaga, K. Tanabe. Genome sequencing of the *P. vivax*-related monkey malaria parasite, *P. cynomolgi* and the comparative genomic analysis. The 10th Awaji International Forum on Infection and Immunity. 2010.9.9.
10. S. Kawai, H. Iseki, N. Takahashi, M. Hirai, K. Tanabe, N. Yokoyama, I. Igarashi. A novel molecular diagnostic tool for zoonotic simian malaria parasite *Plasmodium knowlesi* infection. XIIth International Congress of Parasitology, Melborune, 2010.8.15.
11. 早川敏之, 橘真一郎, 有末伸子, 彦坂健児, 澤井裕美, 田邉和裕. 現生マラリア原虫の起源での急速な多様化 (マラリアビッグバン) 第１２回日本進化学会 (東京), 2010.8.2
12. N. Arisue, Tetsuo Hashimoto, H. Mitsui, A. Kaneko, M. Hasegawa, K. Tanabe, T., Horii. Comparative analyses of the apicoplast genomes from nine Plasmodium species. The 18th meeting of the International society for evolutionary protistology, Kanazawa, Ishikawa, Japan, 2010.7.6.
13. 田邉和裕, 美田敏宏, Nirianne Palacpac, 金子明, 堀部舜, 澤井裕美, 先濱直子, 大前比呂思, 中村正聡, 平山謙二, 堀井俊宏,

岸野洋久，熱帯熱マラリア原虫の遺伝的多様性は東アフリカからの地理的距離によって決定される，第７９回日本寄生虫学会大会, 2010.5.20.

14. 有末 伸子, Nirianne M. Q. Palacpac, 田邉 和裕, 堀井 俊宏, マラリア原虫 *sera* 遺伝子ファミリーの多型解析，第７９回日本寄生虫学会大会, 2010.5.20.
15. 彦坂健児，渡邊洋一，辻尚利，遠山知子，有末伸子，北潔，堀井俊宏，中井裕，五十嵐郁男，田邉和裕，Apicomplexa 生物群におけるミトコンドリアゲノムの進化と多様性，第７９回日本寄生虫学会大会，2010.5.20.
16. 橘真一郎，川合覚，後藤直久，中村昇太，片貝祐子，澤井裕美，東岸任弘，北潔，保富康宏，堀井俊宏，安永照雄，田邉和裕，次世代シーケンサーによる三日熱マラリア原虫近縁サルマラリア原虫 *P. cynomolgi* のゲノム解読，第７９回日本寄生虫学会大会, 2010.5.20.
17. 美田 敏宏，塚原 高広，高橋 延之，遠藤 弘良，田邉 和裕，熱帯熱マラリア原虫はどのような順番でdhps遺伝子に変異を蓄積していくか，第７９回日本寄生虫学会大会2010. 5. 20.
18. 金子 明，Taleo George, Chaves Luis F., Perlmann Hedvig, 江藤 秀顕，竹尾 暁，橘 真一郎，Troye-Blomberg Marita，坪井敬文，Drakeley Chris, 田邉 和裕，島嶼マラリア根絶10年後の三日熱マラリア再燃，第７９回日本寄生虫学会大会, 2010.5.20.
19. 中野 由美子，美田 敏宏，中曽根 英子，田邊 和裕，アーカイブ標本による熱帯熱マラリア原虫における薬剤耐性遺伝子型の同定，第７９回日本寄生虫学会大会，2010. 5. 20.
20. カレトン リチャード，津守 陽子，マセユ ヌデゥンガ，砂原 俊彦，五十棲 理恵，上村 春樹，金子 修，田邉 和裕，Selection for drug resistance in malaria parasites differs between urbanand suburban areas of Brazzaville, Republic of Congo，第７９回日本寄生虫学会大会，2010. 5. 21.
21. 青沼 宏佳，遠山 知子，田原 美智留，Andrabi Syed Bilal Ahmad, 田邉 和裕，永宗 喜三郎，植物ホルモンジベレリン生合成阻害剤はトキソプラズマの増殖を抑制する，第７９回日本寄生虫学会大会，2010. 5. 21.
22. 川合 覚，片貝 祐子，保富 康宏，田邉 和裕，マカク属サル種間にみられるサル・マラリア感染感受性の比較，第７９回日本寄生虫学会大会，2010. 5. 21.
23. 田原 美智留，青沼 宏佳，遠山 知子，田邊 和裕，野崎 智義，永宗 喜三郎，植物ホルモンサイトカイニンのトキソプラズマに及ぼす影響，第７９回日本寄生虫学会大会2010. 5. 21.
24. Tomoko Toyama, Kisaburo Nagamune, Hiroshi Kawaide, Toshihiro Horii, Kazuyuki Tanabe, Inhibitors of gibberellin, a plant hormone, induce swelling and rupture of intraerythrocytic *Plasmodium falciparum.*, 第７９回日本寄生虫学会大会, 2010.5.21

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平成２２年度　分担研究報告書

マラリア流行の血清疫学指標の開発

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研究要旨　コムギ胚芽無細胞タンパク質合成系を用いることにより、コドンを何ら改変することなくゲノムワイドに熱帯熱マラリア原虫組換えタンパク質約1700種を含むプロテインアレイの作製に成功した。我々が確立したハイスループット抗原スクリーニング法（アルファスクリーン法）により、マリ共和国のマラリア流行地から得た10名の免疫血清との反応性を検討した結果、10名全員の血清と反応した抗原８種類、半数（５名）以上の血清と反応した抗原が47種同定された。以上の結果より、コムギ胚芽無細胞タンパク質合成系を用いた熱帯熱マラリア原虫プロテインアレイのハイスループット抗原スクリーニングシステムが樹立され、ゲノムワイドな熱帯熱マラリア原虫新規抗原探索が可能となったと考えられた。

A．研究目的

マラリア流行の解析に有用な新規の血清疫学指標の開発を行うため、マラリア原虫組換えタンパク質の合成に優れているコムギ胚芽無細胞タンパク質合成法を用いて熱帯熱マラリア原虫のゲノムワイドに組換えタンパク質を発現し、その中から新規抗原を同定することを目的に本研究を実施した。

B．研究方法

1) 熱帯熱マラリア原虫メロゾイト期組換えタンパクアレイの作製

コムギ胚芽無細胞タンパク質合成系を用いて、熱帯熱マラリア原虫タンパク質の内、マラリアゲノム情報データベース（PlasmoDB）より、赤血球期完全長cDNAライブラリから各cDNAクローンに特異的なPCRプライマーを用いて約1700種類のcDNAを増幅し、転写用の鋳型DNAを作製した。それらとコムギ胚芽無細胞タンパク質合成系を用いて組換えタンパク質を合成し約 1700 種類の分子を含む熱帯熱マラリア赤血球期プロテインアレイを作製した。

2) ハイスループット抗原抗体アッセイの実施

これらのタンパク質とマラリア感染者血清との抗原抗体反応を検出するために、これまでに確立したアルファスクリーン法を応用した。

3)　マリ共和国において入手したマラリア流行地からの感染者血清の使用

マリ共和国のマラリア流行地において、研究協力者の米国国立衛生研究所三浦憲豊博士の協力の下、成人マラリア免疫血清を入手した。

（倫理面への配慮）

マリ共和国におけるマラリア患者血液の採取に当たっては同国保健省および米国国

立衛生研究所の許可を得、患者への説明を十分行なった上で同意を得て実施したものである。また、本血清試料の利用は坪井が愛媛大学ヒトゲノム・遺伝子解析研究倫理委員会の許可を得ている。

C．研究結果および考察

1) 熱帯熱マラリア原虫赤血球期組換えタンパク質の合成

約 1700 種の熱帯熱マラリア原虫赤血球期プロテインアレイを用いて、マリ共和国のマラリア流行地から得た 10 名の免疫血清との反応性を検討した結果、10 名全員の血清と反応した抗原８種類、半数（５名）以上の血清と反応した抗原が 47 種同定された。以上の結果から、本法のハイスループット免疫スクリーニング系としての有用性が確認された。

3）今後の課題

上記プロテインアレイを用いて、血清疫学指標となりうる抗原の網羅的探索をおこなう。先ず、これまでに入手したタイ、マリ共和国のマラリア免疫血清を用いて実施する。その後、本年度本研究班員の大前博士から入手した太平洋島嶼部等のマラリア流行地から入手した免疫血清を用いて、同様のスクリーニングを行うことにより、地域間の原虫抗原に対する反応性の違いが検討できる。これらの結果が系統的に得られれば、マラリアの感染動向と、各種の原虫抗原に対する抗体価の変動を、大規模に追跡することが出来、将来の流行予知等に有用な血清疫学研究に用いることの出来る新規抗原タンパク質の同定が可能となる。さらに、三日熱マラリアはアジア地域で熱帯熱マラリアと共に広範に流行し、ベクターコントロールや薬剤治療を中心とする対策が実施されているにもかかわらず、熱帯熱マラリアと比較して減少傾向は認められていない。世界的にマラリア撲滅が長期目標として設定された現在、今後は三日熱マラリア原虫にも研究対象を広げる必要性がある。そのため、三日熱マラリア赤血球期原虫のプロテインアレイを作製し、新規抗原の網羅的探索を実施する。

D．結論

コムギ胚芽無細胞タンパク質合成系を用いたハイスループット抗原抗体反応スクリーニングにより、マラリア流行の血清疫学指標となりうる新規抗原タンパク質のスクリーニングがゲノムワイドに可能となると考えられた。

E. 健康危機情報

本研究により、マラリアの感染動向と各種の原虫抗原に対する抗体価の変動を、ゲノムワイドかつ大規模に追跡することが出来、将来の流行予知等に有用な血清疫学研究に用いうる抗原タンパク質セットが準備できる。これにより海外渡航者に向けたマラリア感染危険度の情報を提供できる技術的基盤となる。

E．研究発表

１．論文発表

1) Tsuboi T, Takeo S, Arumugam TU, Otsuki H, Torii M. The wheat germ cell-free protein synthesis system: a key tool for novel malaria vaccine candidate discovery. Acta Trop. 2010, 114: 171-176.

2) Miyata T, Harakuni T, Tsuboi T, Sattabongkot J, Kohama H, Tachibana M, Matsuzaki G, Torii M, Arakawa T. *Plasmodium vivax* ookinete surface protein, Pvs25, linked to cholera toxin B subunit induces potent transmission-blocking immunity by intranasal as well as subcutaneous immunization. Infect Immun. 2010, 78: 3773-3782.

3) Blagborough AM, Yoshida S, Sattabongkot J, Tsuboi T, Sinden RE. Intranasal and intramuscular immunization with baculovirus dual expression system-based Pvs25 vaccine substantially blocks *Plasmodium vivax* transmission. Vaccine. 2010, 28: 6014-6020.

2．学会発表

1) Takeo S, Sakamoto H, Kaneko T, Tachibana M, Miura K, Varma S, Sattabongkot J, Torii M, Tsuboi T.
Identification of novel blood-stage vaccine candidates against *Plasmodium falciparum* by high-throughput immunoscreening.
Adaptive and Innate Immune Responses to Neglected Tropical Diseases, San Diego, USA, January 9-11, 2010.

2) Miura K, Takeo S, Sakamoto H, Kaneko T, Varma S, Torii M, Sirima SB, Tsuboi T.
Identification of *Plasmodium falciparum* blood-stage potential vaccine candidates: high-throughput immunoscreening approach with Burkina Faso children sera.
Adaptive and Innate Immune Responses to Neglected Tropical Diseases, San Diego, USA, January 9-11, 2010.

3) Takeo S, Sawasaki T, Torii M, Sattabongkot J, Endo Y, Tsuboi T.
Functional production of malarial parasites' proteins with wheat germ cell-free system.
Keystone Symposium, Structural Genomics: Expanding the Horizons of Structural Biology, Breckenridge, USA, January 8-13, 2010.

4) Tsuboi T, Tachibana M, Takeo S, Sattabongkot J, Wu Y, Torii M.
Sexual stage parasites and transmission-blocking antibodies.
Keystone Symposium, Malaria: New Approaches to Understanding Host-Parasite Interactions, Copper Mountain, USA, April 11-16, 2010.

5) Chen JH, Jung JW, Wang Y, Lu F, Ha KS, Tsuboi T, Han ET.
Evaluation of putative immunogenic proteins from vivax malaria blood stage by high-throughput screening assays.
Keystone Symposium, Malaria: New Approaches to Understanding Host-Parasite Interactions, Copper Mountain, USA, April 11-16, 2010.

6) Tsuboi T, Tachibana M, Takeo S, Sattabongkot J, Wu Y, Torii M.
(Invited speaker)
Wheat germ cell-free system-expressed Pfs230 is an

effective transmission-blocking vaccine candidate antigen.
12th International Congress of Parasitology, Melbourne, Australia, August 16-20, 2010.

7) Miura T, Takeo S, Otsuki H, Torii M, Tsuboi T.
Erythrocyte proteome screening for interaction partners of malarial merozoite RhopH complex.
12th International Congress of Parasitology, Melbourne, Australia, August 16-20, 2010.

8) Ito D, Han ET, Takeo S, Thongkukiatkul A, Otsuki H, Torii M, Tsuboi T.
Characterization of putative rhoptry neck protein 3 (PfRON3) in *Plasmodium falciparum* merozoite.
12th International Congress of Parasitology, Melbourne, Australia, August 16-20, 2010.

9) Arumugam TU, Takeo S, Thonkukiatkul A, Miura K, Otsuki H, Zhou H, Long CA, Thompson J, Healer J, Crabb B, Cowman A, Torii M, Tsuboi T.
A new Pf merozoite micronemal protein is a novel blood-stage vaccine candidate antigen.
12th International Congress of Parasitology, Melbourne, Australia, August 16-20, 2010.

10) Sakamoto H, Takeo S, Kaneko T, Sattabongkot J, Torii M, Tsuboi T.
A novel blood-stage malaria vaccine candidate with human erythrocyte binding capacity.
The 10th Awaji International Forum on Infection and Immunity, Awaji, Japan. September 7-10, 2010.

11) Arumugam TU, Takeo S, Thonkukiatkul A, Miura K, Otsuki H, Zhou H, Long CA, Sattabongkot J, Torii M, Tsuboi T.
Wheat germ cell-free system facilitated the identification of a novel malaria vaccine candidate.
The 10th Awaji International Forum on Infection and Immunity, Awaji, Japan. September 7-10, 2010.

12) Miura T, Takeo S, Otsuki H, Sawasaki T, Endo Y, Torii M, Tsuboi T.
RhopH complex from mouse malaria parasite interacts with erythrocyte calmyrin.
ASTMH 59th annual meeting, Atlanta, USA, November 3-7, 2010.

13) Arumugam TU, Takeo S, Thonkukiatkul A, Miura K, Otsuki H, Zhou H, Long CA, Sattabongkot J, Torii M, Tsuboi T.
Identification of a novel blood stage vaccine candidate for *Plasmodium falciparum*.

ASTMH 59th annual meeting, Atlanta, USA, November 3-7, 2010.

14)坪井 敬文(シンポジスト)
マラリアワクチン研究の最前線-我が国発の技術による国際貢献
厚生労働省主催、平成 21 年度 地球規模保健課題推進研究事業シンポジウム、東京、3/30、2010。

15) 原國哲也、宮田 健、坪井敬文、Sattabongkot Jetsumon、橘 真由美、鳥居本美、李 長春、渡部久実、松崎吾朗、新川 武
酵母 *Pichia pastoris* 発現ネズミマラリアメロゾイト表面蛋白質 MSP1-19 のワクチン効果
第 79 回日本寄生虫学会大会、旭川市、5/20-21、2010。

16) 新川 武、宮田 健、池原 歩、原國哲也、坪井敬文、Sattabongkot Jetsumon、橘 真由美、鳥居本美、松崎吾朗
新たなワクチンプラットフォーム創製のための三部構成四価免疫賦活複合体(TITs)
第 79 回日本寄生虫学会大会、旭川市、5/20-21、2010。

17) 宮田 健、原國哲也、池原 歩、坪井敬文、Sattabongkot Jetsumon、橘 真由美、鳥居本美、松崎吾朗、新川 武
新たなワクチンプラットフォーム創製のための三部構成五価免疫賦活複合体(TIPs)
第 79 回日本寄生虫学会大会、旭川市、5/20-21、2010。

18) 橘真由美、石野智子、Jenwithisuk Rachaneeporn、Kangwanrangsan Niwat、横内ゆき、Sattabongkot Jetsumon、坪井敬文、鳥居本美
コムギ胚芽無細胞タンパク質合成法を用いた三日熱マラリア伝搬阻止ワクチン候補抗原 Pvs230 の作製
第 79 回日本寄生虫学会大会、旭川市、5/20-21、2010。

19) Jenwithisuk Rachaneeporn、Kangwanrangsan Niwat、橘真由美、石野智子、坪井敬文、鳥居本美
A novel protein is targeted to the crystalloids of *Plasmodium yoelii* ookinetes
第 79 回日本寄生虫学会大会、旭川市、5/20-21、2010。

20) 大槻 均、入子英幸、石野智子、金子 修、福本宗嗣、坪井敬文、鳥居本美
ネズミマラリア原虫赤血球結合タンパク(EBL)の細胞内輸送ドメインの機能解析
第 79 回日本寄生虫学会大会、旭川市、5/20-21、2010。

21) 伊藤大輔、韓 銀澤、竹尾 暁、Thongkukiatkul Amporn、大槻 均、鳥居本美、坪井敬文
熱帯熱マラリア原虫メロゾイト rhoptry neck protein 3 の性状解析
第 79 回日本寄生虫学会大会、旭川市、5/20-21、2010。

22) 北村 圭、熊谷 貴、Bentum Bethel K、三田村俊秀、坪井敬文、太田信生
熱帯熱マラリア原虫におけるオートファジーの役割

第 79 回日本寄生虫学会大会、旭川市、5/20-21、2010。

23) 金子 明、Taleo George、Chaves Luis F、Perlmann Hedvig、江藤秀顕、竹尾 暁、橘真一郎、Troye-Blomberg Marita、坪井敬文、Drakeley Chris、田邉和裕
島嶼マラリア根絶 10 年後の三日熱マラリア再燃
第 79 回日本寄生虫学会大会、旭川市、5/20-21、2010。

24) 大前比呂思、伊藤 誠、中澤 港、亀井喜世子、Bakotee Bernard、Suraj Eka、長岡史晃、坪井敬文、木村英作
ソロモン諸島のマラリア調査における尿診断法の試み
第 79 回日本寄生虫学会大会、旭川市、5/20-21、2010。

25)坪井敬文(招待講演)
マラリアワクチン研究の最前線
第 9 回四国免疫フォーラム、東温市、6/19、2010。

26)坪井敬文(招待講演)
マラリアと人類:歴史は物語る
第 114 回日本医学放射線学会中国・四国地方会、今治市、6/26、2010。

27) 坂本寛和、竹尾 暁、坪井敬文
熱帯熱マラリア原虫メロゾイトの機能未知抗原 PfMSPDBL1 は赤血球侵入に関与するか?
第 18 回分子寄生虫学ワークショップ、草津町、8/2-5、2010。

28) 垰本竜宏、竹尾 暁、坪井敬文
熱帯熱マラリア原虫メロゾイト抗原タンパク質 H103 の機能解明に向けて
第 18 回分子寄生虫学ワークショップ、草津町、8/2-5、2010。

29) Thangavelu U. Arumugam、竹尾 暁、Amporn Thonkukiatkul、三浦憲豊、大槻均、Carole A Long、Jetsumon Sattabongkot、鳥居本美、坪井敬文
マイクロネームに局在する新規熱帯熱マラリア赤血球期ワクチン候補抗原の同定
第 9 回分子寄生虫・マラリア研究フォーラム、長崎市、10/8-9、2010。

30) 加藤晶、竹尾 暁、Jetsumon Sattabongkot、鳥居本美、坪井敬文
熱帯熱マラリア原虫の各侵入型原虫における新規内膜複合体関連分子の同定
第 9 回分子寄生虫・マラリア研究フォーラム、長崎市、10/8-9、2010。

31) 三浦豊和、竹尾 暁、坪井敬文
赤血球寄生期マラリア原虫の RhopH タンパク質複合体:相互作用する宿主赤血球分子の探索
第 33 回日本分子生物学会年会、神戸市、12/7-10、2010。

32) 垰本竜宏、竹尾 暁、坪井敬文
マラリア原虫メロゾイトにおける抗原タンパク質 H103 の機能解明
第 33 回日本分子生物学会年会、神戸市、12/7-10、2010。

Assessment of Malaria Epidemic Situation and its Influential Factors in the Three Gorges Reservoir, China

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Three Gorges Dam Project (TGP) is the world's largest hydropower complex project located in one of the three gorges of the Yangtze River, Xilingxia gorge in Hubei province, China. Previous studies indicated that dams in Cameroon, Kenya and Mali had resulted in an increased malaria burden, a trend that appears to hold for small Ethiopian dams. The area around the Three Gorge Dam has a history of tertian malaria and subtertian malaria epidemic. There was no subtertian malaria after 1960, and the prevalence was controlled by the end of 1980s. However, No systematic, overall, data about malaria epidemics was specified before the completion of the project. In the present study, we carried out a project to assess the malaria epidemic situation in the Three Gorges reservoir and explore its influential factors.

RESULTS AND DISCUSSION

Based on the data collected from six counties, the incidence of malaria in the Three Gorges Reservoir area (0.08～0.64/10 ten thousand) decreased slightly and stabilized at a low level since the construction of The Great Dam, and the average incidence (0.26/10 ten thousand) was lower than that of Hubei Province (4.79/10 ten thousand), as well as that of the national (2.80/10 ten thousand); While significant discrepancy of incidence existed among the different sections in the reservoir with particular missing-report of malaria cases in some areas; The overall percentage of positive response of the same population during post-transmission period was 94.44% (1.40/0.72) higher than that in pre-transmission, an age-related increase in antibody prevalence was evident under 15 years of age and 3.21 times

increase in antibody positive individuals, which the percentage of positive was significant statistically higher ($P<0.05$) than that of pre-transmission periods. The result of antibody levels in the whole population showed that the transmission-blocking has not yet reached during transmission period.

Survey result indicates that the malaria vectors in the Three Gorges Reservoir area was *Anopheles sinensis*, while significant differentiation of its ecological character existed in different sections in this area, the result of this survey for man-biting rate, human blood index and the ratio of multifarious mosquitoes was 0.29～8.92, 0.03～0.21, 0.51～0.66 in turn, and the estimated range of vectorial capacity was 0.01～0.64.

Imported malaria cases in different sections of this area accounts for 25%～100%, with relative high import in some area; while the receptivity index of malaria ranged from 0.01～0.08, with relative high receptivity existing in some region. The stability index of malaria in the Three Gorges Reservoir area was 0.06～0.28, indicating that malaria was unstable in this area; and the actual man-biting rate of Anopheles sinensis in some survey points (Wanzhou, Kaixian and Fuling) was 5.72, 3.37 and 2.87 times that of the critical man-biting rate, confirming that the malaria transmission caused by the *Anopheles sinensis* was still continuing.

Malaria epidemic potential varies in different sections of the reservoir area, and the malaria antibody levels in the local population as well as local incidence were commensurate with its epidemic potential; the stability index showed the malaria was unstable in the reservoir area and the actual man-biting rate much higher than critical biting-rate confirmed the malaria transmission continuing caused by the *Anopheles sinensis*. Therefore, the malaria was unstable with low endemic incidence in the reservoir area.

Diverse ecological habitats of malaria vectors were found in the Three Gorges Reservoir area, therefore conspicuous divergence of epidemic potential among different sections was also presence; and its epidemic potential was closely related to such ecological environments as local crowd behavior (sleeping outdoors) and socio-economic (mosquito net use, livestock) as well as some environmental factors (paddy field area, riparian zones, submerged land area) and others.

According to the trend regression analysis, the coefficient for the density of malaria transmission vectors was statistically significant (b=-0.019, P<0.001), the correlation between the density of malaria vectors and the accumulative temperature of the recent three month was highest (r=-0.375, P=0.003); the total regression analysis showed that the density of malaria vector decreased as the accumulative temperature of the recent three month increased.

Optimal structural equation model has been constructed. The fit indexes are as follows: Ration=5.227, GFI=0.9961, AGFI=0.9987, RMR=0.1094, RMSEA=0.1303, NFI=0.9952, NNFI=0.9929, CN=207, which showed that the model can reflect the relationship of variables; the direct effect of temperature factors, animal factors, humidity factors and breeding environment on the malaria vector density were 0.015、-0.228、0.450、0.516 in turn. The result of this study indicates that the structural equation model is suitable for studying the relationship between the malaria epidemic potential and its related ecological factors.

CONCLUSION

There are different degrees of malaria epidemic potential for different sections of the reservoir area, while relative high potential existing in some regions (Kaixian, Wanzhou, Fuling) and its malaria transmission caused by the *Anopheles sinensis* was still continuing, therefore strengthening the monitoring of malaria vector will provide an important reference for the assessment as well as prediction of the malaria situation in this area.

Since relative high imported malaria in some region (Yubei, Wanzhou) of the reservoir area was observed in our study, strengthening the timely detection and its standard treatment of the malaria cases for shortening the course of disease and improving the recovery rate will have important significance for the control of endemic as well as the malaria elimination in this area.

According to the statistics results, we can conclude that the epidemic situation of malaria in the reservoir area was unstable with low incidence, indicating that transmission trend may be varying with the change of local natural and social factor; the results also showed that the riparian zones was the risk factor for malaria transmission and the humidity factor was the driving force for malaria vectors in the reservoir area. Therefore, we should pay great attention to the impact from the ecological environmental change on the potential transmission of malaria stability and carry out the risk prediction and early warning of malaria transmission for reducing the possible endanger.

Ministry of Health
National Centre for Parasitology,
Entomology and Malaria Control
(CNM)

Kingdom of Cambodia
Nation – Religion - King

The Project Report

ON

The Strengthening & Integrating Of Malaria Control Activities In Newly Developed Area In Kampot Province, Southern Cambodia

Period: September 2010 to February 2011

Date: 28th February, 2011

Submitted by

Dr. Duong Socheat
Dr. Chea Nguon

Supported By

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1. Introduction

Malaria is a major public health problem in Cambodia and a leading cause of mortality and morbidity. It has both short- and long-term consequences for national economic development and has therefore been given high priority by the government and donor agencies. Malaria is the third highest known cause of outpatient attendance (4.6%) and the first cause of hospitalization (13.7%) and hospital death (16%). Real figures are much higher as most malaria cases are either treated first through private clinics and drug sellers or do not seek treatment at all.

In Cambodia the malaria transmission happened in the remote forest with little development or nothing and in the very poor areas that created complexity in controlling that disease as well as problem of providing and receiving the service delivery from the public health sector. The main problems are that those areas were isolated with the complicated geographical barriers, no roads or roads are very bad or very far away from the health facility that provoked hurdle for the intervention, especially in the rainy season. In addition, the dearth of transportation means, the expensive cost of traveling etc...combined with the limited budget provision for malaria control program made those high endemic and secluded areas separated from the public service for many years. Besides the above mentioned reasons, there are still many problems involved and contributed to the low utilization of the public health service.

In order to address to this crucial issue, the Ministry of Health, Welfare and Labor of Japan has funded (A grant on "Research for emerging and re-emerging infections") through the National Institute of Infectious Diseases, Japan, a project in Cambodia entitled "Strengthening and integrating of Malaria Control Activities in newly developed area in Kampot Province, Southern Cambodia" that has been implemented in 10 newly villages in Stung Keo and Chhouk commune in Kampot province.

The main purposes of the project are to get basic epidemiological information based on the comparison of malaria trend in previous and new targeted villages and to compare the trend of genes of malaria parasites among previous and new targeted villages in Kampot province, and other malaria endemic villages in Cambodia, by using sero-epidemiological methods, especially to strengthen the monitoring of the existing volunteer network with the further integration of other operational and feasible interventions to reduce malaria morbidity and mortality in the newly developed area.

2. Objectives of the Project

2.1. Epidemiological comparison in 2 village groups

- To oversee the malaria incidence and epidemiological trend in the villages, especially the male adult and children.

- To oversee the dynamics of the malaria parasites in the villages (including seroepidemiological results).

- To compare the genetic dynamics of malaria parasites in 2 targeted villages.

2.2. Activities of the volunteers and village people in the communities

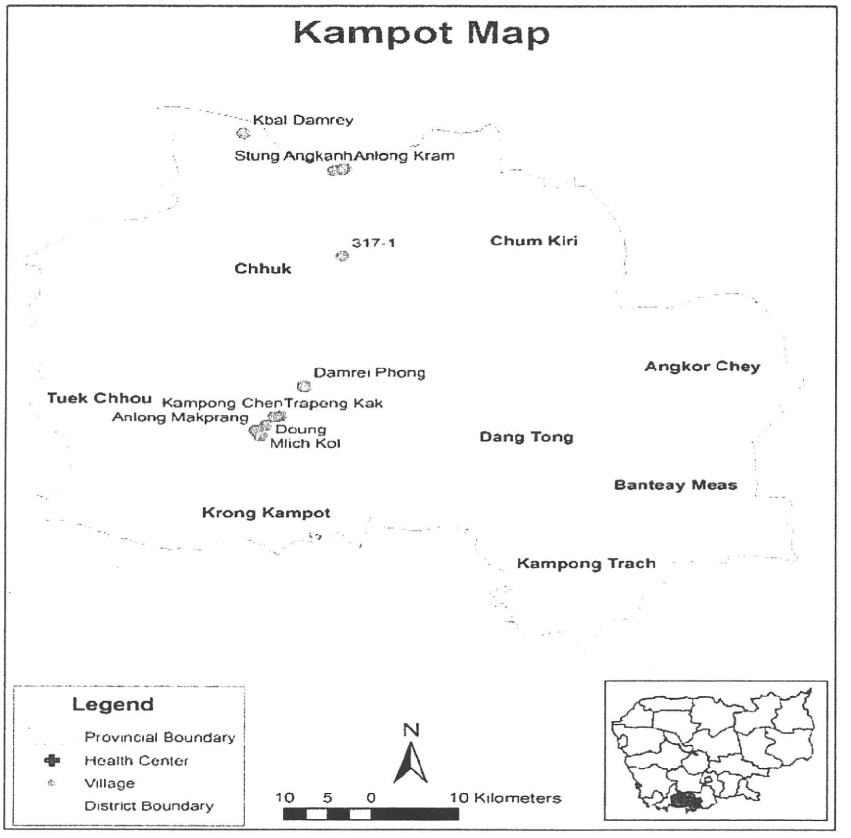
- To integrate and decentralize the re-impregnation activities to volunteers through ITN training with the direct monitoring from the HC, OD and PHD.
- To monitor the volunteers' performance related to the malaria control activities based in the community.
- To strengthen the community's knowledge and practice through the active health education through the community-based network for preventing them from malaria and access them to get the prompt and correct treatment at public health service.

3. Materials and Method

3.1. Village selection

The project sites were located in the two communes of Kampot province: Stung Keo and Chhouk communes that are the two highest malaria communes comparing to other communes in Kampot province. Ten villages of the two above mentioned sites were selected for the study based on the malaria endemicity, remoteness, out of reach by the private providers and the high malaria cases reported by health centers in the catchment areas. Among the ten villages selected villages, six villages (Doung, Malich Kol, Anlong Mac Prang, Trapang Kok, Kampong Chen and Damrei Phong villages) situated in Stung Keo commune and the other four villages(Stung Kbal Domrei, Stung Angkanh, 317, Anlong Krom villages) located in Chhouk commune, Kampot province.

Fig 1: Location of the study villages in the study areas



3.2. Data collection methodology

The team of data collectors for the project were identified and selected through the screening process. Then the training related to the malaria symptom, diagnosis, health education, bed net impregnation, case detection by using rapid diagnostic test, case record, filter paper preparation for the blood spot collection and malaria treatment were also provided to them during the period of the project implementation. All fever and malaria suspected cases in the project villages were screened through the performance of the finger-pricks by using the rapid diagnosis test and the positive test results were treated by antimalarial drug.

4. Results, analysis and discussion

4.1. Monitoring of epidemiological trend in the study villages

4.1.1. Screening cases and gender issue in the study sites

During the implementation of the project, the total of 754 villagers who have fever were screened for malaria in both Koh Sla and Stung Keo study sites and 58% of them were from Stung Keo site, the rest belongs to Koh Sla site. The overall percentage of male tested predominant against female is 74% and 26% respectively.

Table1: Total test cases divided by gender

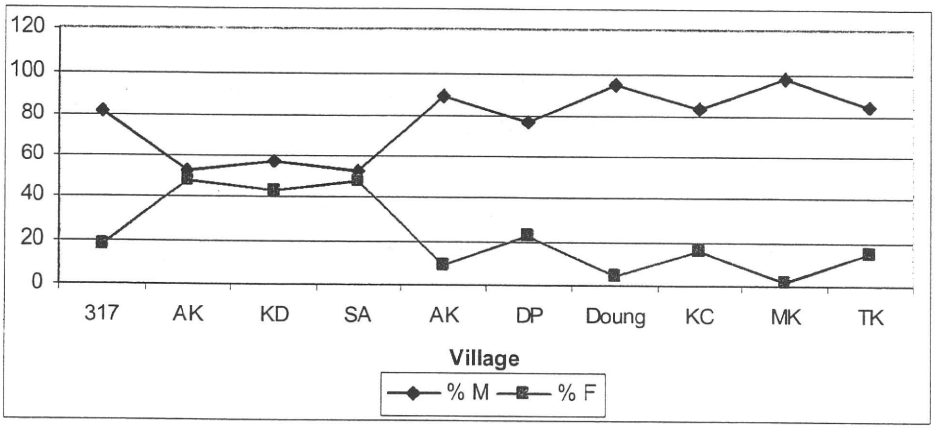
Project sites	Village Name	Total tested	Total test divided by gender			
			Male	%	Female	%
Koh Sla	317	38	31	82	7	18
	Anlong Kram(AK)	124	65	52	59	48
	Kbal Damrey(KD)	44	25	57	19	43
	Stung Angkanh(SA)	110	57	52	53	48
Total		**316**	**178**	**56**	**138**	**44**
Stung Keo	Anlong Makprang(AM)	72	65	90	7	10
	Damrei Phong(DP)	115	89	77	26	23
	Doung	44	42	95	2	5
	Kampong Chen(KC)	70	59	84	11	16
	Mlich Kol(MK)	55	54	98	1	2
	Trapeng Kak(TK)	82	70	85	12	15
Total		**438**	**379**	**87**	**59**	**13**
Grant total		**754**	**557**	**74**	**197**	**26**

Discussion:

- More male villagers were tested for malaria compare to females in Koh Sla (56%) and in Stung Keo (87%). The percentage of male testing in each village ranked from 52% to 82% and 77% to 98% and female testing percentage is ranked from 18% to 48% and 2% to 23% in Koh Sla and in Stung Keo sites correspondingly. The sex ratios (Male/Female) among screening people are 1.29 and 6.42 in Koh Sla and Stung Keo respectively.

- Among 4 villages in Koh Sla site, village 317 has highest males tested (82%) than females (18%) and compare to other 3 villages that their percentages between male and female testing is not a big difference with the variance from 4% to14% (43%-57%). However in Stung Keo, more than 77% tested cases were males in all villagers. (See table below). So men play key role in the daily activity and expose to malaria more than women in the 7 villages of the two project sites.

Fig.2 : Total tested cases divided by gender in project villages

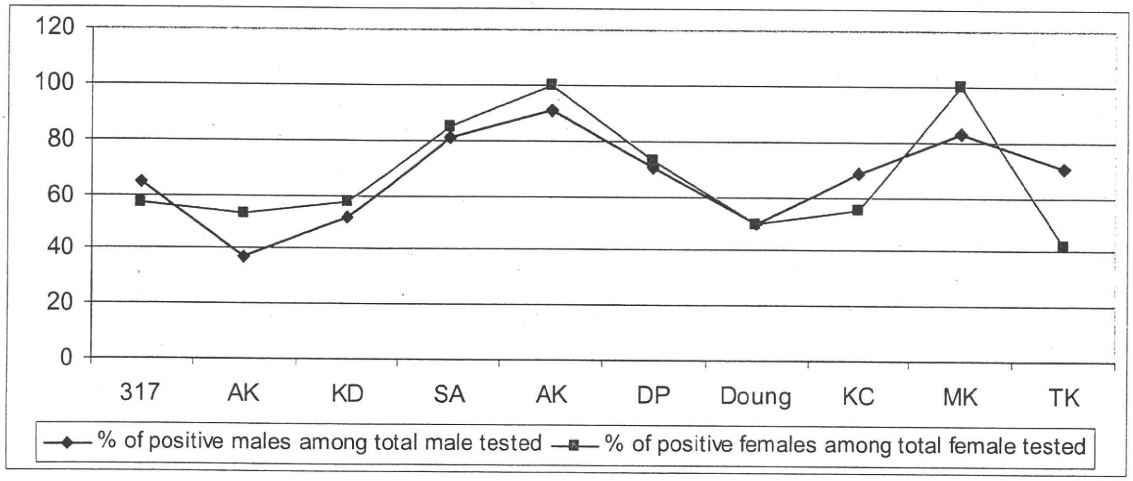


4.1.2. Proportion of male malaria positive among all male tested villagers

There was the high malaria positive result was observed in all men who were tested in both sites. In Koh Sla, the male positive rates among total males tested vary from 37% to 81% and in Stung Keo ranked from 50% to 91%. However the average positive rate of these two sites is 53% and 88% in Koh Sla and Stung Keo consecutively. The percentage of positive female among female testing ranked from 57% to 85% and 42% to 100% in Koh Sla and Stung Keo respectively.

Discussion: The positive rate of male among all male testing is higher than 50% in all villages in both sites except one village in Koh Sla which is only 37%. The same trend was observed for the positive rate of female among all female testing except one village in Stung Keo that has only 42%. The female positive rate among all female testing is higher than male's positive rate in 6 villages and the difference differed from 2% to 17%. However, the male positive rate among all males testing in 3 villages is higher than that of female in 3 villages and it varied from 8% to 29%.

Fig. 3 : Percentage of positive male and female in study villages



4.1.3. Proportion of positive cases among total positive

The data collected from the Koh Sla site showed that male malaria positive rate among total positive tests is very high in village 317(83%) compare to female that is on only 17%. The rest 3 villages showed little difference from one village to another, conversely, in all villages in Stung Keo, the discrepancy of positive rate between male and female are significantly dissimilar as depicted in the table below. The sex ratios (Male/Female) among positive malaria cases are 1.13 and 7.15 in Koh Sla and Stung Keo respectively.

Discussion: There is only 6% of the positive rate difference between male and female among total positive cases in Koh Sla but in Stung Keo positive male counted for 88% and positive female is only 12%. So there is a big difference in that site compare to Koh Sla that little difference. The overall sex ratio male/female among all positive malaria cases in 10 villages is around 3. Among all 512 positive cases in 10 villages, positive male took 75% of the total positive cases.

Table 2: Total positive cases divided by gender

Project sites	Village Name	Total positive	Total positive divided by gender			
			Male	%	Female	%
Koh Sla	317	24	20	83	4	17
	Anlong Kram	55	24	44	31	56
	Kbal Damrey	24	13	54	11	46
	Stung Angkanh	91	46	51	45	49
Total		**194**	**103**	**53**	**91**	**47**
Stung Keo	Anlong Makprang	66	59	89	7	11
	Damrei Phong	82	63	77	19	23
	Doung	22	21	95	1	5
	Kampong Chen	47	41	87	6	13
	Mlich Kol	46	45	98	1	2
	Trapeng Kak	55	50	91	5	9
Total		**318**	**279**	**88**	**39**	**12**
Grant total		**512**	**382**	**75**	**130**	**25**

4.1.4. Number of malaria positive divided by species

The total malaria positive 194 cases in Koh Sla site, 49% of them were accounted by Plasmodium Vivax (PV) and 44% by mix infection and Plasmodium Falciparum (PF) is only 7% and no any case of PF was detected in 317 and Kbal Damrey villages. Whereas, the Stung Keo site, all aspect of spices were identified except the village Mlich Kol that is no PF found. The positive rate in Stung Keo is 28%, 32% and 40% for PF, PV and Mix infection accordingly. There is a bite difference between the PF rate in Stung Keo site that has more PF than in Koh Sla (28% vs 7%), however Koh Sla has 17% and 4% of PV and Mix infection higher than Stung Keo.

In general, the average percentage of these two sites together, PF is responsible for one fifth as for PV and Mix infection is around two fifth each. The data collected could be summarised in the table below.

Table 3: Total malaria positive cases divided by species

Village Name	Total positive	Number of positive by species					
		PF	% of PF	PV	% PV	MIX	% of Mix
317	24	0	0	14	58	10	42
Anlong Kram(AK)	55	3	5	40	73	12	22
Kbal Damrey(KD)	24	0	0	15	63	9	38
Stung Angkanh(SA)	91	10	11	26	29	55	60
Total	**194**	**13**	**7**	**95**	**49**	**86**	**44**
Anlong Makprang(AM)	66	1	2	31	47	34	52
Damrei Phong(DP)	82	52	63	22	27	8	10
Doung	22	7	32	3	14	12	55
Kampong Chen(KC)	47	16	34	4	9	27	57
Mlich Kol(MK)	46	0	0	28	61	18	39
Trapeng Kak(TK)	55	12	22	15	27	28	51
Total	**318**	**88**	**28**	**103**	**32**	**127**	**40**

Discussion:

In Koh Sla: Only 2 among 4 villages that have PF cases. As for the PV rate, three villages have around and above 60% except one village that is less than 30%. For Mix infection, 2 villages have less than 40% and the other 2 have more than 40%.

In Stung Keo: Two villages have PF rate less than 3% and the other 4 villages have more PF rate more than 20%. For PV rate, we observed that 2 villages are less than 20%, 2 villages less than 30% and 2 villages is more than 60%. Regarding for the Mix infection, there is only one village has 10%, the rest 5 villages have more than 40%.

Due to the data collected and analysis, more PF is predominant in Stung Keo(28%) than Koh Sla(7%) in each study site and 4 villages' PF rate ranked from 22% to 63%. However, the PV is prevalent in Koh Sla(49%) than Stung Keo(32%) and all villages ranked from 29% to 73%, whereas Stung Keo 4 villages PV rate less than 30% except 2 villages that have 47% and 61%. Mix infection was found to be more in most of the villages in Stung Keo than Koh Sla.

Fig. 4 : Percentage of PF, PV and Mix infection in study villages