日本熱帯医学会雑誌

第28巻 第1号 平成12年3月

内　　容

JTM FOUNDED 1959

日熱医会誌

日 本 熱 帯 医 学 会

総　説

旅行者によるマラリア診断キット使用の問題

木村　幹男[1*]，大友　弘士[2]，熊谷　正広[2]，廣重　由可[3]

平成11年12月16日受付/平成11年12月20日受理

はじめに

世界におけるマラリアの患者数は年間３－５億人，それによる死亡者は150-270万人と推定されており，他の感染症の撲滅や制御が行われている現代においても，全般的なマラリアの流行状況は少しも改善を見ていない。なかでも，熱帯アフリカのマラリアの殆どを占める他，アジア，オセアニア，南米などにも広く分布する熱帯熱マラリアは脳症，急性腎不全，肺水腫/ARDS，重症貧血，DIC 様出血傾向などを生じて重症化したり死亡する危険のある疾患である。国際交流の活発化，それを可能にする大量航空機輸送の発達により，日本人もマラリア流行地へ出かけることが頻繁となり，帰国後の発症例として年間110-120例程度が知られるが，徐々に熱帯熱マラリアの占める割合が増加しつつあることが危惧される（大友，1998; Suzaki *et al*., 1999)。また日本人の問題として言えば，統計に上がって来ない国外での発症例も多数あり，そこでの邦人の死亡例も散見される。このような状況下で，マラリアから身を守るために旅行者自身でも使えると期待されて，ヨーロッパの数か国で市販もされているマラリア診断キットにつき，検討を加えてみた。

マラリア予防，その中での緊急治療およびその問題点

マラリア予防で全ての場合に行うべき事は蚊に刺されないように工夫することであるが，目的地での熱帯熱マラリアの流行が高度で，旅行者の予想される行動から判断しても罹患の可能性が高い場合，オプションとしての薬物予防が選択される。しかし，副作用が皆無であるはずもなく，たとえば，予防効果に優れるメフロキンを選択した場合には精神神経系の副作用が危惧されている(木村, 藤井, 1997; Schlagenhauf, 1999)。ただし，このような自覚的副作用については服用者の先入観にも大きく作用されるので，無作為割付，二重盲験による判定が望ましいが，現在までに出されているそれらのデータは少数例での検討であり，メフロキンの真の副作用がどの程度であるのかについてのコンセンサスは得られていないのが現状である（木村，橋本，1998)。

別のオプションとして，発熱してマラリアが疑われる状況であれば，旅行者自身の判断で治療薬を服用する緊急治療（自己治療）が挙げられる。世界保健機関 WHO の定義では，発熱して24時間以内に医療機関を受診できない場合に緊急避難的に行うものとするが，あくまで急場しのぎであることの理解が必要であり，緊急治療を行った後もできるだけ医療機関を受診するよう努めるべきとされている(WHO, 1999)。熱帯熱マラリアの流行が高度な地域に一定期間滞在する場合には薬物予防を主体に行うべきとされており，緊急治療が適応となるのは流行度が低い地域へ行く場合，短期間の滞在，短期間の滞在を頻回に繰り返す場合（飛行機の乗務員など）などとされる（Schlagenhauf and Steffen, 1994)。しかし，薬物予防を行っている場合でも薬剤耐性の原虫により罹患することもあるので，緊急治療のオプションを考慮すべきであるとも言える。

緊急治療の問題点として，用法・用量の間違いが挙げられる。内在的な問題として，実際にはマラリアでない場合の使用が多くなる。これは初めから承知のことではあるが，ドイツの調査からすると，マラリアの緊急治療を行った旅行者の中で，後日抗体陽性によりマラリアであったと確認されたのは10.4％のみであった（Nothdurft *et al*., 1995)。この数値は地域，旅行者自身の知識や経験などの因子により様々であろうが，熱帯地域での発熱原因としてはマラリア以外の方が圧倒的に多いことは事実である。マラリアでない場合に緊急治療を行い，高度の副作用が出るなどは非常に不幸なことである。そこで，正確な自己診断および緊急治療を行うために，一般旅行者でも簡便に検査でき，しかも信頼性の高いマラリア診断キットの開発が望まれてきたが（Schlagenhauf *et al*., 1995)，ここ数年その期待がかけられる immunochromatography 法を用いたキットが市販されるようになった。ただし，これらは海外で市場に出回るようになったが，日本国内での入手は現段階では不可能である。

1　東京大学医科学研究所感染免疫内科
2　東京慈恵会医科大学熱帯医学教室
3　海外勤務健康管理センター研修交流部
＊　著者連絡先：〒108-8639 東京都港区白金台４－６－１ 東京大学医科学研究所感染免疫内科
TEL：03-5449-5338，FAX：03-5449-5427，E-mail: mkimura@ims. u-tokyo. ac. jp

Table 1 Summary of the reports on *Para*Sight™ F*

Author	Study country	Performed by	Reference standard	No. HRP-2(+) /No. *P.f.*(+)	Sensitivity (%)	No. HRP-2(−) /No. *P.f.*(−)	Specificity (%)
Banchongaksorn *et al*., 1996	Thailand	Not described	Thick smear	226/242	93.4	659/671	98.2
			PCR	153/167	91.6	351/353	99.4
Caraballo and Ache, 1996	Venezuela	Unclear	Thick smear	268/309	86.7	1,081/1,089	99.3
Humar *et al*., 1997	Canada	Hospital staff	PCR	66/75	88.0	74/76	97.4
Singh *et al*., 1997a	India	Medical laboratory staff	Thick smear	354/382	92.7	785/849	92.5
Kodisinghe *et al*., 1997	Sri Lanka	Not described	Thick and thin smears	202/224	90.2	1,069/1,079	99.1
Kilian *et al*., 1997	Uganda	Health worker (after single day training)	Thick smear	68/78	87.2	98/102	96.1
Bustos *et al*., 1999	Philippines, Japan	Not described	Thick and thin smears	103/103	100.0	313/335	93.4
Current authors, unpublished	Japan	Medical doctor	Thick and thin smears, partially confirmed by PCR	57/57	100.0	239/256	93.4

*Only those published since 1996.

マラリア診断キットの種類，感度，特異度

1）Histidine-rich protein 2（HRP-2）検出系

a．*Para*Sight™ F

Dipstick 形式の *Para*Sight™ F が最初に市販されたが，これは熱帯熱マラリア原虫のみを検出する。モノクローナル抗体を吸着させたスティックの他に試薬として3種類含まれ，それらは初めに血液を溶血させる試薬，スルフォローダミンBを吸着させた当該抗原に対するポリクローナル抗体を含む試薬，最後にスティックを洗浄する試薬などから構成される。必要な血液量は50μl，全体の反応時間は15-20分間，キットは2-30℃での保存が可能である。

感度・特異度に関しては様々なデータが報告されており，低い場合は90%以下であるが，概して90%以上の感度が報告されており，一部では100%の数値も挙げられている（表1）。野外調査で多数検体を扱う場合などには，1例ごとの検討が十分でない可能性があるので，それらのデータの解釈には注意が必要である。例えば，三日熱マラリアの輪状体しか見られない時期での顕微鏡検査で，熱帯熱マラリアと判定される可能性もある。これは，特に厚層塗抹標本の場合に起こりやすい。そして抗原検出では陰性であるので，感度の数値を低下させることとなる。我々も熱帯熱マラリアに関して東大医科研と慈恵医大での症例57例につき検討し，感度100%の成績を得ている（表1）。しかも，その半数以上の症例では18S rRNA 遺伝子をターゲットする PCR 法（Kimura *et al*., 1995）を併用して顕微鏡法でのマラリア診断，原虫種の同定の裏付けを得ている。筆者らの印象として，特に non-immune 患者を対象にする場合には通常一定数以上の原虫数になるので，表1の数値を全体的に見るよりも感度はより優れており，より100%に近いと考えられる。しかし稀ではあるが，原虫数15,000/μl で抗原陰性であった例も報告されている（Humar *et al*., 1997）。

また，抗原検出での感度が原虫感染赤血球数に依存するのは当然であり，1-100/μl の場合71.4%，101/μl 以上であれば95.5-97.1%の感度とするデータ（Kilian *et al*., 1997），21/μl 以上であれば100%とするデータ（Caraballo and Ache, 1996）などが報告されている。

特異度に関しても様々な数値であり，マラリア流行地の住民を対象とする野外調査ではやや低く86-90%程度であり，non-immune を対象とする場合には97-99%と高くなる（WHO, 1996）。これは流行地の場合，例えば1-2週間前に熱帯熱マラリアに罹患し，顕微鏡検査で原虫は検出できなくても抗原が陽性に出る例などがあるためである。また，リウマチ因子陽性者の偽陽性反応が66.7-68.4%に見られている（Laferi *et al*., 1997; Bartoloni *et al*., 1998）。さらに，三日熱マラリアでの交差反応も知られており，我々も52例の三日熱マラリアの中で5例（9.4%）の陽性例を見いだした。しかし，この場合には反応は弱いことが殆どである。このように，特異度は対象の種類に大きく影響されることを考慮する必要がある。

b．ICT Mamaria P.f.（MalaQuick™）

*Para*Sight™ F に3年程遅れて市場に出たキットであり，検出する抗原は同一である。しかし，カード型でしかも試薬は1種類のみであり，操作が簡便であることが特徴で，旅行者が携行するには便利である。溶血のための試薬，金コロイドを吸着させた HRP-2に対する抗体などはパッドの中に固相化されている。キットの保存条件は2-8℃となっているが，熱帯地域での高温にも十分耐えられるとのことである（Schlagenhauf, 私信）。血液量も10μl と少なく，検査所要時間は10分程度で済む。特異度に関しては*Para*Sight™

Table 2 Summary of the reports on ICT Malaria P.f. or ICT Malaria P.f/P.v

Author	Study country	Performed by	Reference standard	No. HRP-2(+) /No. *P.f.*(+)	Sensitivity (%)	No. HRP-2(−) /No. *P.f.*(−)	Specificity (%)
Garcia *et al*., 1996	Solomon Is.	Not described	Thick and thin smears	39/39	100.0	204/212	96.2
Singh *et al*., 1997b	India	Field worker	Thick smear	201/201	100.0	120/142	84.5
Kilian *et al*., 1997	Uganda	Health worker (after single day training)	Thick smear	72/78	92.3	94/102	92.2
Durrheim *et al*., 1998	South Africa	Nurse	Thick smear	68/69	98.6	191/195	97.9
Tjitra *et al*., 1999*	Indonesia	Health worker	Thick and thin smears	236/247	95.5	281/313	89.8
Bustos *et al*., 1999	Philippines, Japan	Not described	Thick and thin smears	102/103	99.0	332/335	99.1
Jelinek *et al*., 1999a	Germany	Not described	Thick and thin smears, partially confirmed by PCR	49/53	92.5	175/178	98.3
Current authors, unpublished †	Japan	Medical doctor	Thick and thin smears, partially confirmed by PCR	25/26	96.2	35/35	100.0

*ICT Malaria P.f/P.v
†Mixed data with ICT Malaria P.f. and ICT Malaria P.f/P.v

F と同程度と報告されることが多いが，感度についてはより優れた数値が出されることが多く，100％あるいは殆ど100％に近い数値も報告されている（表２）。筆者らも少数例の検討で感度96.2％の結果を得ているが（表２），偽陰性の１例は原虫数330/μl の low parasitemia であった。原虫数と感度の関係については，１-100/μl で71.4％，101/μl 以上で100％とする報告（Kilian *et al*., 1997）がある。しかし，稀ではあるが，原虫数が20,000/μl で抗原陰性であった症例も示されている（Jelinek *et al*., 1999a）。

リウマチ因子陽性者での偽陽性反応は問題にならず（Mishra *et al*., 1999），筆者らの印象では三日熱マラリアでの交差反応も殆どないようである。

あるメーカーはこの検査キットにアルコール綿，ランセット，スポイト，バンドエイド，体温計を組み合わせたものを発売しているが，これを用いると他には何も必要としない。

c．ICT Malaria P.f/P.v

最近，前述のカード型抗原検出キットに三日熱マラリアも対象に組み込んだものが発売されるようになり，今後はこれが主に使われることになると推測される。熱帯熱マラリア原虫検出に関しての感度・特異度などは従来の ICT Malaria P.f.の場合と同じであると予想されるが，インドネシアから感度95.5％，特異度89.8％と報告されている（表２，Tjitra *et al*., 1999）。しかし，三日熱マラリア原虫検出の感度は75％と低値であった。

２）原虫由来 pLDH 検出系（OptiMAL®）

これは熱帯熱マラリア原虫，あるいは三日熱マラリア原虫に特異的な酵素 pLDH を識別して検出するキットである。必要血液量は10 μl，検査所要時間は15-20分程度，保存は 2-4℃である。

感度や特異度に関してのデータは HRP-2検出系の場合ほどには得られていないが，熱帯熱マラリアに関しては今のところ感度89-94％，特異度88-98％である（Palmer *et al*., 1998; Jelinek *et al*., 1999a; Cooke *et al*., 1999）。HRP-２検出系では治癒例でも長期間陽性反応が続く傾向があるが，この pLDH 検出系では治癒の場合早期に陰性化するので，治療経過の判定に有用であると期待される（Chiodini, 私信）。

一般旅行者を対象にしたマラリア診断キットの検討

以上のデータは殆どの場合，この種の手技にある程度習熟した者が行って得られたものであるが，一般旅行者にこれらのキットを携行させるアイディアが検討課題となる。発熱を生じてマラリアを疑う状況のときに自己診断を行い，その結果が陽性であれば緊急治療を行い，マラリアによる重症化や死亡の発生を抑えることができるであろうとの期待がもたれる。もちろん，感度が100％でなく熱帯熱マラリアの見逃しが皆無とは言えない。Low parasitemia の場合だけでなく，稀ではあるが一定数以上の原虫数があっても抗原陰性であった例が報告されている。それらの原因として，1）血液サンプル処理の問題，2）反応を抑制する抗体の存在，3）HRP-２の抗原性の変異，などが考えられるが（Humar *et al*., 1997），これらの問題に関しては今後解明されることを期待したい。このようにキットは完璧なものでないにしても，マラリアを疑うときに医療機関へのアクセスがない場合もあり，また現地の医療機関での診断に問題がありうることも考えると，適切に使用すれば相当のメリットをもたらすことができると考えられる。

そこで，一般旅行者が *Para*Sight™ F と MalaQuick™を使って正しく検査し，正しく判定できるかの検討がチュー

リヒで行われたが，予想に反した惨澹たる結果であった（Funk *et al*., 1999）。当人が説明書を読んで理解するだけで，それ以外の説明を与えない状況で自己診断を行わせたが，説明書に述べられた試薬類などを理解できなかった人が40-50%以上に達したり，*Para*Sight™ F の場合には血液量が多いので，採血に困難を感じた人が多く見られた。実際の検査手技についても *Para*Sight™ F の場合には16.9%に，MalaQuick™の場合には8.6%に誤りが見られた。さらに，あらかじめ反応を終わっているスティックが用意され，説明書の内容に沿って旅行者が判定したところ，0.1%以下の low parasitemia の血液で反応を行ったスティックの場合に「陽性」と正しく判定した者は，*Para*Sight™ F で52.1%，MalaQuick™で10.8%に過ぎなかった。逆に 2 %以上の high parasitemia の血液で反応を行ったスティックの場合に「陽性」であると正しく判定した者は，MalaQuick™では96.8%であったが，*Para*Sight™ F では33.8%に過ぎなかった。

また，ロンドンのトラベルクリニックでは帰国後発熱などの症状を呈して受診した患者を対象とし，ICT Malaria P.f.による自己診断を行わせ，流行地で一般旅行者がマラリアの自己診断をするのと同じ状況を再現した。実際に熱帯熱マラリアであった者は21人であったが，そのうち 7 人（33%）はキットを用いて「陰性」との自己診断であった（Behrens *et al*., 1999）。発熱を有する旅行者を対象にして ICT Malaria P.f.による自己診断の検討をケニアで行った時にも，様々な段階での誤りが見られ，結果を判定できない者が31.7%にも達し，熱帯熱マラリア11人の中で正しく診断ができたのは 1 人に過ぎなかった（Jelinek *et al*., 1999b）。

これらの結果から，一般人がキットを使うに際しての問題は無視できないものであることが明らかになった。誤った使い方や判定により，旅行者が害悪を被ってはならないことは言うまでもない。特に，熱帯熱マラリアの陽性反応が出ているのに「陽性」の判定ができなかった例では，実地ではマラリアの緊急治療を行わずに，重症化・死亡に至る危険がある。逆に，本当は陰性なのに「陽性」と誤って判定する間違いでは，マラリアでないのに抗マラリア薬を服用して副作用の危険にさらすこと，他の診断が考慮されることなく重症化する可能性，などの問題点が挙げられる。

マラリア診断キットの今後について

一般旅行者に無差別に診断キットを渡し，説明書通りに行えばマラリアの自己診断が的確に行えるとアドバイスするのは，危険であることが明らかになった。したがって，これだけ多くの問題を有する診断キットなので，一般旅行者での使用はあきらめ，医療機関においてのみ使用すべきなのであろうか？　いや，上述のごとく，感度や特異度において完璧でないにしろ，特に non-immune を対象にする場合には100%に極めて近い優れたものと考えられる。アフリカの奥地などで，医療機関を受診するのに数日間を要する所に滞在する者もある。また，現地でのマラリアの診断は残念ながら信頼できないことも少なくない。前項に述べた旅行者が，自己診断を行う場合の悲観的なデータについては，その後の指導や訓練によって改善する余地が残されている。この様な実際的な問題に取り組むのは旅行医学の役割であり，今後も問題点およびそれらの解決策などが真剣に検討され，旅行者がマラリア診断キットを正しく使えるようになることが望まれる。

具体的には，メーカー側が成すべき事として，特に *Para*Sight™ F の場合には検査の過程をより単純化することが望まれる。判定の仕方については図を用いて陽性と陰性の場合を示してあるが，実際のスティックあるいはカード上で起こった反応を正しく判定するのが困難なこともありうる。陽性のバンドが余りにも強すぎると，逆に陽性と判断されなくなる誤りも見られている（Schlagenhauf, 私信）。また，簡便であると評判の高いカード型キットの場合，血液が濾紙上を一様に上って行かずに端の部分を上がったり，時には全く上がらないこともある。このような時にも，正しい判断をすることが必要となる。スティックあるいはカード上での反応として，実際にありうる様々な状況をカラー写真で示すことなどが不可欠であろう。

また，トラベルクリニックの側では旅行者に説明書を読ませるだけでなく，口頭での説明を加えるべきである。説明書を読むのみで正しく検査を行えた者は75%であったが，口頭での説明を加えたら90%に上昇したとのデータもある（Trachsler *et al*., 1999）。あるいは，ビデオでの学習を取り入れるのも効果的であろう。いずれにしろ前もって最低数回の実習を行わせ，正しく実施し正しく判定できるようになったことを確認することが不可欠である。

さらに，ある程度生物・医学関係の実験の経験のある人の場合には，適切に検査・判定できる可能性が高いので，それらの人達に限って使用を勧めていくのも一案であろう。そして彼らが他の人にも正しく教えれば，マラリア診断キットでメリットを受ける人数が増加していくことが期待できる。同時にやはり，キットを正しく使うことが絶対的に必要であり，しかもそれは一般には簡単でなく，適切な訓練を要することも周知させていく必要がある。

おわりに

マラリア診断キットは完璧ではないにしても，簡便性と高い感度は貴重であり，旅行者自身が利用できる可能性を追求すべきであると考える。ただし，そのためには適切に使うことが必須であり，しかもそれが簡単ではないことも認識しなければならない。実際には，メーカーとトラベルクリニックの両者での様々な具体的な工夫が必要となる。この問題に関する，旅行医学的視点からの進歩が望まれる。

文　献

1）Banchongaksorn, T., Yomokgul, P., Panyim, S., Rooney, W. and Vickers, P. (1996): A field trial of the *Para*Sight™ F test for the diagnosis of *Plasmodium falciparum* infection. Trans. R. Soc. Trop. Med. Hyg., 90, 244-245

2) Bartoloni, A., Strohmeyer, M., Sabatinelli, G., Benucci, M., Serni, U. and Paradisi, F. (1998): False positive *Para*Sight™ F test for malaria in patients with rheumatoid factor. Trans. R. Soc. Trop. Med. Hyg., 92, 33-34
3) Behrens, R.H., Whitty, C. and Armstrong, M. (1999): Comparison of laboratory diagnosis of malaria with immunochromatographic kits when self-administered by symptomatic patients. *In*: Abstracts of the 6th Conference of the International Society of Travel Medicine. p. 60.
4) Bustos, D.G., Olveda, R.M., Negishi, M. and Kurimura, T. (1999): Evaluation of a new rapid diagnostic test "Determine™ Malaria Pf" against standard blood film, *ICT* Malaria *P.f*™ and *Para*Sight™ F. Jpn. J. Trop. Med. Hyg., 27, 417-425
5) Caraballo, A. and Ache, A. (1996): The evaluation of a dipstick test for *Plasmodium falciparum* in mining areas of Venezuela. Am. J. Trop. Med. Hyg., 55, 482-484
6) Cooke, A.H., Chiodini, P.L., Doherty, T., Moody, A.H., Ries, J. and Pinder, M. (1999): Comparison of a parasite lactate dehydrogenase-based immunochromatographic antigen detection assay (OptiMAL®) with microscopy for the detection of malaria parasites in human blood samples. Am. J. Trop. Med. Hyg., 60, 173-176
7) Durrheim, D.N., la Grange, J.J.P., Govere, J. and Mngomezulu, N.M. (1998): Accuracy of a rapid immunochromatographic card test for *Plasmodium falciparum* in a malaria control programme in South Africa. Trans. R. Soc. Trop. Med. Hyg., 92, 32-33
8) Funk, M., Schlagenhauf, P., Tschopp, A. and Steffen, R. (1999): MalaQuick™ versus *Para*Sight F® as a diagnostic aid in travellers' malaria. Trans. R. Soc. Trop. Med. Hyg., 93, 268-272
9) Garcia, M., Kirimoama, S., Marlborough, D., Leafasia, J. and Rieckmann, K.H. (1996): Immunochromatographic test for malaria diagnosis. Lancet, 347, 1549
10) Humar, A., Ohrt, C., Harrington, M.A., Pillai, D. and Kain, K.C. (1997): *Para*Sight® F test compared with the polymerase chain reaction and microscopy for the diagnosis of *Plasmodium falciparum* malaria in travelers. Am. J. Trop. Med. Hyg., 56, 44-48
11) Jelinek, T., Grobusch, M.P., Schwenke, S., Steidl, S., von Sonnenburg, F., Nothdurft, H.D., Klein, E. and Löscher, T. (1999a): Sensitivity and specificity of dipstick tests for rapid diagnosis of malaria in nonimmune travelers. J. Clin. Microbiol., 37, 721-723
12) Jelinek, T., Amsler, L., Grobusch, M.P. and Nothdurft, H.D. (1999b): Self-use of rapid tests for malaria diagnosis by tourists. Lancet, 354, 1609
13) Kilian, A.H.D., Mughusu, E.B., Kabagambe, G. and von Sonnenburg, F. (1997): Comparison of two rapid, HRP2-based diagnostic tests for *Plasmodium falciparum*. Trans. R. Soc. Trop. Med. Hyg., 91, 666-667
14) Kimura, M., Miyake, H., Kim, H.-S., Tanabe, M., Arai, M., Kawai, S., Yamane, A. and Wataya, Y. (1995): Species-specific PCR detection of malaria parasites by microtiter plate hybridization: clinical study with malaria patients. J. Clin. Microbiol., 33, 2342-2346
15) 木村幹男，藤井達也 (1997)：マラリア予防薬としてのメフロキンの副作用の問題．日本醫事新報 №3838，37-41
16) 木村幹男，橋本麻希 (1998)：メフロキン薬物予防における精神神経系副作用の問題．Clin. Parasitol.（日本臨床寄生虫学会誌），9，82-85
17) Kodisinghe, H.M., Perera, K.L.R.L., Premawansa, S., Naotunne, T. de S., Wickramasinghe, A.R. and Mendis, K.N. (1997): The *Para*Sight™ F dipstick test as a routine diagnostic tool for malaria in Sri Lanka. Trans. R. Soc. Trop. Med. Hyg., 91, 398-402
18) Laferi, H., Kandel, K. and Pichler, H. (1997): False positive dipstick test for malaria. N. Engl. J. Med., 337, 1635-1636
19) Mishra, B., Samantaray, J.C., Kumar, A. and Mirdha, B. R. (1999): Study of false positivity of two rapid antigen detection tests for diagnosis of *Plasmodium falciparum* malaria. J. Clin. Microbiol., 37, 1233
20) Nothdurft, H.D., Jelinek, T., Pechel, S.M., Hess, F., Maiwald, H., Marschang, A., Sonnenburg, F.V., Weinke, T. and Löscher, T. (1995): Stand-by treatment of suspected malaria in travellers. Trop. Med. Parasitol., 46, 161-163
21) 大友弘士 (1998)：特集 マラリア再び．日本におけるマラリア．化学療法の領域，14，789-794
22) Palmer, C.J., Lindo, J.F., Klaskala, W.I., Quesada, J.A., Kaminsky, R., Baum, M.K. and Ager, A.L. (1998): Evaluation of the OptiMAL test for rapid diagnosis of *Plasmodium vivax* and *Plasmodium falciparum* malaria. J. Clin. Microbiol., 36, 203-206
23) Schlagenhauf, P. (1999): Mefloquine for malaria chemoprophylaxis 1992-1998: a review. J. Travel Med., 6, 122-133
24) Schlagenhauf, P. and Steffen, R. (1994): Stand-by treatment of malaria in travellers: a review. J. Trop. Med. Hyg., 97, 151-160
25) Schlagenhauf, P., Steffen, R., Tschopp, A., Van Damme, P., Mittelholzer, M.-L., Leuenberger, H. and Reinke, C. (1995): Behavioural aspects of travellers in their use of malaria presumptive treatment. Bull. World Health Organ., 73, 215-221
26) Singh, N., Singh, M.P. and Sharma, V.P. (1997a): The use of dipstick antigen-capture assay for the diagnosis of *Plasmodium falciparum* infection in a remote forested area of central India. Am. J. Trop. Med. Hyg., 56, 188-191
27) Singh, N., Valecha, N. and Sharma, V.P. (1997b): Malaria diagnosis by field workers using an immunochromatographic test. Trans. R. Soc. Trop. Med. Hyg., 91, 396-397
28) Suzaki, A., Kimura, M. and Ohtomo, H. (1999): The current situation of imported malaria in Japan. *In*: Abstracts of the 6th Conference of the International Society of Travel Medicine. p. 84.
29) Tjitra, E., Suprianto, S., Dyer, M., Currie, B.J. and Anstey, N.M. (1999): Field evaluation of the ICT

Malaria P.f/P.v immunochromatographic test for detection of *Plasmodium falciparum* and *Plasmodium vivax* in patients with a presumptive clinical diagnosis of malaria in eastern Indonesia. J. Clin. Microbiol., 37, 2412–2417

30) Trachsler, M., Schlagenhauf, P. and Steffen, R. (1999): Feasibility of a rapid dipstick antigen-capture assay for self-testing of travellers' malaria. Trop. Med. Int. Health, 4, 442–447

31) WHO (1996): A rapid dipstick antigen capture assay for the diagnosis of falciparum malaria. Bull. World Health Organ., 74, 47–54

32) WHO (1999): 5. Health Risks and Their Avoidance. *In*: International Travel and Health. Vaccination Requirements and Health Advice. p. 55–89. World Health Organization, Geneva

THE USE OF MALARIA DIAGNOSTIC TEST KITS BY TRAVELERS

Mikio Kimura, Hiroshi Ohtomo, Masahiro Kumagai and Yuka Hiroshige

Received December 16, 1999/Accepted December 20, 1999

Malaria prophylaxis measures of travelers include, in addition to chemoprophylaxis, stand-by treatment (self treatment) which is indicated in areas of low transmission, for short-term travelers and for those with brief, repeated exposure to malaria. The indication of stand-by treatment has now been expanded in some areas, partly due to an increasing concern about the possible adverse effects of malaria chemoprophylaxis. However, the inappropriate use of stand-by treatment in non-malaria cases may expose persons to a significant drug risk, thus developing simple and reliable self diagnostic tests especially for *Plasmodium falciparum* malaria has been expected. Recently marketed kits for detecting *P. falciparum* histidine-rich protein 2 (HRP-2) or pLDH could be good candidates for malaria self diagnosis by travelers. Especially a card-type kit detecting HRP-2 is characterized by its simplicity and reliability, often showing a sensitivity of $>95\%$ or even sometimes 100%. Although the sensitivity of those tests could not always be 100%, they could be very useful when performed properly, especially in cases travelers develop malaria-like symptoms in areas remote from reliable medical facilities. However, reports of self diagnosis with those kits performed by travelers showed discouraging results. Recent studies highlighted errors of performing these tests correctly and of interpreting previously reacted strips or cards. Travelers should not become a victim of potentially useful malaria diagnostic tests due to their incorrect use. Nonetheless, we still believe that the use of those kits by travelers should not be abandoned, rather they must have potential for application to travelers' malaria. This goal could be accomplished by improving the contents of the kits' instructions and also by giving full oral explanation to travelers at travel clinics.

EPIDEMIOLOGICAL FEATURES OF *STRONGYLOIDES* INFECTION IN OKINAWA, JAPAN: COMPARATIVE STUDY WITH OTHER ENDEMIC AREAS

Jun Kobayashi[1], Yoshiya Sato[1], Hiromu Toma[1], Isao Shimabukuro[1], Tsutomu Tasaki[1], Masahiro Takara[2] and Yoshiyuki Shiroma[3]

Received April 21, 1999/Accepted October 4, 1999

Abstract: The epidemiological features of *Strongyloides stercoralis* infection in Okinawa, Japan, were studied by comparing with those in Thailand, Laos and Brazil. The prevalence rates of *Strongyloides* infection in the present study were 9.6% (133/1,380) in Okinawa, 47.6% (99/208) in Chiang Mai, Thailand, 23.8% (106/445) in Khammouane, Laos, and 12.0% (32/267) in Maceio, Brazil, respectively. The age inclination in aged subjects and sex dominance in males were significant features of *Strongyloides* infection in Okinawa, suggesting that new infection from the environment does not occur in present-day Okinawa. The epidemiological feature was considered to provide a favorable field to investigate therapeutic efficacy unaffected by reinfection from environment after treatment. Absence of helminth infection other than *Strongyloides* was an additional feature in Okinawa. The features may also be convenient to study host response and pathogenicity in the *Strongyloides* infection unaffected by concurrent infection with other helminths.

Key words: *Strongyloides stercoralis,* strongyloidiasis, epidemiology, Okinawa, Thailand, Laos, Brazil

Introduction

Strongyloides stercoralis is a nematode parasite of man with a high prevalence in many tropical and subtropical countries. A warm and moist climate is essential for its natural transmission, and man becomes typically infected through skin contact, by penetration of infective larvae developing in the soil. It has also been well known that the parasite can multiply within host by internal autoinfection. Due to the autoinfection, the parasite can survive for many years in its host and it is frequently imported in temperate regions without outside source of reinfection. The biological nature of the parasite influences not only the clinical manifestation of strongyloidiasis but also epidemiological feature in endemic and non-endemic areas.

Okinawa prefecture lies in the southernmost part of Japan and is the only prefecture located in subtropical zone. Under the circumstance, the inhabitants in Okinawa have been suffering in the past from many important parasitic diseases, such as malaria and filariasis. However, these parasitic diseases have already been almost completely eradicated in recent years and only strongyloidiasis is currently highly prevalent in the prefecture. In the recent surveys, the mean prevalence rate was found to be as high as 10% among the inhabitants over 40 years old (Asato *et al*., 1992). On the other hand, the prevalence in the young generation is extremely low, suggesting that new infection from the environment rarely occurs among inhabitants in present-day Okinawa and that presumably the majority (more than 90%) of cases are long-standing cases acquired the infection in childhood and presisted until adulthood (Sato *et al*., 1986). Under the condition, epidemiological feature of strongyloidiasis in Okinawa is considered to be different from those of other endemic areas where *S.*

1 Department of Parasitology, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0125, Japan
2 Kumejima Family Clinic, 1134 Nakadomari, Gushikawa, Okinawa 901-3124, Japan
3 Izumizaki Hospital, 1-11-2 Izumizaki, Naha, Okinawa 900-0021, Japan
Correspondence to: Jun Kobayashi,
Department of Parasitology, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0125, Japan
E-mail: jun@med.u-ryukyu.ac.jp Fax:+81-98-895-4914

stercoralis infection currently manifested among the inhabitants.

The purpose of the present study was to compare the epidemiological features of strongyloidiasis in Okinawa with those of endemic areas in Thailand, Laos and Brazil, where many parasitic infections are currently prevalent among the inhabitants.

Materials and Methods

Survey Areas and Populations Examined:

Okinawa prefecture, also referred as to the Ryukyu Islands, consisted of about 60 small islands. One of the islands, Kume Island, located about 100 km east of Okinawa Island was selected as survey area in the present study. The similar surveys were also conducted in two countries of Southeast Asia and in a country of South America. In Southeast Asia, a village (Sanpatong village) in Chiang Mai province, northern Thailand, and two villages (Sisamsung and Phavang villages) in Khammouane province, southeast Laos, were selected as survey areas. These areas were located in similar tropical environment, however, socio-economic situation was much different among the areas. Sanpatong village was located in rural area in Chiang Mai province, however, infrastructure of the village was well developed. Although the two rural villages in Laos were self-sufficient in food, incomes of the villagers was very low. The two slums (Vila Brejal and Vila Aratu) in Maceio City, Alagoas State, northern Brazil, were selected as survey areas in South America. The slums were located in the city, health facility in the slums being poorly developed as compared with other areas in the city.

The surveys were carried out on November 1991 in Chiang Mai (Thailand), on November 1993 in Okinawa (Japan), on October 1994 in Maceio (Brazil) and on September 1996 in Khammouane (Laos).

In Table 1, sex and age distributions of the subjects in the present study are shown. In Okinawa, all subjects were adults over 20 years old and more than 90% of them were occupied by the aged subjects over 40 years old. The age distribution pattern in Chiang Mai was similar to that in Okinawa although the aged subjects over 70 years old examined in the area were considerably few in the present survey. On the other hand, in Maceio and Khammouance, more than 50% of the subjects belonged to children and the young under 20 years. The average age (±SD) was 59.6 (±13.4) in Okinawa, 47.9 (±15.1) in Chiang Mai, 22.6 (±16.7) in Maceio, and 25.5 (±20.9) in Khammouane, respectively. Sex ratio of the subjects was almost the same in Chiang Mai and Khammouane but the number of female subjects was greater than that of males in Okinawa and Meceio.

Stool Examination:

The stool examinations were performed by fecal concentration (formalin-ether concentration), Harada-Mori fecal culture and an agar plate fecal culture methods. The last method was recently developed in Okinawa, in which fecal samples (about 3 g) were placed on the primary agar plate for becterial culture and incubated at 28°C for 3 days (Arakaki *et al*., 1988). After the incubation, the surface of the agar plate was examined carefully under a stereoscopic microscope to find out motile larvae that crawled out of the fecal mass on the agar plate. When found, the larvae were differentiated morphologically from those of hookworm and free-living *Rhabditis*. If the tracks left by the larvae were observed but no larvae were found on the agar

Table 1 Number of subjects examined in 4 survey areas

Area	Sex	No. subjects examined (by age group)									
		-9	10-	20-	30-	40-	50-	60-	70-	80-	Total
Okinawa (Japan)	Male	0	0	7	44	78	97	166	116	25	533
	Female	0	0	11	78	110	163	293	162	30	847
	Total	0	0	18	122	188	260	459	278	55	1,380
Chiang Mai (Thailand)	Male	0	2	13	24	13	15	28	8	0	103
	Female	0	0	11	31	13	19	26	5	0	105
	Total	0	2	24	55	26	34	54	13	0	208
Khammouane (Laos)	Male	67	55	18	25	17	23	16	6	2	229
	Female	73	31	33	22	25	19	12	1	0	216
	Total	140	86	51	47	42	42	28	7	2	445
Maceio (Brazil)	Male	24	47	10	6	4	4	4	2	0	101
	Female	34	40	39	20	19	8	5	1	0	166
	Total	58	87	49	26	23	12	9	3	0	267

Table 2 Prevalence of parasitic helminths among the subjects examined in 4 survey areas

	Okinawa (n=1,380)	Chiang Mai (n=208)	Khammouane (n=445)	Maceio (n=267)
No. positive for helminth infection	133 (9.6)	193 (92.8)	384 (86.3)	186 (69.7)
Type of infection:				
Strongyloides stercoralis	133 (9.6)	99 (47.6)	106 (23.8)	32 (12.0)
Ascaris lumbricoides	0 (0)	0 (0)	193 (43.4)	111 (41.6)
Trichuris trichiula	0 (0)	7 (3.4)	112 (25.2)	118 (44.2)
Hookworm*	0 (0)	50 (24.0)	164 (36.9)	77 (28.8)
Enterobius vernicuralis	0 (0)	6 (2.9)	10 (2.2)	2 (0.7)
Opisthorchis viverrini	0 (0)	162 (77.9)	243 (54.6)	0 (0)
Schistosoma mansoni	0 (0)	0 (0)	0 (0)	71 (26.6)
Fasciola hepatica	0 (0)	0 (0)	2 (0.4)	0 (0)
Taenia sp.	0 (0)	4 (1.9)	17 (3.8)	0 (0)
Hymenolepis nana	0 (0)	0 (0)	0 (0)	10 (3.7)

* The species did not identified in the present study.

surface, the presence of larvae was assumed and further appropriate examinations (i.e. fecal concentration and/or Harada-Mori fecal culture) were carried out for a correct diagnosis. For the above stool examination, two stool samples were collected from each subject on 2 different days.

Statistics:

The data were analyzed by the χ^2 (chi-suare) test to determine significance level among the subject groups. A P value for smaller probability than 0.05 and 0.01 was considered to be significant and highly significant, respectively.

RESULTS

In Table 2, the prevalence rates of helminth parasites among the subjects in the present surveys are shown. About 70% or more of the subjects were found to be positive for one or more helminth parasites in the survey areas except in Okinawa. *Ascaris lumbricoides* and *Trichuris trichiura* were dominant infection in Maceio, Brazil. While in Chiang Mai and Khammouane, Southeast Asia, liver fluke, *Opisthorchis viverrini*, showed as the highest prevalence rate as 77.9% in Chiang Mai and 54.6% in Khammouane. *A. lumbricoides, T. trichiura* and hookworm, although the species did not identified in the present study, were also common parasites among the subjects in Khammouane, but *A. lumbricoides* and *T. trichiura* infections were rare in Chiang Mai. A total of 133 subjects (9.6%) were confirmed harboring parasite infection in Okinawa, but *Strongyloides* was the only helminth species detected among the subjects. The highest infection rate of *S. stercoralis* was obtained in Chiang Mai, showing 47.6%, which was followed by 23.8% in Khammouane, 12.0 in Maceio and 9.6% in Okinawa, respectively. Hookworm infection was higher in rate than that of *S. stercoralis* in Maceio and Khammouane, but was only about a half of that of *S. stercoralis* in Chiang Mai.

Table 3 Prevalence of *Strongyloides* infection by age group in 4 survey areas

Age group	Okinawa	Chiang Mai	Khammouane	Maceio
-9	—	—	21/140 (15.0)	5/58 (8.6)
10-	—	1/2 (50.0)	21/86 (24.4)	13/87 (14.9)
20-	0/18 (0)	9/24 (37.5)	16/51 (31.4)	5/49 (10.2)
30-	1/122 (0.8)	26/55 (47.3)	15/47 (31.9)	3/26 (11.5)
40-	4/188 (2.1)	12/26 (46.2)	9/42 (21.4)	1/23 (4.3)
50-	18/260 (6.9)	17/34 (50.0)	13/42 (31.0)	1/12 (8.3)
60-	55/459 (12.0)	25/54 (46.3)	9/28 (32.1)	4/9 (44.4)
70-	46/278 (16.5)	9/13 (69.2)	2/7 (28.6)	0/3 (0)
80-	9/55 (16.4)	—	0/2 (0)	—
Total	133/1,380 (9.6)	99/208 (47.6)	106/445 (23.8)	32/267 (12.0)

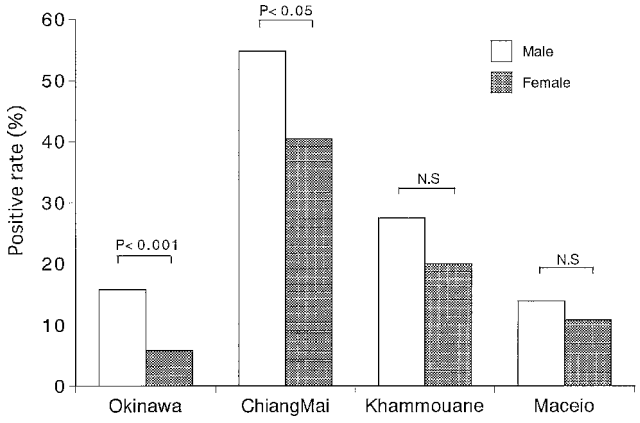


Figure 1 Comparison of positive rate of *Strongyloides* between male and female subjects in 4 survey areas.

The positive rates of *S. stercoralis* by age groups of the subjects are compared among the 4 survey areas in Table 3. In Okinawa, more than 95% of the positive persons were aged subjects over 50 years old, although the number of younger subjects were very few in the present survey. The positive rate increased steadily with the age of the subjects in Okinawa. On the other hand, *Strongyloides* infection was highly confirmed in younger subjects in the other areas; the mean positive rate in the subjects under 20 years old was more than 10% in Khammouane (18.6%) and Maceio (12.4%). Increasing tendency of the *Strongyloides* positive rate with subject's age was not clear in the areas in Southeast Asia and South America.

Fig. 1 shows sex difference of the positive rate in each area. The positive rate in males was consistently higher than that in female subjects. Especially in Okinawa, there was a great difference in positive rate between males and females; the rate in male subjects was three-times higher than that in females, this difference being highly significant ($P<0.001$). On the other hand, sex difference of positive rate was not so significant in the other areas, although male positive rate was significantly higher in Chiang Mai ($P<0.05$).

DISCUSSION

There have been several reports on the prevalence of *Strongyloides* infection in the countries surveyed here; the positive rate reported ranged from 6% to 24% in Thailand (Yamaguchi *et al*., 1982; Bayajian, 1992; Sukhavat, 1994), from 3% to 13% in Laos (Sormmani *et al*., 1974; Chai *et al*., 1998), and from 6% to 40% in Brazil (Dias, 1968; Asami, 1970; Marzochi *et al*., 1978), respectively. These results on prevalence of *Strongyloides* infection, however, may extremely underestimate the true prevalence because of low efficacy of detection methods used in the past. In the previous study in which strongyloidiasis cases were reinfection-checked without treatment several months later, the conventional methods of fecal examination, such as direct smear, fecal concentration and Harada-Mori fecal culture methods, were only 15-24% effective in reconfirming the infection (Sato *et al*., 1995). On the other hand, an agar plate culture method, a newly developed fecal culture method, was confirmed to be much effective for detection of chronic, low-level infection with *Strongyloides*. In the present survey, therefore, the agar plate culture method was applied. The results indicate that *Strongyloides* infection was a major human public health problem in the survey areas. Especially in Chiang Mai, Thailand, positive rate as high as 47% was estimated among the subjects examined. The positive rate of 23% in Khammouane, Laos, was also significantly higher than those reported in the past in this country. On the other hand, the positive rate in Okinawa, Japan, was greatly low as compared to those in the other countries. One of the characteristic epidemiological features of strongyloidiasis in Okinawa, however, was observed in the age inclination of *Strongyloides* positive persons (Sato, 1986; Sato *et al*., 1990). Namely, most of *Strongyloides* positive people (more than 95%) were included in middle to upper age groups over 40 years old in Okinawa. Only one positive case in 140 residents was found under the age of 40. Unfortunately, the subjects under 20 years old were not examined in this survey. However, it was reported that no case of *Strongyloides* infection was found in 205 subjects under 20 years old in Okinawa (Asato *et al*., 1983). Whereas many young subjects were found infected with *Strongyloides* in the survey areas in Southeast Asia and South America. The extremely low incidence in the young generation in Okinawa suggests that the inhabitants have no opportunity to acquire new infection from environment in the island recently. From the epidemiological feature, Okinawa seemed to provide favorable field to investigate therapeutic efficacy of anthelmintics, because the follow-up examination can not be affected by reinfection-infection after treatment. Although there have been many reports on therapeutic effect on strongyloidiasis, the results obtained were considerably different from each other even in the case in which same drug was used in the similar regimen. The inconsistency may be attributed to the several factors. The most effective factor may be difference of applied methods in the follow-up fecal examination. Another factor affec-

ting therapeutic efficacy may be the duration of follow-up examination after the treatment. The *Strongyloides* infection has been known to relapse frequently in several months after seemingly successful treatment. The relapses due to unsuccessful treatment can not be distinguished from reinfection from environment during the follow-up examination. On the basis of the above epidemiological background, the authors could examine the exact efficacy of several anthelmintics for as long as a year after treatment without consideration of reinfection during the follow-up period (Sato *et al*., 1992; Takara *et al*., 1992; Toma *et al*., 1993).

In Okinawa, the infections with soil-transmitted nematodes were highly prevalent until 1950's, but many of them have already been controlled. Ascariasis and trichuriasis were very rare in 1960's. Hookworm infection was still observed in more than 30% of Okinawan people in 1960's, but the prevalence rate also decreased extremely to less than 0.1% in the following two decades (Asato *et al*., 1990). These changes are considered to be caused by the appropriate control measures, socio-economical and agricultural improvements. Apparently, most of *Strongyloides* carriers are also considered to acquire the infection during and/or immediately after the World War II when the standard of living was very low and human excrements were used as fertilizer. Long term of *Strongyloides* infection among aged people without new infection from environment may be due to internal autoinfection of the parasite. Therefore, the other epidemiological feature of strongyloidiasis in Okinawa seems to be that there is no concurrent infection with other helminths occurred among the people with *Strongyloides* infection. Actually, no intestinal helminth infection other than *Strongyloides* was observed in Okinawa in the present study, while helminth infections such as ascariasis and opisthorchiasis were concurrently detected in many subjects positive for *Strongyloides* infection in the other survey areas. This epidemiological feature may also be convenient for the investigation of immune response and clinical features of strongyloidiasis unaffected by concurrent infection with other helminth infection.

Finally, significant dominance of male subjects in prevalence of *Strongyloides* infection in Okinawa was also an epidemiological feature different from those in the other areas. In general, it has been recognized that males were more commonly infected with *S. stercoralis* than females (Soroczan, 1976; Scagalia *et al*., 1984; Walzer *et al*., 1982). The infection rate in males was 3 times higher than that in females in Okinawa in the present study. One of the reasons for the high prevalence in males may be that males have had frequent opportunities for the infection in their life style. It is also possible to consider that males were more susceptible to the infection than females, as known in experimental infection model with *S. ratti* and animal hosts (Katz, 1961; Dawkins *et al*., 1980). In the previous study, the authors also demonstrated that the efficacy of treatment with various anthelmintics was significantly low in male subjects as compared to that in females (Kobayashi *et al*., 1996). Due to the significant resistance to treatment, male subjects harbor the parasite for many years, and the obstinacy in males might result in a significant accumulation of male subject positive for *Strongyloides* under the improved sanitary condition in which persons do not acquire new infection from environment.

In conclusion, *Strongyloides* infection in Okinawa showed a significant age inclination and sex dominance. These epidemiological features may be derived from an environmental situation in which new infection from outside did not occur for many years, and also from the unique property of the parasite to maintain the infection for several decade years beyond the life span of the parasite. These epidemiological features in Okinawa are considered to provide many favorable conditions to investigate the parasite and its disease.

ACKNOWLEDGEMENTS

We are especially grateful to Prof. C. Khamboonruang, Chiang Mai University, and Prof. A.R.C. Dacal, Escola de Ciencias Medicas de Alagoas for their generous help and arrangement. We thank the Institute of Malariology, Parasitology and Entomology (IMPE), Lao PRD, and Japan International Cooperation Agency (JICA) Laos Office for giving the opportunity of the study, and all stuff of malaria net work in Khammouane province, Lao PDR, for their cooperation in this study.

REFERENCES

1) Arakaki, T., Hasegawa, H., Asato, R., Ikeshiro, T., Kinjo, F., Saito, A. and Iwanaga, M. (1988): A new method to detect *Strongyloides stercoralis* from human stool. Jpn. J. Trop. Med. Hyg., 16, 87-90

2) Asami, K., Enomoto, Y. and Miura, S. (1970): Infestations by ancylostomides and *Strongyloides stercoralis* in Pernambuco. Survey based on the identification of larvae. Rev. Inst. Med. trop. São Paulo, 12, 31-35

3) Asato, R., Hasegawa, H., Takai, A. and Ikeshiro, T. (1983): Epidemiological study of strongyloidiasis in Okinawa. Annual Report of Okinawa Prefectural Insti-

tute of Public Health, 17, 58-63
4) Asato, R., Hasegawa, H. and Ikeshiro, T. (1990): Transition in the prevalence of intestinal parasitic infections in Okinawa, Japan, after World War II. *In*: Collected Papers on the Control of Soil-transmitted Helminthiasis, vol. IV, APCO Research Group (ed.), The Asian Parasite Control Organization (APCO), Tokyo, 39-50
5) Asato, R., Nakasone, T., Yoshida, C., Arakaki, T., Ikeshiro, T., Murakami, H. and Sakiyama, M. (1992): Current status of *Strongyloides* infection in Okinawa, Japan. Jpn. J. Trop. Med. Hyg., 20, 169-173
6) Bayajian, T. (1992): Strongyloidiasis on the Thai-Cambodian border. Trans. Roy. Soc. Trop. Med. Hyg., 86, 661-662
7) Chai, J.Y. and Bouasy, H. (1998): A small scale-survey of intestinal helminthic infections among the residents near Pakse, Laos. Korean J. Parasitol., 36, 55-58
8) Dawkins, H.J.S., Grove, D.I., Dunsmore, J.D. and Mitchell, G.F. (1980): *Strongyloides ratti*: susceptibility to infection and resistance to reinfection in inbred strains of mice as assessed by excretion of larvae. Int. J. Parasitol., 10, 125-129
9) Dias, J.C.P. (1968): Observacoes sobre a estrongiloidose no Oeste de Minas Gerais, Brasil. Rev. Inst. Med. trop. São Paulo, 10, 305-311
10) Katz, F.F. (1961): Differences in *Strongyloides ratti* worm burdens in male and female rats. J. Parasitol., 47 (Suppl.), 52
11) Kobayashi, J., Sato, Y., Toma, H., Tasaki, T., Takara, M. and Shiroma, Y. (1996): Sex-related different efficacy of chemotherapy on human strongyloidiasis in Okinawa, Japan. Jpn. J. Parasitol., 45, 350-354
12) Marzochi, M.C. and Carvalheiro, J.R. (1978): Studies on factors involved in the dissemination of intestinal parasites: III. The distribution of some intestinal parasites in 2 social groups of Ribeirao Preto City, São Paulo, Brazil. Rev. Inst. Med. trop. São Paulo, 20, 31-35
13) Sato, Y. (1986): Epidemiology of strongyloidiasis in Okinawa. *In*: Collected Papers on the Control of Soil -transmitted Helminthiasis, vol. III, APCO Research Group (ed.), The Asian Parasite Control Organization (APCO), Tokyo, 20-31
14) Sato, Y., Kobayashi, J., Toma, H. and Shiroma, Y. (1995): Efficacy of stool examination for detection of *Strongyloides* infection. Am. J. Trop. Med. Hyg., 53, 248-250
15) Sato, Y., Shiroma, Y., Kiyuna, S., Toma, H. and Kobayashi, J. (1992): Reduced effect of chemotherapy of strongyloidiasis in patients with concurrent HTLV-1 infection in Okinawa, Japan. Jpn. J. Trop. Med. Hyg., 20, 183-192
16) Sato, Y., Toma, H., Takara, M., Kiyuna, S. and Shiroma, Y. (1990): Seroepidemiological studies on the concomitance of strongyloidiasis with T-cell leukemia viral infection in Okinawa. Jpn. J. Parasitol., 39, 376-383
17) Scaglia, M., Brustia, R., Gatti, S, Bernuzzi, A.M., Strosselli, M., Malfitano, A. and Capelli, D. (1984): Autochthonous strongyloidiasis in Italy: An epidemiological and clinical review of 150 cases. Bull. Soc. Pathol. Exot., 77, 328-332
18) Sornmani, S., Pathammavong, O. (1974): An epidemiological survey of human intestinal parasites in Vientiane, Laos. Southeast Asian J. Trop. Med. Public Health, 5, 541-546
19) Soroczan, W. (1976): *Strongyloides stercoralis* in eastern and southeastern Poland. Wiad. Parazytol., 22, 51-516
20) Sukhavat, K. (1994): Comparative efficacy of four methods for the detection of *Strongyloides stercoralis* in human stool specimens. Ann. Trop. Med. Par., 88, 95-96
21) Takara, M., Toma, H., Kobayashi, J. and Sato, Y. (1992): Effect of concurrent HTLV-1 infection on the efficacy of pyrvinium pamoate treatment of strongyloidiasis. Jpn. J. Parasitol., 41, 202-212
22) Toma, H., Sato, Y., Kobayashi, J., Shiroma, Y., Kiyuna, S., Takara, M. and Ohtomo, H. (1993): Treatment of strongyloidiasis with albendazole in Okinawa, Japan. Jpn. J. Parasitol., 42, 300-307
23) Walzer, P.D., Milder, J.E., Banwell, J.G., Kilgore, G., Klein, M. and Parker, R. (1982): Epidemiologic features of *Strongyloides stercoralis* infection in an endemic area of the United States. Am. J. Trop. Med. Hyg., 31, 313-319
24) Yamaguchi, T., Khamboonruang, C., Inaba, T., Huang, W.H., Ishida, K., Fujimaki, Y., Asano, H., Thitasut, P. and Vajrasthira, S. (1982): Studies on intestinal parasitic infections in Chiang Mai Province, north Thailand. Jpn. J. Parasitol., 31, 447-459

TETRACYCLINE RESISTANT AND POLYMYXIN B SENSITIVE *VIBRIO CHOLERAE* O1 EL TOR ISOLATED FROM THE RECENT EPIDEMICS

MASAAKI IWANAGA[1], SITHAT INSISIENGMAY[2], NAOMI HIGA[1] AND LAY SISAVATH[2]

Received August 16, 1999/Accepted October 4, 1999

Abstract: By examining 99 *Vibrio cholerae* O1 isolates from cholera epidemics in a variety of areas in Laos in 1998, we found two unusual characteristics of the organisms. All except 4 isolates were moderately resistant to tetracycline without having plasmids, and susceptibilities to the other drugs were as expected. Eleven isolates showed the same level of susceptibility to polymyxin B as classical *V. cholerae* O1. With the exception of 4 tetracycline-susceptible strains, all the isolates were resistant to or poorly sensitive to the vibriostatic agent O/129 (2,4-diamino-6,7-di-iso-propyl pteridine phosphate). All isolates, including those susceptible to polymyxin B, produced E1 Tor hemolysin which was neutralized by anti-E1 Tor hemolysin and were resistant to cholera phage IV, indicating that they were of the E1 Tor biotype.

Key words: *Vibrio cholerae* O1, tetracycline, polymyxin B, drug sensitivity, Laos

INTRODUCTION

The current cholera pandemic, which began in 1961 from a focus in South Sulawesi, Indonesia, shows no signs of declining. During the past 4 decades, there have been a variety of changes in the epidemic cholera vibrios; these changes include hemolytic properties of E1 Tor strains, drug susceptibilities, disappearance and reappearance of classical cholera vibrios and the appearance of new serovar *V. cholerae* O139. Tetracycline was originally the first choice for antibiotic therapy and prophylaxis of cholera. The first epidemic due to a tetracycline resistant strain was seen in Tanzania in 1977 (Mhalu *et al*., 1979). Soon after this, a tetracycline resistant strain caused an outbreak in Bangladesh (Glass *et al*., 1980). The vibrios isolated in both countries were plasmid-mediated multiple drug-resistant strains. The first outbreak in Tanzania was long-lasting and spread to Kenya around Lake Victoria (Ehara *et al*., 1983), whereas the outbreak in Bangladesh was limited to the Matrab area and disappeared after 5 months. The emergence of drug-resistant strains in Tanzania was attributed to extensive use of tetracycline for prophylaxis (Towner *et al*., 1980). In Bangladesh, however, prophylaxis was not common, and the source of outbreak could not be determined. Although a few strains of drug-resistant *V. cholerae* O1 have been reported (Kobari *et al*., 1970; Threlfall *et al*., 1993; Yamamoto *et al*., 1995), there have been no large outbreaks other than these two episodes. In 1998, cholera outbreaks due to tetracycline-resistant strains occurred in a wide area of Laos. Here, we describe characterization of the *V. cholerae* O1 isolated in Laos in 1998.

MATERIALS AND METHODS

Bacterial strains: A total of 99 *V. cholerae* O1 strains isolated from patients in Laos in 1998 were

1 Department of Bacteriology, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0215, Japan
2 Center for Laboratory and Epidemiology, Km3, Thadeua Rd., Vientiane, Lao People's Democratic Republic
Corresponding author: MASAAKI IWANAGA
Mailing address: Department of Bacteriology, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0215, Japan
Phone: +81-98-895-3331 Ext. 2285
FAX: +81-98-895-2951
E-mail: iwanaga@med.u-ryukyu.ac.jp

Table 1 Characteristics of isolates and the places of isolation

Place	number of isolates	resistant to tetracycline	resistant to O/129*	sensitive to polymyxin B
Vientiane	82	78	77	3
Xaisettha	43	39	39	2
Xaithani	30	30	29	1
Sisathanak	5	5	5	0
Thaluang	2	2	2	0
Thourakham	1	1	1	0
Maxaythang	1	1	1	0
Khammouan	9	9	9	0
Savanakhet	4	4	4	4
Champasak	4	4	4	4
Total	99	95	94	11

*includes moderately resistant strains

examined. The strains were isolated during the period from May to November 1998 from a wide area in the Southern half of the country as shown in Table 1.

Identification of *V. cholerae* O1: The isolates were identified as *V. cholerae* O1 by routine laboratory tests. Serotype was determined by the slide agglutination method using commercial anti-Ogawa and anti-Inaba sera (Denkaseiken Co. Tokyo).

Production of and susceptibility to phage: Suscepti bility to cholera phage IV, E1 Tor phage 5, kappa-phage, fs-1 and fs-2 phage was examined by the following method. First, 10 m*l* of peptone agar (1% peptone, 0.5% NaCl, 1.5% agar) was poured into a Petri dish (diameter, 9 cm) and solidified. Overnight heart infusion broth culture of each isolate (0.2 m*l*) and 4 m*l* of soft agar (1% peptone, 0.5% NaCl, 0.5% yeast extract, 0.6% agar) kept at 43°C were mixed and poured on to peptone agar plates. Aliquot of 10 μl of phage solution at the routine test dilution were spotted on to the soft agar plates containing the organisms. After 24 hr incubation at 37°C, the vibrios showing plaque formation were regarded as susceptible strains. Production of kappa phage by the organisms was examined as described by Takeya and Shimodori (1963). Briefly, broth cultures of each isolate were treated with chloroform to kill the bacterial cells. The killed culture fluid (0.1 m*l*), a 0.2 m*l* broth culture of an indicator strain H218 (*V. cholerae* O1, serotype Ogawa, biotype cholerae) and 4 m*l* soft agar kept at 43°C were mixed and poured on to peptone agar plates. After overnight incubation at 37°C, plaque formation was observed. To examine productivity of filamentous phage fs-1 and fs-2, the organisms were cultured in HIB with shaking overnight and the cell-free supernatant was spotted on to the indicator strain UDT109 (*V. cholerae* O1 E1 Tor, Inaba).

Cholera toxin production: The organisms were cultured in AKI medium at 37°C for 20 hr in a stationary test tube, after which the cholera toxin in the culture supernatant was titrated using the reversed passive latex agglutination method (Iwanaga and Yamamoto, 1985).

Hemolysin production: The isolates were inoculated on to sheep blood agar plates, and hemolysis around the grown colonies was examined. The isolates were also cultured in heart infusion broth supplemented with 1% glycerol and incubated at 37°C for 24 hr. Washed sheep red blood cells at the final concentration of 0.5% were added to the culture supernatant, incubated at 37°C for 2 hr followed by overnight incubation in a cold room. Neutralization test was carried out by adding 1 to 20 volumes of anti-E1 Tor hemolysin serum to the culture supernatant.

Drug susceptibilities: Susceptibilities of the isolates to ampicillin (ABPC, Meiji), tetracycline (TC, Wako), erythromycin (EM, Dainihon), ofloxacin (OFLX, Daiichi) and polymyxin B (PmxB, Feizer) were determined by multipoint inoculation onto a series of heart infusion agar plates containing doubling dilutions of the drugs from 100 μg/m*l* to 0.0125 μg/m*l*. A 10-fold dilution of overnight broth culture was inoculated in to each plate using Microplanter (Sakuma Co. model MITP # 00257). The susceptibility was expressed as minimum inhibitory concentration (MIC) of each drug.

Isolation of plasmid and electrophoresis: Plasmid DNA was isolated by the method of Kado and Liu (1981) with slight modifications (lysing buffer was added and stand still at room temperatue before phenol extraction) and electrophoresed in 0.8% agarose gel. The gels were stained with ethidium bromide and observed under a UV transilluminator.

RESULTS

All isolates revealed typical biochemical characteristics of *V. cholerae*, but 9 out of the 99 strains did not grow in peptone water without sodium chloride, and 19 strains were negative for Voges-Proskauer reaction. All isolates were toxigenic, and hemolytic on blood agar plates, and the hemolysis was inhibited by anti-E1 Tor hemolysin serum. All isolates belonged to serotype Ogawa.

Phage: Ninety-six of the 99 isolates were susceptible to E1 Tor phage 5, and none was susceptible to cholera phage IV. Kappa phage was produced by 96 isolates. The remaining 3 non-producers were susceptible to this phage; *i.e.* all belong to the Celebes type

Table 2 Production of and susceptibility to phages

phage	productive	sensitive	independent	Total
cholera IV	0	0	99	99
E1 Tor 5	0	96	3	99
kappa	96	3	0	99
fs-1	0	3	96	99
fs-2	0	7	92	99

Table 3 Drug susceptibilities of 99 isolates

μg/ml	TC	ABPC	EM	OFLX	Pmx-B
0.0125≧	0	0	0	86	0
0.025	0	0	0	13	0
0.05	0	0	0	0	0
0.1	0	0	0	0	0
0.2	1	0	0	0	0
0.39	3	0	0	0	2
0.78	0	0	0	0	8
1.56	0	1	0	0	1
3.13	54	26	38	0	0
6.25	41	72	61	0	0
12.5	0	0	0	0	0
25	0	0	0	0	0
50	0	0	0	0	7
100	0	0	0	0	80
100<	0	0	0	0	1

TC: tetracycline, ABPC: ampicillin, EM: erythromycine, OFLX: ofloxacine, Pmx-B: polymyxin B

including 3 cured Celebes. Three and 7 of the 99 isolates were susceptible to the filamentous phage fs-1 and fs-2, respectively. None of the strains produced fs-1 or fs-2 (Table 2).

Drug susceptibilities: Almost all isolates were mod erately resistant to tetracycline, with MIC of 3.13 or 6.25 μg/ml. Only 4 isolates from an area of Vientiane were sensitive to tetracycline with MIC of 0.2 or 0.39 μg/ml. Most of the isolates showed polymyxin B MICs of 100 μg/ml, but 11 showed MICs of 0.39 to 1.56 μg/ml. Polymyxin B-susceptible strains were isolated from Vientiane (3 of 80 isolates), Savanakhet (4 of 4) and Champasak (4 of 4), areas separated by fairly large distance. The susceptibilities to ampicillin, erythromycin, and ofloxacine were similar to the expected values (Tables 2 and 3).

Susceptibility to O/129: The size of the zone of growth inhibition around the disk containing 150 mg of the vibriostatic agent O/129 (2,4-diamino-6,7-di-iso-propyl pteridine phosphate) was variable, therefore, the susceptibilities were defined as follows. Resistance was defined as an inhibition zone of 0 to 1 mm, and susceptibility was defined as a zone of more than 10 mm. Other strains were expressed as intermediate. According to these criteria, 31 strains were resistant and 5 (including 4 tetracycline-sensitive strains) were sensitive to O/129.

Plasmids: No plasmids were detected in 20 randomly selected strains, whereas the tetracycline-resistant strains isolated in Kenya in 1982 examined as positive controls contained plasmids.

DISCUSSION

The present study revealed 2 novel findings in the drug susceptibility pattern of epidemic *V. cholerae* O1. Among the 99 *V. cholerae* O1 E1 Tor isolated in these epidemics, 11 were sensitive to polymixin B with the same MIC as classical cholera vibrios, and 95 were moderately resistant to tetracycline without harboring any plasmids as far as 20 isolates examined are concerned. The MIC of polymyxin B against *V. cholerae* O1 E1 Tor is 50 to 200 μg/ml, whereas that of classical *V. cholerae* O1 is 0.4 to 1.56 μg/ml. Although we found a strain against which the MIC of polymixin B was 6.25 μg/ml in the epidemic in 1994 in Laos (Higa *et al*., 1995), epidemic E1 Tor strains showing the same susceptibilities as classical strains (0.4 to 1.56 μg/ml) were found in the present study for the first time. In Southern Laos, all isolates (8 strains) were polymyxin B-sensitive, whereas 3 of 80 isolates from Vientiane, the capital of Laos. *V. cholerae* O1 E1 Tor were distinguished from classical *V. cholerae* O1 by their productivity of E1 Tor hemolysin. However, the susceptibilities of *V. cholerae* O1 to polymyxin B and cholera phage IV have been reported to correspond to the productivity of E1 Tor hemolysin (Nakasone *et al*., 1987). Therefore, these 2 biotypes are usually distinguished by testing susceptibility to polymyxin B. However, the present study suggested that biotype should be determined by analysis of E1 Tor hemolysin productivity and susceptibility ot cholera phage IV.

All strains of tetracycline-resistant *V. cholerae* O1 reported to date were plasmid-dependent, multi-drug resistant, with a tetracycline MIC of 25 to 200 μg/ml (Coppo *et al*., 1995; Glass *et al*., 1980; Towner *et al*., 1980), except a report by Yamamoto *et al*. (1995). In contrast, the organisms described here did not harbor any plasmids, and were moderately resistant to tetracycline with MICs of 3.13 or 6.25 μg/ml. MICs of EM, ABPC and OFLX remained within the expected range. These isolates were generally resistant to the vibrio static agent O/129. Only 5 isolates including 4 tetracycline-sensitive strains were sensitive to O/129. These observation do not, however, necessarily indicate a close correlation between resistance to O/129 and tetracycline, as that *V. cholerae* O139 is resistant to the former but susceptible to the latter (Albert *et al*., 1997; Higa *et al*., 1995).

Recently, 83 of 93 isolates (89%) of tetracycline sensitive *V. cholerae* O1 E1 Tor collected from a variety

of areas throughout the world were reported to be lysogenized by the phage fs-2 (Ikema and Honma, 1998). However, in the present study, 92 of 99 isolates of tetracycline resistant *V. cholerae* O1 E1 Tor were not associated with fs-2. Considering the phages as the gene vector, the relation between fs-2 and drug sensitivities is now under investigation.

The phenotypic changes in these isolates must have been based on chromosomal mutations as seen in the case of sulfamethoxazole-trimethoprim-resistant *V. cholerae* O139 in which susceptibility was shown to be dependent on chromosomal self-transmissible genetic elements (Waldor *et al*., 1996), since no plasmid was detected in the examined 20 isolates. The phenotypes based on chromosome would be more stable than that associated with plasmids, therefore, careful monitoring of these mutant organisms is required.

REFERENCES

1) Albert, M.J., Bhuiyan, N.A., Talukder, K.A., Faruque, A. S.G., Nahar, S., Faruque, S.M., Ansaruzzaman, M. and Rahman, M. (1997): Phenotypic and genotypic changes in *Vibrio cholerae* O139 Bengal. J. Clin. Microbiol., 35, 2588-2592

2) Coppo, A., Colombo, M., Pazzani, C., Bruni, R., Mohamud, K.A., Omar, K.H., Mastrandrea, S., Salvia, A.M., Rotigliano, G. and Maimone, F. (1995): *Vibrio cholerae* in the horn of Africa: Epidemiology, plasmids, tetracycline resistance gene amplification, and comparison between O1 and non-O1 strains. Am. J. Trop. Med. Hyg., 53, 351-359

3) Ehara, M., Watanabe, S., Ichinose, Y., Shimodori, S., Siongok, T.K.A., Alia, K. and Sang, F.C. (1984): Epidemiological observation of cholera in rural Kenya in 1983. *In*: Proc. The 20th Joint Conference of cholera; US-Japan Cooperative Medical Science Program. Nov. 608, 1984, Nara, Japan.

4) Glass, R.I., Huq, I., Alim, A.R.M.A. and Yunus, M. (1980): Emergence of multiply antibiotic-resistant *Vibrio cholerae* in Bangladesh. J. Infect. Dis., 142, 939-942

5) Higa, N., Iwanaga, M., Utsunomiya, A, Kuyyakanond, T., Sithivong, N., Wasito, E.B., Toma, C. and Yamashiro, T. (1995): Drug sensitivity of *Vibrio cholerae* and *Shigella* species in the world. Jpn. J. Trop. Med. Hyg. 23, 159-164

6) Ikema, M. and Honma, Y. (1998): A novel filamentous phage, fs-2, of *Vibrio cholerae* O139. Microbiol., 144, 1901-1906

7) Iwanaga, M. and Yamamoto, K. (1985): New medium for the production of cholera toxin by *Vibrio cholerae* O1 E1 Tor. J. Clin. Microbiol., 22, 405-408

8) Kado, C.I. and Liu, S.T. (1981): Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol., 145, 1365-1375

9) Kobari, K., Takakura, I., Nakatomi, M., Sogame, S. and Uylangco, C. (1970): Antibiotic-resistant strains of E1 Tor vibrio in the Philippines and the use of Furalazine for chemotherapy. Bull. Wld. Hlth. Org., 43, 365-371

10) Mhalu, F.S., Mmari, P.W. and Ijumba, J. (1979): Rapid emergence of E1 Tor *Vibrio cholerae* resistant to antimicrobial agents during the first six months of fourth cholera epidemic in Tanzania. Lancet, 1, 345-347

11) Nakasone N., Iwanaga, M. and Eeckels, R. (1987): Characterization of *Vibrio cholerae* O1 recently isolated in Bangladesh. Trans. Roy. Soc. Trop. Med. Hyg., 81, 876-878

12) Takeya, K. and Shimodori, S. (1963): Prophage-typing of E1 Tor vibrios. J. Bacteriol., 85, 957-958

13) Threlfall, E.J., Said, B. and Rowe, B. (1993): Emergence of multiple drug resistance in *Vibrio cholerae* O1 E1 Tor from Ecuador. Lancet, 342, 1173

14) Towner, K.J., Pearson, N.J., Mhalu, F.S. and O'Grady, F. (1980): Resistance to antimicrobial agents of *Vibrio cholerae* E1 Tor strains isolated during the fourth cholera epidemic in the United Republic of Tanzania. Bull. Wld. Hlth. Org., 58, 747-751

15) Waldor, M.K., Tschape, H. and Mekalanos, J.J. (1996): A new type of conjugative transposon encodes resistance to sulfamethoxazole, trimethoprim and streptomycin in *Vibrio cholerae* O139. J. Bacteriol., 178, 4157-4165

16) Yamamoto, T., Nair, G.B., Albert, M.J., Parodi, C.C. and Takeda, Y. (1995): Survey of *in vitro* susceptibilities of *Vibrio cholerae* O1 and O139 to antimicrobial agents. Antimicrob. Agents Chemother., 39, 241-244

DESCRIPTION OF A NEW SPECIES OF *SIMULIUM* (*NEVERMANNIA*) FROM JAPAN (DIPTERA: SIMULIIDAE)

HIROYUKI TAKAOKA[1] AND KATSUMI SAITO[2]

Received August 20, 1999/Accepted November 8, 1999

Abstract: A new black-fly species, *Simulium saitoi* sp. nov., is described from female, male, pupal and larval specimens collected from Kanagawa Prefecture, Japan. This new species is assigned to the *feuerborni*-group of the subgenus *Simulium* (*Nevermannia*), and is distinctive within this species-group by having several unusual characters, such as the orange yellow scutum of both sexes of adults, female mandible serrated on both sides, and larval body without any dorsal colored markings. This is the fourth species of the *feuerborni*-group in Japan.

Key words: Simuliidae, *Simulium,* black fly, *Nevermannia,* Japan, new species

Recently, one of us (KS) found a new black-fly species while collecting simuliid pupae and larvae for faunal and ecological studies in Kanagawa Prefecture, near Tokyo, Japan. This species is assigned in the *feuerborni*-group within the subgenus *Simulium* (*Nevermannia*), originally defined by Datta (1973), and is shown to have several characters in the adult and larval stages, which are thought to be unusual for this species-group.

This new species is here described from the reared females and males, pupae and mature larvae. The morphological features and terms used herein follow mostly those of Crosskey (1969), and partially those of Takaoka (1983).

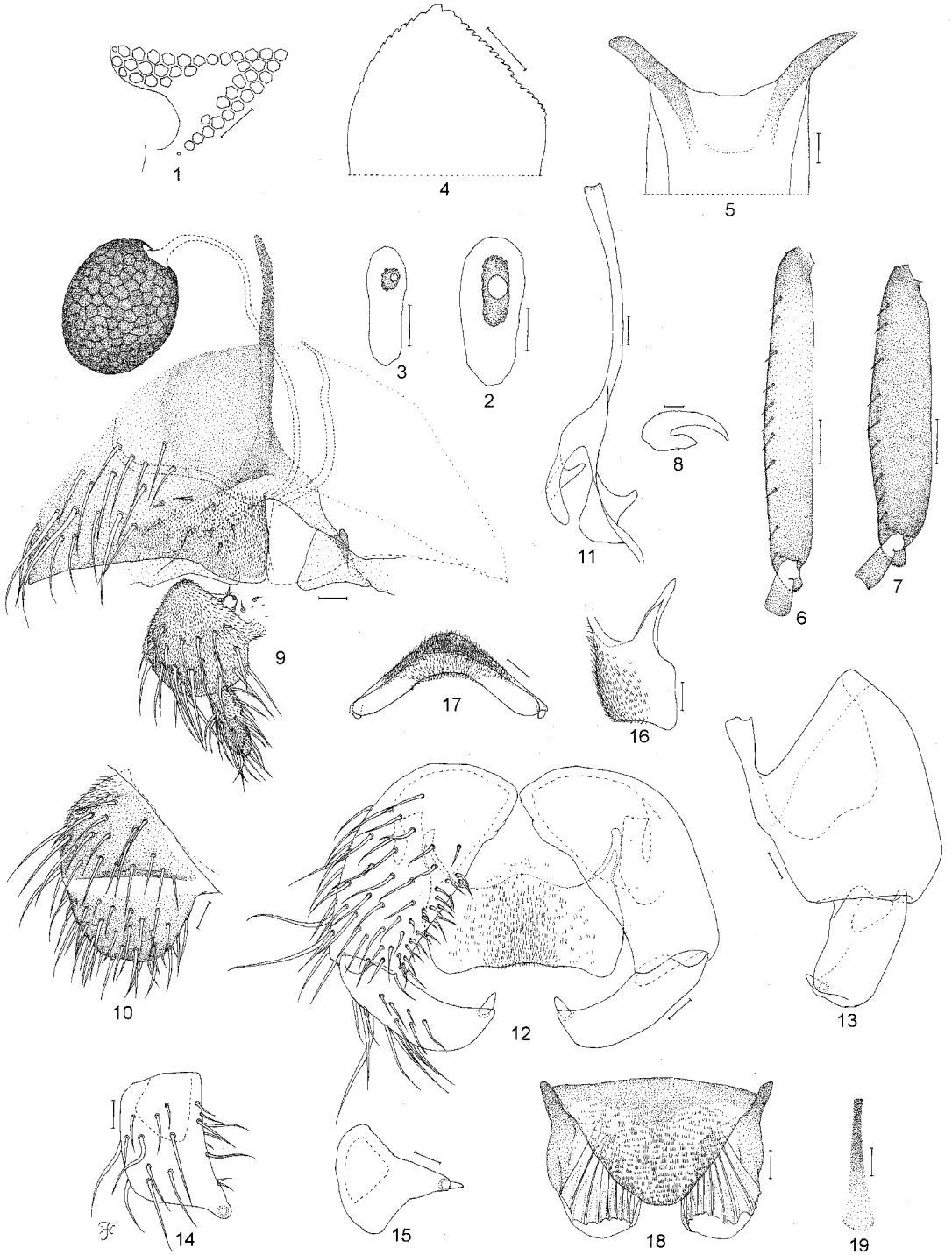
***Simulium* (*Nevermannia*) *saitoi* Takaoka sp. nov.**

DESCRIPTION. **Female**. Body length 3.5 mm. ***Head***. Narrower than thorax. Vertex, frons and clypeus, as well as posterior surface of head, brownish black, not shiny, very densely covered with whitish-yellow, recumbent hairs, interspersed with several dark brown longer and stouter hairs. Frontal ratio 1.8: 1.0: 2.4. Frons-head ratio 1.0:4.7. Fronto-ocular area (Fig. 1) well developed, triangular. Proboscis ca. 0.9× as long as clypeus. Antenna composed of 2+9 segments, pale yellow. Maxillary palp consisting of 5 segments, light to medium brown, proportional lengths of 3rd, 4th and 5th segments 1.0:1.0:1.9; 3rd segment (Fig. 2) much enlarged, with sensory vesicle elongate, ca. 2.3× as long as wide, and ca. 0.5× as long as 3rd segment. Maxillary lacinia with 8-10 inner teeth and 14 outer ones. Mandible (Fig. 4) with ca. 20 inner teeth and 7-10 outer ones. Cibarium (Fig. 5) smooth on posterior margin, with well sclerotized arms directed anterolaterally. ***Thorax***. Scutum mostly orange yellow, with 3 faint narrow dark longitudinal vittae (1 medial and 2 submedial) (in the other female, only 2 submedial vittae visible), and with slightly darker portion on each shoulder (except anterior calli yellow), along each lateral margin and on prescutellar area, densely covered with whitish-yellow, recumbent hairs, and with several dark brown upright hairs on prescutellar area. Scutellum whitish yellow, with many brown upright hairs as well as whitish-yellow, shorter hairs. Postscutellum light brown, shiny, white-pruinose, and bare. Pleural membrane bare. Katepisternum longer than deep, light brown, and bare. ***Legs***. Foreleg: coxa and trochanter whitish yellow; femur light brown with apical cap medium brown; tibia medium brown with base and median large portion paler; tarsus brownish black; basitarsus slightly dilated, ca. 5.9× as long as its greatest width. Midleg: coxa light brown with posterior surface dark brown; trochanter whitish yellow; femur

1 Department of Infectious Disease Control, Oita Medical University, Hasama, Oita 879-5593, Japan
2 Department of Parasitology, School of Medicine, Yokohama City University, Kanazawa-ku, Yokohama City, Kanagawa 236-0004, Japan

yellow basally, slightly darkened toward apex, with apical cap dark brown; tibia whitish yellow on basal 1/3, gradually darkened toward apex, and with apical cap dark brown; tarsus brownish black. Hind leg: coxa and trochanter whitish yellow; femur whitish yellow with apical cap dark brown; tibia yellowish on basal 1/3 or a little more, gradually darkened toward apex, and with apical cap dark brown; tarsus dark brown to brownish black with median large portion of basitarsus slightly paler, and basal 1/2 of 2nd segment whitish; basitarsus (Fig. 6) parallel-sided, ca. 6.8× as long as wide; calcipala well developed, nearly as long as wide; pedisulcus well developed. Claws (Fig. 8) each with large basal tooth, 0.5× as long as claw. All femora and fore tibia covered with shiny whitish yellow hairs on most of outer surface; mid and hind tibiae covered with shiny whitish-yellow hairs on outer and posterior surface of basal 4/5 or a little more. ***Wing***. Length 2.8 mm. Costa with 2 parallel rows of dark short spines as well as dark hairs except basal portion of costa from base to humeral cross vein densely covered with dark hairs on basal 1/3 and with whitish yellow hairs on apical 2/3. Subcosta with dark hairs except near apex bare. Hair tuft on stem vein composed mostly of dark hairs, interspersed with several shiny whitish-yellow hairs. Basal portion of radius fully haired. Basal cell absent. ***Abdomen***. Basal scale dark yellow, with fringe of pale long hairs. Dorsal surface of segment 2 pale yellowish white, densely covered with pale short hairs, interspersed with dark ones near posterior margin; that of remaining segments light brown, densely covered with dark short hairs, interspersed with pale ones; terga of segments 6-8 subshiny when illuminated; segment 7 with large sternal plate medially. ***Genitalia*** (Figs. 9-11). Sternum 8 wide, bare medially but furnished with 20-24 short and long hairs on each side. Anterior gonapophysis triangular, thin, membraneous except inner margin narrowly sclerotized, densely covered with microsetae, interspersed with 5 or 6 short setae. Genital fork of inverted Y- form, with its stem well sclerotized, and with arms very wide at least basally; each arm with wide, round, lobe-like projection directed medioposteriorly and prominent projection directed forward. Paraproct of usual form, only slightly protruding ventrally. Cercus in lateral view rounded posteriorly, ca. 0.5× as long as wide. Spermatheca ellipsoidal, strongly sclerotized except small area around juncture to duct, and duct itself unsclerotized, with distinct reticulate surface pattern, and without internal setae; main spermathecal duct narrow, while both accessory ducts slightly wider than main duct, and with tapered apex.

Male. Body length 3.5 mm. ***Head***. Slightly wider than thorax. Holoptic; upper eye consisting of large facets in 16 vertical columns and 17 horizontal rows. Clypeus black, white-pruinose, moderately covered with pale and dark simple hairs. Antenna composed of 2+9 segments, pale yellow except scape and pedicel light brown; 1st flagellar segment somewhat elongate, ca. 1.5× as long as 2nd flagellar segment. Maxillary palp brown, composed of 5 segments, proportional lengths of 3rd, 4th and 5th segments 1.0:1.0:2.3; 3rd segment (Fig. 3) of moderate size; sensory vesicle small, globular or ellipsoidal, ca. 0.2× as long as 3rd segment. ***Thorax***. Scutum orange yellow, with 3 faint narrow longitudinal vittae (1 medial and 2 submedial) (in the other male no such vittae present) not shiny, densely covered with yellow recumbent hairs, and with several black upright hairs on prescutellar area. Scutellum orange yellow, with many black upright hairs as well as yellow hairs. Postscutellum medium brown, shiny, white-pruinose, and bare. Plueral membrane bare. Katepisternum medium brown, longer than deep, and bare. ***Legs***. Foreleg: coxa and trochanter whitish yellow; femur light to medium brown with apical cap dark brown; tibia dark brown with base whitish yellow and median large portion light brown; tarsus brownish black; basitarsus slender, slightly dilated, ca. 7.0× as long as its greatest width. Midleg: coxa light brown with posterior surface dark brown; trochanter whitish yellow; femur yellow basally, slightly darkened toward apex, with apical cap dark brown; tibia yellow on basal 1/4, gradually darkened toward apex, and with apical cap dark brown; tarsus brownish black. Hind leg: coxa dark yellow; trochanter yellow; femur yellow basally, slightly darkened toward apex, and with apical cap dark brown; tibia medium brown with basal 1/4 or 1/3 dark yellow, and apical cap dark brown; tarsus dark brown to brownish black except basal 1/3 of 2nd segment whitish yellow; basitarsus (Fig. 7) enlarged, spindle-shaped, ca. 4.4× as long as its greatest width, ca. 1.0× and ca. 0.8× as wide as hind tibia and femur, respectively. Calcipala well developed, nearly as long as wide; pedisulcus well developed. Fore femur and tibia covered with shiny yellow hairs on outer surface of all and basal 2/3 of shaft, respectively; mid and hind tibiae covered with shiny yellow hairs on posterior and outer surface of basal 1/3 or a little more of shaft. ***Wing***. As in female except pale hairs on costa present only near juncture to humeral cross vein, and subcosta bare; length 2.5 mm. ***Abdomen***. Basal scale light brown, with fringe of yellow long hairs. Dorsal surface of segment 2 yellowish white except median portion light to medium brown somewhat



Figures 1–19. Female and male adult characters of *Simulium saitoi* sp. nov. 1, fronto-ocular area of female; 2 and 3, 3rd segment of maxillary palp with sensory vesicle (2, female; 3, male); 4, mandible of female; 5, cibarium of female; 6 and 7, hind basitarsus and 2nd tarsal segment (6, female; 7, male); 8, claw of female hind leg; 9, female genitalia *in situ* (ventral view), showing 8th sternite, anterior gonapophyses, genital fork, spermatheca with main and accessory ducts, and right paraproct and cercus; 10, left paraproct and cercus (lateral view); 11, genital fork (lateral view); 12, male genitalia *in situ* (ventral view), showing coxites, styles, ventral plate (parameres, aedeagal membrane and median sclerite omitted); 13, right coxite and style (lateral view); 14 and 15, right style (14, ventrolateral view; 15, end view); 16 and 17, ventral plate (16, lateral view; 17, end view); 18, dorsal plate, aedeagal membrane and parameres *in situ* (dorsal view); 19, median sclerite. Scale bars 0.01 mm for fig. 8; 0.02 mm for figs. 4, 5, 9–19; 0.05 mm for figs. 1–3; 0.1 mm for figs. 6 and 7.

widely on posterior 1/2, and each lateral portion also light brown narrowly just before posterior margin, and covered with pale simple hairs; dorsal surface of remaining segments brownish black, with dark simple hairs; ventral surface of segments 2–4 yellowish white with sternites of segments 3 and 4 light or medium brown, that of remaining segments dark brown to brownish black. ***Genitalia*** (Figs. 12–19). Coxite subquadrate much longer than wide. Style much shorter than coxite, broad, nearly parallel-sided from base to near apex, then abruptly tapered apically and bent inwards, and with distinct apical spine directed inward and forward. Ventral plate lamellate, much shorter than wide, well sclerotized except along anterior margin thin, membraneous, with posterior margin sinuous (when viewed ventrally), and moderately covered with fine short setae on ventral, posterior and dorsal surface; arm of moderate length, stout, and curved inwardly and dorsally. Paramere of normal form, with 9 distinct hooks. Median sclerite simple, club-shaped, narrow, slightly widened toward apex. Dorsal plate broad, thin, medial portion protruding posteriorly. Aedeagal membrane densely covered with spinous microsetae. Cercus small, rounded in lateral view, and with 7 simple hairs.

Pupa. Body length 3.2–3.6 mm. ***Head***. Integument yellow, moderately covered with round tubercles (Fig. 20); frons with 2 short, slender, simple trichomes on each side, while face with 1 long, somewhat stout, simple trichome (ca. 3.2× as long as frontal trichomes) on each side. ***Thorax***. Integument yellow, moderately covered with round tubercles of various sizes, with 3 long, slender, simple trichomes mediodorsally, 2 slender, simple trichomes (1 long, and 1 medium) mediolaterally, 1 medium, slender, simple trichome posterolaterally, and 3 short, slender, simple trichomes ventrolaterally, on each side. Gill (Fig. 21) with 6 long slender filaments arranged in pairs, upper pair with short stalk, arising from basal common stalk of medium length, middle pair sessile or with short stalk, sharing short primary stalk with lower pair which has long stalk; all filaments lying close together, directed forward, grayish brown except basal 1/2 or 3/4 of basal common stalk yellow, subequal in length to one another (length is variable from 3.6–4.8 mm depending upon individual pupae), and somewhat or much longer than pupal body; cuticular surface with distinct annular ridges and furrows (though ridges becoming indistinct on apical 1/2), and densely covered with minute tubercles. ***Abdomen***. Terga 1 and 2 yellowish, moderately tuberculate; tergum 1 with 1 medium, slender seta on each side; tergum 2 with 1 medium, slender seta and 5 short, spinous setae on each side; terga 3 and 4 each with 4 hooks and a few spinous setae on each side; tergum 5 bare; terga 6–8 each with a transverse row of spine-combs directed backward on each side; tergum 9 with a pair of distinct, horn-shaped terminal hooks (Fig. 22). Sternum 4 with 1 simple hook submedially, which is subequal in size to those on sterna 5–7, and a few slender setae on each side; sternum 5 with a pair of bifid hooks submedially and a few slender setae on each side; sterna 6 and 7 each with 1 bifid hook submedially and 1 simple hook laterally, and a few slender setae on each side. ***Cocoon*** (Fig. 23). Simple, wall-pocket-shaped, compactly woven without open spaces in web, very thin, with anterior margin thickly woven, and extending ventrolaterally; 3.8–4.8 mm long × 2.3–3.5 mm wide.

Mature larva. Body length 6.0–7.0 mm. Body creamy white, without any colored markings except 2 brown spots on each side of posterior abdominal segments. Cephalic apotome pale on anterior 2/5, dark yellow to yellowish brown on posterior 3/5, with posterior margin somewhat darkened; head spots basically positive except posterolateral spots usually negative because of relatively darker surrounding areas, in particular a narrow area between two spots; anteromedian longitudinal spot and anterior 1/2 of lateral spots usually faintly positive, while others moderately distinctive; isolated spots below area of eye spots on lateral surface of head capsule absent or indistinct if any. Cervical sclerite composed of 2 small rod-like pieces, not fused to occiput, widely separated medially from each other. Antenna consisting of 3 segments and apical sensillum, longer than stem of labral fan; proportional lengths of 1st, 2nd and 3rd segments 1.0:1.0:0.8. Labral fan with ca. 36 main rays. Mandible (Fig. 24) with 2 usual mandibular serrations; comb-teeth composed of 3 teeth, 1st tooth longest and thickest, 2nd tooth subequal in length to, but somewhat thicker than, 3rd tooth; supernumerary serrations absent (though 1 larva with 2 minute supernumerary serrations posterior to mandibular serrations on left mandible, as shown in Fig. 25). Hypostomium (Fig. 26) with a row of 9 apical teeth; median and corner teeth well developed; median tooth of 3 intermediate teeth on each side smallest; lateral serrations undeveloped; 7 or 8 hypostomal bristles lying parallel to, or slightly divergent posteriorly from, lateral margin on each side. Postgenal cleft (Fig. 27) small, shallow, with anterior margin somewhat variable in shape, usually simple straight but sometimes, sinuous or with minute notch, or arched, and ca. 0.36× as long as

postgenal bridge. Thoracic cuticle bare. Abdominal cuticle bare except last segment covered with colorless short setae on each side of anal sclerite. Rectal papilla of 3 lobes, each lobe with 9–11 finger-like secondary lobules. Anal sclerite of usual X-form, with posterior arms nearly as long as anterior ones; basal portion of arms widely sclerotized. Accessory sclerite absent. Ventral papillae large, conical, placed ventrally, then, well discernible when the larva is viewed laterally. Posterior circlet with ca. 80 rows of up to 15 hooklets per row.

TYPE SPECIMENS. Holotype female, reared from pupa, collected from a small stream, International Camping site of Ichinose, Kiyokawa Village, Kanagawa Prefecture, Japan, 6.VI.1999, by K. Saito. Paratypes, 1 reared female, 2 reared males, 2 pupae, 4 pupal exuviae, 21 mature larvae, same data as holotype. Holotype and most of paratype specimens will be deposited at the Natural History Museum, London, UK.

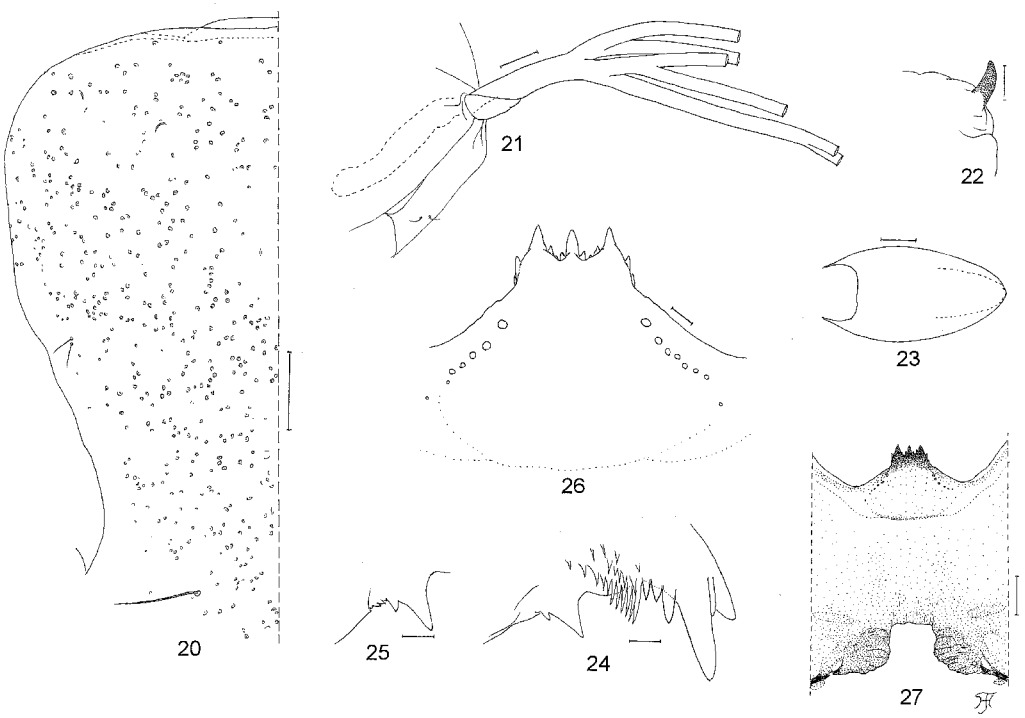
BIOLOGICAL NOTES. Immature stages of *S. saitoi* sp. nov. were found in a small, shallow, very slow-flowing stream partially shaded by shrubbery. The pupae and larvae attached to grasses trailing in the water from the banks. This species was collected together with *S. japonicum* Matsumura, 1931, *S. mie* Ogata and Sasa, 1954, *S. subcostatum* (Takahasi, 1950), and *S. uchidai* (Takahasi, 1950).

DISTRIBUTION. Limited to the type locality in Kanagawa Prefecture, Japan.

ETYMOLOGY. The species *saitoi* is named after Dr. K. Saito in memory of his discovery of this new species, as well as his contribution to knowledge in ecological aspects of Japanese black flies.

REMARKS. The *feuerborni*-group within the subgenus *Simulium* (*Nevermannia*) is a small and relatively homogenous taxon, consisting of 12 named species in the Oriental and Palaearctic Regions (Crosskey and Howard, 1996; Crosskey, 1999). Three of these species have been reported from Japan: i.e., *S. mie, S. morisonoi* Takaoka, 1973, and *S. sasai* (Rubtsov, 1962).

S. saitoi sp. nov. is readily assigned to the *feuerborni*-group, by the combination of the following characters: male genitalia with a simple, lamellate ventral



Figures 20–27. Pupal and larval characters of *Simulium saitoi* sp. nov. 20–23, pupa; 24–27, larva. 20, frontoclypeus (right half only); 21, basal portion of gill filaments (lateral view); 22, terminal hook (lateral view); 23, cocoon (dorsal view); 24, apical part of mandible; 25, mandibular serrations and supernumerary serrations; 26, hypostomium (ventral view); 27, head capsule (ventral view) showing hypostomium and postgenal cleft. Scale bars 0.01 mm for figs. 24 and 25; 0.02 mm for figs. 22 and 26; 0.1 mm for figs. 20, 21 and 26; 1.0 mm for fig. 23.

plate, a short style twisted inward, several parameral hooks, and a simple, narrow median sclerite; pupal gill with six long thread-like filaments per side; and larval head with small, short postgenal cleft.

It is worthwhile to note that *S. saitoi* has the orange yellow scutum in both sexes of adults, while all the other 12 known *feuerborni*-group species have the reddish brown or dark brown scutum. In addition, the female of this new species differs by having the mandible serrated on both sides (Fig. 4) (c.f., mandibles are serrated only on the inner margin and smooth on the outer margin in the other member species). Moreover, in the larva of *S. saitoi*, dorsal colored markings on its body are absent, and isolated spots below the eye spot area on the head capsule are also absent, or indistinct if any; while in the eight other *feuerborni*-group species, of which the larval stage is known, there are distinct dorsal colored markings on the larval body and three marked spots below the eye spot area of the larval head. The untoothed lateral margins of hypostomium in this new species are also unusal for the *feuerborni*-group.

By contrast, no such unusual characters were found in the pupa of this new species. However *S. saitoi* is easily separated from four of eight known species (of which the pupal stage is known): i.e., *S. feuerborni* Edwards, 1934 from Java, Bali, and Malay Peninsula, *S. leigongshanense* Chen and Zhang, 1997 from China, *S. praelargum* Datta, 1973 from India, China and Nepal, and *S. sasai* from Japan, by the simple, wall-pocket-shaped cocoon (Fig. 23), in place of the hooded one. The other four species, i.e., *S. chitoense* Takaoka, 1979 from Taiwan and China, *S. mie* from Japan, China and Korea, and *S. morisonoi* from Japan, and *S. perlucidulum* Takaoka, 1983 from the Philippines, have the similar type of simple cocoon. The six gill filaments of *S. perlucidulum* are not paired and almost sessile, without secondary stalks (Takaoka, 1983), while those of the three other species are arranged in three pairs, as in *S. saitoi*. There seems, however, to be a slight difference in the branching of the gill filaments: i.e., three pairs of filaments divide dichotomously in this new species (Fig. 21), while each of three pairs arises almost independently at the same level from the basal common stalk in the others (Takaoka, 1973, 1976, 1979).

This new species seems to have more similarities to *S. chitoense* and *S. mie* than to *S. morisonoi* because secondary lobules on its larval rectal papillae are finger-like (c.f., thumb-like in *S. morisonoi*) and the projection directed anteriorly on each arm of its female genital fork is markedly developed (c.f., weakly developed in *S. morisonoi*).

Acknowledgements

We thank Dr. D.M. Davies, professor emeritus, McMaster University, Ontario, Canada for reading the manuscript, and giving valuable suggestions. Thanks are due to Dr. R.W. Crosskey, The Natural History Museum, London, who kindly provided valuable refer ences. This study was supported in part by the Grant-in-Aid of the Ministry of Education, Science and Culture, Japan (no. 11670246) to HT.

References

1) Crosskey, R.W. (1969): A re-classification of the Simuliidae (Diptera) of Africa and its islands. Bull. Br. Mus. Natur. Hist. (Entomol.), suppl., 14, 1-195
2) Crosskey, R.W. (1999): First Update to the Taxonomic and Geographical Inventory of World Blackflies (Diptera: Simuliidae). 10 pp., The Natur. Hist. Mus., London.
3) Crosskey, R.W. and Howard, T.M. (1996): A New Taxonomic and Geographical Inventory of World Blackflies (Diptera: Simuliidae). 144 pp., The Natur. Hist. Mus., London.
4) Datta, M. (1973): New species of black flies (Diptera: Simuliidae) of the subgenera *Eusimulium* Roubaud and *Gomphostilbia* Enderlein from the Darjeeling area, India. Oriental Insects, 7 (3), 363-402
5) Takaoka, H. (1973): Descriptions of 2 new species of blackflies, *Simulium* (*Gomphostilbia*) *tokarense* and *S.* (*Eusimulium*) *morisonoi* (Diptera: Simuliidae), from the Tokara Islands, Japan. Jpn. J. Sanit. Zool., 23 (3), 201-207
6) Takaoka, H. (1976): Studies on black flies of the Nansei Islands, Japan (Simuliidae; Diptera) I. On six species of the subgenus *Eusimulium* Roubaud, with the descriptions of *Simulium* (*E.*) *satsumense* sp. nov. and *S.* (*E.*) *subcostatum koshikiense* ssp. nov. Jpn. J. Sanit. Zool., 27 (2), 163-180
7) Takaoka, H. (1979): The black flies of Taiwan (Diptera: Simuliidae). Pacific Insects, 20 (4), 365-403
8) Takaoka,H.(1983):The blackflies (Diptera: Simuliidae) of the Philippines. xi+199 pp., Japan Society for the Promotion of Science, Tokyo.

TAXONOMIC NOTES ON *SIMULIUM SIRIPOOMENSE* FROM THAILAND (DIPTERA: SIMULIIDAE): DESCRIPTIONS OF FEMALE AND PUPA, AND CHANGE OF ITS SUBGENERIC STATUS FROM *BYSSODON* TO *SIMULIUM* S. STR.

HIROYUKI TAKAOKA[1] AND MIR S. MULLA[2]

Received September 13, 1999/Accepted December 13, 1999

Abstract: The female and pupa of *Simulium siripoomense* Takaoka and Saito, 1996, hitherto known only from larval specimens collected from Thailand, are described for the first time. Based upon several diagnostic characters found in the female and pupa, this species, previously placed in the subgenus *Byssodon*, is transferred to the subgenus *Simulium* s. str., and is further assigned to the *malyschevi*-group within this subgenus by the characteristic shape of the female genitalia, the pupal gill with six slender filaments per side, and the fenestrate cocoon. This represents the first record of the *malyschevi*-group in Thailand.
Key words: Simuliidae, *Simulium*, black fly, *Byssodon*, Thailand, change of subgeneric status

Simulium siripoomense Takaoka and Saito was described from two mature larvae collected from northern Thailand, and was tentatively placed in the subgenus *Byssodon* within the genus *Simulium* s. l. (Takaoka and Saito, 1996). The adult and pupal stages of this species remained unknown.

Recently, one of us (MSM) collected several pupae and larvae of *S. siripoomense* from two streams in northern Thailand. Two of the four pupae examined were pharate females with almost develpoed organs of the adult stage including the head, legs and genitalia, and then used for the observation of the morphology of the adult female.

We here describe the female and pupa of this species and, based upon their characters, change its subgeneric status from *Byssodon* to *Simulium* s. str.

***Simulium* (*Simulium*) *siripoomense* Takaoka and Saito, 1996**

Simulium (*Byssodon*) *siripoomense* Takaoka and Saito, 1996: 163–165; Crosskey and Howard, 1997: 28.

DESCRIPTION. **Female**. Body length 2.3 mm. ***Head***. Frons shiny, almost bare except several hairs along both lateral margins; frontal ratio 1.3:1.0:1.0. Frons-head ratio 1.0:3.6. Fronto-ocular area (Fig. 1) moderately developed. Antenna composed of 2+9 segments. Clypeus moderately covered with hairs except central portion of upper 2/3 largely bare. Proboscis short, ca. 0.7× as long as clypeus. Maxillary palp consisting of 5 segments, proportional lengths of 3rd, 4th and 5th segments 1.0:1.1:1.8; 3rd segment (Fig. 2) of moderate size, with oblong sensory vesicle, ca. 0.4× as long as 3rd segment. Maxillary lacinia with 8–10 inner teeth and 11 or 12 outer ones. Mandible with 25 inner teeth and 12 outer ones. Cibarium (Fig. 3) with short medial projection directed upward on posterior margin, and furnished with 3 or 4 teeth on each side near posterior margin. ***Thorax***. Scutum dark, shiny, densely covered with recumbent fine hairs, and with several upright hairs on prescutellar area. Scutellum with many upright hairs as well as fine hairs. Postscutellum bare. Pleural membrane bare. Katepisternum longer than deep, light brown, and bare. ***Legs***. Fore basitarsus strongly dilated, widened toward apex, ca. 4.4× as long as its greatest width. Hind basitarsus parallel-sided; calcipala

1 Department of Infectious Disease Control, Oita Medical University, Hasama, Oita 879-5593, Japan
2 Department of Entomology, College of Natural and Agricultural Sciences, University of California, Riverside, California 92521, U.S.A.

and pedisulcus well developed. Claws (Fig. 4) each with small subbasal tooth. ***Wing***. Length not measured. Costa with 2 parallel rows of short spines as well as hairs. Basal portion of radius bare. ***Abdomen***. Tergite of segment 2 largely shiny, iridescent when illuminated; dorsal surface of other segments dark, moderately covered with short hairs; tergites of segments 6–8 shiny when illuminated; ventral surface of segment 7 with a median cluster of 46 hairs, most of which are forked into 2–6 branches but some are simple (Fig. 5); ventral surface of segment 6 also with a few bifid hairs among simple hairs. ***Genitalia*** (Figs. 6 and 7). Sternite 8 wide, bare medially but furnished with ca. 8 short and long hairs on each side. Anterior gonapophysis short, bluntly truncate posteriorly, densely covered with microsetae except near posterior margin bare or sparsely covered with microsetae, interspersed with ca. 8 short and long hairs; inner margins diverged basally from each other, then parallel-sided posteriorly; posterior margin straight, oblique outwardly. Genital fork of inverted Y-form, with its stem well sclerotized and slender, and with arms of moderate width; each arm with a distinct round projection directed forward. Paraproct of usual form, somewhat protruding ventrally, markedly depressed along anteroinner margin, and with 8 or 9 hairs on ventral and lateral surfaces. Cercus in lateral view semicircular, ca. 0.5× as long as wide, moderately covered with hairs. Spermatheca ellipsoidal, probably well sclerotized except large area around juncture to duct, and duct itself unsclerotized, with many internal setae; main and accessory ducts narrow, subequal in size to one another.

Pupa. Body length 1.9 mm. ***Head***. Integument of frons yellow, almost bare, and with 2 medium, slender, simple trichomes situated close together on each side; face with 1 similar trichome on each side. ***Thorax***. Integument yellow, almost bare except dorsal surface and part of lateral surface of posterior 1/2 moderately covered with small conical tubercles, and with 9 usual trichomes on each side, i.e., 2 long and 1 medium, simple trichomes mediodorsally, 1 long, bifid trichome and 1 medium, simple trichome anterolaterally, 1 medium, simple trichome posterolaterally, and 3 medium, simple trichomes ventrolaterally, all trichomes somewhat stout. Gill (Fig. 8) with 6 slender, thread-like filaments arranged in pairs, all short-stalked, upper and middle pairs sharing very short stalk; all filaments pale, subequal in length to one another (ca. 1.0 mm), also subequal in thickness to one another (though outer filament of middle and lower pairs slightly thinner than inner filament), and gradually tapered toward apex; cuticular surface with annular ridges and furrows, and densely covered with minute tubercles. ***Abdomen***. Terga 1 and 2 pale yellow, without tubercles; tergum 1 with 1 medium, slender seta on each side; tergum 2 with 1 medium, slender seta and 5 short, spinous setae on each side; terga 3 and 4 each with 4 hooks and a few spinous setae on each side; terga 5 and 6 bare; terga 7–9 each with a transverse row of spine-combs directed backward on each side; tergum 9 without terminal hooks. Sternum 4 with a few slender setae on each side; sterna 5–7 each with a pair of bifid hooks and a few slender setae on each side. ***Cocoon*** (Fig. 9). Simple, wall-pocket-shaped, compactly woven, with large anterolateral window on each side; 2.5 mm long × 0.9 mm wide.

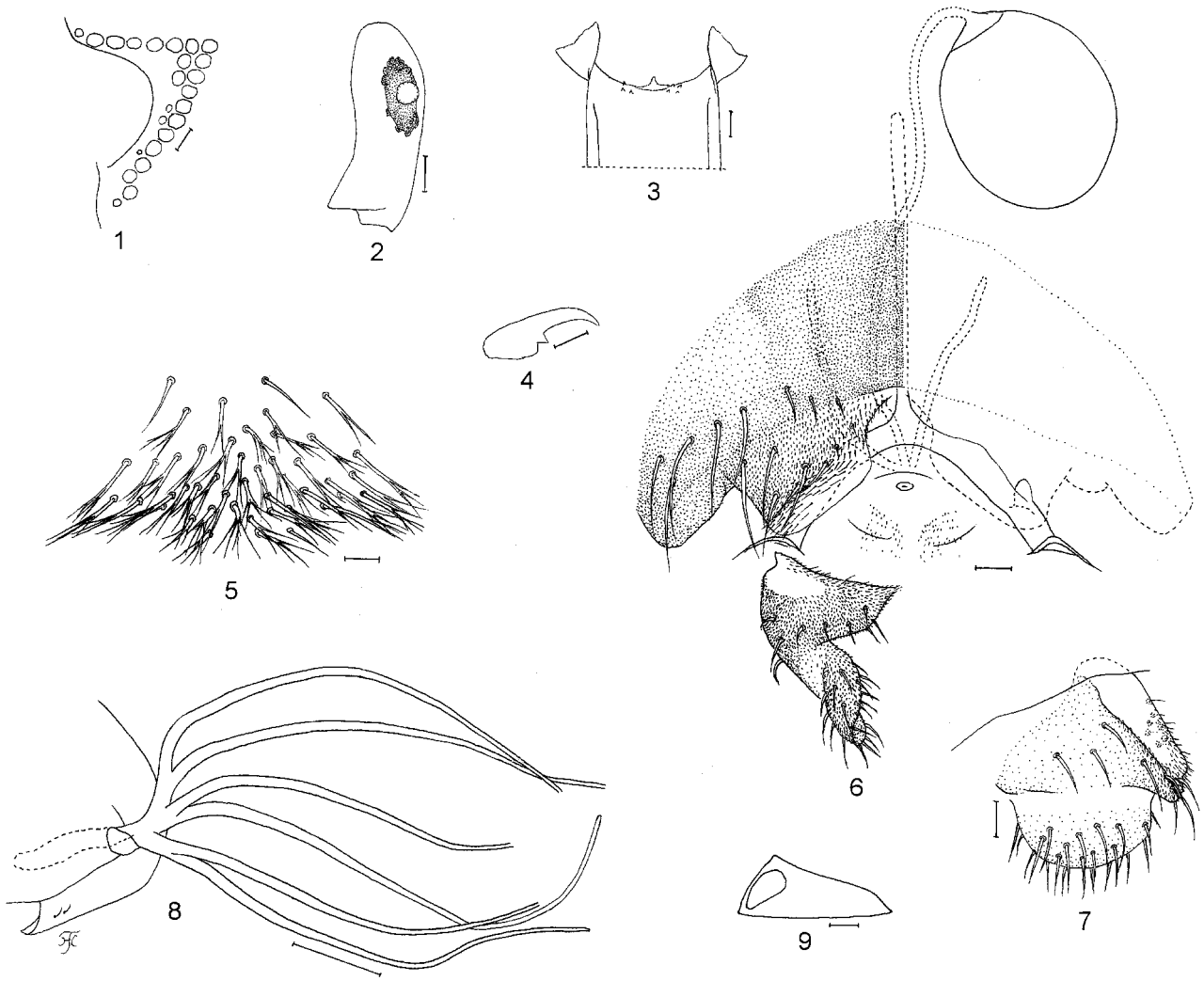
SPECIMENS EXAMINED. 1 female dissected out of pupa, 1 pupa, 1 pupal exuvia and 5 mature larvae, collected from a small stream, Pa Bang, Mae Hong Son, northern Thailand, 5.II.1996, by M.S. Mulla and J. Rodcharoen. 2 pupae (1 pharate female inside), 1 pupal exuvia and 1 mature larva, collected from a stream, Mae Klang Fall, Doi Inthanon, Chiangmai, northern Thailand, 4.II.1996, by M.S. Mulla and J. Rodcharoen.

ECOLOGICAL NOTE. The pupae and larvae of *S. siripoomense* were collected together with *S. chamlongi*, *S. nigrogilvum*, *S. tani*, *S. nakhonense*, and *S. quinquestriatum*.

DISTRIBUTION. Thailand.

REMARKS. Takaoka and Saito (1996) described *Simulium siripoomense* as a new species from the two mature larvae collected from Thailand, and tentatively placed it in the subgenus *Byssodon* within the genus *Simulium* s. l. This subgeneric assignment was based upon the fact that the larva of this species was very similar to that of *S. maculatum* (Meigen), one of the typical species of the subgenus *Byssodon*, by sharing several unusual characters, such as posession of prominent protuberances not only dorsally but also laterally and ventrally on the abdomen, postgenal cleft very large reaching the posterior border of the hypostomium, and last abdominal segment without ventral papillae.

From the material collected by Mulla and Rodcharoen in 1996 in northern Thailand, it is clear that the female and pupa of *S. siripoomense* differ from those of the subgenus *Byssodon* in many diagnostic characters, e.g., in this species, fore basitarsus strongly dilated, cibarium armed with teeth, tarsal claws with a small



Figures 1–9. Female and pupal characters of *Simulium siripoomense*. 1–7, female; 8 and 9, pupa. 1, fronto-ocular area; 2, 3rd segment of left maxillary palp with sensory vesicle; 3, cibarium; 4, tarsal claw; 5, median cluster of simple and branched hairs on ventral surface of abdominal segment 7; 6, genitalia *in situ* (ventral view), showing 8th sternite, anterior gonapophyses, genital fork, spermatheca with main and accessory ducts, and right paraproct and cercus; 7, right paraproct and cercus (lateral view); 8, gill filaments (lateral view); 9, cocoon (lateral view). Scale bars 0.5 mm for fig. 9; 0.2 mm for fig. 8; 0.02 mm for figs. 1–3, 5–7; 0.01 mm for fig. 4.

subbasal tooth, tergites 6–8 shiny, and pupal gill with 6 slender filaments per side, and fenestrate cocoon. These female and pupal characters, as well as others such as both pleural membrane and katepisternum bare, and basal portion of radius bare, well agree with the diagnosis of the subgenus *Simulium* s. str., defined by Crosskey (1969). The larval characters, such as deep cleft and absence of ventral papillae, do not depart from the diagnosis of the subgenus *Simulium* s. str. For these reasons, *S. siripoomense* is here transferred to *Simulium* s. str.

Moreover, this species is assigned in the *malyschevi*-group within *Simulium* s. str., also defined by Crosskey (1987), by the characteristic shape of the anterior gonapophyses in the female genitalia. Among the 28 known species (excluding *S. nujiangense* Xue and *S. saccatum* (Rubtsov) apparently belonging to the *striatum*-group) of the *malyschevi*-group listed by Crosskey and Howard (1997), *S. hirtipannus* Puri from India, *S. jacuticum* Rubtsov from Siberia and China, *S. jieyangense* An, Yan, Yang and Hoa from China, *S. nacojapi* Smart from Japan, Korea, Siberia and China, *S. subvariegatum* Rubtsov from Siberia, Mongolia and China, and *S. kyushuense* Takaoka from Japan are very similar to *S. siripoomense* by having the 6-filamented pupal gill, the fenestrate cocoon, and a cluster of numerous bran-

ched hairs medially on the ventral surface of seventh abdominal segment of the female (though, these branched hairs are absent in *S. kyushuense*, and not known in *S. jieyangense*). However all these related species (except *S. hirtipannus* and *S. jieyangense*, of which larval stage is yet unknown) are separated from *S. siripoomense* by the absence of prominent protuberances on the dorsal, lateral and ventral surfaces of the larval abdomen.

According to the original description and illustrations given by Puri (1932), the female and pupa of *S. hirtipannus* seem to be almost indistinguishable from those of *S. siripoomense*, but there are some differences in the female between the two species, i.e., the number of hairs on the paraproct which is 18 or 19 (vs. 8 or 9 in this species), and also the number (64) of hairs composing a median hair cluster on the ventral surface of the seventh abdominal segment (vs. 46 in this species). There are distinct differences in the shape of female gonapophyses and paraprocts between *S. siripoomense* and *S. jieyangense* (An *et al*., 1994).

This is the first record of the *malyschevi*-group from Thailand.

Acknowledgements

This study was supported in part by the Grant-in-Aid of the Ministry of Education, Science, Sports and Culture, Japan (No. 11670246) to HT, and by the grant from Chulalongkorn University in Bangkok to MSM.

References

1) An, J. Y., Yan, G., Yang, L.X., and Hao, B.S. (1994): A new species of Simuliidae from Guangdong Province, China (Diptera: Simuliidae). Sichuan J. Zool., 13, 4-6 (in Chinese with an English abstract)

2) Crosskey, R.W. (1969): A re-classification of the Simuliidae (Diptera) of Africa and its islands. Bull. Br. Mus. Natur. Hist. (Entomol.) suppl. 14, 1-195

3) Crosskey, R.W. (1987) (issued 1988): An annotated checklist of the world black flies (Diptera: Simuliidae), p. 425-520. *In* Kim, K.C. and Merritt, R.W., (eds), *Black flies: ecology, population management, and annotated world list*. xv + 528 pp., The Pennsylvania State University Press, University Park and London.

4) Crosskey, R.W. and Howard, T.M. (1997): A New Taxonomic and Geographical Inventory of World Blackflies (Diptera: Simuliidae). 144 pp., The Natur. Hist. Mus., London.

5) Puri, I.M. (1932): Studies on Indian Simuliidae. Part IV. Descriptions of two new species from north-east India *Simulium howletti* sp. n. and *Simulium hirtipannus* sp. n., with a note on *S. ornatum* Meigen. Indian J. Med. Res., 20, 505-514

6) Takaoka, H. and Saito, K. (1996): A new species and new records of black flies (Diptera: Simuliidae) from Thailand. Jpn. J. Trop. Med. Hyg., 24 (3), 163-169

Jpn. J. Trop. Med. Hyg., Vol. 28, No. 1, 2000, pp. 29–97

PROCEEDINGS OF JOINT CONFERENCE, 40TH ANNUAL MEETING OF JAPANESE SOCIETY OF TROPICAL MEDICINE AND 14TH ANNUAL MEETING OF JAPANESE ASSOCIATION FOR INTERNATIONAL HEALTH

3–5 September 1999, Tokyo

Presidents

Nobukatsu Ishikawa (Japanese Society of Tropical Medicine)

The Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association

Naoki Furuta (Japanese Association for International Health)

Waseda University

CONTENTS

Symposium III: What Can be Expected in Tropical Medicine and International Health?
Chaired: Fujita, K. and Uehara, N.

1 A thinking in the field work of filariasis in the Mozambique in East Africa
Fujita, K.
2 Multidisciplinary research agenda originating from the tropics Aoki, Y.
3 What can be expected in international health as an academic discipline? Marui, E.
4 Practice and research in international health–disaster relief and epidemiology–
Kunii, O.

Symposium IV "Cross-Fire: Tropical Medicine vs. International Health"
Chaired: Ishikawa, N. and Furuta, N.

Work shop I

Special lecture:
1 What can we do about emerging parasitic disease challenges? Colley, D.G.
2 Japan's global parasitic disease control initiatives and other international initiatives
Kasai, K.

1 Knowledge, attitude and practice test for the evaluation of hygienic education in an intestinal parasitic diseases prevention and control project in Prek Russey Commune, Cambodia Kita, K. *et al.*
2 Differential serodiagnosis of alveolar echinococcosis from cystic echinococcosis in China for a global parasite control Ito, A. *et al.*
3 Global elimination of lymphatic filariasis–strategy and weapons for the operation
Kimura, E.
4 Seroepidemiological survey of schistosomiasis mekongi in Laos and Cambodia
Matsuda, H. *et al.*
5 Schistosomiasis and agricultural development Shimada, M. and Shiratori, K.
6 Biology and control strategy of Chagas' disease vectors in Guatemala Tahara, Y.
7 New epidemy of parasitic diseases in Southeast Anatoria in Turkey
Matsumoto, Y. *et al.*
8 Malaria situation and control program in Khammouane Province, Lao PDR
Kobayashi, J. *et al.*
9 Eradication of malaria on an island of Vanuatu Kaneko, A. *et al.*
10 A successful course of malaria control in the Solomon Islands Laefasia, J. *et al.*

Work shop II

＊ 1 Transition of infectious diseases in Nepal and changing environment Ohara, H.
＊ 2 The health problem due to environmental pollution around the Aral Sea
Hashizume, M. *et al.*
＊ 3 The economic impact of tobacco: a look at Cambodia Marshall, S. *et al.*
＊ 4 The meaning of biomedicine in a community in the Brazilian Amazon flood plain
Matsuoka, H.
＊ 5 Health relief for refugees as an issue of development–the study on Kosovoan refugees in Macedonia Nakamura, Y.
＊ 6 Medical activities for Afgan refugees at JIFF physiotherapy center in Peshawar
Ahmadyar, K. *et al.*
＊ 7 Health status of mothers and children in economic crisis in urban populations in Indonesia Matsuba, H. *et al.*
＊ 8 Impact of long-term environmental changes on malaria vectors and malaria occurrence in Mae Hong Son, northern Thailand Suwonkerd, W. *et al.*
＊ 9 Community malaria control project on Tongoa Island in Vanuatu Hata, K. *et al.*

＊10 Medical support as a motivation for cooperation in protecting the environment
Sawada, Y. *et al.*
＊11 Review of natural disasters in the western pacific region and problems
Asahi, S. *et al.*
＊12 The consciousness of village people drinking arsenic contaminated groundwater in Bangladesh Ushijima, K. *et al.*
＊13 Bacterial contamination of tube well water in Bangladesh Nakamura, T. *et al.*
＊14 Bangladesh floods disaster: its health effects and factors influencing diarrheal episodes Kunii, O. and Nakamura, S.
＊15 Living water analysis in Nepal (2) Ogawa, T. *et al.*

Work shop III

＊ 1 Preventing induced abortion among urban poor in Fortaleza, Brazil: is post-abortion counselling effective? Misago, C. *et al.*
＊ 2 Prospective birth spacing and family planning through the research of pregnancy, birth and children's deaths in Senegal Asano, M.
＊ 3 Traditional birth attendants in Cambodia: preliminary study Shimizu, M. *et al.*
＊ 4 Evaluation of the 'quality' of the target in 'the project for the emergency care' in the Dr. Soetomo Hospital in Surabaya, Indonesia Urabe, D.
＊ 5 A study on utilization of health care services among the residents in a provincial city in Thailand Kitajima, T. *et al.*
＊ 6 Drug use status of revolving drug fund in Vietnam Akiyama, Y. *et al.*
＊ 7 Training course for participatory health administration in autonomous region Ruslim Mindanao through linpage between NGO and JICA
Nakashima, T. and Horimoto, T.
＊ 8 Toward national health policy with infusion of indigenous knowledge-the case of Yemen Hirayama, M. *et al.*
＊ 9 An evaluation of cold chain in Blantyre, Malawi by potency testing of measles vaccine vials collected at each step of the cold chain Yamaguchi, S.
＊10 Clinical features of HIV infected patients-1,500 cases of surgical experience in Malawi- Sugishita, T.
＊11 Study on the practice and the problem of health education in the JOCV dispatched area (1) Oyama, O. *et al.*
＊12 Examination of researches conducted in action-oriented projects
Sakamoto, N. *et al.*
＊13 Oral health behavior of Nepali adult population Yamane, K. *et al.*
＊14 Prevalence of disabilities among children in Nahia village Tateyama, K. *et al.*
＊15 PHC project in Cambodian rural district Ishimatsu, Y. *et al.*

Work shop IV

＊ 1 AIDS prevention and control program in the Philippines Yuasa, M. *et al.*
2 Blood safety on developing countries Yoshihara, N.
3 HIV research at the MRC laboratories, the Gambia, west Africa: the link between the field and the laboratory Ariyoshi, K.
4 Detection of *Escherichia coli* O157 antigen and Shiga-like toxins in clinical specimens using monoclonal antibodies Tongtawe, P. *et al.*
5 Prevalence of enteroaggregative *E. coli* causing children diarrhea in Thailand
Ratchtrachenchai, O-A. *et al.*
6 Establishment of the PCR system specific to *Salmonella* spp. and its application for the inspection of food and fecal samples Makino, S. *et al.*
7 Can we eliminate leprosy? -comparison of the current states between low and highly

endemic countries Namisato, M.
8 Present status and future planning of a leprosy elimination program in Khulna district, Bangladesh –collaboration of a NGO with the local Government– Ishida, Y.
＊ 9 Ploblems on the treatment for Leprosy Nagao, E.
＊10 Twelve years achievements by JICA TB control project in Nepal Yamada, N. *et al.*
11 Improvement of TB smear examination in Cebu City, Philippines Fujiki, A. *et al.*
12 International tuberculosis training couses in Japan Hoshino, H. *et al.*

Poster presentation

＊ 1 HIJ Ear Health Care Project in Indonesia supported by eight Rotary Clubs and Rotary International Foundation (fourth report) Sakai, S. *et al.*
＊ 2 The effect of flouride mouth rinsing program in developing countries Matsuoka, N. *et al.*
＊ 3 The way of dental treatment in developing countries Tokunaga, K. *et al.*
＊ 4 Promotion of primary health care program in rural area in Kenya through participatory action research Eto, K. *et al.*
＊ 5 HOPE partner program (Thailand) Otani, A. and Sumi, A.
＊ 6 School-based health project in Nepal Haruki, K. *et al.*
＊ 7 Comparison of user fee's introduction of donor agencies in Yemen Inaoka, E. *et al.*
＊ 8 Attitude toward traditional healers among villagers in Lombok Island, Indnesia Kawashima, T. *et al.*
＊ 9 Behavioral changes of rural women in Paraguay through participation in focus groups to develop a maternal health record booklet Onishi, M.
＊10 Result of questionnaire survey to the participants of MCH Handbook International Symposium in 1998 Nakahara, S. *et al.*
＊11 Factors affecting antenatal attendance rate. An experience in Ceará State, Brazil Haneda, K. *et al.*
＊12 The growth of Japanese children living abroad Hiroshige, Y. *et al.*
＊13 Breastfeeding in Thailand Hotta, M. *et al.*
＊14 Mother's hygiene behavior in Tayabo Village, Nueva Ecija Province in the Philippines Sakisaka, K. *et al.*
＊15 Activities for maternal and child health at health centers Fukuda, H. *et al.*
＊16 Test study with child development screenings for promotion of primary health care in developing countries Saeki, K. *et al.*
＊17 Inoculations for foreigners living in Japan, seen through phone calls received by AMDA International Medical Information Center Katori, M. and Kobayashi, Y.
＊18 Attitudes towards contraception in Peruvian sex workers Herrera *et al.*
＊19 Spousal communication about family planning and contraceptive use in Bulacan, the Philippines Ishikawa, Y. *et al.*
＊20 Analysis of maternal death in tertiary care hospitals Pakistan Naka, S. *et al.*
＊21 Medicalization of female genital mutilation in Egypt Wakasugi, N. and Ogiwara, R.
＊22 Health and welfare for foreign women in Fukuoka Ohara-Hirano, Y. *et al.*
＊23 The role of X-ray CTs and its cost effectiveness in Nepal Date, T.
＊24 Orthopedic treatment in Bangladesh Hashizume, C. *et al.*
＊25 JOCV nurses' viewpoints in their cooperation to a Nepalese hospital Ito, T. *et al.*
＊26 Technology transfer on oral cancer surgery at JICA Dental Education Project for University of Peradeniya: achievement and problem Sato, J. *et al.*
＊27 Medical support after the big tsunami disaster in Papua New Guinea Kaneo, K. and Shibata, R.

＊28 A view of international medical collaboration in future Tokunaga, S. *et al.*
＊29 The experiences of Cho Ray Hospital Project in Vietnam –how to plan the project in curative medicine field– Akiyama, M. *et al.*
＊30 Simple quality assurance for medical ultrasound image in developing countries and its importance Date, J.
＊31 A study of international cooperation in the clinical laboratory Sato, S. *et al.*
＊32 The role of clinical examinations in antenatal care of developing countries –evaluation of mass-screening of preeclampsia by examining of proteinuria in Cambodia Kudo, Y. *et al.*
＊33 Dental situation and JICA approach in Sri Lanka Nakakuki, K.
＊34 Dental health cooperation in developing country –our experiences for 10 years in Nepal– Nakamura, S. *et al.*
＊35 Training for oral health specialist in Nepal –perspective– Kamachi, S. *et al.*
＊36 GIS for health project management Horikoshi, Y.
＊37 A study on the impact of a condom price reduction program Onuki, D. *et al.*
＊38 Essential Drug Revolving Fund (EDRF) programme within the context of pharmaceutical development of Vietnam Hamada, A. and Umenai, T.
＊39 Assessment of ODA's International Health Projects Searching for collaboration with NGO Suzuki, C. and Mizota, T.
＊40 A study of partnership with local NGOs in International Cooperation Project in Japan Ishii, K. *et al.*
＊41 Visits to Henry Dunant-related Monuments with special reference to the International Red Cross movement education Kahyo, H.
42 The situation and problems of technical support system for NGO nurses Yanagisawa, S. *et al.*
＊43 The trial on midwife training in the maternal and child health project in Cambodia Naito, S. *et al.*
＊44 International health education on medical student: student interest and request Nagamatsu, Y. *et al.*
＊45 The improvement of NGO nurses' knowledge and skills after working experience in developing countries Mori, Y. *et al.*
＊46 A questionnaire survey for university nursing students about International Health Takeuchi, Y. *et al.*
＊47 Study on the practice and the problem of health education in the JOCV, members dispatched area (2) Okuno, H. *et al.*
＊48 Participatory evaluation of leadership development training for community-based health and development workers Yamazaki, M. *et al.*
＊49 Report on study tour in Cambodia Ito, J. *et al.*
＊50 Oral health promotion with Cambodian dentist Suzuki, M. *et al.*
＊51 The process of participatory planning of training of trainers for promoting "humanized childbirth" in Northeast Brazil Mohri, T. *et al.*
＊52 Participatory occupational health promotion through a trade union network in Pakistan Kawakami, T. *et al.*
＊53 Psychiatric disorders amongst Burmese asylum seekers in Japan Ichikawa, M. *et al.*
＊54 Action research and life support for "Starving Koreans" beyond the border Irohira, T. *et al.*
＊55 Report of the difficulty for receiving the request from JDR Yajima, K.
＊56 The role of medical coordinator in Japan disaster relief medical team Kondo, H. *et al.*
＊57 Report from Emerging Infectious Diseases US-Japan Meeting in the Pacific Rim 1999

Mizota, T. and Rakue, Y.

＊58 A case of *Plasmodium falciparum* malaria Kodaira, K. *et al.*

59 A rapidly fatal case of severe falciparum malaria complicated with high-level metabolic acidosis Saito, Y. *et al.*

60 A rescued case of complicated falciparum malaria using continuous hemodiafiltration (CHDF) Kimura, K. *et al.*

61 Current situation of malaria chemoprophylaxis among Japanese residents in Tropical Africa Hamada, A. *et al.*

62 A field study on malaria prevalence in southeastern Laos by polymerase chain reaction assay Toma, H. *et al.*

63 Appropriate technical transfer for control program of parasitic diseases in developing countries Ohmae, H. *et al.*

64 The malaria mathematical model for *Plasmodium vivax* and its computer simulation Ishikawa, H. *et al.*

65 *Plasmodium coatneyi*: cytoadherence of infected monkey erythrocytes to human umbilical vein endothelial cells and C32 amelanotic melanoma cells *in vitro* Kawai, S. *et al.*

66 Examination of phospholipase A2 in sera from malaria patients by ELISA Sato, K. *et al.*

67 Polymorphism of TNF-α promoter region associated with susceptibility to cerebral malaria in Thailand Hirayama, K. *et al.*

68 The role of CR1 (complement receptor 1) on erythrocytes in the development of severe malaria Nagayasu, E. *et al.*

69 Measurement of anti-malarial antibodies for inhabitants in malaria endemic areas using recombinant proteins of *Plasmodium falciparum* as ELISA antigens Matsuoka, H. *et al.*

70 Cultivation of *Plasmodium falciparum* isolates under the AnaeroPack™ gas condition in a portable thermostatic incubator Mizuno, Y. *et al.*

71 Effect of tetracycline and minocycline against *Plasmodium falciparum in vitro* Lin, Q.H. *et al.*

72 Development of new antimalarial drugs -the antimalarial activity of endoperoxide analogs- Ishizuka, Y. *et al.*

73 Development of new antimalarial drugs -*in vivo* antimalarial activity of endoperoxides- Nagai, Y. *et al.*

74 Development of new antimalarial drugs -the semi-synthetic antimalarial alkaloids from *Dichroa febrifuga*- Kim, H-S. *et al.*

75 Studies of antisense oligonucleotide -effects against *Plasmodium falciparum*- Ikemoto, N. *et al.*

76 RNA expression pattern of catalytic subunit of mitochondrial complex II from *Plasmodium falciparum* Takeo, S. and Kita, K.

77 Primary structure of the rhoptry protein, PyRhop100, of *Plasmodium yoelii* merozoite Shirano, M. *et al.*

78 Detection of the sequence polymorphism in *Plasmodium vivax* transmission-bloking vaccine candidate antigens by using PCR-SSCP Tsuboi, T. *et al.*

79 Inhibitory effect of dinitroaniline herbicides on the growth of *Entamoeba histolytica* Makioka, A. *et al.*

80 A stage-specific immunodominant glycoprotein appeared in encysting *Entamoeba invadens* Makioka, A. *et al.*

81 Quantitative change of thiol compounds, with the growth of *Entamoeba histolytica*, in the supernatant of exogeneous cystein free axenic culture medium Nangi, N. *et al.*

82 Congenital Chagas' disease and its environmental factors in Santa Cruz, Bolivia

Miura, S. *et al.*

83 Trial to detect of *Trypanosoma cruzi* DNA from dried feces of triatomine bugs
Hamano, S. *et al.*

84 Variation of proteins among different isolates of *Trypanosoma cruzi*
Mu, J.-B. *et al.*

85 Immunogenetic analysis of Chagas' disease Aida, K. *et al.*

86 Antiprotozoan activity of marine sponge extracts Mizushima, T. *et al.*

87 Nucleotide sequence variations of the cytochrome C oxidase subunit I gene within *Phlebotomus* species, vector insects for leishmaniasis Sanjoba, C. *et al.*

88 PCR-based differentiation of the species complexes in *Leishmania* spp.
Kawazu, S. *et al.*

89 Ultrastructural and pathological study of the lesion of diffuse cutaneous leishmaniasis (DCL) patient Maruno, M. *et al.*

90 A case of cerebral schistosomiasis mansoni probably infected at Maceió, Alagoas, Brazil Ueda, Y. *et al.*

91 Appearances of the echogenic pattern of the network among school children in the highly endemic area with *Schistosoma japonicum* in the Philippines Otake, H. *et al.*

92 Urine cytology in endemic area of schistosomiasis haematobium Ohki, T. *et al.*

93 Vaccination of domestic pig with recombinant paramyosin against *Schistosoma japonicum* in China Hirayama, K. *et al.*

94 Analysis of the eggshell precursor protein of *Schistosoma japonicum* using synthetic peptides and antisera against them Sugiyama, H. and Kawanaka, M.

95 Molecular phylogenetic relationships between the *S. indicum* group and other species of *Schistosoma* Agatsuma, T. *et al.*

96 A case of elephantiasis caused by primary lymphedema, differential diagnosed from filariasis Noguchi, M. *et al.*

97 Effect of PF1022A on Mongolian gerbiles infected with *Brugia pahangi*
Shimizu-Suganuma, M. *et al.*

＊98 A study on people's knowledge and behavior toward lymphatic filariasis in southern Thailand Shiga, F. *et al.*

99 Prevalence of intestinal parasitic infections among the inhabitants in Santo Domingo, Dominican Republic Nakashima, K. *et al.*

100 Monitoring morbidity control of soil transmitted nematodes in Zanzibar-Tanzania
Chwaya, H.M. *et al.*

101 Review of current 139 leprosy patients at Ryukyu University Hospital and a case report of borderline lepromatous leprosy who showed atypical clinical findings
Hosokawa, A. *et al.*

102 WHO leprosy elimination program in federated states in Micronesia
Takashima, J. and Kobori, O.

103 Social implication of leprosy control in Myanmar with an emphasis on the goal of elimination Barua, S. *et al.*

104 Clinical features of 44 cases of dengue virus infection among overseas travelers in Japan Itoda, I. *et al.*

105 The current status of *Aedes albopictus* distribution in northeast Honshu, Japan
Kurihara, T. *et al.*

106 Analysis on chemokine and VEGF in sera from patients with Dengue fever (DF)/ Dengue hemorrhagic fever (DHF) in Phillipines Nagao, T. *et al.*

107 Laboratory diagnosis of dengue virus infection -evaluation of diagnostic methods based on the day of defervescence- Yamada, K. *et al.*

108 Efficacy dengue control using plyset net during an epidemic in Vientiane, Lao PDR
Phommasack, B. *et al.*

109 Comparative sequence analysis of type 2 dengue virus strains from patients with different clinical severities and infection to primary culture human peripheral blood mononuclear cells Igarashi, A. *et al.*

110 Molecular analysis of different infectivity of dengue 1 virus strains in various cell lines Ishak, H. *et al.*

111 The present condition of medical care and management for patients with HIV infection in Iki Island Utsunomiya, Y. *et al.*

112 Clinical study on respiratory infection in HIV-positive patients in northern Thailand Watanabe, H. *et al.*

＊113 Effect of treatment with labre for HIV/AIDS-infected patients in Chiang Mai, Thailand Suprasert, S. *et al.*

＊114 Level of knowledge on AIDS and present situation of sex of pupils (Lycéens 6^{e}) in Côte-d'Ivoire Nakatsuka, J. *et al.*

＊115 A study on relationship between HIV infected people and migration in Thailand Kobori, E. *et al.*

＊116 Evaluation of community based AIDS program, in Thai rural community Sawada, T. *et al.*

＊117 Socioeconomic environment for HIV infected women and children and role of community-based care Takai, A. and Wakasugi, N.

＊118 The role of AIDS volunteers to develop the community-based care in Thailand Mashimo, A. *et al.*

119 Expression of chemokine receptors on human CD4+ lymphocytes of peripheral blood from HIV-infected individuals in Uganda Oishi, K. *et al.*

120 Epidemiological proposal from U.S.A. for TB prophylaxis to HIV-infected patients in Thailand Rakue, Y. *et al.*

121 International assistance to development of community-based TB program by NGO, experience of BRAC in rural Bangladesh Islam, A. *et al.*

122 Characteristic findings of the liver diseases in northern Thailand Toriyama, K. *et al.*

123 An imported case of brucellosis Kato, Y. *et al.*

＊124 Strengthening acute flaccid paralysis surveillance for polio eradication in Lao P.D.R. Murakami, H. *et al.*

125 Haulage methods and diunal habits of the porters in Kathmandu Kaneda, E. and Kosaka, M.

126 International collaboration during the epidemic encephalitis outbreak in Malaysia, 1999 Morita, K. and Igarashi, A.

＊127 Protective effects of colostrum administration on *E. coli* O157 infection Funatogawa, K. *et al.*

128 Immunological investigations on diarrheal diseases control of children in developing countries Nakano, T. *et al.*

129 Process of setting up *Haemophilus influenzae* type B (HIB) Vaccination Project for Bangladeshi children to prevent meningitis Masaki, H. *et al.*

130 Food plant-based vaccines against infectious diseases Arakawa, T. and Sato, Y.

131 Depression of immunological ability by ultraviolet rays and protection from ultraviolet rays Ohwatari, N. *et al.*

＊ Presentation from Japanese Association for International Health

Prize Winner's lecture

JSTM (Japanese Society of Tropical Medicine) Young Investigator Award

CRYPTOSPORIDIOSIS IN INDONESIA: A HOSPITAL-BASED STUDY AND COMMUNITY-BASED SURVEY

TATSUYA KATSUMATA
Department of Parasitology, Institute of Tropical Medicine,
Nagasaki University

Hospital-based and community-based studies were conducted to understand the prevalence and mode of transmission of *Cryptosporidium parvum* infection in Surabaya, Indonesia. In both studies people with and without diarrhea were examined for oocysts. A community-based survey included questionnaires to a community and stool examination of cats. Questionnaires covered demographic information, health status, and hygienic indicators. In the hospital, *C. parvum* oocysts were found in 26 (2.8%) of 917 patients with diarrhea and 15 (1.4%) of 1,043 control patients.

The most susceptible age was less than two years old. The prevalence was higher during the rainy season. A community-based study again showed that *C. parvum* oocysts were frequently detected in diarrhea samples (8.2%), exclusively during rainy season. Thirteen (2.4%) of 532 cats passed *C. parvum* oocysts. A multiple logistic regression model indicated that contact with cats, rain, flood, and crowded living conditions are significant risk factors for *Cryptosporidium* infection.

President's lecture

ETHICS OF TROPICAL MEDICINE: PEOPLE-CENTERED HEALTH PROMOTION

Reflections from 20 years experiences of medical cooperation in Bangladesh

NOBUKATSU ISHIKAWA
The Research Institute of Tuberculosis
Japan Anti-Tuberculosis Association

Based on my own experience and the ethical reflections for the last 20 years for tuberculosis control in Bangladesh, the address was specially made to young people who are interested in studying for the health promotion in the tropics. The following 4 key terms were focused in the talk: 1) Go to the people, 2) Health by the people, 3) Need of Health Systems and Services Research, and 4) Need of Base in Japan and Institutional Capacity.

Ethics of Tropical Medicine

Ethics is an attitude to think of the one's own deeds by standing on the other's standpoint. It can be also an attitude of the dialogue or interaction between the subject and the object of any action. Here lies a basis for hope, peace or social justice. I discuss what tropical medicine is through ethical reflections based on my own experiences in Bangladesh in the area of tuberculosis control.

〈Where is the place for tropical medicine?〉

The experiment in the laboratory or analysis at the study room is one, and the field experiment or field study is the other. Some people spent most of the time in the laboratory under the name of tropical medicine, and others are too busy working in the fields under the same name or international health. But I would say that both are equally needed for tropical medicine.

〈Who is the subject of tropical medicine?〉

The subject or actor of tropical medicine is usually a researcher outside the tropics or community. In this case, local people in the tropics are just an object of the study. However a local researcher, workers and even people should be the subject of any research as well. The external researcher and local people need to cooperate and collaborate equally. There are various kinds of local people, including local researchers, supervisors, community level workers, and community people. We need to clarify for whom and with whom we are working, and to whom we should feed back the outcomes of the research. And we need to be aware that the local rich or local researcher cannot often represent the local people.

〈Who is the owner of the research outcome of tropical medicine?〉

Conventionally tropical medicine has been positivist science. This characteristic is basically important but we need to know its limitation as well. It has a danger that the importance of the knowledge often lies only in the authorship. A new horizon for tropical medicine to overcome this is to strengthen the aspects of applied science, social science or qualitative analysis, and to change of our awareness that the knowledge belongs to the people or the people have the ownership of the knowledge.

In summary, the aims of tropical medicine are; to work or conduct a study for better health collaboratively with the people in the tropics; to share equally the study results; to support the people in the tropics to work and study by themselves for their own health promotion; and to share internationally the health problems in the tropics through our work in the laboratory or publications. For this, we need to strengthen a base i.e., the institutional capacity in Japan.

Keynote lecture

TROPICAL MEDICINE AND INTERNATIONAL HEALTH FOR THE 21ST CENTURY

DAVID J. BRADLEY
London School of Hygiene and Tropical Medicine

We need to look back if we are to plan for the future. The last century has seen many dramatic discoveries but also many changes in the way tropical health is conceptualized. In coping with great epidemics around 1900, first microbiology and then entomology led the field. Some time after, they were followed by epidemiology and then, much later, by molecular biology and the social sciences. All are needed for a coherent and sustainable approach to tropical disease problems. By analysis of success stories in disease control, including oral rehydration, insecticide-treated mosquito nets, smallpox eradication and the management of severe malaria; of mixed experiences as with health care delivery and attempted malaria eradication; and of the new and unexpected problems that have emerged in recent decades, notably HIV/AIDS, multiple drug resistant malaria and bacterial infections, we can gain a picture of the challenges and opportunities ahead.

There are persistent and intractable problems of war, marginalization, forced migration, and above all, poverty. Also there are opportunities, both at the molecular level with the coming of complete genome sequences and also at the global level with improved ability to predict climatic changes in both the short and longer run, together with ability to handle the spatial aspects of epidemiology and to intervene successfully in life-threatening illness. The simpler problems are being solved: we now need to make a better job of the complex ones that require multidisciplinary action.

SYMPOSIUM I

PROGRESSES TO CONTROL INFECTIOUS DISEASES IN THE TROPICS

1 MALARIA –HOW WE CAN MOVE FURTHER AHEAD TOWARDS FREEDOM FROM THE DISEASE

SHIGEYUKI KANO
Department of Appropriate Technology Development and Transfer,
Research Institute, International Medical Center of Japan

I presented the general idea of the current situation of malaria world wide in the last (the 39th) Annual Meeting of Japanese Society of Tropical Medicine held in Okinawa. Following the symposium, I proposed some specific ideas how we can move further ahead towards the control of malaria in the next century. This report summarizes the presentation of the ideas spoken in the 40th Annual Meeting of the Society held in Tokyo.

We sometimes fail to see malaria as the disease which is basically built up with the fabric of lives of parasites, mosquitoes and humans. This ecosystem is, as a matter of fact, very fragile, but people are liable to dwell on or even strengthen the system of the disease. In fact, malaria is not evenly distributed in the area where it is prevalent, but it is highly focal. Therefore we have to investigate epidemiologically first how it is really focal in the certain areas.

Now, the importance of the basic sciences for getting the epidemiological data should be highlighted. Climate changes including the data on precipitation and temperature are really important because it affects the breeding places and biting habits of the mosquitoes. Recently, remote sensing using the artificial satellite is becoming useful for the detection of the geometrical information on the distribution of malarial factors. Parasitological information is also very important; which species are prevalent, how they are susceptible or resistant against antimalarials. Thus, researches for the appropriate drug usage or the vaccine production has to be further strengthened. Our human factors inclusive of socio-economical ones are non-specifically affecting the prevalence of malaria, such as poverty leading to population movement, hunger to malnutrition, hygiene and environmental development to promote vectorial capacity. Worsening of the malaria situation is often achieved directly or indirectly by human behaviors.

Then, what can we do as a part of the ecological system to break the balance of supporting the malaria prevalence? The more specific the way of prevalence in the certain area is, the more important the involvement of the same community becomes. Thus, integration of the malaria control activities into existing primary health care system is mostly needed. Looking back into our history of malaria control in Japan, we can recognize that the measures for the control were mostly conducted under public health activities, such as health education and hygiene control. I believe that more effective control strategies will be feasible if Japanese experiences and techniques are employed together.

2 ROLES OF EPIDEMIOLOGY IN THE CONTROL OF TUBERCULOSIS IN TROPICAL COUNTRIES

TORU MORI
The Research Institute of Tuberculosis,
Japan Anti-Tuberculosis Association

The global estimate of the size of the tuberculosis problem, "tuberculosis burden" as it is called nowadays, is a typical challenge facing epidemiology, especially where there is no well established routine statistical system. Ad hoc prevalence surveys may be the best remedy, and they could be replaced by more practical tuberculin surveys. The latter survey assumes that there is an empirical relationship between the annual risk of infection that is derived from the tuberculin survey data and incidence of smear-positive pulmonary

tuberculosis. Based on this principle with refinements to make the entimates consistent, it is estimated that in the year 2000 more than eight million tuberculosis cases will occur globally, 10% of which is the excess due to HIV epidemics.

In sub-Saharan Africa, the ratio of the HIV-seropositivity of the general inhabitants and that of TB patients is almost 1:5, indicating that TB is drifting to the HIV-infected segment of the population. Also, seroepidemiology shows that where the TB patients' HIV-positivity is 50%, the incidence of TB is doubled. In this way, the HIV epidemic is adding a serious excess to the TB burden.

Almost 100 years after the Koch's discovery of TB bacillus, humankind has developed Directly Observed Treatment, Short-Course (DOTS), a truly curative technology and strategy for fighting tuberculosis. DOTS' efficacy, applicability and cost-effectiveness for the developing countries' conditions have been confirmed with epidemiological as well as economic methods. WHO, in collaboration with the World Bank, adopted the new concept of disability-adjusted life-years (DALY) in order to further advocate the benefit of TB control using DOTS for saving TB-derived BALY per unit of invested dollars.

In addition to the enhanced treatment management that is the nucleus of DOTS, there are other managerial components that are recognized as equally essential. These are drug management and logistics support, advocacy, and political commitment.

This strategy has also been adopted widely in the JICA-collaborating TB programs in Yemen, Nepal and the Philippines. The treatment success rate of these countries used to be less than 50%, but that has been improved to more than 80%, almost meeting the WHO target of 85%. Also, it is being shown that the strategy can be quite robust against unfavorable conditions such as HIV epidemics.

Epidemiology can provide a perstective of the future tuberculosis picture based on model simulation. Recently, WHO announced that if the WHO target of 70% DOTS coverage of patients could be achieved, then the global toll of TB would undergo a historical breakthrough, but the toll would continuously grow if the coverage remained at the current level of 40%.

The next challenge to the global tuberculosis strategy is drug-resistant tuberculosis that is indeed a problem left by the past failure of the control program. This problem is difficult to solve even with DOTS, and an enhanced treatment system with refined examination is needed. This new system is called DOTS+ and may present formidable problems to the developing world such as sustainability, high technical level, and accompanying issues of politics and international dynamics. Such a multi-disciplinary approach will become more important in future TB control programs, where epidemiology is expected to play a new role of coordinating between these disciplines.

3 DENGUE FEVER/DENGUE HAEMORRHAGIC FEVER

KOUICHI MORITA
Department of Virology, Institute of Tropical Medicine,
Nagasaki University

Dengue fever/dengue haemorrhagic fever is the most important mosquito-borne virus disease in tropics in terms of the number of patients and deaths. The World Health Organization (WHO) estimates that half of the world population is living in dengue risk area and approximately twenty million people are infected annually.

In some countries, dengue incidence once declined due to well-planned and implemented national dengue control programmes. In Singapore for example, seropositive rate among the population between the age 15-19 was about 70% in the early 1980s, but declined to 20% in 1993. However, dengue cases dramatically increased from early 1990s in most of the tropical countries including Singapore. Dengue incidence rate in the country was below 20 per 100,000 population in late 1980s but increased to 80 to 120 per 100,000 population after 1990. Many factors are supposed to contribute to the resurgence and increase of world-wide dengue cases: rapid and enormous international travel and trade, urbanization in tropics without sufficient development of infrastructures, increased and more complicated breeding sites of *Aedes aegypti* relating to the economic development (construction sites etc.), global warming. And it is evident that these factors surely continue to exist in the next millenium. As for the development of

dengue vaccines, it will not be widely available for at least next ten years. Also, we have not yet completely understood the mechanism of development of dengue haemorrhagic fever. Incomplete immunization may just increase the incidence of dengue haemorrhagic fever due to antibody mediated enhanced infection phenomenon.

Under the above circumstances, is it possible to control dengue fever and dengue haemorrhagic fever? Author believes that answer is yes. Dengue infection is a self-limiting disease. Dengue virus never causes persistent infection in humans, only one major reservoir of the virus, and it can be survived by human-mosquito infection cycle. In nature, trasovarial transmission of the virus in mosquito is extremely inefficient. In theory, dengue control through vector control would be possible.

Control strategy, however, should be restructured for multi-sectoral approach at national, regional and global levels to cope with the changing situation of tropical countries. New scheme and methods for vector control need to be properly evaluated for the practical use in various social and geographical settings in the tropics.

4 PROGRESS OF POLIO ERADICATION IN CHINA

YASUO CHIBA
International Medical Center of Japan and
China Polio Control Project of JICA

Children with paralytic poliomyelitis suffer from a lifelong physical and social handicap owing to its severe neurological sequelae. Supported by worldwide development of the expanded program of immunization (EPI), the World Health Organization (WHO) launched a global initiative to eradicate poliomyelitis in 1988. Among the six WHO regions, the Pan American Health Organization (PAHO) was certified first as having interrupted poliovirus transmission (1994). Poliomyelitis eradication in a global initiative of infectious disease control, which follows smallpox eradication, and has tremendous benefit for human health.

With the development of EPI, epidemic poliomyelitis reduced greatly by the mid-1980's in China. The outbreaks, however, recurred from 1989: more than 10,000 cases were reported nationwide by the end of 1991. In 1993, coordinated National Immunization Days (NIDs) consisted of two rounds of immunization sessions began targeting about 80 million children. Acute flaccid paralysis (AFP) surveillance, case investigation and stool specimens collection, was also initiated in the early 1990's, achieving excellent performance nationwide recently. The national laboratory in Beijing is responsible for virological diagnosis. Poliomyelitis outbreaks have rapidly subsided after the introduction of NIDs; the last 6 indigenous cases were detected in 1994. Four cases associated with wild poliovirus infections detected in Yunnan province through 1995 to 1996 were those imported from Myanmar. Five years have passed since the last endemic poliomyelitis cases were detected in China. WHO Western Pacific Region (WPR) plans to apply to the regional commission for poliomyelitis eradication certification in the early 2000's.

SYMPOSIUM III

WHAT CAN BE EXPECTED IN TROPICAL MEDICINE AND INTERNATIONAL HEALTH?

KOICHIRO FUJITA[1] AND NARUO UEHARA[2]
Faculty of Medicine, Tokyo Medical and Dental University[1] and
Tohoku University School of Medicine[2]

Prof. Fujita presented, with impressive pictures, his experiences and difficulties encountered in the field work on Filariasis and its vector in Mozambique, referring to interesting scientific findings on immunology of parasite infection as well as the inter-relationship, and its mechanism, of filariasis and malaria infection. Prof. Aoki presented his experience and findings in the project in Kenya on Scistosomiasis control, emphasizing the needs, and fun, of interdisciplinary approach with anthropology, human ecology, public health, sanitary engineering and others. The bench was in the bush in the years around the beginning of twentieth century. Although the research methodologies have changed, we are still learning a lot from fields, including the needs of harmonization of science and technology with human societies. Dr. Marui considers that the principal values of International Health as one of scientific disciplines are; to assess the valid health problems today, to understand cultures, to detect the true causes in biomedical, sociocultural and political economy context and to indicate the road to solutions. "International Cooperation" could be an entry gate to International Health, although we should not see the world only through it, which is rather narrow in view. Dr. Kunii presented his variety of experiences, including those in heize disaster in Indonesia. He reviewed with slides the processes of his work including preparation before dispatch, planning of site survey, considerations taken on political and economic factors, field survey, reporting and so on. He expressed his concern about promotion of scientific approach in International Health.

After presentations of the four speakers, relevant questions and encouraging comments were raised by panelists and audience. In response to the question of Dr. Ishikwa, Prof. Fujita expressed his doubt on the positive effects of parasites' control, while Prof. Aoki opposed to it. In response to the question of Dr. Furuta, Dr. Kunii reemphasized the importance of understanding of cultures and international politics although another view or strategy is required in ODA project management. Several participants gave comments on how they find International Health, saying; "patience is key for success since our work very much depends on the efforts of local people", "younger physician is more suitable for conductor of projects/programs", "it was fun to work together with local staff while just a physician was powerless in front of dying patients in Ethiopia refugee camp", "we are missing data-base to share information/data and knowledge which have been compiled through individual activities in international cooperation", "we are sometimes unaware of the system and situation of our own country", "the association should provide with something like PUB where the young and the old chat together", "research on human behavior is critical as well as fun for international health study", "the association should keep the door always open to the young generation", "we learn many things from the life of people in developing countries", "sometimes researches are abused in development projects", and so on.

1 A THINKING IN THE FIELD WORK OF FILARIASIS IN THE MOZAMBIQUE IN EAST AFRICA

KOICHIRO FUJITA
Faculty of Medicine, Tokyo Medical and Dental University

We studied the field work of filariasis in the Mozambique for 5 years during 1981 and 1986. In the study of the field work, we could learn various things, for example, the filariasis in the Mozambique was mainly transmitted by the mosquito of *Culex pipiens quinquefasciasis*, and the worms was *Wuchereria ban-*

crofti.

But many interesting things appeared during this field work. In this meeting, I would like to report some of them.

1. We tried to eliminate the tropical diseases completely, but is it truly beneficial to the people in tropics?
2. Is it necessary for us to do medical treatments to the people suffered from tropical diseases transiently?
3. Does any threads of global warming to biological diversity bring to the people of the Mozambique?
4. Is the high level of the culture human discovered good for the human himself?

By the discussions above mentioned articles, I would like to tell the younger members of this meeting that the tropical medicine is very fascinating.

2 MULTIDISCIPLINARY RESEARCH AGENDA ORIGINATING FROM THE TROPICS

Yoshiki Aoki
Department of Parasitology, Institute of Tropical Medicine,
Nagasaki University

Tropical diseases are principally infectious diseases of areas where there exists poverty, ignorance, poor housing, substandard hygienic practices and few sanitary facilities. Therefore there is a great need of multidisciplinary research in tropical medicine. Since 1981 we have been carrying out control program of schistosomiasis haematobia in Kenya. Recently we have done RAP (Rapid Assessment Procedure) and KAP (Knowledge, Attitudes and Practices) study in our study area. RAP study describes and understands a culture from the perspective of member of that culture by anthropological or ethnographic method. KAP study collects information about cultural beliefs and local custom by asking a predetermined set of question. Unexpected cultural beliefs and local custom were collected by RAP and some problems were posed by KAP study. The villagers consider haematuria as on of the signs of physical growth. They believe that they will face misfortune if they take ion.

Therefore they do not use tap water. They report schistosomiasis causes the death and destruction of birth canal. When they eat much pepper, they get infection. The biggest social problem to be solved is the crop damaged by elephants. RAP and KAP information facilitates understanding of epidemiology of disease and will modify the medical science and technology so as to be accepted by the community. And it encourages us to conduct the new field of research, such as hidden morbidity of schistosomiasis. The driving force of research at the pioneer period of tropical medicine about 100 years ago was "The bench was in the bush" (Warren, 1981). Multidisciplinary research in the developing countries where diseases exist will stimulate the intensive and extensive studies on tropical medicine.

4 PRACTICE AND RESEARCH IN INTERNATIONAL HEALTH —DISASTER RELIEF AND EPIDEMIOLOGY—

Osamu Kunii
Bereau of International Cooperation, International Medical Center of Japan

My presentation demonstrated both fascinating and challenging aspects of international health, showing a case of emergency assistance of the 1997 haze smoke in Indonesia, to motivate the rising generation.

The disaster resulted from large forest fires in Indonesia, caused by land clearing practices under the dry conditions influenced by the El Niño. As is often the case with emergencies, the request from the Japanese government came suddenly and unexpectedly and time given was only 3 days till the departure of the mission. Within the limited time, it was needed to gather key information, make survey designs setting both best and worst scenarios, collect materials and equipment, and prepare logistics for it. I made most of Internet and the

electric human network of my own. The political mapping also help us to identify the task and role, do and not do, of our mission.

We measured CO, CO_2, SO_2, NO_2, O_3, particulate matters less than 10 microns in diameter (PM10), inorganic ions, and the polycyclic aromatic hydrocarbons (PAHs), known as carcinogens, in both affected and unaffected areas. We also conducted a structured survey to 543 people in 6 sites and a respiratory function test with spirometry for 138 who had respiratory symptom. As a result, CO and PM10 reached 'very unhealthful' and 'hazardous' levels respectively, and the concentrations of the 5–7 ring PAHs were 6 to 14 times higher than that of the unaffected area. In questionnaire, 98.7% developed health problems and 91.1% of those showed respiratory symptoms. In multivariate analysis, gender, history of asthma, and frequency of using mask in staying outdoors were associated with the severity of respiratory problems. Finally, we presented such results and the recommendations for countermeasures to the Indonesian government, and submitted the proposal for further assistance to Japanese government.

The terms of reference given to such government missions are limited, and are seldom a hard task to carry out. However, as public health expert, I often realize the need of actions to raise public's awareness for preparedness, mitigation and prevention of human-made disasters to vitiate humankind's health. Besides, as an epidemiologist, I frequently feel the necessity to facilitate further researches to identify factors associated with mortality and morbidity and the way to minimize them.

International health is a new sphere in Japan, and needs to be developed not only by *Kan* (intuition), *Keiken* (experience) and *Dokyo* (courage) but logical approaches and a pile of evidence.

SIMPOSIUM Ⅳ

"CROSS-FIRE: TROPICAL MEDICINE VS. INTERNATIONAL HEALTH"

Discussants
Tadao Shimao[1], Isao Tada[2], Takeshi Kurata[3], Yasuhide Nakamura[4],
Hiroshi Takahashi[3], Taro Yamamoto[5], Yoshihiro Takayama[6],
Mari Mugitani[7] and Takebumi Furuhata[8]

Chairpersons
Nobukatsu Ishikawa[9] and Naoki Furuta[10]

Japan Association for International Health[1],
Japanese Society of Tropical Medicine[2],
National Institute of Infectious Diseases[3], University of The Tokyo[4],
Institute of Tropical Medicine, Nagasaki University[5],
International Health Forum[6], Ministry of Health and Walfere[7],
Japan International Cooperation Agency[8],
Research Institute of Tuberculosis[9] and Waseda University[10]

The symposium was designed to stimulate the both disciplines and to seek for future direction how the both can cooperate or be united. Firstly several members of both societies discussed their views on the topic, and then comments were given from other groups including students, JICA or Ministry of Health and Welfare, and other attendants on the floor as well. Dr. Shimao representing Japan Association for International Health (JAIH) defined the international health as a discipline to diminish the disparity in health and health care among the areas in the world, through identifying the indices, contributing factors and solutions. Dr. Tada representing Japanese Society of Tropical Medicine (JSTM) noted that the both are complementary with each strengths; tropical medicine is concerned more for the scientific evidence, while international health is more for field application of the scientific knowledge. Dr. Nakamura noted the needs for periodical joint meetings with wider range of groups such as foreign students, trainees researchers from developing countries, domestic health care researchers in Japan, NGO/NPOs, other disciplines including economics, anthropology, social development, or education. These can be steps for building an international school of public health in Japan. Dr. Bradley of London School of Tropical Medicine and Hygiene commented that in the both disciplines, subjects had been changing, and never been fixed. Currently participation by anthropologists or economists in tropical medicine studies is common sense but it was not only few years ago. Flexible way of thinking with broader background is very much needed. Tropical medicine and international health need to learn from each other and ultimately both are one.

Lastly, recommendations/joint statement were resoluted, stressing that both societies need to cooperate each other and exchange the programs through liaison committee members in both executive councils, periodical joint meetings, common training programs, or membership fee reduction.

WORK SHOP

WI-S1 WHAT CAN WE DO ABOUT EMERGING PARASITIC DISEASE CHALLENGES?

DANIEL G. COLLEY
Division of Parasitic Diseases,
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Since 1994 the Centers for Disease Control and Prevention (CDC) has been working with many partners to formulate how to address the threat of emerging infectious diseases and to build the capacity to carry out such plans. In 1998, CDC issued an updated version of its 1994 plan entitled Preventing Emerging Infectious Diseases: A Strategy for the 21st Century. So far in 1999, on the very eve of the 21st Century, occurrences such as an outbreak of fatal encephalitis in Malaysia associated with a previously unrecognized virus (Nipah virus); human disease associated with another avian influenza virus in Hong Kong (Influenza A [H9N2]); epidemic louse-born relapsing fever in Sudan; and the first recognized infectious by Marburg virus in Africa in 12 years, have made it clear that the ongoing challenge of emerging infectious diseases will remain with us and must be confronted. The CDC plan of action to do this is based on a set of four interdependent core systems: Surveillance and Response; Applied Research; Infrastructure and Training; and Prevention and Control. These can be more simply stated as: Watch; Learn; Prepare; and Act. We need to apply these principles to create a solid public health foundation to manage current infectious disease problems, emerging and re-emerging infectious disease problems, and mass casualty infectious disease threats, such as pandemics and bioterrorism.

Some of the major infectious disease threats currently facing the global public health community are parasitic diseases. Emerging parasitic diseases, such as cryptosporidiosis and cyclosporiasis present particularly difficult problems because of their biology or their unknown life cycles, and can pose domestic (in the USA and Japan) as well as global difficulties. The re-emergence of West African trypanosomiasis is a frightening example of what can occur when reasonable control programs are not adequately continued. The most widespread and highly publicized parasitic disease threat to global development and human health is the resurgence of malaria, especially drug-resistant malaria. Some of the foundations and plans of the new WHO-coordinated global effort "Roll Back Malaria" will be discussed. The public health community is also struggling with infectious disease challenges caused by several serious parasitic diseases that are either life-threatening, seriously diminish the quality of life through disfigurement or incapacitation, or interfere with cognitive or physical development. These include control, elimination, and eradication efforts to address Chagas' disease, lymphatic filariasis, dracunculiasis, taeniasis/cysticercosis, onchocerciasis, schistosomiasis, and soil-transmitted helminths. Summary discussions of some of these efforts will also be presented.

WI-S2 JAPAN'S GLOBAL PARASITIC DISEASE CONTROL INITIATIVES AND OTHER INTERNATIONAL INITIATIVES

KEN KASAI
Infectious Disease Control Division, Health Service Bureau,
Ministry of Health and Welfare, Japan

(Abstract not received in time)

WI-1 KNOWLEDGE, ATTITUDE AND PRACTICE TEST FOR THE EVALUATION OF HYGIENIC EDUCATION IN AN INTESTINAL PARASITIC DISEASES PREVENTION AND CONTROL PROJECT IN PREK RUSSEY COMMUNE, CAMBODIA

KAORI KITA[1,2], MACHIKO OKAWA[3] AND YOKO WATANABE[3]
Department of Public Health[1], School of Health Sciences[3],
Faculty of Medicine, Kagoshima University and
Festa TD (NGO)[2]

[Objectives] To evaluate our hygienic education activity performed within an Intestinal Parasitic Diseases Prevention and Control Project in Prek Russey Commune, Cambodia, we did Knowledge, Attitude and Practice test targeted for inhabitants of the field, and for the neighboring village inhabitants as control.
[Methods] The examination targeted for all house hold (about 150) in Prek Angchanh village (PA) and 33 selected household in the control village, and was performed by the commune nurses. The examiners distributed the questionnaire previously to each household, and interviewed to someone (usually mother) to fill out the blanks, when they collected them back. Differences were tested statistically by χ-square test.
[Results] One hundred forty-two household in the PA was answered. For the question "Why do you think you get intestinal parasitic diseases?", 98% of PA and 79% of control answered something. It was significant. For "What do you think you should care about, in order to prevent intestinal parasite infections?", 99% and 67% (significant) answered something respectively. For "If you find you or your family member have got intestinal parasite disease, what do you do?", 96% and 61% (significant) answered "go to hospital", respectively. For "When you defecate in the toilet or outside, do you wear sandals or shoes?", 77% and 73% (not significant) answered "always", respectively. For "Do you wash your hands before cooking or eating?", 87% and 88% (not significant) answered "always", respectively. Thus, the differences between PA and control village were significant about knowledge and attitudes, but not significant about their practices.

W I-2 DIFFERENTIAL SERODIAGNOSIS OF ALVEOLAR ECHINOCOCCOSIS FROM CYSTIC ECHINOCOCCOSIS IN CHINA FOR A GLOBAL PARASITE CONTROL

AKIRA ITO[1], M. NAKAO[1], Y. SAKO[1], K. NAKAYA[1], S. WATANABE[1],
QIU JIAMIN[2], LIU FENGJIE[3], WANG HU[4] AND P.M. SCHANTZ[5]
Department of Parasitology, Asahikawa Medical College[1],
Sichuan Institute of Parasitic Diseases, Sichuan, China[2],
National Hydatid Disease Center, Xinjiang, China[3],
Qinghai Medical College, Qinghai, China[4] and
Centers for Disease Control and Prevention, Atlanta, USA[5]

It has become evident that both alveolar echinococcosis (AE) and cystic echinococcosis (CE) are hyperendemic in some parts of China. Dr. Schantz at CDC, Atlanta, organized an international collaboration project on the survey of AE and CE in Tibetan populations in China. From June 1997 to August 1998, Tibetan populations on the Qinghai-Tibetan Plateau in Qinghai and Sichuan Provinces were surveyed for echinococcosis, either AE or CE or both by abdominal ultrasonogram, chest radiograph and serodiagnostic tests. This is a brief summary of this collaboration project. Pastoralists and the local Tibetan people tend herds of yaks and sheep with smaller populations of cattle, horses, and goats. Although schools serve the populations in villages and towns, more than 98% of the herdsmen and farmer populations are not formally educated. One of the high-risk groups is the woman handling feces of yak.

Abdominal ultrasound revealed suggestive space occupying lesions in the liver, kidneys, spleen or abdominal space of 423 of 7,702 local people examined (5.5%). Upon critical review based on WHO classification of CE, 415 (5.4%) were diagnosed as echinococcosis. They were further classified as CE (305, 4.0%) and AE (110, 1.4%).

For serological tests, we examined ELISA established at Chengdu and Em18-test at Asahikawa. ELISA revealed 385 of 435 cases as echinococcosis but could not differentiate AE from CE at all. Em18-test revealed that 92 of 110 AE (84%) were exclusively positive. However, 19 of 305 CE were also diagnosed as AE by Em18-test. As huge AE liquid cavity sometimes will be confused as CE and some of these CE were confirmed to have been exposed to mixed infections, Em18-immunoblots will be taken as routine test and the positives will be diagnosed as AE.

WI-3 GLOBAL ELIMINATION OF LYMPHATIC FILARIASIS -STRATEGY AND WEAPONS FOR THE OPERATION

EISAKU KIMURA
Department of Parasitology, Aichi Medical University

Lymphatic filariasis is endemic in 73 countries and affects 120 million people, 43 million of whom suffer from lymphedema (including elephantiasis) and hydrocele, and are considered the long-term or permanent disabled. The economic losses incurred by the disease is enormous, losing US$ 1.5 billion annually in India only. In May 1997, the 50th World Health Assembly made a resolution to eliminate lymphatic filariasis from the world.

Basic strategies for the elimination are; (1) to conduct annual single-dose mass treatments covering all people in endemic areas, and (2) to treat clinical cases. The drugs of choice are (a) diethylcarbamazine (DEC), (b) ivermectin (IVM) and (c) albendazole (ALB). DEC can be used alone but a combination of ALB and DEC or ALB and IVM is more effective. DEC-medicated salt may also be used. It is planned that (1) by year 2000, the programme will start in a half of 73 endemic countries, (2) by year 2005, it will start or complete in 3/4 of the endemic countries, (3) by year 2010, it will start or complete in all the endemic countries and that (4) by year 2020, the elimination will have been achieved.

Important factors for the success are; (I) Rapid Assessment Procedures (RAPs) to identify endemic areas, (II) supply of drugs, and (III) drug distribution system. As a RAP, obvious clinical signs can be recorded by means of clinical examination or questioning. Various immunological tests are also available. Recently, we have developed an ELISA which uses urine instead of serum. As for drugs, SmithKline Beecham and Merck decided to donate ALB and IVM, respectively. In areas where health manpower is not sufficient, local people could play a crucial role in the distribution of drugs, treatment and recording.

WI-4 SEROEPIDIMIOLOGICAL SURVEY OF SCHISTOSOMIASIS MEKONGI IN LAOS AND CAMBODIA

HAJIME MATSUDA[1], MASASHI KIRINOKI[1], JUN MATSUMOTO[1],
HIDEHIRO OTAKE[1, 2], KHAMLIANE PHOLSENA[3], DUONG SOCHEAT[4] AND
KAZUO YASURAOKA[1]
Department of Medical Zoology, Dokkyo University School of Medicine[1],
Faculty of Nursing, Kure University[2],
Institute of Malariology, Parasitology and Entomology, Laos[3] and
National Malaria Centre, Cambodia[4]

Schistosomiasis mekongi has been seen from the around Khong island in the southern part of Laos to the northeastern section of the Cambodia along the Mekong River. We conducted malacological survey along the Mekong River from Khong island up to the north of Pakse during the low water periods in April-May 1995 and 1996. As the result, large numbers of gamma *Neotricula aperta,* the intermediate snail host of *Schis-*

tosoma mekongi, were widely observed along the Mekong River extending over 120 km upstream of Khong. In the meantime, we carried out the filter paper blood sampling method for ELISA from children sum total of 810 persons of 10 elementary schools, which is close to the snail collection site. The positive rate showed 25.3% in the Khong island and 16.7% in the 120 km upstream. The mass treatment with praziquantel promoted by WHO/WPRO is carried out since 1989 and brought the infection rate to drop sharply around the Khong island. However, our findings suggest high possibility of the existence of the *S. mekongi* along the Mekong River from Khong up to the north of Pakse, however further studies are required.

On the other hand, there are many uncertain points in recent situation of the schistosomiasis mekongi in Cambodia. We have been investigating to Kratie and Stung Treng provinces since 1997. As a result of the investigation of the snail habitat, the infection in this area could occur in the rocky stream during the period of the lowest water level in dry season. The sero-epidemiological surveys showed that 92.3%–96.7% school children were positive at the basin of 50 km upstream of Kratie town proper while 20% and less IgG positive rates were seen in villages downstream of Kratie. Kampong Cham province was the southern limit of endemic transmission of *S. mekongi* infection in the Mekong River at 1.1%. Antibody positive rate at 8 elementary schools in the Stung Treng province was shown 0%–89.1% with wide differences by the district. Schistosomiasis mekongi exist along not only Mekong River's mainstream but also Sekong and Sesan River, which are Mekong River's tributaries in the province of Stung Treng. Further studies of malacological, epidemiological surveys and the health education and mass treatment will be desired in future.

WI–5 SCHISTOSOMIASIS AND AGRICULTURAL DEVELOPMENT (DOES AGRICULTURAL DEVELOPMENT SPREAD TROPICAL DISEASES?)

Masaaki Shimada[1] and Kiyoshi Shiratori[2]

Information and Reference Center,
Institute of Tropical Medicine, Nagasaki University[1] and
Kilimanjaro Agricultural Training Center, Moshi, Tanzania[2]

It has been pointed out that large-scale construction of irrigation canal and development of paddy field in tropical area increase the prevalence of schistosomiasis and also the increase of the infested area. However, the available information is insufficient so far.

[Purpose] The aim of the study is to determine 1) the difference in the prevalence of schistosomiasis in an area with irrigation canal and in those with less irrigated areas, and 2) what factors influence the difference.

[Study area and Methods] The study was carried out at Lower Moshi in Tanzania, an endemic area of *Schistosoma mansoni* infection, where a large-scale development of paddy field had been implemented. For parasitological study, the Kato-Katz stool examination was used for school children and villagers and a snail survey was carried out for 1 year at the river and paddy field. A preliminary study on the water contact behavior of villagers was carried out in a dry season.

[Results] 1) More vector snails were collected from natural water sources, river and less at artificial water sources, canal, paddy fields and drainages. 2) At natural water sources, a seasonal fluctuation in snail density was observed with a peak from December–February, at dry season. 3) The prevalence of having eggs of *S. mansoni* was 77% at age 10–14 and 42% at age 50–59. A very low prevalence of 5% was observed in the 30–39 age group. This age-prevalence change was rarely observed in other endemic areas. 4) The water contact activities at the irrigation was more frequent in adults of 30–39 years than in children.

[Discussion] The unexpected low prevalence at the age 30–39 may be due to their high frequency of usage of canal water where very few snails were found. The construction of a large-scale irrigation canal does not always bring an increase of cases of *S. mansoni* infection. The change of endemicity of *S. mansoni* due to the agricultural development, if there were, should be evaluated not only by the spread of vector snails but also through the change of standard of living in the area.

WI-6 BIOLOGY AND CONTROL STRATEGY OF CHAGAS' DISEASES VECTORS IN GUATEMALA

YUICHIRO TAHARA
Ex-JICA Expert, IC NET Limited

(Abstract not received in time)

WI-7 NEW EPIDEMY OF PARASITIC DISEASES IN SOUTHEAST ANATORIA IN TURKEY

YOSHITSUGU MATSUMOTO[1], YASUNOBU MATSUMOTO[1], CHIZU SANJOBA[1] AND SHIN-ICHIRO KAWAZU[2]
Department of Molecular Immunology[1],
Department of Global Agricultural Sciences[2],
School of Agricultural and Life Sciences, The University of Tokyo and
International Medical Center of Japan[3]

The southeastern region of Turkey is a part of the cradle of the ancient Mesopotamian civilization. Now a day this area has changed into semidesert and less developed area. Since 1981, Turkey has initiated the southeastern anatolia project (GAP), a comprehensive socioeconomic development project in this region. GAP covers 6 million residents and 75,000 km^2 region corresponding to approximately 10 percent of Turkey's total population and surface area. The water resources development program includes constructions of 22 dams and 19 hydropower plants on the Euphrates and the Tigris and the irrigation network. The GAP brings about not only the socioeconomical development but also significant migratory changes in human as well as changes in ecology of animals, insects and parasites. These changes are leading an increase in the prevalence of parasitic diseases including malaria and leishmaniasis, and threatening public health in this region.

Malaria caused by *Plasmodium vivax* has affected Turkey for centuries. As a result of efforts to control the disease including the malaria eradication campaign that began in 1957, the infection was practically eradicated from Turkey by 1970. With the increase in irrigation the number of malaria cases, however, has increased and in the past ten years a serious malaria epidemic has been building up in the southeastern provinces, reaching at 36,842 cases in 1998 (15-fold rise since 1990).

The cases of Leishmaniasis caused by *Leishmania tropica* has also increased especially in urban area with the increase of population. In Sanliurfa (pop. 200,000), one of the major cities in the GAP area, the number of cases increased from 552 in 1990 to more than 3,000 in 1996. Other parasitic diseases including ameobiasis, cryptosporidiosis, taeniasis, and hymenolepiasis are also endemic and expected to increase in the prevalence, unless strict control measures are undertaken.

WI-8 MALARIA SITUATION AND CONTROL PROGRAM IN KHAMMOUANE PROVINCE, LAO PDR

Jun Kobayashi[1, 2], Simone Nambanya[1], Yoshiya Sato[3], Ichiro Miyagi[4] and Hiroyuki Amano[2]
Institute of Malariology, Parasitology and Entomology (IMPE)[1],
Ministry of Health[2], Lao PDR,
Department of Parasitology[3] and Laboratory of Medical Zoology[4],
Faculty of Medicine, University of the Ryukyus

It was known that Malaria is serious public health problem in Lao PDR, however, only few data for malaria situation was reported because the surveillance system in anti-malaria network has been still weak in this country. JICA Lao PHC Project was carried out from 1992 to 1998, and one of achievement in this project was strengthen of anti-malaria net work in Khammouane Province.

Malaria survey in communities was conducted using this surveillance system among villages located in four different geographical situations. Only few cases of malaria were detected in the flat area along Mekong River. In the villages located in the flat forest, malaria infection rate was 5-10% through the years. The high prevalence (20-50%) was presented in the two different geographical areas. One is the basin area surrounded by rice field, other one is villages located shallow valley located near the Lao-Vietnam border. Anopheline mosquito collection was also carried out in same areas. It is suspected that different vector existed among different geographical situations. The vector survey has been started from 1999.

The control program using by Impregnated Bed Net (IBN) and Health Education has been started from 1996 in two villages. After 1 year setting IBN, malaria parasite rate among the villagers was markedly decreased from 8% to 2%. The expanding of IBN program to nation wide has been started supported by many donors from 1998. After JICA PHC Project was closed, the Japan Government also continues the support by Grant-in-Aid and dispatch expert for evaluation of IBN. The cost effective was strongly considered for expanding the program. After this, scientifically analyze for malaria situation among different geographical situations is expected for planning and evaluation of malaria control program.

WI-9 ERADICATION OF MALARIA ON AN ISLAND OF VANUATU

Akira Kaneko[1, 5], G. Taleo[2], Toshihiro Mita[1], S. Yamar[2],
H. Perlmann[3], P. Perlmann[3], Kazuyo Ichimori[4],
Takatoshi Kobayakawa[1] and A. Björkman[5]
Department of International Affairs and Tropical Medicine,
Tokyo Women's Medical University School of Medicine[1],
Malaria Section, Department Health, Port Vila, Vanuatu[2],
World Health Organization, Honiara, Solomon Island[3],
Department of Immunology, Stockholm University[4], and
Department of Infectious Diseases, Karolinska Institutet,
Karolinska University Hospital[5], Stockholm, Sweden

Vanuatu consists of 80 islands in Melanesia. Malaria is hypo- to mesoendemic and seasonal with occasional epidemics. The isolated island environment however provides conditions suitable for intervention measures leading to sustainable lowering or even elimination of the parasite reservoir. Aneityum with 700 inhabitants in 3 major villages, was selected as a study island for such an undertaking.

In 1991 before the rainy season, mass drug administration (MDA) was conducted during 9 weeks, permethrin-impregnated bed nets were distributed to the people and larvivorous fish was introduced in identified breeding sites of *Anopheles farauti*. The malariometric effects of this intervention were monitored over a 7 year

period and the IgG antibody levels to *P. falciparum* mature stages were determined by enzyme-linked immunosorbent assays at the end of this period. Survey data from two islands, Futuna without malaria transmission and Malakula with rather stable malaria, were used for comparisons.

With a high degree of involvement by the Aneityum community in the intervention activities, a MDA compliance of 88.3%, and a high provision rate of bed nets (ratio of 1.06 villagers/net), the malaria transmission was in principle interrupted. The surveys showed complete absence of *P. falciparum* from 1993 and *P. vivax* from 1996 onwards. The anti-malaria IgG antibody values increased according to age, but this age effect was greatest in Malakula, intermediate in Aneityum, and lowest in Futuna and the main increase in Malakula was from 0-5 to 6-15 and in Aneityum from 16-30 to >30 years of age.

On Aneityum island with low-to-moderate transmission, we have most probably interrupted transmission by combining MDA with impregnated bed nets and achieved sustained and significant gains. The sero-epidemiological results suggest that without antigenic stimulation immunologic memory will still remain for many years in adults previously repeatedly exposed to malaria.

WI-10 A SUCCESSFUL COURSE OF MALARIA CONTROL IN THE SOLOMON ISLANDS

JUDSON LAEFASIA[1], BARNARD BAKOTE'E[1], MASATO KAWABATA[2],
AKIRA ISHII[3] AND TOSHIAKI IKESHOJI[1]
Solomon Islands Medical Training and Research Institute[1],
Kobe University School of Medicine[2] and Jichi Medical School[3]

Malaria is a historically major public health problem in the Solomon Islands involving tardiness of economic development of this country. Since introduction of the first malaria control programme in 1960's, the main strategies have changed two times from Malaria Eradication Programme through Anti-Malaria Programme in 1976 to National Malaria Control Programme (MCP) in 1991 according to evolution with the global trends. Based on the experiences of malaria eradication/control activities and researches carried out at the Solomon Islands Medical Training and Research Institute during the previous two decades, the whole centralized approach to malaria control should be re-oriented towards a more decentralized system to respond to the epidemiological reality of a situation in the current National Malaria Control Programme. The re-orientation process involves restructuring of the programme in order to carry out the policy objectives and activities. The Provincial Director of Health became the responsible officer for the provincial malaria control programme who would be assisted by a senior anti-malaria officer to operate vector and malaria control specific activities and supervision of malaria staff.

The epidemic peaked in 1992 when the country recorded 600 cases per 1,000 population, and Honiara was dubbed the "Malaria Capital of the World" with 1,072 cases per 1,000 population. After the re-orientation of the malaria control programme was applied, the incidence rate steadily fell to 822 in 1994, 332 in 1996 and 260 in 1998. With the decentralized approach, each of the provinces was directly given a grant and other resources to plan and operate appropriate control measures. The decline in Honiara and the provinces contributed to an overall reduction in the incidence of malaria in Solomon Islands for the last 7 years. A good programme management supported by the political initiative of the national and provincial governments might be attributable to this prominently successful malaria control in Solomon Islands. Also, without sustainable supports by the World Health Organization and other agencies including Japan International Cooperation Agency (JICA), a successful course of malaria control would not have been achieved.

WIV-2 BLOOD SAFETY ON DEVELOPING COUNTRIES

NAMIKO YOSHIHARA
AIDS Research Center, National Institute of Infectious Diseases

[Back ground] WHO and UNAIDS have reported 3,600 thousand persons living with HIV, 2,600 thousand AIDS death cases and 5.6 thousand new HIV infected cases in the world during 1999. Most of the cases are the developing countries HIV infection rapidly spread in Sub-Sahara and South-East Asia. By in those countries, it is difficult even for HIV diagnosis, but it is more difficult for medical treatment. Major modes of transmission are sexual intercourse, transfusion with contaminated blood and transmission from infected mothers to newborns. WHO has reported that transmission rates of sexual intercourse, vertical transmission and blood transfusion are 0.1 to 1%, about 30% and higher than 90%, respectively.

[The International training course and making the African network] It is most important that the blood safety is the spread of scientific knowledge and blood testing. The international training course for blood borne viruses is opening against medical doctors of about 15 African's countries for one month at once year at Suez Canal University in Egypt. The course is a assistance of Japan International Agency Cooperation (JICA). I have attended three year as the lectures and the technical transfer concerning AIDS and viral hepatitis. Participants can get the knowledge of up to date about blood borne viruses and safety blood.

We are making a African serum bank in Egypt and the multi African countries network including emerging diseases and re-emerging diseases. It is important that participants understand the health condition of their country and neighboring countries.

[How to collect safety blood] I have been to Malawi in 1995. I have tested their blood borne viruses. Prevalence of them were extremely high. Prevalence of anti-HIV antibody of pregnant women and STDs patients are 33% and 64%, respectively.

Prevalence of blood donor was almost same. I have heard that if there is no test kit, the test on blood donor does not perform in Malawi. Ivory Coast and Thailand are higher HIV infection than Japan. Prevalence of HIV in the initial donor in Ivory Coast and Thailand is 6.4% and 0.9%, respectively. However, prevalence of HIV in the regular donor is 0.43% and 0.3%, respectively. Their countries recommend regular donor. I would like to advice that the selection of high quality donor is useful as same as tests for safety blood.

WIV-3 HIV RESEARCH AT THE MRC LABORATORIES, THE GAMBIA, WEST AFRICA; THE LINK BETWEEN THE FIELD AND THE LABORATORY

KOYA ARIYOSHI
National Institute of Infectious Diseases

UK Medical Research Council (MRC) Laboratories, The Gambia is one of few research institutes in Africa, which has a P2/P3 facility thus it has a capacity of conducting a laboratory oriented basic research close to the site where epidemiological surveys are being conducted. Six years of experiences of the author's working at the institute as an HIV laboratory scientist, will be briefly presented by focusing on the following three on-going HIV cohorts and associated basic research questions:

1) A community-based cohort in HIV-2 highly endemic village in Guinea-Bissau: What are the mechanisms of better prognosis of HIV-2 infection?
2) A cohort of HIV infected pregnant women in The Gambia: Why is the rate of HIV-2 mother-to-child transmission lower than that of HIV-1?/Is there any correlation between the transmission rate and malaria infection?
3) A cohort of commercial sex workers in The Gambia: Is there any commercial sex workers who are resistant to HIV infection?/Does HIV-2 infection protect an individual from HIV-1 infection?

Research can be conducted more productively if basic laboratory work can be combined to field epidemiological studies. Most of basic research has been conducted in a different country from the country where field research was conducted. However the experiences at the MRC laboratories, The Gambia show

that the basic research laboratory could be done effectively in the field and could create training opportunities for local staff researchers and could stimulate to improve local government personnel.

WIV-4 DETECTION OF *ESCHERICHIA COLI* O157 ANTIGEN AND SHIGA-LIKE TOXINS IN CLINICAL SPECIMENS USING MONOCLONAL ANTIBODIES

Pongsri Tongtawe[1], Urai Chaisri[1], Akemi Kai[3], Manas Chongsa-Nguan[1], Pramuan Tapchaisri[1], Hisao Kurazono[2], Hideo Hayashi[2] and Wanpen Chaicumpa[1]
Faculty of Tropical Medicine, Mahidol University, Thailand[1],
Institute of Basic Medical Science, University of Tsukuba[2] and
Tokyo Metropolitan Research Laboratory of Public Health, Tokyo[3]

Hybridomas secreting monoclonal antibodies (MAbs) to lipopolysaccharide (LPS) of *E. coli* O157 and A- and B-subunits of Shigatoxin-1 (Stx-1) and Shiga toxin-2 (Stx-2) were produced. Mono-epitope specificities of the MAbs were screened by hetelogous antigens and homologous antigens. The MAbs, namely MAb to O157 LPS and MAbs to A- and B-subunits of Stx-1 and Stx-2 were used in a dot-blot ELISA for the detection of their respective antigens in stool samples collected from patients with diarrhea. The Mab-based dot-blot ELISA, when performed in double-blind manner, could correctly identified 5 samples of patients with *E. coli* O157 infections while the tests were negative for samples collected from patients with diarrhea caused by other enteric pathogens.

The membrane ELISA is easy to perform and relatively inexpensive compared to the culture and DNA amplification methods. It offers a relatively rapid turn-around time (90 min), can test multi-samples at a single time, does not require special equipment, and does not produce large quantities of contaminated waste. Most of all, the test using a cocktail of MAbs to A- and B-subunits of both toxins offers advantage in detection of their respective antigen(s) in clinal specimens of patients infected with non-O157, Shiga toxin producing bacteria (supported by the Thailand Research Fund and the Grant-in-Aid form Japan).

WIV-5 PREVALENCE OF ENTEROAGGREGATIVE *E. COLI* CAUSING CHILDREN DIARRHEA IN THAILAND

Orn-Anong Ratchtrachenchai[1], Sarayoot Subpasu[1], Ken-ichiro Ito[2] and Hideo Hayashi[3]
National Institute of Health, Ministry of Public Health, Thailand[1],
National Institute of Public Health[2] and
University of Tsukuba[3]

Enteroaggregative *E. coli* (EAggEC) has been reported to be associated with persistent diarrhea in children in many developing countries. Laboratory surveillance of EAggEC was carried out at the enteric laboratory, National Institute of Health, Thailand, during the period from January 1996 to December 1998.

E. coli isolated from children under 7 years old with diarrhea were examined for the presence of genes encoding transcriptional activator of aggregative adherence fimbriae I expression (*aggR*) and EAggEC heat-stable enterotoxin 1 (*astA*) by PCR. One hundred and twenty nine out of 1,204 *E. coli* isolates were found to possess *aggR*. Fifty-four (41.8%) of the 129 isolates contained the *astA*. However, *E. coli* possessing the *astA* without the presence of *aggR* were the most predominant that one hundred and sixty seven of the 1,204 *E. coli* isolates harbored the *astA*. These strains were negative for the virulence factors of the four standards categories of diarrheagenic *E. coli* (enterotoxigenic, -invasive, -pathogenic, and -haemorrhagic).

The role of *astA* possessing *E. coli* in causing diarrheal diseases needs to be further characterized.

WIV-6 ESTABLISHMENT OF THE PCR SYSTEM SPECIFIC TO *SALMONELLA* SPP. AND ITS APPLICATION FOR THE INSPECTION OF FOOD AND FECAL SAMPLES

SOU-ICHI MAKINO[1], MANAS CHONGSANGUAM[2], HISAO KURAZONO[3] AND HIDEO HAYASHI[2]
Obihiro University of Agriculture and Veterinary Medicine[1],
School of Health Science, Okayama University[2] and
University of Tsukuba[3]

Salmonella species are widely distributed in the environments, and cause salmonellosis in humans and animals. Since *Salmonella* often transmit to humans through dairy products *e.g.* meat or egg, it is important to detect and remove *Salmonella*-contaminated foods. We established PCR method using the nucleotide sequences of the enterotoxin gene, *stn*. Total 89 *Salmonella* strains originating from animals with 20 serovars, in Japan, were examined. Among them, 62 isolates were from cattle; 52 from feces, 3 from farms, 3 from organs (liver, kidney and intestine) and 1 from milk. Other 27 isolates were derived from 18 poultry farms in the slaughter houses.

Total of 542 *Salmonella* isolates from humans and foods with 58 serovars, in Thai, were all positive, while other 43 species of Gram-negative and 19 species of Gram-positive bacterial strains were all negative. We examined the practical applications of this PCR system using minced meats and bovine-fecal samples.

Salmonella cells were artificially added to one gram of both samples at the final viable cell number of 0, 1, 10, and 100, respectively. Although any DNA fragments were not amplified by the PCR using the 1st enrichment cultures of bovine fecal samples, 260 bp fragments were amplified in the 2nd enrichment cultures. In minced meats, 260 bp DNA bands were amplified in all culture samples.

We concluded that the sensitivity of this PCR system was defined as 1 cell per gram of the ample. We believe that this PCR system and the enrichment procedures are very useful for the practical detection system for *Salmonella* species in the foods (Cooperation with Hyongil Chun, Saori Suzuki, Toshikazu Shirahata).

WIV-7 CAN WE ELIMINATE LEPROSY? -COMPARISON OF THE CURRENT STATES BETWEEN LOW AND HIGHLY ENDEMIC COUNTRIES

MASAKO NAMISATO
National Sanatorium Tama-Zenshoen

After WHO recommended multidrug therapy (MDT) for leprosy in 1982, the numbers of countries where the prevalence rate was more than 1/10,000 decreased from 122 in 1985 to 32 in 1998. The relapse rate was also proved to be satisfactory low. Based on these results, the 44th World Health Assembly adopted a resolution to decrease the prevalence below 1/10,000 in every country by the year 2000. However, new case (NC) detection rate (NCDR) has not shown enough decrease. Through the comparison of the data between the top 3 endemic countries (X) and the 6 countries (Y) that have achieved global target recently, some crucial factor(s) for leprosy control might be elucidated. Our studies in Myanmar, the 5th endemic country, and Morocco where the NCDR has greatly decreased recently are also shown for typical examples.

Under MDT-system, all cases completed fixed doses of MDT are discharged from registration. Therefor the prevalence rate of X had greatly decreased after MDT-implementation. The NCDR however, has not decreased still now. The prevalence rate and NCDR in Y were already low in 1985 and the latter is decreasing slowly but steadily. In Myanmar the rapid decrease of prevalence rate after MDT-implementation is almost

same as that of X, but the NCDR is fluctuating and even increasing. In Morocco after the adoption of unique control system in 1991, NCDR has greatly decreased. Although the increased NCDR is contributed to aggressive case finding activity, untreated backlog cases must be remaining as infectious sources in endemic areas. In Myanmar more cases were found where MDT was implemented earlier, and in the areas where progressive case finding activity like LEC (leprosy elimination campaign) was performed.

The rate of under 15-year-old (children-rate) in NC in X is decreasing slowly but counts more than 15%. The same in Y has been less than 10% during past 10 years and becoming less than 5%. In Myanmar it has been decreasing after 1993, and became less than 10% last 2 years. In Morocco children-rate has been less than 10% for 10 years with only exception in 1995. Children-rate tends to decrease along with decreasing NCDR. The rate of multibacillary type (MB%) in NC has been around 50% in Y. But in X it had been about 20% until 1992 and then gradually increased. The MB% is known to increase accompanied with decreasing NCDR. The WHO's criteria for MB has changed many times in the past. Therefor it might have reflected in the increased MB% in X. The grade 2 disability rate (G2%) in NC is higher in Y than in X. Generally, G2% is more in MB than in paucibacillary type. So the higher G2% in Y may correlate with higher MB. In X and Myanmar, the G2% dose not correlate with MB%. Presently, we cannot expect reliable data for G2% in these countries. But the greatly increased G2% in Myanmar in 1998, when active LEC was performed, suggests that many backlog cases are waiting for detection and treatment. In endemic countries NCDR will not decrease soon and present case finding effort should be sustained.

WIV-8 PRESENT STATUS AND FUTURE PLANNING OF A LEPROSY ELIMINATION PROGRAM IN KHULNA DISTRICT BANGLADESH —COLLABORATION A NGO WITH THE LOCAL GOVERNMENT—

YUTAKA ISHIDA[1] AND LORELLA PICORINI[2]
Okayama Hakuaikai Hospital[1] and
PIME Sisters Khulna Leprosy Program, Bangladesh[2]

The 5-year long TB and Leprosy eradication project ended in Bangladesh in 1998 and the national leprosy control project has been integrated into the general health service. Some observations on it are presented here from the point of view of the front-line leprosy control project run by NGO. After the 5-year national project ended and resigned the leadership, the collaboration between the local government and vertical NGO projects is important in future for the performance of effective leprosy control work.

[Method] PIME Sisters Khulna Leprosy Program has been doing leprosy control work in Khulna, the south west of Bangladesh since 1986. According to the statistics of case detection there from 1986 to 1998 the recent leprosy prevalence and the impact of the 5-year national project is discussed. The future planning of the collaboration is suggested last.

[Analysis of new case detection] The 3,464 new cases detected in Khulna Metropolitan city, the third biggest one of Bangladesh from 1986 to 1998 are analyzed. After the 13-year case detection the total of yearly new cases was over 200 in 1998 (NCDR was around 2 per 10,000 population/year). It increased in 1992 when intensified control work started (NCDR was around 3.5) and disability grading (G=2) at detection and the MB rate declined accordingly, and the mean age reduced. It has decreased yearly for the last three years, which indicates that the prevalence rate has declined through some years' active case detection. But the number of MB patients has remained almost the same throughout 13 years and the proportion of children (less than 14 yrs old) has remained unchanged or has been even increasing. It indicates that man-to-man transmission has lasted until recently. The project's active case detection was effective for PB leprosy but not so effective for MB leprosy. The number of new cases from "out of Khulna city" has been fluctuating yearly for the last 13 years, so have been disability grading (G=2), the MB rate and the mean age at detection. The high proportion of disability grading (G=2), MB cases and the low proportion of females and children (less than 14 yrs old) indicates less accessibility of a leprosy centre from far.

[Results] The 5-year national project was just the beginning of leprosy control in Khulna district, so it

should be continued or even intensified for decades. The statistics published by the government is far from the reality in the field. The project's proposal for collaborating with the governmental project has been approved by the local government. The staff has already started to train the governmental staff in 3 sub-districts (thana) of 9 in Khulna district. In one sub-district (Rupsha thana) more new cases have been detected in collaboration for a few months than by the Governmental project of the last 5 years. The close collaboration between the local government and NGOs will be expected to make leprosy control work more effective in future.

WIV-11 IMPROVEMENT OF TB SMEAR EXAMINATION IN CEBU CITY, PHILIPPINES

Akiko Fujiki[1], Dorotea M. Bacalso[2] and Shoichi Endo[1]
The Research Institute of Tuberculosis, JATA[1] and
Cebu City Health Department, the Philippines[2]

WHO estimated that 1/3 of the World's population are infected with tuberculosis. An estimated 88 million new cases of TB will occur on third millennium and approximately 30 million people are to die of the disease. Based on this background, WHO advocated DOTS (Directly Observed Treatment, Short course) Strategy and stressed the importance of sputum smear examination. TB sputum smear examination is the pillar of TB control. The most important for the success of TB control is to ensure the quality of TB sputum smear examination. JICA Philippines TB control project has a major component to strengthen the quality of sputum smear examination since 1994 in the Regions 4 and 7 in the Philippines. Cebu City of Region 7 with 700,000 population has a Reference Laboratory and 5 peripheral laboratories. The quality control system has been developed since 1997 in Cebu City as a model approach.

The activities of TB smear examination in Cebu City were analyzed with the results of quality control reported from January 1997 to December 1998 in the 5 peripheral laboratories. Various technical aspects of sputum smear examination and management of laboratory activities were discussed.

Total smear slides checked for quality control in 1997 and 1998 were 4,775 and 4,249 respectively. Comparing the results from first quarter of 1997 and the last quarter of 1998, the improvement was observed in all assessment points of smear preparation; namely sputum quality, staining, smear cleanness, smear area size, smear thickness and evenness. In particular, remarkable improvement was observed in staining from 56.2% to 96.0% and in smear thickness from 48% to 91.2%, which were almost doubled in each assessment point of smear preparation. The marked improvement was noted within 6 months after quality control started. It is suggested that at least 6 months are required and frequent supervisory visit should be made to strengthen the smear preparation. The reading ability of acid-fast bacilli (AFB) was improved and considered to be acceptable and reliable as well. Overall agreement of the reading grade was 81.1% in the first quarter of 1997 and 99.1% in the last quarter of 1998. False(+) and false(−) were from 1.9% to 0.2% and from 2.9% to 0.7%, respectively. Most of the false readings occurred at a reading of (1+) or 1-2 AFB in 300 visual fields. The factors identified for contributing to the improvement are: standardized equipment and regular provision of reagents supply, distribution of binocular microscopes to all laboratories, expansion and maintenance of proper size of the laboratory, and frequent supervisory visits made with a strong leadership by the supervisor.

WIV-12 INTERNATIONAL TUBERCULOSIS TRAINING COURSES IN JAPAN

HITOSHI HOSHINO, AKIRA SHIMOUCHI AND NOBUKATSU ISHIKAWA
Japan Anti-Tuberculosis Association, Research Institute of Tuberculosis

Research Institute of Tuberculosis (RIT) currently runs 3 international group training courses and individual training courses for health personnel working for tuberculosis control program. Three group training courses consist of National Tuberculosis Program Management course for central/regional level medical officers (5 participants for 6 weeks), Tuberculosis Control course for intermediate level medical officers (20 participants for 12 weeks) and Tuberculosis Control Laboratory course for TB laboratory staff (5 participants for 12 weeks).

These training courses have trained totally 1,492 participants (including 1,022 medical officers and 190 laboratory staff) as of September 1999. TB Control course was started in 1963 and jointly organized with WHO since 1967. TB management course started in 1973 for the ex-participants of TB control course. TB laboratory course started in 1975 succeeding TB surgery course for chest surgeons. Participants are from all over the world; 126 participants from Philippines, 112 from Thailand, 111 from Indonesia, 78 from Nepal and Bangladesh, and 52 from Yemen and so no.

Contents of the course for medical officers are lectures, practices, module training, field visits, action plan working and its presentation. The lectures contain basic knowledge on tuberculosis, including pathology and immunology in TB disease, TB epidemiology, statistics, advocacy, HIV, economics, socio-cultural aspects of TB, program management and communication skill. Practices are exercise for statistics, WHO training modules and case conferences.

Field visits to Ministry of Health and Welfare, TB hospitals, public health centers and Japan Anti-Tuberculosis Association are informative to understand NTP in Japan. The TB management course is more focused on program management skill and advocacy. The Laboratory course consists of lectures, field visit and laboratory practices. Laboratory practices are for sputum smear examination, culture examination, sensitivity test, variation studies and so on.

Follow-up visits to countries with many ex-participants have been conducted to supervise and encourage them. Most of ex-participants are working for national TB control program or other related health programs. Many of them are working as director of national TB control program in their own countries.

Poster presentation

59 A RAPIDLY FATAL CASE OF SEVERE FALCIPURUM MALARIA COMPLICATED WITH HIGH-LEVEL METABOLIC ACIDOSIS

YURIKO SAITO[1], MIKIO TAKAMORI[1] AND MIKIO KIMURA[2]
Department of Internal Medicine, Tokyo Kousei Nenkin Hospital[1] and
Department of Infectious Diseases and Applied Immunology,
Institute of Medical Science, The University of Tokyo[2]

A 67-year-old male was admitted presenting with consciousness disturbance (JCS, III-200) after completing a 12-day tour to east Africa without malaria chemoprophylaxis. When he visited the hospital one day prior to admission complaining fever and slight sore throat, he did not mention the travel history. Soon after his travel history was revealed, blood films were prepared which showed abundant ring forms mixed with a small number of trophozoites and schizonts of *Plasmodium falciparum,* with the parasitemia of 26%. Despite intravenous quinine infusion, first that of loading dose, his consciousness state (JCS, III-300), renal and hepatic functions and anemia (Hb, 5.8 g/d*l*) deteriorated progressively. Moreover, metabolic acidosis worsened with the pH of 6.954, HCO_3^- of 6.1 mmol/*l* and BE of 16.1 mmol/*l* by arterial blood gas analysis, although he received a large volume of sodium bicarbonate solution. The patient died on the 4th day of his illness.

According to the literature, it is suggested that the treatment of metabolic acidosis in severe falciparum malaria with sodium bicarbonate could sometimes be harmful, since it can result in sodium overload which may then precipitate pulmonary edema/ARDS. However, alternative treatment regimens have not yet been established. Future investigations on the etiology and the proper treatment of metabolic acidosis associated with severe falciparum malaria are strongly needed.

60 A RESCUED CASE OF COMPLICATED FALCIPARUM MALARIA USING CONTINUOUS HEMODIAFILTRATION (CHDF)

KYOKO KIMURA[1], KAORI NAKAMURA[2], YUUICHI HAMABE[2] AND KENJI OHNISHI[1]
Department of Infectious Diseases[1] and Life Support Emergency Center[2],
Tokyo Metropolitan Bokutoh General Hospital

A 65-year-old woman was admitted to the Life Support and Emergency Center in our hospital with cerebral malaria on October 21, 1998. She had traveled in Niger from September 22 to October 6, 1998. She was comatose (Glasgow coma scale, E4V1M1), hyperpyretic, in respiratory distress, and had acidosis and acute renal failure on admission. Her hemoglobin concentration was 8.2 g/d*l* and her platelet count was 56,000/μl. Blood smears showed ring form of *Plasmodium falciparum* and a parasitemia level of 12.2%.

An antimalarial chemotherapy with quinine (Quinimax^R 700 mg, div, q 8 hr, 2 times plus 350 mg after 8 hr, 1 time), mefloquine (1,000 mg, po plus 500 mg after 8 hr) and artesunate (250 mg, suppo, q 6 hr, 6 times) was performed immediately along with intensive care. But pulmonary edema, acute renal failure, anemia and thrombocytopenia deteriorated and mechanical ventilation and continuous hemodiafiltration (CHDF) were induced on her second day of hospitalization. 800 m*l* of red blood cell concentrates and 10 units of platelet concentrates were transfused through CHDF. Acidosis was quickly corrected within one day and pulmonary edema, acute renal failure, and her level of consciousness gradually improved. *P. falciparum* had disappeared by her 6th day of hospitalization. She was weaned off mechanical ventilation and CHDF on her 7th day of hospitalization. Three hemodialysis sessions were added at two-day intervals. She recovered almost perfectly after one month of rehabilitation and was discharged on December 12, 1998.

CHDF is one of the blood purification teqniques which is used for critically ill patients, such as those with multiple organ failure, in the area of emergency and critical care medicine. It has been proved that

CHDF can not only compensate for renal dysfunction and correct acidosis, electrolyte imbalance, and water balance, but also remove toxic substance, such as cytokines, humoral mediators, lactic acid and improve tissue oxygeneration.

We induced antimalarial drugs to attack the malaria itself and used CHDF to eliminate toxic substances and to manage the overall critically ill situation. This combination was successful and required a minimum amount of blood transfusion, which is much safer than blood exchange. We conclude that CHDF is effective as one of the ancillary treatments of cerebral malaria.

61 CURRENT SITUATION OF MALARIA CHEMOPROPHYLAXIS AMONG JAPANESE RESIDENTS IN TROPICAL AFRICA

Atsuo Hamada, Eiichi Okuzawa, Yuka Hiroshige and Tetsuo Nishikawa
Japan Overseas Health Administration Center, Labor Welfare Cooperation

[Objective] In Japan, malaria chemoprophylaxis is not officially recommended for Japanese staying in the endemic area. However, it is supposed that some Japanese have already taken the measure without any official guideline. Therefore, we surveyed a current situation of malaria chemoprophylaxis among Japanese residents in tropical Africa.
[Methods] The survey was carried out in 1994 and 1998 with a self-administered questionnaire. The questionnaire was completed by 129 (in 1994) and 139 (in 1998) Japanese residents in Tanzania, Nigeria, Ghana and Cote D'lvorie (Ave. age: 24.8 yo in 1994 and 35.9 yo in 1998, Ave. period of stay: 31.8 months in 1994 and 30.5 months in 1998).
[Results] Japanese who took malaria chemoprophylaxis was 23.3% (30) in 1994 and 36.7% (51) in 1998, suggesting that the ratio in 1998 significantly elevated compared to that in 1994 ($p<0.02$). Regarding to the drug regimen, more than 60% of Japanese used chloroquine alone in both 1994 and 1998. Users of chloroquine plus proguanil increased in 1998 (23.5%) compared with that in 1994 (3.3%), although mefloquine users decreased from 16.6% in 1994 to 12.2% in 1998. Failure of chemoprophylaxis was observed in users of chloroquine alone (16.7%) and chloroquine plus proguanil (8.3%) (data in 1998). Mild adverse reactions such as gastroenteric and neurological symptoms were frequently associated with each regimen especially in mefloquine.
[Discussion] Recently, chemoprophylaxis becomes a major preventive measure of malaria among Japanese residents in tropical Africa. It is an urgent need to provide them an official guideline for the chemoprophylaxis in order to carry out the measure correctly.

62 A FIELD STUDY ON MALARIA PREVALENCE IN SOUTHEASTERN LAOS BY POLYMERASE CHAIN REACTION ASSAY

Hiroshi Toma[1], Jun Kobayashi[1, 2], Bouakham Vannachon[2],
Takeshi Arakawa[1] and Yoshiya Sato[1]
Department of Parasitology, Faculty of Medicine,
University of the Ryukyus[1] and
Institute of Malariology, Parasitology and Entomoligy, Lao PDR[2]

An active case detection survey for malaria infection by a routine microscopy and polymerase chain reaction (PCR) assay was conducted on the 336 inhabitants in two villages of Khammouane Province in Lao PDR, in July, 1997. The malaria infection was demonstrated in 58 (17.3%) of the subjects by microscopy and in as many as 117 (34.8%) by the PCR assay. The prevalence rate was highest (50%) in young villagers aged from 11 to 20 years old, showing that more than 50% of the positive villagers were occupied by children under 15 years old. The specimens demonstrated to be positive by both methods were frequent in the young

villagers, suggesting the presence of many latent infections in elderly villagers.

The most abundant etiologic species of malaria parasite was *P. falciparum* (82.9%) in these villages. *P. vivax* occupied 31.6% of the malaria parasites detected by the PCR assay. *P. malariae* and *P. ovale* infections were also demonstrated in 8 and 6 specimens by the PCR assay, respectively. The PCR assay detected mixed infections with two to four malaria species in 27 specimens (23.1%). The results in the present study demonstrated that there are many latent malaria infection with low parasite level among the villagers and the infections with all human malaria species are occurring in the country.

63 APPROPRIATE TECHNICAL TRANSFER FOR CONTROL PROGRAM OF PARASITIC DISEASES IN DEVELOPING COUNTRIES

HIROSHI OHMAE[1] AND KAZUO YASURAOKA[2]
Institute of Basic Medical Sciences, University of Tsukuba[1] and
Department of Medical Zoology, School of Medicine, Dokkyo University[2]

We investigated appropriate technical tranfer for control program of parasitic diseases in developing countries. Since 1981, we have been cooperating a control program of schistosomiasis japonica in Bohol, the Philippines. And since 1991, we have been cooperating a control program of malaria in Guadalcanal, the Solomon Islands.

After the introduction of selective mass treatment, prevalence of *Schistosoma japonicum* infections has reduced from 15% to 5% in the Philippines. A combination control program for schistosomiasis including selective mass treatment and snail control started in Bohol, in 1986. And this program is supported by a Japanese non governmental organization (NGO). Prevalence of *S. japonicum* has reduced from 4.9% in 1984 to 0.1% in 1992. For evaluation of this control program, an immunological technique which had been transferd from Japan in a cooperation project supported by JICA, was very useful.

In the Solomon Islands, we had a JICA's technical cooperation project for malaria control, from 1992 to 1996. Annual incidence of malaria has reduced from 500/1,000 in 1992 to 200/1,000 in 1996. As the first step of malaria control program in the Solomon Islands, insectcide-treated bed nets were distributed. After the distribution of insectcide-treated bed nets, joint program with mass blood survey and selective mass treatment was carried out in primary schools. Following the project supported by JICA, this control activity has been supported by a program of Japanese ministry of health and welfare. For evaluation of this control program, a new microscopic technique which had been transferd from Japan in the cooperation project supported by JICA, was used.

For parisitic control program in developing countries, appropriate technical transfer is very important. And combination of Go and NGO projects is very useful to go on or expand control programs.

64 THE MALARIA MATHEMATICAL MODEL FOR *PLASMODIUM VIVAX* AND ITS COMPUTER SIMULATION

HIROFUMI ISHIKAWA[1], AKIRA ISHII[2] AND MAI SHIMIZU[3]
Department of Environmental and Mathematical Sciences,
Okayama University[1],
Department of Medical Zoology, Jichi Medical School[2] and
Nippon Telegragh and Telephone West Cooperation[3]

To make an estimate of the degree of transmission of *Plasmodium vivax* malaria, and to estimate the incidence of *P. vivax* when the mass drug administration and/or any anti-malarial measures, such as the distribution of permethrin impregnated bed nets, are carried out, we construct a mathematical model for transmis-

sion of *P. vivax,* which is formed as the system of non-linear difference equations, and have carried out various simulations on this model. In addition to direct infection from vectors, the symptom of *P. vivax* is caused by the relapse of a hypnozoite in the liver, and this is a contrast to *P. falciparum*. The patterns of the relapse period are widely distributed around 100 days after the primary infection. We take the cases of relapses into account in our model, which is based on Dietz-Molineaux-Thomas model. The epidemiological parameters are determined by the field data surveyed in the Solomon Islands, 1993.

The simulation carried out shows that in the Solomon Islands, a holoendemic region, about 4-6% of the population hold hypnozoites in their livers. We cannot expect an efficient transmission blocking in the case that a single dose of primaquine 45 mg is put into practice once a year, which is usually used as the mass drug administration for *P. falciparum,* since the relapse of the hypnozoite restores the prevalence of *P. vivax* before long. In the case of the mass drug administration in which the doses of primaquine 15 mg are iterated for 14 days, the trial will succeed in keeping the prevalence at low level, but it will resurge within a few years. If the mass drug administration is accompanied with vector control diminishing vectorial capacity to 40%, the prevalence of *P. vivax* will be preserved at a low level for a long time.

65 *PLASMODIUM COATNEYI*: CYTOADHERENCE OF INFECTED MONKEY ERYTHROCYTES TO HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS AND C32 AMELANOTIC MELANOMA CELLS *IN VITRO*

SATORU KAWAI[1], JUN MATSUMOTO[1], MASASHI KIRINOKI[1],
MASAMICHI AIKAWA[2] AND HAJIME MATSUDA[1]
Department of Medical Zoology, Dokkyo University School of Medicine[1] and
Research Institute of Medical Sciences, Tokai University[2]

Binding assays with the C32 amelanotic melanoma cells (C32 cells) and human umbilical vein endothelial cells (HUVECs) are commonly used as *in vitro* models of adherence of *P. falciparum*-infected erythrocytes to endothelial cells. C32 cells express mainly CD36 and little ICAM-1, while HUVECs express ICAM-1, modulated by cytokines such as TNF, but not CD36. In the present study, we have shown that adhesion events *in vitro* similar to those *P. falciparum*-infected with erythrocytes occur with *P. coatneyi*-infected erythrocytes obtained from Japanese macaques.

P. coatneyi-infected erythrocytes were able to attach to both HUVECs and C32 cells. However, the adherent cell number and the number of adherent parasitized red blood cells (PRBCs) differed significantly between HUVECs and C32 cells. Cytoadherence was significantly higher on C32 cells than on HUVECs. Binding of PRBC to HUVECs were observed in 44 cells per 100 cells (44%), and the mean number of bound PRBC per cell was 1.5, ranging from 1 to 7. Binding of PRBCs to C32 cells were observed in 84 cells (84%) per 100 cells, and the mean number of bound PRBC per cell was 12.8, ranging from 1 to 41. Scanning electron microscopy revealed knob protrusions evenly distributed over the entire surface of adherent PRBC. Higher magnification of the site of attachment revealed that interaction of the PRBCs with cultured cells was mediated by protrusion of knobs.

The specific interactions between host receptors and ligands on monkey erythrocytes infected with *P. coatneyi* have been poorly described. As described for *P. falciparum,* if the parasite antigen as PfEMP1 family are expressed on the surface of *P. coatneyi*-infected erythrocytes, our findings suggest that CD36 is one of the major host receptors for adherence to endothelial cells.

66 EXAMINATION OF PHOSPHOLIPASE A2 IN SERA FROM MALARIA PATIENTS BY ELISA

KUMIKO SATO[1], SHIGEYUKI KANO[2], YOSHITSUGU MATSUMOTO[3],
SORNCHAI LOOAREESUWAN[4], MASAMICHI AIKAWA[5] AND MAMORU SUZUKI[6]
Gunma University School of Health Sciences[1],
Research Institute International Medical Center of Japan[2]
School of Agriculture and Life Sciences, The University of Tokyo[3]
Faculty of Tropical Medicine, Mahidol University[4],
Institute of Science and Technology, Tokai University[5] and
Department of Parasitology, Gunma University School of Medicine[6]

Phospholipase A2 (PLA2) is the first enzyme in the arachidonate cascade, and is found in various mammals as well as insects and snake venom. In 1994, Vadas *et al.* found a rapid increase of levels of circulating PLA2 in children with *P. falciparum,* particularly so in patients with severe disease as manifested by high parasite burden, anemia, coma, and death. They also showed that the PLA2 level was closely related to the TNF level. In this study, we developed a new method of measuring serum PLA2 by ELISA, and investigated the serum PLA2 level in malaria patients using this method. We also investigated the dynamics of the serum PLA2 level through the course of malaria infection.

[Materials and Methods] Sera were collected from Thai *P. falciparum* and *P. vivax* infected symptomatic and parasitemic patients, who were hospitalized in the Hospital for Tropical Diseases, Mahidol University, Thailand. The fluorescence-ELISA for PLA2 measurement was developed using the sandwich method which used anti-human sperm PLA2 (human type II secretory PLA2) as the antigen, rabbit anti-porcine PLA2 antibody as the antibody for the first reaction, and alkaline phosphatase conjugated anti-rabbit antibody as the enzyme-labeled antibody for the second reaction. 4-methyl-umbelliferyl phosphate was used as the enzyme substrate.

[Results and Conclusion] Of 73 falciparum malaria patients, 50 sera (68.5%) showed a higher PLA2 level than that of healthy individuals. Amng patients with vivax malaria, 13 of 15 patients (80%) showed a higher PLA2 level. The frequencies of this PLA2 in the group of patients as classified according to the WHO criteria for severity were 57.1%, 72,7%, and 78.5% in falciparum malaria patients with mild, moderate, and severe, respectively. The PLA2 level also tended to be higher in patients with severe malaria group. Regarding the relation to the disease stage, the PLA2 level was high immediately after hospitalization, and rapidly decreased within one week. We consider that measurement of PLA2 in malaria patients during the early phase provides an important clue to predicting progression of the disease.

67 POLYMORPHISM OF TNF-α PROMOTER REGION ASSOCIATED WITH SUSCEPTIBILITY TO CEREBRAL MALARIA IN THAILAND

KENJI HIRAYAMA[1], SORNCHAI LOOAREESUWAN[2], RATAWAN UBALEE[1, 2],
MIHOKO KIKUCHI[1], O. TASANOR[2], K. NABANGCHANG[2], JUNTRA KARBWANG[2],
M. TAKEDA[1], AKIKATA KIMURA[3], KYOGO ITO[4] AND MASAMICHI AIKAWA[5]
Department of Medical Zoology, Saitama Medical School[1],
Faculty of Tropical Medicine, Mahidor University[2],
Medical Institute, Tokyo Medical and Dental University[3],
Department of Immunology, Kurume University School of Medicine[4] and
Research Institute of Medical Science, Tokai University[5]

To investigate the host genetic factors affecting the clinical course of falciparum malaria, polymorphism of TNF-α promoter region was analyzed in the patients with different clinical severity of malaria. Two hundred and eight Thai patients with falciparum malaria at the Faculty of Tropical Medicine, Mahidol University in

Bangkok and Two hundred and fifty-four Myanmar's patients with falciparum malaria at the Mae Sod Malaria clinic in Mae Sot located at the border between Thailand and Myanmar, were adopted. Among the Thai patients 76 were asymptomatic, 89, severe and 43, cerebral malaria, and among the Myanmar's patients (128 from Karen, 116 from other Burmese) 210 were asymptomatic and 44, cerebral malaria. Those patients were diagnosed by the WHO criteria. After the diagnosis, all the subjects were immediately treated. TNF-α5′-flanking region which is located within class III region of HLA, was revealed to be polymorphic in -238, -308, -857, -863, -1031 positions from the initiation codon of TNF-α. In the patients there were at least 7 linkage groups identified and one of the groups -857C, -863C, -1031T was significantly increased in the cerebral malaria. We also analyzed frequency of the promoter region polymorphism of TNF-α in Japanese and Thais and found that the susceptible type was dominant in Japanese (Af=82.3) compared with Thais (Af=7.5).

Our data suggested the TNF-α production at the infection is controled by the polymorphic promoter region of TNF-α gene.

68 THE ROLE OF CR1 (COMPLEMENT RECEPTOR 1) ON ERYTHROCYTES IN THE DEVELOPMENT OF SEVERE MALARIA

Eiji Nagayasu[1], Mamoru Ito[2], Y. Nakano[1], Minoru Kimura[3],
Sornchai Looareesuwan[4] and Masamichi Aikawa[1]
Research Institute of Science and Technology[1],
Central Institute for Experimental Animals[2],
The Institute of Medical Science[3], Tokai University and
Faculty of Tropical Medicine, Mahidol University[4]

Complement receptor 1 on erythrocytes (E-CR1) shows an inherited numerical polymorphism (CR1 density polymorphism) which correlates with a RFLP of the CR1 gene. E-CR1 plays an essential role in the clearance of immune complexes from the circulation. On the other hand E-CR1 is also known as a receptor for erythrocyte rosetting (adhesion of *P. falciparum* infected erythrocytes to uninfected erythrocytes). To investigate the relationship between CR1 density polymorphism and disease severity, we typed 117 acute falciparum malaria patients for their genotypes of this polymorphism.

Patients diagnosed as having acute falciparum malaria were separated into severe or mild group according to the WHO criteria. Their genotypes were determined by PCR amplification of CR1 gene followed by restricton enzyme digestion (PCR-RFLP).

We have observed a significant higher frequency of homozygotes of CR1 low density allele in severe group as compared to mild group. Our results suggest that a genetically determined low CR1 density may be a risk factor for developing a more severe form of malaria because of their defective ability of the clearance of immune complexes.

69 MEASUREMENT OF ANTI-MALARIAL ANTIBODIES FOR INHABITANTS IN MALARIA ENDEMIC AREAS USING RECOMBINANT PROTEINS OF *PLASMODIUM FALCIPARUM* AS ELISA ANTIGENS

Hiroyuki Matsuoka, Kuni Iwai, Shigeto Yoshida, Meiji Arai,
Enjie Luo and Akira Ishii
Department of Medical Zoology, Jichi Medical School

We have produced recombinant malarial proteins using baculovirus-insect expression systems. So far we succeeded to express recombinant MSP-1 (rMSP-1: *Plasmodium falciparum* merozoite surface protein, block 17) in silkworm hemolymph, and purified it using affinity chromatography with monoclonal antibody. Recom-

binant SERA (rSERA: *P. falciparum* Serine Repeat Antigen) was produced in *Trichoplusia ni* cell expression system using no-protein culture medium. These recombinant proteins have been under evaluation for malaria vaccine candidates. We, on the other hand, tested these proteins as ELISA antigens for measuring anti-malarial antibodies in malaria endemic areas.

Native malaria antigen was extracted from cultured *P. falciparum* as a control antigen (nPf). Two recombinant proteins (rMSP-1 and rSERA) and one native protein (nPf) were diluted and filled in ELISA plates. Diluted sera from malaria endemic areas and non-endemic areas were reacted in the plates, and anti-human IgG conjugated with horse raddish peroxidase was added as the 2nd antibody. Titres of anti-nPf and anti-rMSP-1 in individual sera were positively correlated. Titres of anti-nPf and anti-rSERA were correlated as well. These results indicate that recombinant proteins we produced can be used as ELISA antigens. High antibody titres to rMSP-1 and rSERA were obtained from persons whose age were 10 years or more. This suggests that antibodies to these antigens are induced after repeated malaria infection.

70 CULTIVATION OF *PLASMODIUM FALCIPARUM* ISOLATES UNDER THE ANAEROPACK™ GAS CONDITION IN A PORTABLE THERMOSTATIC INCUBATOR

YASUTAKA MIZUNO[1,2,3], TOSHIMITSU HATABU[2], SHIN-ICHIRO KAWAZU[2],
HIROSHI OHTOMO[3], MAMORU SUZUKI[4] AND SHIGEYUKI KANO[2]
Department of Pediatrics[1] and Department Tropical Medicine[3],
Jikei University School of Medicine,
Research Institute International Medical Center of Japan[2] and
Department of Parasitology, Gunma University School of Medicine[4]

Cultivation of *Plasmodium falciparum* (*P.f.*) *in vitro* requires lower oxygen concentration and appropriate pH condition. AnaeroPack™ Malaria culture kit (SUGIYAMA-GEN Co., Ltd.) was devised as a portable cultivation system which can maintain such proper conditions for *P.f.* parasites to grow in the specific sealed jar (AnaeroPack™ Kakugata jar, 2.5 *l*). It was reported that the culture-adapted strains of *P.f.* could grow nicely in this system (Onda *et al.*). We examined if this system could also be used for the cultivation of newly isolated parasites from the patients. Infected erythrocytes taken from four patients (2 Japanese men, 1 Japanese woman and 1 Zambian man) were incubated under the following three different gas conditions; Mix gas (5% CO_2, 5% O_2, 90% N_2), AnaeroPack™ CO_2 (15% O_2, 6% CO_2) and AnaeroPack™ plus (5% O_2, 5% CO_2). The culture dishes were kept in the portable incubator, Super duo cargo (SUGIYAMA-GEN Co., Ltd.), which can maintain a stable temperature inside at 37°C conditioned by a specific thermostat. The culture medium was changed every 24 hr and an AnaeroPack™ gas generator was replaced by a new one.

We made slide smears from the culture suspension when the medium was changed, and counted the parasites under the microscopic observation. The parasite proliferation curves of all conditions were proved to be showing parallel curves each other. This finding showed that AnaeroPack™ Malaria culture system with the portable thermostatic incubator was an appropriate method not only for the cultivation of culture-adapted strains but also for that of newly isolated parasites. Important data on the epidemiology of drug resistant malaria will be collected by applying this new method to the field survey.

71 EFFECT OF TETRACYCLINE AND MINOCYCLINE AGAINST *PLASMODIUM FALCIPURUM IN VITRO*

QUING HUA LIN[1], MIHO OHUE[1], TOMOKO ONDA[1], KEN KATAKURA[1],
SHIGEYUKI KANO[2] AND MAMORU SUZUKI[1]
Department of Parasitology, Gunma University School of Medicine[1] and
Research Institute of International Medical Center of Japan[2]

The emergence and spread of drug resistant *Plasmodium falciparum* is a major problem for chemotherapy of malaria. Development of potent antimalarials is important, but it is also necessary to investigate the possible use of existing antimicrobial agents, which have already been approved for the treatment of other infectious diseases. In this study, we examined effects of tetracycline (TC) and minocycline (MC) on the *P. falciparum* strains, which show different sensitivity to chloroquine (CQ), mefloquine (MQ) and pyrimethamine (PM) *in vitro*.

Parasites used in this study were the SGE-1 strain showing CQ sensitive (CQS), MQ sensitive (MQS) and PM sensitive (PMS), the K1 strain exhibiting CQ resistance (CQR), a clinical isolate exhibiting MQ resistance (MQR), and other clinical isolate showing both CQ resistance (CQR) and PM resistance (PMR). *In vitro* drug susceptibility test was carried out by semi-microtechnique. Parasites were cultured for up to 96 hr in the presence of different concentrations of drugs and 50% inhibitory concentrations (IC_{50}) of the drugs were determined. The IC_{50} of TC and MC against the SGE-1 strain at 96 hr of culture were 1.07 ± 0.74 (n=3) and 0.19 ± 0.09 (n=3) $\mu g/ml$, respectively. A similar drug susceptibility was obtained with CQR, MQR and PMR parasites. Overall, highly lipophilic MC was about five times more effective than TC against *P. falciparum* proliferation.

In the presence of TC or MC, no matured schizonts but trophozoites with a nuclear shrinkage were observed at 72 hr in Giemsa-stained smear preparations. Electron microscopy revealed that the endoplasmic reticulum and the mitochondria were dilated and that the cytoplasm contained numerous electron-dense vesicles with a single membrane. In addition, decreased accumulation of Rh123 dye was observed in the TC or MC-treated *P. falciparum,* suggesting damage of the mitochondria membrane of the parasites.

72 DEVELOPMENT OF NEW ANTIMALARIAL DRUGS —THE ANTIMALARIAL ACTIVITY OF ENDOPEROXIDE ANALOGS—

YUKI ISHIZUKA[1], HYE-SOOK KIM[1], YUSUKE WATAYA[1], YOSHIAKI HAMADA[2],
KAORU TSUCHIYA[2], ARAKI MASUYAMA[2] AND MASATOMO NOJIMA[2]
Faculty of Pharmaceutical Sciences, Okayama University[1] and
Faculty of Engineerings, Osaka University[2]

Based on the ancient Chinese herbal medicine, a new class of nonalkaloidal antimalarial compound quinghaosu was identified by organic chemists in the 1970s; characteristic of these potent and fast-acting antimalarial is their chemically unusual 1,2,4-trioxane pharmacophore unit. Clinically used examples of such trioxanes derived from *Artemisia annua* (quinghao) are natural artemisinin (quinghaosu) and it's derivatives which has stimulated extensive synthetic and mechanistic research into their novel mode of action. Thus, organic chemists have synthesized a large number of structurally diverse artemisinin analogs and several simplified 1,2,4-trioxanes have been also found to show remarkable activities against *Plasmodium* infections *in vivo* in animals. These discoveries prompted us to investigate new structures of antimalarial endoperoxides.

Now, we have confirmed the potential of 1,2,4,5-tetraoxacyclo- alkanes as a new class of simple peroxide antimalarial drugs. In the preliminary studies, some 1,2,4,5-tetraoxacycloalkanes have been found to be available for chloroquine resistant *P. falciparum in vitro*. For instance, 1,2,6,7-tetraoxaspiro [7.11] nonadecane (N-89) has a potent antimalarial activity for *P. falciparum* (K1 strain), the EC_{50} value of which is 2.6×10^{-8} M. In contrast, the 50% inhibitory concentra-

tion of against mouse mammary FM3A cells is 8.0×10^{-6} M, demonstrating tht the selective toxicity (307) is also remarkable. It should be noticed that these compounds are similar effective of artemisinin (the EC_{50}'s against *P. falciparum* and FM3A cells were 7.8×10^{-9} M and 1.0×10^{-5} M, respectively). An *in vitro* test, morphological changes of malaria parasites during the treatment of N-89 against *P. falciparum* was studied. In these results, we have observed a condensation of parasite nuclei, shrunken cytosol in trophozoites and inhibition of schizont formation by micoscopy after treatment with 100 times the EC_{50} value of N-89 (2.5×10^{-6} M) for 24 hr. The morphological changes in parasites induced by N-89 may be a consequence of several phenomena including inhibition of protein synthesis.

Our results may be helpful in the design of better chemotherapeutic endoperoxide in the worldwide fight against malaria.

73 DEVELOPMENT OF NEW ANTIMALARIAL DRUGS —*IN VIVO* ANTIMALARIAL ACTIVITY OF ENDOPEROXIDES—

Yukiko Nagai[1], Kanako Ono[1], Hye-Sook Kim[1], Yuki Ishizuka[1], Yusuke Wataya[1], Yoshiaki Hamada[2], Kaoru Tsuchiya[2], Araki Masuyama[2] and Masatomo Nojima[2]
Faculty of Pharmaceutical Sciences, Okayama University[1] and Faculty of Engineerings, Osaka University[2]

1,2,4,5-Tetraoxacycloalkanes as analogs of artemisinin, were studied as antimalarial agents against *Plasmodium falciparum in vitro* and *P. berghei in vivo*. In our experiments, 1,2,6,7-tetraoxaspiro[7.11]nonadecane (N-89) has potent antimalarial activities and has been found to be available for chloroquine resistant *P. falciparum* (K1 strain), the IC_{50} value of which was 2.6×10^{-8} M. The IC_{50} value of against mouse mammary FM3A cells was 8.0×10^{-6} M, demonstrating that the selective toxicity (307) was also remarkable.

We investigated the *in vivo* antimalarial activities of N-89 by 4-day suppressive test. The ED_{50} value of the compound has shown that 12 mg/kg/day (ip), which was required to cause 50% suppression of *P. berghei* in mice. In the experiments, the dose of 50 mg/kg/day of N-89 causes cure (for 4 mice in 5 tested mice), in which malaria parasites were not observed in circulating blood after 60 days. Furthermore, no side effects such as diarrhea, body weight loss and mortality were observed during treatment with N-89 at doses by 1,600 mg/kg (ip).

As a control, mice treated with artemisinin (50 mg/kg/day, ip) were not cured and mice died due to *P. berghei* infection. These results may be helpful in the design of better chemotherapeutic 1,2,4,5-Tetraoxacycloalkanes in the worldwide fight against malaria.

74 DEVELOPMENT OF NEW ANTIMALARIAL DRUGS —THE SEMI-SYNTHETIC ANTIMALARIAL ALKALOIDS FROM *DICHROA FEBRIFUCA*—

Hye-Sook Kim[1], Yuki Ishizuka[1], Yusuke Wataya[1], Yoshiaki Takaya[2], Hidehisa Tasaka[2] and Yoshiteru Oshima[2]
Faculty of Pharmaceutical Sciences, Okayama University[1] and Faculty of Pharmaceutical Sciences, Tohoku University[2]

A number of medicines such as chloroquine and quinine are available for treatment of malaria, but the rapid development of drug resistance are a serious problem. Medicinal agents based on novel mechanisms of action are, therefore, required to overcome emergence of resistance and to control an ever-increasing number of epidemics caused by the malaria parasite. In China, the roots of *D. febrifuga* have been employed against malaria fevers. Febrifugine and isofebrifugine were isolated as potential antimalarial agents. The febrifugine was once submitted to clinical test, however, it is reported that the compounds have serious adverse

reactions such as vomiting and diarrhea.

As part of a multidisciplinary research program on antimalarial natural products, we are screening plant extracts that are alleged to have antimalarial activity. As the results, we found the semi-synthetic compounds, Df-1 and Df-2 (C-252), from febrifugine and isofebrifugine. Df-1 and Df-2 have potent antimalarial activities with higher therapeutical selectivity than febrifugine against *P. falciparum in vitro* and *in vivo*.

75 STUDIES OF ANTISENSE OLIGONUCLEOTIDE —EFFECTS AGAINST *PLASMODIUM FALCIPURUM*—

NAOMI IKEMOTO[1], HYE-SOOK KIM[1], MAKIKO KANAZAKI[2], YOSHIHITO UENO[2], SATOSHI SHUTO[2], AKIRA MATSUDA[2] AND YUSUKE WATAYA[1]
Faculty of Pharmaceutical Sciences, Okayama University[1] and
Graduate School of Pharmaceutical Science, Hokkaido University[2]

Synthetic oligodeoxynucleotides (ODNs) have been shown to inhibit viral and cellular gene expression by sequence-specific antisense (AS) hybridization. AS ODNs inhibit specific protein synthesis and cell growth. Previously we designed and synthesized nuclease-resistant ODNs, phosphorothioate (PS) ODNs and ODNs containing 4'α-*C*-(2-aminoethyl)thymidines (4'-amino ODNs). In this study, we investigated inhibition of growth and mRNA levels of the malaria parasite, *Plasmodium falciparum, in vitro* culture using those AS ODNs in order to search for new target of antimalarial agent.

We examined antimalarial activity of AS ODNs (20-30 mer) targeting mRNA encoded plasmodial enzymes such as dihydrofolate reductase (DHFR), dihydropteroate synthetase (DHPS) and succinate dehydrogenase (SDH) of *P. falciparum* (FCR-3 strain). AS ODN targeting mRNA of SDH iron-sulfur subunit (IP AS ODN) showed most effective inhibition of growth of *P. falciparum in vitro*. Our results suggested that IP should be essential for parasite growth, and therefore it would be a good target for anti-malarial agents. Furthermore we found that 4'-amino AS ODNs have less non-specific cytotoxicity compared with PS AS ODNs. We performed quantitative RT-PCR assay to determine the IP mRNA level in the parasites after the 24 hr treatment with IP PS AS. As the results, IP mRNA level was strikingly decreased by treatment with IP AS ODN. We guessed that the antimalarial activity of IP AS ODN was resulted in specific decrease of the complementary mRNA level. From the results, we concluded that IP was essential for parasite growth.

These results suggest that SDH is one of the candidates of the target for novel antimalarial drugs. For search and assessment of new drug targets rather than for therapeutic drugs, the combination of AS ODNs and quantitative RT-PCR methods is effective in the case of disease having economical problems such as malaria.

76 RNA EXPRESSION PATTERN OF CATALYTIC SUBUNIT OF MITOCHONDRIAL COMPLEX II FROM *PLASMODIUM FALCIPARUM*

SATORU TAKEO AND KIYOSHI KITA
Department of Biomedical Science, Graduate School of Medicine,
The University of Tokyo

Chemotherapy of malaria using chloroquine and alternative compounds has been followed by the appearance of resistant parasites as shown in many epidemiological studies. The major reason for this vicious cycle is that the modes of action of many antimalarials are poorly understood. This lack of knowledge would be efficiently overcome by exploring the biology of *Plasmodium* in the asexual erythrocytic stage and focusing on preferential targets.

The morphological difference and the favorable effect of low O_2 level on the *in vitro* cultivation of *Plasmodium falciparum* suggests that the physiological

role of malarial mitochondria, including that in energy metabolism, is different from human host. The studies so far did not support the existence of complete TCA "cycle" in *Plasmodium,* but suggested the functional parasite electron transport system. For elucidating the role of this organelle, we have focused on complex II (succinate dehydrogenase, SDH) because it is exclusively mitochondrial enzyme and plays a unique role as a direct link between these two major mitochondrial systems. And furthermore, this enzyme functions as fumarate reductase (FRD), opposite to SDH, under anaerobic condition in some parasites such as adult *Ascaris suum*. Complex II is generally composed of four subunits, and we have already cloned the genes for two catalytic subunits (flavoprotein subunit: Fp and iron-sulfur subunit: Ip) from *P. falciparum*. Northern analysis and RT-PCR also demonstrated the expression of two genes at intraerythrocytic stage parasite.

In the present study, the expression pattern of the transcripts of the gene for Fp was analyzed using gametocyte-producing clone of *P. falciparum* (3D7) cultivated *in vitro*. The pattern of RNA level is most abundant in gametocyte, the sexual stage responsible for malaria transmission to the mosquito vector. In asexual stages (3, 21, 32, 43, 50 hrs after synchronization by sorbitol), transcript level is relatively high at ring form and down-regulated at trophozoite and schizont stages. This result is consistent with that from mitochondrial adenylate translocase (ADP/ATP transporter). The expression pattern of plasmodial 6 Kb element-encoding cytochrome *c* oxidase (electron transport chain enzyme complex IV) subunit I (COI) and cytochrome *b* of ubiquinone-cytochrome *c* oxidoreductase (complex III) (CYb) is also similar to Fp in asexual stages, but they are down-regulated in gametocyte. However, this result of two proteins encoded on the putative malarial mitochondrial DNA is quite different from that reported by Feagin *et al.* (Exp. Parasitol., 80, 430-440, 1995). The result encourages us to confirm the expression pattern using FCR3 strain which rarely produces gametocytes, and analysis is now in progress. It is also interesting from the viewpoint of the co-expression of mitochondrial proteins encoded on different genetic elements because complex II catalytic subunits and ADP/ATP transporter are encoded not on mitochondrial DNA, but on parasite nucleus.

77 PRIMARY STRUCTURE OF THE RHOPTRY PROTEIN, RyRhop100, OF *PLASMODIUM YOELII* MEROZOITE

Michinori Shirano, Takafumi Tsuboi, Mayumi Tachibana and
Motomi Torii
Department of Molecular Parasitology,
Ehime University School of Medicine

All invasive forms of Apicomplexa possess a complex of apical organelles, which comprise rhoptries, dense granules and micronemes. The rhoptries are pear-shaped, electron-dense, membrane-bound organelles and are considered to have roles in host cell attachment, invasion and formation of parasitophorous vacuole membrane. We have developed monoclonal antibodies (mAb) against rhoptry proteins of *Plasmodium yoelii* merozoites. mAb # 25 reacted against 140/125 kDa antigens and mAb # 32 reacted against 100 kDa antigen, respectively. Subcellular localization of the antigens was confirmed by IFA and immunoelectron microscopy.

These antigens appeared on the rhoptries of the *P. yoelii* merozoites. We affinity purified 140 kDa and 100 kDa *P. yoelii* rhoptry proteins by using the affinity column conjugated with mAb # 25. After partial purification, we analyzed the partial amino acid sequences of the *P. yoelii* RhopH proteins. We synthesized several sets of degenerate oligonucleotides based on the amino acid sequences of peptides obtained from the 100 kDa-rhoptry protein of *P. yoelii*. By using these primers, we partially cloned the gene which encode PyRhop100 from the genomic DNA and cDNA of *P. yoelii* merozoites. Then, we synthesized three synthetic peptides based on the deduced amino acid sequences of PyRhop100. By using the mouse immune-sera against these synthetic peptides, we detected the positive IFA-staining of *P. yoelii* merozoite rhoptries. We also detected that the homologue of PyRhop100 protein was a 105 kDa RhopH3 of *P. falciparum* by the database search in about 40% amino acid sequence identities.

In comparison with these amino acid sequences of PyRhop100 and RfRhopH3, almost all the cysteine residues were conserved between PyRhop100 and

PfRhopH3. These results suggest that the tertiary structure of the PyRhop100 and PfRhopH3 proteins have a biological importance on the merozoite invasion into the erythrocytes.

78 DETECTION OF THE SEQUENCE POLYMORPHISM IN *PLASMODIUM VIVAX* TRANSMISSION-BLOKING VACCINE CANDIDATE ANTIGENS BY USING PCR-SSCP

Takafumi Tsuboi, Miyuki Yano, Mayumi Tachibana and Motomi Torii
Department of Molecular Parasitology,
Ehime University School of Medicine

In many malarious regions outside of Africa, development of transmission-blocking vaccine will require activity against both *Plasmodium falciparum* and *P. vivax* if the vaccine is to have a large impact on the overall morbidity due to malaria in that region. We have recently cloned the genes, Pvs25 and Pvs28, encoding two major surface proteins on *P. vivax* zygote/ookinetes, which are malaria transmission-blocking vaccine candidates. Polymorphism of malaria parasite antigens makes the determination of their amino acid sequence diversity in field isolates prior to vaccine trials a prudent exercise. The ookinete surface proteins of *P. falciparum,* Pfs25 and Pfs28, which are thought to be expressed predominantly while the parasite resides in the mosquito, are usually highly conserved for malaria surface antigens. Only two conserved amino acid substitutions were found in Pfs25 and one conserved substitution in Pfs28 to date.

In order to characterize the sequence polymorphism of Pvs25 and Pvs28, we PCR-amplified and determined the DNA sequence of these two genes in genomic DNA of *P. vivax* extracted from a laboratory strain, Sal1, an Indian isolate and several Bangladesh isolates. In Pvs25, we found only three point mutations that would result in amino acid substitutions. We also succeeded to distinguish the four gene types results from the above point mutations of Pvs25 by using PCR-SSCP.

In contrast, the Pvs28 gene had more point mutations. The most striking variation we detected in Pvs28 was the four to seven tandem repeats of GSGGE/D. We also tried to distinguish the sixteen gene types results from the above point mutations of Pvs28 by using PCR-SSCP, however, we could not distinguish these gene types by using PCR-fragments in full length (694 bp). Finally, we succeeded to distinguish the twelve Pvs28 gene types out of sixteen by using PCR-fragments digested with BsmI (125/336/233 bp).

79 INHIBITORY EFFECT OF DINITROANILINE HERBICIDES ON THE GROWTH OF *ENTAMOEBA HISTLYTICA*

Asao Makioka[1], Masahiro Kumagai[1], Hiroshi Ohtomo[1],
Seiki Kobayashi[2] and Tsutomu Takeuchi[2]
Department of Tropical Medicine, Jikei University School of Medicine[1] and
Department of Tropical Medicine & Parasitology,
Keio University School of Medicine[2]

Effect of dinitroaniline herbicides oryzalin and trifluralin on the growth of *Entamoeba histolytica* was examined. Oryzalin inhibited the growth of *E. histolytica* strain HM-1:IMSS. Trifluralin was less effective than oryzalin for this parasite. *E. histolytica* was more resistant to these dinitroanilines than other parasitic protozoa examined so far, including *Leishmania* species, *Trypanosoma brucei, Plasmodium falciparum, Toxoplasma gondii* and *Cryptosporidium parvum.* Colchicine, a potent microtubule inhibitor of animal cells, was much less effective for *E. histolytica* even at very high concentrations.

A reptilian parasite *E. invadens* strain IP-1, examined for comparison, was more resistant to these dinitroanilines than *E. histolytica.* Accumulation of *E. histolytica* trophozoites at mitotic stage was observed

during culture with 100 μM of oryzalin. The inhibitory effect of oryzalin on the growth of *E. histolytica* was abrogated by removal of the drug that was exposed to trophozoites at a concentration of 100 μM for 2 days.

In parallel to the recovery of growth after removal of the drug, percentage of trophozoites at mitotic stage reduced to normal level. Thus the results indicate that treatment of trophozoites with oryzalin arrests their mitosis and its effect is reversible. Therefore, oryzalin is a useful molecular tool for studies relating to cell cycle of this parasite.

80 A STAGE-SPECIFIC IMMUNODOMINANT GLYCOPROTEIN APPEARED IN ENCYSTING *ENTAMOEBA INVADENS*

ASAO MAKIOKA[1], MASAHIRO KUMAGAI[1], HIROSHI OHTOMO[1],
SEIKI KOBAYASHI[2] AND TSUTOMU TAKEUCHI[2]
Department of Tropical Medicine, Jikei University School of Medicine[1] and
Department of Tropical Medicine & Parasitology,
Keio University School of Medicine[2]

Appearance of cyst-specific proteins in encysting *Entamoeba invadens* and their immunogenicity were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting using an axenic encystation system *in vitro*. A rabbit antiserum against trophozoites of *E. invadens* reacted with a number of proteins of cysts after 1-4 days of encystation. Thus, a number of cyst proteins remained antigenically unchanged as common antigens of the two forms after transformation from trophozoites to cysts.

A rabbit antiserum against cysts also reacted with the trophozoite proteins as well as the cyst ones. The most interesting results were that the rabbit anti-cyst serum reacted predominantly with an 88 kDa protein of cysts after 1 day of encystation. The 88 kDa protein reacted with the anti-cyst serum absorbed with trophozoite proteins so that it was cyst-specific. Reactivity of the 88 kDa protein of cysts with the absorbed anti-cyst serum decreased as encystation proceeded.

When soluble and particulate fractions prepared from cysts after 1 day of encystation were examined by electrophoresis and immunoblotting, the 88 kDa protein that reacted with the absorbed anti-cyst serum was found to be present in the particulate fraction which was rich in the cell wall fragments and stained with the periodic acid-Schiff's reagent, indicating that it is a glycoprotein. The results indicate that encystation is accompanied by appearance of the cyst-specific 88 kDa glycoprotein which is immunodominant and most abundantly expressed in cysts after 1 day of encystation and appears to be associated with the cyst wall.

81 QUANTITATIVE CHANGE OF THIOL COMPOUNDS WITH THE GROWTH OF *ENTAMOEBA HISTLYTICA*, IN THE SUPERNATANT OF EXOGENOUS CYSTEINE FREE AXENIC CULTURE MEDIUM

NOBUKO NANGI, MASANORI KOBAYASHI AND TSUTOMU TAKEUCHI
Department of Tropical Medicine & Parasitology,
Keio University School of Medicine

Cysteine is known as an essential and specific compound for the growth of *Entamoeba histolytica* (E.h) in TYI-S-33 axenic culture system (Gillin and Diamond, 1981, 1984). And in this culture system, E.h cannot grow without one of either exogenous cysteine (or cystine) or ascorbic acid. We designed and established a newly system [C&A (−) system] removed exogenous cysteine (or cystine) and ascorbic acid from filter sterilized modified YI-S medium (Diamond, 1995), for analysis of cysteine synthesized by E.h itself. Exogenous ascorbic acid is known as the only compound to make successful culture of axenic E.h possible in exogenous cysteine (or

cystine) free TYI-S-33 medium (Gillin and Diamond, 1981).
[Characteristics of amebae in C&A (−) system] 1) The doubling time of trophozoite of E.h (HM-1:IMSS cl 6) growing in C&A (−) system did need about two times as long as that in filter sterilized YI-S medium. 2) Intracellular vacuoles of HM-1:IMSS cl 6 (Clone 6) diminished the size electron microscopically as compared with same strain of amebae in TYI-S and filter sterilized YI-S medium.
[Results of SH quantitative assay in C&A (−) system] 1) Amount of SH compounds in the supernatant of ameba (Clone 6) growing medium increased with the growth of Clone 6. 2) Released cysteine proteinase from Clone 6 seemed form a part of this SH compounds. Because activity of cysteine proteinase in the supernatant also increased with the growth of Clone 6.
[Discussion] E.h can grow without exogenous cysteine (or cystine) or ascorbic acid. However, the E.h grown in C&A (−) system were broken easily when transferred into isotonic buffer system as compared with E.h grown in cysteine containing medium. It seems that E.h requires plenty of cysteine for the stable growth, and exogenous cysteine is most available supplement.

82 CONGENITAL CHAGAS' DISEASE AND ITS ENVIRONMENTAL FACTORS IN SANTA CRUZ, BOLIVIA

SACHIO MIURA[1], KEIICHI FUKUDA[2], KENSUKE SEKI[3],
MASAHIRO HORIO[4] AND TSUTOMU TAKEUCHI[1]
Department of Tropical Medicine & Parasitology[1] and
Department of Internal Medicine[2], Keio University School of Medicine
Department of Environmental Health,
School of Health Sciences, Kyorin University[3] and
Department of Parasitology and Tropical Public Health,
University of Occupational and Environmental Health[4]

The reported birth number of congenital infection of Chagas' disease in Bolivia shows marked difference compared with its neighbor countries. We researched the inhabited environment of patient's and the living condition of triatomine bugs to prevent Chagas' disease.

In the prefectural maternity hospital in Santa Cruz, Bolivia, the antibody against *T. cruzi* of pregnant women were examined by IHA and congenital Chagas' disease of newborn were diagnosed by Strout method using umbilical cord blood. We researched about the following, inhabited environment of the newborn which were diagnosed as congenital Chagas' disease; the living condition of *Triatoma* around the house, the structure of the house, the inhabited condition of other blood-sucking insects and the possibility of contact with reservoir host. We also studied about awareness for Chagas' disease and serodiagnosis by IHA to inhabitant and researched about the prevalence of Chagas' disease in this area.

The newborn babies of congenital infection were 31 cases out of 1,936, and more than 20 cases were detected *T. cruzi*-DNA by PCR. Half of the congenitally infected babies live in the environment where the vector inhabits in without any treatment, and need immediately prevent measure against Chagas' disease.

83 TRIAL TO DETECT OF *TRYPANOSOMA CRUZI* DNA FROM DRIED FECES OF TRIATOMINE BUGS

SHINJIRO HAMANO[1], MASAHIRO HORIO[2], SACHIO MIURA[3], HIROO HIGO[1],
KANAMI NODA[1], ISAO TADA[1] AND TSUTOMU TAKEUCHI[3]
Department of Microbiology (Parasitology),
Graduate School of Medical Sciences, Kyushu University[1],
Department of Parasitology, Faculty of Medicine,
University of Occupational and Environmental Health[2] and
Department of Tropical Medicine & Parasitology,
Keio University School of Medicine[3]

From a viewpoint of public health, it is important to examine the distribution of triatomine and the infection rate of triatomines living in the houses. However, collecting live triatomes in the field involves a great deal of trouble and risk. In this study, we tried to detect *T. cruzi* DNA in Triatomine dried feces collected from the wall of a brick-built house in Bolivia, where is endemic area for Chagas' disease.

The primers (P35/P36) were chosen to amplify the conserved resion within the minirepeats of *T. cruzi* kinetoplast DNA minicircles. The primers were confirmed to amplify 330 bp long product from different strains of *T. cruzi* with typical zymodem patterns, including isolates both from human and triatomine and from Central and South America. Triatoma infestans naturally infected with *T. cruzi* in Bolivia allowed to excrete feces on filter paper. The filter papers with fresh feces were preserved in the incubator at 25°C for 2 weeks to 6 months or at 40°C for 4 weeks. *T. cruzi* DNA was detected in the dried feces by PCR even after the exposure to an artificial environment for 6 months. Furthermore, DNA of *T. cruzi* was actually detected in the dried feces collected from the wall of a brick-built house in Bolivia. The results indicate that PCR can be a good tool for surveillance of the infection rate of triatomines which live in crevices in the walls, roofs and holes of poor houses.

84 VARIATION OF PROTEINS AMONG DIFFERENT ISOLATES OF *TRYPANOSOMA CRUZI*

JIANG-BING MU[1], TOSHIO SONE[1], TETSUO YANAGI[2], CARIOTA MONTROY[3],
MIHOKO KIKUCHI[1], ISAO TADA[4] AND KENJI HIRAYAMA[1]
Department of Medical Zoology, Saitama Medical School[1],
Department of Protozoology, Institute of Tropical Medicine,
Nagasaki University[2],
Department of Cytohistology, Faculty of Chemical Science and Pharmacy,
San Carlos University, Guatemala[3] and
Department of Parasitology, Faculty of Medicine, Kyushu University[4]

Trypanosoma cruzi is a causative parasitic protozoon for Chagas' disease in South and Central America. Previous studies on isozyme pattern and DNA polymorphism analyses have been carried out about various isolates of *T. cruzi,* and the genetic variations among individual zymodemes have been reported. To estimate the variability within the species of *T. cruzi,* two-dimensional gel electrophoresis (2D-PAGE) of the proteins extracted from 21 isolates of epimastigote stage of *T. cruzi* was performed.

Epimastigote form of *T. cruzi*, isolated in Guatemala, Colombia, Brazil, Peru and Chile, was grown in Liver infusion tryptose (LIT) medium. Those were harvested and were lysed by PBS containing 2% Triton X-100. Soluble proteins obtained were examined to separate in the gel by 2D-PAGE according to the method of O'Farrell. The polypeptide spots were detected on the gel after staining with coomassie brilliant blue R-250.

Move than 400 polypeptide spots were detected in

each of 21-gel profile. We compared the presence or absence of the spots between the two isolates in all combinations to calculate percent difference. The values obtained were divided into two groups, one showed over 6% and another was less than 3%. Twenty-one isolates were clearly classified into three groups, in case that the isolates showing 3% spot difference were clustered into the same group. *T. cruzi* among the other groups may be genetically more variable than those in a same group.

85 IMMUNOGENETIC ANALYSIS OF CHAGAS' DISEASE

KAYANO AIDA[1], SANDRA JUARES[1], MIHOKO KIKUCHI[1], KENJI HIRAYAMA[1],
TETSUO YANAGI[2], MARIA PAULA LEON[3], SATOSHI KANEKO[4], OSCAR AYAU[4], TOSHIO SONE[1],
AKIKATA KIMURA[5], KYOGO ITO[6] AND ISAO TADA[7]
Department of Medical Zoology, Saitama Medical School[1],
Department of Protozoology, Institute of Tropical Medicine,
Nagasaki University[2],
Department of Histocytology, School of Pharmacy,
San Carlos University, Guatemala[3], JICA Guatemara[4],
Department of Molecular Pathogenesis, Division of Adult Disease,
Medical Research Institute, Tokyo Medical and Dental University[5],
Department of Immunology, Faculty of Medicine, Kurume University[6] and
Department of Parasitology, Faculty of Medicine, Kyushu University[7]

Chagas' disease is caused by a protozoan parasite *Trypanosoma cruzi*. Whether the infected individuals are able to eliminate the organism or develop chronic Chagas' disease with cardiopathy is unpredictable. In the present study, to reveal the host genetic factors that influence susceptibility to Chagas' disease, we typed 44 unrelated, seropositive Chagas' patients at the Department of Cardiology, Zacapa National Hospital, Guatemala, and 138 seronegative controls for polymorphisms of HLA-DQB1 and DPB1.

Thirty-one alleles of HLA-DPB1 were detected in the subjects. The frequency of HLA-DPB1*5101 was increased in the patients ($\chi^2=7.31$, P=0.0068, Pc=0.19, OR=3.43, 95%Cl 1.36-8.68). Twenty alleles of HLA-DQB1 were detected, however, there was no association between HLA-DQB1 alleles and seropositivity. We have reported that HLA-B35 and MICA-A5 increased the risk for Chagas' disease, and that the effects of the two alleles on susceptibility were synergistic.

In the present study, there was positive linkage disequilibrium between HLA-DPB1*5101 and HLA-B35. In conclusion, we suggest that HLA class I influences the susceptibility for Chagas' disease more than HLA class II.

86 ANTIPROTOZOAN ACTIVITY OF MARINE SPONGE EXTRACTS

TAKESHI MIZUSHIMA[1], YUKO KATAKAI[1], SUKUMAWATI BASUKI[1], YASUYUKI GOTO[1],
YOICHI NAKAO[2], SHIGEKI MATSUNAGA[2], NOBUHIRO FUSETANI[2],
TAKASHI ONODERA[1] AND YOSHITSUGU MATSUMOTO[1]
Department of Molecular Immunology[1] and Department of Marine Biochemistry[2],
School of Agricultural and Life Sciences, The University of Tokyo

Discovery of new therapeutic agents against protozoan diseases is in urgent need. Marine sponges have received much attention as potential sources of such new agents. In this study, antiprotozoan activity of marine sponge extracts were examined using *in vitro* culture of erythrocytic stages of *Plasmodium falciparum* FCR-3 and promastigotes of *Leishmania donovani* (MHOM/IN/80/DD8) and *L. amazonensis* (MPRO/BR/72/M1845). Ninety three sponge specimens were obtained from the East China Sea. Hydrophilic and

hydrophobic fractions were prepared by partition between chloroform and water of ethanol extract from each specimen. To analyze *in vitro* inhibitory effects of samples on proliferation of *Leishmania* parasites, Tetracolor One proliferation assay kit (Seikagaku Corporation, Japan) was used. For evaluation of malarial parasite growth, direct counting of infected erythrocytes were performed using Giemsa stained smears. Two hydrophobic samples (E23 and E65) showed 100% growth inhibitory effect to *P. falciparum* at the concentration of 1 μg/m*l*. On the other hand, the 50% growth inhibitory concentrations (IC_{50}s) of E34, E45 and E65 to *L. donovani* were 0.8, 0.3 and 0.3 μg/m*l*, respectively. E34 and E65 were from *Theonella* sp. and *Mycale* sp., respectively, and the sponge from which E45 was extracted is unidentified. Cytotoxity to p388 murine leukemia cells (JCRB17) was also examined. IC_{50}s of E23, E34, E45, and E65 to p388 were 5 μg/m*l*, 2.2 μg/m*l*, 2.2 μg/m*l*, and 0.004 μg/m*l*, respectively. These samples were further separated on ODS short columns with stepwise gradient solvent systems {50%, 75%, 100% methanol and chloroform-methanol-water (7:3:0.5)}. 75% methanol eluted fractions were the most effective in each sample (IC_{50} of E65 to *P. faliciparum* = 2 ng/m*l*, IC_{50}s of E34, E45, and E65 to *L. donovani* = 100 ng/m*l*, 30 ng/m*l*, and 8 ng/m*l*, respectively). These results suggest marine sponge contain antiprotozoan chemicals and might be a potent source of new antiprotozoan agents.

87 NUCLEOTIDE SEQUENCE VARIATIONS OF THE CYTOCHROME C OXIDASE SUBUNIT I GENE WITHIN *PHLEBOTOMUS* SPECIES, VECTOR INSECTS FOR LEISHMANIASIS

CHIZU SANJOBA[1], MIKIKO YAMAUCHI[1], YUSUF OZBEL[2], TETSUHIKO SASAKI[3], YASUNOBU MATSUMOTO[1], TAKASHI ONODERA[1] AND YOSHITSUGU MATSUMOTO[1]
Department of Molecular Immunology,
School of Agricultural and Life Sciences[1],
Department of Biological Sciences[3], The University of Tokyo and
Department of Parasitology, Ege University[2]

Leishmaniasis is transmitted by vector insects belonging to the genus *Phlebotomus* in Old World and the genus *Lutzomyia* in New World. Morphological identification of species within the genus *Phlebotomus* is complex and causes controversy sometimes. In an attempt to characterize objectively the various species, the nucleotide sequence of a region of the mitochondrial cytochrome C oxidase subunit I (COI) gene was determined for four species of *Phlebotomus, i.e., Phlebotomus papatasi, P. sergenti, P. major* and *P. simici*. Total DNA was extracted separately from three individuals from each species which were collected in Turkey and identified morphologically by Dr. Yusuf Ozbel. PCR was carried out using 2.5 μl of extract DNA as the template, 25 pmol of the primers, 100 μM dNTPs, 1 mM $MgCl_2$, 2.5 U Taq DNA polymerase in the buffer recommended by the manufacturer.

The primers were designed based on the COI sequence published for *Drosophila yakuba*. After amplification, the amplified products were purified and cloned into the vector pT7 Blue. Sequencing of the clones was performed by the dideoxy chain-termination method. The sequence of *D. yakuba* was also examined for comparative analysis. The COI gene sequence obtained was the same in length (314 bp) among all specimens. The nucleotide sequence analysis of the COI gene showed that *P. simici, P. papatasi* and *P. sergenti* are 84.3% (265/314 bp), 85.3% (268/314 bp) and 83.4% (262/314 bp) identical to *P. major,* respectively. *P. papatasi* and *P. sergenti* are 84.0% (264/314 bp) and 83.1% (261/314 bp) identical to *P. simici,* respectively. *P. papatasi* and *P. sergenti* have the lowest homology at 80.8% (254/314 bp). The four species of *Phlebotomus* were clearly distinguished based on the nucleotide sequences. The identification of *Phlebotomus* species using COI gene nucleotide sequences were correspondent with the morphological identification. We thus conclude that nucleotide sequences of the COI gene can be used for reliable identification of *Phlebotomus* species.

88 PCR-BASED DIFFERENTIATION OF THE SPECIES COMPLEXES IN *LEISHMANIA* SPP.

Shin-ichiro Kawazu[1], Ken Katakura[2], Koichi Nagakura[3], Toshimitsu Hatabu[4], Mamoru Ito[5], Yutaka Nakai[6], Masamichi Aikawa[7], K.-P. Chang[8], Tsugihiko Kamio[9], Shigeyuki Kano[1] and Yoshitsugu Matsumoto[4]
Research Institute of International Medical Center of Japan[1],
Department of Parasitology, Gunma University School of Medicine[2]
Tokai University School of Medicine[3],
School of Agriculture and Life Science, The University of Tokyo[4],
Central Institute of Experimental Animals[5],
Department of Animal Microbiology and Parasitology, Tohoku University[6],
Research Institute of Medical Science and Technology,
Tokai University[7] and
Finch University of Health Sciences/The Chicago Medical School[8] and
National Institute of Animal Health[9]

Leishmaniases are vector-borne and largely zoonotic diseases caused by parasitic protozoa in the genus *Leishmania*. The genus comprises some 30 species of mammalian parasites, of which 21 are known to infect humans. As a matter of clinical convenience, they are often classified into four assemblages (species-complex) according to both the geographic origin (Old World and New World) and the clinical types of the disease they produce (inapparent infections to various forms of cutaneous and visceral diseases). The classification of the parasites based on the species-complex often provide useful information for understanding the epidemiology of the disease, for example when characterize the parasite stocks isolated from sandfly vectors or the reservoir animals.

The gene encoding ζ-crystallin/NADPH oxidoreductase homologue (p36) is single-copied per haploid genome highly conserved in the *Leishmania* spp. The p36 gene has been reported to be useful marker to classify the parasite stocks into the four species-complexes. In the present study, we PCR-amplified 976 bp of this ~1 kb gene from 7 isolates, representing *L. tropica* complex or *L. donovani* complex. Following amplification, the genes were clearly differentiated using the restriction enzymes sites that were not shared between them. The result indicated that the p36 gene was suitable for differentiation of the 2 species-complexes that coexisted in the disease-endemic foci in Eurasian and African continents.

89 ULTRASTRUCTURAL AND PATHOLOGICAL STUDY OF THE LESION OF DIFFUGE CUTANEOUS LEISHMANIASIS (DCL) PATIENT

Motoyoshi Maruno[1], Noor Mohammed Kaskhely[1], Atsushi Takamiyagi[1], Hiroshi Uezato[1], Shigeo Nonaka[1], Gomez E.A. Landries[2] and Yoshihisa Hashiguchi[3]
Department of Dermatology, Faculty of Medicine,
University of the Ryukyus[1], Deparatmento de Medicina Tropical,
Universidad Catolica Santiago de Guayaquil, Ecuador[2] and
Department of Parasitology, Kochi Medical School[3]

We examined the ultrastructural and pathological difference of skin lesion between improving and worse period of a male DCL patient in Ecuador, South America. Together, we compared with LCL (localized cutaneous leishmaniasis) skin lesion. Skin samples were taken from the same lesion in both period and cut into two pieces for light and electron microscopic examination.

Immunohistochemical study using anti-human lysozyme antibody (Dako Kyoto, Japan) was carried

out following the instruction manual. Electron microscopic examination was performed using JEOL 2000EX electron microscope (JEOL, Japan).

Staining intensity to anti-human lysozyme antibody of the specimen was weak in skin lesion of both period. Number of positive macrophage cells to the antibody was a bit increased in improving period than worse period. On electron microscopic examination, large parasitophorous vacuole (PV) formation of macrophages was markedly observed in both period. In worse period, PVs containing several *leishmania* parasites were filled with electron dense materials and surrounded by single membrane. The cell organelli of the macrophages were well developed. In contrast, PVs in improving period showed very few *leishmania* parasites within them and also be transparent higher than worse period, losing single PV membrane. Cytoplasm of the macrophages appeared with degeneratic change as we couldn't distinguish the cell organelli. Collagen fibers were increased between the macrophages in improving period. PV formation was unclear in LCL specimen. Macrophages showed degeneratic cytoplasm as like breaking up, with dense, dark nucleus. These findings suggested that PV appearance was likely to be correlative with disease condition and/or prognosis.

90 A CASE OF CEREBRAL SCHISTOSOMIASIS MANSONI PROBABLY INFECTED AT MACEIÓ, ALAGOAS, BRAZIL

YASUAKI UEDA[1], TAKAYUKI KAWANO[1], MASAYUKI NITTA[1], MASATO KOCHI[1], YUKITAKA USHIO[1], MASATAKA KORENAGA[2] AND YOSHIHISA HASHIGUCHI[2]
Department of Neurosurgery, Kumamoto University of Medical School[1] and Department of Parasitology, Kochi Medical School[2]

Central nervous system (CNS) may be involved in chronic schistosomiasis. It is fairly common in chronic *Schistosoma japonicum* infection but any schistosome species may affect CNS. Although it is rare, *S. mansoni* may involve CNS by embolization of eggs or ectopic migration of the adult worms. This paper demonstrated a case of cerebral schistosomiasis caused by *S. mansoni* in a 21-year-old Japanese male, who was in Maceió, Alagoas of Brazil for 3 years to study association football after graduation of junior high school. The patient presented on July 7, 1998 with headache for 3 days. Computerized tomography (CT) showed a hyperdense, enhancing lesion located in the left temporal lobe. Magnetic resoance imaging (MRI) of brain showed iso-intensity areas within a low intensity area on T1-weighted images and iso-intensity areas within a high intensity area on T2-weighted images. Contrast enhancement MRI revealed enhancing multipartite lesions in the same area.

Magnetic resonance angiography did not show tumor in the lesions; only a displacement was observed above the left middle cerebral artery. The patient was hospitalized with a suspected case of high grade glioma. Neurological findings were normal except slightly increased tendon reflexes. Both blood picture and blood chemistry were within normal limits. Peripheral blood eosinophil count was 5.1%. The temporal lobotomy was performed and biopsy showed parasitic eggs in the tissue sections without having malignancy; hence only apparent lesions were removed. After lobotomy the patient did not declare palsy, aresthesia, or logopathy. The removed tissue was examined and diagnosed as granuloma caused by *S. mansoni* eggs. The patient was treated with praziquantel (600 mg, 3T 3×1). In the follow-up of the patient, no abnormality was detected in liver CT and no parasite egg was found in feces.

91 APPEARANCES OF THE ECHOGENIC PATTERN OF THE NETWORK AMONG SCHOOL CHILDREN IN THE HIGHLY ENDEMIC AREA WITH *SCHISTOSOMA JAPONICUM* IN THE PHILIPPINES

HIDEHIRO OTAKE[1,2], YUICHI CHIGUSA[2], HIROSHI OHMAE[3], MASATAKA HAYASHI[4], EUNICE ILAGAN[5], MASASHI KIRINOKI[2] AND HAJIME MATSUDA[2]
Faculty of Nursing, Kure University[1], Dokkyo University School of Medicine[2], Institutes of Basic Medical Sciences and Clinical Medicine, University of Tsukuba[3], Kofu City Hospital[4] and Schistosomiasis Control Team, Oriental Mindoro, Philippines[5]

We have not been reported for the new patient with *S. japonicum* after 1980 when the ultrasonographic investigation was introduced for schistosomiasis cases in Japan. It means ultrasonographic examinations were done for the treated, non-reinfected and adult cases in Japan. Several investigations have been followed in some endemic countries for adult cases since then.

All those reports had showed the adult cases of the network pattern. Therefore, the echogenic pattern of the network could appeared at the only adult patients who had kept for the long standing infection. Our investigation showed the echogenic pattern of the network on 11 young patients (9 to 14 years old) among school children at the highly endemic area in Mindanao Island, Philippines.

92 URINE CYTOLOGY IN ENDEMIC AREA OF SCHISTOSOMIASIS HAEMATOBIUM

TOMOHARU OHKI[1], HIDEHIKO YAMAUCHI[1], MASANOBU ANAMI[2], MASACHIKA ISEKI[3], D.K. MIGWI[4], F.B.M. KILLIKU[4], N.D. MUHOHO[4], TATSUYA KATSUMATA[1] AND YOSHIKI AOKI[1]
Department Parasitology[1], Department of Pathology[3], Institute of Tropical Medicine, Nagasaki University, Department of Pathology, Nagasaki University Hospital[2] and Kenya Medical Research Institute[3]

Schistosomiasis haematobium is classified as group 1 carcinogens to humans by International Agency for Research on Cancer (WHO). However only one study on the incidence of bladder cancer associated with schistosomiasis haematobium has been reported in Egypt (1980). Therefore We initiated the urine cytology to know if schistosomiasis haematobium influences the incidence of bladder carcinoma and squamous metaplasia in Kenya.

The urine cytology was done from the individuals who were all 20 years old and above from an endemic area of Tserezani where no antibilharzia control measures have been carried out and from a non-endemic area of Kiambu. For the cytological diagnosis, the Papanicolau classification (class I to class V) was adopted. In Tserezani the urine examination for *Schistosoma haematobium* was also carried out by nucleopore filtration method.

In Tserezani, the prevalence and intensity of infection were 41.6% and 1.1 eggs per 10 ml of urine in 261 individuals examined (74 males and 187 females, mean age 37.4±14.5 year old). Among them 4 cases were diagnosed by urine cytology as class III and 7 as class II. Nine out of these 11 cases were accompanied with squamous metaplasia. All the cases were females below the age of 50 years old. In five of the nine cases the eggs were identified in the urine.

In Kiambu, all were diagnosed as class I without any cases of squamous metaplasia in 294 individuals examined (78 males and 216 females, mean age 46.4± 17.6 years old).

Thirty three cases (12.6%) in Tserezani and 16 cases (5.4%) in Kiambu were suspected urinary tract infection by the presence of neutrophilia in the urine.

In conclusion, the cases with squamous metaplasia and the suspected cases with urinary tract infection were found to be significantly higher from the endemic area than those from the non-endemic area ($p<0.05$).

These results suggest that schistosomiasis haematobium might influence the incidence of squamous metaplasia and urinary tract infection.

93 VACCINATION OF DOMESTIC PIG WITH RECOMBINANT PARAMYOSIN AGAINST *SCHISTOSOMA JAPONICUM* IN CHINA

KENJI HIRAYAMA[1], TAKESHI NARA[2], HONGGEN CHEN[3], XIANJUN ZENG[3], MASAO SATOH[1], S. ZHANG[3] AND SOMEI KOJIMA[4]
Department of Medical Zoology, Saitama Medical School[1],
Department of Parasitology, Juntendo University School of Medicine[2],
Jiangxi Provincial Institute of Parasitic Diseases[3] and
Department of Parasitology, Institute of Medical Science,
The University of Tokyo[4]

Paramyosin (PM), a myosin-like protein is a major antigen on *Schistosoma japonicum* (Sj). We reported that passive transfer of a monoclonal IgE SjE18ε.1 which recognizes PM of Sj (SJPM), partially protected mice from challenge infection. In the present study, we developed an experimental model system of schistosomiasis japonica with domestic pigs in China and used it for the evaluation of vaccination with recombinant SJPM (rSJPM). Sixteen-week-old pigs were successfully infected by dermal penetration of 120 cercariae of a domestic strain of Sj (50-60% worm recovery 11 weeks after challenge).

The pigs vaccinated with 400 UV attenuated cercariae showed a reduction of worm recovery (49-53%, $p < 0.001$). The experimental groups were immunized intradermally with rSJPM and alum or TiterMax and were partially protected against the challenge infection (32-35% reduction).

94 ANALYSIS OF THE EGGSHELL PRECURSOR PROTEIN OF *SCHISTOSOMA JAPONICUM* USING SYNTHETIC PEPTIDES AND ANTISERA AGAINST THEM

HIROMU SUGIYAMA AND MASANORI KAWANAKA
Department of Parasitogy, National Institute of Infectious Diseases

We have isolated a cDNA clone encoding part of the 34 kDa eggshell protein of *Schistosoma japonicum* from an adult female library with a rabbit antiserum raised against the 34 kDa female worm fraction (Int. J. Parasitol., 27, 811, 1997). This clone, designated Sj23A, contains an open reading frame of 230 nucleotides coding for a polypeptide of 76 amino acids. According to the deduced amino acid sequence of Sj23A, overlapping peptides were synthesized. They represented the following amino acid residues of the deduced polypeptide: 7 to 26 (peptide 1), 10 to 29 (pep. 2), 19 to 38 (pep. 3), 27 to 46 (pep. 4), 29 to 48 (pep. 5), 38 to 57 (pep. 6) and 45 to 64 (pep. 7). An extra cysteine residue was added to the amino terminus of each synthetic peptide. Each of the synthetic peptides was tested by ELISA for its binding ability to the anti-34 kDa female protein antiserum, which was employed to screen Sj23A. Of the 7 peptides, peptide 4 showed the highest binding activity to the antibody.

Antisera against the peptides were produced by hyperimmunization of rabbits. With the antiserum against peptide 4, the band of 34 kDa was recognized in the female worm extracts by SDS-PAGE and immunoblotting analysis. By immunohistochemical analysis, a positive reaction was observed in the vitelline cells of the vitelline glands of the female worms. These data indicated that the antibody against the synthetic peptide (peptide 4) is available to analyze the eggshell gene product, namely, the eggshell precursor protein of *S. japonicum*.

95 MOLECULAR PHYLOGENETIC RELATIONSHIPS BETWEEN THE *S. INDICUM* GROUP AND OTHER SPECIES OF *SCHISTOSOMA*

TAKESHI AGATSUMA[1], MORITOSHI IWAGAMI[2], R.P.V.J. RAJAPAKSE[3] AND M.M.H. MONDAL[4]
Department of Environmental Health Science, Kochi Medical School[1],
Department of Biological Resource Science,
Obihiro University of Veterinary Medicine and Agriculture[2],
Department of Veterinary, Para-Clinical Studies,
University of Perageniya[3] and
Department of Veterinary Medicine, Bangladesh Agricultural University[4]

Traditionally, *Schistosoma* species have been arranged in groups based on egg morphology and the genus or family of intermediate host. The *S. japonicum* group have eggs with a minute spine and develop in the operculate snails, like the *Oncomelania* species. On the other hand, species of the *S. mansoni* group has eggs with a lateral spine and utilize pulmonate snails, *Biomphalaria,* while the *S. haematobium* group has eggs with a terminal spine and develop in the genus, *Bulinus*. On the other hand, members of the *S. indicum* group (*S. indicum, S. spindale, S. nasale* and *S. incognitum*) develop in snails of Planorbiidae and Lymnaeidae, having subterminal-spined eggs. In the present paper, phylogenetic relationships between members of the *S. indicum* group and other Asian species of *Schistosoma* was examined using DNA sequence data from part of the mitochondrial cytochrome C oxidase subunit I (COI) gene and the second internal transcribed spacer (ITS2) of the ribosomal gene repeat.

The African schistosomes tend to form well resolved groups on the tree, strongly supported by bootstrapping, and the isolates of *S. japonicum* always form a single clade. In all analyses, *S. malayense* is associated with *S. mekongi* rather than with *S. japonicum* (Blair *et al.,* 1997). On the other hand, *S. sinensium* was placed within the Asian groups, and positioned to near the root of the tree. Tree analysis also showed that *S. incognitum* appeared as the sister group to all remaining species, and surprisingly all other three species, *S. indicum, S. spindale* and *S. nasale* were included into the Arican cluster. The present study suggested that Asian species of *Schistosoma* comprised of several genetically distant groups which may have migrated into the Asian continent at the different periods as well as at the different ways.

96 A CASE OF ELEPHANTIASIS CAUSED BY PRIMARY LYMPHEDEMA, DIFFERENTIAL DIAGNOSED FROM FILARIASIS

MIKIMASA NOGUCHI[1], CHIE KAN[1], MITSUHIRO KAMIYOSIHARA[2], HISANORI YAJIMA[3], OSAMU ISHIKAWA[4] AND MAMORU SUZUKI[5]
Division of Dermatology[1], Division of Cardiovascularsurgery[2] and
Division of Urology[3], Maebashi Red Cross Hospital,
Department of Dermatology[4] and Department of Parasitology[5],
Gunma University School of Medicine

A 32-year-old man with elephantiasis caused by primary lymphedema was reported, who presented with 14-years history of severe edema in both of his legs. His past history included protenuria at the age of 10, but his family history was noncontributory. He has no experience to go abroad or extra-prefecture. He had been diagnosed as nephrosis and treated by oral predonisolone without any improvement. The patient admitted our hospital for further examinations on June 17, 1998.

On physical examination, elephantiasis is remarkable in both of his legs. The laboratory tests revealed slight protenuria, but white blood cell and eosinophil count within normal, as were results of screening of hepatic and renal function. Serologically, antifififilarial antibodies were negative and pathological findings

revealed no filaria. The patient was diagnosed as having primary lymphedema, and treated by surgical excision. Elephantiasis occure not only by filariasis but also by another lymphedemic disorders. Primary lymphedema is one of the differential diagnosis from filarial elephantiasis, especially in the epidemic area. We, clinitian, should keep this in our mind.

97 EFFECT OF PF1022A ON MONGOLIAN GIRBILES INFECTED WITH *BRUGIA PAHANGI*

Masumi Shimizu-Suganuma[1], Kazuhiro Shichinohe[1], Katsuya Haga[2], Tetsuo Iedokoro[2], Setsuko Tsukidate[2] and Koichiro Fujita[2]
Department of Laboratory Animal Sciences, Nippon Medical School[1] and
Department of Medical Zoology, Faculty of Medicine,
Tokyo Medical and Dental University[2]

PF1022A is a new anti-nematode drug isolated from a microflora on leaves of plant, which has a cyclic depsipeptide structure. The substance has a superior action against various intestinal nematodes and some effects were reported on *Angiostrongylus costaricensis, A. cantonensis* and microfilariae of *Brugia pahangi*. We investigated effect of oral administration of PF1022A on the third- (L_3) or the forth-stage larvae (L_4) of filaria using Mongolian gerbils infected with *B. pahangi*. Male gerbils were infected subcutaneously into the inguinal region with 100 L_3 of *B. pahangi*. The animals were orally administered at doses of 10, 20 or 100 mg/kgBW/day PF1022A with oral cream from 7 to 9 days after infection.

Numbers of microfilariae and of eosinophils in blood were lower in the gerbils treated with 20 and 100 mg/kg than the infected control animals. Body weight loss was observed in the gerbils of infected control and 10 mg, but not in those of 20 and 100 mg/kg. Adult worm recovery rate at 25 week after infection was significantly lower in the 100 mg group than in the infected control. These results suggest that the oral cream of PF1022A is effective on L_3 or L_4 and suppress body weight loss induced by filarial infection. PF1022A has potential activity against filariasis.

99 PREVALENCE OF INTESTINAL PARASITIC INFECTIONS AMONG THE INHABITANTS IN SANTO DOMINGO, DOMINICAN REPUBLIC

Kazutoshi Nakashima[1], Hideo Hasegawa[2] and Akira Miyata[2]
Department of Microbiology[1] and Department of Biology[2],
Oita Medical University

[Materials and Methods] Prevalence study of intestinal parasites was conducted among people who live in Santo Domingo (SD), Dominican Republic from October 1995 to August 1996. We visited them house to house to collect facal specimens, and also conducted questionnaires concerning income, drinking water and others related to sanitation. We also conducted the same study in two primary schools in SD. Kato-Katz method and MGL method were performed for detecting intestinal parasites from stools. We collected 1,070 fecal specimens among 1,635 persons who replied the questionnaire. All specimens were microbiologically examined, and results were given back to the people with free therapeutic drugs if needed. Among them, we analyzed 783 samples, of which information of inhabitation was available, and further in comparison with data obtained from people living in the urban-type slum La Cienaga (LC) in SD under the previous study (Hasegawa *et al.*, 1995).

[Results] We categorized people into two groups such as higher class (CH) and lower class (CL) depending upon the area of their inhabitation. In CH, age of the people was 34.5 ± 2.1 (mean$\pm$SD), number of family members was 6.0 ± 2.1 and median of monthly income of

home was US$692. In CL, they were 21±17.2, 6.4±3.1, 230, respectively.

[Discussion] Soil-transmitted helminths and water-transmitted protozoa were highly prevalent among the inhabitants. The prevalence of the helminths was related to people's inhabitation. On the other hand, no obvious tendency was observed in the prevalence of water-transmitted protozoa, therefore contamination of the protozoa seemed to be widely spreaded. Although routine anti-parasitic medication was conducted in the schools where we studied, students living in LC were more likely to be infected with helmintos. Comprehensive approach to control parasitic infection in SD might be needed.

100 MONITORING MORBIDITY CONTROL OF SOIL TRANSMITTED NEMATODES IN ZANZIBAR-TANZANIA

H.M. CHWAYA[1,2], R.J. STOLTZFUS[3], M. ALBONICO[4], J. TIELSCH[3],
K.S. ALAWI[2], A. MONTRESOR[5] AND SAVIOLI LORENZO[5]
Department of Parasitology, Institute of Tropical Medicine,
Nagasaki University[1],
Ministry of Health, Zanzibar, Tanzania[2],
Center for Human Nutrition, The Johns Hopkins School of Public Health, USA[3],
Ivo De Carneri Foundation, Italy[4] and
SIP Unit, Division of Control Tropical Diseases, WHO, Geneva[5]

In Zanzibar morbidity due to soil transmitted nematodes is recognized as an important public health problem. A community survey (N=2,200), undertaken on Pemba island, one of the two major islands forming archipelago of Zanzibar showed the prevalence of *Ascaris lumbricoides, Trichuris trichiura* and hookworm infection to be 48% 76% and 61%, respectively. Both prevalence and intensity were found to peak in school age-group. This was also further confirmed by a school survey (N=3,605) which showed the prevalence of *A. lumbricoides, T. trichiura* and hookworm infection to be 72%, 96% and 94%, respectively. The school survey showed that 99% of the children were infected with at least one of the three major soil transmitted nematodes prevalent in Zanzibar. The documented associated morbidity of the infection include growth faltering, iron deficiency anaemia and poor cognition as manifested by low level of school performance and absenteeism.

Community-based intervention programmes aimed at reducing morbidity by regular chemotherapy of the high risk groups have now become of priority in areas where these infections are endemic.

We report the results of twelve months follow up of school-based deworming programme focusing on hookworm infection in iron deficiency and anaemia in children where the incidence of severe anaemia was reduced by 39% prevalence of iron deficient erythropoises was reduced by 16% that of depleted iron stores by 20% and non-wasting and stunting observed in these children improved by 16% and 20%, respectively.

In this environments of intense helminth transmission 3×/year deworming with single dose mebendazole is necessary to achieve the best impact of iron status. The control of iron deficiency in Zanzibar will require the integration of multiple interventions of which deworming is one essential component of this strategy and that monitoring of periodic chemotherapy has provided evidence of the short term cost effectiveness of the school-based deworming programme. However, long term monitoring is essential for the purpose of targeting sustainability and adjusting control strategies.

101 REVIEW OF CURRENT 139 LEPROSY PATIENTS AT RYUKYU UNIVERSITY HOSPITAL AND A CASE REPORT OF BORDERLINE LEPROMATOUS LEPROSY WHO SHOWED ATYPICAL CLINICAL FINDINGS

Atsushi Hosokawa, Atsushi Takamiyagi, Hiroshi Uesato and Shigeo Nonaka
Department of Dermatology, Faculty of Medicine, University of The Ryukyus

The aim of our presenting study is to evaluate the paients with leprosy visited to Ryukyu University Hospital, located at Okinawa Prefecture, where the leprosy had show gradually epidemilogicaly improvements over the past 15 years (1982–1996).

The number of each type of leprosy patients examined at our hospital during the past 15 years were as follows; indeterminate leprosy, 11 (8.1%); tuberculoid leprosy (TT), 48 (35.6%); borderline tuberculoid leprosy, 36 (26.7%); middle borderline leprosy, 14 (10.4%); borderline lepromatous leprosy (BL), 10 (7.4%); lepromatous leprosy, 13 (9.6%); purely neural type, 2 and primary lesion, 1. Though number of new patients and percentage of TT has decreased, relatively a large number of middle-aged cases was detected.

The patient was a 47-year-old female. She had been suffered from aortitis syndrome and was treated with Prednisolone 15–30 mg/day for 20 years. Since 7 years, a reddish freckle appeared on her forearm. The lesion was diagnosed as dermatitis and treated with steroid ointment for 5 years. At the time of her visit to our hospital, she was diagnosed as BL. This case was considered to be iatrogenic BL which had been downgraded from TT and showed atypical cutaneous changes by the treatments with steroid hormone.

From the results obtained, it was suspected that small number of leprosy patients may still be seen in Okinawa in the future. In addition, such misdiagnosed case as above mentioned BL may be encountered because this disease became very rare in Japan.

102 WHO LEPROSY ELIMINATION PROGRAM IN FEDERATED STATES IN MICRONESIA

Junya Takashima and Ohichirou Kobori
International Medical Center of Japan

The Federated States of Micronesia has one of the highest prevalence rate in the world despite implementation of multidrug therapy (MDT) with high coverage since 1983 onwards. With this situation, a two-year project on leprosy elimination was launched in 1996 and was completed. The writer worked for the first round of the project as a WHO consultant.

[Objectives] The objective is to reduce the prevalence rate in the Federated States of Micronesia to less than one case per 10,000 population by the year 2000.

[Methods] The project includes two rounds of total population screening for leprosy case detection as intensive case finding, to provide MDT to all cases and propose preventive therapy to all non-cases, which consists of a combination of rifampicin, ofloxacin and minocycline (ROM) a single dose a year for two years. After completion of the project, all cases detected during 1996–1998 were reviewed.

[Results] 75,886 people (72% of the total population) and 77,199 people (73%) were screened during the first round and second round respectively. The preventive therapy was administered to 73,516 people (70%) and 75,865 people (72%) in each round. The coverage of people receiving 2 doses of preventive therapy was 54% of the total population. During the first round, a total of 322 new cases was detected of which 67 (21%) were MB and 116 (36%) were below 15 years. During the second round, a total of 80 new cases were detected of which 38 (47%) were single lesion, 28 (35%) were MB and 31 (39%) were below 15 years, which indicated a 75% reduction from the first to the second round. Between 1996 and 1997 a total of 488 cases completed MDT treatment, as consequence, the prevalence rate dropped from 41 per 10,000 in 1996 to 15 per 10,000 in 1997. Preliminary data of 1998 indicates a further reduction of prevalence down to 8 per 10,000.

[Conclusions] This project made a contribution to the sharp decline of the prevalence rate in Federated States of Micronesia. This was also due to the implementation of the new WHO simplified MDT regimens, which involved one single dose of ROM for single skin lesion and the shortening to 12 months of the treatment for MB

cases. However, due to the geographical and communication constraints, the case holding is still poor. Further management should be reinforced.

103 SOCIAL IMPLICATION OF LEPROSY CONTROL IN MYANMAR WITH AN EMPHASIS ON THE GOAL OF ELIMINATION

S. Barua[1], S. Wakai[2], T. Shwe[3] and Takusei Umenai[1]
Department of Health Policy and Planning[1],
Department of Community Health, Faculty of Medicine[2],
The University of Tokyo and
National Leprosy Elimination Program, Department of Health, Yangon, Myanmar[3]

Myanmar (population; 46 million) is significant in leprosy endemic and included by WHO in a list of the top 16 major leprosy endemic countries. Multi Drug Therapy (MDT) was introduced in Myanmar in 1988. MDT coverage over the registered cases sharply increased from less than 20% in 1988 to 100% in 1995 and the Reg. PR was reduced from 39.9 in 1988 to 2.91 per 10,000 population in 1997.

The present study was undertaken to investigate: public health and social implications of the loprosy control program of the country. The study was focused on social-factors influencing community-based case detection such as awareness on leprosy, knowledge and attitude towards the disease, existing social stigma of the community leaders and the patients. 3 Townships were selected by the stratified random samplingas high, medium and low leprosy endemic areas according to the 1997 Reg. PR of leprosy and interviewed all newly diagnosed and registered cases (284) from January to December 1997 and one or more community leaders (156) from each village with leprosy case.

The study showed that 1) the higher the knowledge-score on leprosy, the more cases are diagnosed by passive case detection method to diagnose the patients interviewed; 2) the MDT of leprosy infection-load and showed its epidemiological implication as well as its impact on public health of the country. Increasing knowledge, and awareness, improved attitude and reduced social stigma towards leprosy among the interviewed patients and the community leaders reflect in the increasing trend or passive case detection proportion among the new cases detected.

Based on these findings, Myanmar may formulate a national target with a long-term action-plan that would be necessary by considering the long incubation period of the disease.

104 CLINICAL FEATURES OF 44 CASES OF DENGUE VIRUS INFECTION AMONG OVERSEAS TRAVELERS IN JAPAN

Ichiro Itoda[1], Gohta Masuda[1], Atsushi Ajisawa[1], Akifumi Imamura[1],
Masayoshi Negishi[1], Ken-ichiro Yamada[2], Sadao Yabe[2],
Tomohiko Takasaki[2] and Ichiro Kurane[2]
Department of Infectious Diseases,
Tokyo Metropolitan Komagome Hospital[1] and
National Institute of Infectious Diseases[2]

[Object] To determine the clinical features of imported dengue virus infections in Japan.
[Method] We investigated 44 cases of imported dengue virus infection who were admitted to Tokyo Metropolitan Komagome Hospital between 1985 and 1998.
[Cases and Results] We identified 44 cases of serologically confirmed dengue virus infectiou. 33 cases were male and 11 were female. Age distribution was 18 to 62 years old (mean age 32 years old). Subsequent to 1991, we applied a polymerase chain reaction for each cases to determine its subtype. We identified subtypes in 8 cases (1 case of DEN1, 5 cases of DEN2, 1 case of

DEN3, 1 case of DEN4). 13 cases were presumed infected in Thailand, 10 cases in India, 7 cases in Indonesia, 4 cases in Philippines. Nearby all cases were presumed to originate in Asia, with the majority therein from Southeast Asia. Clinical manifestations revealed fever (100%), chills (40%), headache (88%), retoroorbital pain (29%), myaliga (71%), profuse sweating (56%), diarrhea (53%), skin rash (82%).
Laboratory examinations revealed thrombocytopenia (between 10,000–298,000/μl, mean at 92,000/μl, leukocytopenia (between 1,000 to 8,500/μl, mean at 2,730/μl), elevation of AST and LDH levels. One case was considered as dengue haemorrhagic fever Grade II according to WHO dengue haemorrhagic fever grading severity.
[Conclusion] Dengue fever should be taken into differential diagnosis when we see patients with fever among travelers from tropical areas in addition to malaria, typhoid fever, paratyphoid fever etc. Lacking typical symptoms (*i.e.* retroobital pain), we should consider dengue virus infection when thrombocytopenia, leukocytopenina, and elevation of AST and LDH levels are seen.

105 THE CURRENT STATUS OF *AEDES ALBOPICTUS* DISTRIBUTION IN NORTHEAST HONSHU, JAPAN

TAKESHI KURIHARA, NAOKO NIHEI AND MUTSUO KOBAYASHI
Department of Entomology, National Institute of Infectious Diseases, Japan

The published records of *Aedes albopictus* in the northeast of Honshu island, Japan, were from Taira (Ishii *et al.,* 1944), Fukushima (Ara, 1949), Niigata (Omori, 1958) and Sendai (Kamimura, 1968) cities. We have examined the status of distribution of this mosquito at 26 cities in northern Japan since 1993 (Kurihara *et al.,* 1997), and subsequently extended the survey to cover 26 cities located in northeast Honshu. The survey focused on finding the larvae and biting adults in districts which were densely populated and also where there were many water-filled containers. The temperature data were obtained from the Meteorological Agency.

As of September 1998, *Ae. albopictus* was found at 22 out of the 52 cities examined. The mosquito was also found in abundance in the cities initially recorded. Then 18 new locations were identified, namely, Honjou, Yonezawa, Furukawa, Sakata, Aizuwakamatsu, Kitagata, Nagano, Matsumoto, Ueda, etc. It was suggested that the first three cities which are located between latitude 37°50′ N to 39°24′ N constitute the northermost front of this mosquito distribution at present. The mean annual temperature of these 22 cities ranged from 10.7 to 13.5°C: the daily mean January temperature ranged from −1.2 to 3.1°C and the daily minimum January temperature ranged from −6.1 to −0.5°C. It is apparent that such cold winter conditions do not deter the establishment of *Ae. albopictus*. The number of days of daily mean temperature under 10°C, that is, the putative threshold temperature for mosquito development, was observed to be 143 to 177 in a year.

However, no *Ae. albopictus* was found at the other 30 cities. The range of annual mean temperature, daily mean January temperature and daily minimum January temperature of these cities were 7.6 to 12.0, −4.3 to −1.0 and −9.5 to −4.5°C, respectively. The number of days of daily mean temperature of less than 10°C was from 169 to 224 in a year. In respect of the climatic conditions no significant difference is at present noted in the presence or absence of *Ae. albopictus*. However, some cities which are now free of *Ae. albopictus,* are potentially at high risk of future colonization in view of the morquito's ability to expand their distribution and the improved means of transportation. Of the 30 cities, 10 cities including Nikko, Karuizawa, Komoro etc., are free of the mosquito although they are located at around 37°N or farther south. These cities are located in mountainous areas at 400 to 950 m above sea level, and the population density is less than 120/km².

106 ANALYSIS ON CHEMOKINE AND VEGF IN SERA FROM PATIENTS WITH DENGUE FEVER (DF)/DENGUE HEMORRHACGIC FEVER (DHF) IN PHILIPPINES

TAKAYOSHI NAGAO[1], KAZUNORI OISHI[1], FUTOSHI HASEBE[2], KOUICHI MORITA[2],
AKIRA IGARASHI[2] AND TSUYOSHI NAGATAKE[2]
Department of Internal Medicine[1] and Department of Virology[2],
Institute of Tropical Medicine, Nagasaki University

[Objective] To determine the involvement and roles of chemokine and vascvlar endothelial growth factor (VEGF), which is known as vascular permeability factor, in sera from patient with DF/DHF.
[Methods] At the St. Lukes Medical Center of San Lazaro Hospital in Phillippines, we enrolled patients with Dengue viral infection confirmed by the methods of IgM ELISA or RT-PCR using serum sample. Subjects were classified into dengue fever (DF) and dengue hemorrhagic fever (DHF) by the criteria of WHO. The chemokine (IL-8 and MIP-1α) and VEGF in the sera of DF/DHF patients and healthy volunteers (HV) were measured by ELISA and each value were compared and statistically analysed.
[Results] The value of mean IL-8 in sera from HV, DF and DHF were 0, 692, 805 (pg/ml), the value of mean MIP-1α were 0, 1,880, 2,006 (pg/ml) and the value of mean VEGF were 9, 62, 75 (pg/ml), respectively. The value of both chemokine and VEGF in DF/DHF patients were significantly higher than those in HV. There was no difference in serum levels of chemokine and VEGF between DF and DHF.
[Conclusions] We demonstrated significant increases of IL-8, MIP-1α, and VEGF in sera from DF/DHF, compared with sera from HV. None of these mediators in sera, however, correlated with disease severity. These results may be due to inaccuracy of grading in DHF in Phillippines. Further study will be required to determine if these mediators correlate with disease severity under careful clinical diagnosis and grading in DHF.

107 LABORATORY DIAGNOSIS OF DENGUE VIRUS INFECTION —EVALUATION OF DIAGNOSTIC METHODS BASED ON THE DAY OF DEFERVESCENCE—

KEN-ICHIRO YAMADA[1], TOMOHIKO TAKASAKI[1], SADAO YABE[1],
MASARU NAWA[2] AND ICHIRO KURANE[1]
Department of Virology 1, National Institute of Infectious Diseases[1] and
Department of Microbiology, Saitama Medical School[2]

Dengue virus infections are a major public health ploblem in many tropical and sub-tropical countries of the world. Dengue is occasionally imported by travelers who visit tropical areas and become infected with dengue virus. Laboratory diagnosis is necessary for confirming the diagnosis of dengue. Detection of specific IgM by IgM-capture enzyme-linked immunosorbent assay (ELISA) and of dengue virus genome by reverse transcriptase polymerase chain reaction (RT-PCR) has recently been used for laboratory diagnosis of dengue.

In the present study, we tested serum specimens from dengue-suspected Japanese cases, by IgM-capture ELISA, RT-PCR, HI and virus isolation. Serum samples collected before or on the day of defervescence were positive by RT-PCR, and there were no PCR-positive samples which were obtained after the day of defervescence. IgM-capture ELISA was positive as early as 4 days after onset of illness, and all the samples but one were IgM-positive when collected on after onset of illness 5 or later. We, therefore, recommend to perform both RT-PCR and IgM-capture ELISA, irrespective of the stage of dengue illness. Combination of RT-PCR and IgM-capture ELISA increases the ability to diagnose dengue virus infection, even when only single serum specimen is available.

108 EFFICACY OF DENGUE CONTROL USING PLYSET NET DURING AN EPIDEMIC IN VIENTIANE, LAO PDR

B. Phommasack[1], M. Saito[2], T. Sisouk[3], K. Bounlu[3],
S. Insisiengmay[3], Y. Makino[4], T. Itoh[5] and T. Fukunaga[2]
Department of Public Health, Vientiane Municipality[1],
Faculty of Medicine, University of the Ryukyus[2]
Center of Laboratory and Epidemiology, Lao People's Democratic Republic[3],
Oita Prefectural Institute of Health and Environment[4] and
Sumitomo Chemical Co. LTD.[5]

Olyset Net is a new type of polyethylene net incorporated with insecticide, permethrin during manufacturing. A field study was carried out to evaluate the efficacy of dengue control using the Olyset Net in Xaisettha district, one of dengue epidemic urban areas in Vientiane Municipality, Lao PDR, by hanging down or covering at any opening of all houses in a village in May, 1996. *Aedes aegypti* population decreased (adult density index 1.00 to 0.00, larval House Index 12.00 to 3.13, Breteau Index 16.00 to 3.13) after setting up the Olyset Net and kept low level until the end of monitoring, December, 1996. In the year of 1996, dengue fever (DF)/dengue hemorrhagic fever (DHF) epidemic occurred in Xaisettha district with 441 cases and the highest morbidity rate (597.5) in all districts in Vientiane.

In the Olyset Net treatment village no DF/DHF case was reported while 14 cases including 3 DHF cases were reported in the control village (6 km away from the treatment village, in Xaisettha). The sero-conversion rate of anti-flavivirus IgG antibody before and after epidemic season in children in the treatment village was significantly less than that of the control village. Thus dengue virus transmission was interrupted by Olyset Net during dengue epidemic. The interview survey showed that the community accepted well the Olyset Net as a simple and comfortable method for dengue control. The results of this study indicate that Olyset Net should be effective and applicable in other villages of Lao PDR and possibly elsewhere.

109 COMPARATIVE SEQUENCE ANALYSIS OF TYPE 2 DENGUE VIRUS STRAINS FROM PATIENTS WITH DIFFERENT CLINICAL SEVERITIES AND INFECTION TO PRIMARY CULTURE HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS

Akira Igarashi, Basu Devu Pandey, Beti Ernawati Dewi Soemanto,
Futoshi Hasebe and Kouichi Morita
Department of Virology, Institute of Tropical Medicine,
Nagasaki University

In order to obtain direct evidence for the virulent virus theory regarding the pathogenesis of dengue hemorrhagic fever (DHF), nucleotide and deduced amino acid sequences were analyzed on 19 strains of dengue type 2 (D2) virus isolated from patients with different disease severities, in Nakhon Phanom, Northeast Thailand in 1993. The results were compared with clinical symptoms and serological response of the patients. Three representative strains were infected to primary culture human peripheral blood mononuclear cells (PBMC) obtained from 3 different donors to measure infection rate as well as cytokine levels released into the culture supernatant.

Amino acid sequence analysis classified 19 isolates into 3 subtypes. A single strain of subtype I uirus was isolated from a dengue shock syndrome (DSS) case showing secondary type antibody response. Thirteen strains of subtype II viruses were isolated from DHF cases showing secondary type antibody response, whereas two strains of subtype II viruses were from mild dengue fever (DF) case showing primary type antibody response. On the other hand, all 3 strains of subtype III viruses were isolated from mild DF patients, whose antibody response was primary type for 2 cases and secondary type for a remaining case, respectively. Infection rate of the virus to human PBMC was differ-

ent from donor to donor, but highest for the subtype I, followed by subtype II, and lowest for subtype III virus, for each donor. Similar trend was observed for some cytokine levels (TNF-alpha and IL-2) released into culture supernatant of infected PBMC.

The results indicated that both molecular structure of the virus and serological response of the patients determines pathogenesis of DHF indicating following possibilities. (1) Secondary infection with subtype I virus could be very severe (DSS). (2) Secondary infection with sybtype II virus could be quite severe (DHF), but its primary infection could be mild (DF). (3) Infection with subtype III virus could be mild (DF) irrespective of the serological response, either primary or secondary. These possibilities should be confirmed with other virus strains isolated in different epidemic areas during different epidemic seasons.

110 MOLECULAR ANALYSIS OF DIFFERENT INFECTIVITY OF DENGUE 1 VIRUS STRAINS IN VARIOUS CELL LINES

HASANUDDIN ISHAK[1,2], KIYOSHI KAMIMURA[2] AND TSUTOMU TAKEGAMI[1]
Medical Research Institute, Kanazawa Medical University[1] and
Department of Biodefence Medicine, Faculty of Medicine,
Toyama Medical and Pharmaceutical University[2]

Dengue virus causes a lot of the dengue fever in tropical area. The infection with dengue virus sometimes leads to serious cases like hemorrhagic fever and dengue shock syndrome. The mechanism of such severe diseases is still unknown, although two possibilities, *i.e.* immune enhancement and the viral virulence are thought. Here we started to compare the difference in viral virulence between two strains of dengue type 1. We used two dengue type 1 strains, A88 (isolated in Indonesia) (Fujita *et al.*, 1997) and Mochizuki (isolated in Nagasaki, Japan) (Kimura and Hotta, 1943).

For the virus multiplication, C6/36 (mosquito cells), Vero (African green monkey kidney cell line) and HepG2 (human hepatoma cell line) were used. The viral growth rate and yield of A88 strains in mosquito C6/36 cells were almost same as those of Mochizuki strain viruses. However, when we used mammalian cells (Vero and HepG2), the multiplication of A88 were 100 times lower than that of Mochizuki. In addition, the infectivity of A88 strains was decreased after the storage at −80°C or heat treatment at 50°C. These results suggest that the envelope (E) protein of A88 seems to be sensitive against temperature. To know the variation of A88 E protein, we analyzed the sequence. RT-PCR using specific primers and direct sequencing analysis were performed. Nucleotide sequencing analysis indicated that 19 amino acids of E protein of A88 strain were substituted. Now, the study on the difference in virulence between both strains is undertaken.

111 THE PRESENT CONDITION OF MEDICAL CARE AND MANAGEMENT FOR PATIENTS WITH HIV INFECTION IN IKI ISLAND

YOSHIAKI UTSUNOMIYA[1,2], KAZUNORI OISHI[2], TSUYOSHI NAGATAKE[2] AND
KEIZO MATSUMOTO[2]
Internal Medicine, Iki Public Hospital[1] and
Department of Internal Medicine, Institute of Tropical Medicine,
Nagasaki University[2]

We discuss the marginal capacity and the possibility of the medical care and management for patients with HIV infection in the isolated island Iki through our experiences in Iki Public Hospital. The Iki island is located in Genkai-nada, the northern sae of Kyushu. The size of the island is 18 km north and south, 13 km east and west. The population is about 35,000. We have had three male patients of hemophilia A infected with

HIV. Case no.1 affected AIDS in 1987 and died in the following year due to opportunistic infections. At that time AIDS was incurable and prevention for this infection was not established, we accomplished terminal care for this patient without confusion and guarded his privacy. But in case no.2 and 3 the relationship among patients and hospital staffs became worse after the responsible doctor for these patients moved. The patients were forced to visit a hospital in Fukuoka. With the joining of the new doctor to our hospital, the relationship among patients and staffs become good and better.

Followings are the unique difficulties in isolated islands on the present condition of medical care and management for patients with HIV infection were revealed through our experience. 1) The community of islands in a narrow closed area, there is disadvantage for patients with special disease such as HIV infection. 2) The hospital in islands is poorly equipped for the medical examination and treatment for HIV infection in general. 3) The medical staffs including doctors of the hospital in islands are inexperienced. 4) The condition of management of patients with HIV infection should be variable for better or worse by the responsible doctor. 5) It is difficult to secure capable and expert doctors everytime in isolated islands. 6) Transportation and information network get more advanced, more patients intend to visit a big hospital in a big city. On the other hand, we point out the following possibilities and assignment of medical care and management for patients with HIV infection in isolated islands. 1) We have demonstrated the appropriate care and management for HIV infected patients with enthusiasm. 2) We have to study the HIV infection and it's complications, and prepare to care and to manage the HIV infected patients including staffs, medicine and technique with simulation in a case of operation, delivery and emergency. 3) We expect mutual understanding among patients and medical staffs will be more developed by advance of transportation and information network.

112 CLINICAL STUDY ON RESPIRATORY INFECTION IN HIV-POSITIVE PATIENTS IN NORTHERN THAILAND

Hiroshi Watanabe[1], Shinobu Kobayashi[1], Kiwao Watanabe[1],
Kazunori Ohishi[1], Tipaya Sancyai[2], Khemrassamee Kunsuikmengrai[2]
Weerayut Kositsakulchai[2], Sumpun Kahintapong[2], Prasit Tharavichitkul[3],
Thira Sirisanthana[3] and Tsuyoshi Nagatake[1]
Department of Internal Medicine,
Institute of Tropical Medicine, Nagasaki University[1],
Nakorn Ping Hospital[2] and Faculty of Medicine, Chaing Mai University[3]

We studied the respiratory infection of 82 HIV-positive patients (83 episodes, 51 males and 31 females, mean age 33.2 y.o.) in northern Thailand. Mean (CD4 (CD4/CD8) in 34 patients was 48.8/mm^3 (0.08). Diagnosis were 49 pneumonia, 12 pulmonary mycobacteriosis, 10 *Pneumocystis carinii* pneumonia-like interstitial pneumonia, etc. Organisms detected from sputum were 12 *Haemophilus influenzae,* 8 *Pseudomonas aeruginosa,* 4 *Rhodococcus equi,* 3 *Streptococcus pneumoniae,* etc. And more than 2 organisms were detected from sputum and/or blood in 9 cases (10.8%).

Five *Penicillium marneffei* infection, 4 meningitis (cryptococcal 3 and tuberculous 1) and 2 septicemia (*Staphylococcus aureus* 1 and *Pseudomonas* spp. 1) occurred as severe complication, and 14 patients (16.9%) were dead. In conclusion, there are a variety of organ isms and complications on respiratory infection in HIV-positive patients in northern Thailand. It is important to diagnose and treat early on the basis of our results.

119 EXPRESSION OF CHEMOKINE RECEPTORS ON HUMAN CD4+ LYMPHOCYTES OF PERIPHERAL BLOOD FROM HIV-INFECTED INDIVIDUALS IN UGANDA

Kazunori Oishi[1], Hiroyuki Yoshimine[1], Kazushi Motomura[1],
Atsushi Kumatori[2] and Tsuyoshi Nagatake[1]
Department of Internal Medicine[1] and Department of Biochemistry[2],
Institute of Tropical Medicine, Nagasaki University

To evaluate the expression of co-receptors for HIV-1, and to determine if a dynamic change in expression of CCR4 on CD4 lymphocyte, which has been shown to be Th2 cells, in African individuals, we developed a cross-sectional study in which we examined the expression of CXCR4, CCR5, and CCR4 on CD+ lymphocytes in the whole blood samples from HIV-1 infected and uninfected Ugandan adults by flowcytometry. We also examined the plasma viral load in HIV-infeceted individuals. We demonstrated the up-regulation of CCR5 and CCR4 expression, but there was no down-regulation of CXCR4 expression on CD4+ lymphocytes in the peripheral blood from African adults with HIV-1 advancing disease.

Plasma HIV-1 viremia significantly correlated with the percent CD4 on lymphocytes, but did not correlate with the degree of CCR5 and CCR4 expression on CD4+ lymphocytes in the peripheral blood from HIV-1 infected individuals. A lack of down-regulation of CXCR4 expression on CD4 lymphocytes may be due to immune activation in African individuals, and the up-regulation of CCR4 expression on CD4 lymphocytes may indicate a polarized type 2 state in HIV advancing disease (Collaborators: Masashi Hayano, Kouji Matsushima. Department of Molecular Preventive Medicine, School of Medicine, University of Tokyo. Peter Mugenyi. Joint Clinical Research Center, Uganda).

120 EPIDEMIOLOGICAL PROPOSAL FROM U.S.A. FOR TB PROPHYLAXIS TO HIV-INFECTED PATIENTS IN THAILAND

Yasuyuki Rakue[1], Scott F. Micheal[1], Orasa Suthienkle[2] and
Prayong Radomys[2]
Department of Tropical Medicine, Tulane Medical Center,
SPH & TM, LA., U.S.A.[1] and
Mahidor University Bangkok, Thailand[2]

Tuberculosis represents one of the leading opportunistic infections in HIV-infected patients in developing countries. The close relationship between HIV infection and clinical tuberculosis has widely observed. The progression of recent TB incidence is now climbing alarmingly. By preventing tuberculosis will also help to reduce transmission of tuberculosis in Thailand. I propose to examine the impact of TB chemorprophylaxis for patients who are HIV-infected with positive tuberculin skin tests (TST+) in Thailand.

[Study Design] Prospective, randomized, placebo-controlled, unmasked trial

To compare 3 arms: Isoniazid 300 mg PO QD+Vitamin B6 25 mg PO QD×6 months

Rifampin 600 mg PO QD+Ethambutol 1,500 mg PO QD×2 months

[Inclusion Criteria] HIV seropositive (2 HIV-1 EIA or 1 HIV-1 EIA followed by Western blot), Chest X-ray with no evidence of active TB

[Exclusion Criteria Study Population] Active tuberculosis (pulmonary or extrapulmonary)

Prior TB chemoprophylaxis

[Sample Size] Baseline incidence of TB in HIV+ untreated patients of 8% per person per year, Screen 1,500 HIV-infected patients followed in Mahidol University Hospital infectious disease clinics in Bangkok with tuberculin skin testing

[Sample Size Calculation Analysis] p_1=proportion of patients in placebo group who develop=TB 0.08. p_2=proportion of patients in treatment group who develop TB=0.02

[Conclusion] The proportion of these developing active TB is statistically smaller in the group getting prophylaxis than in the group getting placebo.

121 INTERNATIONAL ASSISTANCE TO DEVELOPMENT OF COMMUNITY-BASED TB PROGRAM BY NGO, —EXPERIENCE OF BRAC IN RURAL BANGLADESH

AKRAMUL ISLAM[1], NOBUKATSU ISHIKAWA[2], SUSUMU WAKAI[1] AND YASUHIDE NAKAMURA[1]
The University of Tokyo[1] and
The Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association[2]

[Aim] Tuberculosis remains a major public health problem in Bangladesh, despite the efforts of the government program. We aim to discuss the role of NGOs and international assistance for successful TB program.
[Methodology] The experience of Bangladesh Rural Advancement Committee (BRAC), one of the biggest NGOs for community development in Bangladesh is reviewed.
[Results and Discussion] BRAC started a pilot community based tuberculosis program in 1984. It covered a rural sub-district (thana) with 220,000 population, where about 200 community health workers (CHWs) were involved in educating community, identifying symptomatic patients, giving them anti-TB drugs until cure. Currently this model has been extended to 60 thanas, covering 14.6 million population in collaboration with the new government TB program. From January 1995 to June 1998, 13,014 patients were diagnosed, and 5,076 (86.3%) out of 5,882 new sputum positive patients by June 1997 were cured by CHWs. This program has been supported financially by various international agencies such as DFID (UK), SIDA, SDC and UNICEF. The Research Institute of Tuberculosis (RIT) of Japan has made technical guidance and support, which is crucial for the success of the program. WHO also provides support through the government. Involving the community development NGOs like BRAC for the national TB program is proved to be effective. International assistance to this kind of approach is essential in developing countries.
[Conclusion] International resources both financial and technical need to be mobilized more towards community based NGOs for TB control.

122 CHARACTERISTIC FINDINGS OF THE LIVER DISEASES IN NORTHERN THAILAND

KAN TORIYAMA, MASACHIKA ISEKI AND HIDEYO ITAKURA
Department of Pathology,
Institute of Tropical Medicine, Nagasaki University

We have performed a hospital-based histopathologic and virologic study of the liver diseases in northern Thailand. The most common liver disease among 275 biopsy cases is hepatocellular carcinoma (HCC;62), followed by chronic viral hepatitis (CH;36), cholangiocellular carcinoma (CCC;34), penicilliosis (21), fatty metamorphosis (13), adenocarcinoma with unknown primary site (12), ascending cholangitis (12), metastatic adenocarcinoma (11) and others. Comparing with the situation of the liver diseases in Japan, CCC showed a high incidence peculiarly and *Opisthorchis viverrini* infection, which is related to the life styles of the inhabitants in this area, is supposed to play a major role in CCC.

The dominant causative virus in HCC cases is HBV (81%), followed by non-B, non-C virus (NBNCV;11%) and HCV (8%). Among CH cases, HBV showed the highest prevalence (52%), followed by HCV (29%) and NBNCV (19%). It is well known that northern Thailand has witnessed an explosive rise in the number of HIV infected populations. The most common and characteristic hepatic manifestation of the opportunistic infections among HIV infected individuals is penicilliosis, caused by *Penicillium marneffei*. In addition, several liver diseases such as opportunistic infections, ascending cholangitis, cholelithiasis, intrahepatic cholestasis and alcoholic liver damage are suggested to be related to the geographical situation, socio-economic and hygienic conditions, and cultural practice in northern Thailand.

123 AN IMPORTED CASE OF BRUCELLOSIS

YASUYUKI KATO[1], GOTA MASUDA[1], ICHIRO ITODA[1], FUMIAKI IMAMURA[1],
ATSUSHI AJISAWA[1], MASAYOSHI NEGISHI[1] AND MORIO KOIKE[2]
Department of Infectious Diseases[1] and Department of Pathology[2],
Tokyo Metropolitan Komagome Hospital

A 64-year old Japanese man was admitted to our hospital with one-month-history of fever and low back pain. He had been good in health and visited Baghdad, Iraq three months before. He was given cephalosporins with no improvement. Careful questioning revealed that he had eaten sheep's milk cheese in Iraq. At the time of admission, his temperature was 38°C. Examination revealed hepatosplenomegaly and spinal tenderness. Laboratory studies revealed a WBC count of 8,400/μl and the erythrocyte sedimentation rate of 67 mm/h. Levels of hepatic enzymes were raised. Chest X-ray film was normal. A lumber puncture was performed. The CSF had normal levels of glucose and protein concentration, but lymphocytic pleocytosis was present. Brucella agglutinin titer was 1:800, and cultures of blood and intervertebral disk subsequently yielded *B. melitensis*. Biopsy specimens of the liver revealed noncaseating granuloma and mononuclear cell infiltraton.

A magnetic resonance imaging revealed diffuse high signal intensity of the L3-L5 vertebrae and a paraspinal mass at this level, which were thought to represent vertebral osteomyelitis and epidural abscess, respectively. Treatment with doxycycline, rifampicin and streptmycin for four weeks resulted clinical improvement. This was followed by rifampicin, trimethoprim-sulfamethoxazole and tosfloxacin for a total of five months. His wife who did not visit Iraq with him developed fever one month later. Cultures of her blood also yielded *B. melitensis*. The organism was thought to transmit from him to his wife.

125 HAULAGE METHODS AND DIUNAL HABITS OF THE PORTERS IN KATHMANDU

EIKO KANEDA AND MITSUO KOSAKA
Department of Environmental Physiology,
Institute of Tropical Medicine, Nagasaki University

The mountainous nature of most of Nepal restricts the development of vehicular transportation. People have to depend on human power even today, especially porters who use a number of different transport styles.

The aim of this study were to document (1) the characteristics of haulage methods in relation to the geographical conditions, and (2) the diurnal activities and health of porters in Kathmandu.

First, according to the geographical features two places were selected for each of the three different geographical areas: Terai zone, Mountain zone and Himalayan zone. In each of these places, observations were made on the tribes of the porters and on the techniques employed for carrying their loads. As the result, the porters in Nepal to carry loads were divided into four main classes: (1) in baskets on a yoke across the shoulders, (2) by handcart, (3) on the top of the head, and (4) on the back using a rope. The method of carrying a load on the back with a rope was most commonly observed, although this style might cause damage to the spinal vertebrae.

Secondly, the study of the diurnal activities and health condition of porters was limited to those in Kathmandu. For this purpose seven porters were interviewed orally. The results can be summarised as follows: (1) luggage of about 60 kg to 110 kg could be carried, (2) six of the seven porters habitually smoked tobacco and drank alcohol, (3) many porters wore cloth tightly twisted around their waist, and (4) complaints of severe neck pain were not made, but all porters complained of knee and/or back pains.

On the other hand, X-ray photographs of the cervical and lumbar spine regions of the porters were taken to investigate possible changes in the vertebrae. The ratio of the front length to the back length for each vertebral body was calculated and compared. However, X-ray examination showed no significant differences ($P > 0.05$) in the vertebral bodies of either the cervical or the lumbar regions of the porters examined in this study. This appears to be because the muscles support-

ing their cervical vertebrae were very well trained and developed. Even if there were some unobserved degenerative changes in the spines of these porters, it would be difficult to prove a causal relationship between occupation and injury, because cervical spondylosis and degenerative intervertebral disks also occur with ageing.

126 INTERNATIONAL COLLABORATION DURING THE EPIDEMIC ENCEPHALITIS OUTBREAK IN MALAYSIA, 1999

KOUICHI MORITA AND AKIRA IGARASHI
Department of Virology, Institute of Tropical Medicine,
Nagasaki University

[Background] An epidemic encephalitis outbreak occurred in northern part of Peninsular Malaysia, Japanese encephalitis (JE) endemic area, since late 1998. Epidemic area expanded to southern part of the peninsular by February in 1999 and disease intervention activities for JE was strengthened in the affected areas. However, the number of patients increased in March. In middle of March, a novel virus was isolated in University of Malaya from cerebro-spinal fluids of patients and soon identified as Hendra-like virus by CDC in the USA. The virus was confirmed as the pathogen and named Nipah virus after the name of village of the patients. Swine in the farm were considered as source of pathogen to the humans and the government immediately started nation-wide destruction of pigs in the infected areas. Under these circumstances, the authors were dispatched to the country by JICA in order to collaborate in the disease control programme in middle of April 1999.
[Purpose] The purpose of the visit was to overview the situation of the epidemic encephalitis possibly due to Nipah virus and JE virus and to make recommendations for control of the diseases.
[Method] The authors made a visit to the epidemic areas, Malaya University, Ministry of Health and a governmental reference laboratory for JE laboratory diagnosis in IMR (Institute for Medical Research) in Kuala Lumpur.
[Findings] Nipah virus encephalitis cannot be distinguished from Japanese encephalitis by clinical manifestations and other clinical laboratory data. However, epidemiological features were clearly distinct for each diseases due to different mode of transmission (JE: mosquito-borne, Nipah: direct contact). Authors confirmed JE cases among the patients during December 1998 as diagnosed by IgM-Capture ELISA. On the contrary, most of the patients after February 1999 were considered not JE. These patients were diagnosed as Hendra-like virus infection by CDC team. The epidemic was completely contained after the government destroyed nearly one million pigs by the end of April, 1999. Reservoir of the virus in nature is not yet clear.
[Discussion] Foreign research institutions, particularly CDC, played a key role in virus identification and disease surveillance during emergency situation. Relevant institutions in Japan should strengthen suitable capacity to respond to emerging infectious disease outbreaks at an international level.

128 IMMUNOLOGICAL INVESTIGATIONS ON DIARRHEAL DISEASES CONTROL OF CHILDREN IN DEVEROPING COUNTRIES

TAKASHI NAKANO[1], HITOSHI KAMIYA[1] AND B.D. AKANMORI[2]
Department of Pediatrics, National Mie Hopital[1] and
Noguchi Memorial Institute for Medical Research, Ghana[2]

Diarrhoea is one of the most serious diseases burdens of children in developing countries. Infective agents, host immunity, nutritional status interact one another. We investigated and analysed these factors to establish effective control measures for diarrhoeal diseases.

A prospective study was carried out in an urban slum, Nima-Maanobi near Accra, the capital city of Ghana from 1996-1998. The target population were under 3 years old and 601 children were recruited. Diarrhoea occurred to 355 cases (59%), but 246 (41%) didn't have diarrhoea. Acute diarrhoea accounted 460 epiosodes (83%) and 97 (17%) were persistent diarrhoea which lasted more than 14 days. Acute diarrhoea had its peak of occurrence between 7 and 12 months old, and persistent diarrhoea occurred frequently in younger (4-6 months old) infants.

Microbiological investigations were performed for acute diarrhoea, persistant diarrhoea, and healthy children. No particular agent was isolated with a significantly high rate from acute and persistent diarrhoeal cases. The causative candidates for persistent diarrhoea such as enteroaggregative *E. coli, Cryptosporidium, Giardia lamblia* were not isolated significantly high from persistent diarrhoea group.

Surface marker analysis of lymphocytes indicated that $CD19^+$ cells, CD4/8 ratio, $CD16^+/56^+$ cells were decreased in persistent diarrhoea group compared with acute diarrhoea group. Malnourished children also had decreased number of the same subpopulation of lymphocytes. Analysis of blood samples from healthy Ghanaian children revealed that $\gamma\delta$T cells were increased.

These observations can become an introduction to clarify immunological backgrounds of severe or intractable diarrhoea. We should aim at persistent diarrhoea, and study for virulence facors and host factors on each case. These examinations will be beneficial for establishing more effective strategies of diarrhoea diseases control.

129 PROCESS OF SETTING UP *HAEMOPHILUS INFLUENZAE* TYPE B (HIB) VACCINATION PROJECT FOR BANGLADESHI CHILDREN TO TREVENT MENINGITIS

HIRONORI MASAKI[1], NAOTO RIKITOMI[1], K. AHMED[1], KIWAO WATANABE[1], KAZUNORI OISHI[1], TSUYOSHI NAGATAKE[1], KEIZO MATSUMOTO[1], S.K. SAHA[2], M. HUSSAIN[2], M. RUHULAMIN[2], M. HANIF[2], N. KHAN[2] AND M. ISLAM[2]
Department of Internal Medicine,
Institute of Tropical Medicine, Nagasaki University[1] and
Dhaka Shishu (Children) Hospital, Bangladesh[2]

Haemophilus influenzae Type b (Hib) is the most important pathogen in acute purulent meningitis. In our previous study, it was demonstrated that one-third of these patients die. In developing countries, the effectiveness of Hib vaccine for both children and adults has not yet been fully evaluated. We report here the process of setting up of Hib vaccine project for Bangladeshi children. The aim of the Hib vaccine project is to improve child health in developing countries.

[Setting] Kaligonj in Gazipur district, located at about 40 km from Dhaka city, Bangladesh.

[Materials and Methods] Hib vaccine will be given for about 1,000 children, under 5 years old (mainly 2 years old), utilizing government thana health center in Kaligonj. Expanded programme of immunization (EPI) is still working there. Therefore running cost for vaccination and its follow-up study will not be high. Hib vaccine will be purchased by donation from Rotary Club of Nagasaki, Rotary Club of Dhaka, and International Rotary Club. Follow-up study of Hib vaccine will be supported by Japan International Cooperation Agency (JICA). During the study period, patients with severe illness will be transported to Dhaka Shishu (Children) Hospital for further examination and treatment.

[Results and Conclusion] Hib vaccine project will be initiated from April, 2000. We are looking forward for further collaboration with persons who are interested in our project.

130 FOOD PLANT-BASED VACCINES AGAINST INFECTIOUS DISEASES

TAKESHI ARAKAWA AND YOSHIYA SATO
Department of Parasitology, Faculty of Medicine, University of the Ryukyus

A gene encoding the cholera toxin B subunit (CTB), fused to an endoplasmic reticulum retention signal (SEKDEL), was inserted adjacent to the bi-directional mannopine synthase P2 promoter in a plant expression vector containing a bacterial luciferase marker gene linked to the P1 promoter. Potato plants were transformed by *Agrobacterium tumefaciens* carrying the vector and kanamycin-resistant plants were regenerated. The CTB-SEKDEL fusion gene was identified in the genomic DNA of bioluminescent plants by polymerase chain reaction amplification. Immunoblot analysis indicated that plant-derived CTB was antigenically indistinguishable from bacterial CTB, and that pentameric CTB molecules (M_r～50 kDa) were the dominant molecular species isolated from transgenic potato tissues. The maximum amount of CTB was approximately 0.3% of total soluble plant protein. Enzyme-linked immunosorbent assay indicated that plant-synthesized CTB bound specifically to G_{M1}-ganglioside, the natural membrane receptor of cholera toxin (CT). In the presence of the SEKDEL signal, CTB accumulates in potato tissues and is assembled into an pentameric form which retains native biochemical and immunological properties.

Feeding transgenic potato tissues to mice induced both serum and intestinal anti-CTB antibodies. Mucosal antibody titers declined gradually after the last oral immunization but were restored following an oral booster of transgenic potato tissues. Following intra-ileal injection with CT, the plant-immunized mice showed up to a 60% reduction in diarrheal fluid accumulation in the small intestine. Protection against CT was based on inhibition of enterotoxin binding to the cell surface receptor G_{M1}-ganglioside. The experimental results demonstrate the ability of transgenic food plants to generate protective immunity in mice against a bacterial enterotoxin, which may open the way for development of food plants as production and delivery systems for mucosal immunization.

131 DEPRESSION OF IMMUNOLOGICAL ABILITY BY ULTRAVIOLET RAYS AND PROTECTION FROM ULTRAVIOLET RAYS

NOBU OHWATARI, YU CAO, EIKO KANEDA AND MITSUO KOSAKA
Department of Environmental Physiology,
Institute of Tropical Medicine, Nagasaki University

Exposure to ultraviolet rays (UV) influences on ability of immunity, and induces tissue degeneration. Furthermore, long term exposure of UV causes skin cancer. Immunity has important role to protect against infectious disease, so we investigated relationship of dose responses of UV-B and ratio of CD4 to CD8. Additionally, protections from UV in wild mammals is shown in this study.

Whole body of mouse (DDY mouse, N=16) were irradiated UV-B of 0 J/m² (Control), 40 J/m², 400 J/m², 2,000 J/m² at two times with one week interval. CD4 and CD8 measured on 3, 7, 14 days after the second UV-B irradiation with 100 μl of heart drawing blood. On irradiation at 400 J/m², CD4/CD8 decreased 3.42±0.28 to 2.05±0.18 on 7th day after the last irradiation, which was small on indvidual deference in mice and was lowest compared with other irradiation mice. The 400 J/m² of UV-B was equal to exposure by solar energy for 2 min and 32 sec at 12 o'clock in May in Nagasaki City.

On protections from the ultraviolet rays in wild mammals, the study used Yellow rats (N=9), Squirrels (N=12), Jirds (N=15) and Pikas (N=17) with black layers, and Wistar rats (N=12) for controls. Wild mammals has a two color structure. It is black near the body, and is body color the end. The black part of the hair (black layer) may be effective in absorbing UV.

1) Transmittance of UV through the black layer is significant low compared with rats, because of black layer absorbed UV efficiently. So, the black layer has an effective role to protect the skin tissue from UV.
2) These wild mammals possess the protecting ability

with black layer of hair and/or thick epidermis to protect skin from UV, and environmental conditions influence the protecting ability.

3) A little UV-B changed the ratio of CD4/CD8 easily, therefore we must consider the influence of UV on infection in tropical zone in which human without long hair are exposed to severe UV irradiation.

ISSN 0304-2146

JAPANESE JOURNAL OF TROPICAL MEDICINE AND HYGIENE

VOL. 28 NO. 1 MARCH 2000

CONTENTS

JTM FOUNDED 1959

Jpn. J. Trop. Med. Hyg.

JAPANESE SOCIETY OF TROPICAL MEDICINE