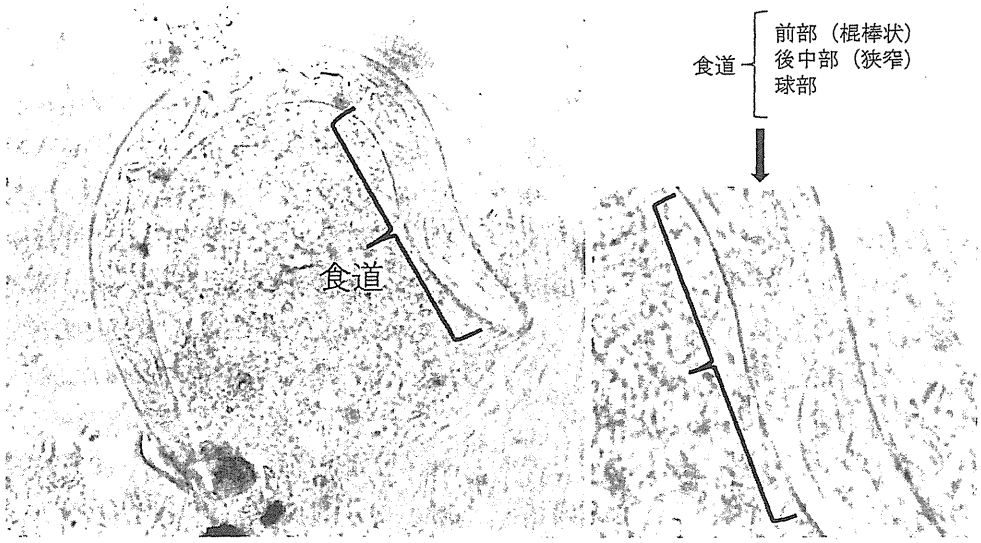
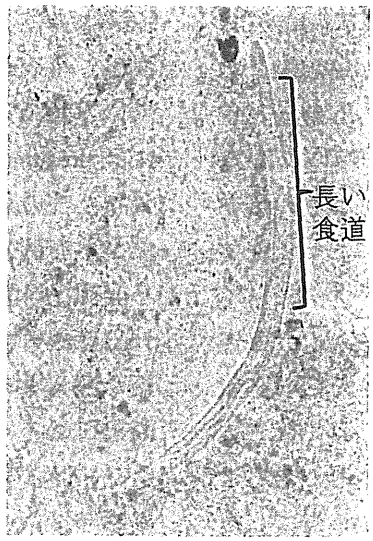


A: R 型幼虫疑い



B: F 型幼虫疑い

図 1: 本症例で検出した虫体

なため特徴的な尾端を確認できなかった（図 1）.

経過：写真にて，糞線虫症を扱う専門医療機関にコンサルテーションをおこなった．形態学的に糞線虫症の可能性が高いとのことで，ご本人に濾紙培養または寒天培養を勧告するも早期の治療を望まれた．2009 年 1 月 7 日と 21 日にイベルメクチン 12 mg を投与し，1 月 26 日の便より線虫は検出されなかった．

考察

本症例の診断と治療における問題点は，診断確定ができなかったことと感染経路が不明であった二点が挙げられる．まず観察された虫体は形態的に糞線虫のR型幼虫とF型幼虫と考えられたが，濾紙培養と寒天培養を施行できず診断の確定ができなかった．鑑別として鉤虫の幼虫または土壌

線虫（桿線虫）の contamination が考えられた．治療後の便より虫体は検出されなかったが，糞線虫症として治療を行ったため治療が効果的であったと断定することはできない．次に感染地域であるが，頻回の海外渡航と野菜が好きとのことで海外における感染の可能性を第一に考えたが，沖縄県出身とのことで区別が不可能であった．常に海外旅行に同行するという親友も検便を行ったが虫体は検出できなかった．

旅行者の糞線虫症に関して現時点で重要なことは，「①旅行者がリスク行動をあまり認知していない．②帰国後診療において医師が鑑別診断に想起することが少ない．」の二つである．

浜辺における行動のリスクは不明であるが，流行地で裸足やサンダルで歩くことはリスクがある[1]．最近出版された渡航医学の教科書では，「悪性腫瘍，ステロイド投与中，HTLV-1 感染などのリスクのある旅行者は裸足で歩かず特にぬかるみを避けるべきである[2]．」，「淡水，土壌との接触のある長期滞在した帰国者（若者）にとって重要な疾患[3]」との記載がある．最近のカナダ，スイスの調査より旅行者の例が増加しているが報告によりばらつきがある[4)-6)]．理由として便の直接塗抹法の感度が低く，また他の感度の高い検査法も普及していないため，診断確定が困難であることが考えられる．2008 年に新婚旅行から帰国したイタリア人 2 人が蕁麻疹様の皮疹，高熱，咳嗽，倦怠感にて入院となった．一人の便中に R 型幼虫が検出され血清学的に陽性となり，さらに二人とも便培養にて陽性であったため急性糞線虫症の輸入例と診断された．リスク行動としてたった一度のみタイのサムイ島にてバンガロー周囲の草地を裸足で歩行しており，それが原因として最も疑われた．同論文の考察では，今まで安全と考えられてきた地域での感染であり渡航前アドバイスの必要性を議論すべきであると提案されている[7]．

次にこの疾患を医師がどの程度認識しているかについて文献を検索すると，米国，ブラジル，シンガポール，タイの 363 名の研修医を対象とした調査において，研修医の糞線虫症の知識は乏しく特にその傾向は米国の研修医に顕著に見られた．喘息の既往がなく肺野に笛声音を聴取し好酸球増多を認める典型的な糞線虫症のシナリオが提示され，米国の研修医の 9%が寄生虫のスクリーニングが必要と答え，23%は更なる精査を行わずステロイドの使用を主張した．一方，その他の国の研修医の答えた比率はそれぞれ 56%，7%であり米国の研修医に比較して有意に良い成績であった（それぞれ $p<0.001$，$p=0.005$）[8]．我が国ではこのような調査は行われていないが，糞線虫症の啓蒙を行った方が適切と推測される．本疾患は診断確定が難しくステロイド投与などにて顕在化し致死的となるため，多くの専門家は，潜在的に重症糞線虫症を発症するリスクのある患者には presumptive treatment を推奨している[9, 10]．我々もこの点を踏まえ，診断確定は不可能であったが本症例に治療を行った．

今回，帰国者の診療において糞線虫症と考えられる症例を経験した．トラベルクリニックの帰国後診療において流行地からの帰国者では鑑別診断の一つとして検査を積極的に行うべきだが，スクリーニング検査をどの程度まで行うか明確な基準がないのが現状である．しかし一度感染すると潜在的に危険な疾患であるため積極的にスクリーニングを行っていく必要があると考えられる．また帰国後診療にあたる医師が診断を想起できるように生涯教育に組み入れ，さらに渡航前相談の際に旅行者にリスク行動について情報を提供するべきであると考える．

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病院薬剤師として服薬指導を行った アフリカ帰り卵形マラリアの一例

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Key Words: 卵形マラリア，メフロキン，プリマキン，病院薬剤師，渡航医学専門薬剤師

諸言

我が国のマラリア輸入症例は年間 52－109 例（2001-2008 年）であり，治療は特殊であるため，一般に医師・看護師をはじめとする医療従事者は治療に慣れていない．今回，我々は病院薬剤師が治療に介入することで医師・看護師・患者との情報の共有を達成し薬物治療の安全性を高めることに貢献した．一連の治療経過を報告し病院薬剤師が抗マラリア薬の服薬指導を行う際の一助としたい．

症例

46 歳男性

主訴：発熱，悪寒戦慄
既往：腎癌にて左腎摘出（35 歳）
職業：エンジニア（国際医療協力）

現病歴：

2011 年 5 月 4 日－31 日，ザンビアに滞在した．5 月 28 日より発熱あり 30 日に現地の医療機関を受診し迅速診断キットにてマラリア陰性，何らかの投薬を受けた．6 月 3 日に聖隷横浜病院トラベルクリニックを受診した．薄層塗抹標本にてマラリア原虫を認め（図 1），渡航地域と原虫の形態から卵形マラリアあるいは四日熱マラリアを考え入院となった．

A case of *Plasmodium ovale* malaria acquired in East Africa: the medication was supervised by the hospital pharmacist specialized in travel medicine

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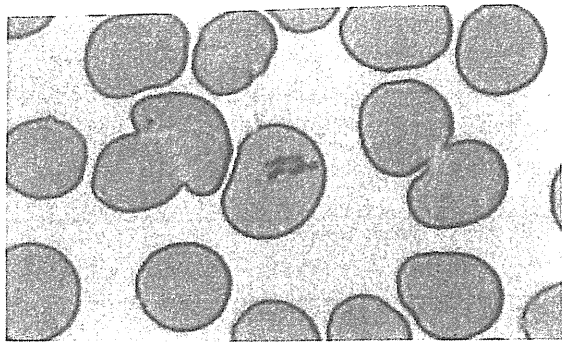
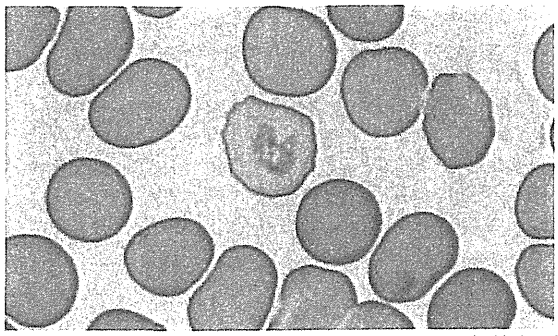


a) 早期栄養体



b) 後期栄養体（感染赤血球は卵形で鋸歯状縁を認めた.）

図1　本症例で観察された原虫の形態（×1000）

身体所見（入院時）：

体温 40.3℃，顔面の軽度浮腫あり，肝脾は触れず

検査所見（入院時）：

WBC:3870 /μL, RBC:396×10^4/μL, Hb:13.5 g/dL, Plt:3.3 × 10^4/μL, AST:29 IU/L, ALT:31 IU/L, γ-GTP:16 IU/L, ALP:163 IU/L, LDH:333 IU/L, CRP:3.1 mg/dL, BUN:15.2 mg/dL, Cre:1.39 mg/dL, parasite density:1857.6 /μL, 赤血球の原虫感染率:0.0469 %, 尿蛋白:1+, 尿潜血:陰性

経過と薬剤師の介入：

治療開始にあたり主治医と薬剤師でクロロキン入手について検討した．当院採用の抗マラリア薬はメフロキンのみであったこと，受診時の情報では服薬歴より熱帯熱マラリアとの混合感染が完全に否定できなかったことから，メフロキンで急性期治療を行い赤血球内の G6PD 活性を確認後プリマキンで根治療法を行うこととした．

メフロキン 275 mg 錠（塩基として 250 mg）を 3 錠－2 錠－1 錠と 8 時間おきに内服し症状は改善した．治療に伴い，parasite density の低下を認めた．メフロキン投与にあたり薬剤師は再度患者に既往歴と併用薬を聴取し禁忌事項の除外を行った．患者に本剤での治療の必要性，副作用の初期症状，十分な薬効を保つために食後に大量の水で服用することの重要性を伝えた．また主治医了承のもと看護師に副作用モニタリングとして心電図モニター装着，片腎であるため尿量と尿所見の確認を依頼した．

ヒト感染性マラリア原虫 4 種鑑別 nested PCR を行い卵形マラリア原虫の単純感染と診断された．根治療法に先立ち，赤血球内の G6PD 活性の測定を依頼し正常の活性であると確認した．プリマキンの使用に関しては当院の倫理委員会に治療内容を提示し承認された．12 月 19 日よりプリマキン塩基 15 mg/日を 14 日間投与し合併症は認められなかった．プリマキン投与にあたり薬剤師は患者に溶血発作のモニタリングとして尿の色を観察し尿が濃くなったら必ず受診すること，消化器症状副作用軽減のために必ず食後に服用することを指導した．

考察

本症例ではクレアチニン 1.39 mg/dL と腎機能の低下が問題となった．前述のごとく機能低下により溶血のリスクが高まるが 14 日間投与の後にとくに問題を認めなかった．頻繁に流行地に滞在する職種であり再度感染する可能性が高いと評価された．問診により今まではマラリア予防（防蚊対策，薬物予防）は行ってこなかったことが判明した．プリマキンの使用とマラリア罹患自体が

腎機能を悪化させる可能性があり，今後は防蚊対策と高度流行地では予防内服をおこなうように説明し服薬指導をおこなった．

プリマキンの副作用で最も重要なものは G6PD 欠損症の罹患者に起こる溶血発作である．溶血の程度は G6PD 欠損症の重症度とプリマキンの用量に依存する．発作は通常は self-limited に治癒に至るが，G6PD 活性が低いクラスⅡ（活性＜10%）の Gd^{Canton} 変異，クラスⅢ（10－60%の活性）の $Gd^{Mediterranean}$ 変異，GdA-変異では重症化することがあり注意が必要である [1]．日本人では G6PD 欠損症の頻度は 0.1%と非常に低く，臨床症状を伴う例はさらに稀である [2]．アフリカ，東南アジア出身者では G6PD 欠損症の頻度が高く注意が必要である．肝機能，腎機能の低下と他の溶血を促す薬剤との併用によりさらに溶血をおこしやすくなる [1]．ときに G6PD 活性の正常者においても溶血が起こることがある [3]．服薬指導の観点からは尿の色を観察し濃くなった場合は診察を受けるように指導をおこなう．さらに服薬期間が 14 日と長いことと悪心，嘔吐，腹痛などの消化器症状のためコンプライアンスの低下を招きやすい．消化器症状は空腹時に服用すると現れやすいので食後に服用するように服薬指導をおこなうとコンプライアンスを改善することができる．

今回，病院薬剤師として特殊な感染症に対する薬剤の管理と治療に参加し，より安全な治療を行うことに貢献できた．日頃から感染症専門医・指導医の在籍する医療機関と連携をとっておくことも大切である．渡航医学に興味がありスキルアップを計るならば日本渡航医学会のホームページを利用するのも良い [4]．スイス，ドイツのように薬剤師の渡航医学専門資格を設置している国もある．渡航医学と輸入感染症は今後我が国において薬剤師が力を発揮できる新しい分野であると考えられる．

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木村幹男先生（結核予防会新山手病院内科），狩野繁之部長（国立国際医療研究センター研究所熱帯医学・マラリア研究部），早川枝李先生，松岡裕之教授（自治医科大学医学部感染・免疫学講座医動物学分野）

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症　　例

形態学的な診断が困難であった卵形マラリアの１例

日　谷　明　裕*1　大　槻　和　花*2　高　谷　　周*3
党　　雅　子*4　春　木　宏　介*5

A Case of *Plasmodium Ovale* Malaria
－Morphological Diagnostic Difficulty and Utility of Rapid Diagnostic Tests－

Akihiro HITANI, MD*1, *Waka OTSUKI**2, *Shu TAKAYA*, MD, PhD*3,
Masako TO, MD, PhD*4 and *Kosuke HARUKI*, MD, *5

A 46-year-old Japanese man was referred to our travel clinic because of high fever for the past 7 days. He worked as an engineer for a month in Zambia and returned to Japan 2 days ago. He had a high-grade fever of 40.5℃. Examination of the palpebral conjunctiva showed no evidence of anemia. Liver and spleen were not palpable. Blood sample was collected at the time of the febrile paroxysm. Malaria parasites were detected by examination of Giemsa-stained thin blood films. The dominant feature of parasite was early trophozoit with a low parasitemia (0.0469%, 1,857.6/μL). The James' stippling was absent. Schizonts and gametocytes were scarce. As ring morphology was quite variable, identification of species might not be possible. Identification of species is more difficult than usual, on the grounds that: 1) the blood sample contains rare early trophozoites, 2) the level of parasitemia is low, and 3) it is quite possible for parasites to be transformed due to the inappropriate treatment. Finally, the diagnosis was confirmed by nested PCR.

Examination of Giemsa-stained blood films is the "gold standard" for detection and identification of organisms. However, in non-endemic countries, trained laboratory personnel are scarce and the most may be inexperienced in malaria diagnosis. It is recommended that personnel continue to gain experience by participating in external quality assurance schemes, and that routine laboratories utilize rapid diagnostic tests (RDTs) concurrently. The availability of simple and accurate RDTs could aid the diagnosis in no-endemic countries. 【Case Report】

[Rinsho Byori **61** : 32～37, 2013]

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【Key Words】 *Plasmodium ovale* malaria（卵形マラリア），early trophozoit（早期栄養体），rapid diagnostic test（迅速診断キット）

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マラリアは致死的にもなりうる重要な感染症であるため帰国者の診療時に早急な診断確定が必須である。しかし一般医療機関の医師，臨床検査技師にとっては診断が非常に困難である。標準的な診断法は光学顕微鏡を用いたギムザ染色による。我が国では症例数が少なく臨床検査技師の大多数は日常の業務でマラリア原虫に遭遇することがほとんどなく原虫の形態に熟知することができない。顕微鏡による診断法を補助するものとして迅速診断キット(以下，RDT と略)と，ある程度時間を費やす PCR による遺伝子検査があるが両者とも保険適応はなく普及していない。一般的に RDT の有用性は明らかであるが我が国で認可された検査法ではないので，これを用いて診断を確定することはできない[1]。

今回，我々は形態学的に診断に苦慮した卵形マラリアの一例を経験した。その理由として幼若な栄養体が多数を占めたこと，低い原虫血症，現地における不完全な治療の可能性などが考えられた。診断確定の補助として RDT を使用し迅速に治療を開始することができた。RDT の有用性について考察したので報告する。

I. 症例(46 歳男性)

主　訴：発熱，悪寒戦慄

既　往：腎癌にて左腎摘出(35 歳)

職　業：医療機器エンジニア

現病歴：2011 年 5 月 4 日より 31 日までザンビア東部のチピタに国際医療協力の専門家として滞在した。滞在中，深夜以降に屋内で蚊に刺された。28 日より発熱あり，30 日に現地の医療機関を受診しマラリアの検査を行ったが陰性であった。6 月 3 日に聖隷横浜病院トラベルクリニックを受診し末梢血の薄層塗抹標本にてマラリア原虫を認めた(**Fig. 1**)。ほとんどの原虫が幼若な栄養体であり，赤血球の感染率は 0.0469%，感染赤血球は正常赤血球よりやや大きく卵形で鋸歯状縁を認めた。また斑点は認められなかった。30 分程の観察の後，ようやく分裂体と雄性生殖母体をわずかに観察した。渡航地域が東アフリカである事と原虫の形態から卵形マラリアを最も疑い入院となった。

身体所見(入院時)：体温 40.3℃，顔面の軽度浮腫あり，肝脾は触れず。

画像所見(入院時)：腹部超音波検査では肝腫大なく脾腫を認めた(Spleen index: 21.9)。入院時検査所見を **Table 1** に示す。

Table 1　Laboratory study at admission

WBC	3,870/μL
neutrophil	77.5 %
RBC	396×10⁴/μL
Hb	13.5 g/dL
Ht	37.1 %
Plt	3.3×10⁴/μL
CRP	3.1 mg/dL
Parasite density	1,857.6/μL
Parasitemia percentage	0.0469 %
AST	29 IU/L
ALT	31 IU/L
γ-GTP	16 IU/L
ALP	163 IU/L
LDH	333 IU/L
BUN	15.2 mg/dL
Cre	1.39 mg/dL
Urinary protein	1+
Occult hematuria test	Negative

経　過：メフロキン 275mg 錠を 3 錠，2 錠，1 錠と 8 時間おきに内服し症状は改善した。ヒト感染性マラリア原虫 4 種鑑別 nested PCR を依頼し卵形マラリア原虫の単純感染と診断した。PCT_{10}(治療開始後に原虫数が初期値の 10%に低下するまでの時間)は 60 時間，FCT(治療開始後に 37.5℃未満に解熱するまでの時間)は 6 時間であった。根治療法に先立ち赤血球内の G6PD(glucose-6-phosphate dehydrogenase)の活性測定を依頼し正常の活性であると確認した。プリマキンは熱帯病治療薬研究班(略称)より供与を受け，使用に関しては聖隷横浜病院倫理委員会に承認された。プリマキン塩基 15mg/日を 14 日間投与し合併症は認められなかった。

II. 考　　察

我々が苦慮した理由として，以下四つの理由が考えられる。まず幼若な栄養体が多数を占めたことが挙げられる。分裂体の破裂とともに多数のメロゾイトが放出され新たな赤血球に感染する[2]が，熱発作は分裂体の破裂に一致する(**Fig. 2**)。*Plasmodium vivax*(以下，Pv と略)，*Plasmodium ovale*(以下，Po と略)，*Plasmodium malariae*(以下，Pm と略)ではほとんどの場合，種々の発達段階が同時に観察されるが，採血の時期により一定の段階の形態が優勢となる[2]。すなわち悪寒戦慄の時期には分裂体が最も多

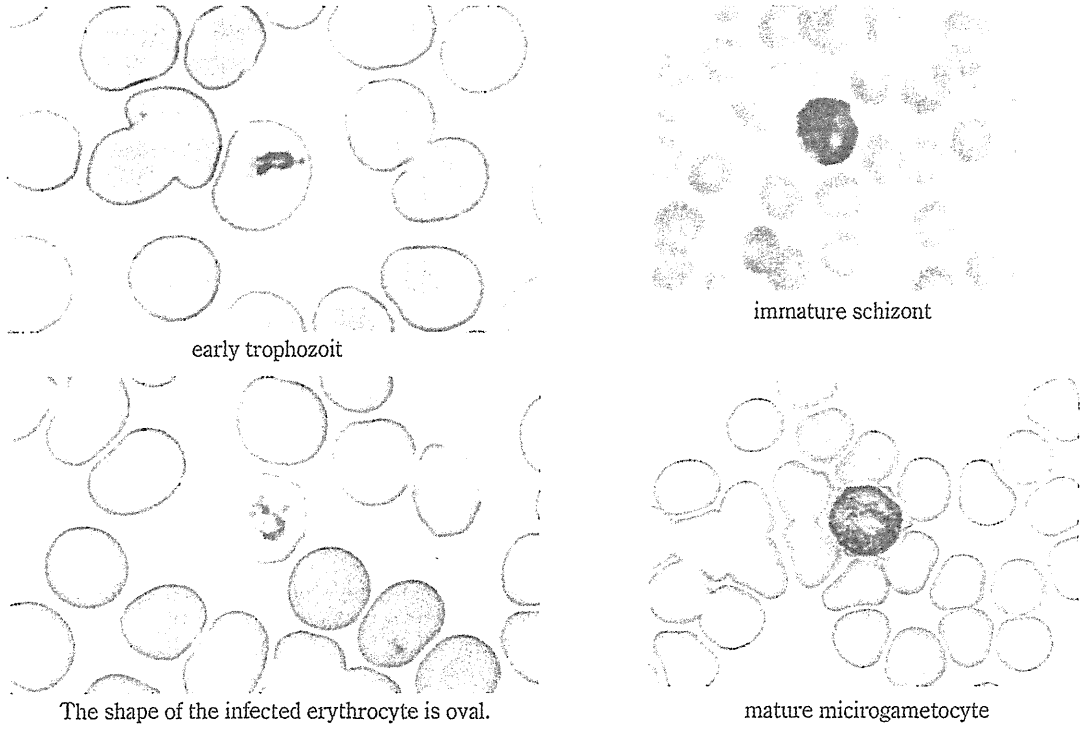

Figure 1 Photomicrographs of *Plasmodium ovale* in this case (×1,000)

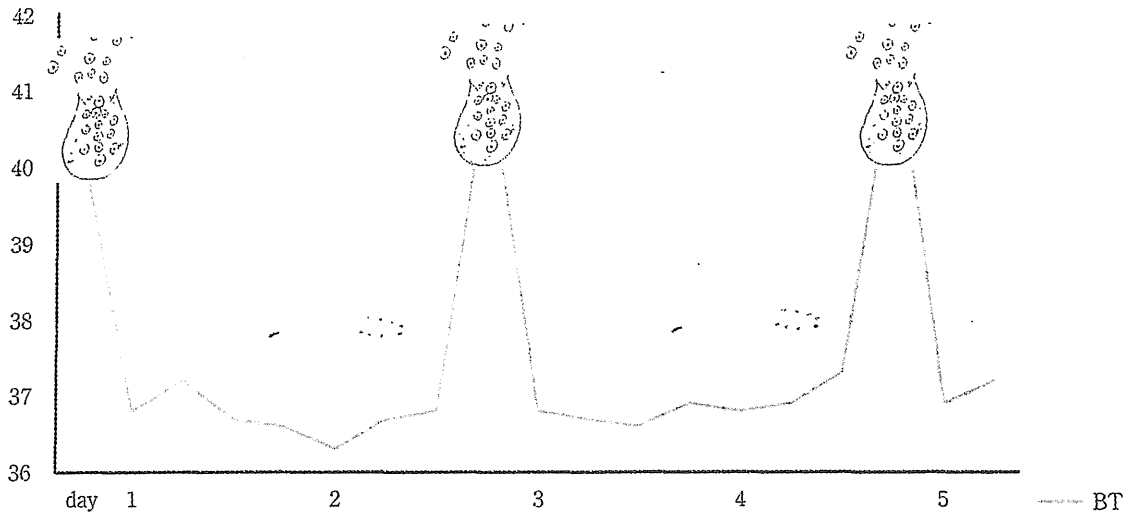

Figure 2 Life cycles of *P. ovale* and malaria paroxysms.

Within erythrocytes, merozoites develop from ring forms into trophozoites and then into schizonts over 48 hours (*P. ovale*). The classic malaria paroxysm lasts several hours, can occur with a regular periodicity coinciding with the synchronous rupture of blood schizonts.

(*This drawing is truly original and illustrated by the first author.)

く観察される。本症例は熱発作の直後に採血を行っており，このため観察しうる形態のほとんどが幼若な栄養体であったと考えられる。**Fig. 3** はそれぞれの原虫の発育段階を特色がわかるように筆者がスケッチしたものである。幼若な早期栄養体は種の区別がむずかしいことがわかる。さらに Po の栄養体には James' stippling (Schüffner 様斑点) があるとの先入観が強く，その考えが診断に至る過程を難しくした。

次に低い原虫血症が挙げられる。low parasitaemia の定義は薄層塗抹標本にて 0.5%未満の感染率と定

義され[3]，本症例では0.0469%であり，この基準を満たしていた。受診時の標本の観察にかけた時間は30分程度であり，帰国後診療の多忙な外来の合間にこれ以上の時間は費やせなかった。以下に，原虫血症が低い場合の診断の困難さについて記載された教科書の一部を引用する。少数の幼若な栄養体のみを含む標本では種の同定は不可能と推測される。リング状の形態は変異に富み，*Plasmodium falciparum*（以下，Pfと略）の早期栄養体はPmの早期帯状体またはPvの早期アメーバ体によく似ていることがあり，リング状の形態だけを基にPfを除外しないことが大切である[4]。

三番目の理由として現地の医療機関にて投薬を受けていることが挙げられる。本人に確かめたところ，現地の医師は“解熱鎮痛薬”と話したそうだが，抗マラリア薬を100%服用していないと断言できなかった。医療教育を受けていない人が通訳をした場合，意図的ではなく医師の話す言葉の意味を変えるかも知れないからである[5]。たとえばスワヒリ語のhomaは「発熱」と「マラリア」という二つの意味があり間違えやすい。このため不完全な治療により原虫の形態が変化している可能性も考慮せねばならなかった。

最後の理由として，ザンビア滞在の前にベナン，ソロモン諸島などマラリア流行地へ頻繁な滞在があった。したがって混合感染の可能性も考慮しなければならなかった。混合感染は実際より低く見積もられているがサハラ砂漠以南のアフリカではPfの感染はしばしばPoまたはPmと同時に起こる[6]。

最終的に，分裂体と雄性生殖母体をわずかに観察した事と患者の滞在地域よりPoあるいはPmによる感染を推測したが上述の四つの理由により確定はできなかった。Pfとの混合感染を完全に否定できなかったため受診時に塩酸メフロキンの投与を開始し，同時に補助診断法としてRDTを使用した。さらにnested PCRの検査を依頼しPoの単純感染と診断した。

マラリア診断の遅れは，まず問診により医師が疾患を思い浮かべないことが挙げられる。次に診断のgold standardはギムザ染色によって濃塗標本と薄層塗抹標本を作製し観察することに尽きるが，いかに犀利な感覚であっても熟練しなければ原虫を検出し種の鑑別を行うことは難しい。初診でどの程度の診断の間違いがあるかについて文献的に調べると，少し古い引用ではあるが，1979年から1993年の間にカリフォルニア州ロサンジェルス市のER（救命救急外来）を受診した20例の熱帯熱マラリア患者で初期に診断確定されER内で抗マラリア薬の投与ができたものは4例であったという報告がある[7]。また，1992年の英国における267例のマラリア患者を対象とした調査では，4つの種のなかでも特に卵形マラリアの診断がむずかしく，17例の卵形マラリア患者のうち最初の検査室で正確に診断できたのは5例ほどであった[8]。

診断確定するまでの時間を調査した我が国の報告では，国立国際医療研究センターを2005～2010年に受診した輸入マラリア50例を対象とした調査[9]において，同施設を最初に受診した者の診断確定までの平均日数は2.8±1.2日であった。一方，他院を受診後に同院を受診した者の日数は4.9±2.5日であり診断の遅れが認められた。

マラリア非流行地にて，診断の間違い・遅れを改善するためには，①医師，技師へ生涯教育を行う，②専門家へ早急に相談できるシステムを利用する，③補助診断法を活用する，などが挙げられる。WHOにおいても光学顕微鏡と臨床診断の限界を克服するために簡単で費用効果の高いマラリア検査の必要性が強調されている（WHO 1996）。

補助診断法においてはマラリア抗原を検出するキットは多種類あり入手可能であるが，研究用試薬の範疇に入り，我が国では法的にこれを用いて診断することはできない[1]。キットに用いられる原虫特異蛋白としては，①Pf原虫（栄養体および早期生殖母体）に存在する可溶性蛋白histidine-rich protein 2（HRP-2），②plasmodium lactate dehydrogenase（pLDH）（これには血中のPf原虫に特異的なもの，Pv原虫に特異的なもの，Pf以外の3種の原虫に共通なものがある。），③4種の原虫が共通して保有するaldolaseの三つが挙げられる。これらが組み合わされたいくつかのキットが販売されている。

本症例では上述の①と②に対するモノクローナル抗体を用いたSD BioLine Malaria Antigen P.f/Pan®（STANDARD DIAGNOSTICS社），Entebe Malaria Cassette®（Laboratorium Hepatika社）を使用した。前者はHRP-2と4種の原虫すべてに共通するpLDHを検出しPfとその他の原虫との鑑別が可能である。後者はHRP-2とPvに特異的なpLDHを検出することが可能でPfとPvの鑑別が可能である。

Fig. 4のごとくSDキットにおいてMalaria Antigen P.f/Panの4種の原虫すべてのpLDHを検出する

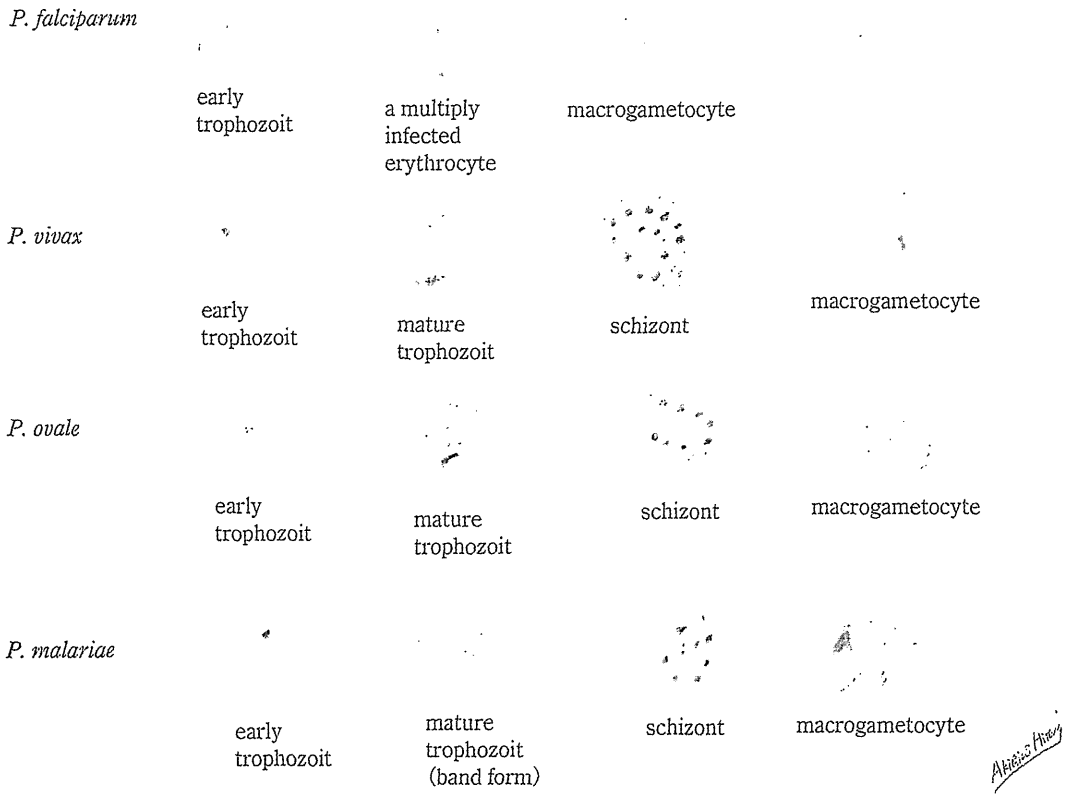

Figure 3 *Plasmodium* parasites: morphology of successive developmental stages in Giemsa-stained thin blood smears. (*This drawing is truly original and illustrated by the first author.)

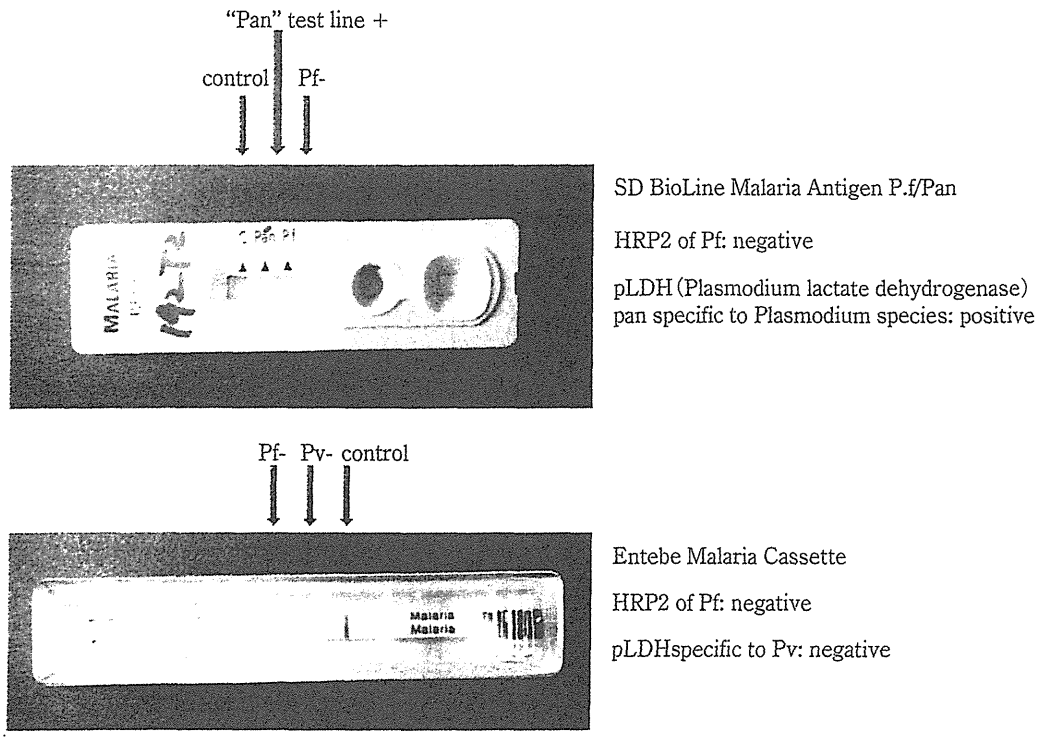

Figure 4 Reactivity to *Plasmodium ovale* malaria parasite in SD BioLine Malaria Antigen P.f/Pan and the Entebe Malaria Cassette.

バンドのみが薄く陽性に検出された。すなわちSDキットではPf陰性でPanが陽性，Entebeキットではpf とPvはともに陰性であった。したがってRDTのみによる解釈では，Pf とPvではないマラリアということになる。この結果はnested PCRによる最終的な診断と一致した。本症例では，このようにRDTを組み合わせることにより，形態学的診断が難しいPoの診断の推測が可能であった。

最近，期待がもたれているRDTとしては，Binax NOW®がある。HRP-2とヒトに感染を起こす4種の原虫すべてに共通するaldolaseを検出する。このキットはサルマラリア原虫*Plasmodium knowlesi*のヒト感染の際もaldolaseを検出したとの報告がある[10]。他のRDTの操作も簡便であるが，このキットはさらに取扱いが易しく，判定までの時間は15分程度である。さらに試薬が一つであることが検査者の負担を軽減すると思われる。添付文書上，Pfの感度99.7%，特異度94.2%である。興味深いことに，Pfの原虫血症が高度な場合，それを半定量的に検出するマーカーとしての有用性が検証されつつある[11]。オランダのvan Goolらによる257名の海外渡航後のPf患者を対象とした研究によれば，栄養体が50,000/μl(赤血球の感染率＞1%)をこえる患者(n＝23)のすべてに，aldolaseとHRP2の両方が陽性に検出され，aldolase陰性でHRP2のみが陽性の場合は，「赤血球の感染率＞1%」を除外する信頼できるマーカーとなると示されている。本キットは我々も参加している「国内未承認薬の使用も含めた熱帯病・寄生虫症の最適な診療体制の確立」に関する研究班において既に使用されており，現在我が国の輸入症例に対して評価が行われている。帰国後診療を行う医療機関において将来的に導入することが望ましいと考えられる。

III. 結　　語

本症例のように種々の条件によりマラリア原虫の形態は変化に富む。今回我々は採血時期により早期栄養体が優勢に観察され形態学的診断が困難であった卵形マラリアを経験し，RDTの組み合わせにて診断を推測しnested PCRにて最終診断を行った。一般的にRDTの有用性は明らかであり顕微鏡法と組み合わせると診断能力の向上を図ることができる。これから増えていくと予測される帰国後診療を行う医療機関において，形態学的診断の更なる教育とRDTの導入を提案する。

nested PCRは国立国際医療研究センター研究所熱帯医学・マラリア研究部，狩野繁之先生にお願いしたので，ここに感謝する。

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Cerebral Schistosomiasis Due to *Schistosoma haematobium* Confirmed by PCR Analysis of Brain Specimen[▽]

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The case of a 25-year-old Japanese male who had cerebral schistosomiasis caused by *Schistosoma haematobium* is reported here. Although serum antibody tests showed a cross-reaction with other helminths and no ova were excreted in urine or feces, the existence of *Schistosoma haematobium* in the brain was confirmed by PCR analysis.

CASE REPORT

In October 2009, a 25-year-old Japanese man was admitted to a local community hospital in Japan with a 1-week history of mild headache and sporadic paraphasia. He had worked as an agricultural consultant in the Republic of Malawi from April 2007 to June 2009. During his stay, he lived with local residents, consumed water from a well, and had swum in a lake at least twice. He had been in excellent health until October 2009, except for a *Giardia lamblia* infection in 2008. At the community hospital, a computed tomography (CT) scan of the patient's head showed four hyperdense and edematous lesions in the left parietal lobe, and these lesions were suspected to be related to tropical infectious diseases due to the fact that the onset of his symptoms appeared soon after his return from the Republic of Malawi. Subsequently, the patient was referred to our institution for further workup.

Upon presentation to our institute, the patient's temperature was 36.8°C, his pulse was 60 beats per minute (bpm), and his blood pressure was 120/70 mm Hg. Although the patient was alert and appropriate at a glance, verbal paraphasia was occasionally observed. Laboratory evaluation revealed the following: white blood cell count, 8,780/μl (67.5% neutrophils, 25.0% lymphocytes, 1.5% eosinophils); serum C-reactive protein, 0.03 mg/dl; IgE, 18 U/ml; HIV antibody negative; toxoplasma IgM and IgG negative; and *Entamoeba* antibody negative. A magnetic resonance imaging (MRI) scan of the brain with gadolinium enhancement showed a couple of ill-defined, heterogeneously enhancing lesions. They were each approximately 10 mm in diameter, in the left parietal lobe, with increased intensity of the signal on the T1-weighted image (Fig. 1). A lumbar puncture was not performed. The patient's headache and nausea worsened rapidly, and we were obliged to relieve his symptoms as soon as possible. Based on the clinical presentation and characteristic imaging finding, we clinically concluded that the cerebral lesions were neurocysticercosis. Albendazole (15 mg/kg of body weight per day) was administered with dexamethasone (0.1 mg/kg per day) for a total of 8 days. The patient's headache and nausea then subsided, and the verbal paraphasia disappeared. The findings from an MRI scan of the brain were improved but still remained.

One week after the initiation of treatment, the results of the commercially available serum enzyme-linked immunosorbent assay (ELISA; SRL, Tokyo, Japan), which can detect IgG antibody for 12 helminthic diseases as a screening (22), were reported: *Spirometra erinacei* (also known as *Spirometra mansoni*) antibody on admission was positive, whereas *Taenia solium* antibody was negative. *Schistosoma* species are not included in this screening ELISA. Repeated microscopic examination of urine and stool specimens disclosed no ova or parasites. An enhanced CT scan from the neck to the pelvis was unremarkable, without evidence of subcutaneous nodules. From these findings, cerebral sparganosis, which is due to *Spirometra* species, was strongly suspected as the cause of the cerebral lesions. Cerebral sparganosis responds best to surgical excision of the parasite, because praziquantel has limited success or no effect on adult worms (14, 17). Eleven days after admission, subtotal excision of the nodules at left parietal lobe was achieved by a craniotomy. No live worms or degenerative worms were observed in the surgical field. Pathological examination of the specimen revealed gliosis and multiple necrotizing granulomas scattered within the parenchyma of the brain,

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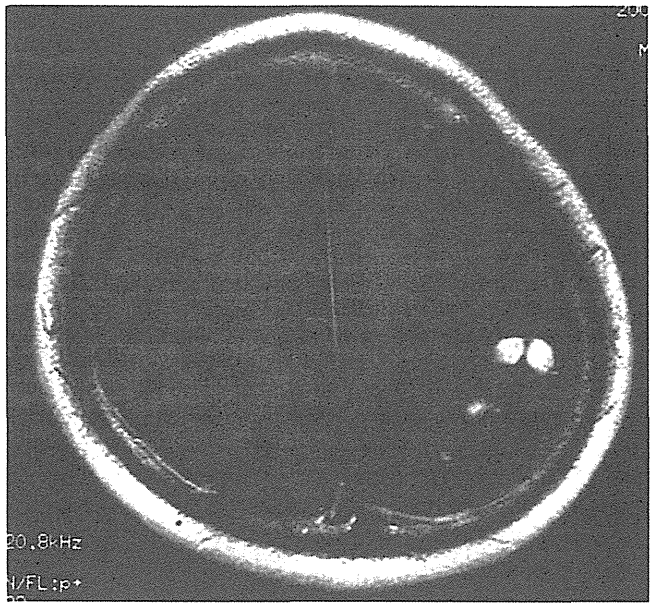

FIG. 1. T1-weighted MRI scan with enhancement, obtained at admission, showed tumor-like lesions in the left parietal lobe with the presence of edema.

with deposits of helminth ova in the center of these granulomas (Fig. 2a). Granulomas had multinucleated giant cells around these ova, which seem to have a prominent terminal spine (Fig. 2b). These morphological characteristics suggested that the helminth ova were eggs of the *Schistosoma* species, particularly *S. haematobium*. To identify *Schistosoma* species, we performed serological tests in our laboratory in the Section of Environmental Parasitology, Tokyo Medical and Dental University (Tokyo, Japan). The result of ELISA revealed increases in serum IgG antibodies against the ova of *S. haematobium* and *Schistosoma mansoni* (*S. mansoni*) and against the larvae of *Spirometra erinacei*. The serum IgG antibodies against *S. haematobium* and *S. mansoni* increased to a level higher than those against *S. erinacei*. For the treatment for the residual lesion, oral praziquantel was commenced at a dose of 20 mg/kg twice a day for a total of 3 days. An MRI scan of the brain with gadolinium enhancement 3 months after the excision and the

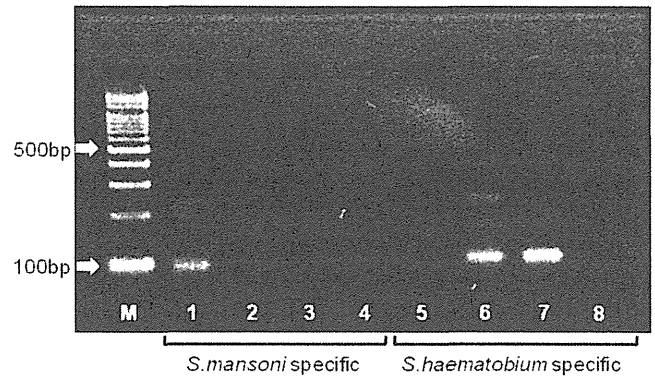

FIG. 3. PCR assay for *Schistosoma haematobium* and *S. mansoni*. M, marker; lanes 1 and 5, *S. mansoni* DNA; lanes 2 and 6, *S. haematobium* DNA; lanes 3 and 7, patient DNA; lanes 4 and 8, no DNA; lanes 1 to 4, *S. mansoni*-specific 121-bp tandem repeat sequence; lanes 5 to 8, *S. haematobium*-specific DraI sequence.

chemotherapy showed a significant reduction in the high signal change. The patient remained in stable condition without clinical complications 4 months after completion of the therapy.

In order to make the definitive diagnosis, the brain specimen was tested by PCR assays. DNA extraction from a paraffin-embedded section of the brain specimen was carried out by using a PCR template preparation kit (TaKaRa DEXPAT Easy; TaKaRa, Shiga, Japan). DNA was amplified with two PCR assays utilizing distinct primer pairs. The first primer targeted to the 97-bp repeated DNA sequence, DraI, which is specific to *S. haematobium* (10). The second primer targeted to the 121-bp tandem repeated DNA sequence, which is specific to *S. mansoni* (11, 25). These specific DNA sequences are not contained in the DNA of *S. japonicum*. As shown in Fig. 3, the PCR amplification using the brain specimen showed an intense band of *S. haematobium* DNA; however, there was no band specific to *S. mansoni* DNA. Therefore, we finally diagnosed the patient's cerebral lesions as cerebral schistosomiasis due to *S. haematobium*.

Diagnosis of a focal lesion in the brain of patients with a

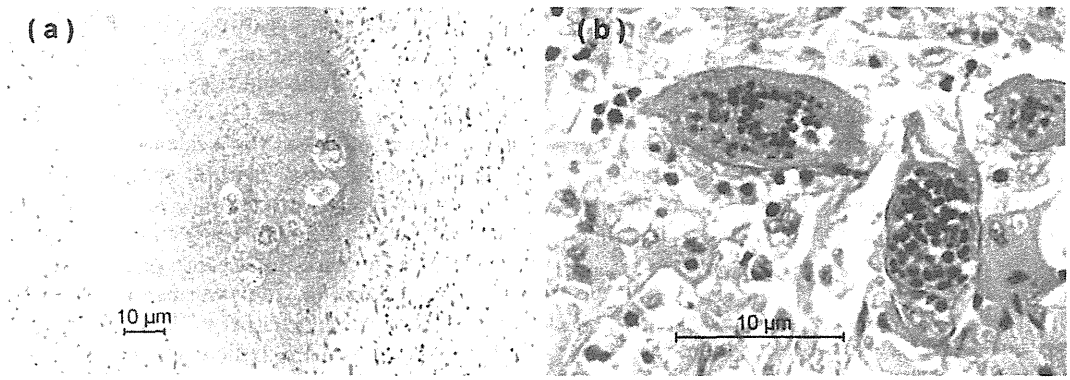

FIG. 2. (a) Photomicrograph showing nodular granulomas within the parenchyma of the brain containing deposits of *S. haematobium* ova in the center of the granulomas (hematoxylin-eosin stained; magnification, ×100). (b) Ova of *S. haematobium* with a characteristic prominent terminal spine (hematoxylin-eosin stained; magnification, ×400).

recent history of a stay in sub-Saharan Africa is usually challenging. Since there were no systemic findings characteristic of a specific tropical disease in this patient, we were obliged to rely on epidemiologic and brain MRI features to establish the provisional diagnosis. The patient's final diagnosis was made by PCR assay using a brain specimen from the surgical excision. Although the prevalence of central nervous system (CNS) invasion in schistosomal infection has been considered to be low (20), CNS involvement in *S. haematobium* infection may be underdiagnosed. An autopsy study in Africa showed that over half of patients infected with *S. haematobium* in the bladder had brain lesions (8). Another pathological study in Africa has found scattered ova of *S. haematobium* or *S. mansoni* in the brain at autopsy in over a quarter of 150 unselected cadavers (1).

Among 22 previous cases of cerebral schistosomiasis due to *S. haematobium*, 7 cases were diagnosed by ovum excretion in urine or feces (15, 23), and 15 cases were diagnosed by immunological testing (18). However, the detailed mechanism of egg deposition in the brain remains unknown. The presence of egg deposits may reflect either an aberrant migration of worms or the embolization of eggs from a remote location (19, 27). In this case, no worms were detected in the brain specimen, and portal hypertension or liver cirrhosis, which could increase the possibility of worm migration to the CNS (26), was not observed. These findings suggest that cerebral schistosomiasis in this case was caused by translocated eggs from a remote location rather than by eggs from ectopically parasitizing adult worms in the brain.

Diagnosing cerebral schistosomiasis can be difficult, since neurological symptoms and radiological findings are nonspecific. In some reported cases of neuroschistosomiasis, brain tumors, such as meningioma and glioma, had been suspected initially (2, 13, 23). Moreover, as in the present case, patients with cerebral schistosomiasis may have no clinical evidence of systemic disease (21, 28). The presence of parasite ova in the urine and/or stool can be detected in only 40 to 50% of neuroschistosomiasis patients (7). Antibody-based assays are quite sensitive but cannot distinguish a history of exposure from acute infection; they can also cross-react with other helminths (3, 9), such as *T. solium* (12). In our patient, the elevated IgG antibody of *Spirometra erinacei* screened by the commercial ELISA led to a presumptive diagnosis of cerebral sparganosis, which in radiological findings mimics cerebral schistosomiasis. The further ELISA performed after histopathological analysis of the brain specimen revealed that IgG antibody titers of *S. haematobium*, *S. mansoni*, and *Spirometra erinacei* were increased. An elaborate examination to screen the infectious focus outside the brain could not detect any abnormality. We then tried to detect the parasite DNA directly from the brain specimen and successfully amplified *S. haematobium* DNA by PCR assay (Fig. 3). These results strongly suggest that PCR assays are helpful means of confirming the results of serum ELISAs for schistosomiasis. Identification and differentiation of human schistosomiasis by PCR in the laboratory setting (16) and in clinical specimens (24, 26) have been reported.

Praziquantel, a pyrazinoisoquinoline derivative, is the mainstay of the treatment for human schistosomiasis. While the standard regimen for chronic systemic schistosomiasis is 40 to 60 mg/kg of praziquantel in divided doses for 1 day, as described in the Centers for Disease Control and Prevention database (http://www.cdc.gov/parasites/schistosomiasis/health_professionals/index.html), the length of the treatment for cerebral schistosomiasis has not been clearly established (4). To reduce the severity of the inflammatory reaction in the brain parenchyma, corticosteroids are commonly used for CNS invasion. Repeated courses of praziquantel and corticosteroids may be required to reduce neurological symptoms in a severe case (13). Our patient responded well to 40 mg/kg of praziquantel in two divided doses for a total of 3 days after surgical excision of the nodules (5, 6).

In conclusion, we report the first case of cerebral schistosomiasis due to *S. haematobium* that was diagnosed by molecular methods. We successfully treated the patient with surgical excision and oral praziquantel. PCR assay is a promising method for definitive diagnosis and species identification of cerebral schistosomiasis when *Schistosoma* ova in urine and/or stool are absent.

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ORIGINAL PAPER

Effect of Mirazid in *Schistosoma japonicum*-infected mice: parasitological and pathological assessment

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Abstract Conflicting reports are found in the literature about the antischistosomal efficacy of Mirazid (MZ), which is a special formulation of myrrh obtained from the stem of the plant *Commiphora molmol*. This initiated the present study to assess this drug for the first time in experimental schistosomiasis japonicum. Mice were divided into four groups: infected untreated control (I); infected treated with MZ, 500 mg/kg (II); infected treated with MZ, 250 mg/kg (III); and infected treated with praziquantel (PZQ), 200 mg/kg (IV). The drugs were given 7 weeks post-infection for five successive days. All animals were killed 3 weeks post-treatment. Results showed no signs of antibilharzial activity of MZ. Total worms, total tissue egg load, egg developmental stages, and granuloma area were not affected by any of the MZ treatment regimens as compared to the infected untreated group ($P > 0.05$ for all variables). These results were in contrast to those obtained in PZQ-treated animals in which 82.82 % total worm reduction, 94.62 % egg reduction, and 86.35 % granuloma area reduction were observed. Also, it significantly increased the percentage of dead ova and decreased the percentage of mature ova with complete absence of immature ones in comparison with the control group ($P < 0.01$ for all variables). In conclusion, the results of the current study raise serious doubts about the antischistosomal activity of MZ.

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Introduction

Schistosomiasis is a chronic debilitating disease that continues to rank, following malaria, at the second position of the world's parasitic diseases in terms of prevalence, morbidity, and mortality rates. Currently, over 200 million people are estimated to be infected, while close to 800 million individuals are at risk of contracting the disease (Steinmann et al. 2006). At least 200,000 people die each year of schistosomiasis. The estimated burden of the disease is up to seven million disability-adjusted life years (King and Dangerfield-Cha 2008).

Due to the unavailability of a vaccine that is practically applicable to humans, the use of chemotherapy is the mainstay of schistosomiasis-associated morbidity control (Abdul-Ghani et al. 2009). For more than two decades, praziquantel (PZQ) has remained as the drug of choice for the treatment of the three common schistosome species, *Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma japonicum* (Doenhoff et al. 2008). Concern is mounting in public health medical circles that heavy reliance on a single drug for schistosomiasis control may promote the selection and spread of drug-resistant parasites (Caffrey 2007). There has been evidence of resistance to the praziquantel-based therapy and reports of acute disease manifestation; therefore, other drugs affecting different stages of the schistosome parasites life cycle and alternative therapeutic regimens should be developed and become accessible (Ribeiro-dos-Santos et al. 2006). As a result, the

need to develop alternative antischistosomal drugs has been stressed.

Mirazid (MZ) is a new antischistosomal drug introduced in the Egyptian market since 2001, in the form of gelatinous capsules produced by Pharco (Alexandria, Egypt). It is prepared from myrrh, which is an oleo-gum-resin obtained from the stem of the plant *Commiphora molmol* (Greene 1993). Myrrh contains the resin myrrhin (23–40 %), the volatile oil myrrhol (2–8 %), gum (40–60 %), and a bitter unidentified component (Claeson et al. 1991). It is a safe, natural flavoring substance and has been approved by the US Food and Drug Administration (Ford et al. 1992).

Myrrh is one of the oldest known medicines and was widely used by Ancient Egyptians (Badria et al. 2001). Traditionally, it has been used by Sumerians and Greeks to treat worms (Michie and Cooper 1991), by Chinese to relieve pain and swelling due to traumatic injury (lee and lam 1993), and by Somalians to treat stomach complaints, diarrhea, and wounds (Claeson et al. 1991; Michie and Cooper 1991). Tincture of myrrh is used for therapy of aphthous ulcer, treatment of sore throat and pharyngitis (Claeson et al. 1991), and reduction of cholesterol and triglycerides (Michie and Cooper 1991; Jain 1994). In experimental studies, the antitumor potential of myrrh was comparable with that of the standard cytotoxic cyclophosphamide (Al Harbi et al. 1994).

Many reports stated the efficacy of myrrh (Mirazid) as anthelmintic drug (Massoud et al. 2001, 2003, 2004, 2007; Sheir et al. 2001; Soliman et al. 2004; Al-Mathal and Fouad 2004; Haridy et al. 2004; Fathy et al. 2005) and antiprotozoa (Baghdadi and Al-Mathal 2010). Also, it has a molluscicide and cercaricide action (Allam et al. 2001; Massoud and Habib 2003) and mosquito larvicide action (Massoud and Labib 2000).

Over the last years, a debate is raised regarding the schistosomicidal effectiveness of MZ. Most of the published data documenting its antischistosomal activity against *S. mansoni* and *S. haematobium* consist of papers written by the discoverers of these properties (Badria et al. 2001; El Baz et al. 2003; Abo-Madyan et al. 2004; Massoud et al. 2004; Sheir et al. 2001; Soliman et al. 2004; Hamed and Hetta 2005). The mechanism of action of myrrh on the schistosome worms, as suggested by the manufacturer, is related to permanent loss of musculature of the worms leading to separation of male and female couples and their shift to the liver where destruction and phagocytosis take place (Badria et al. 2001). Only four groups of investigators reported low cure rates (Botros et al. 2004; Barakat et al. 2005; Ramzy et al. 2010; Osman et al. 2010).

Due to great controversy concerning the effect of myrrh on *S. mansoni* and *S. haematobium*, the current study was designed to assess the antischistosomal activity of MZ; in *S. japonicum*-infected mice in comparison with PZQ. Drug efficacy was evaluated on the basis of some parasitological and pathological criteria.

Materials and methods

Animals, parasites, and infection

All animal studies presented here were approved by the committee of animal rights and ethics, Tokyo Medical and Dental University, Tokyo, Japan, based on the institutional and national regulations for animal experimentation. Six-week-old female C57BL/6 mice were obtained from Japan SLC and used in this study. The animals were maintained on a standard commercial pellet diet and kept in air-conditioned animal house at 20–22 °C.

The life cycle of *S. japonicum* (Japanese strain) is maintained in our laboratory using ICR mice and *Oncomelania hupensis nosophora* (*Yamanashi* strain). Mice were percutaneously infected with 40 *S. japonicum* cercariae/mouse applied to the shaved abdomen.

Drugs

MZ (Pharco Pharmaceuticals Co., Alexandria, Egypt) was tested after resuspension of the content of the resinous capsules in 2 % Cremophore-EL (Sigma Chemical Co., St. Louis, MO, USA). Praziquantel (PZQ; Shin Poong Pharmaceutical Co., Ltd, Kyonggi, South Korea) was given as a freshly prepared suspension in 2 % Cremophore-EL.

Animal groups

In the present study, mice were randomly allocated into four groups each of seven mice:

Group I: Infected control and received the vehicle only.
Group II: Infected and treated with MZ 500 mg/kg.
Group III: Infected and treated with MZ-reduced dose 250 mg/kg.
Group IV: Infected and treated with PZQ 200 mg/kg.

All mice were deprived of food 1 h before treatment, drugs or vehicle were administrated orally using a ball-tipped feeding needle in a volume of 200 μl/mouse, and they were allowed to eat 1 h after treatment. The dosing protocols were given 7 weeks post-infection (p.i.) for 5 days. All animals were killed 3 weeks posttreatment. They were anesthetized using sodium thiopental and given heparin injection.

Study of parasitological criteria

After the mice were killed, hepatic and portomesentric vessels were perfused, using citrated saline. Recovered

schistosomes from each mouse were sexed and counted (Smithers and Terry 1965). The number of eggs per gram of hepatic and intestinal tissues was counted (Cheever 1968). Percentage of the different egg developmental stages (oogram pattern) was examined (Pellegrino et al. 1962).

Hepatic granuloma measurement

Liver specimens were fixed in 10 % formalin and processed to paraffin blocks. Sections (4 μm thick) were stained with hematoxylin and eosin. The size of granulomas was measured (30/mouse) using a VM-30 video micrometer (Olympus, Tokyo, Japan) and NIH image software (National Institute of Health, Bethesda, USA). Average granuloma area (square micrometer) was calculated for each mouse.

Statistical analysis

Comparison was made between each treated group and untreated control. The percentage of reduction between the treated group and the untreated control group was assessed using the formula: (mean value of the untreated group − mean value of the treated group) × 100/mean value of the untreated group. SPSS software version 17.0 was used for data analysis. Descriptive statistics including the mean ± standard deviation (SD) were used. Nonparametric Mann–Whitney test was used to test for significant differences between groups. The data were considered significant if P values were less than 0.05.

Results

Parasitological studies

Tables 1 and 2 show the effect of Mirazid using 500 and 250 mg/kg×5-dosing regimens in mice infected with the Japanese strain of *S. japonicum*. MZ did not show significant decrease in total females (mean ± SD=9.00±1.63 and 9.14±2.27 versus 8.29±1.98) or total worms (20.57±4.04 and 19.71±3.77 versus 19.15±4.22) when compared to the untreated control group, respectively. PZQ at a dose of 200 mg/kg×5 produced a highly significant female and total worm reduction (89.63 and 82.82 %, respectively). MZ regimens did not cause significant reduction in either the hepatic tissue egg count (55.02±20.82 and 82.74±22.83 versus 50.91±18.37/mg of tissue) or intestinal tissue egg count (199.52±113.44 and 189.33±62.26 versus 204.67± 90.55/mg of tissue) when compared to the untreated infected mice, respectively. While, PZQ reduced them significantly by 90.30 and 95.69 %, respectively. Eggs of all developmental stages were observed with MZ regimens. In the PZQ-treated group, no immature eggs were found, with marked reduction in mature eggs (9.86 %) and a marked increase in dead eggs (90.14 %) when compared with parallel values in the untreated controls (55.86, 35.0, and 9.14 %, respectively).

Granuloma measurements

Liver sections of infected untreated controls showed several cellular granulomas. Alternatively, when MZ was given using 500 mg/kg×5 regimen or the reduced dose, the liver pathology was similar to the infected untreated controls, with mean granuloma area of 150.14±8.07, 160.48±11.70, and 150.94±6.78 mm^2, respectively. However in the PZQ-treated group, the granulomas were less numerous and cellular compared with the control group, with highly significant reduction in mean granuloma area (20.60±1.33 78 mm^2), with percentage reduction of 86.35 % (Table 2).

Discussion

This work showed a striking discrepancy between the antischistosomal activity observed in this study and that reported by previous investigators for other schistosome species: *S. mansoni* (Badria et al. 2001; Sheir et al. 2001; Abo-Madyan et al. 2004; Massoud et al. 2004; Hamed and Hetta 2005) and *S. haematobium* (El Baz et al. 2003; Abo-Madyan et al. 2004). In the present study, we have tested two MZ dosing protocols (500 and 250 mg/kg for five consecutive days) in mice infected with *S. japonicum* (Japanese strain), and as a control it was compared with PZQ. Animals were treated 7 weeks p.i. and killed 3 weeks following treatment, which should allow the death of all drug-damaged worms.

Table 1 Effects of Mirazid (MZ) in comparison to praziquantel (PZQ) on worm burden in *S. japonicum*-infected mice 3 weeks posttreatment

Animal groups	Drug dose (mg/kg)	Total males	Total females	Total worms
Infected control	Vehicle	10.86±2.61	8.29±1.98	19.15±4.22
Infected + MZ	500×5	11.57±2.64*	9.00±1.63*	20.57±4.04*
Infected + MZ	250×5	10.57±1.72* (2.67)	9.14±2.27*	19.71±3.77*
Infected + PZQ	200×5	2.43±0.98** (77.62)	0.86±1.21** (89.63)	3.29±2.06** (82.82)

Values are expressed as means ± SD. Numbers between parentheses indicate the percentage reduction from infected control group

*P<0.01 (significant difference from PZQ 500×2 mg/kg); **P<0.01 (significant difference from infected control)

Table 2 Effects of Mirazid (MZ) in comparison to praziquantel (PZQ) on ova counts, oogram patterns, and granuloma area in *S. japonicum*-infected mice 3 weeks posttreatment

Animal groups	Drug dose (mg/kg)	Hepatic ova count×10^3	Intestinal ova count×10^3	Total tissue egg load×10^3	% immature ova	% mature ova	% dead ova	Granuloma area ($\mu m^2 \times 10^3$)
Infected control	Vehicle	50.91±18.37	204.67±90.55	255.58±90.54	55.86±2.41	35.00±1.91	9.14±1.46	150.94±6.78
Infected + MZ	500×5	55.02±20.82*, **	199.52±113.44** (2.52)	254.54±126.96 (0.41)	60.43±1.27**	29.57±0.53**	10.00±1.00**	150.14±8.07** (0.53)
Infected + MZ	250×5	82.74±22.83**, ***	189.33±62.26** (8.10)	272.07±73.47	59.29±1.11**	34.43±0.98**	6.30±0.95**	160.48±11.70**
Infected + PZQ	200×5	4.94±7.72**** (90.30)	8.82±14.28**** (95.69)	13.76±22.00**** (94.62)	0±0****	9.86±1.35****	90.14±1.35****	20.60±1.33**** (86.35)

Values are expressed as means ± SD. Numbers between parentheses indicate the percentage reduction from infected control group

*$P<0.05$ (significant difference from MZ 250×5 mg/kg); **$P<0.01$ (significant difference from PZQ 500×2 mg/kg); ***$P<0.05$; ****$P<0.01$ (significant difference from infected control)

In present work, no signs of antischistosomal activity of MZ were seen. No alterations in either the *S. japonicum* total worm burden or total tissue egg load were observed. Moreover, MZ failed to induce any alterations in the oogram pattern or in the granuloma area in comparison to those in untreated animals. These results are in agreement with few previous reports. Indeed, a multicenter investigation of the potential antischistosomal activity of different derivatives of the resin including the commercial preparation MZ was tested in mice and hamsters infected with Egyptian, Puerto Rican, or Brazilian *S. mansoni* strains. The drug was found toxic for mice at high doses and produced modest or no worm reduction at lower doses and the authors stated that they could not recommend the use of this drug in human cases of schistosomiasis (Botros et al. 2004). Moreover, Ramzy et al. (2010) reported that MZ given in a dose 500 mg/kg for 6 days to *S. haematobium*-infected hamsters showed very slight 3.4 % worm reduction. No change in the number of ova in tissues and slight reduction of 18.3 % in the number of stool eggs were found. Scanning electron microscopic examination of *S. haematobium* worms revealed intact tubercles, spines, and sensory bulbs and no effect of the ventral side

Our findings are in contrast with those of Badria et al. (2001), who reported 76 and 75 % worm reduction upon treatment of mice infected with an Egyptian strain of *S. mansoni* with myrrh at doses 250 and 500 mg/kg twice a day for 3 days. These investigators reported that these treatment regimens induced worm uncoupling and hepatic shift of female worms in a dose-dependent manner. They also revealed a marked increase (93 %) in mature eggs in MZ-treated mice with diminution up to complete absence of some of the immature egg stages. They did not report on the percentage of dead eggs, an increase of which can be considered as a hallmark effect for effective antischistosomals (Pellegrino et al. 1977). The percentages of dead eggs were 90.14 % in our PZQ-treated animals compared with 9.14 % in untreated controls and 6.30–10 % in those receiving MZ regimens. In other studies, when myrrh in a dose of 500 mg/kg/day for 5 days was given to *S. mansoni*-infected mice on the 21st or 45th day p.i., the percentage reduction of worm burden was 76.92 and 98.46 %, respectively, with marked reduction in the egg count in tissues. Significant decrease in the mean number and size of granulomas, paucity of eosinophils, decreased fibrosis and reticular fibers, and the restoration of the glycogen content in the hepatocytes were also reported (Massoud et al. 2004). Furthermore, oral dose of MZ as 600 mg/kg/day for 3 days, given to infected mice, also caused significant reduction in worm and ova count of 81.10 and 73.07 %, respectively (Hamed and Hetta 2005).

Although many observations concerning the antischistosomal activity of MZ appeared promising, we have failed to detect any antischistosomal activity with the commercially