特集 子どもの薬―私なら今これをこう使う
各論 小児に日常よく使われる薬とその使い方

5．駆虫薬
―小児の寄生虫駆除の問題点―

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はじめに

日本では戦後官民を挙げて寄生虫対策事業を推進した結果，もはや寄生虫症は消滅したかのような印象が社会一般のみならず，医療者においてさえも抱かれる今日である。しかし，土壌伝播性の腸管寄生線虫の感染率はほぼゼロを達成したものの，条虫や一部の吸虫感染には大きな変化が見られず，また輸入寄生虫症の増加には十分な対応ができていない。一部の原虫感染症では初期対応を誤れば予後に重大な影響をもたらすことも銘記すべきである。

寄生虫症治療の原則は正確な診断に基づく駆虫薬服用にある。しかし今日の薬価収載駆虫薬は限られており，12薬剤，適応疾患も15種にすぎない（表1）。WHOのessential drugも十分にはカバーされていない。さらに輸入寄生虫症治療に不可欠な治療薬の多くが国内未承認薬であることに加えて，小児に用いる場合のガイドラインも未確定であり，臨床医として対応に苦慮することは想像に難くない。一部の例外を除いて，今日の小児科診療において寄生虫症治療は対応が困難な分野と考える方が妥当であろう。本稿では国内で遭遇しうる寄生虫症について，駆虫薬・治療薬の処方についての基本的な考え方と注意点を紹介したい。

Ⅰ．蠕虫駆虫薬

ヒト寄生の蠕虫には線虫，吸虫，条虫の3群が含まれ，それぞれに診断・治療のアプローチが異なる。さらに本来はヒトの寄生蠕虫ではないものが感染することによって起こる幼虫移行症は日本人の生活様式の変化に伴って今後さらに問題が拡大すると予想されている。

1．腸管寄生線虫の駆虫薬

第二次大戦後の日本では国民の7割が何らかの腸管寄生線虫に感染していた。わが国の寄生虫症の減少とは腸管寄生虫の減少のことである。今日小児で最も頻度が高い腸管寄生線虫は蟯虫であるが，自然農法で糞便を肥料に用いる家庭菜園愛好者で回虫の家族内感染

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表1　国内承認の抗寄生虫薬剤

薬剤名	適応
メファキン (メフロキン)	マラリア
ファンシダール (スルファドキシン+ピリメサミン)	マラリア
塩酸キニーネ	マラリア
サントニン	回虫症
スパトニン (ジエチルカルバマジン)	糸状虫症
ストロメクトール (イベルメクチン)	糞線虫症
メベンダゾール	鞭虫症

薬剤名	適応
コンバントリン (パモ酸ピランテル)	蟯虫症，回虫症，鉤虫症，東洋毛様線虫症
ビルトリシド (プラジカンテル)	肝吸虫症，肺吸虫症，横川吸虫症
エスカゾール (アルベンダゾール)	エキノコックス症
フラジール (メトロニダゾール)	膣トリコモナス症
ハイシジン (チニダゾール)	膣トリコモナス症

が見られることもある。沖縄県では風土病である糞線虫の感染も起こりうるが，若年者には稀な寄生虫になった。

1) コンバントリン

ヒトの腸管寄生線虫のうち鞭虫と糞線虫を除けばコンバントリンで効果的に駆虫ができる。一般医家はまず本剤を念頭におき，それが有効な寄生虫か否かを考えればよい。小児に用いるにあたっては過敏症に留意すれば大きな問題はない。寄生虫の神経系に障害を与え，糞便中に虫体が排出される。駆虫には10 mg/kgの単回投与を行う。本剤は幼虫には無効であるため，蟯虫の駆虫に際しては感染宿主体内に幼虫と成虫が混在している可能性が高いことを念頭において，2週間程度の間隔を置いて再度同量を服用することが必要である。

2) メベンダゾール

コンバントリンで駆虫ができない腸管寄生線虫の代表が鞭虫である。国内にも局所的な流行地区が残っていたが本剤の導入により1980年代には消滅した。成人では200 mg/日，分2で3日間連用するが20 kg以下の小児では半量とする。シメチジンとの併用で重篤な薬剤アレルギーの危険性がある。

3) ストロメクトール

2002年に糞線虫症治療薬として国内承認がされたが，本来はフィラリア治療薬として世界で広く用いられてきたものである。日本人の適切な用量も成人症例での検討データしかないため体重15 kg以下の小児には投与を控えるように指導している[1]。体重15～24 kgの小児では3 mg錠を1錠／日，朝食1時間前に服用し，自家感染が起こることを考慮して2週後に同量を服用する。

2．吸虫症の治療薬

今日国内で遭遇する吸虫感染症の大半は横川吸虫によるものであり，主な感染源であるアユを好んで食べる地域に多い。ヒトの腸管に限定して寄生するので多数寄生にならない限り無症状である。肺吸虫症も中間宿主のカニよりも待機宿主であるイノシシ肉の生食から感染するという最近の傾向のために小児の感染例は少なくなった。輸入感染症として住血吸虫症が稀に見られるが，その多くはアフリカからの帰国者である。吸虫症の駆虫薬はプラジカンテルであり，肝蛭を除いてほぼ全ての吸虫に強い駆虫効果がある。国内では横川吸虫，肝吸虫および肺吸虫の駆虫だけに保険適用は限られている。

1) プラジカンテル

1970年代に安全で効果の高い吸虫症治療薬として実用化されたもので，住血吸虫症治療に劇的改善をもたらした。吸虫類体表のイオンチャンネル系に障害を与えて殺滅する。軽度の消化器症状が副作用としてみられる場合があるが重大なものは少ない。吸虫の種類によって用量が異なるので処方に際して注意を要する。小児に対する安全性にも重大な特記事項の報告はない。本剤は成虫を殺滅する一方で幼虫に対する効果は低いので，成虫と幼虫の混在が予想される場合には服用スケジュールを考慮する。

2) エガテン

肝蛭がプラジカンテル治療に抵抗することは知られていたが，代替薬がないことが問題であった。国内では山間部の畜産業地域に感染が見られるため治療薬備蓄の必要性が論じられ，WHOが推奨する本剤を厚生労働省研究班で保管している[2]。しかし乳児には安全性が確立しておらず処方は控える。

3．条虫症治療薬

条虫の成虫はすべて終宿主の腸管に寄生する。ヒトを本来の終宿主とする条虫には体長10 mにも及ぶ巨大なものもあるが，症状は一般に軽微で肛門からの虫体排泄によって気づく場合が多い。感染幼虫を含んだ食品摂取により感染する。成人に多く見られるが，同じ食品を通じて家族全員が感染した事例がある。一方，小型条虫，縮小条虫などは昆虫の誤飲で感染するので小児のリスクが高いが，国内での症例報告は少ない。有鉤条虫とそれ以外の条虫で駆虫方法の選択を行う必要があり，正確な診断が重要である。

1) プラジカンテル

条虫の駆虫は駆虫剤を投与して下剤により虫体を回収し，頭節が排出されたことを確認することで完了する（図）。頭節の排出がないと必ず再生するので患者への説明が必要である。プラジカンテルは有鉤条虫を除いた全てのヒト寄生条虫の駆虫に有効であるが保険適用ではない。治療当日の朝に排便させた後，プラジカンテルを20 mg/kg服用させる。2時間後に下剤を内服させ，極力我慢させて一気に排便させて排出虫体の頭節を確認する。複数虫体寄生の場合もあるのでそれぞれの頭節を確認する。小型条虫，縮小条虫で寄生個体数が多い場合，すべての虫体排出は確認できないが，小型条虫では自家感染を起こすので10日後に再度駆虫することが望ましい。

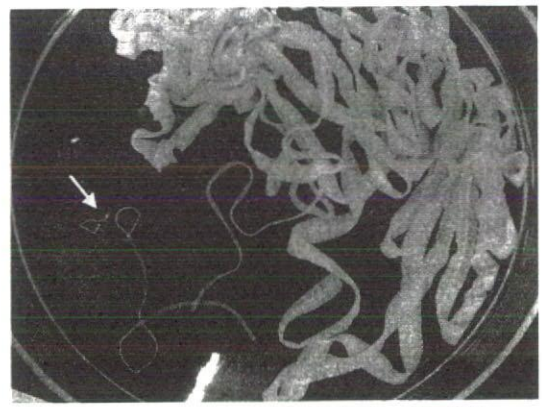

図　駆虫した日本海裂頭条虫（→が頭節）

有鉤条虫だけはプラジカンテルによる駆虫は禁忌である。有鉤条虫の虫体が破壊されて虫卵がヒト体内で散布されるとその幼虫である嚢虫がヒトの組織中に形成されるからである。これを有鉤嚢虫症とよび，全身に皮下結節が形成されて，脳内病変も起こすことがある。代替手段としてX線透視下に造影剤を注入して有鉤条虫を排出させる方法が取られる。

4．幼虫移行症の治療薬

幼虫移行症はヒトを本来の宿主とはしない線虫または条虫の虫卵または幼虫を取り込んだ結果，発育が止まった幼虫がヒト体内を移行することによる疾患である。ペットや野生動物との接触の濃密化が今日の発生数の増加と多様性を招いている。診断自体困難であ

り，薬物治療が可能なものと困難なものとがあり，一部は重症化するなど今日の寄生虫疾患では最も問題が大きい[3]。

1）駆虫が可能な幼線虫移行症

今日薬物による駆虫が試みられるのはイヌ／ネコ回虫症だけである。本症は幼虫がまれに眼球内に至り，ぶどう膜炎の原因になるほか，網膜芽細胞腫と所見が似るため眼球が摘出される事例が過去にあった。ペットとの接触に加えて公園砂場の虫卵汚染を指摘する報告もある[4]。血清診断には幼虫の分泌抗原を用いる特殊検査となる[5]。アルベンダゾールが駆虫薬であるが，服用が2週間以上にわたり，肝機能障害も起こりやすいなど小児への処方は慎重にする必要がある。放置した場合は数年の経過で幼虫は自然に死滅する。

2）駆虫ができない幼線虫移行症

代表的なものにネズミの寄生虫による広東住血線虫症があり，好酸球性髄膜炎の原因になる。有効な駆虫薬はなく対症療法で自然に治癒するが，最近沖縄県で女児の死亡例があった[6]。イヌ糸状虫症は肺の coin lesion として偶然発見される。診断が確定しても積極的な治療法はない。最近の問題は各種動物回虫の幼虫移行症であり，特にアライグマ回虫症は神経系を侵して死亡率が高い[7]。日本各地のアライグマの増加は懸念材料である。

3）条虫による幼虫移行症の駆虫

イヌ科動物の腸管寄生条虫の幼虫によるエキノコックス症が代表例で，ヒトが偶発的に虫卵を取り込むと幼虫が活発に増殖して悪性腫瘍に似た病態が引き起こされる。主に肝臓で包虫と呼ばれる幼虫が増殖し，包虫の胚層が血行性／リンパ行性に他組織に転移する。治療は早期発見による外科的摘除であるが，手術適応にならない場合にプラジカンテルを用いる。抗原放出によるアナフィラキシー予防のためにステロイドを併用する。

有鉤嚢虫症も外科的な摘出が原則であるが手術適応にならない場合にプラジカンテルを試みる。同様にアナフィラキシー防止のためにステロイドを併用する。エキノコックス症も有鉤嚢虫症も未治療で放置した場合には自然治癒することはないので，積極的に治療を図る。

II．原虫症の駆虫薬

原虫は宿主体内で増殖して寄生個体数を増加させて重症化するために適切な薬剤による駆虫が必要である。国内で駆虫薬処方が要求される原虫感染症としてはトキソプラズマ症，赤痢アメーバ症，ジアルジア症，マラリアなどが考えられる。一方で駆虫薬が未開発の原虫感染症も多く，クリプトスポリジウム症やサイクロスポーラ症などは対症療法が主となる。

1．トキソプラズマ症の駆虫薬

トキソプラズマ症は先天感染と後天感染とがあり，小児科領域で治療対象となるのは先天性トキソプラズマ症である。胎児期の感染がありかつ症状を伴っている場合はピリメタミンとスルファヂアジンを葉酸製剤とステロイドとの併用にて1年間服用を継続する。ステロイドは症状軽快の時点で中止してよい。ピリメタミンとスルファチアジンは国内未承認薬であり，厚労省の熱帯病稀用薬保管研究班を通じて入手する。

2．マラリアの治療

マラリアは熱帯地方の代表的な感染症でわが国でも年間100例内外の輸入症例がある。小児では容易に重症化するので成人患者より迅速な処置が必要となる（表2）。特に熱帯熱マラリアでは進行が速いので適切な薬剤選択と全身管理を行う。小児は嘔吐しやすく，内服困難な重症例では坐薬を考えてもよい。国内認可薬であるメフロキンは合併症を伴わない熱帯熱マラリアの治療に用いるが，東南アジアを中心にメフロキン耐性マラリアが拡

表2　小児と成人の重症マラリア比較

	小児	成人
咳そう	初期にはしばしばあり	頻度低い
けいれん	脳マラリアか低血糖のサイン，ただし熱性のものも含む	脳マラリアか低血糖のサイン
重症化までの期間	通常1～2日	数日（4～6日？）
黄疸	まれ	しばしば
治療開始後，昏睡から回復までの期間	通常1～2日	2～4日
低血糖	しばしば	頻度少ないが，キニン治療時にhyperinsulinemia
肺水腫	まれ	しばしば
薬剤副作用	成人に比べて頻度高い	少ない
腎不全	まれ	しばしば
神経学的続発症	約10%で見られる	まれ

表3　熱帯病治療治療薬開発研究班による保管薬剤一覧

薬剤名	一般名	対象	剤形
Alinia	nitazoxanide	クリプトスポリジウム	20mg/mℓ（60mℓ）
Arsobal	melarsoprol	アフリカトリパノソーマ	180mg/5mℓ vial
Humatin	paromomycin	赤痢アメーバ	250mg，錠剤
Egaten	triclabendazole	肝蛭	250mg，錠剤
Flagyl Inj	metronidazol	赤痢アメーバ	注射液，0.5%100mℓ/bag
Germanin	suramin	アフリカトリパノソーマ	1g（10%solution）
Malarone	atovaquone/proguanil	マラリア	1錠＝250/100mg
Nivaquine	chloroquine sulfate	マラリア	150mg塩基錠
Ornidyl	eflornithine hydrochloride	アフリカトリパノソーマ	注射液，100mℓ/vial
Pentstam	sodium stibogluconate	リーシュマニア	注射液，100mℓ/vial
Impavido	miltefocin	内蔵リーシュマニア	50mg錠剤
Alinia	nitazoxanide	クリプトスポリジウム	20mg/mℓ（60mℓ，500mℓ）
Sulfadiazine	Sulfadiazine	トキソプラズマ	500mg錠剤
Plasmotrim	artesunate	マラリア	200mg坐薬，200mg錠
Promaquine	primaquine phosphate	マラリア	7.5mg塩基錠
Quinimax	quinidine gluconate	マラリア	注射液，250mg/vial
Riamet	artemether/lumefantorine	マラリア	20/120mg錠

散している[8]。本剤は新生児，乳児への投与は行わない。小児に対する安全性も確立していないので保護者への説明を慎重に行う。しかし何らかの処置をしないと生命予後は著しく悪化するので無用の躊躇は許されない。三日熱マラリアではクロロキンが第一選択薬で，さらに根治療法としてプリマキン処方を追加する。いずれも国内未承認薬であるので

前記研究班を通じて入手する。

3．赤痢アメーバの駆虫薬

国内では知的障害者施設で赤痢アメーバ感染の集団発生事例があるため，施設内生活の小児で注意する必要がある[9]。防疫上重要なのはシストを排出する無症候性キャリアであり，その治療薬選択と用量について議論がある。腸炎患者ではメトロニダゾールが第一選択薬であり小児では 20 mg/kg/日で 10 日間連用が推奨されているが，本剤は保険適用外である。効果には個人差が大きいので薬剤投与を繰り返す必要があり得ることを説明した方がよい。無症候の嚢子排出者には研究班からパロモマイシンを供給しているが新生児への投与は控え，それ以外の小児では 30 mg/kg/日で 10 日間が一応の標準処方とされる。

おわりに

駆虫薬の今日的問題を列挙した。すべての寄生虫症の駆虫薬が開発されている状況ではなく，また多くはいわゆる neglected disease に含まれるので，国際的にも対応が困難な状況にある。わが国では輸入寄生虫症のうち，国内未承認薬の国内備蓄が必要なものについて研究費ベースで確保しており，確保薬剤は適宜見直しをしているが，現段階の保管薬剤は**表 3** に記した。寄生虫疾患は診断，治療方針とも特殊な領域にあることは事実であり，各地の大学の寄生虫病関連部署に相談されるか，日本寄生虫学会のホームページを通じてコンサルトをされる方も多いので活用いただきたいと思う。

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Parasitology in Japan

Disease burden and epidemiology of soil-transmitted helminthiases and schistosomiasis in Asia: the Japanese perspective

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The disease burden due to soil-transmitted helminthiases (STH) and schistosomiasis is not well documented in Asia. Both STH and schistosomiasis are chronic diseases but case detection is not easy because of the absence of clinical symptoms. STH and schistosomiasis are, however, endemic in Asia and their burden is significant. At the preparatory meeting for the Hashimoto Initiative in Japan in 1997, STH and schistosomiasis were categorized as Group 2 diseases. Parasitic infections in this category were well understood at the time but sophisticated control strategies were lacking. Japan has promoted comprehensive collaborative projects to reduce the burden of STH and schistosomiasis throughout Asia, creating an international network to collect epidemiological information and to implement and improve disease control, thus extending the school-based control method that had proved so successful in Japan.

Evaluation of disease burden

Helminth infections are important health problems in many parts of Asia but an exact evaluation of disease burden due to soil-transmitted helminthiases (STH) and schistosomiasis is not available because they are so-called 'neglected diseases'. There is no registration system for STH and schistosomiasis in Japan or other Asian countries. The current status of STH and schistosomiasis varies across the region. A recent increase in trade and human migration within Asia has highlighted the need to evaluate the epidemiological status of STH and schistosomiasis.

Japanese researchers have been proactive at building partnerships with Asian parasitologists to find applicable and effective strategies for parasite control. In the Hashimoto Initiative for global parasite control (HI) of 1997, STH and schistosomiasis were designated as Group 2 diseases [1]. The HI working group categorized parasitic diseases into three groups: Group 1 diseases require investment from the basic research stage to develop new treatments. Group 2 (which includes the filariases, in addition to STH and schistosomiasis) is a group of diseases for which control drugs already exist, and the priority is to establish a mechanism to deliver them to endemic areas. Group 3 falls between Group 1 and Group 2. This means that applied or operational research is needed, rather than basic research, for implementing disease control for STH and schistosomiasis. To discuss the Japanese perspective on STH and schistosomiasis in Asia, it is important to understand the current status of these parasitic infections in Japan and in East or Southeast Asian countries.

STH and schistosomiasis in Japan: then and now

Heavy disease burden due to STH and schistosomiasis in the first half of the 20th century was a strong driving force for parasitology research in Japan. In 1949, the incidence of STH peaked at 73% of the population [2] but the picture regarding schistosomiasis remains unclear. In 1950, only 1.6% of residents in the Kofu basin, which is in the central Yamanashi part of Japan, tested positive for *Schistosoma japonicum* in stool examinations using the direct smear method [3]. This relatively low incidence was probably a gross underestimation because a positive rate of *S. japonicum* infection of 44.2% was reported in the same area when some, but not all, health centers tested stools of schoolchildren using the centrifugal concentration (AMS III) method. Furthermore, single testing results in underestimates because repeated testing of fecal samples from residents of the Kofu basin raised the positive rate to more than twice that detected by the merthiolate-iodine-formaldehyde concentration (MIFC) method [4].

The most common STH in Japan in the first half of the 20th century was *Ascaris lumbricoides* infection, followed by *Necator americanus* infection. Night soil was widely used as a fertilizer for cultivation, resulting in contaminated vegetables, which were the main source of

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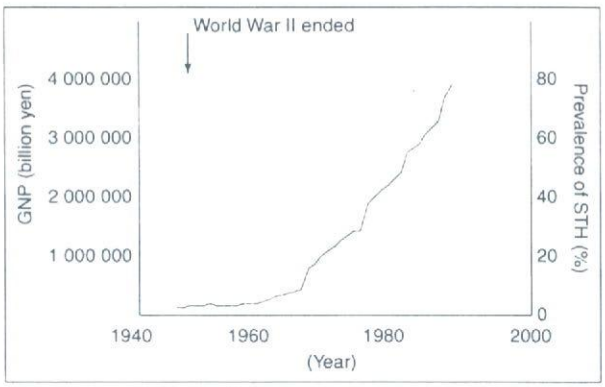

Figure 1. Control of parasitic diseases and economic development in Japan. The GNP (blue line) of Japan started to rise in the mid-1960s, whereas the prevalence of STH (green line) decreased rapidly before the economic growth in Japan. Reproduced, with permission, from M. Shimada, Nagasaki University.

STH. With the rapid improvement in quality of life in Japan (including the cessation of night soil use), the prevalence of STH fell rapidly and reached a negligible level in the late 1970s (Figure 1). Despite rapid economic growth in Japan in the 1960s, economic development was not the main factor behind successful STH control. Japan created a unique and effective scheme for parasite control after World War II, which was led by the private sector, the Japan Association of Parasite Control (JAPC) and supported by local governments [5]. JAPC implemented a school-based approach, in which teachers had the main role in health education and deworming, that was backed by the Japanese Government via proclamation of the School Health Law in 1958. The Japanese Organization for International Cooperation in Family Planning (JOICFP: http://www.joicfp.or.jp/) was also important and was established based on the unique idea of integrating STH control with family planning and nutritional improvement [6]. The parasite control activities of JAPC and the integrated programs contributed substantially to health promotion, not only in schools but also in communities because parasite control has proven to be a good starting point for encouraging community participation. Members of academia also had important roles in parasite control activities, not only by providing scientific guidance but also by evaluating the efficacy of the control interventions.

Currently, a small number of sporadic cases of STH infection still occurs in Japan. Several factors contribute to this: (i) there is a movement to use non-chemical fertilizer for vegetation, such as night soil, the use of which places more people at risk of *A. lumbricoides* infection; (ii) a specific group at risk comprises those who have lived for prolonged periods in other endemic countries, with >10% of people returning from Africa bringing back STH [7]; (iii) recently, food-borne STH from fresh food imported from endemic countries has also been identified [8]; and (iv) strongyloidiasis is still endemic in Okinawa and other southern Japanese tropical islands. A recent survey reported that the incidence of *Strongyloides stercolaris* infection is 5–10% in Okinawa, although infections were observed mainly in older age groups [9]. A strong association between strongyloidiasis and adult T-cell leukemia virus has been reported [10,11], although the biological mechanisms of the association remain to be elucidated. In addition, there is a small number of cases of opportunistic infection with *S. stercolaris* in immunocompromized people in Okinawa [12].

Schistosomiasis japonica was endemic in Japan, with two Japanese pathologists, Katsurada and Fujinami, discovering the causative parasite, *S. japonicum*, in 1904 [13]. Nine years later, Miyairi discovered *Oncomelania nosophora*, which is the intermediate host snail for *S. japonicum* [14]. These discoveries enabled the implementation of schistosomiasis control in the early 20th century [15]. Schistosomiasis is prevalent in several foci where intermediate snail host colonies exist. Although the intermediate host snails in Japan are of a single species, *S. japonicum* in each endemic focus in Japan has adapted only to *O. nosophora* of the same geographical origin [16,17]. This indicates that imported strains of *S. japonicum* are not readily introduced into Japan.

Since 1977, no newly infected cases of schistosomiasis have been reported in Japan and, in 1996, the local government in Yamanashi (Japan) declared that transmission had ceased. Since then, only imported cases have been reported, most of which were schistosomiasis from Africa. The disease control strategy for schistosomiais in Japan comprised three main approaches: (i) control of the snail intermediate host; (ii) treatment of all infected people; and (iii) concreting over the wetland habitat of the snail host [18]. Because schistosomiasis japonica is zoonotic, health checks of human residents and sampling of wild mice were the methods used for case detection. At a health check, intradermal skin tests were used to screen for schistosomal infection [19]. Although the cause is unclear, the hepatitis C virus appeared earlier in schistosomiasis-endemic areas than in schistosomiasis-free areas [20]. A similar association was reported in Egypt [21], where schistosomiasis is also endemic. *S. japonicum* infection might be carcinogenic [22], with epidemiological studies showing significant association between rectal and hepatic cancers and *S. japonicum* infection in Japan [23,24]. Cercarial dermatitis has also been reported, which is caused by schistosomes of birds (e.g. *Gigantobilharzia sturniae*). Rice farmers, in particular, are at risk because there are snails in paddy fields, and birds that feed on the snails perpetuate the life cycle of the parasite.

STH in Southeast Asia and China

STH and schistosomiasis are the most common helminth infections worldwide, especially in poor communities in Southeast Asia. STH are widely distributed throughout the region (Figure 2). It is estimated that, in 2003, 33.9 million people in Vietnam and 74.7 million people in the Greater Mekong Subregion (GMS) countries were infected with *A. lumbricoides*, and 17.6 million people in Vietnam and 32.9 million people in the GMS were infected with *Trichuris trichiura* [25,26].

There is only a small amount of recent published data about the disease burden and epidemiology of STH, and data are not available from many countries. Brooker *et al.*

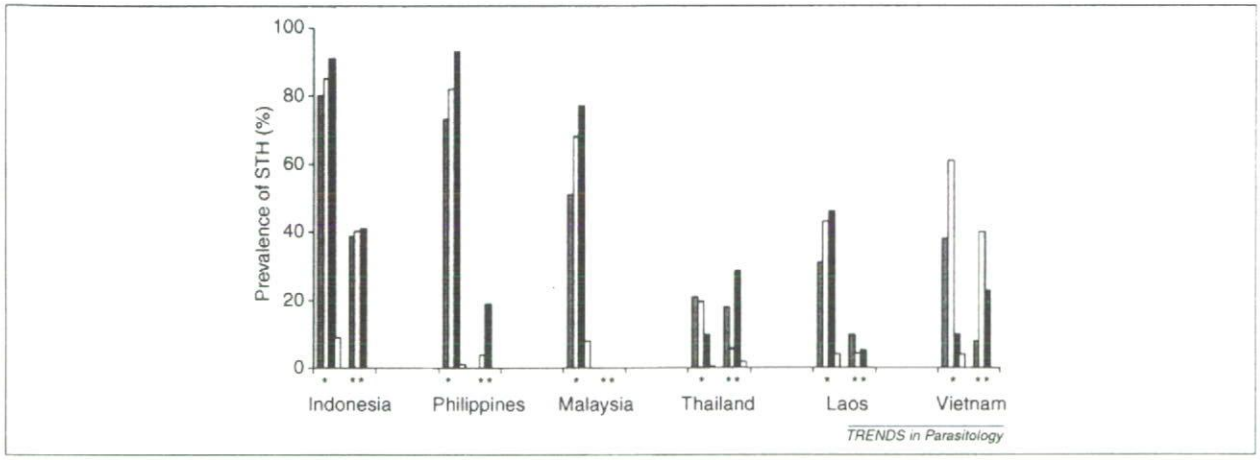

Figure 2. Prevalence of STH in Southeast Asia. The prevalence of STH is high in the south-central part (*) compared with the northern part (**) of the countries shown. Key: *Ascaris lumbricoides*, orange bars; hookworm, green bars; *Strongyloides stercoralis*, unfilled bars; *Trichuris trichiura*, blue bars.

[26] used a geographical information system (GIS) to collate and map STH distribution in Southeast Asia and found a distinct geographical variation throughout the region. In Vietnam, for instance, the prevalence of STH infection declined from the north to the south of the country [25], whereas higher prevalence occurred in the south of Thailand than in other parts of the country [27]. A report from Bali showed that wet highland had significantly higher STH prevalence than did wet lowland, dry highland or dry lowland [28]. In Malaysia, high levels of STH infection were reported in Orang Asli, an aboriginal tribe resettlement village [29]. This variation in STH occurs because transmission is strongly related to environmental and host behavioral factors (Table 1).

A national survey was carried out in China in 2003 to analyze the epidemiological status of helminthic infections [30]. Results showed that the infection rate of STH was 19.6%, of which ascariasis accounted for 12.7% (86 million people), followed by hookworm infections with 6.12% (39 million people) and whipworm infections with 4.63% (29 million people). One-third of infected individuals carried two or more parasites, with some infected by six species of parasite at the same time. The highest infection rate, with >10 000 hookworm and/or whipworm eggs per gram and >50 000 *A. lumbricoides* eggs per gram, was noted in Hainan Province (54.7%), with other southern areas such as Guizhou, Sichuan, Guangxi and Hunan showing an infection rate of at least 30%. STH in northern areas were less frequent, with the lowest infection rate reported in Xinjiang Province (0.72%). STH are most common in primary-school children and illiterate people in China, indicating that the STH infection rate decreases with higher education level. The helminthic infection rate in China seems to be declining, with heavy infections of *A. lumbricoides* and hookworm found in fewer than 2% of infected people in 2003.

Table 1. Factors influencing the transmission of STH and schistosomiasis

STH	Schistosomiasis
Environmental	
Tropical climate	Tropical climate
High humidity	Water (e.g. river)
Unhygienic sanitation	Unhygienic sanitation
Land surface temperature	Snail intermediate host
Night soil fertilizer	Reservoir hosts
Behavioral	
Toilet usage	Toilet usage
Personal cleanliness	Water contact
Occupation (e.g. farmer)	Occupation (e.g. farmer, fisherman)
Wearing shoes	

Schistosomiasis in Southeast Asia and China

Schistosoma blood flukes in Southeast Asia belong to the *S. japonicum* complex, which comprises three different species: *S. japonicum*, *Schistosoma mekongi* and *Schistosoma malaysiensis* [31]. They differ in morphology, geographical distribution, snail intermediate host and enzyme polymorphisms. Approximately 60 000 Laotians and 80 000 Cambodians are estimated to be at risk from schistosomiasis mekongi [32], and ~6.7 million Filipinos are at risk from schistosomiasis japonicum [33]. The best-known species is *S. japonicum*, which is found in China, the Philippines [34] and certain areas of Sulawesi, Indonesia [35]. In China, endemic foci occurred in seven provinces, and 843 000 infected individuals were reported in 2003. Two distinct types of schistosomiasis are endemic in China: marshland type and hill type. Approximately 11 million people are at risk from the marshland type and 5 million people are at risk from the hill type. Marshland schistosomiasis occurs in the mid- or lower reaches of the Yangtze and is under the direct influence of the environmental conditions of both the river and the surrounding lakes. Hill-type schistosomiasis occurs in western China. Although the endemic foci are not big, disease control is difficult because the foci are located in remote areas.

S. mekongi occurs in the Khong District of Laos and along the Mekong in Cambodia [32]. Up to 150 000 inhabitants were at risk of *S. mekongi* infection in the 1980s. However, the recent situation in the endemic area is much improved because of a mass treatment program led by the Cambodian Government, the World Health Organization (WHO: http://www.who.int) and Medicins sans Frontieres (http://www.msf.org). The program started in 1995, with a Japanese non-governmental organization (NGO), the

Sasakawa Memorial Health Foundation (http://www.sasakawa-igaku.or.jp), joining in 1997 [36].

S. malayensis infects various indigenous tribes in the upper Rejang river basin, Sarawak, Malaysia [37]. Among animal schistosome species, *Schistosoma spindale* is a common cause of cercarial dermatitis in humans in Indonesia, Malaysia, Thailand and Vietnam. This dermatitis is strongly associated with farmers and fishermen working in rice paddies [38].

More than ten species of mammal, including water buffalo, wild pigs, deer, horses, dogs, cats and rodents, are reservoir hosts of *S. japonicum*. Water buffalo and cattle are the most important hosts of this parasite in China, whereas dogs, but not water buffalo, are important hosts in the Philippines [32]. In the case of *S. mekongi*, 12.2% of pigs in Laos and 0.3–3.6% of dogs in Cambodia are hosts [39–41]. *Oncomelania hupensis hupensis* and *Oncomelania hupensis quadrasi* are the snail hosts for *S. japonicum* in China and the Philippines, respectively; however, apparent strain differences are noted within *O. h. hupensis* [42]. *Neotrichura aperta* is the only species of snail known to be a host of *S. mekongi* [43], and *S. malayensis* uses *Robertsiella kaporensis* as its intermediate host [37]. The restricted distribution of these snails limits the endemic areas of schistosomiasis. A study of rats in proximity to snail colonies showed that 95.5% of rats caught within a snail colony were positive for schistosomiasis, 56.5% of rats caught within 100 m of a snail colony were positive and no rat caught $\geq$1 km from a snail colony was positive for schistosomiasis. Existing control programs in endemic areas aimed at improving sanitation and reducing both the number and the size of snail habitats have led to decreased infection rates among rats and snails [44]. Common locations relating to snail breeding sites that increase the presence of the disease are irrigation networks and agricultural land [45] (Table 1).

Results of studies on the epidemiology and immunology of schistosomiasis in the Philippines indicate that the individuals who are most vulnerable to rapid reinfection are 5–14-year-old children. In China, however, high incidence is observed even in adults [46]. A drop in incidence at age 15–19 years and decreased intensity of infection at this age and in older Filippino people indicate the development of immunity [47]. Schistosomes, *T. trichiura* and hookworms cause anemia, and co-infections of these species increase the likelihood of anemia, particularly in 5–14-year-old children. Carcinogenesis associated with *S. japonicum* has also been found in China, and specific effects on mutagenicity have been suggested [48].

Schistosomiasis control in China has been implemented since 1955, when endemic situations were serious in areas along the Yangtze, including Shanghai, Wuhan and other big cities. Although the endemic situation has improved, a renewed effort to eliminate schistosomiasis was mounted as a collaborative project with the World Bank (http://www.worldbank.org) in 1992. Over eight years, a nationwide mass chemotherapy program was implemented and one endemic province, Zejiang, declared the disease to be eradicated in 1996. During the program, >200 research projects, both applied and operational, were promoted and new therapeutics, diagnostics, epidemiological techniques and cost-effective operational approaches were investigated [49]. China is considered to be in the final stage of disease eradication; however, there are several obstacles to overcome before reaching this goal [50]. Despite the intensive control program, a warning was recently issued by the Chinese Center for Disease Control and Prevention (http://www.chinacdc.net.cn) that there is a reemergence of schistosomiasis japonica in China. A nationwide survey was carried out in 2005, the results of which will be made public in the near future. Construction of the Three Gorges Dam will be completed in 2006 and the distribution of endemic foci is anticipated to change because of the changing water levels of the Yangtze. Because selective chemotherapy is undertaken during low disease prevalence in China, it is important to develop a sensitive, but cost-effective, case-detection system. Intensive surveys that use new tools and techniques are needed to create a new strategy for schistosomiasis control in China.

The viewpoint in Japan

Historically, the transmission of STH has been related to social infrastructure, including water supply, toilet facilities and sanitation, lifestyle, cultivation techniques and food distribution. Therefore, STH are considered to be a socioeconomic matter. However, in the case of Japan, the economy did not have an important role in the control of STH. Instead, the gross national product increased in Japan just after the successful control of infectious diseases such as STH (Figure 1). This means that improved public health conditions preceded economic growth. Attitude changes, based on improved knowledge and experiences, resulted in successful parasite control. Control is not expensive, yet the presence of STH and schistosomiasis is an inhibitory factor for socioeconomic development, and this is the most obvious consequence of disease burden due to helminth infection.

Japanese scientists have sought to build a close relationship with researchers in other Asian countries in both basic and applied research into parasitic diseases. Bilateral cooperative overseas aid orchestrated by the Japan International Cooperation Agency (JICA: http://www.jica.go.jp) has helped to strengthen the training aspects needed for parasite control, which are based on the lessons learned during previous success in Japan. One of the successful programs promoted by JICA is the HI [51]. In 2000, JICA established the Asian Center of International Parasite Control (ACIPAC: http://www.tm.mahidol.ac.th/en/seameo/thailand.htm) at Mahidol University, Bangkok, as the first center within the HI. Training courses for the school-based control of malaria and STH for program managers were organized by ACIPAC for health personnel and educators from central to provincial levels. Approximately 111 personnel, mostly from GMS countries, were trained between 2001 and 2005. Small-scale pilot projects (SSPPs) on school-based STH control, supported by JICA, have been conducted in Cambodia, Laos, Myanmar and Vietnam.

The contribution made by Japanese NGOs to parasite control should also be emphasized. The JAPC supported the Asian Parasite Control Organization (APCO), which was established in 1974. Between 1977 and 1999, an APCO

training course that used school-based STH control to gain entry into the community was conducted at Mahidol University and its partner institutions (the Faculty of Public Health and the Ministry of Public Health). More than 530 health personnel in Asia have been trained and evaluation of the training, conducted after the 20th course, showed that >50% of ex-participants continue to work in the field of parasite control (J. Waikagul, unpublished). Another bilateral cooperation scheme is the US–Japan Cooperative Medical Science Program, which was established in 1964 to focus research on diseases that are prevalent in Asia, with parasitology research being one of the main subjects. Through these cooperation schemes, the exchange of scientific information among Asian parasitologists is increasing and, by combining various bilateral cooperation programs, the Federation of Asian Parasitologists was officially established in 2001. More recently, the Japanese Government launched a new strategy for the research and control of infectious diseases by collecting biological and epidemiological information in Asia and Africa. In addition, three centers were established in Thailand, Vietnam and China as cooperative projects with Osaka University (http://ww.osaka-u.ac.jp), Nagasaki University (http://www.nagasaki-u.ac.jp) and the University of Tokyo (http://www.u-tokyo.ac.jp), respectively.

Future perspectives

Collaborative projects between Japan and other Asian countries have been ongoing in the field of basic research into parasitology and disease control, and will increase in number in the future. In particular, the development of vaccines and new therapeutics is an urgent research subject for *S. japonicum*. Several groups from Japan are, with Chinese colleagues, undertaking research projects on schistosomiasis vaccine development. Paramyosin and calpain of *S. japonicum* were tested as vaccine candidates [52–54], and partial but significant vaccine effects were observed in a field trial using domestic pigs [55,56]. Qinhaosu derivatives have been intensively investigated as new therapeutic and/or prophylactic drugs for Asian schistosomiasis [57,58]. Artemether was also shown to have prophylactic effects on various schistosome species [59]. Artesunate was also effective, not only against *S. japonicum* but also against *S. mansoni* infection; however, the optimal protocols for artesunate treatment were different between *S. japonicum* and *S. mansoni* infections [60]. Side effects were not observed and complete cure rate was confirmed.

The monitoring of intermediate host snails by remote sensing was investigated as a novel epidemiological tool for schistosomiasis. In the Philippines, a research group from Japan proposed a monitoring system that uses digital maps of Landsat images to which epidemiological information is added [61]. A similar system was developed to monitor the reemergence of schistosomiasis japonica in a former endemic focus of the disease in Japan [62].

Cooperative projects are being intensively promoted for STH and schistosomiasis control. Children of primary-school age are most affected by STH and schistosomiasis. Deworming is a preventive control measure but, to keep reinfection rates as low as possible, preventive education must be implemented widely and continuously throughout the region. A regional training program of effective education for trainers is necessary but the program remains in a low profile at present. ACIPAC activities are continuing as a collaborative project between Japan and Thailand, and SSPPs in the surrounding countries are encouraged to develop into country-level projects. In fact, as a result of this activity and the support of other international organizations, a National Intestinal Parasite Control Program has been started in Cambodia and Laos [63].

Although the prevalence of STH has decreased compared with the prevalence in the 1980s, these diseases remain a major public health problem in Southeast Asia. Extensive training programs are still needed in the region to support national programs for parasite control. ACIPAC, together with collaboration from Japan, is ready to be a partner of other international and local organizations and agencies to provide training based on the successful Japanese model of school-based control programs for parasites and other infectious diseases.

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Review

Toxocariasis in Japan

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Abstract

Toxocariasis has long been considered a parasitic disease affecting pet owners and children who often play in sandboxes at public parks. Recent cases of this animal-borne infection, however, indicate that its clinical manifestations and etiologies are changing. In this article, we will describe the critical characteristic features of toxocariasis alongside the contributions of Japanese researchers to a better understanding of the disease.

Keywords: *Toxocara canis*; *Toxocara cati*; Toxocariasis; Visceral larva migrans

Contents

1. Introduction

Among animal-borne diseases, toxocariasis is one of the most popular parasitic infections in the world, caused by the larval stage of *Toxocara* spp. Humans are infected mainly by the tiny developmental stage of the parasite, which belong to the family Ascaridoidea, through their pet dogs and cats. Other natural hosts include wild Canidae for *Toxocara canis* and wild felines for *Toxocara cati*. Symptoms depend on organs affected and the magnitude of infection. It is usually a non-fatal disease, but the larvae migrate through the eyes and can cause severe vision disability or even blindness.

In 1950, Dr. Wilder, an American ophthalmologist, histopathologically identified a nematode of unknown etiology in the retinas of 26 out of 46 enucleated eyes with retinoblastoma [1].

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Two years later, Beaver et al. [2] recognized the same parasite in the liver of three young children. Shortly afterwards, the parasite was correctly identified as an infectious stage larva of *T. canis* [3–5]. Since then, many clinicians and biologists have been accumulating knowledge of *Toxocara* and toxocariasis.

In this review article, we describe the lesser-known contributions of Japanese researchers to the understanding of *Toxocara* and toxocariasis. This articles builds on the work of Kondo [6], focusing on the topics that he did not cover in his review and on new findings since his publication.

2. Toxocariasis in humans

2.1. Clinical cases

Toxocariasis is clinically classified into four types: visceral, ocular, neurologic, and covert [7,8]. In 1963, the first report on toxocariasis in Japan was presented orally at the 32nd Annual Meeting of the Japanese Society of Parasitology by Fushimi et al. [9]. A 14 year-old boy was admitted to a university hospital because of fever, hepatomegaly and persistent eosinophilia. The patient died from severe anemia six months later. Though no autopsy or serological examinations were performed, the patient was strongly suspected to have suffered from visceral toxocariasis. In the early 1960s, immunological tests for parasitic infections, especially for helminthiasis, had only just begun, and antigen for the diagnosis of toxocariasis was not yet known.

Just as in other parasitic infections, direct demonstration is the only way to make definite diagnosis of toxocariasis. However, it is difficult to find the larva in either tissue biopsies or autopsies due to its very small size. So far in Japan, one morphologically and two pathologically confirmed cases have been reported [10–12]. Two additional reports, both of ocular toxocariasis, were doubtful because of the lack of characteristic features of the parasite; the authors nevertheless reproduced the microscopic findings of the purported larva in their papers [13,14]. One of these two cases showed increased antibody production in vitreous fluid against *Toxocara* antigen prepared from larval excretory–secretory product (LES), suggesting that the case might be attributable to ocular toxocariasis.

Serology is an alternative method for the diagnosis of toxocariasis. A method has been established for *in vitro* cultivation of the larvae, with LES prepared from the culture medium serving as an antigen. Detection of specific antibodies against LES provides evidence of *Toxocara* infection in individual patients and useful tool for understanding the epidemiological characteristics of this disease. The first serological survey in Japan was reported by Matsumura and Endo [15] using sera of 83 clinically healthy children. In their sample, 3.6% tested were positive for LES. In another study, Matsumura and Endo [16] demonstrated that 20 of 530 adults possessed the IgG antibody to LES. The positive individuals were thought to have a latent or past infection. In a large-scale seroepidemiolgical survey, Kondo et al. [17] collected 3277 sera from 14 prefectures in Japan and tested for LES antibodies. Antibodies were confirmed in 52 individuals (1.6%), but geographical patterns were notable: the highest prevalence rate was observed in Miyagi Prefecture (6.1%), and the lowest was in Ibaragi Prefecture (0.5%). The researchers concluded that the overall seroprevalence rate was in good agreement with those reported from other countries [17–19].

Based on improvements in the field of serology, diagnosis of toxocariasis is usually made by detection of the specific antibody to LES, along with clinical manifestations such as eosinophilia, eosinophilic pneumonia, or ophthalmoscopic findings.

2.2. Characteristic features of toxocariasis

2.2.1. Toxocariasis as a food-borne infectious disease

Using serological methods, there were nearly 200 reports of toxocariasis in the database of Japana Centra Revuo Medicina, and almost 300 cases have been diagnosed in Japan in the past two decades. Among these cases, some significant reports have provided a new perspective on the pathogenic mechanisms of toxocariasis.

Since Beaver et al. [2] introduced the concept of visceral larva migrans, characterized by chronic eosinophilia with granulomatous lesions in the liver, toxocariasis was regarded as a disease in children who were infected by soil contaminated with embryonated eggs [20]. In 1983, Sakai et al. [21] reported a case toxocariasis after ingestion of raw chicken liver. The 57-year-old man was admitted to a hospital due to cough, fever and weight loss. Complete blood count revealed a marked increase in eosinophils in peripheral blood with leukocytosis, and serum antibody against *T. canis* was strongly positive. Before onset, he and his friends had eaten raw chicken livers derived from his poultry and boar farm. Soon after the meal, they experienced abdominal pain, vomiting and diarrhea, but the symptoms improved within two days after ingestion. One month later, his chief complaints emerged. Two similar cases were subsequently reported by the same group [22].

These cases clearly indicates that the disease should be considered a food-borne parasitic infection. Four additional papers describing six patients were published in Japan in the 1980s [22–25]. These patients, all male and between 22 and 51 years of age, had a history of eating raw meat or liver of fowl and/or cattle before onset of symptoms. The possibility that raw liver of domestic animals can transmit the pathogens of human visceral larva migrans was substantiated by Lee et al. [26] of Yonsei University College of Medicine in Korea. They found that a dietary habit of raw liver was much more frequently seen in males than in females, especially in the 31–40 age group. Experimental studies revealed that chicken, cattle and swine were able to act as paratenic hosts for *T. canis* [27–29]. Most of the adult cases reported in recent years in Japan are categorized as this type of infection [30].

2.2.2. Respiratory illness and toxocariasis

In animal models in rodents, hatched larvae migrate into the lungs through the liver after ingestion, resulting in liver dysfunction and pneumonia [31–33]. In humans, similar manifestations are well documented in the literature [30,34–36]. Pulmonary lesions appear on computed tomography as multifocal subpleural nodules with halos or ground-glass

opacities and ill-defined margins. Additionally, transient pulmonary infiltrates are a characteristic finding. Morimatsu et al. [30] recently reported a familial case of visceral toxocariasis after consumption of raw chicken livers. In this case, the patients, a father (71 years old) and his son (45 years old), ate raw chicken livers three weeks before onset and then developed mild fever, general fatigue, headache and respiratory disorder. The specific antibody to LES was identified both in their serum samples and in bronchoalveolar lavage fluid (BALF). *T. canis* larvae were recovered from chicken liver from the same source as that ingested by the patients. These cases showed that BALF is a reliable specimen to demonstrate LES antibodies when the patient shows respiratory illness.

2.2.3. Urticaria-like skin lesions and toxocariasis

Parasitic infection is often said to be associated with chronic urticaria [37]. This is still a controversial issue, but acute urticaria is certainly associated with infection with larva from the marine fish parasite, *Anisakis simplex* [38]. Japanese have long tradition of eating raw fish, sashimi and sushi, and anisakidosis is a common parasitic infection in Japan. It is well documented that urticaria is closely related to the infestation of *Anisakis* larva [38,39]. As with anisakidosis, an allergic reaction could be elicited by the invasion of *Toxocara* larvae and result in skin rash that looks like hives. These skin manifestations might occur as a result of immunological response to larval metabolites [40,41].

In 1999, the first confirmed case of toxocariasis with larva in subcutaneous tissue was reported [11]. A 26-year-old female with fever, headache, and dry cough was admitted to a university hospital. Her peripheral blood smear showed an eosinophilia (61%) and her chest radiograph revealed multiple nodules. A diagnosis of visceral toxocariasis was made after detection of LES antibodies. During her hospitalization, several brown itchy nodules, which were thought to be prurigo, developed on her legs. Histological examination showed *Toxocara* larva in the center of an eosinophilic and lymphocytic abscess. The patient admitted frequently eating raw beef liver almost one year before her hospitalization for its purported health benefits. We can learn from this case that larvae migrating into subcutaneous tissue directly elicit pruriginous skin lesions.

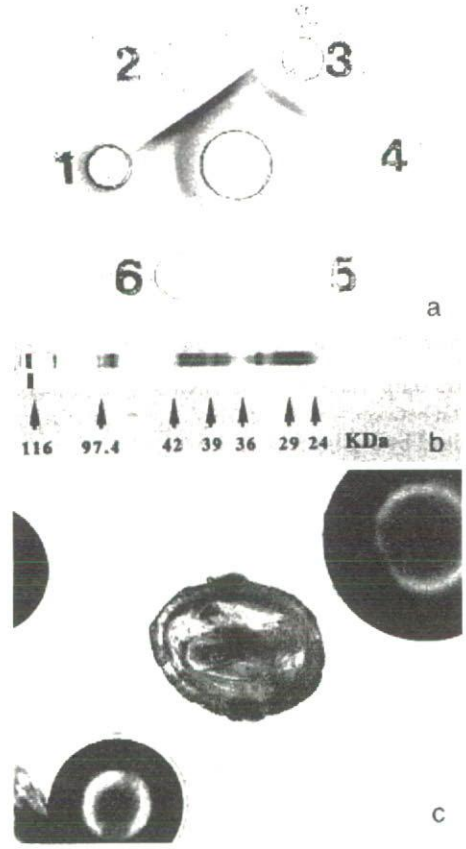

Fig. 1. The results of double gel diffusion (a) and western blot (b) tests of a patient of visceral toxocariasis. Strong precipitin bands were obviously observed between larval excretory–secretory products (LES) of *Toxocara canis* and patient's serum by means of double gel diffusion test. Antigens used in this test were adult worm extract (AEX) of *T. canis* (1), LES of *T. canis* (2), AEX of *Dirofilaria immitis* (3), AEX of *Ascaris suum* (4), LES of *Anisakis simplex* (5) and AEX of *Ascaris lumbricoides* (6). Western blot test shows a whole range of LES molecules were reacted with the patient's serum (upper strip) but not with a normal control serum (bottom strip). An embryonated egg recovered from the soil in the park where the patient often played (c). A fully developed and live *Toxocara* larva was found in the egg.

2.2.4. Toxocariasis is a disease that affects adults rather than children

Many reviews from western countries indicated that children under 12 years old, who often play outside, are the most affected age group for toxocariasis [42,43]. They are accidentally infected with *T. canis*/*T. cati* eggs, which expelled in feces puppies and fully develop in the surrounding environment within two to four weeks. Therefore, contaminated soil is the most important etiological source for toxocariasis [44,45]. Hori et al. [46] reported a case of visceral toxocariasis in a 1.5-year-old girl with fever, hepatomegaly, and eosinophilia (73%). The patient had a history of pica, particularly eating soil from a nearby park where she frequently played with her brother. Serological examination strongly suggested that she was suffering from *Toxocara* infection (Fig. 1a, b). They also found many embryonated eggs from the soil in the park that contained a live larva closely resembling *T. canis* eggs (Fig. 1c). Fortunately, her brother showed a negative result in serological tests.

In a review article of Barriga [47], the average age of visceral toxocariasis was 9.5 years, and only 18% of patients were adults. However, in recent investigations, adults rather than young children were more frequently affected by this parasite. This tendency is particularly true for ocular toxocariasis. Yoshida et al. [48] described that, among 38 Japanese cases of ocular toxocariasis, 34 (89%) were older than 20 years of age, and suggested that clinical features observed in these patients were somewhat different from those of previously reported cases [49]. Therefore, ocular toxocariasis is no longer merely a disease of young children, but affects any age group having a risk factor such as consumption of raw meat or close contact with contaminated soil.

As of the end of 2006, 584 clinically suspected cases of toxocariasis (112 of visceral type and 472 of ocular type) have been referred to our laboratory for detection of the anti-*Toxocara* antibody. We omitted 109 cases from this study due to a lack of description of the patient's age and sex. In visceral toxocariasis, the male-to-female ratio in the remaining sample was 2.04 (male: 53, female 26). The average age was 39.2± 21.7 (range, 0–83 years old) in male and 31.3±23.9 8range, 0.5–82 years old) in female. On the other hand, the male-to-female ratio in ocular toxocariasis group was 1.16 (male: 213, female: 183). The average age was 39.3±18.5 among males (range, 2–83 years old) and 37.6±18.2 among females (range, 2–74 years old). There were no significant differences in age distribution between males and females (Fig. 2). A similar result was obtained by Fujino et al. in 1998 [50].

2.2.5. Myelitis and toxocariasis

According to the case-control study by Magnaval et al. [51], migration of *T. canis* larvae in the human brain does not frequently induce recognizable neurological signs, but is possibly responsible for repeated low-dose infections. These light parasitic burdens usually do not appear to elicit a special clinical symptom, but in some cases, sever neurological disorders such as encephalitis, myelitis and meningitis are manifested [52]. In Japan, Ota et al. [53] reported a case of eosinophilic meningo-encephalo-myelitis due to *Toxocara* infection. The patient, a 21-year-old woman, showed frontal headache, low-grade fever and convulsion. She had a long history of close contact with her pet dog. Immunological tests were strongly positive for LES antigen in both her serum and cerebrospinal fluid. Based on clinical evidence and characteristic features in similar patients, Kira and his colleagues proposed a new disease entity: "atopic myelitis" or "parasitic myelitis." They assumed that allergic reaction to LES might be involved in this neurologic disorder [54]. Interestingly, most of the patients lived in Kyushu District, in the south of Japan, suggesting that myelitis due to *Toxocara* infection might be a regional clustering disease.

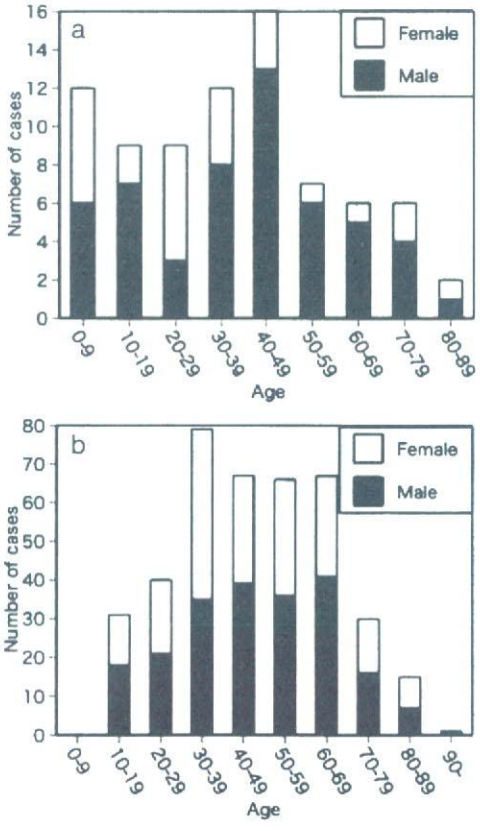

Fig. 2. Age distribution of suspected cases of visceral (n=79)(a) and ocular toxocariasis (n=396)(b) referred to our laboratory from August 1994 to December 2006.

2.3. T. cati

Because morphological differences between *T. canis* and *T. cati* in the adult stage are apparent [55], *T. cati* is easy to identify when patients expel adult worms. It has been suggested that *T. cati* could develop in children through the ingestion of the immature worm of *T. cati* [56]. More than 26 cases were reported so far [56,57], but there was only one case was reported from Japan. A 5-year-old male boy was admitted to a hospital due to a complaint of vomiting 3 worm-like foreign bodies. These worms were morphologically identified as two female and one male immature worms [58].

On the contrary, there are few reports of human intestinal infection with adult worms of *T. canis* [59], and many of these are believed to be erroneous observations [60]. Serological discrimination between toxocariasis canis and toxocariasis cati, however, is not so apparent, because of complete cross-reactivity between the two LESs, although *T. cati*-specific LES has been identified [61]. Therefore, distinguishing between *T. canis* and *T. cati* is even more difficult if somatic antigens are used in the serological diagnosis [62–64]. For the precise serodiagnosis of toxocariasis, a great deal of additional research effort is needed to obtain *T. cati*-specific LES antigens.

3. Advances in serological diagnosis

3.1. Antigens

As mentioned above, the most reliable and suitable antigen for the diagnosis of toxocariasis is LES from *T. canis*. Once the larvae are cultivated *in vitro*, they are viable for up to two years. During this period, no morphological changes have been observed, but chemosusceptibility to some compounds were found to have changed [65], suggesting that the physiological natures of the larva do change over this time period. The nature of LES was extensively studied by Maizels and colleagues [61,66–68]. Around the same time, Sugane and Oshima demonstrated that LES had an ability to induce not only IgG and IgM antibodies, but also IgE antibody in mice. Allergenic activity was lost when LES was treated with guanidine hydrochloride and 2-mercaptoethanol. LES also showed a cross-reaction with serum from *Ascaris suum*-infected mice

[69]. In addition, studies have identified numerous lectin-specific glycoconjugates on the surface of the larvae [61,66–68,70–73], and these have been found to dynamically change during the course of infection in murine [74] and rabbit models [75].

Although the antigenicity and specificity of LES is fairly high, cross-reaction to other parasites, especially nematode parasites, have been observed [76]. To overcome this problem, Yamasaki et al. [77] produced a recombinant antigen that reacted with serum from patients with toxocariasis but not from those with roundworm or hookworm infections.

3.2. Rapid diagnostic test for toxocariasis

For many years, numerous diagnostic measures, such as the double gel diffusion test, immunoelectrophoresis, indirect hemagglutination test, latex agglutination test, plate-based ELISA, membrane-based dot-ELISA, etc., have been employed to detect specific antibodies against LES. However, these tests require 1.5 hours or more to obtain an accurate result. In 1997, a new rapid diagnostic test kit for the detection of anti-LES antibody was introduced by us [78]. The test is based on the antigen-sensitized nitrocellulose membrane-based assay. It is easy to perform, does not require any sophisticated apparatus or expertise and the results can be obtained within 3 min. This test kit can even detect the antibody in intraoccular fluid.

4. Conclusion

In this review article, we present an overview of human toxocariasis in Japan. Due to space limitations, we do not describe in detail the aspects of experimental investigations concerning biology, immunology and molecular biology using animal models. However, we briefly pay special attention to Japanese investigators who contributed to advance the understanding of toxocariasis. In early studies, Oshima established a standard method for the oral inoculation of eggs, in which the albuminoid coat of the egg is first removed in order to prevent the adhesion of eggs onto glassware [79]. Sugane is a longtime co-worker of Oshima, and his colleagues are actively engaged in the field of immunology [80–88]. They demonstrated many examples of cellular immunity to *Toxocara* infection in mice. The late Dr. Tsuji made pioneering efforts to develop immunodiagnostic techniques for toxocariasis [50,89,90]. Recently, Mongolian gerbils, *Meriones unguiculatus* have been established as a suitable animal model for experimental ocular and neurologic toxocariasis [91–94].

Human toxocariasis is a public health hazard not only in children but also in adults, both in developing and developed countries. There are still questions to which we have no answers: How does ocular toxocariasis develop? Why do nearly half of ocular toxocariasis patients not produce detectable antibody to LES? What is the pathogenesis of neurologic toxocariasis? What mechanisms are involved in the reemergence of *Toxocara* larvae during pregnancy both in definitive and undefinitive hosts? In addition, we have not yet established an effective anthelmintic against *Toxocara* parasites in the tissue stage, especially for the ocular toxocariasis. Continuous efforts should be made to address these issues. Finally, toxocariasis is a disease that afflicts two of the very best and oldest friends of humans: dogs and cats. Therefore, we must continue to study this puzzling disease both for the sake of humans, and for that of our animal friends.

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AN IMPROVED METHOD FOR RECOVERY OF MUSCLE-STAGE LARVAE FROM MICE INFECTED WITH *TOXOCARA CANIS*

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ABSTRACT: We report a modified digestion method that improves the recovery rate of *Toxocara canis* larvae from skeletal muscle. Minced muscle tissue from infected mice was incubated in artificial gastric juice for 48 hr at 37 C, with ethanol added for the second 24 hr. This procedure allowed the larvae to be identified and counted more quickly than with the standard digestion method. This method allows measurement of the total number of larvae present in muscle tissue following oral inoculation of embryonated eggs, although it does not permit counting of live larvae.

Following oral inoculation of embryonated eggs, infectious-stage *Toxocara canis* larvae migrate into skeletal muscle tissue via the systemic circulation. Muscle-stage larvae tend to increase in number after infection: almost half of all recovered larvae come from skeletal muscles beyond the 10th day of infection (Havasiova-Reiterova, et al., 1995; Oshima, 1961). These larvae are able to survive for long periods in muscle tissue. If an anthelmintic drug is effective against migrating larvae, the number of larvae appearing in skeletal muscle will be reduced. Therefore, for an anthelmintic trial, the number of muscle-stage larvae is a good indicator of efficacy (Fok and Kassai, 1998; Horiuchi, et al., 2005; Hrckova and Velebny, 2001; Satou, et al., 2005).

Both the Baermann technique and the digestion method using artificial gastric juice are use to detect larvae in skeletal muscle. The Baermann technique,