していたフィリピンとタイから初めて報告された．その後1970年までは9カ国からしか報告がなかったが，現在では40カ国以上の国で患者の発生をみている．WHOの報告では，全人類の40％が感染のリスクに曝され，毎年5千万人が罹患しているという．また，2001年だけで南北アメリカ各国から60万人以上の感染者が報告され，うち1万5千例がデング出血熱であった．これは1995年の報告と比べると患者数は2倍の増加であった．このようにデング熱は過去数年，急激にその感染域を広げつつある．

1）症　状

デング熱はインフルエンザに似た熱性疾患で，3-14日(通常5-7日)の潜伏期間後突然の発熱で発症する．発熱は3-5日間続き(しばしば二峰性)，頭痛，眼窩痛，関節痛を伴い，発症後しばらくすると体幹部，胸部を中心に発疹が出現し，四肢，顔面へと広がるが，通常1週間で何ら後遺症を残すことなく回復する．しかし，幼児では症状が重い場合がある．4種血清型のデングウイルスの1種に感染し回復すると，同じ血清型のデングウイルスに対しては終生免疫を獲得するが，残り3種の血清型ウイルスに対してはほとんど交差反応を示さず，デング出血熱の発症リスクを高めるという報告さえある．

デング出血熱はデング熱と同様の経過で発症した患者が急激な体温上昇と顔面紅潮のあと突然の出血傾向と循環不全状態に陥る病態で，肝腫大もみられ，適切な治療が施されない場合，患者は急速にショック状態に陥り半日から1日以内に死亡することもある．

2）診　断

血清中のデングウイルスに対するIgM抗体を検出する．この診断キットは国内でも入手可能である．

3）治　療

デングウイルスに対する特異的な治療法はなく，対症療法および脱水予防が中心となり，抗血小板作用のあるアスピリン類の投与は禁忌である．デング出血熱の場合も同様であるが，循環血液量減少を補完するための輸液療法が重要である．また血小板減少がみられるときには血小板輸血も考慮する．

b. 黄　熱

デング熱と同じフラビウイルスに属する黄熱ウイルスによって起きる熱性出血性疾患で，主にネッタイシマカによって媒介される．森林型黄熱，都市型黄熱，中間型黄熱の3つの流行形式に区別される．アフリカの熱帯雨林地方では3つの型すべてが，また南アフリカでは森林型と都市型の流行がみられる．

森林型黄熱は熱帯雨林に生息するサルと蚊(主要媒介蚊は*A. africanus*)の間で流行サイクルが回っているが，木材などの切り出しのために森林に入ったヒトが偶然に感染し，多くは散発例として報告される．中間型はアフリカ中央部に分布するサバンナ気候区の農村部で流行がみられ，ヒトとサルの両方に感染が起こっており，近年患者が増加している．都市型は都市の生活用水，水たまり，雨水で繁殖する昼間吸血性のネッタイシマカが媒介し，ヒト→蚊→ヒトの感染経路をとり，しばしば大規模な流行を起こす．

1）症　状

潜伏期間は3-6日．発症後の経過によって軽症黄熱と重症黄熱に分けられる．軽症黄熱では発熱，背部痛，頭痛，振戦，食欲不振，嘔気・嘔吐がみられる．発熱しているのにもかかわらず徐脈を呈する場合がしばしばみられる．3-4日で完全に回復する．

黄熱の患者のうち15％ほどが24時間以内に重症黄熱に移行する．黄疸が急速に悪化し，歯肉出血，鼻出血，下血，結膜出血がみられる．更には蛋白尿がみられ，腎不全が進行し，ついには乏尿となる．2週間以内に半数の患者は死亡するが，残りの患者は重篤な後遺症もなく回復する．

2）診　断

血清中の黄熱ウイルスに対するIgM抗体の検出，PCR法による病原体遺伝子の検出，ペア血清を用いた中和抗体法による抗体価の陽転のいずれかによって診断を確定する．ウイルス分離は発症後3日以内の血液を用いて行われるが，設備の整った施設で熟練した技術者によって行

表2 節足動物媒介感染症流行に及ぼす気温の影響(文献[2]より改変)

	病原体が生存可能な最低気温(℃)	病原体が生存可能な最高気温(℃)	媒介動物	媒介動物が生存可能な最低温度(℃)
熱帯熱マラリア	16-19	33-39	ハマダラカ	8-10
三日熱マラリア	14.5-15	33-39	ハマダラカ	8-10
アメリカトリパノソーマ症	18	38	サシガメ	20
住血吸虫症	14.2	>37	淡水産貝	5(最適範囲25±2)
デング熱	11.9	データなし	ヤブカ	6-10

わなければならない．黄熱は四類感染症であり，診断した医者は届出が義務づけられている．

鑑別診断としては，マラリア，腸チフス，発疹チフスなどのリケッチア感染症，ラッサ熱などウイルス性出血熱，デング熱，レプトスピラ症，ウイルス性肝炎，薬物中毒などがあげられる．

3) 治　療

特異的な治療方法はなく，対症療法のみである．それゆえ，黄熱の流行国への入国に際しては10年以内のワクチン接種が義務づけられている．黄熱ワクチンの効果は99%終生免疫が得られるが，生後9カ月以下では投与せず，4カ月以下は禁忌である．

c. マラリア

人体に寄生するマラリア原虫には三日熱マラリア原虫，四日熱マラリア原虫，卵形マラリア原虫，熱帯熱マラリア原虫の4種類あり，いずれもハマダラカ(*Anopheles*)属の蚊によって媒介され固有のマラリアを起こす．年間3-5億人が感染し，毎年200万人以上が死亡している．死亡者の多くはサハラ砂漠以南に暮らすアフリカの幼小児である．また，流行地を旅行する非流行国からの旅行者にとっても危険な感染症であり，治療が遅れると死亡する．形態学的な分類では，世界中には50種類ほどのハマダラカが分布しているが，同胞種も独立種として数えると400種類近くのハマダラカ属のうちマラリアを媒介するものは約90種類に上る．しかし，流行地における媒介蚊は1，2種類に限定されていることが多い．例えば，かつて日本にもマラリアが流行していた時期があり，このときの主要な媒介蚊はシナハマダラカであった．また，1951年米国からマラリアが駆逐されるまでの主要な媒介蚊は*A. freeborni*と*A. quadrimaculatus*であったという．

マラリア原虫はハマダラカの体内で有性生殖を行い，ヒトへの感染源となるスポロゾイトを形成するが，その発育は気温の影響を受け，16℃以下では発育できないといわれている(**表2**)．これはほぼ現在の宮古八重山諸島の平均最低気温に匹敵する．温暖化が進めば鹿児島県南部がこの気温になると予想されている．

マラリアは感染症新法の四類感染症として扱われ，診断した医師は届出を要する疾患に指定されている．国内においては平成11年からの6年間の届出数は686例で，そのうち297例が悪性の熱帯熱マラリアであった．デング熱，黄熱も四類感染症で届出が必要である．

1) 症　状

発熱，貧血，脾腫を三大主徴とする．特有の熱発作は第5病日頃からみられ，病因原虫の赤血球内での分裂周期に一致して三日熱マラリアと卵形マラリアでは48時間ごと，四日熱マラリアは72時間ごとの周期熱となるが，熱帯熱マラリアでは原虫の発育周期に同調せず熱型不規則な高熱が持続する．しかし，他のマラリアでも発病の初期や他種マラリアとの混合感染時には熱型が規則的ではない例もある．

発熱は40-41℃に達し，頭痛，背部痛を伴う(灼熱期)．発熱の直前には悪寒戦慄を覚える(悪寒期)．次いで大量の発汗が2，3時間続いたあと急速に解熱する(発汗期)．これを熱発作といい8-12時間で症状は消失する(無熱期)．

潜伏期間はマラリアの種類によって異なる．三日熱マラリアと卵形マラリアでは1-2週間，

四日熱マラリアは2週間以上で通常1カ月以内に発症するが，1年以上経過して突然発症することもある．熱帯熱マラリアの潜伏期間は5-10日で，通常蚊の刺咬後1カ月以内に発症する．

熱帯熱マラリアでは悪寒期を欠くことが多く，灼熱期も半日から1日以上も持続する．治療が遷延すると肺水腫，腎不全，黄疸など全身管理を必要とする重篤な合併症を伴う．そして，意識障害，けいれん，錯綜などの意識障害が起こり，昏睡から死に至る．

2) 診　断

ギムザ染色(希釈にはpH 7.2のリン酸緩衝液を使用)を施した血液塗抹標本を作製し原虫を検出するのが標準法である．一度の検査で原虫が見つからない場合でも時間をおいて複数回の検査を行う．また，治療開始後も経時的に検査を行い，治療効果の判定や使用薬剤の感受性を確認する．なお，特異抗原(HRP-II)検出キットも有用性が高い．

3) 治　療

現在，世界各地の流行地で従来の抗マラリア薬に対する耐性株の出現が問題になっている．また，感染したマラリア原虫の種類や病状によっても治療法は異なってくる．熱帯熱マラリアの重症例では全身的な管理が必須である．

標準的な治療法，薬剤耐性マラリアが疑われるときの治療法，副作用，重症マラリアの際の全身管理などについては，紙面の関係上，最近出版された'日本の旅行者のためのマラリア予防ガイドライン(マラリア予防専門家会議編，2005)'[5]および「熱帯病に対するオーファンドラッグ開発研究」班(編)寄生虫症薬物治療の手引き—2003—，改訂第5版[6]を参照いただきたい．

文　献

1) 堂本暁子，岩槻邦男(編)：温暖化に追われる生き物たち　生物多様性からの視点，p414，築地書館，1997.
2) IPCC: Infectious disease. In: Climate Change 2001: Working Group II, Impacts, Adaptation and Vulnerability, Chapter 9.7, p462-472, 2001.
3) 松林　格：地球温暖化と感染症，デング，マラリア．臨床と微生物　33: 373-377, 2006.
4) 津田良夫：地球温暖化に伴う蚊媒介性疾患の分布拡大の可能性について，2001. (http://www.aa.tufs.ac.jp/~gisr/iryo/koen13t.htm)
5) マラリア予防専門家会議(編)：日本の旅行者のためのマラリア予防ガイドライン，p43，(株)フリープレス，東京，2005.
6) 厚生科学研究費補助金「熱帯病に対するオーファンドラッグ開発研究」班：寄生虫症薬物治療の手引き—2003—，改訂第5版，p1-5，2003.

変貌するトキソカラ症と新しい動物モデル（1）

東京医科歯科大学大学院
国際環境寄生虫病学分野准教授　**赤尾 信明**

はじめに

動物由来感染症の中で、イヌやネコに寄生する回虫――イヌ回虫 Toxocara canis あるいはネコ回虫 Toxocara cati―によって起きるトキソカラ症はペット由来の寄生虫感染症の中でも最もよく知られたもののひとつである。本症は、これらの回虫の感染期幼虫がヒトの体内に侵入して起きる感染症であり、原因となる寄生虫が、どちらもトキソカラ属の回虫であることから、最近ではトキソカラ症と呼ばれることが多い。症状は、幼虫が寄生した臓器や侵入した幼虫の多寡によってさまざまであるが、通常、死に至ることはない。しかし、幼虫が眼に寄生すると視力障害や失明の原因となることがある。

米国の眼病理の専門家であったであったワイルダー女史が、幼小児に多く見られる網膜の悪性腫瘍のひとつである網膜芽細胞腫の診断で眼球摘出術を受けた子供の眼球を精査していたところ、46の眼球組織のうち26眼から、幼虫の断端を見つけ出した(1)。その２年後に、当時米国の寄生虫学会の大御所であったビーバー博士が、同じ断端構造を持つ寄生虫を３名の幼い黒人の子供の肝臓内に見いだし、『内臓幼虫移行症』という疾病概念を提唱した(2)。その後まもなくして、この寄生虫がイヌ回虫の感染期の幼虫であることが明らかになり、ペット由来の回虫幼虫が幼小児に深刻な健康被害を起こすことが明らかになった。

爾来、トキソカラ症はペットとの接触機会の多い幼小児に感染する小児感染症であると考えられてきた。ところが、近年になり成人の感染例が増え、その病型にも明らかな変化が見られるようになってきた。ここでは、近藤(3)の総説以降に、主として日本人の手によって明らかにされてきたトキソカラ症の新しい感染経路やこれまであまり注目されていなかった病態について解説する。

トキソカラ症の臨床像と診断

これまでトキソカラ症は、内臓型、眼型、神経型、潜在型の４型に分類されてきた(4、5)。内臓型が日本で最初に報告されたのは1963年で、患者は発熱と肝腫大、好酸球増多症等の典型的な症状が見られた14歳の男児であった。当時はまだトキソカラ症の血清診断法が確立される前であり、抗体検査などは実施されていないが、その臨床経過から国内での第１例であると考えられている。これ以降、数多くの症例が国内からも報告されてきたが、虫体それ自体を検出できた例は少ない。

寄生虫感染症の診断では、寄生している虫体を直接見つけ出すことが最も確実な診断法である。しかし、トキソカラ症の場合、ヒトに寄生する幼虫の大きさは体長が400μm、体幅が20μm程度と非常に小さく、病理組織学的な検査で見つかることは稀である。そこで、トキソカラ症では血清中の抗体検査が補助的診断法として実施されている。検査法としては寒天ゲル内二重拡散法、カウンター免疫電気泳動法、各種の凝集反応、ＥＬＩＳＡ等が開発されてきた。しかし、こうした検査法が開発された当初に用いられていた抗原が成虫から抽出した粗製タンパク質であったこともあって、非特異的な反応が強く、結果の判定に苦慮する場合も少なくなかった。しかし、1975年 de Savigny (6)によって幼虫を in vitro で長期間飼育する方法と、その飼養液がトキソカラ症の診断抗原として優れていることが報告されて以来、この幼虫飼養液から作製した幼虫排泄物抗原（ＬＥＳ）を用いた抗体検査が実用化されている。幼虫は通常の細胞培養液（ダルベッコ変法イーグル培地）の中で２年間生存可能であり、その間ＬＥＳを排泄し続ける。

このＬＥＳを用いてトキソカラ症の血清疫学調査を国内で初めて行った報告によれば、83名の臨

床的に健康な人のうち３名で抗体が陽性であったという(7)。同じ著者による別の研究でも530名のうち20名でＬＥＳに対するＩｇＧ抗体が検出され、これらの抗体陽性者は既感染あるいは潜伏感染者であろうとされている(8)。さらに大規模な疫学調査では一般住民のうち1.6％が抗体保有者であると結果が報告されている(3)。これらの不顕性感染者の割合は諸外国での調査成績とほぼ同じであったという。

トキソカラ症の新しい臨床像

前章ではトキソカラ症をその寄生部位の違いから生じる病型に沿って分類したが、トキソカラ症をその感染経路から分類してみると、本症の特徴がよく理解できると思う。これはトキソカラ症が虫卵で汚染された砂場などで感染する小児の感染症であるというこれまでの概念を改める必要があることを明確に示すものである。

ＬＥＳを用いた血清診断法の進歩により、過去20年に300例以上の症例が国内から報告されている。これら中で、トキソカラ症の発症病理を理解する上で重要と考えられる症例について、筆者が直接関わった症例を中心に、いくつか例を挙げて解説する。

食品媒介感染症としてのトキソカラ症

1983年、酒井らはニワトリの生肝を食べた後に咳嗽と発熱、体重減少を訴えた57歳の男性の例を報告した(9)。血液検査で好酸球増多が指摘され、血清中のＬＥＳに対する抗体も強陽性反応を示した。発病前に自宅で飼育していたニワトリの生肝を友人と一緒に食べ、食後数時間してから腹痛、嘔気、下痢が見られた。これらの食中毒様症状は数日のうちに収まったという。しかし、１ヶ月後に呼吸器症状が現れて入院となった。同じような経過を辿った２症例がその後同じグループによって報告されている。

これらの症例は、トキソカラ症が「食品媒介寄生虫感染症」であることを明確に示している。さらにその後、６症例が追加報告されている。これらの患者に共通しているのは、年齢が22歳から51歳までの男性で、発症前にウシやニワトリの生肉あるいは生肝を食べた食歴を有していることであった。家畜の生肝がトキソカラ症の原因となる可能性についてはすでに韓国の研究者が1976年に報告していた(10)。それによると、韓国内で市販されていたウシやブタ、ニワトリ、イヌの肝臓を食べたことのある人は女性よりも男性で優位に多く、ウシからはウシ回虫が高率に分離され、実験的にニワトリにイヌ回虫を感染させると肝臓から幼虫が回収できたという。実験的にはブタやウシもイヌ回虫の待機宿主になることが知られている(11-13)。近年日本国内で感染する成人のトキソカラ症は大部分がこのような食品媒介によるものだと考えてよい。

呼吸器症状で発病するトキソカラ症

マウスなどを使った動物実験では、消化管内で孵化した幼虫は粘膜を穿通して門脈から肝臓を経て肺臓に至る。そのため、ヒトでも肝機能障害や肺炎が起きる。近年の画像診断技術の進歩によって、幼虫が肝臓や肺臓を通過する際に起きる炎症像をとらえることが出来るようになってきた。特に、肺臓通過時には咳嗽や発熱などの呼吸器症状

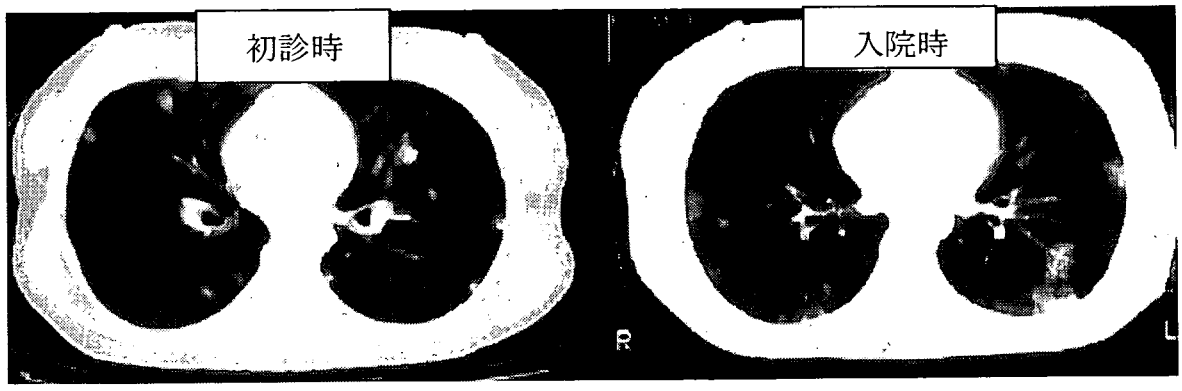


図１　呼吸器症状を呈したトキソカラ症患者（26才女性）の胸部ＭＲＩ

を伴うことから、このような時期に病院を訪れてＭＲＩ検査が行われると、多発性の小さな結節が移動するのが観察される（図１）。Morimatsu らが報告した症例では、ニワトリの生肝を食べた親子（75歳と45歳）が３週間後に発熱と倦怠感、頭痛、呼吸困難を訴えて国立熊本病院呼吸器科に入院した(14)。二人とも肺野に多発性小結節陰影が見られ、時間の経過と共に肺炎病巣が移動していったという。気管支洗浄液中に多数の好酸球が見られ、血清のみならず気管支洗浄液中にもＬＥＳに特異的な抗体が検出され、トキソカラ症の診断が下された。

皮膚病変とトキソカラ症

ある種の寄生虫感染では皮膚病変を伴うことが知られている。例えばオンコセルカ症では発疹や皮膚の肥厚、色素沈着が生じる。これは皮下組織に寄生するミクロフィラリアによるものと考えられている。また、アニサキス症では蕁麻疹様の皮疹を伴うことが知られている。トキソカラ症の場合も皮疹が生じることが報告されているが、その機序は幼虫の皮膚への移行による直接的な結果ではなく、もっぱら感染に伴う免疫応答の結果だと考えられていた(15,16)。しかし、1991年にAraganeらが報告した症例では、皮膚に生じた痒疹の生検で、イヌ回虫幼虫を直接検出し、この幼虫が皮下組織にも移行して皮膚炎が生じることを証明した(17)。患者は26歳の女性で、健康のためと信じてウシの肝臓を生でしばしば食べていたという。この例も先のMorimatsuらの症例と同様に、発熱と咳嗽を主訴に呼吸器科を受診し、治療中に皮疹が生じている。

トキソカラ症における皮膚病変の成立機序についてはこれまでほとんど研究されたことがなかった。今後このさらに詳しい検討が必要であると考えている。

（次号につづく）

文 献

1. Wilder HC. Nematode endophthalmitis. Trans Am Acad Ophthalmol Otolaryngol 19502 ; 55 : 99-109.
2. Beaver PC、Synder CH and Carrera GM. Chronic eosinophilia due to visceral larva migrans. Pediatrics 1952 ; 9 : 7-19.
3. 近藤力王至、日本における寄生虫学の研究、大鶴正満、亀谷了、林滋生編、東京：目黒寄生虫館；1999：465-474.
4. Glickman LT and Magnaval JF. Zoonotic roundworm infections. Infect Dis Clin North Am 1993 ; 7 : 717-732.
5. Taylor MR、Keane CT、O'Connor P、Mulvihill E and Holland C. The expanded spectrum of toxocaral disease. Lancet 1988 ; I : 692-695.
6. de Savigny H. In vitro maintenance of Toxocara canis larvae and a simple method for the production of Toxocara ES antigen for use in serodiagnostic tests for visceral larva migrans. J Parasitol 1975 ; 61 : 781-782.
7. Matsumura K and Endo R. Enzyme-linked immunosorbent assay for toxocariasis、its application to the sera of children. Zentralbl Bakteriol Mikrobiol Hyg [A] 1982 ; 253 : 402-406.
8. Matsumura K and Endo R. Seroepidemiological study on toxocaral infection in man by enzyme-linked immunosorbent assay. J Hyg (Lond) 1983 ; 90 : 61-65.
9. 酒井健二、岡島泰一郎、大内和弘．鶏肝の生食により発症したと考えられる内臓幼虫移行症の１例、内科 1983 ; 51 : 963-967.
10. Lee KT、Min HK、Chung PR and Chang JK. Studies on the inducing possibility of human visceral larva migrans associated with eating habit of raw liver of domestic animals. Korean J Parasitol 1976 ; 14 : 51-60.
11. Taira K、Permin A and Kapel CM. Establishment and migration pattern of Toxocara canis larvae in chickens. Parasitol Res 2003 ; 90 : 521-523.
12. Taira K、Saeed I、Permin A and Kapel CM. Zoonotic risk of Toxocara canis infection through consumption of pig or poultry viscera. Vet Parasitol 2004 ; 121 : 115-124.
13. Takakura Y. An epidemiologgical study of food-borne toxocariasis : Fowl and catle as paratenic hosts of Toxocara canis. J Juzen Med 1993 ; 102 : 828-935 (in Japanese with English abstract).
14. Morimatsu Y、Akao N、Akiyoshi H、Kawazu T、Okabe Y and Aizawa H. A familial case of visceral larva migrans after ingestion of raw chicken livers: appearance of specific antibody in bronchoalveolar lavage fluid of the patients. Am J Trop Med Hyg 2006 ; 75 : 303-306.
15. Humbert P、Niezborala M、Salembier R et al. Skin manifestations associated with toxocariasis: a case-control study. Dermatology 2000 ; 201 : 230-234.
16. Piarroux R、Gavignet B、Hierso S and Humbert P. In Toxocara: The Enigmatic Parasite、ed. C V Holland HVS、Toxocara : The Enigmatic Parasite : CABI International ; 2005.
17. Aragane K、Akao N、Matsuyama T、Sugita M、Natsuaki M and Kitada O. Fever、cough、and nodules on ankles. Lancet 1999 ; 354 : 1872.

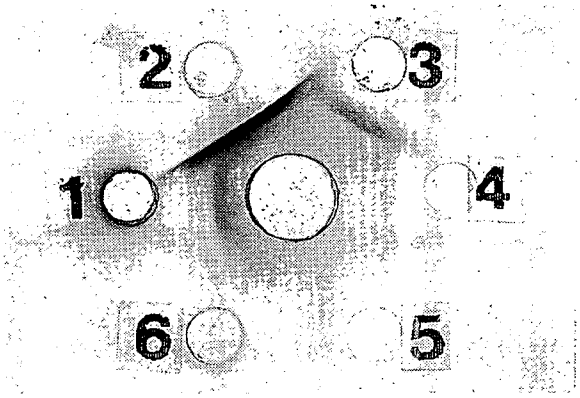
変貌するトキソカラ症と新しい動物モデル（2）

東京医科歯科大学大学院
国際環境寄生虫病学分野准教授　**赤尾 信明**

成人型トキソカラ症

これまでトキソカラ症は砂場などの戸外で遊ぶ機会の多い12歳以下の子供に多い感染症であると考えられてきた。イヌ回虫やネコ回虫の虫卵が糞便と共に排泄されると２ないし４週間で完全に発育し、虫卵内に感染幼虫が形成される。このような虫卵に汚染された土壌が重要な感染源と考えられてきた。堀らが1997年に報告した１歳６ヶ月の女児の場合が典型的な症例である(18)。発熱と肝腫大と好酸球増多を主訴に開業医から某市立病院に紹介のあった患児で、入院時末梢血好酸球の比率は73%であった。患児には以前から、しばしば土などを口に入れる、いわゆる異食症が見られたという。血清学的検査からトキソカラ症が強く疑われた（図２）。患児が兄と共によく遊んでいた近所の公園の砂場からはイヌ回虫卵によく似た虫卵が多数回収でき、さらにこの虫卵内の幼虫は卵殻内で動いており、生きていることが確認された。幸いなことに、兄の抗体検査では感染は証明されなかった。

図２　肝腫大と好酸球増多症を呈した１歳６ヶ月女児の寒天ゲル内二重拡散法による血清抗体検査



１：イヌ回虫成虫抗原
２：イヌ回虫幼虫排泄物（ＬＥＳ）抗原
３：イヌ糸状虫成虫抗原
４：ブタ回虫成虫抗原
５：アニサキス幼虫排泄物抗原
６：ヒト回虫成虫抗原

Barrigaの総説によると、トキソカラ症の好発平均年齢は9.5歳で、成人例は18%しか見られなかったとしている(19)。しかし、最近の傾向としてはむしろ成人の感染例が増えてきている。Yoshidaらの報告でも、38例の眼トキソカラ症患者の89%（34例）は20歳以上の成人であった(20)。Wilderが26例の小児の眼トキソカラ症を報告して以来、半世紀以上にわたって眼トキソカラ症は小児に多い疾患であると考えられ続けてきたが、眼トキソカラ症は生肉の喫食や汚染した土壌に触れる機会のあるどんな年齢層にも発症する感染症であると考えなければならない。

我々の研究室に2006年末までにトキソカラ症を疑われた584症例のうち年齢と性別が判明している475例について集計してみると、内臓型では男性53例に対して女性26例であった、また、男性の平均年齢は39.2±21.7歳、女性は31.3±23.9歳（0.5歳から82歳）であった。眼型では男性39.3±18.5歳、女性37.6±18.2歳と、いずれも両群に有意な差を認めなかった。年齢分布を見ると、内臓型では9歳以下の患児と40から49歳までの成人に患者の集積を見る二峰性の分布を示していた。

脊髄炎とトキソカラ症

マウスを使った動物実験では、感染した幼虫の約40%は脳内に移行する。この脳内に寄生した幼虫によってマウスに異常行動が見られることが知られているが、ヒトを対象とした症例対照研究では神経症状を呈する例はそれほど多くない(21)。しかし中には脳炎や脊髄炎などの激しい神経症状を伴ったトキソカラ症も報告されている(22)。日本でも太田らが、イヌ回虫幼虫によると思われる好酸球性の髄膜脊髄脳炎の１例を報告している(23)。患者は21歳の女性で、前頭部頭痛と発熱、痙攣を認めた。患者の脊髄液と血清中のＬＥＳに対する抗

体は陽性であった。九州大学の吉良らはこのような症例について『アトピー性脊髄炎』あるいは『寄生虫性脊髄炎』という新しい疾患概念を提唱している。彼らはこの疾患がＬＥＳに対するアレルギー反応によって起きると考えている。現在のところこの疾患は九州地方だけに見られることから地域集積性のある疾患であるとも見なされている。

ネコ回虫によるトキソカラ症

イヌ回虫とネコ回虫の成虫はその頭部に見られる頚翼の形態から比較的簡単に区別することが出来る。ネコ回虫の頚翼は大きく張り出している。これまでヒトがネコ回虫の成虫を吐出あるいは排泄したという報告はいくつかある(24,25)。国内でも、５歳の男児が３隻の虫体を吐出した例が報告されている(26)。しかし現在までにイヌ回虫成虫を吐出したという報告はあるにはあるが、いずれも誤った観察に基づいたものであると考えられている(27)。これらの報告から、ネコ回虫はヒトの体内である程度発育可能ではないかと推測されている。

一方、両種感染幼虫を形態学的に分類することはそれほど容易ではない。さらに免疫学的にイヌ回虫感染とネコ回虫感染を区別することはもっと容易ではない。なぜなら両種感染幼虫由来ＬＥＳの抗原性はきわめて類似しており、その差はほとんどないに等しい(28)。それ故、これまで成虫抗原を使ってネコ回虫による感染であると報告されているいくつかの症例については今後見直す必要があるかもしれない。

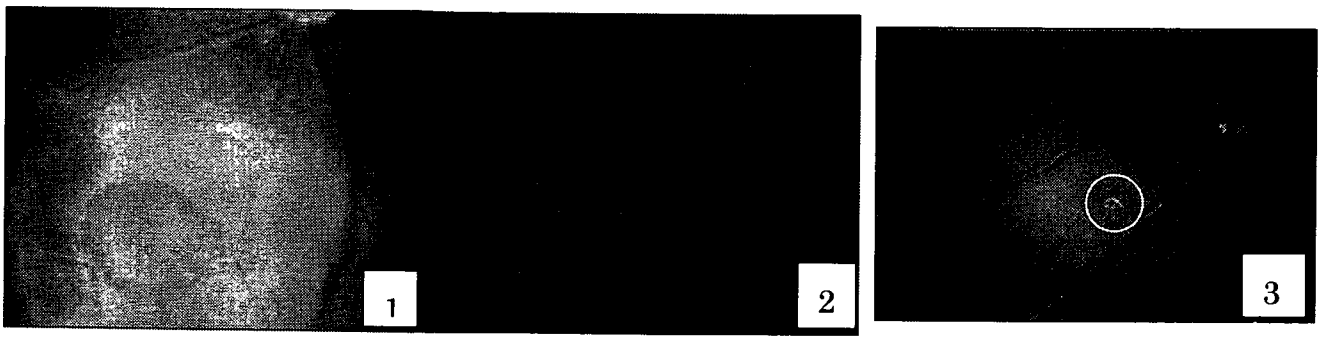
トキソカラ症の新しい動物モデル

トキソカラ症の病態を理解する上で動物モデルの果たしてきた役割は非常に大きい。マウスのモデルからは幼虫の体内移行経路の解明や肝臓内で幼虫が捕捉くされる機序が明らかにされた。ウサギのモデルからは病態生理学的解析や血清抗体の推移、モルモットを用いた実験系からはIgE抗体の消長が明らかにされてきた。しかし、眼型トキソカラ症のモデルとなりうるような動物モデルはこれまで知られていなかった。ヒトに近いカニクイザルやアカゲザルを用いても、経口感染では眼病変を惹起するには至らなかった。マウスでは眼内に幼虫は移行するがその頻度はきわめて低く、動物モデルといえるほどの有用性は認められていなかった。

1998年に我々はスナネズミが眼トキソカラ症を効率よく発症することを初めて明らかにした(29)。それまでスナネズミを用いたトキソカラ症の研究はBurren (1972) の研究が唯一のものであり、彼がその論文の中で、眼内に幼虫は見いだせなかったと記載して以降、誰も追試をしていなかった(30)。我々がスナネズミを使って実験したところ、イヌ回虫幼虫包蔵卵を経口投与して３日目以降、さまざまな網膜病変が出現すると共に、幼虫も眼底に観察できることがわかった（図３)。幼虫は感染させたスナネズミの70%以上に見られ、出血や血管炎などの病変は95%以上の個体で観察できた。また、イヌ回虫だけでなくネコ回虫卵の経口投与によっても同様の病変を惹起することが出来た(31)。

スナネズミ体内に侵入した幼虫は、マウスと同様に中枢神経系をはじめとして全身に分布する(32)。しかしマウスと異なり、脳内に侵入した幼虫によって不可逆性の神経症状を発症することもスナネズミを使った実験で明らかになってきた(33)。また

図３　イヌ回虫幼虫包蔵卵経口投与後にスナネズミの眼底に出現する病変。



１：硝子体出血層　　２：血管炎　　３：網膜上を移動する幼虫（円印内）

中枢神経系に侵入した幼虫が視神経を介して網膜内に出現することも明らかにすることができ、幼虫の眼内への移行経路には視神経を介するものがあること証明できた(34)。

このように、スナネズミはイヌ回虫やネコ回虫の感染に対して感受性が高いばかりでなく、ヒトにおけるトキソカラ症の病態を理解する上できわめて有用な動物モデルである。現在、スナネズミを用いた薬物治療モデルの開発を行っている。

おわりに

イヌ回虫は先進国や開発途上国を問わず、世界中に分布する寄生虫であり、私達にとってはごく身近な存在であるペットから感染する寄生虫症でもある。この病気が広く知られるようになって50年あまりが過ぎようとしているが、いまだ解明されていない問題が多く残されている。幼虫の母子間移行機序や眼トキソカラ症の半数以上の患者で血清抗体が上昇しないのはなぜか。あるいは神経型トキソカラ症の発症機序に関わるLESの意義など、まだまだわからない点が残されている。今後これらの問題をひとつでも解決できるよう微力を尽くしていきたいと考えている。

謝　辞

本稿の内容は平成19年３月１日に東京恵比寿の日仏会館で行われた日仏獣医学会での特別講演で発表した内容を基に、補足してまとめた。

文　献

18. 堀　司、吉田雅喜、布施茂登 et al. 著明な好酸球増多を呈した犬回虫症の１例. 臨床小児医学　1997 ; 45 : 157-161.
19. Barriga OO. A critical look at the importance, prevalence and control of toxocariasis and the possibilities of immunological control. Vet Parasitol 1988 ; 29 : 195-234.
20. Yoshida M, Shirao Y, Asai H et al. A retrospective study of ocular toxocariasis in Japan: correlation with antibody prevalence and ophthalmological findings of patients with uveitis. J Helminthol 1999 ; 73 : 357-361.
21. Magnaval JF, Galindo V, Glickman LT and Clanet M. Human Toxocara infection of the central nervous system and neurological disorders: a case-control study. Parasitology 1997 ; 115 (Pt 5): 537-543.
22. Eberhardt O, Bialek R, Nagele T and Dichgans J. Eosinophilic meningomyelitis in toxocariasis: case report and review of the literature. Clin Neurol Neurosurg 2005 ; 107 : 432-438.
23. Ota S, Komiyama A, Johkura K, Hasegawa O and Kondo K. Eosinophilic meningo-encephalo-myelitis due to Toxocara canis. Rinsho Shinkeigaku 1994 ; 34 : 1148-1152 (in Japanese).
24. Eberhard ML and Alfano E. Adult Toxocara cati infections in U.S. children : report of four cases. Am J Trop Med Hyg 1998 ; 59 : 404-406.
25. von Reyn CF, Roberts TM, Owen R and Beaver PC. Infection of an infant with an adult Toxocara cati (Nematoda). J Pediatr 1978 ; 93 : 247-249.
26. 上野良樹、蓮井正樹、近藤力王至、小松原彰. ネコ回虫成虫の生体を吐出した５歳男児例. 臨床寄生虫学雑誌 1999 ; 10 : 54-56.
27. Beaver PC, Jung RC and Cupp EW. Clinical Parasitology. Philadelphia, Lea & Febiger, 1984.
28. Kennedy MW, Maizels RM, Meghji M, Young L, Qureshi F and Smith HV. Species-specific and common epitopes on the secreted and surface antigens of Toxocara cati and Toxocara canis infective larvae. Parasite Immunol 1987 ; 9 : 407-420.
29. Takayanagi TH, Akao N, Suzuki R, Tomoda M, Tsukidate S and Fujita K. New animal model for human ocular toxocariasis: ophthalmoscopic observation. Br J Ophthalmol 1999 ; 83 : 967-972.
30. Burren CH. The distribution of Toxocara canis larvae in the central nervous system of rodents. Trans R Soc Trop Med Hyg 1972 ; 66 : 937-942.
31. Akao N, Takayanagi TH, Suzuki R, Tsukidate S and Fujita K. Ocular larva migrans caused by Toxocara cati in Mongolian gerbils and a comparison of ophthalmologic findings with those produced by T. canis. J Parasitol 2000 ; 86 : 1133-1135.
32. Cho S, Egami M, Ohnuki H et al. Migration Behaviour and Pathogenesis of Five Ascarid Parasites, Toxocara canis, Baylisascaris procyonis, B. transfuga, Ascaris suum, and A. lumbricoides in the Mongolian gerbil, Meriones unguiculatus. Journal of Helminthology 2007 ; 81 : 43-47.
33. Akao N, Tomoda M, Hayashi E et al. Cerebellar ataxia due to Toxocara infection in Mongolian gerbils, Meriones unguiculatus. Vet Parasitol 2003 ; 113 : 229-237.
34. Hayashi E, Akao N and Fujita K. Evidence for the involvement of the optic nerve as a migration route for larvae in ocular toxocariasis of Mongolian gerbils. J Helminthol 2003 ; 77 : 311-315.

Available online at www.sciencedirect.com
ScienceDirect
Parasitology International 56 (2007) 87–93
www.elsevier.com/locate/parint

Review

Toxocariasis in Japan

Nobuaki Akao *, Nobuo Ohta

Section of Environmental Parasitology, Graduate School of Tokyo Medical and Dental University, Yushima 1-5-45, Bunkyo-ku, Tokyo, 113-8519 Japan

Received 27 December 2006; received in revised form 16 January 2007; accepted 17 January 2007
Available online 26 January 2007

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].

* Corresponding author.
E-mail address: ocha.vip@tmd.ac.jp (N. Akao).

1383-5769/$ - see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.parint.2007.01.009

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.

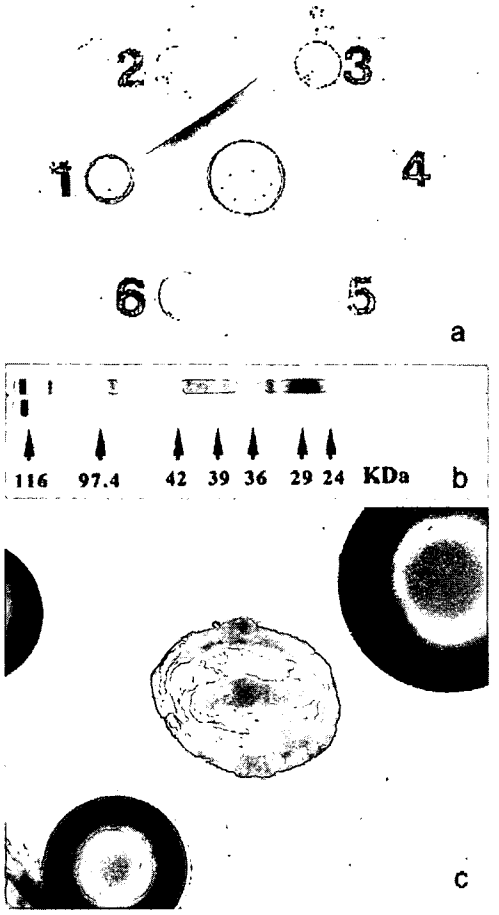


Fig. 1. The results of double gel diffusion (a) and western blot (b) tests of a patient of visceral toxocariasis. Strong precipitin bands were obviously observed between larval excretory–secretory products (LES) of *Toxocara canis* and patient's serum by means of double gel diffusion test. Antigens used 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.

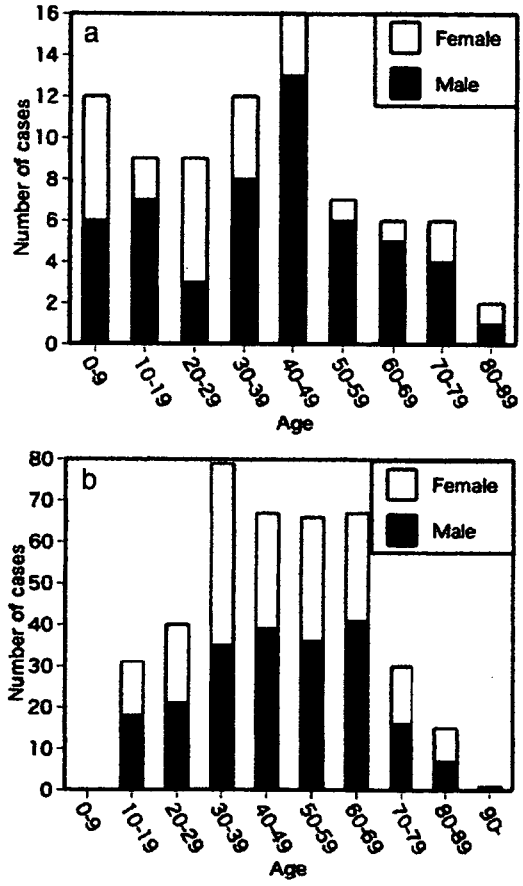


Fig. 2. Age distribution of suspected cases of visceral (n=79)(a) and ocular toxocariasis (n=396)(b) referred to our laboratory from August 1994 to December 2006.

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.

Acknowledgements

We wish to acknowledge the late Dr. Kaoru Kondo, the former Professor of the Department of Parasitology, School of Medicine, Kanazawa University, who gave us an opportunity for the study of *Toxocara* and toxocariasis. We also thank Drs K. Aragane, T. Suzuki, T. Hori, and Y. Morimatsu for providing us the patient's samples and information.

References

[1] Wilder HC. Nematode endophthalmitis. Trans Am Acad Ophthalmol Otolaryngol 1950;55:99–109.
[2] Beaver PC, Synder CH, Carrera GM. Chronic eosinophilia due to visceral larva migrans. Pediatrics 1952;9:7–19.
[3] Smith MHD, Beaver PC. Persistence and distribution of *Toxocara canis* larvae in the tissues of children and mice. Pediatrics 1953;12:491–7.
[4] Nichols RL. The etiology of visceral larva migrans. 1. Diagnostic morphology of infective second-stage *Toxocara* larvae. J Parasitol 1956;42:349–57.
[5] Sprent JFA. Observation on the development of *Toxocara canis* (Werner, 1782) in the dogs. Parasitology 1957;48:184–93.
[6] Kondo K. *Toxocara* infection and toxocariasis. In: Kamegai S, Hayashi S, editors. Progress of Medical Parasitology in Japan Tokyo: Meguro Parasitological Museum; 2003. p. 475–84.
[7] Glickman LT, Magnaval JF. Zoonotic roundworm infections. Infect Dis Clin North Am 1993;7:717–32.
[8] Taylor MR, Keane CT, O'Connor P, Mulvihill E, Holland C. The expanded spectrum of toxocaral disease. Lancet 1988;1:692–5.
[9] Fushimi J, Nishimura T, Murakami K. On a case which is considered as human toxocariasis cured by dithiazanine iodine. Jpn J Parasitol 1963;12:303–4.
[10] Yoshioka H. Nematode endophthalmitis, possibly due to *Toxocara canis*. Report of a case. Jpn Clin Ophthalmol 1966;20:149–54.
[11] Aragane K, Akao N, Matsuyama T, Sugita M, Natsuaki M, Kitada O. Fever, cough, and nodules on ankles. Lancet 1999;354:1872.
[12] Akao N, Nishi-Nakagawa K, Nishi O. Ocular toxocariasis: recovery of intraocular larva-like foreign body from a uveitis patient. Clin Parasitol 2003;14:71–3 [in Japanese].
[13] Ijuin N, Shimizu T, Fukuhara J, Nawa Y, Hara Y, Saishin M, Nishiyama T. Demonstration of causative organism by vitrectomy in a case of ocular toxocariasis. Jpn J Clin Ophthalmol 1999;53:1305–7 [in Japanese].
[14] Haruta Y. Toxocariasis. Practical Ophthalmol 1993;8:136–9 [in Japanese].
[15] Matsumura K, Endo R. Enzyme-linked immunosorbent assay for toxocariasis, its application to the sera of children. Zentralbl Bakteriol Mikrobiol Hyg A 1982;253:402–6.
[16] Matsumura K, Endo R. Seroepidemiological study on toxocaral infection in man by enzyme-linked immunosorbent assay. J Hyg (Lond) 1983;90:61–5.
[17] Kondo K, Akao N, Ohyama T, Okazawa T. Sero-epidemiological investigation of toxocariasis in Asian area. In The 5th Asian-Pacific Congress for Parasitic Zoonoses. In: Yamaguchi T, Araki T, Chen ER, editors. The Organizing Committee of Asian-Pacific Congress for Parasitic Zonooses; 1998. p. 65–70.
[18] Uga S, Ono K, Kataoka N, Hasan H. Seroepidemiology of five major zoonotic parasite infections in inhabitants of Sidoarjo, East Java, Indonesia. Southeast Asian J Trop Med Public Health 1996;27:556–61.

[19] Rai SK, Uga S, Ono K, Nakanishi M, Shrestha HG, Matsumura T. Seroepidemiological study of *Toxocara* infection in Nepal. Southeast Asian J Trop Med Public Health 1996;27:286–90.
[20] Overgaauw PA. Aspects of *Toxocara* epidemiology: human toxocariasis. Crite Rev Microbiol 1997;23:215–31.
[21] Sakai K, Okajima Y, Ohuchi K. A case of visceral larva migrans due to the ingestion of raw hen liver. Naika 1983;51:963–7 [in Japanese with English abstract].
[22] Ito K, Sakai K, Okajima T, Ouchi K, Funakoshi A, Nishimura J, et al. Three cases of visceral larva migrans due to ingestion of raw chicken or cow liver. J Jpn Soc Int Med 1986;75:759–66 [in Japanese].
[23] Nakatsuji Y, Shigemoto S, Kojiro N, Nanahoshi M, Masaki S. Brother cases of serologically diagnosed visceral larva migrans. J Jpn Soc Int Med 1989;78:35–40 [in Japanese].
[24] Nagakura K, Tachibana H, Kaneda Y, Kato Y. Toxocariasis possibly caused by ingesting raw chicken. J Infect Dis 1989;160:735–6.
[25] Mitsugi K, Umei T, Inoue T, Sumida I, Hanada M. Visceral larva migrans by *Toxocara cati* with multiple nodules in liver. J Jpn Soc Int Med 1988;77:1742–3 [in Japanese].
[26] Lee KT, Min HK, Chung PR, Chang JK. Studies on the inducing possibility of human visceral larva migrans associated with eating habit of raw liver of domestic animals. Korean J Parasitol 1976;14:51–60.
[27] Taira K, Permin A, Kapel CM. Establishment and migration pattern of *Toxocara canis* larvae in chickens. Parasitol Res 2003;90:521–3.
[28] Taira K, Saeed I, Permin A, Kapel CM. Zoonotic risk of *Toxocara canis* infection through consumption of pig or poultry viscera. Vet Parasitol 2004;121:115–24.
[29] Takakura Y. An epidemiological study of food-borne toxocariasis: fowl and cattle as paratenic hosts of *Toxocara canis*. J Juzen Med 1993;102:828–935 [in Japanese with English abstract].
[30] Morimatsu Y, Akao N, Akiyoshi H, Kawazu T, Okabe Y, Aizawa H. A familial case of visceral larva migrans after ingestion of raw chicken livers: appearance of specific antibody in bronchoalveolar lavage fluid of the patients. Am J Trop Med Hyg 2006;75:303–6.
[31] Epe C, Sabel T, Schnieder T, Stoye M. The behavior and pathogenicity of *Toxocara canis* larvae in mice of different strains. Parasitol Res 1994;80:691–5.
[32] Kayes SG, Jones RE, Omholt PE. Use of bronchoalveolar lavage to compare local pulmonary immunity with the systemic immune response of *Toxocara canis*-infected mice. Infect Immun 1987;55:2132–6.
[33] Kayes SG. Nonspecific allergic granulomatosis in the lungs of mice infected with large but not small inocula of the canine ascarid, *Toxocara canis*. Clin Immunol Immunopathol 1986;41:55–65.
[34] Inoue K, Inoue Y, Arai T, Nawa Y, Kashiwa Y, Yamamoto S, et al. Chronic eosinophilic pneumonia due to visceral larva migrans. Intern Med 2002;41:478–82 [in Japanese].
[35] Hayashi K, Tahara H, Yamashita K, Kuroki K, Matsushita R, Yamamoto S, et al. Hepatic imaging studies on patients with visceral larva migrans due to probable *Ascaris suum* infection. Abdom Imaging 1999;24:465–9.
[36] Ishibashi H, Shimamura R, Hirata Y, Kudo J, Onizuka H. Hepatic granuloma in toxocaral infection: role of ultrasonography in hypereosinophilia. J Clin Ultrasound 1992;20:204–10.
[37] Demirci M, Yildirim M, Aridogan BC, Baysal V, Korkmaz M. Tissue parasites in patients with chronic urticaria. J Dermatol 2003;30:777–81.
[38] Del Pozo MD, Audicana M, Diez JM, Munoz D, Ansotegui IJ, Fernandez E, et al. *Anisakis simplex*, a relevant etiologic factor in acute urticaria. Allergy 1997;52:576–9.
[39] Fernandez de Corres L, Audicana M, Del Pozo MD, Munoz D, Fernandez E, Navarro JA, et al. *Anisakis simplex* induces not only anisakiasis: report on 28 cases of allergy caused by this nematode. J Investig Allergol Clin Immunol 1996;6:315–9.
[40] Humbert P, Niezborala M, Salembier R, Aubin F, Piarroux R, Buchet S, et al. Skin manifestations associated with toxocariasis: a case-control study. Dermatology 2000;201:230–4.
[41] Piarroux R, Gavignet B, Hierso S, and Humbert P. Toxocariasis and the skin. In: editors. *Toxocara*: The Enigmatic Parasite *Toxocara*: The Enigmatic Parasite: CABI International, 2005.
[42] Woodruff AW. Toxocariasis. Br Med J 1970;3:663–9.
[43] Parasitic zoonoses. Report of a WHO expert committee with the participation of FAO. World Health Organ Tech Rep Ser 1979:1–107.
[44] Uga S, Matsuo J, Kimura D, Rai SK, Koshino Y, Igarashi K. Differentiation of *Toxocara canis* and *T. cati* eggs by light and scanning electron microscopy. Vet Parasitol 2000;92:287–94.
[45] Uga S. Prevalence of *Toxocara* eggs and number of faecal deposits from dogs and cats in sandpits of public parks in Japan. J Helminthol 1993;67:78–82.
[46] Hori T, Yoshida M, Fuse S, Igarashi C, Fujita S, Yoshida Y, et al. Prominent eosinophilia in a patient with toxocariasis. J Clin Pediatr 1997;45:157–61 [in Japanese].
[47] Barriga OO. A critical look at the importance, prevalence and control of toxocariasis and the possibilities of immunological control. Vet Parasitol 1988;29:195–234.
[48] Yoshida M, Shirao Y, Asai H, Nagase H, Nakamura H, Okazawa T, et al. A retrospective study of ocular toxocariasis in Japan: correlation with antibody prevalence and ophthalmological findings of patients with uveitis. J Helminthol 1999;73:357–61.
[49] Wilkinson CP, Welch RB. Intraocular *Toxocara*. Am J Ophthalmol 1971;71:921–30.
[50] Fujino T, Haruki K, Matsui T, Yokota N, Kobayashi F, M. T. The serodiagnostic examination for helminthiasis (1991–1996). J Ky Med Assoc 1998;29:581–4.
[51] Magnaval JF, Galindo V, Glickman LT, Clanet M. Human *Toxocara* infection of the central nervous system and neurological disorders: a case-control study. Parasitology 1997;115(Pt 5):537–43.
[52] Eberhardt O, Bialek R, Nagele T, Dichgans J. Eosinophilic meningomyelitis in toxocariasis: case report and review of the literature. Clin Neurol Neurosurg 2005;107:432–8.
[53] Ota S, Komiyama A, Johkura K, Hasegawa O, Kondo K. Eosinophilic meningo-encephalo-myelitis due to *Toxocara canis*. Rinsho Shinkeigaku 1994;34:1148–52 [in Japanese].
[54] Osoegawa M. Diagnosis and treatment of CNS parasite infection with special reference to parasitic myelitis. Rinsho Shinkeigaku—Clin Neurol 2004;44:961–4 [in Japanese with English abstract].
[55] Fisher M. *Toxocara cati*: an underestimated zoonotic agent. Trends Parasitol 2003;19:167–70.
[56] Eberhard ML, Alfano E. Adult *Toxocara cati* infections in U.S. children: report of four cases. Am J Trop Med Hyg 1998;59:404–6.
[57] von Reyn CF, Roberts TM, Owen R, Beaver PC. Infection of an infant with an adult *Toxocara cati* (Nematoda). J Pediatr 1978;93:247–9.
[58] Ueno Y, Hasui M, Kondo K, Komatsubara A. A case report of infant infected with adults of *Toxocara cati*. Clin Parasitol 1999;10:54–6 [in Japanese].
[59] Bisseru B, Woodruff AW, Hutchinson RI. Infection with adult *Toxocara canis*. Br Med J 1966;1:1583–4.
[60] Beaver PC, Jung RC, Cupp EW. Clinical parasitology. Philadelphia: Lea & Febiger; 1984.
[61] Kennedy MW, Maizels RM, Meghji M, Young L, Qureshi F, Smith HV. Species-specific and common epitopes on the secreted and surface antigens of *Toxocara cati* and *Toxocara canis* infective larvae. Parasite Immunol 1987;9:407–20.
[62] Nishikata H, Hirata Y, Shimamura R, Dohmen K, Kudo J, Ishibashi H, et al. A case of visceral larva migrans by *Toxocara cati* infection with multiple liver granuloma. Nippon Shokakibyo Gakkai Zasshi 1991;88:2697–702 [in Japanese with English abstract].
[63] Shimokawa H, Nakashima T, Akagi K, Omae T, Tsuji M. Visceral larva migrans by *Toxocara cati*. Fukuoka Igaku Zasshi 1982;73:64–9.
[64] Takeda M, Tanabe K, Nishi Y, Tsuji M, Iwanaga Y. Familial cases of *Toxocara cati* infection. Nippon Rinsho 1975;33:3558–65.
[65] Akao N, Goto Y, Kondo K, Tsuda Y. Changing chemosusceptibility in the second-stage larvae of *Toxocara canis* by long-term incubation. J Helminthol 1993;67:145–50.
[66] Maizels RM, de Savigny D, Ogilvie BM. Characterization of surface and excretory–secretory antigens of *Toxocara canis* infective larvae. Parasite Immunol 1984;6:23–37.
[67] Maizels RM, Page AP. Surface associated glycoproteins from *Toxocara canis* larval parasites. Acta Trop 1990;47:355–64.

[68] Page AP, Maizels RM. Biosynthesis and glycosylation of serine/threonine-rich secreted proteins from *Toxocara canis* larvae. Parasitology 1992;105 (Pt 2):297–308.
[69] Sugane K, Oshima T. Purification and characterization of excretory and secretory antigen of *Toxocara canis* larvae. Immunology 1983;50:113–20.
[70] Loukas A, Doedens A, Hintz M, Maizels RM. Identification of a new C-type lectin, TES-70, secreted by infective larvae of *Toxocara canis*, which binds to host ligands. Parasitology 2000;121(Pt 5):545–54.
[71] Gems D, Maizels RM. An abundantly expressed mucin-like protein from *Toxocara canis* infective larvae: the precursor of the larval surface coat glycoproteins. Proc Natl Acad Sci U S A 1996;93:1665–70.
[72] Page AP, Rudin W, Maizels RM. Lectin binding to secretory structures, the cuticle and the surface coat of *Toxocara canis* infective larvae. Parasitology 1992;105(Pt 2):285–96.
[73] Page AP, Rudin W, Fluri E, Blaxter ML, Maizels RM. *Toxocara canis*: a labile antigenic surface coat overlying the epicuticle of infective larvae. Exp Parasitol 1992;75:72–86.
[74] Akao N, Kondo K. Glycoconjugates of excretory–secretory antigens of second stage larvae of *Toxocara canis*: analysis of their reactivity to lectins. Jpn J Parasitol 1986;35:395–401.
[75] Akao N, Kondo K, Okamoto T, Yoshimura H. Antigenic analysis of excretory–secretory products of second sate larvae of *Toxocara canis* and the antigen recognition in the course of infection. Jpn J Parasitol 1983;32:541–8 [in Japanese with English abstract].
[76] De Andrade Lima Coelho R, De Carvalho LB, Perez EP, Araki K, Takeuchi T, Ito A, et al. Prevalence of toxocariasis in northeastern Brazil based on serology using recombinant *Toxocara canis* antigen. Am J Trop Med Hyg 2005;72:103–7.
[77] Yamasaki H, Araki K, Lim PK, Zasmy N, Mak JW, Taib R, et al. Development of a highly specific recombinant *Toxocara canis* second-stage larva excretory–secretory antigen for immunodiagnosis of human toxocariasis. J Clin Microbiol 2000;38:1409–13.
[78] Akao N, Chu AE, Tsukidate S, Fujita K. A rapid and sensitive screening kit for the detection of anti-*Toxocara* larval ES antigens. Parasitol Int 1997;46:189–95.
[79] Oshima T. Standardization of techniques for infecting mice with *Toxocara canis* and observations on the normal migration routes of the larvae. J Parasitol 1961;47:652–6.
[80] Hiratochi M, Takamoto M, Tatemichi S, Sugane K. Inhibition of interleukin 5 production with no influence on interleukin 4 production by an anti-allergic drug, tranilast, in *Toxocara canis*-infected mice. Int J Immunopharmacol 2000;22:463–71.
[81] Takamoto M, Wang ZX, Watanabe N, Matsuzawa A, Nariuchi H, Sugane K. Eosinophilia, IgE production, and cytokine production by lung T cells in surface CD4-deficient mutant mice infected with *Toxocara canis*. Immunology 1998;95:97–104.
[82] Takamoto M, Isobe M, Sugane K. The role of ICAM-1/LFA-1 and VCAM-1/VLA-4 interactions on T helper 2 cytokine production by lung T cells of *Toxocara canis*-infected mice. Immunology 1998;95:419–26.
[83] Hokibara S, Takamoto M, Isobe M, Sugane K. Effects of monoclonal antibodies to adhesion molecules on eosinophilic myocarditis in *Toxocara canis*-infected CBA/J mice. Clin Exp Immunol 1998;114:236–44.
[84] Takamoto M, Ovington KS, Behm CA, Sugane K, Young IG, Matthaei KI. Eosinophilia, parasite burden and lung damage in *Toxocara canis* infection in C57Bl/6 mice genetically deficient in IL-5. Immunology 1997;90: 511–7.
[85] Sugane K, Kusama Y, Takamoto M, Tominaga A, Takatsu K. Eosinophilia, IL-5 level and recovery of larvae in IL-5 transgenic mice infected with *Toxocara canis*. J Helminthol 1996;70:153–8.
[86] Takamoto M, Kusama Y, Takatsu K, Nariuchi H, Sugane K. Occurrence of interleukin-5 production by CD4-CD8-(double-negative) T cells in lungs of both normal and congenitally athymic nude mice infected with *Toxocara canis*. Immunology 1995;85:285–91.
[87] Kusama Y, Takamoto M, Kasahara T, Takatsu K, Nariuchi H, Sugane K. Mechanisms of eosinophilia in BALB/c-nu/+ and congenitally athymic BALB/c-nu/nu mice infected with *Toxocara canis*. Immunology 1995;84:461–8.
[88] Takamoto M, Sugane K. Mechanisms of eosinophilia in *Toxocara canis* infected mice: in vitro production of interleukin 5 by lung cells of both normal and congenitally athymic nude mice. Parasite Immunol 1993;15:493–500.
[89] Tuji M. Comparative studies on the antigenic structure of several helminths by immunoelectrophoresis. Jpn J Parasitol 1975;24:227–36.
[90] Tuji M. On the immunoelectrophoresis for helminthological researches. Jpn J Parasitol 1974;23:335–45.
[91] Akao N. Critical assessment of existing and novel systems of toxocariasis. In: Holland CV, HVS, editors. *Toxocara*: the enigmatic parasite. London: CABI International; 2005. p. 74–85.
[92] Akao N, Takayanagi TH, Suzuki R, Tsukidate S, Fujita K. Ocular larva migrans caused by *Toxocara cati* in Mongolian gerbils and a comparison of ophthalmologic findings with those produced by *T. canis*. J Parasitol 2000;86:1133–5.
[93] Takayanagi TH, Akao N, Suzuki R, Tomoda M, Tsukidate S, Fujita K. New animal model for human ocular toxocariasis: ophthalmoscopic observation. Br J Ophthalmol 1999;83:967–72.
[94] Akao N, Tomoda M, Hayashi E, Suzuki R, Shimizu-Suganuma M, Shichinohe K, et al. Cerebellar ataxia due to *Toxocara* infection in Mongolian gerbils, *Meriones unguiculatus*. Vet Parasitol 2003;113:229–37.

Journal of Helminthology (2007) **81**, 43–47 DOI: 10.1017/S0022149X07212118

Migration behaviour and pathogenesis of five ascarid nematode species in the Mongolian gerbil *Meriones unguiculatus*

S. Cho[1], M. Egami[2], H. Ohnuki[2], Y. Saito[1], S. Chinone[1], K. Shichinohe[3], M. Suganuma[3] and N. Akao[2]*

[1]Department of Veterinary Parasitology, Azabu University, Fuchinobe 1-17-71, Sagamihara 229-8501, Japan: [2]Section of Environmental Parasitology, Graduate School, Tokyo Medical and Dental University, Yushima 1-5-45, Bunkyo-ku, Tokyo 113-85119, Japan: [3]Division of Laboratory Animal Science, Nippon Medical School, Sendagi 1-1-5, Bunkyo-ku, Tokyo 113-9602, Japan

Abstract

To understand the characteristic features of the Mongolian gerbil, *Meriones unguiculatus*, as an animal model of ascarid infections, the migration behaviour and pathogenesis of larvae were investigated in experimentally infected gerbils. Embryonated eggs from each of *Toxocara canis*, *Baylisascaris procyonis*, *B. transfuga*, *Ascaris suum*, and *A. lumbricoides* were orally inoculated into gerbils and larvae were recovered from various organs at designated periods. In *T. canis*-infected gerbils, larvae were present in the liver 3 days after infection and in the skeletal muscle and brain via the heart and lungs at a similar rate. In *B. procyonis*- and *B. transfuga*-infected gerbils, larvae were present in the lungs within 24 h after infection, with some having reached the brain by that time. After 24 h, larvae of *B. procyonis* tended to accumulate in the brain, while those of *B. transfuga* accumulated in skeletal muscles. In *A. suum*- and *A. lumbricoides*-infected gerbils, larvae remained in the liver on day 5 post-infection and elicited pulmonary haemorrhagic lesions, which disappeared 7 days after initial infection. Thereafter, no larvae of any type were recovered. Ocular manifestations were frequently observed in *T. canis*- and *B. procyonis* infected gerbils, but were rare in *B. transfuga*-infected gerbils. In the cases of *A. suum* and *A. lumbricoides*, migration to the central nervous system and eyes was extremely rare, and larvae had disappeared by 2 weeks post-infection. Fatal neurological disturbances were observed in *B. procyonis*-infected gerbils, whereas irreversible non-fatal neurological symptoms were observed in the case of *B. transfuga*.

Introduction

Larval stages of ascarid nematodes elicit severe tissue damage when they invade hosts which are not normally the definitive host. The racoon roundworm, *Baylisascaris procyonis*, is particularly prone to cause a fatal neurological disturbance (Huff *et al.*, 1984; Kuchle *et al.*, 1993; Moertel *et al.*, 2001; Wise *et al.*, 2005). The dog roundworm, *Toxocara canis*, and the cat roundworm, *T. cati*, are also responsible for the visceral larva migrans syndrome (VLM) in humans (Glickman & Magnaval, 1993; Fisher, 2003). The VLM caused by these two roundworms is commonly known as toxocariasis, which is considered a disease of infants and children, although adults are also infected (Glickman *et al.*, 1987; Aragane *et al.*, 1999; Yoshida *et al.*, 1999). Moreover, outbreaks of VLM due to the pig roundworm, *Ascaris suum*, have been reported from the southern part of Japan (Maruyama *et al.*, 1996; Sakakibara *et al.*, 2002). Meningitis can occur when *Ascaris* larvae migrate into the central nervous system

*Author for correspondence
Fax: +81 3 5684 2849
E-mail: ocha.vip@tmd.ac.jp

(Osoegawa *et al.*, 2001). However, a precise diagnosis of these animal-borne ascarid infections is not always possible and an adequate anthelminthic therapy against these infections was not available. The lack of a comprehensive study using animal models for ascarid infections has also prevented progress in this field.

The Mongolian gerbil *Meriones unguiculatus* is known to be susceptible to a variety of parasites including *Brugia pahangi* (Ash & Riley, 1970), *Strongyloides stercoralis* (Nolan *et al.*, 1993), *Nippostrongylus brasiliensis* (Horii *et al.*, 1993), and *Entamoeba histolytica* (Chadee & Meerovitch, 1984). Akao *et al.* (2000) and Takayanagi *et al.* (1999) demonstrated that gerbils could serve as an animal model for ocular toxocariasis due to both *T. canis* and *T. cati*. The occurrence of retinal haemorrhages including larval invasion into the retina was found to be quite high compared with that in mice after oral inoculation of infective eggs. However, no information is available on the migration route, the final site of infection, or on the pathogenesis of ascarid larvae in gerbils. Here, we present our findings on the characteristic features of ascarid infections in gerbils as they relate to the pathogenesis of VLM in humans.

Materials and methods

Mongolian gerbils *Meriones unguiculatus* ranging between 2 and 3 months of age, were raised in the Animal Centre of Nippon Medical University and were maintained under pathogen-free conditions. Only male gerbils with black hair were used and all experiments were carried in accordance with the guidelines of the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University.

Recovery of ascarid eggs

Eggs of *T. canis* were obtained from the uteri of adult worms collected from faeces following the administration of anthelmintics to naturally infected puppies. Worms of *B. procyonis* were recovered from the intestine of infected racoons (Sato *et al.*, 2002), and those of *B. transfuga* collected from the faeces of infected bears. Worms of *B. transfuga* were naturally expelled from bears during their fasting period just prior to hibernation. Adult worms of *A. suum* were obtained from slaughtered pigs and *A. lumbricoides* were collected after treatment with anthelmintics from naturally infected humans. Female worms of each species were isolated, and matured embryonated eggs were prepared following the method of Oshima (1961).

Fully embryonated eggs were treated with 50% hypochlorous acid for 10 min to remove their proteinous membranes. After repetitive washing with distilled water, 1000 eggs of each ascarid were inoculated into each gerbil through a gastric tube under light anaesthesia.

Recovery of larvae

After ophthalmological observations (Takayanagi *et al.*, 1999), four gerbils from each group were sacrificed using sodium pentobarbital at predetermined intervals. One gerbil from each group was examined histopathologically and the remainder were used for larval recovery from the gastrointestinal tract, liver, lungs with heart, and skeletal muscle including bone and genital organs except for the skin. The contents of the gastrointestinal tract were collected in a conical tube at 6 and 12 h after inoculation. Each organ was minced and digested with artificial gastric juice (0.5% 1:10,000 pepsin, 0.7% hydrochloric acid) for 2 h at 37°C along with vigorous agitation. After digestion, the fluids were sieved with a tea strainer, and centrifuged at 320 × g for 5 min. The supernatant was discarded and a small amount of distilled water was added to the tube. The sediment was then spread out on glass, and larvae in the fluid were counted using a stereoscopic microscope. Examination of the brain tissue was performed as follows: each brain including the olfactory bulb, cerebrum, cerebellum, and pons was enucleated and minced into small pieces (approximately 2 mm^3) on a slide glass using forceps, and these were then covered with another slide glass. Migrating larvae were then counted using a microscope with eight or nine slide glasses being examined in each sample. Recovery rates were calculated from a mean of three gerbils at each period.

Histopathology

Tissue samples of liver, lungs, femoral muscle, brain, and gastrointestinal tract including the stomach, duodenum, ileum, caecum and rectum were fixed in 10% neutral formalin solution. Serial sections were prepared and stained with haematoxylin and eosin or periodic acid Schiff haematoxylin.

Results

Changes in the recovery rates of larvae from various organs after oral inoculation of the five ascarid species were recorded (fig. 1) and recovery rates arranged in the order of the migration route shown in fig. 2. In *Toxocara canis*-infected gerbils, almost all larvae were recovered from the intestinal wall up to 24 h after infection. Thereafter, larvae began to appear in the liver and lungs (by day 3), and then in the skeletal muscle and brain. The number of larvae in the muscle and brain were approximately equal. Macroscopically, haemorrhagic lesions which were observed in the lung 3, 5, 7 and 14 days after infection gradually disappeared. Ophthalmoscopically, a motile larva was observed in the retina 14 days after infection.

In *B. procyonis*- and *B. transfuga*-infected gerbils, the recovery rates of larvae were significantly lower than that in the case of *T. canis*. Both species of larvae had migrated into the lungs through the liver within 24 h after infection, and some had already arrived in the brain by this time, resulting in small haemorrhagic foci in the brain (fig. 3). With *B. procyonis*, ocular invasion by the larvae was observed and neurological disorders such as rotational and involuntary movement or paraplegia emerged, resulting in the mortality of gerbils between days 15 and 20 post-infection. Macroscopically, the gerbils exhibited fresh petechial haemorrhages of the lung 1 day after infection, but no new haemorrhagic lesions were evident beyond that time. Ophthalmic examination

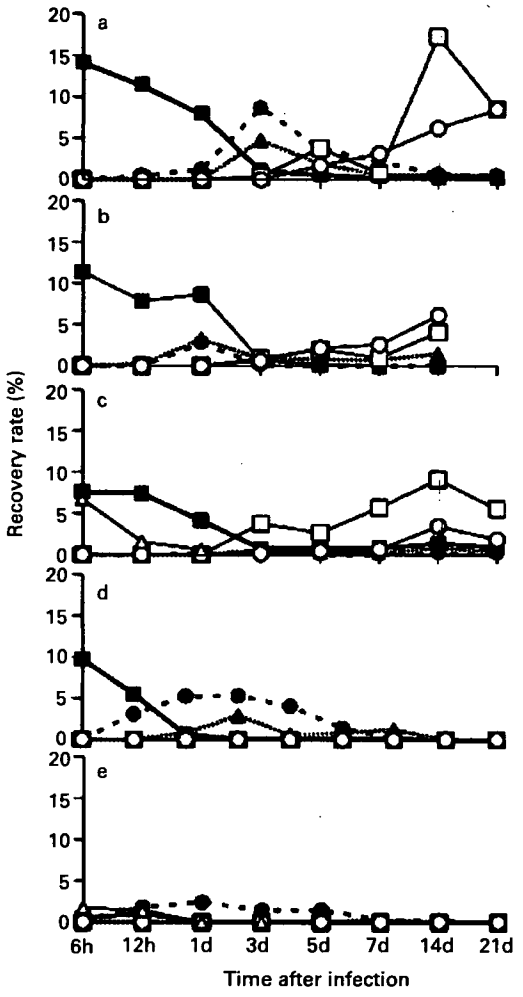


Fig. 1. Recovery rates (%) of larvae from various organs in gerbils after oral inoculation with five ascarid species up to day 21 post-infection. a, *Toxocara canis*; b, *Baylisascaris procyonis*; c, *B. transfuga*; d, *Ascaris suum*; e, *A. lumbricoides*; ■, gastrointestinal tract; ●, liver; ▲, lung and heart; □, muscles; ○, brain; △, intestinal contents.

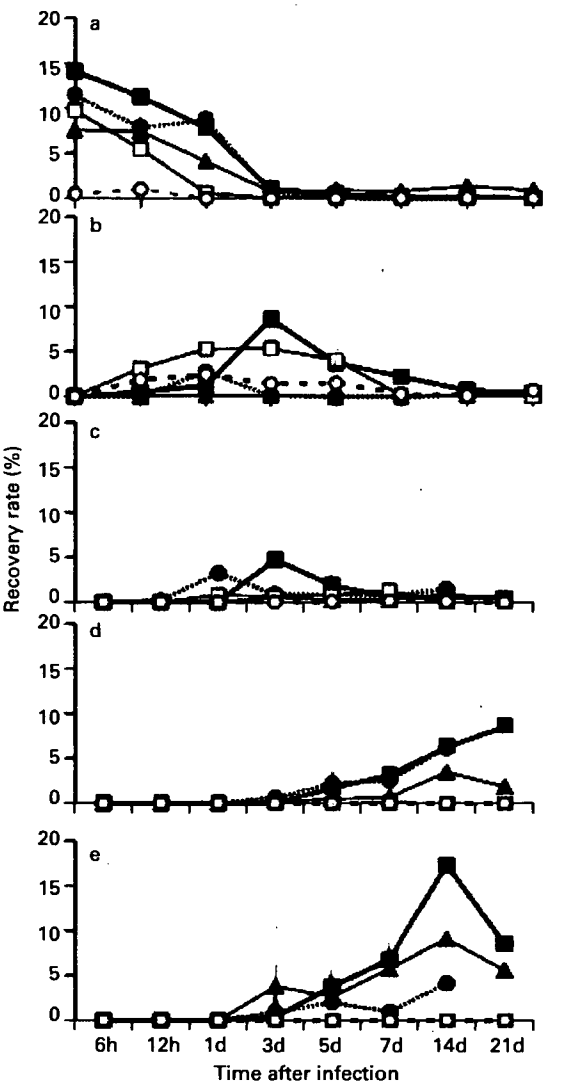


Fig. 2. Recovery rates (%) of larvae in gerbils after oral inoculation with five ascarid species to show the route of migration. ■, *Toxocara canis*; ●, *Baylisascaris procyonis*; ▲, *B. transfuga*; □, *Ascaris suum*; ○, *A. lumbricoides*; a, gastrointestinal tract; b, liver; c, lung and heart; d, brain; e, muscles.

demonstrated motile larvae in the retina and severe chorioretinitis 7 days after infection. The number of migrating larvae of *B. procyonis* in the brain was higher than that of *B. transfuga*-infected gerbils at all times after the infection. By day 3 post-infection an average of 6.3 larvae (range 6–7) in *B. procyonis*-infected gerbils and 1.7 larvae (range 1–2) in *B. transfuga*-infected gerbils were found. By day 7, an average of 26.0 larvae (range 23–31) in *B. procyonis*-infected gerbils and 6.7 larvae (range 5–8) in *B. transfuga*-infected gerbils were recovered. Ophthalmic and neurological abnormalities were less severe than those observed with *B. procyonis*, although gait difficulty and circulatory movements in the same direction were presented by day 10 post-infection. The number of *B. transfuga* larvae in the skeletal muscle was higher than that in the brain; i.e. an average number of larvae recovered were 37.7 (range 21–67) in muscle and 1.7 (range 1–2) in brain at the day 3 post-infection, and 91.0 (range 95–100) in muscle and 34.7 (range 24–49) in brain at the day 14 post-infection.

With *A. lumbricoides*, the average recovery rate of larvae after 6 h of infection was 2.3% (range 1.7–2.6%) compared with about 10% or higher in other ascarid parasites (14.1% in *T. canis*, 11.4% in *B. procyonis*, 16.3% in *B. transfuga* and 9.7% in *A. suum*; fig. 1a). Migrating larvae were present in the liver 12 h after infection, and remained there for up to 5 days after infection in the case of *A. lumbricoides* and

Fig. 3. A fresh haemorrhagic lesion in the granular layer of the cerebellum in gerbils 24 h after infection with *Baylisascaris procyonis*; larva (arrowed) in the molecular layer of cerebellum immediately beneath the pia mater.

7 days in the case of *A. suum*. On day 7, haemorrhagic lesions in the lungs were the most prominent feature in gerbils infected with both species, although these lesions gradually disappeared and no larvae were recovered thereafter from any organ.

As far as the migration route is concerned, the highest recovery rate in the gastrointestinal wall occurred in *T. canis*-infected gerbils followed by *B. procyonis*-, *A. suum*-, and *B. transfuga*-infected gerbils 6 h after infection. Larvae of both species of *Ascaris* immediately migrated away from the intestinal wall to the liver within 24 h after infection, with *Toxocara* and *Baylisascaris* larvae remaining there until the end of experiment. *Ascaris lumbricoides* larvae were minimally recovered from not only the gastrointestinal tract but also from other organs throughout the experiment. The recovery rate of ascarid larvae from the brain was high in the case of *T. canis* (3.1%) and *B. procyonis* (2.6%) at day 7 post-infection as compared with *B. transfuga* (0.7%), even though *B. procyonis*-infected gerbils did not survive until the end of the experiment. On the other hand, no *A. lumbricoides* larvae were found and only one *A. suum* larva was observed on day 7 post-infection. The recovery rate from skeletal muscles was high in the case of *T. canis* and *B. transfuga*, although the number of muscle stage larvae of *B. transfuga* was always higher than that in *B. procyonis*.

Discussion

Takayanagi *et al.* (1999) demonstrated that the Mongolian gerbil is a suitable animal model for ocular toxocariasis because of the high incidence of ocular invasion by the larvae. However, little is known about the migratory behaviour or pathogenesis of ascarid larvae in gerbils. In the present study, *T. canis* larvae migrated to the liver within 3 days after infection, and were thereafter distributed equally in skeletal muscles and the brain. These results are similar to those of Olson (1962) and Sprent (1952), suggesting that the migration route and final site of infection have little influence on the development of ocular toxocariasis in gerbils.

In the present study, *B. procyonis* larvae more so than *B. transfuga* were likely to accumulate in the brain and all gerbils infected with *B. procyonis* died from severe neurological disturbances within 2 weeks after infection. On the other hand, gerbils infected with *B. trasfuga* survived throughout the duration of the experiment, despite exhibiting neurological disorders. The number of *B. transfuga* muscle stage larvae was always higher than in *B. procyonis*-infected gerbils. Sato *et al.* (2004) reported that the *B. procyonis* and *B. transfuga* larvae that had migrated into the brain of gerbils were larger than those of *T. canis*; however, no significant differences in larval size were observed between *B. procyonis* and *B. transfuga*. These results suggest that severe neurological disorders caused by *B. procyonis* could be attributed to the total amount of larvae in the brain. Additionally, these findings suggest that *B. procyonis* larvae may have a neurotropism, whereas *B. transfuga* larvae may have an affinity for muscular tissue. Further studies are needed to better understand the pathogenetic differences between *B. procyonis* and *B. transfuga* larvae in the brain of infected gerbils. Ophthalmologically, the lesions elicited by both species closely resembled each other although the incidence was extremely low in *B. transfuga*-infected gerbils. These results indicate that *B. transfuga* should not be used as an alternative parasite for studying diffuse unilateral subacute neuroretinitis induced by *B. procyonis* in gerbils (Akao *et al.*, 2003).

In the present study, the infectivity of *A. suum* and *A. lumbricoides* in gerbils was very low, with migration to the central nervous system being minimal and no ophthalmological changes were found. Therefore, *A. suum* and *A. lumbricoides* are considered inappropriate parasites for studying ophthalmological and neurological disorders in gerbils. Severe to mild pulmonary haemorrhagic lesions were common in infected gerbils, although a complete healing of these lesions occurred in the case of *A. suum* and *A. lumbricoides*. Interestingly, no larvae were recovered from any organs of these gerbils beyond 14 days post-infection. Mouse models have shown a similar pattern (Slotved *et al.*, 1997, 1998). To further document the migratory behaviour of *A. suum* and *A. lumbricoides* larvae in gerbils after 7 days of infection, the contents of the gastrointestinal tract were examined daily between days 8 and 13 post-infection because we assumed that the larvae might return to the intestine via the larynx and pharynx. However, no larvae were detected (data not shown), suggesting their rapid expulsion from infected gerbils.

Further studies are needed to more fully elucidate the migration behaviour and pathogenesis of *T. cati* so that we may potentially improve the therapy against this important zoonotic parasite of human VLM (Akao *et al.*, 2000; Fisher, 2003).

Acknowledgements

The authors are grateful to Drs Masanori Kawanaka, Hiroshi Sugiyama, and Yasuyuki Morishima for providing some of the *B. procyonis* eggs in this study.

References

Akao, N., Takayanagi, T.H., Suzuki, R., Tsukidate, S. & Fujita, K. (2000) Ocular larva migrans caused by