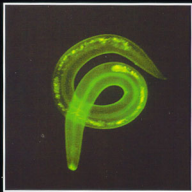


Toxocara

The Enigmatic Parasite



Edited by
C.V. Holland and H.V. Smith



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Edited by

Celia V. Holland

*Department of Zoology
Trinity College
University of Dublin
Ireland*

and

Huw V. Smith

*Scottish Parasite Diagnostic Laboratory
Stobhill Hospital
Glasgow
UK*

CABI Publishing

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CABI Publishing
CAB International
Wallingford
Oxfordshire OX10 8DE
UK

Tel.: +44 1491 832111

Fax: +44 1491 833508

E-mail: cabi@cabi.org

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CABI Publishing
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Tel.: +1 617 395 4056

Fax: +1 617 354 6875

E-mail: cabi-nao@cabi.org

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Contributors

Nobuaki Akao, Section of Environmental Parasitology, Graduate School of Tokyo Medical and Dental University, Yushima 1-5-45, Bunkyo-ku, Tokyo 113-8519, Japan. ocha.vip@tmd.ac.jp

Christine M. Budke, Institute of Parasitology, University of Zurich, Winterthurerstrasse 266a, CH-8057 Zurich, Switzerland.

Deborah M. Callister, Institute of Immunology and Infection Research, Ashworth Laboratories, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK. Deborah.Callister@ed.ac.uk

Neil B. Chilton, Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon SK Canada S7N 5E2. neil.chilton@usask.ca

Jan Dormans, Laboratory for Toxicology, Pathology and Genetics, National Institute of Public Health and the Environment (RIVM), P.O. Box 1, 3720 BA Bilthoven, The Netherlands.

Christian Epe, Institute of Parasitology, Centre for Infectious Diseases, Hannover School of Veterinary Medicine, Buenteweg 17, Hannover, D-30559 Germany. Christian.Epe@tiho-hannover.de

Robin B. Gasser, Department of Veterinary Science, The University of Melbourne, 250 Princes Highway, Werribee, Victoria 3030, Australia. robinbg@unimelb.EDU.AU

Béatrice Gavignet, Department of Mycology, University Hospital Jean Minjoz, Besançon cedex, France. beatrice.gavignet@univ-fcomte.fr

Lawrence T. Glickman, Department of Veterinary Pathobiology, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907–2027, USA. ltg@purdue.edu

Clare Hamilton, Department of Zoology, Trinity College, University of Dublin, Dublin 2, Ireland. hamiltcm@tcd.ie

Stéphanie Hierso, Department of Dermatology, University Hospital Saint Jacques, Place Saint Jacques, 25030 Besançon Cedex, France.

Celia V. Holland, Department of Zoology, Trinity College, University of Dublin, Dublin 2, Ireland. cholland@tcd.ie

Gabriela Hrková, Parasitological Institute, Slovak Academy of Sciences, Hlinkova 3, 040 01 Kosice, Slovak Republic. hrcka@saske.sk

Min Hu, Department of Veterinary Science, The University of Melbourne, 250 Princes Highway, Werribee, Victoria 3030, Australia.

Philippe Humbert, Department of Dermatology, University Hospital Saint Jacques, Place Saint Jacques, 25030 Besançon Cedex, France.

Dennis E. Jacobs, Department of Pathology and Infectious Diseases, The Royal Veterinary College, University of London, North Mymms, Hatfield AL9 7TA, UK. DJacobs@rvc.ac.uk

-
- Stephen G. Kayes**, Department of Cell Biology and Neuroscience, College of Medicine, University of South Alabama, 307 University Boulevard Mobile, AL 36688-0002, USA. kayes@sungcg.usouthal.edu
- Malcolm W. Kennedy**, Environmental and Evolutionary Biology, Institute of Biomedical and Life Sciences, Graham Kerr Building, University of Glasgow, Glasgow G12 8QQ, UK. malcolm.kennedy@bio.gla.ac.uk
- John W. Lewis**, School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey TW20 0EX, UK. j.w.lewis@rhul.ac.uk
- Jennifer L. Liang**, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Mailstop F22, 4770 Buford Highway, Atlanta, GA 30341, USA.
- Jean-François Magnaval**, Department of Parasitology, Rangueil University Hospital, 31059 Toulouse 9, France. magnaval@cict.fr
- Rick M. Maizels**, Institute of Immunology and Infection Research, Ashworth Laboratories, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK. rick.maizels@ed.ac.uk
- Hanna Mizgajska-Wiktor**, Department of Biology and Environmental Protection, Eugeniusz Piasecki University, School of Physical Education, Krolowej Jadwigi 27/39, 61-871 Poznan, Poland. mizgajska@awf.poznan.pl
- Gavin Nicoll**, Institute of Immunology and Infection Research, Ashworth Laboratories, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK. 0406316N@student.gla.ac.uk
- Rahmah Noordin**, Institute for Research in Molecular Medicine, Suite 110, Eureka Complex, Universiti Sains Malaysia, 11800 Penang, Malaysia.
- Renaud Piarroux**, Department of Mycology, University Hospital Jean Minjoz, 25030 Besançon cedex, France. renaud.piarroux@ufc-chu.univ-fcomte.fr
- Elena Pinelli**, Department of Parasitology and Mycology, Diagnostic Laboratory for Infectious Diseases and Perinatal Screening, National Institute of Public Health and the Environment (RIVM), P.O. Box 1, 3720 BA Bilthoven, The Netherlands. Elena.Pinelli@rivm.nl
- Irma Schabussova**, Institute of Immunology and Infection Research, Ashworth Laboratories, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK. irma.schabussova@ed.ac.uk
- Peter M. Schantz**, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Mailstop F22, 4770 Buford Highway, Atlanta, GA 30341, USA. pms1@cdc.gov
- Huw Smith**, Scottish Parasite Diagnostic Laboratory, Stobhill Hospital, Glasgow G21 3UW, UK. Huw.Smith@NorthGlasgow.Scot.nhs.uk
- Wilma A. Starke-Buzetti**, Departamento de Biologia e Zootecnia, Faculdade de Engenharia, Universidade Estadual Paulista 'Júlio de Mesquita Filho' (FEIS/UNESP), Campus de Ilha Solteira, 56- Av. Brasil, 15385-000, SP, Brazil. starke@bio.feis.unesp.br
- Mervyn Taylor**, Departments of Paediatrics and Zoology, Trinity College, Dublin 2, Ireland. mrhtaylor@doctors.org.uk
- Paul R. Torgerson**, Institute of Parasitology, University of Zürich, Winterthurerstrasse 266a, CH-8057 Zürich, Switzerland. paul.torgerson@access.unizh.ch
- Shoji Uga**, Department of Medical Technology, Faculty of Health Sciences, Kobe University School of Medicine, 7-10-2 Tomogaoka, Sumaku, Kobe 654-0142, Japan. ugas@ams.kobe-u.ac.jp
- Irma van Die**, Department of Molecular Cell Biology and Immunology, VU University Medical Center, FdG Postbus 7057, 1007 MB Amsterdam, The Netherlands.
- Corey S. Watts**, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Mailstop F22, 4770 Buford Highway Atlanta, GA 30341, USA.
- Xing-Quan Zhu**, Laboratory of Parasitology, College of Veterinary Medicine, South China Agricultural University, 483 Wushan Street, Tianhe District, Guangzhou 510642, Guangdong Province, People's Republic of China. xingquanzhu@scau.edu.cn

Introduction

The literature on *Toxocara* is extensive, and the purpose of this book is not to review the field comprehensively, but to present chapters by selected experts who were asked to review a particular area and to place it within the context of the wider field (Figure I.1). We sincerely hope that this book will contribute to a wider understanding of this truly enigmatic parasite and stimulate better insights into *Toxocara* biology.

Toxocara canis and *Toxocara cati* are cosmopolitan gastrointestinal parasites of canids and felids, respectively. Infective ova excreted by these highly fecund parasites contaminate the environment extensively exposing paratenic hosts, including humans, to the risk of infection. Many seroprevalence studies worldwide indicate that exposure to infection in humans can be high, particularly within certain geographical locations, but the relationship between seropositivity and disease is far from clear. In paratenic hosts, including humans, larvae do not develop to maturity, but undergo migration through the liver and lungs to reside, in an arrested developmental state, in various soft tissues and organs.

A variety of disease states are described but some symptoms are not well understood and require further elucidation. Of particular public health concern is larval invasion of the eye (ocular larva migrans) and the potential for visual impairment. Central nervous system involvement also occurs in paratenic hosts, although the public health significance of small numbers of larvae in, for example, a child's brain, remains unclear. *Toxocara* larvae accumulate in the murine brain causing behavioural alterations, including impairment in learning and memory, which are dependent upon the intensity of infection. The mouse model of cerebral toxocariasis may prove useful for interpreting the impact of chronic helminth infection on cognitive development, a relationship that has been very difficult to unravel in humans.

The association between *Toxocara* infection, lung and skin involvement and the consequent allergic responses (e.g. asthma, cutaneous manifestations) have received particular attention recently. Clearly, these relationships are very important, but there have been few appropriately rigorous, case-controlled studies in humans. Experimental models of human infection and disease will help determine the immunological, pathophysiological and molecular basis of these disease processes, while further insight into the nature and biology of parasite-derived molecules should expand our understanding of parasite-driven immuno-modulation of immune responses in both paratenic (e.g. human) and definitive hosts.

With the exception of overwhelming infections, the widespread distribution of small numbers of tissue-dwelling larvae dictates that serology is the laboratory tool of choice for diagnosing human infection. Yet, the interpretation of serological data, both at the level of the individual patient and at the population-based level, is fraught with difficulties including the choice of cut-off levels. Cross-reactive ascaridid epitopes reduce serodiagnostic specificity in poly parasitized communities, antigenaemias are

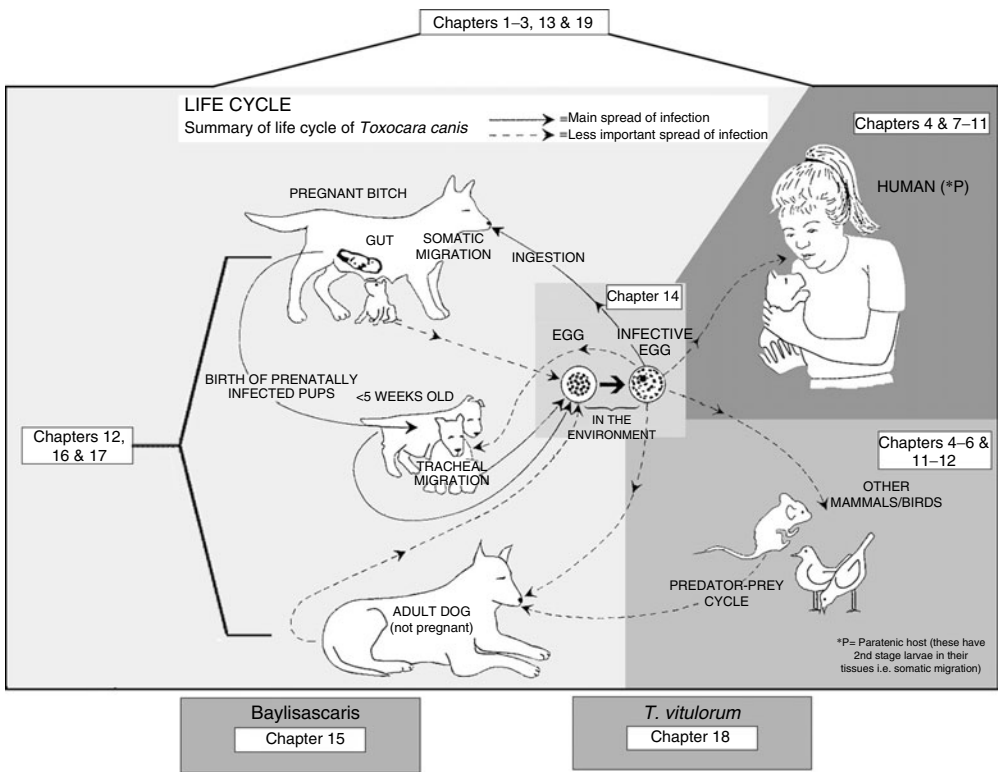


Fig. I.1. Illustration of the relationship between the life cycle and biology of *Toxocara canis* and the chapters in this book.

transient due to the release of highly immunogenic antigens and circulating antibodies can be below the sensitivity of detection of the diagnostic assay in disease-causing, low-level infections.

The influence of genetic type in causing infection and disease, whether through ingesting infective ova from definitive hosts, or arrested state larvae from paratenic hosts, is currently unclear, although sensitive molecular typing and subtyping methods should be capable of addressing these issues. Furthermore, whether specific genotypes or subtypes are organ-specific or become arrested larvae, rather than mature intestinal adults, should also be determined, thus advancing our knowledge of their specific roles in maintaining the *Toxocara* life cycle. The development of molecular typing and subtyping methods should increase the public and veterinary health usefulness of surveillance and extend our insight into the micro- and macro-epidemiology of infection and disease.

Various vertebrates serve as laboratory models for human disease and have provided us with important data on pathophysiology, the immunobiology of infection and disease and host-based selection. The novel Mongolian gerbil model is particularly susceptible to ocular invasion, while the advantages of murine models of immunobiology and neuropathophysiology are frequently voiced. Critical evaluation of the relative merits of different paratenic model systems is required, including their capacity to remain as sources of infection under natural conditions.

Infection with other *Toxocara* spp. such as *Toxocara vitulorum* impacts on the economic cost of buffalo production, a cost-effective, lean meat source. Yet, few studies have evaluated the burden of human disease and its economic cost, partly because toxocariasis is not reportable, partly because of the lack of

pathognomonic signs and symptoms and partly because of the difficulty in associating positive serology with specific disease presentations. Despite these challenges, the cost of disease, based upon disability-adjusted life years, is required.

Strategies for the control and management of this complex disease in humans, and other paratenic and definitive hosts, is an obvious challenge which needs to be tackled from a number of perspectives. Veterinary public health awareness is in the vanguard here, with extensive information on pet care, minimizing the risk of infection for both pet and owner, drug dosing regimes and pet and owner hygiene. Appropriate and effective chemotherapy of companion animals is a significant intervention, which reduces environmental contamination, although the issue of drug resistance must be borne in mind. Vaccination is a further option for control, but requires further research investment. Humans are infected from environmental sources, primarily, and reducing environmental contamination, through chemotherapeutic and educational interventions, are significant control interventions. Chemotherapy for humans is generally unsatisfactory and further rigorous case-control studies are required to test the efficacy of existing and novel agents.

An overarching need for all aspects of the biology of *Toxocara* and toxocariasis is the collection and collation of appropriate epidemiological data. To understand fully the biology of this enigmatic zoonosis, we require clinical, serological, environmental, molecular and other epidemiological parameters for both definitive and paratenic hosts. Web-based bioinformatics systems offer the advantages of rapid, controlled access to researchers, worldwide, and data logging for macro- as well as micro-epidemiological studies and should provide a better global understanding of the significance of this enigmatic parasite.

Interest in this enigmatic zoonosis encompasses a wide variety of disciplines including veterinarians, clinicians, biologists and public health professionals. In addition, *Toxocara* provides a unique model system to explore further, fundamental questions in nematode biology such as the role of parasite molecules and the nature of host-parasite interaction and its relationship with disease states. It is apparent that the veterinary and public health significance of *Toxocara* and toxocariasis can only be solved by combining effective interdisciplinary approaches with recent developments in detection technologies. Much depends upon a better understanding of all the disciplines involved in preventing disease transmission and no one approach may be effective across the variety of situations encountered. While multidisciplinary approaches offer the most effective solutions, apparently diverse approaches may well become powerful partners in our efforts to understand and address the significance of this enigmatic zoonosis.

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Celia Holland
Huw Smith
Dublin and Glasgow, April 2005

Dedication

This book is dedicated to Rory, Kate and Roísín.

Section 1

Biology of *Toxocara*

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1 Molecular Biology and Immunology of *Toxocara canis*

Rick M. Maizels, Irma Schabussova, Deborah M. Callister
and Gavin Nicoll

*Institute of Immunology and Infection Research, Ashworth Laboratories, University of
Edinburgh, UK*

Introduction

Toxocara canis is a widespread gastrointestinal nematode parasite of dogs and a causative agent of zoonotic disease in humans (Glickman and Schantz, 1981; Lewis and Maizels, 1993; Despommier, 2003). Larval stages of this parasite have an obligatory tissue-migratory phase, which can extend for months or years, giving rise to a drug-resistant reservoir in dogs, and to visceral larva migrans in infected humans. With a high prevalence in both domestic dogs and wild canids such as foxes, *T. canis* is present not only in tropical regions but also in the temperate climate of Europe and North America. Although the incidence of human pathology appears to be low, exposure of the human population, judged by serological surveys, is likely to be extensive (van Knapen *et al.*, 1983; Gillespie, 1988; Kenny *et al.*, 1995; Lokman Hakim *et al.*, 1997; Radman *et al.*, 2000).

Infection normally results from the ingestion of *T. canis* eggs from the environment (see Chapter 14, this volume). In the canine host, the larvae emerge in the intestine and burrow through the epithelial wall to migrate to the lungs, as is the case for other ascarid parasites in general (Schacher, 1957; Sprent, 1958). From the lungs, larvae climb the trachea, enter the oesophagus and re-

turn to the intestinal tract to mature as reproductive adult worms. However, if mice or humans ingest the eggs, emerging larvae migrate to muscle and neurological tissues, where they can remain for many years in an arrested state without growth, differentiation or reproduction. In mice, dormant tissue larvae can re-enter the transmission cycle if predation by a canid carnivore takes place. In humans, migrating larvae can invade visceral tissues, causing eosinophilia and non-pathognomic malaise, as well as the eye in cases of ocular larva migrans (Schantz, 1989; Good *et al.*, 2004) (see Chapter 9, this volume). There is also evidence of central nervous system invasion (Magnaval *et al.*, 1997), extending to the brain (Hill *et al.*, 1985) (see Chapter 5, this volume). Although it is difficult to estimate the frequency of such episodes, the link between *Toxocara* seropositivity and increased risk of epilepsy (Nicolletti *et al.*, 2002) causes further concern.

The immune response to infection presents a number of paradoxical features: there is a generalized inflammation evidenced by eosinophilia, stimulation of Th2-type cytokines and high titres of anti-*Toxocara* antibodies. However, inflammatory responses do not appear effective against the parasites themselves. Some investigators have suggested that the mobility of larvae confers the

ability to 'flee the scene of the crime', leaving inflammatory mayhem behind (Parsons *et al.*, 1986); part of this scenario may be a highly labile surface coat, which is shed, if and when antibodies and granulocytes bind (Smith *et al.*, 1981; Fattah *et al.*, 1986; Badley *et al.*, 1987a; Smith, 1993); but part of the success of this parasite in evading effective immunity is also likely to be specific down-modulation of those arms of the response most capable of providing protective immunity (see Chapters 3 and 11, this volume).

Set against this backdrop, it is clear that *T. canis* presents many fascinating aspects to biologists, and many practical difficulties to clinicians and veterinarians. Often these are two sides of the same coin: the developmental flexibility of larvae, which allows them to arrest in the tissue stage, is also the basis of pathology in humans (Gillespie, 1987), and renders them relatively inaccessible to anthelmintic treatment in dogs (Lloyd, 1993). Equally remarkable is the ability of *T. canis* larvae to traverse the placenta and infect pups *in utero*, while the tropism of the same larvae in humans for neurological and ocular tissue is of major concern for public health. Laboratory scientists are also intrigued by the larva's capacity to survive for many months *in vitro*, while producing quantities of biologically active glycoproteins. For these reasons, *T. canis* has become a unique model system for parasitic nematodes, as well as a clinical problem which requires renewed effort for control and eradication (Maizels *et al.*, 1993).

Genomics and Genetics

Genomics is transforming helminth biology, starting with the completion of the genome sequence of the free-living nematode *Caenorhabditis elegans* in 1998 (The *C. elegans* Genome Consortium, 1998). This achievement ushered in a wealth of new tools and approaches to analysis, isolation and testing of genes from nematode organisms. In the near future, major human pathogenic species are likely to be scheduled for complete genome sequencing, including *Ascaris*. Thus, even without complete sequencing of *T. canis* itself, the availability of related sequences and the feasibility of functional experiments in *C. elegans* opens important new opportunities in the molecular biology of *Toxocara*.

The genome of *T. canis* is known to be organized into 18 chromosomes within the germ-line cells (Walton, 1959), and is assumed to be similar in size to that of *Ascaris*, around 3×10^9 bp (Müller *et al.*, 1982). However, as in *Ascaris*, somatic cells of *T. canis* undergo a process of chromosome fragmentation and diminution in early embryonic development, prior to gastrulation. Around 20% of germ-line DNA is lost at this stage, and only germ-line progenitors retain the full genetic complement of the species (Goldstein and Straus, 1978; Davis and Carter, 1980; Streeck *et al.*, 1982; Aeby *et al.*, 1986).

Ribosomal and mitochondrial genes from *T. canis* have confirmed traditional taxonomy in placing this species within the ascarid group (Nadler and Hudspeth, 2000), together with the filarial nematodes within the spirurid subphylum of nematodes, classified as Clade IV (Blaxter *et al.*, 1998). Although no sequence comparisons of species within the genus *Toxocara* have been made, immunological and biochemical indications are that *T. canis*, *Toxocara cati* and *Toxocara vitulorum* (a parasite of cattle) are extremely closely related (Kennedy *et al.*, 1987; Nadler, 1987; Page *et al.*, 1991).

Detailed studies of individual coding genes of *T. canis* commenced in the early 1990s, using PCR techniques with small amounts of parasite material. Some of the first insights were gained by amplifying mRNA with a 5' primer designed to the conserved nematode 22 nt spliced leader (SL), as well as an oligo-dT primer which anneals to the poly-A 3' tail. Because a significant proportion of all nematode mRNAs bear an identical 5' 22 nt segment, trans-spliced during gene transcription (Bektesh *et al.*, 1988; Blaxter and Liu, 1996), this approach was expected to amplify a wide range of parasite mRNAs. However, SL-dT amplified cDNA showed two dominant bands rather than a polydispersed range of sizes. Analysis of these bands revealed two abundant SL-bearing mRNA transcripts which encoded the *Toxocara* excretory-secretory (ES) proteins TES-26 and TES-120 (Gems *et al.*, 1995; Gems and Maizels, 1996). A fuller account of both genes is given below, but the isolation of TES-120 cDNA at this stage was an important step in understanding the surface structure of *T. canis* larvae, as well as the composition of the ES material.

Where complete genome analyses are not (yet) available, valuable insights into many parasitic nematode genes have been gained from expressed sequence tag (EST) projects (Parkinson

et al., 2003). In this approach, clones from cDNA libraries are randomly picked and sequenced, usually as a single pass, to generate informative, if incomplete, information on the maximal number of genes from any particular organism. Thus far, for *T. canis*, over 4000 clones from several cDNAs have been sequenced from different cDNA libraries and grouped into 1466 clusters or presumed products of distinct genes, according to sequence identity (Parkinson *et al.*, 2004). The majority of these sequences are derived from adult worm cDNA, chosen for the purposes of phylogenetics and drug target discovery, and the complete set of clustered genes is available at the following URL: http://nema.cap.ed.ac.uk/nematodeESTs/pg_blast.php?organism=TCC. Within this dataset, 23% of identified *T. canis* genes are unique to the species (absent from other nematodes including other members of the Spirura). At the other extreme, 47% of gene sequences had recognizable similarity with known genes outside the Nematoda (Parkinson *et al.*, 2004).

At the initial phase of this EST survey, we focused on the larval stage, which we selected from the perspective of identifying genes potentially involved in parasite immune evasion. From a larval *T. canis* cDNA library, 129 new genes were identified from a total of 266, sequenced from both 5' and 3' ends. All of these are included in the totals given above; some of the more important gene families found are summarized in Table 1.1, and described in more detail below. Remarkably, just seven genes accounted for more than a quarter of the cDNA clones. Three of these seven, are secreted lectins and mucins, but the remaining four genes were completely novel, with no counterpart even in the free-living *C. elegans* (Tetteh *et al.*, 1999). The latter subset were named the abundant novel transcript (*ant*) genes, and are described in greater detail below.

***Toxocara* : a Model for the Study of Nematode Surface Coats**

Infective-stage *T. canis* larvae remain viable not only in paratenic hosts such as mice, but also *in vitro* in simple tissue culture medium (de Savigny, 1975). Indeed, they maintain a motile and metabolically active state in serum-free culture medium for many months, in presumably the

same arrested stage as may be found in the tissues of infected mammalian hosts. Thus, the infective stage *in vitro* is a steady-state model system reflecting the long-lived, viable form which causes human disease. Because larvae are hatched in the laboratory from isolated eggs, and cultivated in a defined, serum-free medium, *T. canis* also offers a molecular system devoid of possible host contaminants. The studies discussed below on the structure and dynamics of the larval surface can therefore be considered in the knowledge that the organisms are fully viable rather than senescent, and that all components are derived from within the parasite itself.

The first indications of the labile nature of the *T. canis* surface came from immunofluorescent assays of polyclonal antibody binding to live larval parasites, in which shedding of fluorescent aggregates was observed, unless parasites were held at 0°C in the presence of azide antimetabolite (Smith *et al.*, 1981). Typically, bound antibodies would be completely shed within 3 h at 37°C (Smith *et al.*, 1983). Subsequently, the attachment of eosinophils to the larval surface was observed to occur in the presence of specific anti-*Toxocara* antibodies, but if maintained at 37°C, adherent cells were sloughed off in a matter of hours leaving free, undamaged parasites (Fattah *et al.*, 1986; Badley *et al.*, 1987a).

The development of monoclonal antibodies (mAbs) to *T. canis* ES provided further tools for this analysis. In particular, mAbs to the TES-120 antigens gave strong immunofluorescent binding to the larval surface, if kept on ice, but fluorescence was shed at 37°C (Maizels *et al.*, 1987). Interestingly, newly hatched larvae were not bound by these antibodies. Evidence that the TES-120 antigens appear on the surface over the 48 h following hatching favoured the conclusion that they were not integral components of the larval cuticle.

The same anti-TES-120 mAbs were then applied to immuno-electron microscopy (EM), to explore the relationship of these antigens to the larval cuticle. Unexpectedly, using conventional protocols, no surface reactivity was observed. These protocols used step-wise ethanol dehydration as part of the gentle fixation process, before application of antibodies to thin sections of tissue. However, ethanol-dehydrated sections lacked a crucial feature seen with harsher stains such as osmium tetroxide, usually avoided for immuno-EM: a fuzzy coat 10–20 nm thick, lying approximately 10 nm

Table 1.1.

(A) Known excretory–secretory (ES) proteins from larval *Toxocara canis*

ES antigen	Gene	Full gene name	Protein size (aa)	Reference
TES-26	PEB-1	Phosphatidylethanol-amine-binding protein-1	263	Gems <i>et al.</i> (1995)
TES-32	CTL-1	C-type lectin-1	219	Loukas <i>et al.</i> (1999b)
TES-70	CTL-4	C-type lectin-4	288	Loukas <i>et al.</i> (2000a)
TES-120	MUC-1	Mucin-1	176	Gems and Maizels (1996)
	MUC-2	Mucin-2	182	Loukas <i>et al.</i> (2000b)
	MUC-3	Mucin-3	269	Loukas <i>et al.</i> (2000b)

(B) Other genes of interest expressed by larval *T. canis*

Gene	Full gene name	Protein size (aa)	Reference
ANT-3	Abundant novel transcript-3	272 and 490	Callister and Maizels (unpublished)
ANT-5	Abundant novel transcript-5	473	Callister and Maizels (unpublished)
ANT-30	Abundant novel transcript-30	844	Callister and Maizels (unpublished)
ANT-34	Abundant novel transcript-34	609	Callister and Maizels (unpublished)
AQP	Aquaporin	310	Loukas <i>et al.</i> (1999a)
CPL	Cathepsin (cysteine protease) L	216	Loukas <i>et al.</i> (1998)
CPZ	Cathepsin (cysteine protease) Z	307	Falcone <i>et al.</i> (2000)
MUC-4	Mucin-4	191	Loukas <i>et al.</i> (2000b)
MUC-5	Mucin-5	316	Doedens <i>et al.</i> (2001)
PRO	Prohibitin	274	Loukas and Maizels (1998)

outside the cuticle itself (Page *et al.*, 1992b). When specialized cryo-EM techniques were applied, which protect the sample and do not require ethanol dehydration, strong binding of mAbs was observed to the fuzzy surface coat. This was the first evidence that the ability of *T. canis* to shed antibodies and granulocytes bound to the surface was due to the sloughing of the surface coat.

Histochemical studies indicated that the surface coat was highly glycosylated, from staining with ruthenium red, and highly negatively charged, by binding to cationized ferritin. Sequence analysis of cloned TES-120 antigens then revealed them to be secretory mucins (Gems and Maizels, 1996; Loukas *et al.*, 2000b), from which we inferred that the surface coat was a negatively charged, mucin-rich, glycocalyx-like structure.

The *Toxocara* larva illustrates in detail many properties noted across a broad range of parasitic

nematodes. Many species are known to release surface antigens when complexed with host antibodies (Vetter and Klaver-Wesseling, 1978, Page *et al.*, 1992b), although the physical basis for this was not understood. The example of *T. canis* is likely to be paralleled in other parasitic nematode species for which ultrastructural evidence for a surface coat exists, such as *Brugia* and *Nippostrongylus* (Blaxter *et al.*, 1992), as well as, possibly, the free-living *C. elegans*.

***Toxocara* ES Antigens**

In culture, the larvae secrete a complex set of glycoproteins, which can be collected in some quantity and analysed biochemically. These secreted products have been named TES (*Toxocara* ES products), and individual proteins classified according to their

apparent molecular weight on SDS-PAGE (e.g. TES-32, TES-120, etc.), as shown in Fig. 1.1. A close relationship between TES molecules and surface antigens of larval parasites was evident from early molecular comparisons (Maizels *et al.*, 1984). The identity between the surface coat and ES mucins became more formally apparent with the development of monoclonal antibodies to TES-120 (Maizels *et al.*, 1987). The broad overlap between surface and secreted antigens allows us to study the function of molecules common to both compartments, using TES as a soluble form for experiments *in vitro* and *in vivo* in the absence of infection.

***Toxocara* as a Model for ES Antigen Investigations**

A central interest around the TES products is the hypothesis that they may directly modulate or divert sections of the immune system. It is known that both live *Toxocara* infection and the soluble TES glycoproteins elicit a highly polarized Th2 immune response in the mammalian host. For ex-

ample, cloned TES-specific human T cells are classical Th2 phenotype (Del Prete *et al.*, 1991), while in mice, infection with live larvae induces strong Th2-type responses (Kuroda *et al.*, 2001) and eosinophilia (Sugane and Oshima, 1980; Takamoto and Sugane, 1993), which can be reproduced by the injection of TES alone in the absence of live parasites (Sugane and Oshima, 1984).

The molecular nature of the TES products has been carefully scrutinized and the key features are well established (Sugane and Oshima, 1983; Maizels *et al.*, 1984, 1987; Sugane *et al.*, 1985; Meghji and Maizels, 1986; Badley *et al.*, 1987b; Maizels and Page, 1990; Khoo *et al.*, 1991; Page *et al.*, 1992a; Page and Maizels, 1992; Loukas *et al.*, 2000b). There are, compared with ES products of other species, relatively few distinct components: less than 50 spots on a two-dimensional gel, and approximately ten major molecules. Nearly all are glycosylated, some very heavily, resulting in 40% of TES by weight being carbohydrate (Meghji and Maizels, 1986). A fuller account is given below.

TES products have also played a crucial role in the practical diagnosis of human toxocariasis. The introduction by de Savigny of a simple ELISA

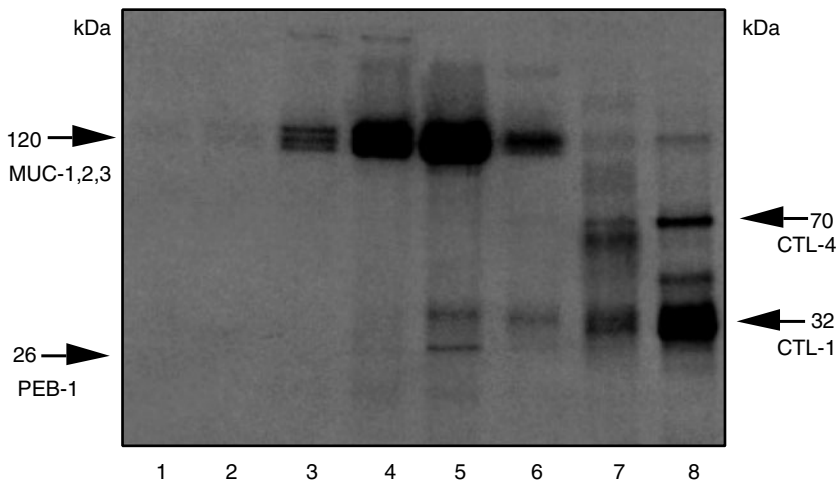


Fig. 1.1. Multiple mucins and lectins in the TES from *Toxocara canis* larvae cultured *in vitro*. The figure shows eight fractions of TES separated by strong anion exchange (Q-Sepharose) chromatography, and analysed by SDS-PAGE. Numbers represent successive elution fractions. Glycoproteins were labelled by ^{14}C carboxymethylation and visualized by autoradiography. (Figure reproduced from Loukas *et al.*, 2000b.) TES-120 contains three mucins: MUC-1 (the central band), -2 (upper band) and -3 (lower band). TES-70 is C-type lectin (CTL)-4, while TES-32 is CTL-1. A 26 kDa band corresponding to phosphatidylethanolamine-binding protein (PEB-1) is also shown.

antibody test using TES material (de Savigny *et al.*, 1979) provided a relatively sensitive and specific test, although it is not free of cross-reactions with other nematode species (Kennedy *et al.*, 1987, 1989; Page *et al.*, 1991; Smith, 1991). Because production of TES for diagnosis is limited to the capacity of parasite culture, a recombinant protein antigen is likely to offer significant benefits for future diagnosis (Yamasaki *et al.*, 2000).

Routes of Secretion: Glandular and Trans-cuticular

The classification of parasite-released antigens as 'excretory/secretory' reveals that their physiological origin is not known. If the ES products are functional modulators of the host, then it is likely that they are actively secreted to promote parasite survival. However, there may also be somatic constituents which leach out from live parasites in culture which may not necessarily be immunologically relevant. It is therefore important to analyse the origin of the major ES components and to ascertain whether the organism selectively secretes these macromolecules.

Nematodes have specialized secretory structures, often involving ducts and pores on the cuticular surface, as well as oesophageal glands, from which products can egress through the buccal opening (Wu *et al.*, 2004). However, few defined molecules have been identified which may be secreted by this route. At the same time, there has been interest in how surface molecules are exported to the cuticular surface, and whether secretory proteins can be released through a trans-cuticular route (Marshall and Howells, 1986; Selkirk *et al.*, 1993). The steady-state viability of *T. canis* larvae *in vitro*, coupled with specific monoclonal antibodies to TES products and a refined immuno-electron microscopy technique, allowed these questions to be analysed in detail (Page *et al.*, 1992a).

The mAbs to TES-120 were shown to bind strongly to two large secretory organs within the larva: the mid-body secretory cell and the anterior oesophageal gland (Page *et al.*, 1992a). Differential staining was observed, with Tcn-2 binding the former and Tcn-8 the latter. As mentioned above, these antibodies also bind to the fuzzy coat, but not the cuticle itself (Page *et al.*, 1992b).

Thus, it was suggested that the TES-120 (mucin) components are secreted physiologically through the buccal cavity and the secretory pore, and that a proportion of the secreted material remains loosely attached to the surface in the form of the fuzzy coat. The coat mucin may itself be shed or sloughed off in the event of immune attack.

In contrast, the mAb to TES-32 did not stain any internal structures, but was localized to the cuticle itself (Page *et al.*, 1992a). Because this product is relatively abundant in TES, we hypothesized that it is exported through the cuticle. If so, then both secretory gland-derived and surface-released ES products are present in the secretions of the *T. canis* larva.

Mucins

Mucins are major components of secretory (e.g. mucosal) surfaces of both vertebrate and invertebrate organisms (Theodoropoulos *et al.*, 2001). *T. canis* expresses a family of mucins which are closely associated with the surface coat or glycocalyx of the larval parasite (Gems and Maizels, 1996). They typically migrate around 120 kDa on SDS-PAGE and hence are collectively termed TES-120. Their mobility on SDS-PAGE does not accord well with their actual mass values of 40–45 kDa, due to extensive glycosylation (Loukas *et al.*, 2000b). To date, five distinct mucins have been isolated (Table 1.1). A combination of monoclonal antibody analysis and proteomics with native TES products has established that MUC-1 and MUC-3 are the most prominent, and are present together with MUC-2 in the TES-120 complex (Fig. 1.1). No evidence for MUC-4 secretion has yet been found, and the amino acid composition of MUC-5 suggests that it also is not one of the secreted mucins; hence there is some selectivity of expression and export within the mucin family.

Mucins typically contain separate segments, a glycosylated domain rich in serine/threonine (the acceptor residues for *O*-glycosylation), and a non-glycosylated cysteine-rich tract. *Toxocara* mucins are similarly constructed, but share a distinctive non-glycosylated structure with two or four repeats of a six-cysteine motif designated NC6 (Gems *et al.*, 1995) and also named SXC (Blaxter, 1998). No mucin from any other organ-

ism is associated with this motif, which currently has no known function.

The 36-amino acid SXC domain is, however, widely distributed in a range of genes from other nematode organisms: for example there are numerous copies within the *C. elegans* genome. Homologues also exist outwith the Nematoda, and the three-dimensional structure of an SXC from a sea anemone has allowed a homology model to be made of a *T. canis* MUC-1 SXC domain in which the three disulphide bonds can be located (Loukas *et al.*, 2000b).

A further characteristic of the SXC family is the preponderance of paired domains. For example, all five mucins have a pair of SXC domains at their C terminus, while both MUC-3 and MUC-5 also have paired N-terminal SXC domains. Paired SXC domains are also found in other *T. canis* proteins otherwise unrelated to the mucins, such as the TES-26 phosphatidylethanolamine-binding protein (Gems *et al.*, 1995) and a protein designated venom allergen/*Ancylostoma* secreted protein-like (VAL), which is similar in its non-SXC region to the ASP family of hookworm antigens (Maizels and Murray, unpublished).

The bipartite structure of the parasite mucins, containing a heavily glycosylated composite paired with a glycan-free globular domain, suggests that the immune system may react with either or both the carbohydrate epitopes of the mucin domain, and/or the cysteine-rich non-glycosylated domain. This former proposition is supported by data indicating human serological reactivity to synthetic glycans corresponding to those attached to the mucins (Shabussova *et al.*, unpublished). Significantly, it has also been demonstrated that human patients mount antibody responses to the non-glycosylated form of the TES-120 mucin expressed in recombinant bacteria (Yamasaki *et al.*, 2000), although it has not been established whether the anti-protein antibodies are reactive with the cysteine-rich domain or with part of the peptide backbone of the mucin domain.

Lectins

Over the past 5 years it has been established that at least two of the major TES glycoproteins are members of the C-type (calcium-dependent) lectin

family (Table 1.1). This was an intriguing discovery, as C-type lectins (CTLs) are known to be central players in pathogen recognition and cellular activation by the mammalian innate immune system (Dodd and Drickamer, 2001). Ancestral members of the CTL family are found in invertebrate organisms as diverse as poriferans (sponges) (Gundacker *et al.*, 2001), planarian worms (Shagin *et al.*, 2002), arthropods (Osta *et al.*, 2004) and ascidians (sea squirts) (Sekine *et al.*, 2001). Thus, it is to be expected that CTLs are represented in nematode genomes, and more than 100 related genes have been identified in *C. elegans* (Drickamer and Dodd, 1999). Functionally, *C. elegans* CTLs may act against infectious agents, as bacterial infection of this nematode stimulates production of CTL proteins (Mallo *et al.*, 2002).

Host lectins bind pathogen carbohydrates at the initial point of infection, and are subsequently involved in host–host cross-talk in the induction of inflammation. In the latter context, lymphocyte surface membrane lectins such as E-selectin recognize carbohydrate ‘danger’ signals expressed on endothelial cells as the first step of extravasation and tissue infiltration (Varki, 1994). By secreting soluble CTLs, parasites may compete with host defence molecules for carbohydrate binding sites and thereby impede the inflammatory process. If so, perhaps *Toxocara* has turned the tables and employs CTL homologues as ‘offence’ molecules which are secreted to promote pathogen survival!

TES-32 (CTL-1) is one of the most prominent larval proteins, present not only as a major TES constituent (Maizels *et al.*, 1984), but also in the cuticular matrix of the larva (Page *et al.*, 1992a). The 219-amino acid sequence shows a carbohydrate-recognition domain with similarity to mammalian CTLs, and recombinant TES-32 has been shown to bind to monosaccharides (mannose or galactose) in a calcium-dependent manner (Loukas *et al.*, 1999b). In addition to CTL-1, two related variants (CTL-2 and -3, with 83–87% amino acid identity) have been identified from ESTs. Currently, it is not known if these variant lectins are secreted, although the SDS-PAGE profile of TES in the region of TES-32 certainly suggests that additional proteins are present (Fig. 1.1).

A fourth lectin, CTL-4, corresponds to the 70-kDa protein also known as TES-70 (Fig. 1.1). This is produced in smaller quantities than TES-32, and is a larger protein due to the duplication

of the non-CRD segment. The specificity of this lectin has not yet been determined, but it is known to bind mammalian cells (Madin–Darby canine kidney cells) (Loukas *et al.*, 2000a). In current studies, TES-70 has also been shown to activate murine dendritic cells in a manner which strongly implies a surface-bound ligand on cells of this type (Schabussova, Nicoll and Maizels, unpublished).

The preponderance of active lectins, and the likelihood of additional lectins being present in the ‘secretome’ of *T. canis*, highlights the critical role of glyco-recognition in the immune response to this parasite. Interestingly, *T. canis* releases not only carbohydrate-binding lectins, but also abundant carbohydrates as discussed below.

Other TES Products

At least 50 distinct molecules can be identified by two-dimensional SDS-PAGE analysis of TES, including many interesting proteins in addition to the mucins and lectins. Major products include TES-26/PEB-1, a functional phosphatidylethanolamine-binding protein with homology to a similar mammalian testis protein (Gems *et al.*, 1995); TES-45 and TES-55; TES-400, a proteoglycan-like material which is predominantly carbohydrate; and a number of active enzyme molecules which have yet to be assigned to specific genes: these include superoxide dismutase (Matzilevich *et al.*, unpublished); acetylcholinesterase (Robertson *et al.*, 1987); and a metalloprotease (Robertson *et al.*, 1989).

Abundant Novel Transcripts

EST analysis identified four novel and relatively large (>1.6 kb) genes, which together represented 18% of all cDNAs recovered from the larval stage (Tetteh *et al.*, 1999). These show no sequence similarity to any existing protein, and no resemblance to each other except, surprisingly, in the 5' and 3' untranslated regions (UTRs) in which significant tracts show conservation (Callister and Maizels, unpublished). Conserved non-coding regions are likely to represent control elements such as transcriptional enhancers; in addition, 3' UTR motifs have often been associated with

post-transcriptional control such as mRNA localization and suppression of translation (Kuersten and Goodwin, 2003). The fact that the four *ant* genes encode unrelated products yet may share control elements suggests that the parasite requires co-ordination of expression of these genes of as yet unknown function.

Each gene contains a sizeable open reading frame (Table 1.1), although only *ant-3* includes a consensus signal sequence at the N terminus which would indicate a secretory fate for the protein. Surprisingly, one of the genes (*ant-3*) appears to encode a bicistronic transcript, with two separate open reading frames (ORFs). Such bicistronic gene organization is highly unusual among eukaryotic organisms, although not entirely unprecedented (Gray *et al.*, 1999). However, we have been unable to confirm that *ant-3* encodes two distinct proteins, as in no case were proteins corresponding to those predicted from the *ant* gene sequences found in TES or larval extract when probed with antibodies to recombinantly expressed antigens.

It therefore seems likely that the common UTR elements contained within these transcripts act to suppress protein translation in the arrested larval stage. To test this proposition, we transfected *C. elegans* with a green fluorescent protein (GFP) reporter construct containing the conserved *ant* 3' UTR, and found suppression of GFP translation (Callister, Page and Maizels, unpublished). Our interpretation of these data is that the *ant* transcripts may be necessary for survival *in vivo*, and are only translated on activation of larvae under conditions which cannot be reproduced *in vitro*. If so, this would be the first confirmation that parasites studied *in vitro* do not fully represent the form which exists in either the paratenic or the definitive host.

Glycans: *Toxocara* as a Model for Glycobiology

The TES products are heavily glycosylated, with around 400 µg of carbohydrate per mg of protein (Meghji and Maizels, 1986), with a high proportion of galactose and *N*-acetylgalactosamine attached to proteins through *O*-linkages. The predominant carriers of *O*-linked glycans in TES are the mucins (Loukas *et al.*, 2000b). Mass spectrometric analysis identified two major *O*-linked trisaccharide structures, both containing fucoses,

galactose, and *N*-acetylgalactosamine (Khoo *et al.*, 1991, 1993). Unusual methylation is found on the fucose and galactose sugars, although the latter modification occurs in only 50% of the molecules. Trisaccharides containing a single methylation site on fucose were found to be a *T. canis*-specific structure, as in *T. cati* the corresponding carbohydrate is 100% methylated on galactose.

The *O*-methylations represent the only difference between *T. canis* trisaccharides and the mammalian blood group antigen H (or type O). There is also some evidence for minor unmodified blood group-like oligosaccharides (Khoo *et al.*, 1993), and we have found infection of mice with *T. canis* generates antibodies not only to the methylated sugars but also to unmodified blood group H. Whether this represents a form of molecular mimicry, or whether the methyl modifications interfere with normal carbohydrate recognition in the host, remains to be determined.

N-linked oligosaccharides are found on lower molecular weight TES proteins, including TES-26, TES-32 and TES-70. The carbohydrates have been determined as biantennary trimannosylated structures linked to a chitobiose-asparagine core (Khoo *et al.*, 1993); these are similar to glycans in other invertebrate organisms such as insects.

The methylated oligosaccharides of *T. canis* have now been chemically synthesized (Amer *et al.*, 2003). We have found that these are strongly recognized by antibodies from human and animal *Toxocara* infection, including infection with *T. cati* and *T. vitulorum* (Schabussova, Kosma and Maizels, unpublished). Moreover, two monoclonal antibodies which were previously shown to react with periodate-sensitive epitopes on the TES mucins (Tcn-2 and Tcn-8; Maizels *et al.*, 1987) recognize the synthetic oligosaccharides but not blood group H. While the methylated glycans are therefore immunogenic in natural infection, it is notable that antibodies are restricted to the IgM isotype, indicating no T-cell involvement in the response (Schabussova, Kosma and Maizels, unpublished).

Immune Responses and Immunopathology

The infected host mounts a vigorous antibody response to *T. canis* which can readily be detected against TES antigens, hence the use of the latter

in immunodiagnostic tests (see Chapter 7, this volume). Strong T-cell reactivity can also be detected in infected mice, and is clearly of the Th2 type (Kuroda *et al.*, 2001). Consistent with this, peripheral human lymphocytes from a normal donor who demonstrated TES reactivity were used to isolate T-cell clones which were of a typical Th2 phenotype (Del Prete *et al.*, 1991). Overall, the co-existence of *T. canis* infection and Th2 responses indicates that the parasite is adept at evading this mode of immunity (Grieve *et al.*, 1993), as discussed further below.

There is also considerable interest in how *Toxocara* infections may initiate or modulate other immunopathological reactions, in particular asthma. A good number of epidemiological reports have associated positive *Toxocara* serology with diagnosis of asthma (Taylor *et al.*, 1988; Buijs *et al.*, 1994, 1997; Lokman Hakim *et al.*, 1997; Chan *et al.*, 2001). Coupled with findings that *T. canis* infection of mice generates a pro-allergic histological response (Pinelli *et al.*, 2001), it is plausible that the parasite may exacerbate airway hyper-responsiveness in susceptible individuals (see Chapter 4, this volume).

In this respect, *Toxocara* appears to act in a manner distinct from the classical, high-intensity nematode infections such as onchocerciasis and lymphatic filariasis in which host responsiveness is often down-regulated and a large proportion of those infected may be long-term asymptomatic carriers (Maizels and Yazdanbakhsh, 2003). Perhaps, as a result of the extraordinary resilience of *T. canis* larvae to immune attack, this species has not developed a strong measure of immune suppression. Consequently, it can be suggested that in the presence of an acute or recent *T. canis* infection, reactivity to bystander antigens (such as aeroallergens) could be amplified, rather than down-regulated, as appears to be the case for many other helminths (Yazdanbakhsh *et al.*, 2001).

Immune Responses: Vaccination and Immunity

Vaccination against toxocariasis may be appropriate for use in dogs, rather than in the human population, in whom the incidence of disease is low. In particular, an anti-larval vaccine would fill the gap left by current anthelmintics, which clear

intestinal worms but do not kill tissue larvae. Surviving larvae are able to migrate across the placenta to infect unborn pups, and vaccination may offer the only means of interrupting transmission and ensuring parasite-free litters are born (see Chapter 12, this volume).

Despite the potential for disease control, very little has been done to identify potential vaccine antigens for *T. canis*. A few investigators have tested either TES antigens (Nicholas *et al.*, 1984; Sugane *et al.*, 1996), or crude larval extracts (Barriga, 1988) and found partial protection in mouse models. One group reported that ultraviolet-irradiated eggs provided a protective effect (Abo-Shehadeh *et al.*, 1991). No reports have yet been published on the use of the new generation of recombinant antigens or defined glycans in stimulating protective immunity against the larval stage.

We are not, however, close to a clear vision of how immunity operates against the tissue stages of *T. canis*, although presumably it is very different from that observed in gastrointestinal nematodes (Finkelman *et al.*, 1997). Eosinophils are prominent in the response to *T. canis* (Beaver *et al.*, 1952; Sugane and Oshima, 1980; Glickman and Summers, 1983; Kayes *et al.*, 1985), and detection of eosinophil cationic protein (ECP) may therefore be a useful diagnostic tool (Magnaval *et al.*, 2001). As few as five larvae can stimulate an eosinophilic response (Kayes *et al.*, 1985), which is elicited even in CD4-deficient mice (Takamoto *et al.*, 1998), confirming earlier observations that interleukin (IL)-5 is produced by both CD4⁺ T cells and non-T-cell populations during infection (Takamoto *et al.*, 1995). The eosinophil is not, however, associated with immunity – even IL-5-overexpressing, hyper-eosinophilic mice are not resistant to *T. canis* infection (Sugane *et al.*, 1996; Dent *et al.*, 1999), and the efficacy of TES vaccination is equal whether vaccinated mice are wildtype or IL-5 overexpressing (Sugane *et al.*, 1996). Moreover, IL-5-deficient mice (Takamoto *et al.*, 1997), and anti-IL-5-treated animals (Parsons *et al.*, 1993), show equal susceptibility

to *T. canis* infection as wild-type controls, although the latter display a greater degree of tissue pathology. This body of evidence that eosinophils cannot kill *T. canis* larvae stands in contrast to the strong link between eosinophils and nematode larval killing (Behm and Ovington, 2000; Meeusen and Balic, 2000; Maizels and Balic, 2004), and quite possibly reflects the ability of larvae to slough their surface coat when under attack from eosinophils (Fattah *et al.*, 1986; Badley *et al.*, 1987a).

Conclusion

In many molecular and immunological respects, *Toxocara* is truly the 'enigmatic parasite'. The larvae defy the onslaught of the immune system with an unusual physiological strategy involving a labile surface coat, but one suspects that this is only the beginning of the story of toxocaral immune evasion. Surely, the conversion of natural defence proteins, the lectins, into active products aimed at frustrating host immunity must play a part in their long-term survival. The developmental aspects of the life cycle combine some exceptional features of hypobiosis and responsiveness to host cues, few of which we are near to understanding. Finally, the molecular details become more fascinating, and more unusual, as further details emerge of gene structure, evolution and control, all properties which underwrite the remarkable biology of *Toxocara*.

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2 Molecular Genetic Characterization of Members of the Genus *Toxocara* – Taxonomic, Population Genetic and Epidemiological Considerations

Robin B. Gasser,¹ Xing-Quan Zhu,² Min Hu,¹ Dennis E. Jacobs³
and Neil B. Chilton⁴

¹*Department of Veterinary Science, The University of Melbourne,
Werribee, Victoria, Australia*

²*Laboratory of Parasitology, College of Veterinary Medicine, South China Agricultural
University, Guangzhou, Guangdong Province, People's Republic of China*

³*Department of Pathology and Infectious Diseases, The Royal Veterinary College,
University of London, Hatfield, UK*

⁴*Department of Biology, University of Saskatchewan, Saskatoon, Canada*

Summary

The molecular genetic characterization of species of ascaridid nematodes, irrespective of their developmental stage, has important implications for diagnosis and for detailed studies of their life cycles, systematics (i.e. taxonomy and evolutionary relationships) and epidemiology. This applies particularly to species within the genus *Toxocara*, some of which are transmissible to humans, where they can cause serious clinical diseases, such as ocular larva migrans (OLM) and visceral larva migrans (VLM). Traditional parasitological approaches can have significant limitations with regard to the specific identification of larvae and other developmental stages of *Toxocara*. This chapter gives an account of molecular methods for the specific identification of species of *Toxocara* and their differentiation from other ascaridids with zoonotic potential, for the diagnosis of infections and for the analysis of genetic variation within and among individual nematodes

and their populations. Prospects for the use of these tools for investigating epidemiological and population genetic questions are discussed.

Introduction

Parasitic nematodes of the superfamily Ascaridoidea infect all major groups of vertebrates (Hartwich, 1974; Sprent, 1983, 1992; Fagerholm, 1991), and some genera and species are of major socio-economic significance (e.g. Cheng, 1982; Bernardo and Dohoo, 1988; Crompton, 1988; Stewart and Hale, 1988; Kazacos and Boyce, 1989; Dick *et al.*, 1991). A particularly important genus is *Toxocara*, because some species are transmissible from animals to humans (i.e. are zoonotic) and can cause significant clinical disease (e.g. Magnaval *et al.*, 2001; Pawlowski, 2001; Despommier, 2003; Fisher, 2003). For instance, the larvae of *Toxocara canis* are capable of invading human tissues and causing OLM, VLM, eosinophilic meningoencephalitis

and/or covert toxocariasis (CT) (e.g. Beaver, 1969; Cypess, 1982; Schantz, 1991; Taylor, 1993; Vidal *et al.*, 2003) (see Chapters 8 and 9, this volume). Larvae of *Toxocara cati*, *Toxocara vitulorum* and *Toxascaris leonina* can invade the tissues of laboratory animals, but there has been some uncertainty as to what extent they are implicated in human disease (Smith, 1993; Miyazaki, 1994; Thompson, 1994) (see Chapter 6, this volume). None the less, a recent review provides evidence that *T. cati* represents an underestimated zoonotic agent (Fisher, 2003). It is also important to appreciate that a number of other ascaridids can invade human tissues (and cause disease) and that they can be difficult or impossible to differentiate from species of *Toxocara* using morphological criteria, particularly given their small size. For example, the larvae of *Baylisascaris procyonis* (an ascaridid of the racoon) can cause OLM, VLM and cerebrospinal nematodosis in humans (Kazacos and Boyce, 1989; Smyth, 1995) (see Chapter 15, this volume). Larvae of some anisakid species from fish can also infect humans and provoke acute abdominal distress (e.g. van Thiel *et al.*, 1960; van Thiel, 1962; Smith and Wootton, 1978; Cheng, 1982; Dick *et al.*, 1991; Yagi *et al.*, 1996). Moreover, *Ascaris* is of public health importance in many countries (e.g. Crompton, 1988; Peng *et al.*, 1998, 2003). While *Ascaris* undergoes hepato-pulmonary migration in humans, it is not usually considered an aetiological agent for human VLM. However, there are some suggestions that *Ascaris suum* can cause VLM in humans in Japan (e.g. Maruyama *et al.*, 1996; Sakakibara *et al.*, 2002).

Based on this information, the precise identification of species of *Toxocara* and their differentiation from other ascaridids has important implications for investigating their life cycles, epidemiology and population biology, and for the specific diagnosis of toxocariasis. Traditionally, ascaridid species have been identified based on morphological features and their predilection site in a particular host species (Bowman, 1999). Sprent (1983) assessed the taxonomic relevance of features, such as the labial structures, oesophagus, intestinal caecum, excretory system and male tail. There are various other reports describing diagnostic features for specific identification (Nichols, 1956; Olson *et al.*, 1983; Bowman, 1987; Averbeck *et al.*, 1995). For example, Nichols (1956) described the diagnostic morphology of *T. canis* and *T. cati* larvae, and Averbeck *et al.* (1995) described criteria for the differentiation of *T. canis* from *T. leonina*

and *B. procyonis* based on the morphology of the egg and adult stages. Despite existing descriptions and keys, there can be considerable limitations in the identification of some ascaridid stages to species.

Approaches such as karyotyping and electrophoretic techniques have been used to assist in addressing diagnostic and taxonomic problems within the Ascaridoidea (e.g. Kurimoto, 1974; Mutafova, 1983; He *et al.*, 1986; Kennedy *et al.*, 1987). Multilocus enzyme electrophoresis (MEE) has been applied widely to the specific identification of ascaridids and for studying their systematics and genetics (Nascetti *et al.*, 1979; Paggi *et al.*, 1985; Orecchia *et al.*, 1986; Nadler, 1987, 1990; Andrews and Chilton, 1999). For example, using this technique, genetically distinct but morphologically similar ('hidden' or 'cryptic') species have been detected within *Parascaris equorum*, *Anisakis simplex*, *Contracaecum osculatum* and *Pseudoterranova decipiens* (see Biocca *et al.*, 1978; Bullini *et al.*, 1978; Paggi *et al.*, 1985, 1991; Nascetti *et al.*, 1986, 1993; Orecchia *et al.*, 1986, 1994; Mattiucci *et al.*, 1997). Although very useful, MEE relies on the use of relatively large amounts of fresh parasite extract and cannot be used for the analysis of tiny, individual larvae, such as those of *Toxocara* from human tissues.

DNA approaches, such as restriction fragment length polymorphism (RFLP) analysis combined with DNA hybridization, have been applied, for example, to the differentiation of *T. canis* from *T. cati* (see Turcekova and Dubinsky, 1996), and to delineate between the larvae of *Anisakis* and *Contracaecum* species (Sugane *et al.*, 1989). Polymerase chain reaction (PCR) (Saiki *et al.*, 1988) techniques are now widely used because of their ability to specifically amplify DNA from minute (pg to fg) amounts of parasite material (e.g. single nematode eggs and tiny sections of worms) (reviewed by Gasser, 1999). For example, the technique of random amplification of polymorphic DNA (RAPD) (Welsh and McClelland, 1990; Williams *et al.*, 1990) has been used for the genetic characterization of ascaridids, including *Toxocara* (see Nadler, 1996; Siles *et al.*, 1997; Wu *et al.*, 1997; Epe *et al.*, 1999). However, RAPD can have some limitations, such as reduced reproducibility, artefacts and/or non-specific amplification, due to the low-stringency conditions usually employed in the PCR and the random nature of the primers used (e.g. Ellsworth *et al.*, 1993; MacPherson *et al.*, 1993).

This chapter provides an account of PCR-based approaches, employing genetic markers in the nuclear ribosomal DNA, for the specific identification of species of *Toxocara* and their differentiation from other ascaridid species with zoonotic potential, for the diagnosis of infections and for the analysis of genetic variation within and among individual nematodes and among populations. It also describes the prospects of using genetic markers in the mitochondrial genome and genetic fingerprinting approaches for studying the systematics and population genetics of *Toxocara*.

Specific Identification of Ascaridids Using Ribosomal DNA Markers

Central to the PCR-based identification of parasites to species is the choice of the appropriate DNA target region (genetic marker). As different genes evolve at different rates, the DNA region chosen should contain sufficient sequence variation to allow the identification of parasites at the taxonomic level needed. For specific identification, the target DNA should differ enough in sequence to allow the differentiation of species, with minor or no variation within a species. In contrast, for the purpose of identifying 'strains' (i.e. genetic variants), a considerable degree of sequence variation should exist within a species. Various target regions, including repetitive DNA, mitochondrial DNA (mtDNA) and nuclear ribosomal DNA (rDNA), have been employed to achieve the identification of parasites to species or strains (reviewed by Gasser, 1999).

In the 1990s, studies demonstrated that the first (ITS-1) and second (ITS-2) internal transcribed spacers (ITS) of rDNA provide reliable genetic markers for the identification of a wide range of bursate nematodes (order Strongylida) to species (reviewed by Gasser, 1999). Intraspecific variation in the ITS-1 and ITS-2 was usually reported to be low. Only in some species was sequence and/or length heterogeneity present in these spacer regions, which was interpreted to reflect population variation. Importantly, the ITS-1 and ITS-2 sequences have also been shown to provide reliable genetic markers for species of *Toxocara* and a range of other ascaridids (e.g. Jacobs *et al.*, 1997; Zhu *et al.*, 1998a,b, 1999, 2000a,b, 2001a,b, 2002; Peng *et al.*, 2003).

Jacobs *et al.* (1997) first demonstrated that morphologically well-defined adults of *T. canis*, *T. cati* and *T. leonina* could be distinguished by their different ITS-2 sequences. The sequence differences (~26–50%) between the species were substantially greater than the variation (0–0.6%) within each species, showing that ITS-2 provides species-specific genetic markers. These authors established two PCR assays for the differentiation of *T. canis* and *T. cati* from one another and from other ascaridids which can occur in human tissues. The specificity of the PCR-based RFLP and the direct PCR (employing species-specific primers in combination with a conserved primer) was assessed using DNA from *Ascaris* of human or pig origin, *B. procyonis* and *T. vitulorum*, which are potentially zoonotic (Kazacos and Boyce, 1989; Miyazaki, 1994; Thompson, 1994). The PCR-RFLP could be used to delineate the three canid and feline ascaridids by amplification of the ITS-2 and use of the restriction endonuclease *Hinf*I or *Rsa*I. Digestion with *Hinf*I differentiated *T. canis*, *T. cati* and *T. vitulorum* from other ascaridids which might be found in human tissues. The ITS-2 of the three *Toxocara* spp. remains undigested with this enzyme. Endonuclease *Rsa*I was used to distinguish between *T. canis* and *T. cati*, displaying a diagnostic banding pattern for each. The fragment sizes detected on the agarose gels were in accordance with predicted values in all cases except for *B. procyonis* where a proportion of the ITS-2 amplicon remained undigested with *Hinf*I (which was likely to be due to sequence heterogeneity within an individual worm). Host DNA was not amplified by PCR. Thus, diagnostic PCR could be performed on homogenized tissue, thereby potentially circumventing the need for localizing larvae by means of serial histological sectioning. Such an approach could be readily evaluated, for example, in the *T. canis*/mouse or *T. canis*/pig model systems (see Chapters 5 and 6, this volume). Both PCR-based assays produced consistent results when tested against a panel of homologous DNA samples from different hosts and/or different geographical regions (e.g. *T. canis* samples originated from British dogs and Australian foxes). This finding was consistent with results of a study from Germany (Epe *et al.*, 1999), showing that *T. canis* from the red fox possesses the same ITS-2 sequence, RAPD patterns and similar biological characteristics compared with those from dogs from the same locality.

Mutation Scanning for Specific Identification and Detection of Population Variation

In order to enable the accurate analysis of sequence heterogeneity in the ITS-2 amplicons within and among individual ascaridids, Zhu and Gasser (1998) evaluated the use of single-strand conformation polymorphism (SSCP) approaches. The principle of SSCP is that the electrophoretic mobility of a single-stranded DNA molecule in a non-denaturing gel is highly dependent on its size and structure (Orita *et al.*, 1989; Hayashi, 1991) and permits the high-resolution analysis of genetic variation within and between individual nematodes (reviewed by Gasser, 1997; Gasser and Chilton, 2001; Gasser *et al.*, 2002). The SSCP analysis of ITS-2 amplicons allowed *T. canis*, *T. cati* and *T. vitulorum* as well as *T. leonina*, *B. procyonis*, *A. suum* and *P. equorum* to be identified to species by their single-strand profiles (Zhu and Gasser, 1998). While no variation in such banding profiles was detected between individual nematodes within some species, four distinct sequence variants of ITS-2 could be displayed among individual *T. cati* from Australia, thus revealing genetic variation within this species (Zhu and Gasser, 1998). This finding was reinforced by results showing that the SSCP was able to detect a single point mutation in ITS-2 (amplicon size: ~530 bp) between *T. cati* samples from Malaysia and Australia (Zhu *et al.*, 1998a). Based on these and other results (reviewed in Gasser and Zhu, 1999), SSCP analysis of ITS-2 amplicons provided a powerful molecular tool for taxonomic and population genetic investigations.

Molecular Evidence for Cryptic Species of *Toxocara*

Interestingly, two reports from Malaysia (Rohde, 1962; Lee *et al.*, 1993) described the occurrence of a parasite of cats, which was identified as *T. canis* based on the presence of an oesophageal ventriculus and spear-shaped cervical alae in the adult. This parasite differed from *T. cati*, which possesses arrow-shaped cervical alae, and from *T. leonina*, which lacks a ventriculus (see Sprent, 1956, 1958, 1959; Sprent and Barrett, 1964). As *T. canis* has

been found only rarely in cats elsewhere (Calero, 1951; Hitchcock, 1953; Ash, 1962; Sprent and Barrett, 1964; Parsons, 1987; Baker *et al.*, 1989), the question arose as to the specific identity of this parasite from Malaysian cats and its genetic make-up.

An investigation was undertaken to genetically characterize specimens of this parasite, designated *Toxocara* sp. cf. *canis* (see Zhu *et al.*, 1998a). Comparison of ITS-1 and ITS-2 sequences showed that *Toxocara* sp. cf. *canis* differed from *T. canis* and *T. cati* by 9.4–15.7 and 11–26.1%, respectively. Also, the magnitude of sequence difference between *T. canis* and *T. cati* in both ITS-1 (9.4–11.3%) and ITS-2 (26.1%) was much greater than the variation detected within each species (0–2.9% for ITS-1 and 0–0.3% for ITS-2) using individual worms from disparate areas of the world (i.e. the UK and Australia for *T. canis*; Australia and Malaysia for *T. cati*). The 14 nucleotide differences in the ITS-1 and the substitution in the ITS-2 detected between *T. cati* specimens from Malaysia and Australia were interpreted as representing population variation. The higher degree of within-species variation in the ITS-1 sequence compared with the ITS-2 suggests that the latter sequence may provide more reliable species markers for ascaridid nematodes, although this did not imply that different species will not differ in their ITS-1 sequences.

Toxocara sp. cf. *canis* differed in both ITS sequences from both *T. canis* and *T. cati* by 11–24.2%. The magnitude of difference in the ITS-1 was greater than that between *T. canis* and *T. cati*. The difference in the ITS-2 sequence between *Toxocara* sp. cf. *canis* and *T. canis* was similar to that between *T. canis* and *T. cati*, and the difference in the ITS-2 sequence between *Toxocara* sp. cf. *canis* and *T. cati* from Malaysian cats was substantially greater than the geographical variation detected in the ITS-2 within *T. cati* (0.3%) and *T. canis* (0%). Thus, based on the molecular evidence presented, *Toxocara* sp. cf. *canis* from Malaysian cats was considered to represent a separate species to *T. canis* and *T. cati*. It was genetically more similar to *T. cati* than to *T. canis*. Based on the sequence difference, the SSCP could be employed effectively for the genetic differentiation of *Toxocara* sp. cf. *canis* from both *T. canis* and *T. cati*. The conclusion based on molecular analyses was supported by a subsequent morphological study of ascaridids from Malaysia

(Gibbons *et al.*, 2001), which included specimens from cats used for molecular study. Three features (for lips, alae and spicules) were identified which consistently differentiated *Toxocara* sp. cf. *canis* from *T. canis*, *T. cati* and other members of the genus, such as *T. tanuki* (from canids), *T. apodemi* and *T. mackerrasae* (from rodents), *T. paradoxura* and *T. sprengi* (from viverrids), *T. vajrasthira* (from mustelids) and *T. pteropodis* (from bats). Hence, the findings from the molecular and classical systematic studies supported the conclusion that *Toxocara* sp. cf. *canis* represented a distinct species. The parasite was described and named *Toxocara malaysiensis* (Gibbons *et al.*, 2001).

T. malaysiensis could have zoonotic potential. Since *T. canis* can cause OLM and/or VLM, it is suspected that other members of the genus, such as *T. cati* and *T. vitulorum*, may be involved in human disease, although it remains uncertain to what extent (Smith, 1993; Miyazaki, 1994; Fisher, 2003) (see Chapter 18, this volume). A survey of cats in Kuala Lumpur (Lee *et al.*, 1993) revealed a prevalence of 11% for *T. malaysiensis*. Thus, there is a significant opportunity for this parasite to infect humans. This possibility requires investigation, including studies of the infectivity of mice and pigs with *T. malaysiensis* eggs and epidemiological surveys using the molecular tools established (in particular SSCP analysis of ITS-2, combined with DNA sequencing). Demonstrating the existence of a cryptic species of *Toxocara* in cats in Malaysia raises some doubt as to the specific identity of ascaridids considered to represent *T. canis* from cats in other geographical regions, including South Africa, Panama, the USA and Czech Republic (cf. Calero *et al.*, 1951; Hitchcock, 1953; Ash, 1962; Sprent and Barrett, 1964; Parsons, 1987; Baker *et al.*, 1989; Scholz *et al.*, 2003), which provides a stimulus to genetically characterize and compare a wide range of *Toxocara* species from different hosts and geographical origins of the world.

The Potential of Mitochondrial (mt) Gene Markers

In addition to rDNA markers, there is significant potential for the use of mtDNA markers for investigating the taxonomy and population genetics of *Toxocara*, given that cryptic species (such as

T. malaysiensis) have been detected within the Ascaridoidea (e.g. Biocca *et al.*, 1978; Bullini *et al.*, 1978; Paggi *et al.*, 1985, 1991; Nascetti *et al.*, 1986, 1993; Orecchia *et al.*, 1986, 1994; Mattiucci *et al.*, 1997; Zhu *et al.*, 1998a,b, 1999; 2000a,b, 2001a). Mt DNA is maternally inherited and is more variable in sequence within a species than nuclear ITS sequences because it evolves (mutates) at a higher rate (Brown, 1985). A range of different mtDNA regions has been employed for studying the population genetics of parasitic nematodes (e.g. Anderson *et al.*, 1993, 1998; Blouin *et al.*, 1995, 1997; Viney, 1998; Blouin, 2002; Hu *et al.*, 2002b, 2004). However, surprisingly, relatively little is known about the mt genomes of parasitic nematodes (also as a source of genetic markers for population genetic studies) (Hu *et al.*, 2004), which appears to have related mainly to technical limitations and the cost associated with mt genome sequencing. Hence, to overcome such constraints, Hu *et al.* (2002a) established a simple and effective long PCR approach for the amplification and subsequent sequencing of the mt genome (in two overlapping fragments of ~5–10 kb each) from small amounts of total genomic DNA purified from single nematodes representing 13 species from three orders of secernentean nematodes, including the ascaridids *A. suum*, *P. equorum* and *C. osculatum*. Given the range of species tested, this approach was considered to be applicable to a relatively wide range of nematodes. Indeed, preliminary evidence (Zhu *et al.*, unpublished results) indicates that this method is also applicable to *Toxocara* species. The availability of the first complete mitochondrial genome sequences for a species of *Toxocara* could thus provide a foundation for addressing population genetic, ecological and epidemiological questions. Conserved primers can be rationally and selectively designed to regions of the mt genome considered to be most informative (based on mt genome sequencing of a small number of individuals representing particular populations, or genetic variants detected using ITS-1 and/or ITS-2). Using such primers, SSCP analysis can be applied to pre-screen large numbers of individuals representing different populations and based on the 'pre-screen', samples representing the entire spectrum of haplotypic variability can be selected for subsequent sequencing and analyses.

Hu *et al.* (2003) applied this approach to hookworms (order Strongylida: Ancylostomatidae). Based on studies of the ITS-1 and/or ITS-2

(cf. Gasser *et al.*, 1998; Romstad *et al.*, 1998), it was proposed that *Necator americanus*, a hookworm of humans, represents a complex of at least two species. Hu *et al.* (2002b, 2003) examined this question further by mt genome sequencing and SSCP analyses of mt gene markers. The mt genome determined from an individual of *N. americanus* from Togo was compared with another from China to estimate the magnitude of genetic variability between them (Hu *et al.*, 2003). The comparative analysis revealed sequence differences of 3–7 and 1–7% (at the nucleotide and amino acid levels, respectively) in the 12 protein-coding genes, with *nad4L* being the most conserved gene and the *nad1* being the least conserved. Nucleotide differences were also detected in 14 of the 22 *tm* genes (~1–13%), the AT-rich region (~8%), the non-coding regions (8–25%) and in the two small (*rmS*) and large (*rmL*) subunits of mt *rn* genes (~1%). Sequence comparison of the *rmL* (a long and conserved) gene from multiple individuals revealed nine unequivocal differences among all of the specimens of *N. americanus* from Togo and those from China. This study thus provided evidence for substantial genetic variation in *N. americanus* and supported clearly the ‘cryptic species hypothesis’ (cf. Romstad *et al.*, 1998), having implications for transmission and control in that each different gene pool of *N. americanus* may possess different biological and ecological characteristics. The investigations by Hu *et al.* (2002b,c, 2003) reinforced the usefulness of mt genome and gene data sets for addressing systematic and population genetic questions in the Nematoda. They suggest that this approach (combined with mutation scanning and sequence analyses of selected mt genes) is readily applicable to species of *Toxocara*. This is particularly relevant given that population variation and cryptic species have been detected within *Toxocara* (Zhu and Gasser, 1998; Zhu *et al.*, 1998a; Gibbons *et al.*, 2001).

Genetic ‘Fingerprinting’ Approaches for Investigating Genetic Diversity Within Species and Population Structures

In addition to the PCR-based analysis of particular loci from the mt or nuclear genome, ‘fingerprinting’ methods (cf. Monis *et al.*, 2002) are frequently applied to estimate genetic diversity

within a species, to detect genetic variants (‘strains’ or cryptic species) and/or to establish the genetic structures of populations. Such methods include RAPD, amplified fragment length polymorphism (AFLP) (Vos *et al.*, 1995) and microsatellite analysis.

RAPD is based on the screening of entire genomes (usually using single, short primers), without the need for any prior DNA sequence information. Although there has been some reservation about the reproducibility of results using this method (e.g. Ellsworth *et al.*, 1993; MacPherson *et al.*, 1993), it has been applied effectively to investigate genetic variation in a range of species of parasitic nematodes (reviewed by Gasser, 1999). Importantly, reliable and reproducible results can be achieved when an increased stringency (i.e. an annealing temperature of >45°C) is employed in the PCR and amplicons are subjected to denaturing polyacrylamide gel electrophoresis (e.g. Gasser *et al.*, 1998). This was also shown, for example, in a recent study of genetic diversity within the nodule worm *Oesophagostomum bifurcum* (Strongylida) from different human and non-human primate hosts (including the Mona monkey, Patas monkey and Olive baboon) from various geographical regions in Ghana (de Gruijter *et al.*, 2004). Multiple decamer primers were selected based on their ability to efficiently and reproducibly amplify (at an annealing temperature of 48°C) products from individual nematodes. Electrophoretic analysis of the amplicons by denaturing polyacrylamide gel electrophoresis defined a large number ($n = 326$) of informative RAPD bands. Cluster analysis of the RAPD data (based on pairwise comparison of banding profiles) revealed that *O. bifurcum* from humans was genetically distinct from *O. bifurcum* from the three other species of primate, which contrasted with previous findings using the mt cytochrome *c* oxidase subunit 1 gene (de Gruijter *et al.*, 2002), but which were concordant with results achieved using AFLP analysis (de Gruijter *et al.*, 2005).

AFLP is based on the selective, high-stringency amplification of restriction fragments produced from genomic DNA and the subsequent display by denaturing gel electrophoretic analysis. This fingerprinting approach permits the specific co-amplification of large numbers (typically 50–150) of restriction fragments from DNA of any origin or complexity. While it has found relevant uses to study various animal groups,

including plant parasitic nematodes (e.g. Marche *et al.*, 2001; Esquibet *et al.*, 2003; Srinivasan *et al.*, 2003), there has been limited application to socio-economically important parasitic nematodes of animals. Some recent reports demonstrate its attributes and effectiveness for assessing genetic diversity or studying population genetic structures of different bursate nematodes of animals, such as the barber's pole worm of small ruminants, *Haemonchus contortus*, and the bovine lungworm, *Dictyocaulus viviparus* (see Otsen *et al.*, 2001; Höglund *et al.*, 2004; Roos *et al.*, 2004). Clearly, this method shows major promise for investigating the population genetics of species of *Toxocara*.

For genetic studies, attention has also been focused on the analysis of satellite DNA (Tautz and Renz, 1984; Tautz, 1993). Minisatellites and microsatellites are both abundant and ubiquitous in the genomes of all eukaryotes, and represent tandem repeats of short motifs which appear to be randomly dispersed throughout the genome. They are usually non-transcribed and maintain polymorphism as a consequence of the accumulation of mutations. The variation in their repeat number allows the alleles present at a locus to be scored by size on electrophoretic gels. The satellites are characterized by allelic ('hyper-') variability in repeat length and consequently have been utilized to study the genetic structure of populations as well as for linkage analysis and genetic mapping (e.g. Tautz, 1989; Love *et al.*, 1990; Bell and Ecker, 1994; Goldstein and Clark, 1995). There have been some applications to nematodes, including *H. contortus* (see Hoekstra *et al.*, 1997), *Strongyloides ratti* (see Fisher and Viney, 1996), entomopathogenic nematodes (Grenier *et al.*, 1996, 1997) and the sugarbeet cyst nematode, *Heterodera schachtii* (see Plantard and Porte, 2004). More recent results now indicate the applicability of microsatellites to ascarids, such as *Ascaris* (Anderson *et al.*, 2003; Roberts *et al.*, unpublished data). By utilizing fluorescently labelled primers designed to flank sequences, multiple microsatellites can be amplified simultaneously and subsequently subjected to automated electrophoresis and computer analyses (e.g. using GENESCANTM and GENOTYPERTM software, Applied Biosystems) (see Anderson *et al.*, 1999; Nair *et al.*, 2003). Such high-throughput systems also provide unique prospects for larger scale studies of *Toxocara* species.

Concluding Remarks

Molecular methods provide a platform for tackling diagnostic problems and biological questions relating to species of *Toxocara*, provided careful consideration is given to sampling procedures, genetic markers and analytical approaches employed (see Anderson, 2001; Constantine, 2003). This chapter emphasizes the utility of PCR-based tools for studying the taxonomy of members within the genus *Toxocara* and for investigating their population genetics, ecology and epidemiology. Such tools are useful for the diagnosis of toxocarosis through the specific detection of *Toxocara* DNA in tissues from naturally or experimentally infected hosts (Rai *et al.*, 1997). Using molecular tools, in combination with traditional parasitological and serological methods, it should also be possible to characterize, in detail, experimental infections in 'model host systems' (e.g. mouse or pig). Real-time PCR (cf. Monis *et al.*, 2002) provides a means to quantitate or semi-quantitate nucleic acids (for particular genetic loci) in environmental samples or tissues from experimentally infected hosts, which may have merit for estimating infection intensity (via the amplification of parasite-specific genomic DNA) and/or parasite viability (via measuring specific RNA message). The development of improved techniques for the isolation and amplification of specific genetic loci from *Toxocara* DNA from tissue or soil samples (Kramer *et al.*, 2002; Ishiwata *et al.*, 2004) will assist biological and epidemiological studies. However, there is no panacea for problems with the isolation of minute amounts of larval or egg DNA from different biological samples (soil, faeces or histological tissue blocks) or for the removal of inhibitory substances, such that methods need to be optimized in each case (cf. Gasser, 1999). From an epidemiological and ecological perspective, it would be interesting to confirm or rule out, employing a DNA method, the involvement of *Toxocara* in human VLM cases in Japan, presently considered to be caused by *A. suum* based on serological evidence (Maruyama *et al.*, 1996; Sakakibara *et al.*, 2002). It would also be relevant to establish whether specific genotypes of *T. canis* have a particular affinity to the human host and/or predilection sites in tissues to cause

different types of toxocariasis and whether there are specific subpopulations of *T. canis* which undergo arrested development in tissues. Interestingly, a recent study of hookworms revealed genetically distinct subpopulations of *Ancylostoma caninum* in dogs from Townsville, Australia (Hu *et al.*, 2002b). As previous morphological and clinical studies in this geographical area had shown that this hookworm is not specific to dogs but is also capable of infecting cats and humans, causing eosinophilic enteritis in the latter (Prociv *et al.*, 1994; Stewart, 1994), it has been speculated that certain genotypes of *A. caninum* can selectively infect humans (Hu *et al.*, 2002b). Thus, genetic comparison of *T. canis* from domestic dogs in endemic areas with those from humans affected, for instance, by OLM and/or VLM would be informative and could provide improved insights into the transmission of toxocariasis, having implications for disease 'tracking' and control. Mutation scanning tools provide the opportunity for detailed genetic characterization and comparison

of a wide range of *Toxocara* species from different hosts and geographical origins globally. Also, in particular, the application of the approaches of AFLP and microsatellite analysis should provide new and exciting insights into and enable improved understanding of the population genetics and epidemiology of members within the genus *Toxocara*, thus assisting in the prevention and control of toxocariasis.

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3 The Larval Surface

Malcolm W. Kennedy

*Environmental and Evolutionary Biology, Institute of Biomedical and Life Sciences,
Graham Kerr Building, University of Glasgow, UK*

Introduction

The surface of the *Toxocara canis* larva is in direct contact with the tissues of its host, and has therefore received considerable attention for its potential role in pathology, immunity and immune avoidance. The relationship between surface antigens of the parasite and its excretory–secretory (ES) products has long been established using a range of techniques that have revealed, broadly, a source in glandular systems, subsequent association with the larval surface and then rapid loss of surface materials, thereby contributing to the principal components of ES. The rapidity with which *T. canis* larvae undergo this surface shedding (Smith *et al.*, 1981) is possibly only matched by the larvae of the related parasite of cats, *Toxocara cati* (Kennedy *et al.*, 1987b). Both species appear to exhibit surface antigen turnover events that are a common feature of larval nematodes, and have therefore been highly informative of the processes applicable to many if not all nematodes. The rate at which *T. canis* larvae manufacture, install and then shed their surface materials is remarkable, and is perhaps a reflection of this species's default transmission strategy. That is, the larvae can survive for several years even in non-canine hosts until ingested by a dog, such that entry into an inappropriate host is not necessarily an endpoint. And in such paratenic/transfer hosts, larvae migrate freely within soft tissues for periods of years, without undergoing a static phase in a specialized site, as does the larva of

Trichinella spiralis. Such a strategy must have had implications for the evolution of a larval form that cannot specialize to one particular host species, but must master nutrient acquisition and evasion of innate and adaptive defence mechanisms in many species of mammal.

The tissue contact surface of *T. canis* larvae comprises an outer layer, the surface coat, which is glycoprotein-rich and the source of shed antigens (Meghji and Maizels, 1986; Maizels *et al.*, 1987; Page and Maizels, 1992; Page *et al.*, 1992a,b,c). Beneath this is the epicuticle, which is the outermost layer of the extracellular cuticle of these organisms, and is integral to the cuticle. The epicuticle is difficult to study in isolation, and has been examined *in situ* using a range of fluorescent lipid probes and biophysical methods. The materials of the surface coat have been the subject of exhaustive protein and carbohydrate analysis, and cloning of cDNA encoding the major components has been successful (Smith *et al.*, 1981; Maizels *et al.*, 1984, 1987; Page *et al.*, 1992b). The latter has revealed that the larvae shed proteins into their environment of unexpected kinds, including a C-type lectin, mucin-like glycoproteins and a phosphatidylethanolamine-binding protein (Maizels and Loukas, 2001). The first two of these may be involved in countering host defence reactions. A curious feature of several secreted and surface-associated antigens of *T. canis* is the possession of a six-cysteine domain that is now being found widely among nematodes, both parasitic and free-living, and also members of other phyla, but with no function yet ascribed (Maizels and Loukas, 2001).

The surface coat is frequently the target of high-level antibody responses by infected humans, and also activates complement directly via the alternative pathway (Kennedy *et al.*, 1987a; Kennedy and Kuo, 1988) (nothing is yet known about activation of the lectin pathway). This chapter will cover some questions about possible intraspecific variation of *T. canis* larvae and the relationship between surface coat and epicuticle, and will include some unpublished results.

Should the Surface Antigens of the Infective Larvae of Parasitic Nematodes be Antigenically Diverse?

Counter questions would include: Why should they be? Is there any evidence for such diversity? If they are invariant, do different infected people respond to them differently? The arguments for the evolution of within-species diversity include that it would be a selective advantage to an individual parasite to be antigenically different from members of its species that have preceded it in sensitizing a host individual. On the other hand, antigenic homogeneity might arise as a parasite species optimizes its antigenic makeup for success in a host species (and, in the case of *T. canis*, many species). But, survival in a pre-sensitized host could still act as a selective force for diversification.

The immune repertoire of mammalian hosts is highly diverse due to polymorphisms in immune genes of both innate and adaptive systems, such as complement and the major histocompatibility complex (MHC). This should render it difficult for a species of parasite to optimize its antigenic makeup to minimize immune attack in all members of the host species. On balance, therefore, it might again be advantageous to a parasite to produce antigenically diverse offspring, a sustainable proportion of which should survive in a host population whose immune repertoire cannot be predicted.

As discussed extensively elsewhere, there is growing evidence for genetic diversity (alleles and possibly gene number) in helminth parasites, some of which extends to the products that interact with hosts, such as secreted enzymes (Maizels and Kurniawan-Atmadja, 2002; Jasmer *et al.*, 2004). Strains of a single species of nematode have already been shown to differ in their immunobiology (Bellaby *et al.*, 1995, 1996; Wakelin and Goyal, 1996; Wakelin *et al.*,

2002). Although so far only found in schistosome larvae, even cloned organisms can differ dramatically in the expression of surface antigens, both within and between clones (Jones and Kusel, 1989; Vieira and Kusel, 1991; Al-Adhami *et al.*, 2001). Surface antigens have been shown to be genetically variable, with phenotypes inherited in Mendelian fashion (Poltz *et al.*, 1987, 1990, 1993; Hemmer *et al.*, 1991; Politz and Philipp, 1992; Grenache *et al.*, 1996; Hoflich *et al.*, 2004), but diversity could also be achieved by a variety of genetic effects such as incomplete penetrance.

It may, however, prove difficult to demonstrate that antigenic polymorphisms are meaningful in terms of parasite survival and transmission. Part of the difficulty might arise from the need to account for local adaptation of an antigenically diverse worm population to a local host population. This represents a significant barrier, given our dependency on available species and strains of nematode parasites that can be investigated in the laboratory. This is compounded by the genetic homogenization likely in laboratory-maintained strains through successive rounds of inbreeding, plus a genetically limited founder population. In this context, it might be useful to examine the case of a nematode related to *T. canis* and whether it might provide a useful guide.

Is *Ascaris* a Useful Guide?

Ascaris lumbricoides is an ascaridid along with *T. canis*, but unlike *T. canis*, it is a natural parasite of humans in which it can complete its life cycle to adulthood. In this species, it has been demonstrated that serum antibody from chronically infected people binds heterogeneously to the surfaces of infective larvae; that is, serum antibody from an individual human will bind strongly to some larvae and weakly or not at all to other larvae in the same batch (Fraser and Kennedy, 1991). The inference from this observation is that the infective larvae of *A. lumbricoides* differ in the antigens they present to their hosts. With *Ascaris*, however, the situation is complicated by the fact that cultured infective larvae are in the process of moving on to the next stage of their life cycle, thus making it difficult to produce a developmentally synchronous and antigenically representative sample of larvae for experimentation. The original report on this went to some lengths

to circumvent this problem, but there is still a clear need for the phenomenon of polymorphism in the surface antigens of infective larvae to be investigated further in this species and others. Interestingly, the effect was not observed using the same serum set but with larvae from *Ascaris suum* (the species infecting pigs) instead (McMonagle and M.W. Kennedy, unpublished). So, investigation of meaningful antigenic diversity in infective larvae of nematodes will require studying fully interacting parasite and host populations.

Thus, there are two selective forces that might result in differential responses to the surface of tissue-invasive nematode parasites: first, heterogeneity among larvae in the antigens that they express on their surfaces (both qualitative and quantitative), and secondly, diversity in immune recognition of surface antigens by the hosts themselves (see below). The latter would likely be due to a combination of genetic restriction to the immune repertoire among humans and prior exposure to antigenically cross-reactive pathogens. So, it would be important to know whether *T. canis* larvae are heterogeneous in their surface antigens, and interesting if they are not.

Are the Surfaces of *T. canis* larvae Antigenically Diverse?

This could be answered in a number of ways. First, a broad sample of parasites could be examined for diversity in the genes encoding the protein backbone of surface antigens. But these antigens are highly glycosylated and the target epitopes include sugars. Diverse epitopes would therefore not be revealed by merely examining these genes, but examination of glycosyltransferases, gene promotor regions, etc. would be required. Next, monoclonal antibodies to the surface (and ES) antigens of *T. canis* that are already available (Kennedy *et al.*, 1987b; Maizels *et al.*, 1987) could be used to establish whether individual larvae differ in the degree to which they bind, using quantitative methods. For now, it might be pertinent to revisit the kind of experiments on *A. lumbricoides* mentioned above with *T. canis* larvae.

Figure 3.1 shows a series of experiments where cultured larvae of *T. canis* were exposed to serum from different toxocariasis patients from the UK, and the binding of antibody to individual larvae measured by quantitative immunofluorescence.

This shows that different patients exhibit different levels of binding (as anticipated from their ELISA antibody values – see legend), but that the recognition of individual larvae follows a reasonable normal distribution, but with some outliers. These outliers (some of which showed less than half-maximum binding), plus the broad distributions, could represent some degree of diversity, although this is much less than that observed for *A. lumbricoides* larvae. In the latter case, many more patient sera were available, so it is conceivable that a larger sample of sera from toxocariasis patients would reveal heterogeneity similar to that found for *Ascaris*, although there has been no sign of this to date.

Is there Genetic Control of Host Immune Repertoires to *T. canis* Antigens?

This question is again prompted by findings with *Ascaris*, in that the immune response of a mammal does not respond to every foreign protein, but is restricted by the peptide binding capacities of the (MHC-encoded) cell-surface proteins involved in antigen presentation. In the case of *Ascaris*, some infected people fail to respond strongly to certain antigens of the parasite to which others mount strong antibody responses (Fraser *et al.*, 1993; McSharry *et al.*, 1999). The effect can be modelled in laboratory mice and rats, in which the controlling factors were indeed found to be alleles of the MHC, the class II region specifically (Tomlinson *et al.*, 1989; Kennedy *et al.*, 1990b, 1991a; McSharry *et al.*, 1999). Moreover, the effect is now known to be broadly applicable to nematode infections, and the restriction of the immune repertoire appears to apply despite the large loads of foreign antigens presented by these parasites (Tomlinson *et al.*, 1989; Else *et al.*, 1990; Kennedy *et al.*, 1990a, 1991b; Kwanlim and Maizels, 1990).

Again in contrast to the situation in infections with *Ascaris* and other parasites, no evidence has yet appeared that people, mice, rabbits or dogs respond differentially to the ES or surface antigen components of *T. canis*. This is perhaps due to the extensive glycosylation of *T. canis* ES and surface antigens such that surface and ES antigens are so epitope-rich, or cross-reactive, that any underlying restriction of the immune repertoire is obscured.

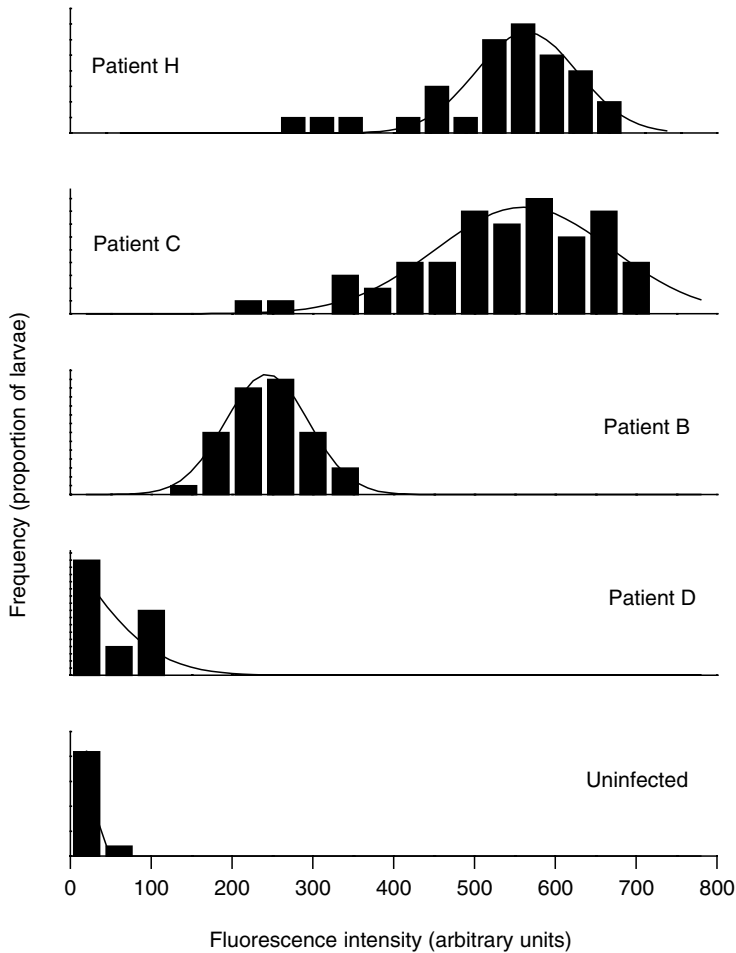


Fig. 3.1. Diversity in binding of serum antibody from toxocariasis patients. *Toxocara canis* larvae were incubated with serum from four toxocariasis patients (and one seronegative control) from the UK for 30 min, washed three times in phosphate buffered saline (PBS), and incubated with fluorescein (FITC)-labelled rabbit anti-human IgG for 30 min, and finally washed in PBS. All these steps were carried out on ice and with ice-cold buffers containing 0.1% w/v sodium azide to prevent surface shedding during the labelling and observation processes. After the final washing, the larvae were suspended in 0.1% sodium azide to immobilize them, placed on a microscope slide with a cover slip supported by a ring of silicon grease, and the fluorescence intensity of a rectangular area (approximately $400 \mu\text{m}^2$) of the surface recorded from between 23 and 57 individual larvae using a Leitz MPV2 quantitative microscope fluorescence system, with appropriate background subtraction. The curves are Gaussian distributions fitted to the data. The number of measurements taken, for the histograms top to bottom, were 32, 57, 43 and 30. The ELISA titres were 1:800, 1:1600, 1:400 and 1:100 for patients H, C, B and D, respectively (H.V. Smith, unpublished).

So, it seems (for the moment) that the larvae of *T. canis* do not appear to be detectably heterogeneous to any significant degree in the exposed surface antigens, either quantitatively or qualitatively.

It therefore appears that an infected human will respond similarly to all the larvae with which he/she is infected, aside from how they would be attacked in different tissues (brain, eye, muscle, liver, etc.).

Surface Lipid Binding by *T. canis* Infective Larvae

Due to the ease with which *T. canis* larvae can be cultured for long periods *in vitro*, they have been particularly useful in investigating the peculiar surface possessed by nematodes, and its lipid binding characteristics in particular. These biophysical investigations showed that the surface to which fluorescent lipids bind is highly unusual among biological surfaces in that it is highly selective of the types of lipids that will bind, and also that those that do bind are highly restricted

in the freedom with which they are able to diffuse laterally in the plane of the surface (Kennedy *et al.*, 1987a; Proudfoot *et al.*, 1993a,b). One particular fluorescently tagged lipid, 5-*N*-(octadecanoyl)aminofluorescein (AF18), has proved to be particularly useful, and appears to be confined to the epicuticle (and possibly also the ducts of the secretory gland; L. Proudfoot, A. Prescott and M.W. Kennedy, unpublished) of the worm as observed both by confocal microscopical observations (Fig. 3.2) and by methods that will quench the fluorescence of surface-restricted probes (Kennedy *et al.*, 1987a; Proudfoot *et al.*, 1993a,b).

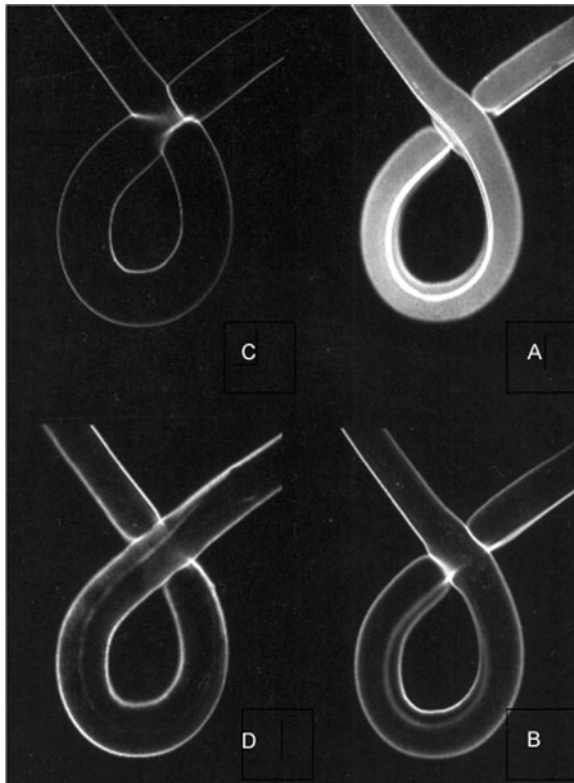


Fig. 3.2. Surface fidelity of labelling with fluorescent lipid. Larvae were labelled with AF18 as for Fig. 3.3 (see below), and one that had crossed over itself imaged by confocal microscopy. The four frames were taken at different planar depths (optical sections) through the specimen from full contact with the cover slip (A) through an intermediate step (B) to a cross section of the worm at midpoint (C), and then on to a lower section (D). The bright line most clearly seen in (A) is the ala. This illustrates that the label is confined to the surface even at high resolution. The surface restriction of AF18 can be further evidenced by viewing labelled worms held in trypan blue solution, which quenches the fluorescein fluorophore if within ~4 nm by energy transfer (Kennedy *et al.*, 1987a).

Post-hatch Changes in Lipid-binding Properties

It is already known that *T. canis* larvae alter their surfaces rapidly after they hatch from their eggs. Freshly hatched larvae do not possess the surface antigens that are known from long-term cultures, as detectable by surface radioiodination or immunofluorescence with monoclonal or polyclonal antibodies (Kennedy *et al.*, 1987b; Maizels *et al.*, 1987). This is presumably because the expression of this set of surface materials is inappropriate within the egg, but is essential for survival within mammalian tissue, particularly in defence against attack by antibody, cells and complement. Is this rapid change in surface antigens after hatching also reflected in an affinity for lipids?

Figure 3.3 shows that there are indeed changes in the affinity of the larval surface for lipids following hatching. Relative to the intensity of fluorescent lipid binding to larvae from a stable, long-term culture (3 months), newly hatched larvae exhibit a considerably higher binding capacity for AF18 (2 h). This is even more apparent 24 h later, but after 7 days the lipid-binding propensity settles to that of long-term cultures. These differences presumably reflect the rapid re-organization of the surface of the larvae underway following emergence from the egg, invasion of host intestinal tissue and establishment of a chronic tissue infection with continuously migrating larvae. In other species of human-parasitic nematode, it has been observed that infective larvae fail to bind AF18 until exposed to mammalian culture conditions (Proudfoot *et al.*, 1993a,b; Modha *et al.*, 1997). In these cases, however, the infective larvae concerned derive from arthropod vectors or from resting stages encapsulated in muscle. It is not clear, therefore, why egg-emergent *T. canis* larvae immediately exhibit high levels of AF18 binding, unless the time taken to artificially hatch and isolate the larvae for experimentation is such that a non-binding phase is missed. Nevertheless, it is clear that newly hatched *T. canis* larvae possess surfaces that will probably absorb lipid from host tissues, and that this phase probably takes a few days to subside, that is, if we can assume that the behaviour of cultured larvae reflects that of larvae fully exposed to host tissue and defence reactions.

Separation Between Lipid-binding Layer and Surface Coat

It has not been easy to examine the relationship between these two entities for any nematode, because of the need to use intact living larvae in the fluorescence-based investigation of the lipid-binding layer, presumed to be the epicuticle, and the sheddable surface coat detectable with antibodies. *T. canis* larvae have again been useful for this due to the ease with which they can be cultured and the rapidity with which surface events occur and are observable.

Figure 3.4 shows the results of an experiment in which *T. canis* larvae were simultaneously labelled with the AF18 fluorescent lipid and rabbit antibody to ES material. The AF18 fluorescence emission is green and the bound antibody is here visualized using a red-emitting rhodamine antibody conjugate. The larvae were immobilized, but the temperature was then raised so that surface coat shedding would occur. The paired images are of the same worms observed under filters appropriate for excitation of, and emission by, AF18 and rhodamine, in which it is clear that the lipid-binding layer remains stable while the antibody-binding layer undergoes aggregation and is lost. This further supports the idea that the lipid-binding layer is the epicuticle and lies beneath the sheddable surface coat. The question of how precisely the surface coat adheres to the epicuticle, and is then separated from it, remains to be answered.

Concluding Remarks

Despite the arguments laid out at the beginning of this chapter (predicting that infective larvae of nematodes should be antigenically diverse), it appears that the surface of *T. canis* larvae is much more uniform between larvae than is found with the related parasite *A. lumbricoides*. If this homogeneity proves to be confirmed on further investigation at the immunological and gene levels, then it needs explaining. It could be, for instance, that the antigenic nature of *T. canis* surfaces has indeed been optimized by selection to a uniform type. Possible explanations for this might lie in the strategy used by these parasites for long-term

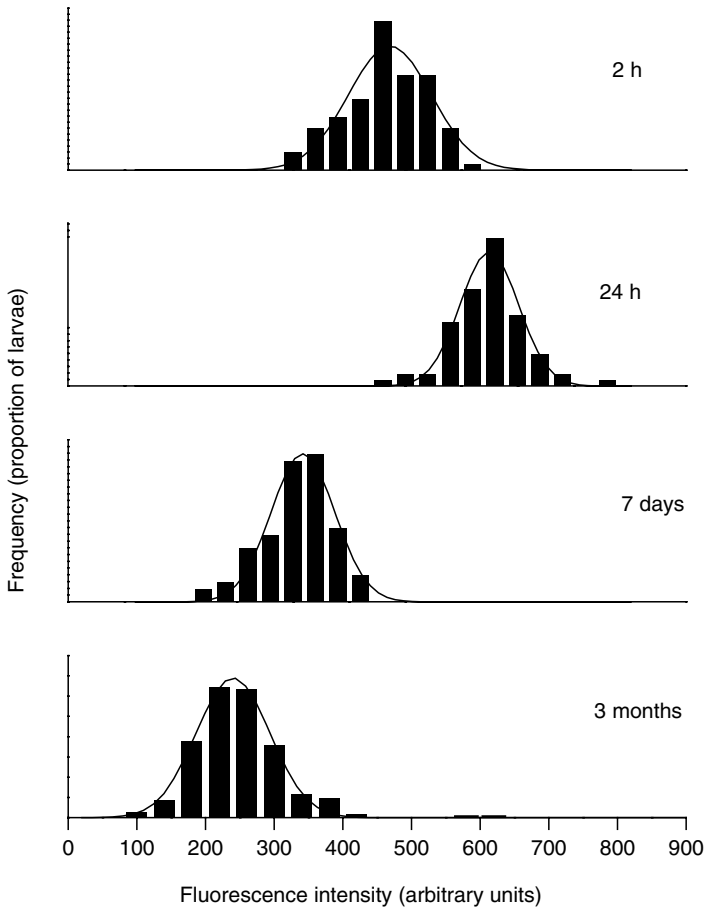


Fig. 3.3. Post-hatch changes in lipid binding to intact *Toxocara canis* larvae with time after hatching. Larvae were hatched from eggs by standard methods and cultured in serum-free medium for 2 h (the minimum required for hatching and purification from debris by migration through a cotton wool plug), 24 h or 7 days. Larvae were labelled with the fluorophore-tagged fatty acid 5-*N*-(octadecanoyl)aminofluorescein (AF18) as follows. A layer of 5 μ l of a 2 mg/ml solution of AF18 in ethanol was layered on top of 1 ml PBS containing the worms, and the suspension was rapidly mixed. After 10 min at 37°C, the worm suspension was then washed several times in PBS/0.1% sodium azide, and the fluorescence intensities at the worm surface quantified as for Fig. 3.1, the fluorescence of 50–96 individual worms per group being measured in this experiment. At each time point, a sample of larvae from a 3 month culture was assayed in parallel. The fluorescence counts for the 2 h, 24 h and 7 day samples were normalized against the mean values for the 3 month cultures examined at the same time, so as to yield data from each time point relative to the 3 month culture as standard. The number of measurements taken, for the histograms top to bottom, were 96, 72, 81 and 241, the last being the cumulative values for the three occasions upon which 3 month cultures were assayed and used to normalize all the data.

survival embedded in host tissue, namely the rapid shedding of a surface coat in order to remove adherent antibodies, complement and cells. The very antigenicity of the shed material could therefore be advantageous by, for example, acting as a diversion or smokescreen to immune effector

molecules and cells. Nevertheless, the infection event is clearly associated with dramatic changes in the surface of the larvae, both in terms of its surface coat antigens and the lipid-binding characteristics of the epicuticle with which the surface coat must interact.

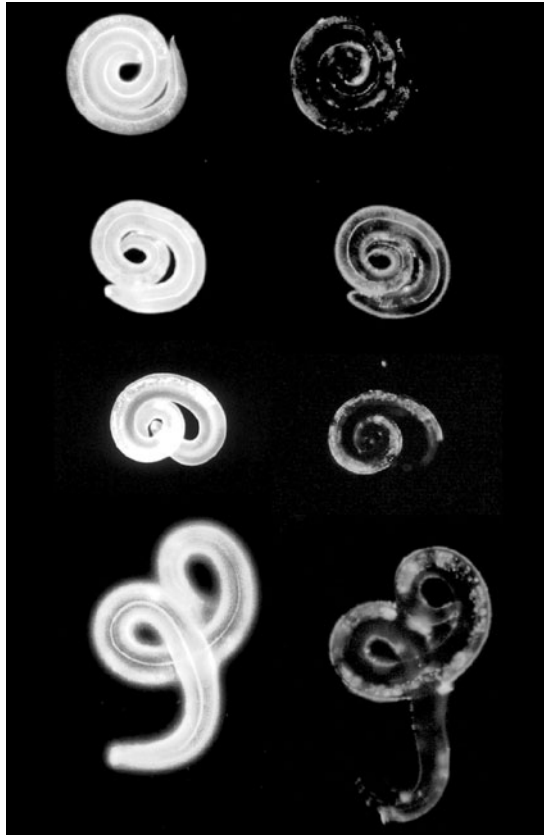


Fig. 3.4. Separation between the lipid-binding layer and the sheddable surface coat. Larvae were simultaneously labelled with AF18 and with rabbit antibody to *Toxocara canis* ES antigens, binding by the latter being visualized with rhodamine-labelled secondary antibody to rabbit immunoglobulin. All washing steps were carried out in cold PBS containing no metabolic inhibitors. The larvae were then incubated at 37°C for 1 h, and viewed under ultraviolet excitation and visible light observation filters appropriate for each of the two fluorophores. The photographs show typical results showing that the AF18 lipid-labelled (left column) layer remained smooth and intact whilst the red antibody-labelled surface coat (right) aggregated and was lost from the surface. Shorter incubations showed that both green- and red-labelled layers were evenly distributed. Longer incubations showed complete loss of the antibody-labelled layer (red) while the lipid-binding layer (green) remained intact. In some of the AF18-labelled worms, the fluorophore had bleached under ultraviolet illumination and autofluorescent internal structures can be seen, as found in unlabelled worms. For a colour version of these pictures, see <http://www.gla.ac.uk/ibls/DEEB/mwkimages/>

Acknowledgements

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due to maturing senses of social and family responsibilities. We are most grateful to Dr Lorna Proudfoot and Alan Prescott, then of Dundee University, for the confocal microscope pictures, and thanks are also due to Lorna and to the irrepressible John Kusel for fruitful discussions. All the work was carried out using facilities and consumables provided under various research grants to Malcolm W. Kennedy from the Wellcome Trust.

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4 *Toxocara* and Asthma

Elena Pinelli,¹ Jan Dormans² and Irma van Die³

¹*Department of Parasitology and Mycology, Diagnostic Laboratory for Infectious Diseases and Perinatal Screening, Bilthoven, The Netherlands*

²*Laboratory for Toxicology, Pathology and Genetics, National Institute of Public Health and the Environment, Bilthoven, The Netherlands*

³*Department of Molecular Cell Biology and Immunology, VU University Medical Center, Amsterdam, The Netherlands*

The incidence of allergic diseases is increasing worldwide and although it is not clear how, several explanations, including exposure to infections, have been proposed (reviewed in Bresciani *et al.*, 2005). Evidence from epidemiological studies suggests that infection with *Toxocara* worms stimulates atopic diseases.

Toxocara canis and *Toxocara cati* are the roundworms of dogs and cats, respectively. Humans can acquire these helminths by ingesting their infective eggs, which are usually found in soil contaminated by dog or cat faeces (see Chapter 14, this volume). Although the worms cannot complete their life cycle in humans, hatched larvae can penetrate the intestine and invade the mucosa and migrate via blood and lymph all over the body causing visceral larva migrans (Kayes, 1997). Larvae remain most frequently located in the liver and the lungs but can also cause serious ocular damage by migrating into the retina (ocular larva migrans) (see Chapter 9, this volume). Although frequently asymptomatic, clinical signs of human toxocariasis include cough, fever, general malaise, weakness, rash, headache, facial oedema and abdominal pain. Severe manifestations include myocarditis, encephalitis and pneumonia. In infected individuals, the larvae may remain in the tissues for long periods of time, inducing a chronic inflammatory response. Infection with this parasite can also result in asthmatic like manifestations such as wheezing, coughing and episodic

airflow obstruction, which have been associated with hypereosinophilic syndrome (Feldman and Parker, 1992).

Allergic asthma is a chronic inflammatory disorder of the airways that is characterized by an increased serum IgE after exposure to allergens and by eosinophilic inflammation, mucus hypersecretion and bronchial hyperreactivity (Holt *et al.*, 1999). Asthmatic complaints include shortness of breath, wheezing and forced inhalation. This disorder is controlled by Th2 cells that secrete interleukin-4 (IL-4), IL-5 and IL-13, which mediate synthesis of IgE, recruitment of immune cells, degranulation of eosinophils and hyperreactivity of the airways (Akbari *et al.*, 2003). Common features in allergic asthma and toxocariasis are inflammation of the airways, accumulation of eosinophils and induction of IgE production (see Chapter 11, this volume).

This chapter aims to review the epidemiological, clinical and experimental data concerning the association between helminth infections and allergy with particular emphasis on infection with *T. canis* and asthma.

Epidemiological Studies

T. canis is widely distributed in most regions of the world where dogs and also feral animals such as

foxes and wolves are infected (Gutierrez, 2000). Human infection with *Toxocara* spp. has been surveyed throughout the world, mostly done in a cohort representing a country, state or village. The recorded seroprevalence varies with age and among countries or even within countries. In Ireland, *Toxocara* seroprevalence in schoolchildren is comparatively high (31%) and rises with age (Holland *et al.*, 1995). The exposure to *Toxocara* spp. in The Netherlands based on serological surveys has been reported to be 19% on average, with 4–15% in people younger than 30 years and 30% for the age group older than 45 years (Overgaauw, 1997). In a study carried out on Dutch schoolchildren aged 4–6 years, *Toxocara* seroprevalence was found to be 6% in the city of Rotterdam and 11% in the city of The Hague (Buijs *et al.*, 1994a). In the state of Connecticut, USA, seroprevalence varied from 6.1% in New Haven to 27.9% in Bridgeport (Sharghi *et al.*, 2001). Several risk factors have been reported which differ among countries. In the USA, an association with living in an urban area, race and family income has been described. In this study, no association was found with owning dogs or puppies and gender (Sharghi *et al.*, 2001). In Ireland, however, males were significantly more frequently infected than females, as well as children attending rural schools versus urban schools (Holland *et al.*, 1995).

In Sri Lanka, a sero-epidemiological study performed among children aged 1–12 years showed a seroprevalence of 43%. Children between the age of 7 and 9 years were found to be at highest risk (Iddawela *et al.*, 2003). In an epidemiological study carried out in Nepal among people aged 14 and older, the seroprevalence was 81%. Males showed higher (85%) antibody-positive rates than females (77%) (Rai *et al.*, 1996). Children are at most risk of infection because of their frequent contact with contaminated soil in yards and sandpits and especially when there is a history of pica.

The association between allergic manifestations and infection with helminths has been proposed by several authors. In 1971, Tullis reported that more than 90% of patients in an asthmatic clinic in Canada were infected with *Ascaris lumbricoides* (Tullis, 1971). Desowitz and colleagues reported in 1981 on the prevalence of antibodies to *T. canis* and *Dirofilaria immitis* in asthmatic and non-asthmatic children born and raised in Hawaii. They found a significantly higher prevalence of parasite-specific IgE antibodies in the asthmatic compared with the non-asthmatic population (Desowitz *et al.*, 1981).

Table 4.1 summarizes recent studies on the association between *Toxocara* seroprevalence and allergic manifestations, including asthma. In a hospital in Kuala Lumpur, Malaysia, the exposure to *Toxocara* infection and bronchial asthma was investigated among children. Using a *Toxocara*-specific ELISA, the seroprevalence of *Toxocara* infections was measured among asthmatic patients. Results indicated a positive relationship between *Toxocara* seroprevalence and asthma (Hakim *et al.*, 1997). In Cairo, a serological study was performed among children aged between 4 and 6 years suffering from idiopathic bronchial asthma or chronic urticaria as well as healthy age-matched children. Results showed that the *Toxocara*-seropositive allergic children presented significantly increased total IgE and eosinophil levels when compared with controls. A contribution of toxocariasis to allergic sensitization was suggested (Oteifa *et al.*, 1998).

More recently, a study on *Toxocara* seroprevalence and childhood asthma carried out among Malaysian children showed that children with bronchial asthma were found to have higher *Toxocara* seropositivity than the non-asthmatic controls (Chan *et al.*, 2001). Buijs and co-workers have carried out cross-sectional studies among elementary schoolchildren in The Netherlands and have concluded that allergic manifestations occur more often in *Toxocara*-seropositive children. In one study, Buijs *et al.* evaluated the relationship between *Toxocara* seroprevalence and allergic asthma in Dutch schoolchildren aged 4–6 years (Buijs *et al.*, 1994a). The authors found that occurrence of asthma/recurrent bronchitis and hospitalization due to asthma/recurrent bronchitis were significantly associated with seroprevalence. A marginally significant relation with eczema was also found. In this study, IgE specific for inhaled allergens occurred significantly more often in the *Toxocara*-seropositive group. The authors suggest that *Toxocara* worms, among other environmental factors, may stimulate polyclonal IgE production, including allergen-specific IgE, and thus may contribute to the manifestation of allergic asthma and possibly eczema in children predisposed to allergy. In another study, Buijs *et al.* investigated differences in *Toxocara* seroprevalence, allergic manifestations and the associations between these two, in children from urban and rural environments. In this study, *Toxocara* antibodies, eosinophil numbers, total IgE concentrations and the

Table 4.1. Epidemiological studies on the association between *Toxocara* seroprevalence and allergic manifestations.

Country/city	Age of studied population (years)	Sex of studied population	Inclusion of age-matched healthy controls	Definition of <i>Toxocara</i> seropositivity using ELISA	Allergic manifestation confirmed by physicians	Statistical analysis	Association between allergic manifestations and <i>Toxocara</i> seroprevalence	Reference
Malaysia/Kuala Lumpur	1–10	Not mentioned	Yes	OD-negative sera 0.20 ± 0.15 OD-positive cut-off value $0.625 + 3 \times \text{SD}$	Bronchial asthma	Not mentioned	Positive	Hakim <i>et al.</i> (1997)
Malaysia	Older than 5	Males and females	Yes	Serum dilution starting at 1:100; absorbance values above 0.25 considered positive	Bronchial asthma	Not mentioned	Positive	Chan <i>et al.</i> (2001)
Netherlands/The Hague and Rotterdam	4–6	Males and females	Yes	1:20	Asthma/recurrent bronchitis/eczema	Logistic regression	Positive	Buijs <i>et al.</i> (1994)
Netherlands/Utrecht and Eindhoven	4–12	Males and females	Yes	1:20	Asthma/recurrent bronchitis/eczema	Logistic regression	Positive	Buijs <i>et al.</i> (1997a,b)
USA/New Haven and Bridgeport	2–15	Males and females	Yes	1:32	Asthma	Stepwise logistic regression	No association	Sharghi <i>et al.</i> (2001)

SD, standard deviation

occurrence of inhaled allergen-specific IgE were measured. Questionnaires investigating respiratory health and putative risk factors for infection were used. Results from this study indicated that the means of total serum IgE levels and blood eosinophils were significantly higher in the *Toxocara*-seropositive than in the seronegative group. Other results from this study were that inhaled allergen-specific IgE and asthma/recurrent bronchitis occurred significantly less often in rural than in urban areas, and significantly less often among girls than among boys. These authors concluded that allergic manifestations occur more often in *Toxocara*-seropositive children and that a relationship with an already existing allergic condition is plausible (Buijs *et al.*, 1997).

Sharghi *et al.* (2001) carried out a case-control study in the cities of New Haven and Bridgeport, to examine whether *Toxocara* infection is associated with asthma in children. The authors collected blood samples from children aged 2–15 years and *Toxocara* antibodies were measured using ELISA. Risk factors for asthma and *Toxocara* infection were assessed by a questionnaire. Significant associations were found between asthma and risk factors and between *Toxocara* infection and risk factors but not between *Toxocara* infection and asthma.

All the studies mentioned here focus upon the possible association between *Toxocara* infection and allergic manifestations, and although several of them come to the same conclusion, namely a positive association, others do not find any association at all. Several important differences among these studies can be pointed out (Table 4.1). It is particularly important to mention the serological assay employed by the different groups. Although it has been well documented that *Toxocara*-specific ELISA using the excretory-secretory (ES) antigen derived from L2 larva has a high specificity and sensitivity, the different groups have different definitions of seropositivity. This is still a problem, not only for epidemiological studies but also for clinical diagnosis. It is important to standardize the *Toxocara* ELISA employed, including the definition of seropositivity.

The age of the studied population is another important factor in the *Toxocara* allergy association. While the studied populations described by some groups include children from 4 to 6 years of age, others include children up to 15 years or do not specify the age. It has been proposed that

heavy and continuous immune stimulation that occurs during chronic worm infections leads to the generation of an anti-inflammatory response that enhances parasite survival, minimizes immunopathology and simultaneously reduces excessive inflammatory reactions to allergens (Wilson and Maizels, 2004). An acute versus a chronic infection may determine whether *Toxocara* exacerbates or on the contrary protects from atopic diseases, including asthma. So far, there are no studies addressing this question. Additional epidemiological, clinical, but also experimental studies are required to address questions such as the effect of acute versus chronic, light versus heavy and sporadic versus regular *Toxocara* infection in relation to well-defined allergic manifestations. An understanding of any possible contribution of *Toxocara* infections to the pathogenesis of childhood asthma is important, particularly since it would provide a potential avenue for prevention.

Animal models for toxocariasis

Murine models for toxocariasis have been used to study the immunopathology caused by infection with this nematode. Most studies report on infection with *T. canis* and different strains of inbred mice such as BALB/c, C57BL/c, CBA/J and C57BL/6J. Results from these studies indicated that after infection the larvae are distributed throughout the body of the mice within 24–72 h. After a week, few if any larvae migrate to the lungs and there is evidence of accumulation in the brain. Granulomas develop within a week and can be found throughout the musculature, in the liver, kidneys, heart and sometimes in the eye (Kayes, 1997). An analysis of cell composition in BAL (bronchoalveolar lavage) indicates that at 2 weeks post-infection (p.i.) eosinophils account for more than 75% of the recovered cells compared with 25% in peripheral blood. This is in contrast to infection with *A. lumbricoides*, which results in high eosinophil levels that disappear when the larvae leave the lungs (Kayes *et al.*, 1987). Pulmonary inflammation develops as soon as 48 h after infection and can persist up to 3 months (Buijs *et al.*, 1994b; Kayes, 1997). The presence of a persistent pulmonary inflammation in the absence of few, if any, larvae in the lungs is intriguing. Bowman *et al.* reported that mice infected with

T. canis have high levels of circulating antigens that remain in the circulation for up to 8 months after infection. The authors suggest that persistent eosinophil accumulation in the lungs in the absence of larvae is due to these circulating antigens (Bowman *et al.*, 1987).

The number of embryonated eggs administered to mice is an important criterion in determining the quality and quantity of the immune response mounted against this helminth. The parasite-specific antibody response peaks around 14 days after infection, but it depends on the load of administered eggs and the strain of mouse used (Kayes *et al.*, 1985). Okada *et al.* observed that in *T. canis*-infected rats the number of eosinophils in peripheral blood and BAL increased markedly after infection, peaking at 12 days and 2 weeks, respectively. The authors suggest that LTB₄ (leukotriene B₄), PAF (platelet-activating factor) and IL-5 contribute to the accumulation of eosinophils in the lungs of rats infected with *T. canis* (Okada *et al.*, 1996).

However, IL-5 does not seem to play an important role in protective immunity, since previous studies have shown that the number of larvae in tissue does not change in IL-5 transgenic compared with non-transgenic mice (Dent *et al.*, 1999). Other studies have shown that mice that are genetically deficient in IL-5 carry similar numbers of larvae compared with those of wild type mice (Takamoto *et al.*, 1997). Furthermore, treatment of mice with anti-IL5 suppresses both blood and tissue eosinophilia but does not influence the number of larvae recovered from tissue (Parsons *et al.*, 1993). The increased production of IL-5 and eosinophilia observed in *Toxocara*-infected animals may play an important role in pathology. Eosinophil accumulation results in significant thickening of the mucosal smooth muscle layers of the airways. These cells secrete oxygen radicals that may contribute to airway and vascular damage (Yoshikawa *et al.*, 1996). A decrease in lung damage has been reported in *T. canis*-infected mice that are genetically deficient in IL-5 and have fewer eosinophils, suggesting a role for IL-5 and eosinophils in pathology (Takamoto *et al.*, 1997).

***Toxocara* versus allergic asthma**

Studies on the association between *Toxocara* infections and allergic asthma in animal models are limited. Few studies describe the effect of *T. canis*

infection on pulmonary pathology and lung function. In a study using tracheal contractility assays in mice and guinea pigs, the authors observed tracheal hyperreactivity within 24 h after *T. canis* infection followed by a hyporeactive state that persisted for more than 4 weeks p.i. (Buijs *et al.*, 1990). In another study using unconscious ventilated rats, Yoshikawa *et al.* showed that infection with *T. canis* leads to airway hyperresponsiveness at 14 days p.i. which coincided with the peak of eosinophil lung infiltration (Yoshikawa *et al.*, 1996).

Recently, we have measured airway reactivity *in vivo* after conscious unrestrained mice were exposed to increasing concentrations of the bronchoconstrictive agent methacholine. Results from this study showed that infection of BALB/c mice with 1000 embryonated eggs resulted in hyperreactivity of the airways that could be measured as early as 4 days p.i. and persisted up to 30 days p.i. At 60 days p.i. the reactivity of the airways decreased to background levels; however, pulmonary inflammation as well as increased levels of IgE and eosinophils in BAL persisted up to 60 days p.i. Evaluation of parasite burden revealed that *T. canis* larvae were still present in the lungs of infected mice at 60 days p.i. The presence of larvae in the lungs could explain the persistent pulmonary inflammation, airway hyperreactivity, eosinophilia and increased IgE production observed after a single infection of BALB/c mice with *T. canis*. (Pinelli *et al.*, 2005).

In a comparative study using a murine model for toxocarasis and a murine model for allergic asthma, we showed that pulmonary inflammation, eosinophilia and IgE production are induced, both after infection of BALB/c mice with 1000 embryonated *T. canis* eggs or after sensitization/challenge with ovalbumin (OVA) (Pinelli *et al.*, 2001). The murine model for allergic asthma described in this study has been shown to display immunological and pathological features similar to those observed in patients with allergic asthma (Hessel *et al.*, 1995). Increased pulmonary inflammation, eosinophilia and IgE production were also observed when C57BL/6 mice were infected with *T. canis* or treated with OVA; however, the intensity of the observed parameters were less pronounced. It was concluded that BALB/c is a better strain than C57BL/6 mice for further studies on the mechanisms involved in the

possible association between *T. canis* infection and asthma.

Using BALB/c mice we have observed that the severity of the pathological changes induced after infection is related to the infective dose. One or two weeks after infection of mice with 500 *T. canis* eggs, a slight peribronchiolitis with moderate perivascularitis was present. Eosinophilic granulocytes constituted a large part of this inflammation (Fig. 4.1a) which persisted up to 60 days after infection. Alveolitis, mainly comprising large alveolar macrophages with phagocytized material and eosinophils, was moderate. Hypertrophy of the mucous bronchiolar epithelium was strong (Fig. 4.1b) compared with uninfected controls. In time this response diminished slightly. At 60 days after infection, a clear inflammatory response and a moderate hypertrophy of the mucous cells was still present. The histological response was clearly dose related, as an infection with ten eggs did not result in an effect, while an infection with 1000 eggs was stronger.

To evaluate the effect of *T. canis* infection on the development of allergic asthma we have combined two experimental models, namely, a murine model for toxocariasis and a murine model for allergic asthma (Pinelli *et al.*, 2001). Table 4.2 shows how infection of BALB/c mice with 100 embryonated *T. canis* eggs followed by sensitization/challenge with OVA results in the enhancement of pulmonary inflammation when compared with mice that were only *T. canis* infected or with mice that were only OVA sensitized/challenged. A significant increase in perivascular infiltration and eosinophil influx was observed in mice infected with *T. canis* followed by OVA treatment compared with mice that were *T. canis*-infected only. Results so far indicate that a single low-dose infection can lead to exacerbation of pulmonary inflammation observed in BALB/c mice with experimental allergic asthma. Evaluation of the timing and frequency of infection on allergic asthma is currently underway.

Underlining Mechanism in the *Toxocara* Asthma Association

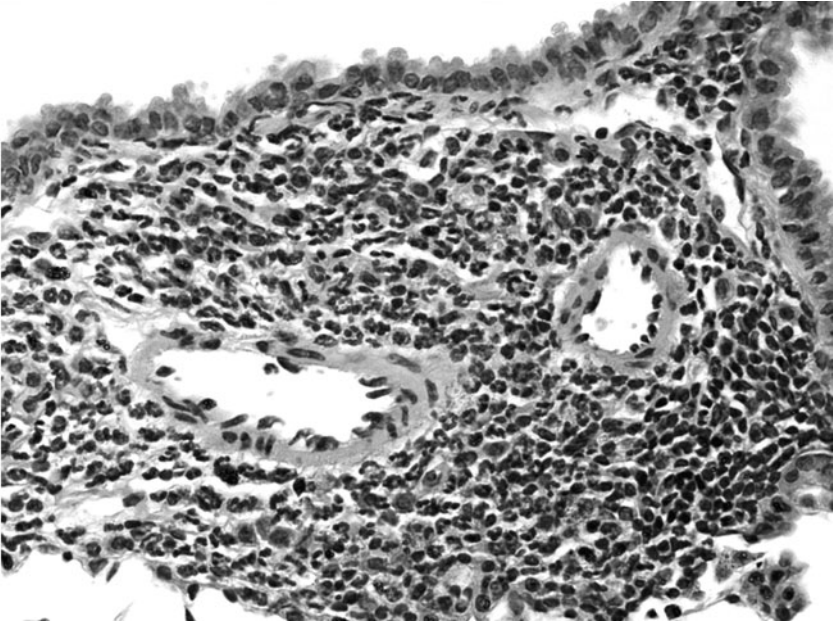
The mechanism by which infection with *Toxocara* spp. can result in allergic manifestations is probably related to the induction of a T-helper (Th2)

type of immune response, characteristic of both a helminth infection and allergic manifestations. Figure 4.2 shows a model for the possible association between *Toxocara* infection and allergic asthma. After infection, the induction of Th2 cells producing IL-4, IL-13 and IL-5 takes place. This type of immune response will be maintained locally for long periods of time due to the presence of living or dead larvae and to circulating parasite antigens. As a consequence, infiltration of eosinophils, macrophages and mast cells will take place. In addition, total IgE levels (only a minority is *Toxocara*-specific) are enhanced due to the induction of IL-4 and IL-13. In predisposed allergic individuals, an infection with this nematode may also enhance the production of allergen-specific IgE. After allergen challenge, IgE will interact with the allergen and bind to high- and low-affinity receptors on mast cells, eosinophils and macrophages that secrete several mediators involved in the induction of the early (PAF, leukotriene C₄ (LTC₄)) and late (prostaglandin (PG), LTC₄) asthmatic response. Based on this model, the immune response induced after *Toxocara* infection can cause enhanced allergic manifestations in allergic individuals.

The Role of Glycans in Cross-reactivity Between Helminth Parasites and Allergens

Current views are that glycan antigens are potent inducers of Th2 immune responses, and that they are responsible for the strong Th2 responses observed in helminth infections (Faveeuw *et al.*, 2003; van Remoortere *et al.*, 2003; Nyame *et al.*, 2004; Thomas and Harn, 2004). One of the factors that may contribute to the association between atopic disease and *Toxocara* infection is that *Toxocara* shares glycan antigens (cross-reactive antigens) with particular allergens derived from plants or other invertebrates. Cross-reactivity has been observed for many years using immunoassays among plant, arthropod, helminth and mollusc extracts. In particular, the highly immunogenic core α 3-fucose and/or β 2-xylose in N-glycans are shared among several organisms, and contribute to the observed cross-reactivity (Fig. 4.3 and Table 4.3) (Aalberse *et al.*, 1981;

(a)



(b)

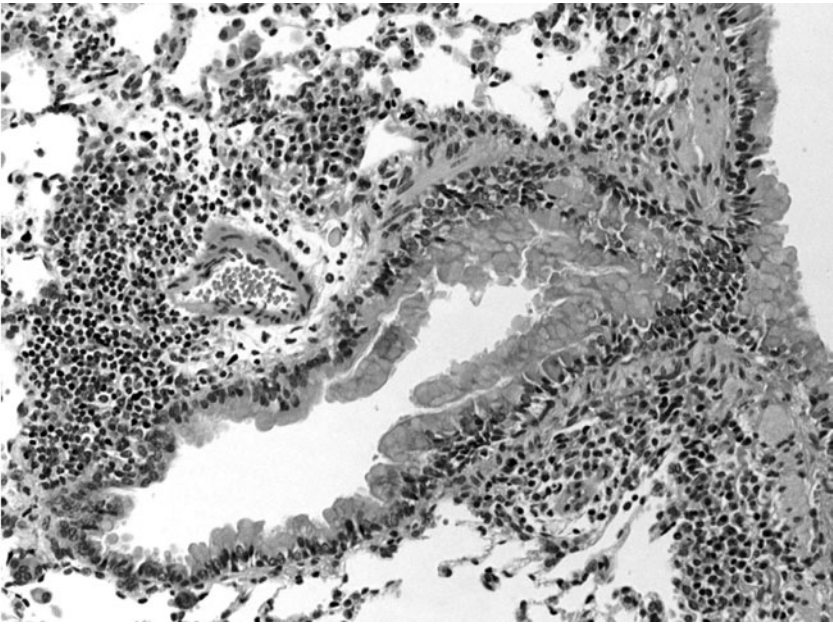


Fig. 4.1. Lung of mouse, 14 days after infection with 500 *Toxocara canis* eggs. (a) A moderate perivascular inflammation, mainly consisting of eosinophilic granulocytes, is lying next to a bronchiole (top and right) with slight hypertrophy of bronchiolar mucous cells. HE, 740 \times . (b) Hypertrophy of the bronchiolar mucous cells is present while peribronchiolitis (bottom right) and perivascularitis (centre left) are moderate. HE, 370 \times .

Table 4.2. Summary of pathological changes in the lung of BALB/c mice. Mice were either infected with 100 embryonated *Toxocara canis* eggs (*Toxocara*) or were *T. canis* infected followed by sensitization/ challenge treatment with ovalbumin (*Toxocara* + OVA) or sensitized/challenged with OVA only or PBS treated only (controls). Histological analysis was carried out at 60 days post-infection. Data are means of histological scores of five mice/group. *Indicates values significantly different ($P < 0.05$) from *T. canis*-infected mice. Histopathological lesions were scored: absent (0), minimal (1), slight (2), moderate (3), marked (4) and severe (5).

	<i>Toxocara</i>	<i>Toxocara</i> + OVA	OVA	Controls
Peribronchiolitis	1.0 ± 0.9	2.3 ± 1.0	1.7 ± 0.8	0
Perivascular infiltrate	2.0 ± 1.1	3.7 ± 1.2*	2.5 ± 1.5	0.5 ± 0.5
Hypertrophy bronchiolar mucous cells	2.8 ± 0.8	3.7 ± 0.8	2.8 ± 1.3	0
Alveolitis	0.3 ± 0.5	1.0 ± 0.6	1.2 ± 1.0	0.5 ± 0.5
Eosinophil influx	0.8 ± 0.8	3.3 ± 1.4*	2.0 ± 2.1	0

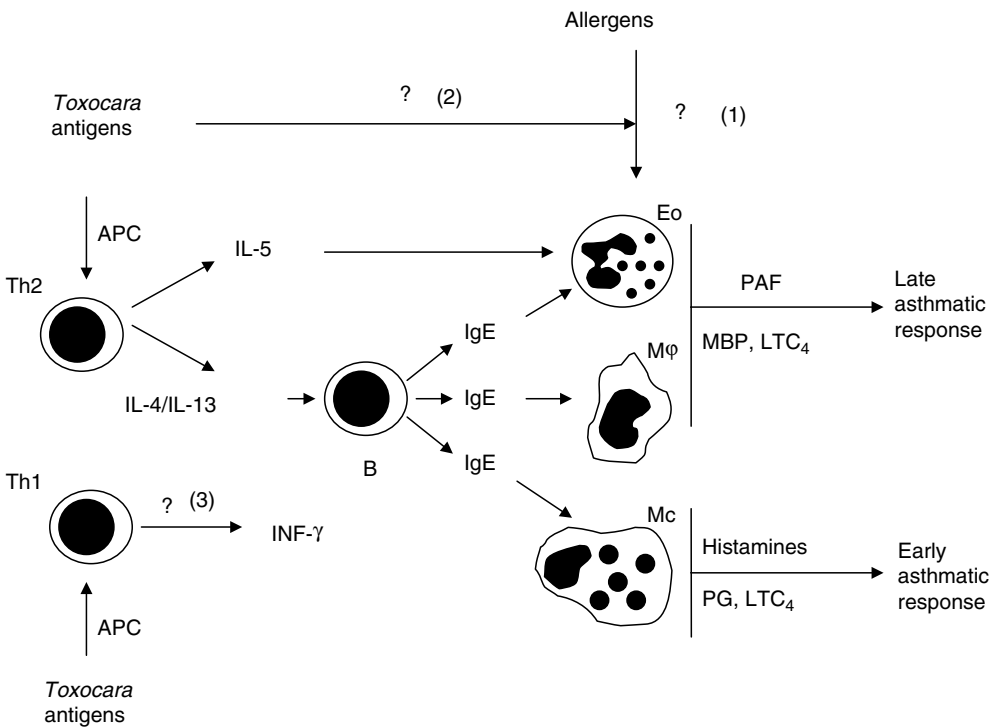


Fig. 4.2. Proposed mechanism underlining the association between *Toxocara* infection and allergic asthma. Infection with *Toxocara* spp. results in the induction of T-helper 2 (Th2) cells producing cytokines such as interleukin-4 (IL-4), IL-13 and IL-5. Due to larval migration to the lungs and the Th2 type of immune response induced, infiltration of eosinophils (Eo), macrophages (Mφ) and mast cells (Mc), in addition to increased levels of IgE, takes place. After allergen challenge, IgE will interact with specific allergen and bind to high- and low-affinity receptors on mast cells, eosinophils and macrophages, which secrete several mediators involved in the induction of the early (platelet activation factor (PAF), leucotriene C4 (LTC₄)) and late (prostaglandin (PG), LTC₄) asthmatic response. Several questions still remain, such as (1) Which are the allergens that trigger asthmatic responses? (2) Are *Toxocara* antigens also allergens? (3) Are parasite-specific Th1 cells induced locally (in the lung) and if so, what role do they play in the induction, maintenance or suppression of allergic asthma? MBP, major basic protein; INF, interferon.

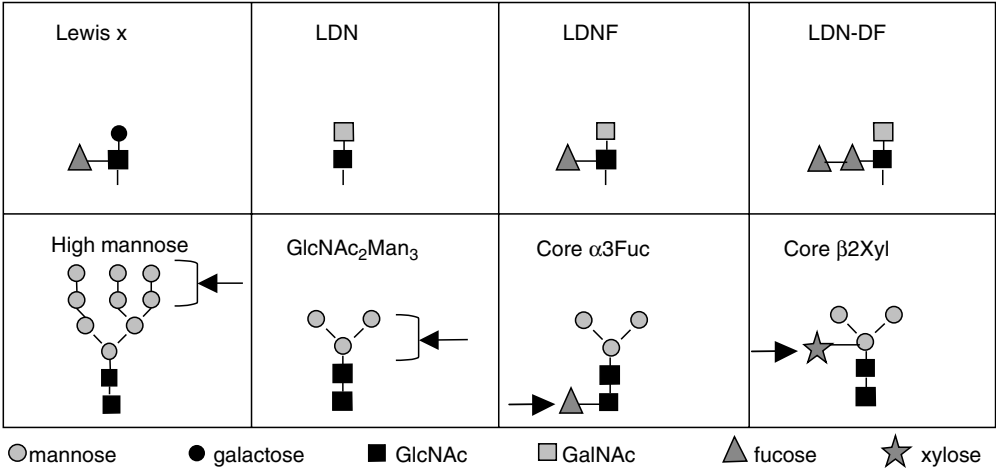


Fig. 4.3. Structures of glycan antigens occurring on parasitic helminths. The upper row shows terminal glycan antigens that can be part of a variety of complex-type protein- and/or lipid-linked glycans. The lower row shows high-mannose-type or oligomannose-type *N*-glycans. The arrows indicate the antigenic glycan moieties.

Table 4.3. Cross-reactivity between extracts and/or proteins derived from different organisms through common glycan antigens. Monoclonal antibodies (mAb) and immunopurified polyclonal antibodies (pAb) recognize glycan antigens within glycoproteins of the trematode *Schistosoma mansoni*, the nematode *T. canis*, the snail *Lymnaea stagnalis*, the plant protein horseradish peroxidase (HRP) or phospholipase A2 (PLA2) from honeybee venom.

Proteins or extracts derived from different organisms		LDNF	SMLDNF1	Man ₃ GlcNAc ₂ 100-4G11-A	mAb ^a	mAb ^b	Core-α3Fuc	pAb ^c	Core-β2Xyl pAb ^c
<i>S. mansoni</i>	Helminth (trematode)	+		+			+		+
<i>T. canis</i>	Helminth (nematode)	–		+			–		+
<i>L. stagnalis</i>	Snail	+		+			+		+
HRP	Plant	NT		+			+		+
PLA2	Honeybee	+		+			+		–

NT, not tested; –, not detected.
^aNyame *et al.* (2000).
^bVan Remoortere *et al.* (2003)
^cFaye *et al.* (1993).

Rasmussen *et al.*, 1985; Dissous *et al.*, 1986; Grzych *et al.*, 1987; Faye and Chrispeels, 1988; Aalberse and Van Ree, 1997; Thors and Linder, 1998; van Die *et al.*, 1999). In some cases it has been established that IgE antibodies from patients allergic to honeybee venom or plant substances such as cereal flour proteins bind to core α3-fucose- and/or β2-xylose-containing *N*-glycans (Weber *et al.*, 1987; Tretter *et al.*, 1993; Garciasado *et al.*, 1996; van Ree *et al.*, 2000). Core α3-fucose glycan

antigens are also commonly found on helminth glycoproteins, and IgE antibodies from sheep infected with the nematode *Haemonchus contortus* bind to this glycan epitope (van Die *et al.*, 1999). This stresses the importance of the conserved core α3-fucose epitope as a potential ‘pan-allergen’ (Wilson *et al.*, 1998) that may be involved in both helminth-mediated Th2-type immunity and in atopic diseases. This may perhaps also be the case for other shared immunogenic glycan antigens, such as

the Man₃GlcNAc₂ antigen that is found in plant, insect, snail and helminth species (van Remoortere *et al.*, 2003) (Fig. 4.3, Table 4.4). Several important questions, however, remain. It is not known whether carbohydrate-specific IgE has the potential to bind to their glycan antigens with sufficient affinity to trigger the activation and proliferation of mast cells and eosinophils (i.e. type I hypersensitivity reactions). If not, this IgE may have a protective function by blocking IgE binding sites on target cells, and so prevent the binding of potentially biologically active IgE. In addition, the molecular mechanisms that govern the production of carbohydrate-specific, or non-specific, IgE are not at all clear. It appears that, although glycan antigens generally induce strong Th2-associated humoral responses, this is not always accompanied by production of significant amounts of carbohydrate-specific IgE (Faveeuw *et al.*, 2003; Vervelde *et al.*, 2003).

The structures of *T. canis* glycan antigens are only partly characterized. The major glycan antigens of *T. canis* ES products (TES) have been shown to be *O*-methylated trisaccharides with resemblance to human blood group glycan antigens of the ABO system (Khoo *et al.*, 1991). In addition, it has been shown that the truncated *O*-linked GalNAc α -Ser/Thr structure (Tn antigen), a common tumour antigen, is present within TES products, and is also found on several other helminth parasites (Meghji and Maizels, 1986; Casaravilla *et al.*, 2003; Freire *et al.*, 2003). Preliminary screening of *T. canis* glycoproteins with a panel of mono- and polyclonal anti-glycan antibodies showed that the cross-reactive core α 3-fucose antigen that is found on many other helminths could hardly be detected. Antibodies against the core β 2-xylose and Man₃GlcNAc₂ epitopes bound to a few

restricted *Toxocara* glycoproteins within adult worms were found, suggesting the presence of these *N*-glycan-linked epitopes (van Die *et al.*, 1999; van Remoortere *et al.*, 2003). As described for other helminth infections, glycans account for a large proportion of the humoral immune response in *T. canis*-infected hosts (Kennedy *et al.*, 1987; Maizels *et al.*, 1987).

Recognition of Glycan Antigens by the Innate Immune System

Since carbohydrate molecules can only modulate immune responses through interaction with other molecules, more insight into the molecular interactions of parasite glycans with surface molecules on host immune cells, and their downstream signalling pathways, are pivotal to understanding their possible role in helminth infections and atopic diseases.

Antigen presenting cells (APCs), such as dendritic cells and macrophages, are crucially involved in the initiation and control of innate and adaptive immune responses (Janeway and Medzhitov, 2002). Glycan antigens linked to proteins or lipids are recognized by APCs through interaction with lectins, specific receptors that contain carbohydrate recognition domains (Drickamer, 1999; Figdor *et al.*, 2002). The family of C-type lectins, which require Ca²⁺ for binding their carbohydrate ligands, contains at least 15 known members on mammalian APCs (Figdor *et al.*, 2002) that are involved in the specific recognition of self and/or non-self carbohydrate antigens. To date, several C-type lectins have been found to function in pathogen recognition (Geijtenbeek

Table 4.4. APC lectins that recognize potential glycan antigens within parasitic helminths.

Lectin	APC	Ligands (putative) in helminths	References
DC-SIGN	Dendritic cell	Le ^x , LDNF	Van Die <i>et al.</i> (2003)
L-SIGN	Liver-sinosoid endothelial cell	(High mannose)	Feinberg <i>et al.</i> (2001) and Van Liempt <i>et al.</i> (2004)
Galectin-3	Macrophage	LDN	Van den Berg <i>et al.</i> (2004)
hMGL	Dendritic Cell/macrophage	LDN, LDNF, (α GalNAc)	van Vliet <i>et al.</i> (2005)

and van Kooyk, 2003; van Die *et al.*, 2004), whereas only a few have been shown to recognize self antigens (Geijtenbeek *et al.*, 2004).

One of the best-studied C-type lectins is DC-SIGN (Geijtenbeek *et al.*, 2000), which shows affinity for fucose as well as mannose (Feinberg *et al.*, 2001). DC-SIGN binds to different pathogens and glycan ligands, indicating that specificity is obtained through distinct requirements in the way in which the sugars are composed and presented (Appelmelk *et al.*, 2003; Guo *et al.*, 2004; van Liempt *et al.*, 2004). The functional consequences of lectin–glycan interactions are complex and not yet fully understood. They may depend on multiple factors, such as bound glycan antigen, or cross-talk with other receptors. After lectin-mediated binding of the glycan antigens, APCs can internalize these components to allow antigen processing and presentation, and thus initiate adaptive immune responses. Recognition and capture of glycosylated antigens by lectins on APCs (receptor-mediated uptake) can facilitate a much more efficient presentation of captured antigens as compared with soluble antigens (Salustio *et al.*, 1995; Engering *et al.*, 1997). C-type lectins can also interact with self glycoproteins to promote tolerance (Geijtenbeek *et al.*, 2004), and some pathogens are reported to target C-type lectins to escape immune surveillance (Geijtenbeek and van Kooyk, 2003).

Recently, we have identified several helminth glycans that are recognized by APC lectins (van Die *et al.*, 2003; van den Berg *et al.*, 2004; van Liempt *et al.*, 2004; van Vliet *et al.*, 2005) (Table 4.4). DC-SIGN expressed on immature dendritic cells (DCs) binds Lewis x (Le^x) antigens within *Schistosoma mansoni* egg glycoproteins and rapidly internalizes Le^x glycans into lysosomal compartments (Appelmelk *et al.*, 2003; van Die *et al.*, 2003). In addition to its presence in different schistosome species (Nyame *et al.*, 1998), Le^x has been demonstrated in the nematode *Dictyocaulus viviparus* (Haslam *et al.*, 2000). In a murine schistosome model, immunization with human serum albumin (HSA) protein carrying Le^x was over 1000-fold more potent in inducing antibody production as compared with HSA alone, and produced significantly higher levels of total IgE as well as HSA-specific IgG, IgG1 and IgE. Although Le^x glycans were required for IgE induction, they appeared not to be targets of the induced IgE response (Okano *et al.*, 2001), indi-

cating that the presence of Le^x on a particular protein may modulate DC maturation resulting in the development of an effector DC subset that selectively promotes Th2 responses. This may be mediated by the interaction of Le^x with Le^x -binding lectins, such as DC-SIGN in humans (van Die *et al.*, 2003) or macrophage galactose-type lectin 1 (mMGL1) in mice (Tsuiji *et al.*, 2002). MGL is a C-type lectin that is expressed on immature DCs and macrophages in skin and lymph node of mouse and man (Suzuki *et al.*, 1996; Valladeau *et al.*, 2001; Higashi *et al.*, 2002; Tsuiji *et al.*, 2002). Whereas mMGL1 is reported to recognize Le^x , both mouse MGL2 (mMGL2) and human MGL (hMGL) seem to show specificity for terminal GalNAc (Tsuiji *et al.*, 2002; van Vliet *et al.*, 2005). We therefore expect that the presence of α -GalNAc (Tn) antigens on *T. canis* ES may allow their recognition through mMGL2 and hMGL, a topic that is currently under investigation.

Summarizing, these data indicate that particular glycan antigens are strong inducers of Th2-cell-associated humoral responses and may be involved in the induction of specific or non-specific IgE. Further characterization of the glycan antigens that induce such IgE responses in different mammalian species, unravelling the underlying mechanisms and investigation into whether this IgE contributes to activation and proliferation of mast cells and eosinophils will be relevant for understanding and treatment of atopic diseases.

Concluding Remarks and Future Directions

The incidence of asthma is increasing worldwide, particularly in Western countries. Evidence from epidemiological studies suggests that infection with *Toxocara* worms contributes to the development of atopic diseases, including asthma. However, the parasite load, frequency of exposure and the genetic background are all factors that may influence the relationship between allergy and *Toxocara* infection. Common features in allergic asthma and toxocarasis are the induction of a Th2-cell-mediated immune response including the production of high levels of IgE, inflammation of the airways and the accumulation of eosinophils.

To establish and understand the relationship between *Toxocara* infection and allergic asthma, the

immunological and molecular mechanisms that can explain the observed association clearly need to be further investigated. Murine models have proved very valuable in investigating the possible factors that contribute to the observed association between these two disorders. Future studies should focus on questions such as: (i) Does infection with *Toxocara* induce, or, in contrast, protect against allergic manifestations? Could both possibilities be feasible? And if so, which are the circumstances that favour one or the other? (ii) Which are the allergens that trigger asthmatic responses in *Toxocara*-infected individuals? Do common structures, such as cross-reactive glycans within *Toxocara* antigens and (un)known allergens play a role? (iii) Which are the receptors on immune cells that recognize the parasite antigens/allergens and how

do they trigger the allergic responses? (iv) Once a definitive Th2 response is induced in *Toxocara*-infected individuals, can re-exposure to *Toxocara* further enhance allergen-specific IgE? (v) Are parasite-specific Th1 cells induced locally (in the lung) and if so, what role do they play in the induction, maintenance or suppression of allergic asthma? and (vi) Are cytokines such as IL-10 and transforming growth factor- β produced? Do these cytokines play any role in regulating the induced pathology? Results from studies that will provide answers to these and other questions will be essential to understanding the relationship between allergic asthma and *Toxocara* infections and will contribute to the development of alternative means to combat or prevent these diseases.

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5 The Significance of Cerebral Toxocariasis

Celia V. Holland and Clare Hamilton

Department of Zoology, Trinity College, University of Dublin, Dublin, Ireland

Introduction

The ability of a parasite to enter the brain of its host, and the consequences of such invasion, has always been a source of fascination for parasitologists. *Toxocara canis* demonstrates this facility not in its definitive host, the dog or fox, but in some of its paratenic or transport hosts, in particular the mouse. Humans are also known to harbour *Toxocara* larvae in their brains but the number of cases of cerebral infection described in the literature is small. This indicates that, in humans at least, the effects of brain involvement are likely to be cryptic and not easily observed or explained. Nevertheless, the significance of cerebral toxocariasis is multifaceted and has a number of ecological, evolutionary and public health implications and associations.

T. canis infects a wide range of paratenic or transport hosts – what is the ecological and evolutionary significance of these foci of infection? What is their relative capacity to maintain sources of infection under natural conditions? Which of these hosts are suitable animal models for human infection under laboratory conditions? What is the clinical and public health significance of low numbers of *Toxocara* larvae in the brain of a child?

The mouse model has provided us with good evidence that a range of murine behaviours is altered in *Toxocara*-infected mice and the magnitude of some of these alterations is related to larval burden in the brain. Furthermore, this model system may also provide insights into the impact

of chronic geohelminth infection on cognitive development, something that has proved to be very difficult to investigate in human subjects. In this chapter, we assess the available evidence concerning cerebral toxocariasis in paratenic hosts, including mice and humans. We also discuss how development of the mouse model can be used to explore the relative contributions of host genotype, behaviour and the immune response to brain involvement.

Toxocara Syndromes and Sources of Infection

The major features of the epidemiology and impact of toxocariasis in humans are summarized in Fig. 5.1 (adapted from Glickman, 1993). Visceral larva migrans (VLM) (Beaver *et al.*, 1952), ocular larva migrans (OLM) (Shields, 1984) and covert toxocariasis (Taylor *et al.*, 1988) are now well-recognized clinical entities associated with toxocariasis. Cerebral or neurological toxocariasis is much less well established clinically and its effects, in humans, are considerably less well understood.

Infected definitive hosts produce large numbers of eggs that contaminate the environment. The major route of transmission to humans is through the ingestion of embryonated ova from soil or soil-contaminated hands or food (Glickman and Schantz, 1981, Glickman, 1993). Recently, Woolf and Wright (2003) provided preliminary

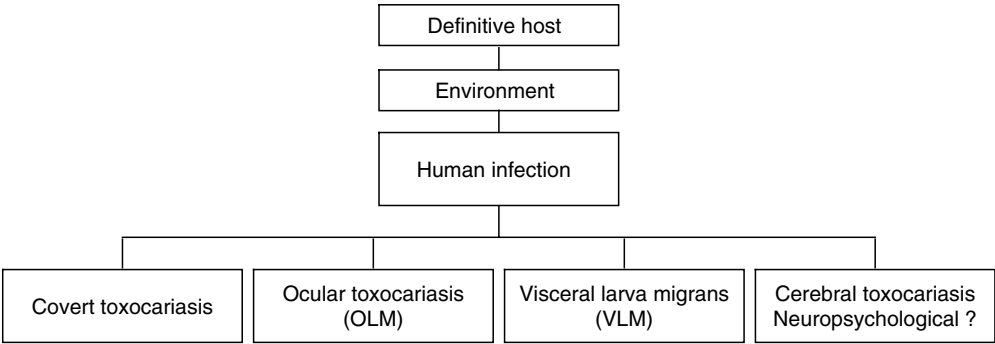


Fig. 5.1. The consequences of *Toxocara* infection in humans (adapted from Glickman, 1993).

data to demonstrate that potentially infective eggs can be detected in the fur of dogs, indicating that direct contact with dogs may be an underestimated route of transmission. Furthermore, infection may also occur as a consequence of ingesting *Toxocara* larvae from undercooked meat, including chicken and lamb (Nagakura *et al.*, 1989; Salem and Schantz, 1992).

The Significance of Paratenesis

Widespread environmental contamination with *Toxocara* ova also facilitates the infection of a wide range of ‘abnormal’ hosts, including mice and humans. In these abnormal or paratenic hosts, the immature second-stage larvae of the parasite undergo a somatic migration through the organs of the body but fail to reach maturity as adult worms in the intestine. What are the important functions and consequences of paratenesis?

Bush *et al.* (2001) define a paratenic host as a host in which development does not occur, but may serve to bridge an ecological, or trophic, gap in a parasite’s life cycle. Furthermore, paratenic hosts may help disseminate infective stages of the parasite or aid these stages in avoiding unfavourable conditions such as the temporary absence of a definitive host. In the case of *Toxocara*, definitive hosts such as dogs, cats and foxes release large numbers of potentially infective eggs into the environment. Some of these eggs may be ingested directly by other definitive hosts, others will desiccate and die in the soil or be consumed by paratenic hosts.

Our knowledge of the relative capacities of a range of vertebrate and invertebrate hosts to act as

paratenic hosts under natural conditions is virtually non-existent. In an important study, Dubinsky *et al.* (1995) examined a range of small mammals for the presence of *Toxocara*-specific antibodies in sera, and larvae in the brain and hind leg femoral muscles. The highest seroprevalence (32%) was detected in the common house mouse, *Mus musculus*, and the intensity of larvae was greater in mammals from suburban locations (Fig. 5.2). The authors concluded that small mammals could act as important foci for the circulation and maintenance of *Toxocara* in the environment and as indicators of environmental contamination.

In contrast to the paucity of data from feral hosts, a range of animals has been infected with *Toxocara* (predominantly *T. canis*) under laboratory conditions and the larval migration of the parasite has been determined. Animals include mice, rats, guinea pigs, hamsters, gerbils, chickens, quail, pigeons, rabbits, pigs, monkeys and earthworms. These studies reveal variations in experimental protocol, including infective dose and its administration, duration of infection and types of tissues and organs processed, making comparison between species difficult.

These animals may act as model systems for human infection, and their relative merits as models for ocular disease in particular have been evaluated by Fenoy *et al.* (2001). Some of these species are unlikely to function as paratenic hosts under natural conditions and therefore the ecological significance of their infection is questionable. A comparison of the different host–parasite systems is shown in Table 5.1, and particular attention has been paid to whether larvae were detected in the brain and any evidence for larval

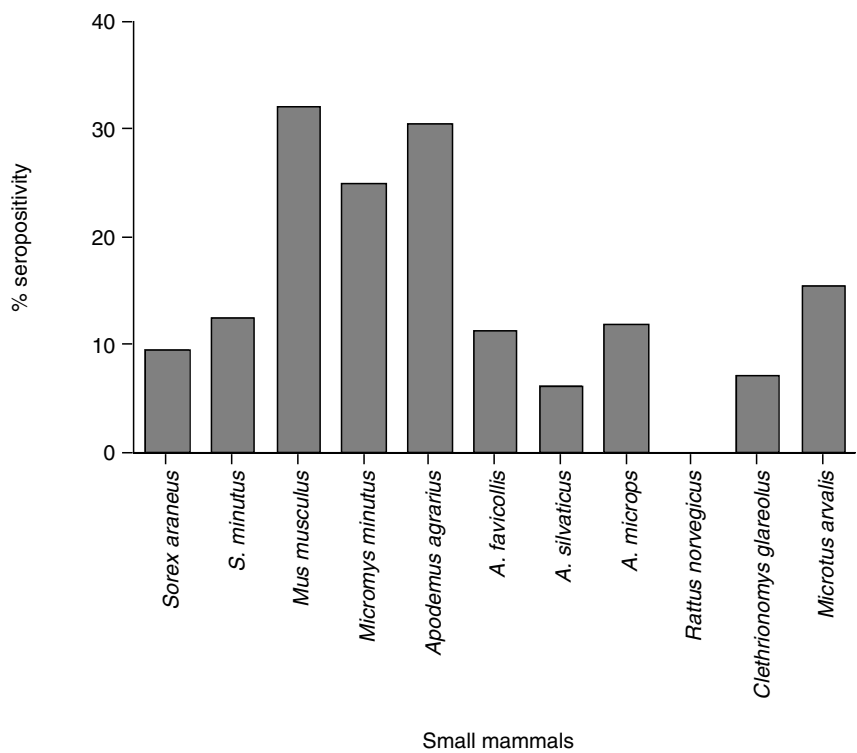


Fig. 5.2. The seroprevalence of *Toxocara canis* in a range of small mammals trapped in Slovakia (adapted from Dubinsky *et al.*, 1995).

accumulation over the course of infection. Where quantitative data are available, it is clear that larger numbers of larvae are found in the murine brain compared with other hosts. Evidence for accumulation was not sought in the majority of experiments undertaken in animals other than mice. It is of interest to note that accumulation does not appear to occur in gerbils.

Novel Model Systems for the Investigation of Larval Toxocariasis

Recently, the merits of two animal models for larval toxocariasis have been evaluated. They are the novel host, the Mongolian gerbil (Takayanagi *et al.*, 1999; Akao *et al.*, 2000; Alba-Hurtado *et al.*, 2000) and the pig, which was investigated originally by Done *et al.* (1960) and more recently by Helwich *et al.* (1999) and Taira *et al.* (2003) (Table 5.1) (see also Chapter 6, this volume).

Mongolian gerbils exhibit a high susceptibility to ocular infection by *T. canis* compared with mice, rabbits, guinea pigs and monkeys (Takayanagi *et al.*, 1999). Furthermore, the dark grey fundi of these animals made detection and observation of living *Toxocara* larvae much easier.

A quantitative assessment of VLM in the Mongolian gerbil has recently been described by Barrett and Holland (unpublished observations). The numbers of larvae detected in all organs and tissues was lower than that recovered from mice and there was no evidence of accumulation in the brain over the course of infection. Nevertheless, larval numbers in the brain remained stable whereas they declined in the other organs. In contrast, Akao *et al.* (2003) described cerebellar ataxia in gerbils infected with *T. canis*. Forty-nine per cent of gerbils (6/13) showed progressive neurological disorders from day 50 of infection, with severe degenerative changes being observed in the cerebellum of some of the infected gerbils.

Table 5.1. *Toxocara* infection in experimental animals: evidence for brain involvement.

Host	Dose (number of ova) (<i>n</i>)	Duration of infection	Presence in brain	Quantitative (number of larvae)	Accumulation	Reference
Primate Cynomolgus monkeys	45,000 (20)	Day 220	Yes	NA	NA	Glickman and Summers (1983) ^a
Pig	50,000 (35)	Day 49	Yes	Low numbers 0–10	None	Taira <i>et al.</i> (2003) ^b
Chicken	1,500 (17)	Day 142	Yes	Low numbers 0–6	None	Galvin (1964) ^c
Pigeon	1,500 (21)	Day 142	Yes	Low numbers 0–12	None	Galvin (1964) ^d
Quail	1,500 (27)	Day 200	Yes	Low numbers 0–10	None	Nakamura <i>et al.</i> (1991) ^e
Rabbit	50,000 (4)	Day 159	Yes	NA	NA	Kunishige (1964)
Rabbit	100,000 (4)	Day 66	Yes	NA	NA	Church (1975) ^f
Gerbil	1,000 (13)	Day 50	Yes	NA	NA	Akao <i>et al.</i> (2003) ^g
Gerbil	100, 1,000 1,000t, 3,000	Day 120	Yes	0–33	None	Barrett and Holland (unpublished observations) ^h
Rat Wistar	3,000 (12)	Day 28	Yes	NA	NA	Burren (1972) ⁱ
Hamster	2,000 (17)	Day 84	Yes	NA	NA	Burren (1972) ^j
Mouse	2,500 (13)	Day 13	Yes	$x = 90.15$ $x = 115$	NA	Sprent (1955) ^k

Continued

Table 5.1. (*Continued*)

Host	Dose (number of ova) (<i>n</i>)	Duration of infection	Presence in brain	Quantitative (number of larvae)	Accumulation	Reference
Mouse						
Canberra	1,000 (80)	Day 122	Yes	$x = 6.9$ (day 4) to 48.7 (day 122)	Yes	Dunsmore <i>et al.</i> (1983)
Canberra	5,000 (24)	Day 65	Yes	$x = 156.5$ (day 8) to 281.3 (day 65)	Yes	Dunsmore <i>et al.</i> (1983)
C57BL	1,000 (30)	Day 50	Yes	$x = 16.5$ (day 4) to 90.2 (day 50)	Yes	Dunsmore <i>et al.</i> (1983)
LACA	100, 1,000 3,000 (45)	Day 26	Yes	Day 4 $x = 24$ Day 14 $x = 82$ Day 26 $x = 94$	Yes	Skerrett and Holland (1997) ⁱ

^aLarval penetration occurred in all infected monkeys; severe lesions in cerebellum: 3/20 neurological symptoms.

^bNo abnormal behaviour; most larvae in lungs.

^cMost larvae in liver.

^dMost larvae in liver.

^eMost larvae in liver; no neurological symptoms.

^fMore larvae in cerebral hemispheres versus cerebellum.

^gNeurological symptoms in 6/13; severe lesions in the cerebellum.

^hNo behavioural abnormalities; numbers in brain stabilized over time.

ⁱMost larvae in the cerebellum; 11/12 had larvae in the brain.

^jPattern of distribution differed from rat; all hamsters had larvae in the brain.

^kMore larvae in brain compared with other ascarids.

^lSignificant individual variation.

It is interesting to note that in the observations of Barrett and Holland (unpublished observations) where some of the gerbils received the same dose as those studied by Akao and colleagues (1000 ova), no neurological symptoms were observed up until day 120 of infection. Akao *et al.* (2003) argue that no suitable animal model for neurological toxocariasis exists at present and the development of irreversible brain damage after chronic infection with *T. canis* suggests that the gerbil is a suitable/alternative model. We suggest that the evidence in humans outlined below indicates that effects in humans are subtle and cryptic and the effects in mice may be more appropriate. Furthermore, from the ecological point of view, the gerbil is unlikely to play as important a role as a paratenic host under natural conditions, which may explain the marked pathogenicity observed.

Recent observations by Sato *et al.* (2004), who infected both Mongolian gerbils and mice (outbred ICR and inbred BALB/c) with the ascarid *Baylisascaris transfuga*, revealed a marked pathogenicity associated with infection in gerbils compared with mice. In mice, the parasite caused symptomatic latent larva migrans of limited extent and duration, whereas the infection was fatal in gerbils. The host reaction in the brain differed between the two hosts: in mice, larvae were immobilized in granulomatous reactions; whereas in gerbils, the larvae were free of host reaction and mobile, causing extensive malacia. These observations underline how the course of infection may differ radically between laboratory models and the need to select the model with care based upon its specific relevance (i.e. to humans and/or other paratenic hosts).

Taira *et al.* (2003) investigated the pig, as a paratenic host and as an appropriate model for human infection. Pigs exhibit physiological similarities to humans, in addition to body size, and have been used as an animal model for other human helminth infections (Boes and Helwich, 2000). *Toxocara* larvae were eliminated early in infection, either before or after leaving the lungs, and only a few larvae reached the eye or the brain (Helwich *et al.*, 1999; Taira *et al.*, 2003). Therefore, pigs would appear to be less satisfactory paratenic hosts compared with mice or chickens (Taira *et al.*, 2003). Furthermore, unlike mice, detection of larvae in their tissues and organs is very time-consuming and they do not form part of the life cycle

under natural conditions. Nevertheless, consumption of undercooked pork may pose a potential zoonotic risk for humans.

Cerebral Toxocariasis in Humans

The evidence for cerebral toxocariasis in humans is patchy but there are some indications that larval involvement in the human brain may have some subtle public health implications. Magnaval *et al.* (1997), after reviewing the English literature from the early 1950s to 1997, reported on only 12 published cases of neurological toxocariasis as determined by finding *Toxocara* larvae in the cerebrospinal fluid (CSF) or in the brain and/or by immunodiagnosis of the CSF. This included one case of a child that died as a result of non-accidental injury. The child was said to have cried incessantly and larvae were detected in the pons, right frontal lobe and white matter of the cerebellum (Hill *et al.*, 1985).

Magnaval and colleagues (Magnaval *et al.* 1997) sought to characterize a recognizable cerebral or neurological syndrome associated with *Toxocara* infection among adults in France. Seropositive cases, with neurological symptoms in the absence of an aetiological diagnosis, were compared with controls that were matched for age, sex, ethnicity and travel history outside the European community. The authors concluded that migration of *Toxocara* larvae in the brain does not induce a recognizable neurological syndrome but is correlated with several risk factors including exposure to dogs, rural residence and dementia. It should be noted that this study was confined to adults, even though children may be at greater risk as a consequence of their smaller brain size and higher potential exposure.

In contrast, two American studies have specifically focused upon the relationship between *Toxocara* seropositivity and neuropsychological parameters in young children. While Worley *et al.* (1984) failed to demonstrate a relationship between *Toxocara* seropositivity and cognitive abnormalities after controlling for social class, Marmor *et al.*'s (1987) findings suggest a role for toxocariasis in subtle effects on cognition. Sera were obtained from over 4000 children aged 1–15 years submitted as part of the New York City Department of Health, Lead Screening

Programme. In a complex and detailed investigation, 155 case-control pairs (matched by age, sex, lead category and time of sampling) were compared in terms of a range of parameters including neuropsychological examinations. More parents of cases reported hyperactivity in their children than did parents of controls, and furthermore, cases performed less well on several measures of neuropsychological tests.

The authors highlight some difficulties associated with their findings, including the difficulty in establishing the sequence of events in terms of exposure to lead and/or *Toxocara* and the causality of the sequelae described. Gillespie (1993) suggested that the relationship between *Toxocara* infection and neurological deficits is likely to remain obscure until there are such studies, which follow individual children, with mild or asymptomatic infection, through the course of their infection, and examine the changes in cognitive function in comparison with matched controls. Nevertheless, as recently stated by Maizels *et al.* (2000), if the evidence of impaired neurological function is correct, the extent of *Toxocara*-induced pathology may be much wider than currently documented.

Another piece of evidence concerning neurological phenomena was provided by a recent study of ocular toxocariasis. The prevalence of consultant-diagnosed ocular toxocariasis was determined in over 120,000 Irish schoolchildren ranging in age from 3 to 19 years (Good *et al.*, 2004). A case-control study (four matched controls by age, sex and rural/urban status by school and county) revealed a strong association between having had a convulsion and ocular toxocariasis.

To conclude, evidence from humans is clearly fragmentary and indicates that if a recognizable neurological syndrome exists, its effects are likely to be cryptic and difficult to detect in human subjects. In our view, this strengthens the choice of the mouse as the most appropriate animal model to explore the significance of cerebral toxocariasis. As described earlier, the severe lesions and sequelae associated with cerebral infection in the gerbil and the monkey would not appear to reflect the characteristics of human infection. Furthermore, mice also provide ease of manipulation (with the wide availability of inbred and knockout strains), they have small organs for easy detection of larvae and they form a natural part of the *Toxocara* life cycle.

The *Toxocara* Mouse Model: Brain Involvement, Accumulation and Behavioural Alterations

Early work by Sprent (1955) described the abundance of *Toxocara* larvae in the murine brain compared with other ascarid larvae. Burren (1971) was interested in the location of *Toxocara* larvae within the murine brain. He divided the brain into cerebral hemispheres, brain stem and cerebellum and counted the number of larvae by means of brain squashes. It was observed that the average weight of murine cerebral hemispheres was heavier than the cerebellum by a factor of 3.6, yet the former contained fewer larvae per unit of tissue. The author concluded that, in general, the number of larvae per 50 mg of tissue was higher in the cerebellum relative to other regions.

Over a decade later, Dunsmore *et al.* (1983), in an important paper, established quantitative evidence for the accumulation of *T. canis* larvae in the brains of mice (Fig. 5.3). The authors reinforced the observations of Sprent (1955) and Burren (1971) that larvae in the brain are not encapsulated or surrounded by any cellular reaction. It is suggested that it is advantageous for the larvae to accumulate in a site of immune privilege where they are protected from the host lymphoid system. Furthermore, the larvae remain alive and can therefore be transmitted to an appropriate definitive host upon ingestion of murine cerebral tissue.

Other workers have demonstrated significant variation in the numbers of larvae detected from individual outbred mice, particularly in mice receiving a high infective dose (Skerrett and Holland, 1997; Cox and Holland, 2001a). For example, outbred LACA mice receiving a dose of 3000 ova harboured an average of 94 (\pm SD 106.4) larvae per brain with a range of 270 ($n = 5$ mice) (Skerrett and Holland, 1997). This variation indicates the probable contribution of host heterogeneity in genetics and immunological response. Bardon *et al.* (1994) in a longer-term experiment described the distribution of *T. canis* larvae in BALB/c mice at 63 days and 1 year post-infection. The numbers of larvae were higher in the brain compared with the carcass upon both dates but did not differ significantly over time for either part of the body (Fig. 5.4).

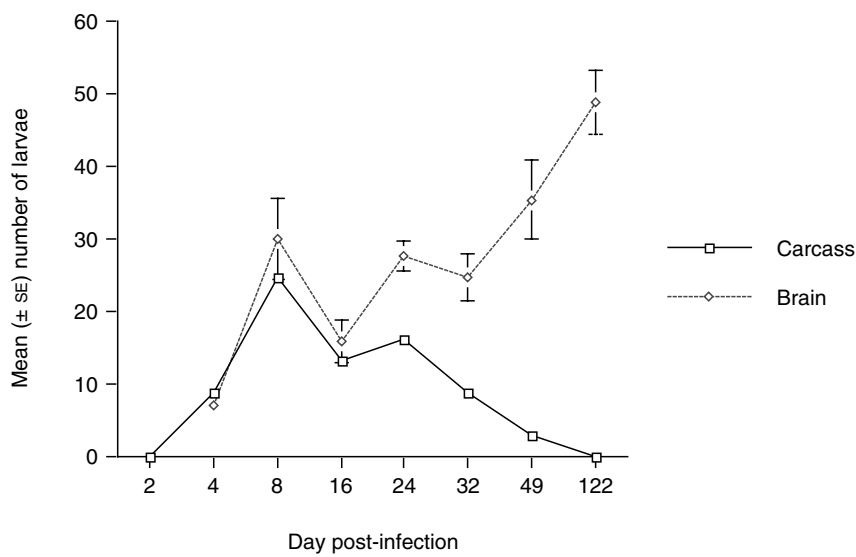


Fig. 5.3. The mean number of larvae of *T. canis* (± SE) recovered from the carcass and brain of Canberra mice sacrificed over 122 days post-infection (adapted from Dunsmore *et al.*, 1983).

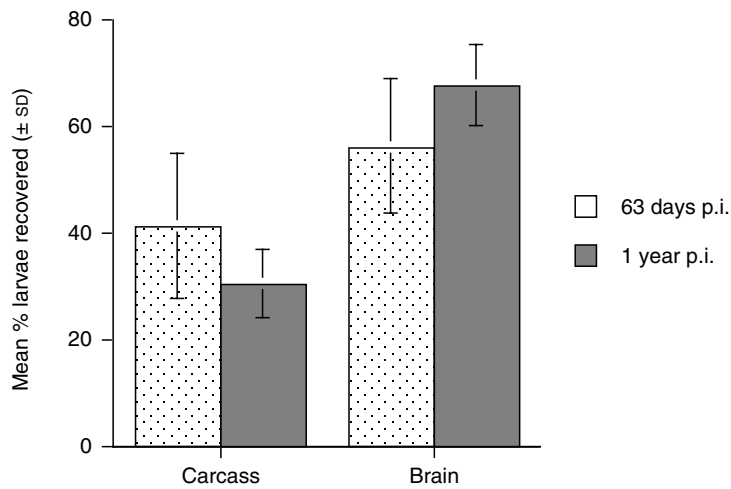


Fig. 5.4. The mean % of *T. canis* larvae (± SD) recovered from BALB/c mice on day 63 and 1 year post-infection (adapted from Bardon *et al.*, 1994).

As stated earlier, virtually no data are available on the larval migration of *T. canis* in feral, paratenic hosts. This is unfortunate, both in terms of establishing what hosts are actually important as reservoirs of infection and to give a realistic assessment of larval burden, under natural conditions. Dubinsky

and colleagues recorded the number of larvae in the brains of naturally infected small mammals, which ranged from 1 to 13 larvae per animal (Dubinsky *et al.*, 1995). These numbers are similar to the ranges observed in experimental animals after a dose of 100 ova (Cox and Holland, 2001a).

Neuropathology

The migration of *T. canis* larvae through the tissue of the CNS results in, as one might imagine, varying degrees of pathology. Summers *et al.* (1983) noted necrosis, cavitation and perivascular cuffing in the brains of mice infected with 1000 *T. canis* eggs. Moreover, foci of parasites were often found in close proximity to the lesions. The lesions described were most frequently noted in the heavily myelinated tracts of the brain, including the corpus callosum, internal and external capsules, cerebellar peduncles and the cerebellar medulla. Inflammatory cells were also observed in the areas surrounding parenchymal necrosis, with the predominant cells being lymphocytic, although plasma cells, eosinophils and neutrophils were also noted. Despite the cellular activity, the larvae appeared to be viable at the time of sacrifice. As part of a larger study, Dolinsky *et al.* (1985) noted the neuropathological effects of *T. canis* larvae in the brains of infected mice over the course of infection. Those mice sacrificed at 10 days post-infection had numerous focal haemorrhages on the dorsal surface of the cerebrum and cerebellum. Although fibre degeneration and the presence of larvae were noted in

the brain on day 51 post-infection, the most severe pathological changes were noted on day 86 post-infection, where there was marked degeneration of fibre pathways, including the corpus callosum, cerebellar peduncles and dorsal cerebellar medulla (Fig. 5.5). It is interesting that in both studies, larvae showed an affinity for the white matter tracts, which may reflect a selective tropism for myelin (providing nutritional advantages), or simply the pathway of least resistance through the brain (Summers *et al.*, 1983). In a study of several different inbred strains and one outbred strain of mouse, Epe *et al.* (1994) reported demyelization, focal malacia and mixed-cell infiltration in the brains of infected mice. Larvae were found in all areas of the brain except the bulbi olfactorii, but there was an obvious absence of granuloma or capsule-forming inflammation in all areas.

In humans, the frequency and localization of *T. canis* larvae in the brain is largely unknown. Of the few cases reported, larvae have been recorded in the leptomeninges (Dent *et al.*, 1956), grey and white matter of the cerebrum and cerebellum (Moore, 1962; Hill *et al.*, 1985), and the thalamus (Beautyman *et al.*, 1966) and spinal cord (Dent *et al.*, 1956). In a recent paper, Moreira-Silva *et al.* (2004) reviewed all available literature on human neurological toxocariasis, and summarized the

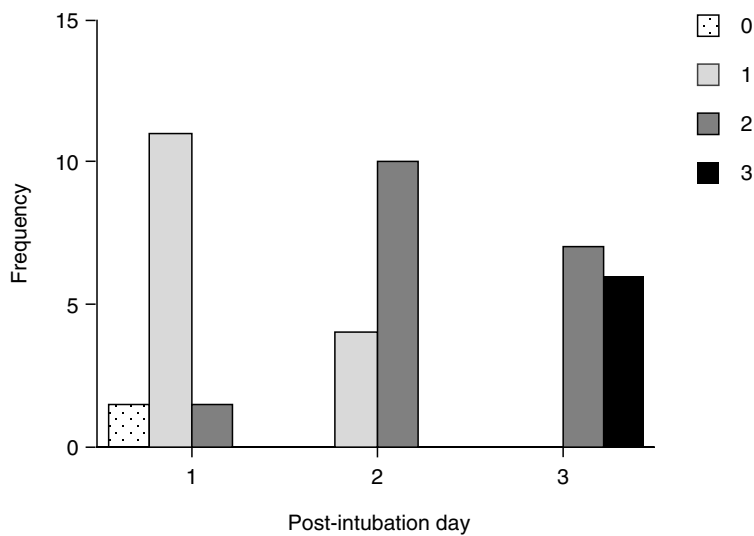


Fig. 5.5. The frequency of the pathology rating scores (0–3) in each *Toxocara* group: post-intubation day 1 = 10 days, post-intubation day 2 = 51 days and post-intubation day 3 = 86 days (adapted from Dolinsky *et al.*, 1985).

main observations in each case. Of nine cases studied at autopsy, all reported granulomas containing larvae in various regions of the CNS, including the cerebellum, medulla, thalamus and pons. In another case, lesions were noted in the white matter. In two suspected cases of neurological toxocariasis described in the paper, each reported neurological lesions, with one case reporting lesions in the spine-bulbar border and in the pedunculus cerebellaris.

The clinical significance of cerebral toxocariasis in humans remains unclear since there are usually other factors involved when the patient presents such as epilepsy, dementia and mental retardation (Glickman *et al.*, 1979; Magnaval *et al.*, 1997; Kaplan *et al.*, 2004). In mice, however, the picture is somewhat clearer, and there appears to be an evident effect of cerebral infection.

Behavioural Alterations

There is now considerable evidence to show that laboratory mice, both outbred and inbred, exhibit a range of altered behaviours after infection with *T. canis*. Behaviours explored include baseline activity, exploration, response to novelty, anxiety, learning, memory and social behaviour. Furthermore, the magnitude of the change can, in some cases, be correlated with larval burden in the brain (Cox and Holland 1998, 2001a,b). A recent review concluded that these effects are unlikely to be an example of a specific, and hence adaptive, parasite-altered host behavioural change, but more a pathological side effect of infection (Holland and Cox, 2001).

Effects on learning and memory may be of particular interest with respect to human infection, and also as regards the use of the mouse model to explore the effects of chronic helminth infection on cognitive development. Preliminary data were obtained from outbred LACA mice exposed to a water-finding apparatus (Cox and Holland, 2001b). The experiment was designed to assess the ability of mice to gather information from a novel environment and to remember the location of a specific resource within that environment. The latency to relocate the resource, in this case a water tube, after a period of deprivation was considered to be an indication of memory capacity of the mice. Mice with moderate and

high-intensity larval numbers in the brain showed latency to enter the alcove, find the water tube and drink from it compared with controls, although this did not reach statistical significance (Fig. 5.6a). In addition, the control mice contained the highest percentage of mice that actually drank once they found the water tube compared with the infected groups in which the proportion that drank was much lower (Fig. 5.6b).

Effects on exploration and motor performance were also noted. An early study by Dolinsky *et al.* (1981) reported that *T. canis*-infected mice demonstrated decreased exploratory behaviour, motor performance and spatial awareness compared with uninfected mice. In a similar study, by the same authors, infected mice displayed a range of altered behaviours over the course of infection, including decreased exploration, sensorimotor skills and shock avoidance, with changes being more pronounced in chronic infection (Dolinsky *et al.*, 1985). Other authors have noted that *T. canis* infection increases hyperactivity (Hay *et al.*, 1985, 1986), and decreases motor performance (Hay and Aitken, 1984) in mice, and affects learning and memory in rats (Olson and Rose, 1966). The effects on hyperactivity may be of particular interest with respect to infection in children.

Extending the Mouse Model

The abundance of data available on the effects of *T. canis* larvae in the brain on paratenic host behaviour (particularly the mouse) highlights the need for further investigation into the significance of cerebral infection.

As stated earlier, a number of studies have reported the presence, and accumulation, of *T. canis* larvae in the brains of infected mice (Sprent, 1955; Burren 1971; Dunsmore *et al.*, 1983). In a more recent study, Epe *et al.* (1994) examined the behaviour and pathogenicity of second-stage *T. canis* larvae in four different inbred mice strains and one outbred strain. They reported the presence of larvae in the brains of all mice from weeks 4 to 21 post-infection, with BALB/c mice carrying the highest larval burden between 8 and 21 weeks post-infection. They also reported CNS symptoms (lethargy, kyphosis, paresis, lack of coordination and tremor) of the mice, and demonstrated that as many as

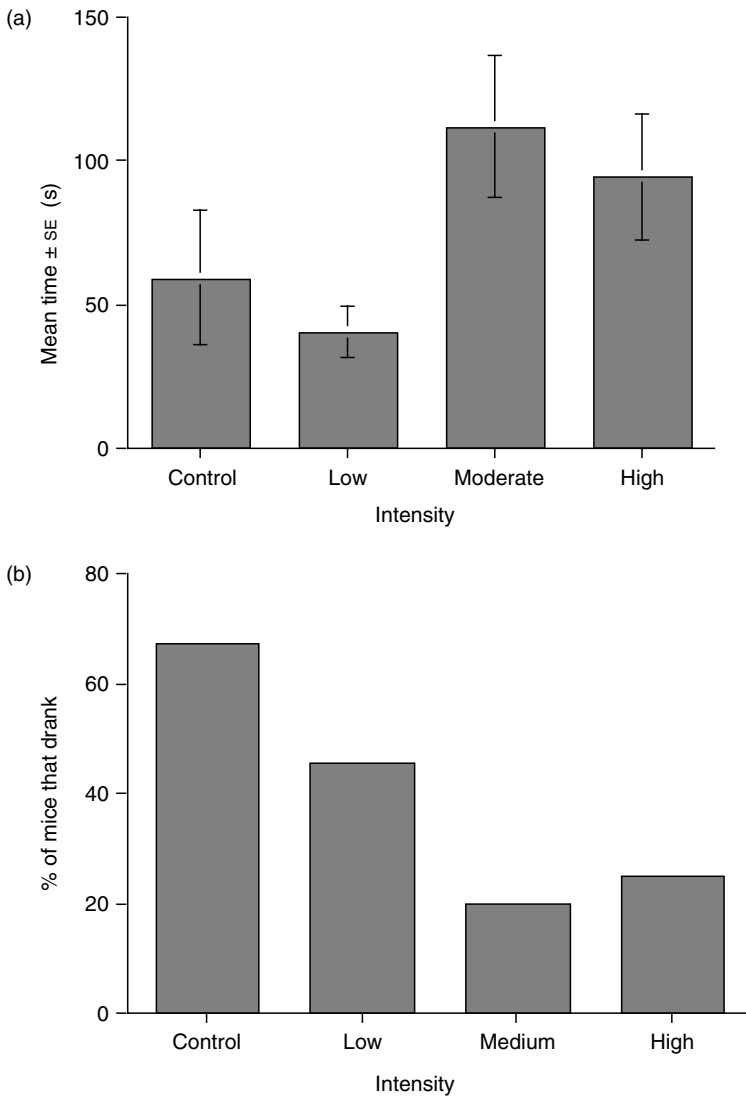


Fig. 5.6 (a) The mean time (\pm SE) in seconds spent by control, low, moderate and high-intensity outbred LACA mice to find the drinking tube in the water-finding task (adapted from Cox and Holland, 2001a). (b) The percentage of control, low, moderate and high-intensity outbred LACA mice that drank from the water tube in the water-finding task (adapted from Cox and Holland, 2001a).

80% of the mice were affected by week 17 post-infection, and that increasing numbers of mice died at this time. Earlier studies, cited in the same paper, reported that the onset of CNS-symptoms in *T. canis*-infected mice generally coincide with the beginning of chronic infection, and that these symptoms may be attributed to

tissue damage caused by migrating larvae in the brain.

Previous studies have reported variation in the number of *T. canis* larvae recovered from the brains of both inbred and outbred mice (Epe *et al.*, 1994; Skerrett and Holland, 1997; Cox and Holland, 2001a,b), suggesting a role of immunity,

and perhaps host genetics, in the establishment of cerebral infection. During migration, *T. canis* larvae release excretory–secretory (ES) antigens, and shed surface components stimulating an inflammatory response (Buijs *et al.*, 1994). Helper T cells are important in the control of parasitic infections, either by directly mediating cellular responses or by regulating antibody production (Scott *et al.*, 1989). They are generally classified into two groups – Th1 and Th2 – based on their different functions in immunity. Th1 cells tend to provoke cell-mediated immunity, and are defined by their production of the cytokines interleukin (IL)-2, interferon (IFN)- γ and tumor necrosis factor (TNF)- β . Th2 cells, on the other hand, provoke more of a humoral response, and are defined by their production of IL-4, IL-5, IL-6, IL-10 and IL-13 (Mosmann and Coffmann, 1987). Both types of T-helper cells can influence and regulate each other by the cytokines they secrete (Janeway *et al.*, 2001).

T. canis infection has been reported to induce a Th2 systemic immune response, with the production of the antibodies IgG1 and IgE, and the cytokines IL-4, IL-5 and IL-10 (Del Prete *et al.*, 1991; Wang *et al.*, 1995; Kuroda *et al.*, 2001). The immune response against *T. canis* larvae in the brain, however, has received much less attention. Dunsmore *et al.* (1983) found that larvae in the brains of infected mice were not encapsulated by any cellular reaction, and that they remained alive, as verified by observing their movement in tissue preparations. This finding was in contrast to the larvae found in other organs such as the kidney and muscles, which were rapidly encapsulated by a host reaction. The apparent lack of immune response in the brain begs the question, do the larvae migrate to this organ to seek a site of immune privilege? Studies on other parasitic infections of the CNS have demonstrated that this may not be the case, and in fact, often, the cytokines produced in response to infection are actually responsible for the induction of pathology.

Neurocysticercosis (NCC) is a common infection of the CNS, acquired when humans ingest eggs from the environment or food containing live cysticerci. The control of this disease has been shown to involve both a Th1 and Th2 profile. Cerebrospinal fluid (CSF) from 14 patients with NCC was shown to contain elevated levels of IL-5 and IL-6, but decreased levels of the pro-inflammatory mediator TNF- α (Evans *et al.*, 1998). The raised levels of IL-5 were suggested to coincide

with the increased levels of circulating eosinophils, since one of the main actions of IL-5 is to stimulate eosinophil maturation, migration and activation. A similar study of CSF from 22 patients with NCC showed raised levels of IL-5 and IL-10, and decreased levels of IFN- γ and TNF- α , indicating a dominant Th2 immune response (Rodrigues *et al.*, 2000). However, an earlier study on brain tissue collected from four patients who underwent a craniotomy for clinical complications due to suspected NCC demonstrated the presence of Th1 cytokines IL-2, IL-12 and transforming growth factor (TGF)- β , and lack of IL-4 and IL-6, indicating a Th1 immune response (Restrepo *et al.*, 1998). The lack of eosinophils and mast cells, and the presence of natural killer cells and macrophages also suggested a Th1 response.

Murine cerebral toxoplasmosis, caused by the protozoan parasite *Toxoplasma gondii*, is known to induce a Th1 immune response in the CNS (Suzuki *et al.*, 2000; Suzuki, 2002a,b; Schluter *et al.*, 2003). A study on C57BL mice demonstrated increased expression of the Th1 cytokines IL-2 and IFN- γ , and decreased expression of the Th2 cytokines IL-4 and IL-5, in the brain (Gazzinelli *et al.*, 1993). There was also increased expression of TNF- α and inducible nitric oxide synthase (iNOS) and, in the later stages of infection, IL-10 and IL-6, indicating a shift toward a Th2 response. *In vivo* neutralization of IFN- γ resulted in dramatic down-regulation of TNF- α , which in turn resulted in decreased expression of iNOS, leading to severe encephalitis and increased mortality. A more recent review reported that the induction of a pro-inflammatory Th1 cytokine response is a key event in the initiation of immunity to *T. gondii* (Sarciron and Gherardi, 2000). An increase in the number of cerebral cysts, induced by IL-4, leads to an increase in IFN- γ , TNF- α and IL-6, and subsequent control of the parasite. The production of IL-12 from activated macrophages also leads to the activation of natural killer cells and T cells, and the production of IFN- γ , and thus control of the parasite.

Cerebral malaria (CM) is a life-threatening complication of infection with the protozoan parasite *Plasmodium falciparum*. Investigations using different mouse models have provided evidence that some cytokines produced in response to infection actually contribute to the pathology of CM, leading to cerebral dysfunction (Kossodo *et al.*, 1993; Jennings *et al.*, 1997; Brown *et al.*,

1999; Hunt and Grau, 2003.). There is convincing evidence that pro-inflammatory cytokines mediate neurodegeneration in murine CM, with TNF- α playing a central role (Grau *et al.*, 1987; Kern *et al.*, 1989; Rudin *et al.*, 1997). Treatment with anti-TNF- α antibodies has been shown to significantly prolong the lives of CBA/Ca mice infected with *Plasmodium berghei* and prevent the development of neurological symptoms (Grau *et al.*, 1989).

In conclusion, the immune system is key to the control of parasitic infections, but as mentioned above, more often this control can lead to pathology and hence neurological symptoms. Careful characterization of the cerebral immune response in *T. canis* infection would provide important insights into the disease process and the consequences of such infection. As demonstrated in this chapter, there is considerable evidence to

show that, in mice at least, *T. canis* infection results in cerebral larval accumulation, and a range of altered behaviours. Whether or not these changes are an adaptive manipulation by the parasite, or merely a side effect of immunopathology remains to be deciphered. One suspects, however, that the basis for the behavioural changes is not straightforward, and most probably involves a number of complex interacting factors. In light of this, we are developing the mouse model further in an attempt to address some of the questions surrounding cerebral toxocariasis. By infecting mice of different genetic background, and investigating behavioural and immune responses, we hope to shed some light on the significance of cerebral infection, and open doors for future experimentation (Hamilton *et al.*, 2004).

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6 Critical Assessment of Existing and Novel Model Systems of Toxocariasis

Nobuaki Akao

Section of Environmental Parasitology,
Graduate School of Tokyo Medical and Dental University, Tokyo, Japan

Introduction

Toxocariasis is a disease caused by the larval stage of *Toxocara* sp., and predominantly involves *Toxocara canis* and *Toxocara cati*. The infectious-stage larvae, which develop in the egg within 2 weeks after their excretion to the surrounding environment and mature 4 weeks after excretion, can migrate through the entire body of either a definitive or paratenic host. In paratenic hosts including human beings, the larvae cause tissue damage as either a direct or an indirect effect of their presence. For example, some level of visual impairment may occur when larvae in the retina pass over the macular region, and neurologic disturbance may appear when larvae reside in the brain for a long period of time. In addition, larvae in the retina may elicit an inflammatory response resulting in serious ocular disease such as chorioretinitis or uveitis. These medical problems have been well known since 1952, when Beaver *et al.* (1952) proposed a disease syndrome characterized by chronic eosinophilia with granulomatous lesions in the liver, as reported in three young children. Since then, much effort has been invested in understanding the pathogenesis of this parasite using animal models.

Toxocariasis in Humans

Toxocariasis is clinically divided into four types of disease: visceral, ocular, neurological and covert types (Taylor *et al.*, 1987; Glickman and Magnaval, 1993). Visceral toxocariasis is associated with the migratory behaviour of the larvae in the early stage, in which they penetrate the intestinal wall, reach the liver, and then the lung, from where they are then distributed throughout the entire body of the host. Ocular toxocariasis is a specific form of the visceral type. This syndrome is not always accompanied by a systemic disorder, but is the type in which the disseminated larvae emerge in the retina. In some patients, a full-body or a part of a larva has been recovered from the vitreous fluid after vitrectomy (Maguire *et al.*, 1990). However, it is still unclear just how the larvae enter or the time course for this invasion after infection. Regarding neurological involvement, some previous studies have shown that neurological defects or epilepsy may be associated with *Toxocara* infection. Children who have a history of epilepsy showed a statistically significant increase in antibody against *Toxocara* antigens. Additionally, meningoencephalitis with eosinophilia and increased antibody in the cerebrospinal

fluid is another clinical manifestation of the disease. These findings are common in neurological toxocariasis. In contrast, the concept of covert toxocariasis is less well established. In the Midi-Pyrénées region of France and in Ireland, patients who had relatively non-specific symptomatology including fatigue, abdominal pain, nausea, fever, lymphadenopathy, etc., with or without accompanying moderate eosinophilia, showed positive results for an anti-*Toxocara* antibody test (Glickman *et al.*, 1987; Taylor *et al.*, 1988). It is increasingly accepted that *Toxocara* infection could account for this syndrome. Although the variety of symptoms in human cases is a characteristic feature of the infection, our knowledge about *Toxocara* pathogenesis is fairly limited. For this reason, *Toxocara* infection has long held the attention of both parasitologists and immunologists.

Experimental Toxocariasis: Existing Animal Models

Mice

Both inbred and outbred strains of mice are commonly used in studies of infectious disease. An outbred strain was first used in a study of the migratory behaviour of *Toxocara* larvae in 1952 (Sprent, 1952), soon after Beaver *et al.* (1952) introduced the notion of 'visceral larva migrans' by *T. canis*. Since then, many attempts were made to clarify the distribution pattern after oral administration of embryonated eggs. Embryonated eggs hatch in the upper gastrointestinal tract and then the infectious-stage larvae penetrate the enteric mucosal membrane. Most of the larvae remain there until 6 h after infection, and migrate to the liver by way of the portal vein. They then remain in the liver for some time before migrating to the lung. Typically, the larvae migrate to the lung and heart; however, with repeated infection or pre-sensitization treatment with *Toxocara* antigen, the larvae accumulate in the liver in both outbred and inbred mice. These findings, along with the fact that trapping of the larvae in the liver does not occur in congenitally athymic mice, suggest that the host immune response plays an important role in this phenomenon (Sugane and Oshima, 1982; Conception and Barriga, 1985; Parsons and Grieve, 1990a,b). Thus, the mouse is a useful model for

determining why the parasite is so often found in biopsy specimens of the human liver.

In general, different strains of mice show different larval distribution patterns and pathophysiological courses (Koizumi and Hayakawa, 1984). Among the inbred mice strains, BALB/c mice, but not C57BL/6 mice, are the best suited for investigations of a possible connection between allergic asthma and *Toxocara* infection (Pinelli *et al.*, 2001).

When they leave the lung, the larvae enter the systemic circulation, from which they reach the skeletal muscles and central nervous system. Interestingly, *Toxocara* larvae tend to accumulate in brain tissue and can remain alive and motile for years, resulting in behavioural changes in affected mice (Summers *et al.*, 1983; Holland and Cox, 2001). These mice also show a reduced ability in maze learning. However, little information is available on the relationship between the site of the larvae in the brain and behavioural changes in the host (Donovick and Burright, 1987; Cox and Holland, 1998). Additionally, there is no correlative evidence regarding the site where the larva was detected and a possible clinical syndrome in these mice. In fact, these findings suggest that mice are not a suitable model for neurological toxocariasis. In spite of having the same major histocompatibility complex (MHC) haplotype background, BALB/c and DBA mice reacted quite differently in terms of their allergic inflammation in the brain, indicating that the host response to an infection is not dictated by MHC haplotype alone (Epe *et al.*, 1994).

Studies of ocular toxocariasis have also been conducted with outbred mice (Olson, 1976; Rockey *et al.*, 1979; Ghafoor *et al.*, 1984). After oral administration of eggs, mouse eyeballs were crushed and observed microscopically. *Toxocara* larvae were observed and inflammatory changes were confirmed histologically, but the incidence was very low. Thus, the use of a mouse model for ocular toxocariasis is not recommended, since it is time-consuming to determine the migration route of the larvae to the retina and the pathogenesis of the larvae, even though useful information has been obtained from some experiments using mice.

The influence of maternal infection on offspring has been the subject of study with murine toxocariasis. In mice infected during pregnancy, larvae were found in the uterus, placenta and fetus (Lee *et al.*, 1976), and there was a predictable decrease in litter size in female mice with

Toxocara infection (Akao *et al.*, 1990; Reiterova *et al.*, 2003).

Numerous immunological and immunopathological studies of *Toxocara* infection in mice have also been performed in the last two decades. Among them, larval trapping in the liver of pre-sensitized hosts is an interesting phenomenon (Sugane and Oshima, 1983; Concepcion and Bar-riga, 1985; Kayes, 1997). This event might remind us why *Toxocara* larvae are frequently observed in the liver of human visceral toxocari-asis. Eosinophilic granuloma formation in the liver was found to be regulated by the host Th1/Th2 response, and eosinophils play an essential role in the pathology of infected C57BL/6 mice (Taka-moto *et al.*, 1997). However, eosinophils do not play a significant role in the expulsion and killing of *T. canis* larvae in infected mice (Sugane *et al.*, 1996). Furthermore, the presence of IgE antibody to excretory-secretory (ES) products of *T. canis* has been monitored during infection, and allergic asthma in murine models has been studied (Buijs *et al.*, 1994; Dent *et al.*, 1997).

To interpret the findings from these experi-mental studies, it is very important to know the precise count and administration method of the eggs in each experiment. In this context, the work done by Oshima (1961) was an important ad-vance in this field. Oshima described a standard method for the oral inoculation of eggs and spec-ified that all equipment used in their preparation should be siliconized and that the albuminoid coat of the egg should be removed. It is also important that the number of eggs be counted in a statisti-cally valid manner so that this and other tech-niques, taken together, will ensure reproducible results.

In conclusion, while mice provide a very informative model for studying the contribution of genetic diversity to *Toxocara* infection and the distribution of larvae after infection, the mouse model cannot provide a complete understanding of all aspects of *Toxocara* infection.

Rats

The utility of the rat model is similar to that of the mouse model; however, the reports on experimen-tal toxocariasis of rats are limited. The pattern of migration of larvae in rats is similar to that in mice

(Lescano *et al.*, 2004) and in one study, rats infected with *T. canis* showed a decline in learning ability in a maze (Olson and Rose, 1966). Rats infected with *Toxocara* have also been used to demonstrate eosinophilic chemotactic activity in bronchoalveolar lavage fluid and eosinophil-me-diated cardiomyopathy (Fujimoto *et al.*, 1990; Schaffer *et al.*, 1992; Okada *et al.*, 1996). Ocular infections have also been reported in infected rats (Burren, 1972), but occurred less commonly than in mice.

Guinea pigs

In allergic asthmatic children, a high prevalence of antibody to *Toxocara* antigens has been reported worldwide (Oteifa *et al.*, 1998). To understand better the factors involving the onset of this dis-ease, guinea pigs are frequently used due to their high responsiveness of bronchial refraction to antigen (Buijs *et al.*, 1995). Collins and Ivey (1975) reported that IgE antibody in infected guinea pigs was evident using homologous passive cutaneous anaphylaxis tests. Ocular inflammation was induced by intravitreal infection (Rockey *et al.*, 1979); however, guinea pigs are considered to be an inappropriate model for the study of ocular toxocariasis due to their atypical immune re-sponse (Ghafoor *et al.*, 1984; Fenoy *et al.*, 2001).

Hamsters

Very little information is available on toxocariasis in the hamster (Burren, 1972). Since hamsters are frequently used to investigate airway hyperrespon-siveness or inflammation to foreign materials, it would be helpful to understand their possible al-lergic response to *Toxocara* infection.

Rabbits

Since, with rabbits, blood samples can easily be taken once or twice a week, they have fre-quently been used to investigate the time course of antibody production during infection. Specific IgG antibody against ES antigens of *T. canis* was first detected in the serum after the 5th day of

infection and reached its peak at 2 weeks post-infection. Thereafter, the level of antibodies remained high for a long period of time (Fernando, 1968; Kondo *et al.*, 1981; Smith *et al.*, 1982). By contrast, eosinophil counts in the peripheral blood reached their peak at 4 weeks after infection, and decreased gradually to the normal level after 10 weeks of infection. Immunoblot analysis has also been performed in rabbits to examine changes in the antigen recognition in infected rabbits and to identify the specific antigen moieties in larval ES products (Akao *et al.*, 1982).

Primates

The genetic homology between human beings and primates has made the primate model of toxocariasis an attractive option for studies of the pathogenesis of toxocariasis (Fernando *et al.*, 1970; Fernando and Soulsby, 1974; Tomimura *et al.*, 1976; van Knapen *et al.*, 1982). In the cynomolgus macaque, *Macaca fascicularis*, the haematological and serological changes were similar to those observed in children with visceral larva migrans (VLM), and some individuals (three out of 16 macaques) developed neurological signs such as ataxia and nystagmus (Glickman and Summers, 1983). Despite intensive studies using oral inoculation of eggs, intraocular lesions associated with larval migration have not been observed, although intraocular inoculation with larvae did cause inflammatory changes. Histopathologically, *Toxocara* larvae can survive for at least 10 years after infection in rhesus monkeys (Beaver, 1969).

Despite these advantages over other animals, primates tend to be nervous and difficult to handle for experimental purposes. Moreover, studies using primates are much more expensive and controversial than those using other animals.

Chickens, pigs and other mammals

Visceral toxocariasis was thought to be a disease affecting younger children who accidentally ingested *Toxocara* eggs, even though ocular toxocariasis can occur in older children or in individuals of any age (Glickman and Magnaval, 1993). In 1989, a new infection route of toxocariasis was

reported (Nagakura *et al.*, 1989). Twin brothers, aged 21 years, were admitted to hospital due to fever, nausea and myalgia with urticaria of both lower legs. They had eaten raw chicken liver and meat 12 days before admission. Eosinophilia, elevation of total IgE and *T. canis*-specific IgG antibodies were confirmed by laboratory examination. In another case, a 26-year-old woman presented to the hospital complaining of fever, headache and a dry cough. Laboratory examination revealed eosinophilia, an elevated concentration of IgE and *T. canis*-specific IgG. A *Toxocara* larva was detected in a small brown itchy nodule on her left ankle (Aragane *et al.*, 1999). Before the onset, the patient had a history of eating raw beef liver. Similar cases have been reported from Switzerland (Sturchler *et al.*, 1990), North America (Salem and Schantz, 1992) and Spain (España *et al.*, 1993). In addition, experimental studies revealed that *Toxocara* larvae tend to accumulate in the liver of chicken (Taira *et al.*, 2003a) and quail (Pahari and Sasmal, 1990; Maruyama *et al.*, 1994). We assume, therefore, that table fowls play an important role in the transmission of toxocariasis.

In a pig model, Taira *et al.* (2003b, 2004) demonstrated that no clinical signs developed in infected pigs, although most of the larvae were recovered from the lungs and there were numerous white spots in the liver due to the continuous migration of the larvae. Although few in number, the larvae were detected in various organs and tissue. Therefore, they suggested that the experimental infection of pigs may be a useful model of covert toxocariasis in humans (Taira *et al.*, 2004). Furthermore, Helwich *et al.* (1999) stated that the pig was a useful non-primate model for human VLM, since *T. canis* larvae migrated well and induced a strong immunological response in the pig.

A New Model for Human Toxocariasis: Mongolian gerbils, *Meriones unguiculatus*

Mongolian gerbils are known to be susceptible to a variety of parasites, including *Brugia pahangi*, *Strongyloides stercoralis*, *Nippostrongylus brasiliensis* and *Entamoeba histolytica* (Hori *et al.*, 1993; Nolan *et al.*, 1993; Campbell and Chadee, 1997). However,

with the exception of the study of Burren (1972), no studies have evaluated the usefulness of the Mongolian gerbil as an animal model of toxocariasis. Unfortunately, since Burren was unable to detect larvae in the ocular chamber, he concluded that the Mongolian gerbil was an unsuitable animal model for ocular toxocariasis, and since then, no similar report has been published on this species. Several species of animals, including mice, rabbits, guinea pigs and monkeys, have been evaluated pathologically; however, the incidence of ocular infection is low and eosinophilic infiltration is rarely observed through oral inoculation.

In 1998, Mongolian gerbils assumed a more important role in *Toxocara* and toxocariasis research when they were found to have a high susceptibility to ocular infection not only by *T. canis* (Takayanagi *et al.*, 1998; Takayanagi *et al.*, 1999), but also by *T. cati* (Akao *et al.*, 2000). After oral inoculation of eggs (approximately 1000 eggs per gerbil), the retinas of gerbils were observed

with an ophthalmoscope, which was specifically adapted for observing the fundi of small animals. This new tool provided valuable insight into the pathogenesis of *Toxocara* infection.

Ocular toxocariasis in Mongolian gerbils

A motile larva was clearly observed in the retina as early as 3 days after infection in Mongolian gerbils, and the incidence of retinal involvement was at least 80% in infected gerbils. A maximum of three migrating larvae was seen in one eye at the same time, and on rare occasions, migrating larvae were found bilaterally. Once a larva appeared in the eye, it was present until the end of the observation period, 158 days after inoculation.

Haemorrhagic lesions and exudative lesions with or without migrating larvae were consistently found in gerbils after 3 days of infection. Figure 6.1 shows typical ophthalmoscopic findings. In haemorrhagic lesions, four different types of changes:

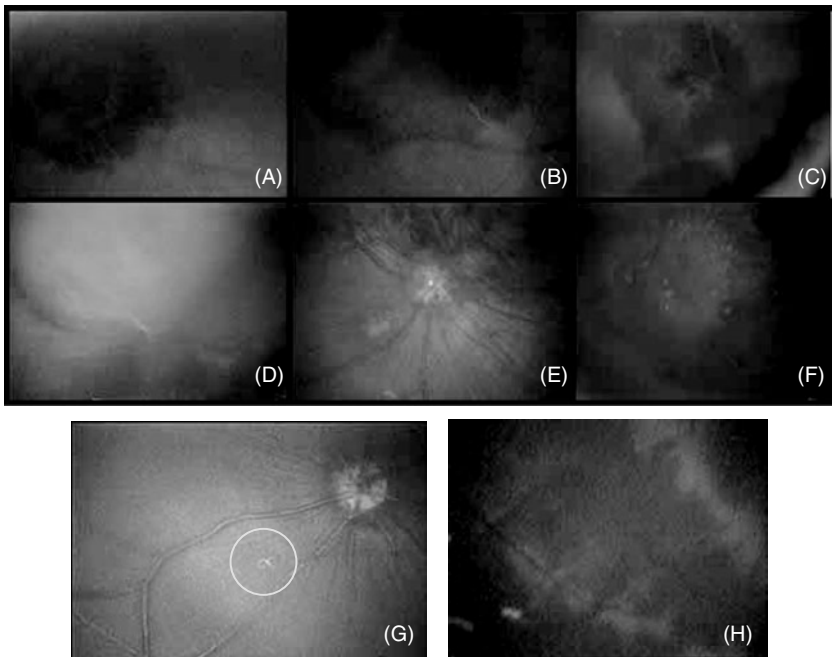


Fig. 6.1. Haemorrhagic changes of the retinas in Mongolian gerbils infected with *Toxocara canis*. (A) Deep-seated retinal haemorrhage. (B) Superficial retinal haemorrhage. (C) Three deep-seated haemorrhages in the peripheral region. (D) Optic papilla is covered with a large vitreous haemorrhage. (E) Optic papilla is covered with a superficial haemorrhage. (F) White-centred small retinal haemorrhages. (G) A motile larva on the retina. (H) White exudative lesions around vessels. For a colour version of all figures, see <http://www.glc.ac.uk/DEEB/mwkimages>.

vitreous haemorrhage, superficial retinal haemorrhage, deeply seated retinal haemorrhage and white-centred small retinal haemorrhage, were seen in the fundi. Histopathologically, haemorrhagic lesions and proliferative changes of the retina were observed (Fig. 6.2). White exudative lesions around the vessel walls suggest vasculitis consisting of eosinophils and lymphocyte infiltration. Table 6.1 shows the results of ophthalmoscopic observations and the incidence of lesions in 46 gerbils. Migrating larvae just beneath the retina often left bright, whitish-yellow restiform traces on the retina. A large vitreous haemorrhage was absorbed within 7 days and left behind small, brilliant, yellowish particles. Fortunately, the dark-grey fundi of the gerbils made it easy to detect the

motile white larvae of *T. canis* on the retina (Fig. 6.1). In contrast, ophthalmological changes are difficult to detect in BALB/c mice, since their albino fundi made the larvae difficult to identify. Figure 6.3 shows the predilection sites of the haemorrhagic lesions that consist of large (larger than one optic disc diameter) and small (smaller than one optic disc diameter) sizes. There was no significant difference in the incidence of lesions between the right and left eyes, but the lesions appeared to emerge more in the peripheral region than in the central region, and more in the horizontal region than in the vertical region.

A variety of lesions were found in gerbil eyes after infection; however, no eosinophilic

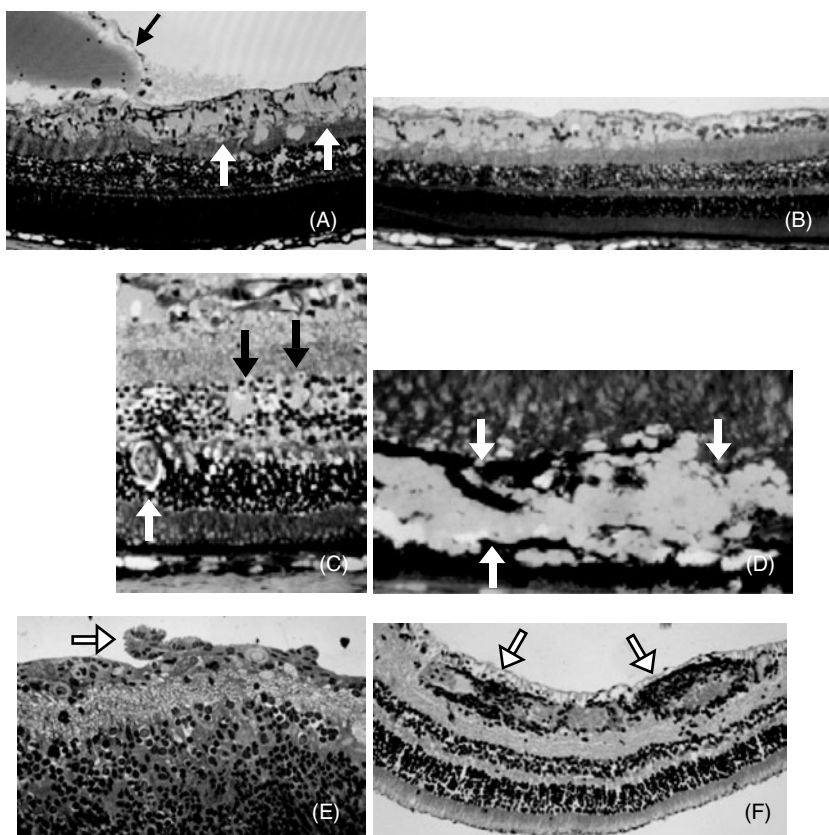


Fig. 6.2. Histopathological changes of the retinas in Mongolian gerbils infected with *Toxocara canis*. (A) Vitreous (white arrows) and superficial (black arrow) haemorrhages. (B) Diffused superficial haemorrhage. (C) Haemorrhage of outer nuclear layer (black arrows) and a transverse section of *T. canis* larva (white arrow). (D) Haemorrhage in the pigment epithelium (white arrows). (E) Proliferative change (arrow) of nerve fibre layer. (F) Vasculitis with lymphocyte and eosinophil infiltration (arrows).

Table 6.1. Ophthalmoscopic characteristics and the frequency of incidence of lesions in gerbils infected with *Toxocara canis**.

	Within 7 days	Until 35 days
Larvae	30 (65)	37 (80)
Vitreous haemorrhage	1 (2)	5 (11)
Superficial retinal haemorrhage	19 (41)	27 (59)
Deep-seated retinal haemorrhage	33 (72)	41 (89)
Exudative lesions	21 (46)	37 (80)
Vasculitis	3 (7)	25 (54)

*46 infected gerbils were observed. Numbers in parenthesis indicate the % of affected gerbils.

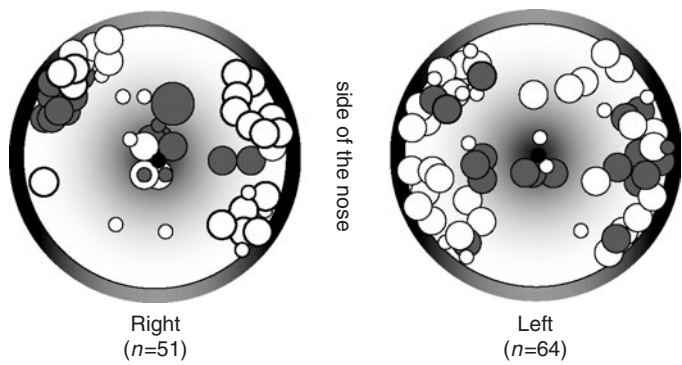


Fig. 6.3. Predilection sites of haemorrhagic lesions in infected gerbils. White circles indicate deep-seated haemorrhage and grey circles indicate superficial retinal haemorrhage. The size of circle represents the diameter of the lesion when the lesion is smaller than that of one optic disc and larger.

granuloma, which is the most frequent finding with human ocular toxocarasis, was observed either ophthalmoscopically or histopathologically. Alba-Hurtado *et al.* (2000) examined gerbil eyes histopathologically after oral inoculation of eggs and found granulomatous lesions in the retina 60 days after infection, which was their last day of observation. This finding is in marked contrast to our own. We found that, once the larvae entered the eye, they survived and were observable under an ophthalmoscope for at least 158 days post-infection. Thus, we suggest that granulomatous lesions would not occur as long as the larvae are motile.

It has been hypothesized that the migration of larvae to the eye occurs via the following routes: (i) through the arteries from the internal carotid artery to the ophthalmic artery, retinal central artery, or ciliary artery; (ii) through the brain to the optic nerve; and (iii) through the brain to the cerebrospinal fluid space, and then to the choroids. We observed a larva that emerged from the

edge of the ora serrata. Additionally, since choroidal haemorrhage was the most frequent observation in the early ocular findings and was often observed simultaneously with motile larvae, the third route is the most likely to be used. To assess the possibility of the second route of migration, we tested whether larvae could arrive in the eye via the optic nerve if motile larvae were directly inoculated into the brain. Approximately 300 larvae that were maintained aseptically in a culture medium were inoculated intracranially through the cranial bone using a 23-gauge needle (Hayashi *et al.*, 2003). From 6 days after inoculation, either vitreous or choroidal haemorrhages were found in the gerbils by ophthalmoscopy. These lesions were sometimes accompanied by a larva. Pathological examination confirmed that larvae were migrating in the optic nerve of the gerbils 6 days after inoculation and two larvae were found in the optic chiasma (Fig. 6.4). These results clearly indicated that *Toxocara* larvae are able to migrate from the

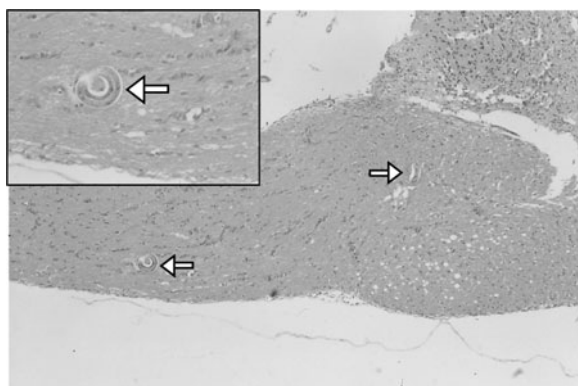


Fig. 6.4. Histopathological observation of the optic chiasma of the Mongolian gerbil following intracranial inoculation with *Toxocara canis* larvae: two migrating larvae (arrows) in the optic chiasma and one at higher magnification (arrow).

central nervous system to the eye via the optic chiasma (see Chapter 9, this volume).

Neurologic toxocariasis in Mongolian gerbils

Mongolian gerbils infected with *Toxocara*, both *T. canis* and *T. cati*, show gait difficulty and progressive ataxia (Akao *et al.*, 2003). The onset of disease occurred 50 days post-infection. Neurological abnormalities developed in six of 13 gerbils (46%) infected with *T. canis*, and in five of seven gerbils infected with *T. cati* (71%). Clinical signs included swinging gait while attempting to stand on their hind legs, circulating movement in the same direction, difficulty in normal positioning of the head, paraplegia of the hind limbs and urinary incontinence. Despite severe illness, they showed a good appetite until they lapsed into a coma.

Histopathologically, the cerebellum was the most affected area of the brain in these gerbils. Loss of Purkinje cells, glial nerve fibres and nerve sheaths were characteristic and common findings. There were no apparent pathological changes in the brain except in the cerebellum. Clearly, these morphological changes could be responsible for the neurologic disorders observed. Migrating larvae were seen in the affected cerebellum, but larvae and lesions also existed independently, suggesting that some of the degenerative changes might be the result of indirect effects of the larvae. Future investigations should include an analysis of

the interaction of nerve cells with the ES products of *T. canis* *in vitro*.

Conclusions

Toxocara spp. are ubiquitous parasites in both developed and developing countries, and are responsible for one of the most challenging zoonotic parasitic infections worldwide. Further more, many of the issues concerning the pathogenesis of *Toxocara* infections, such as the reactivation mechanism of arrested larvae in skeletal muscle and the therapeutic advantage of steroid use in ocular toxocariasis, are poorly understood or controversial. It is hoped that the present gerbil model will contribute to the development of improved diagnostic and therapeutic approaches for toxocariasis, since this model allows us to test these approaches experimentally. Our ongoing research will continue to focus on human toxocariasis and will add to our understanding of the basic process of host–parasite relationships in nematode parasites.

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Section 2

Clinical aspects and public health

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7 Diagnostic Limitations and Future Trends in the Serodiagnosis of Human Toxocariasis

Huw Smith¹ and Rahmah Noordin²

¹Scottish Parasite Diagnostic Laboratory, Stobhill Hospital, Glasgow, UK

²Institute for Research in Molecular Medicine, Universiti Sains Malaysia, Penang, Malaysia

Introduction

The common nematode parasites of canines and felines, *Toxocara canis* and *Toxocara cati*, respectively, are the aetiological agents of human toxocariasis (Beaver *et al.*, 1952; Smith, 1993; Petithory *et al.*, 1994). The life cycle of *T. canis* is complex (Fig I.1) and can involve direct, trans-uterine, colostral or paratenic host transmission. Separate sex, adult worms live in the proximal small intestine of canines, and can produce up to 200,000 eggs per day (Glickman *et al.*, 1979), which can result in extensive environmental contamination (Glickman and Schantz, 1981). Importantly, fertilized eggs are not infectious to other hosts when faeces are voided, and require a period of time in the environment to develop to infectivity. For example, at a temperature of 15–35°C and a relative humidity of 85%, eggs become infective between 2 and 5 weeks after release from the uterus (Glickman and Schantz, 1981). Larvae moult once inside the eggs to become the infective second-stage larvae, although there remains controversy as to whether some larvae may become third stage (Araujo, 1972).

Infective ova hatch in the intestine, releasing their second-stage larvae (L2), which migrate through the soft tissues of the body for prolonged

periods of time. Larval development progresses no further than this infective stage. Human toxocariasis was first recognized by Beaver *et al.* (1952) when he reported finding *T. canis* larvae in eosinophilic granulomata removed from three children at laparotomy. Prior to this, Wilder (1950) noted that the eyes of some patients with suspected retinoblastoma exhibited granulomata and eosinophilic abscesses. Histological sections of these granulomata demonstrated the presence of nematode larvae, which Nichols, in 1956, identified as being the second-stage larvae of *T. canis*.

T. canis and *T. cati* Toxocariasis in Humans

Our perception is that *T. canis* is more important than *T. cati* in causing human infection and disease because cats, but not dogs, bury *Toxocara* ova-contaminated faeces, making infectious ova less accessible to susceptible individuals. Further reasons include: (i) the fact that morphometry of larvae in histological sections and early serology, using cross-reactive antigens, failed to implicate *T. cati* over *T. canis* in human cases; and (ii) the lesser tendency for brain involvement in the murine model of *T. cati* toxocariasis (e.g. Havasiová-Reiterova *et al.*, 1995).

Based on such generalized exclusions, *T. canis* was deemed to be the more important aetiological agent of human toxocariasis.

Yet, *T. cati* toxocariasis is a disease of humans, and its occurrence is probably underreported (Fisher, 2003). Both visceral larva migrans and ocular toxocariasis, caused by migrating larvae, as well as adult *T. cati* infections of humans have been described (Fisher, 2003). Petithory *et al.* (1993) reported that antibody in three of ten samples of human intraocular fluid reacted more strongly to *T. cati* antigens than to *T. canis* antigens, leading to the suggestion that *T. cati* should be recognized as a cause of ocular larva migrans. *T. cati* has also been implicated in a case of decreased visual acuity caused by a macular lesion of the eye, where the patient's serum was more reactive to *T. cati* antigens than to *T. canis* antigens (Sakai *et al.*, 1998). Adult *T. cati* infections of humans have been reported, based on the presence of their characteristic arrow-shaped alae (Eberhard and Alfano, 1998).

Uga *et al.* (1996a) stated that contact between cats and humans was closer than contact between dogs and humans. Furthermore, in Islamic countries, dogs are avoided for religious reasons, while cats are favoured pets. Therefore, the role of *T. cati* in human toxocariasis should not be underestimated and should be investigated fully.

Transmission Routes for Human Toxocariasis

A variety of non-canine hosts, including humans, are paratenic hosts for *T. canis* and become infected following the ingestion of infective eggs. The major route of transmission is the ingestion of infective ova present in soil and on herbage. *T. canis* ova can occur commonly in the environment. Exposure and environmental contamination is discussed in Chapter 14 (this volume). Their numbers will vary according to the frequency of contamination, the sward and soil profile and the permeability of the sward and soil to water. Children in the first decade of life are most susceptible to infection because of their geophagic habits and their mouthing of objects. Minor routes include eating undercooked meats, including liver and

snails. Transmission following dispersion of ova by filth flies should also be included. Flies ingest 1–3 mg faeces over 2–3 h (Greenberg, 1973), and can ingest *Toxocara* ova (Pegg, 1971). *Toxocara* ova have been found on and in 2.4 and 2.1% of wild-caught naturally infected flies in Nigeria (Umeche and Mandah, 1989). However, *Toxocara* ova will require a period of embryonation before being infective, although flies could deposit ova on material that could be ingested later. Being of similar size to *Ascaris* ova, which have been detected in filtered air, inhalation of infective windborne ova can also occur with *Toxocara*, as can waterborne transmission, following their accidental ingestion during immersion watersports, or following inhalation from aerosols.

Clinical Manifestations of *T. canis* Infection

Human toxocariasis can be divided into three clinical syndromes, namely visceral larva migrans (VLM), ocular larva migrans (OLM) and covert toxocariasis (CT) (Table 7.1). More accurately, VLM and OLM should be renamed toxocaral VLM (or visceral toxocariasis) and toxocaral OLM (or ocular toxocariasis) because larva migrans can be caused by larval stages of diverse metazoan parasites. Here, we use the terms VLM or OLM to refer only to those larva migrans syndromes which have a toxocaral aetiology.

Diagnosis

Definitive diagnosis is by histopathological examination and morphological and morphometric identification of L2 in tissue samples or by PCR and RFLP or sequencing of *Toxocara* nuclear ribosomal DNA (rDNA), mitochondrial DNA (mtDNA) or repetitive DNA (see Chapter 2, this volume) extracted from tissue, but the difficulty in acquiring biopsy material, far less biopsy material containing larvae, seriously compromises this approach. The most effective laboratory diagnostic approach for confirming *Toxocara* as the aetiological agent of disease in clinically suspected cases is by serology (Smith, 1993; Magnaval *et al.*, 2001).

Table 7.1. Some signs and symptoms of human toxocariasis.

Visceral larva migrans	Ocular larva migrans	Covert toxocariasis
Fever	Visual loss	Cough
Pallor	Strabismus	Abdominal pain
Malaise	Retinal granuloma	Headache
Irritability	Pars planitis	Sleep disturbance
Weight loss	Endophthalmitis	Behaviour disturbance
Skin rash	Choroidoretinitis	
Hepatomegaly	Uveitis	
Asthma	Retinal detachment	
Hypergammaglobulinaemia		
Respiratory symptoms/signs		
Nervous symptoms/signs		
Myocarditis		
Persistent eosinophilia		
Leucocytosis		
Elevated anti-A and -B isohaemagglutinins		

Global Seroprevalence

Toxocariasis is a cosmopolitan zoonotic disease (Barriga, 1988) and has been reported from a variety of countries, including Argentina, Malaysia, USA, Canada, Brazil, Nepal, Indonesia, France, Australia, Korea and The Netherlands. Table 7.2 shows the global seroprevalence of toxocariasis. Seroprevalence is often lower in developed than in developing countries. In France, 2–5% of apparently healthy adults from urban areas were *Toxocara* seropositive compared with 14.2–37% of adults from rural areas (Magnaval *et al.*, 1994), whereas 63.2% *Toxocara* seropositivity was reported in Bali (Chomel *et al.*, 1993) and 20% in Malaysia (Hakim *et al.*, 1993).

Serodiagnosis also remains the best option for epidemiological studies of prevalence of infection/disease. Since *Toxocara* infection is commonly underdiagnosed, it may be erroneously thought to be an insignificant cause of morbidity in populations (Park *et al.*, 2000; Rahmah *et al.*, 2005). In Malaysia, for instance, the perception is that toxocariasis is not of public health importance. This is due to two reasons, first, the scarcity of reports on *Toxocara* seroprevalence (Hakim *et al.*, 1992, 1993, 1997; Patrick *et al.*, 2001) and secondly, the confidence that can be placed in the results and conclusions of serological and seroepidemiological studies, because of the unsatisfactory specificity of the test employed. For example, to discriminate

between cases and cross-reactions, Hakim *et al.* (1993) required a high serological cut-off value (an absorbance of 0.836 units in an ELISA), which reflects the cross-reactivity of the antigen used in this prevalence study.

The availability of a highly specific and sensitive serological diagnostic assay will enable more accurate seroepidemiological studies to be performed, worldwide, with effective quality control and quality assurance. This should lead to the gathering of more reliable information on the prevalence and sources of the infection in a population, which, in turn, will enable health-related personnel to provide better diagnosis and treatment and governments to improve population-based, public health programmes. A sensitive and specific sero-diagnostic test is also a useful tool for monitoring herd immunity, ensuring that there is no increase in endemicity due to factors such as closer association between animals and man caused by changing life styles, economic activities and climate.

Larval Burden and the Serological Response

Not all *Toxocara* infections will be detected serologically. *Toxocara* antibody levels in dogs are strictly dose related (Glickman *et al.*, 1981), and this may also be the case in humans. Except for ocular disease, very low larval burdens do not cause

Table 7.2. Global seroprevalence of human toxocariasis.

Country/region	% Seroprevalence (n)	Reference
La Reunion, Indian Ocean	92.8	Magnaev <i>et al.</i> (1994)
Kathmandu, Nepal	81	Rai <i>et al.</i> (1996)
Bali, Indonesia	63.2	Chomel <i>et al.</i> (1993)
Sidoarjo, East Java, Indonesia	63	Uga <i>et al.</i> (1996b)
Hawaii, USA	45 (IgE); 17.5 (IgG)	Desowitz <i>et al.</i> (1981)
Kuala Lumpur, Malaysia	57.8	Hakim <i>et al.</i> (1997)
La Plata, Buenos Aires, Argentina	39	Radman <i>et al.</i> (2000)
Espirito Santo, Brazil	39	Moreira-Silva <i>et al.</i> (1998)
Resistencia, Subtropical City, Argentina	37.9	Alonso <i>et al.</i> (2000)
Campo Grande, Mato Grosso, Brazil	35.6	Matos <i>et al.</i> (1997)
Cordillera Province, Bolivia	34 (73/216)	Cancrini <i>et al.</i> (1998)
Peninsular Malaysia	31.9	Hakim <i>et al.</i> (1992)
Jos, Plateau State, Nigeria	29.8	Ajayi <i>et al.</i> (2000)
Salamanca, Spain	29.4–33.1	Conde Garcia <i>et al.</i> (1989)
Slovak Republic	27.4	Havasiova <i>et al.</i> (1993)
Campinas, Sao Paolo, Brazil	23.9	Anaruma Filho <i>et al.</i> (2002)
Durham, North Carolina, USA	23.1	Ellis <i>et al.</i> (1986)
Harnett County, North Carolina, USA	23.1	Worley <i>et al.</i> (1984)
Kuala Lumpur, Malaysia	21.2	Patrick <i>et al.</i> (2001)
Malaysia	19.6	Hakim <i>et al.</i> (1993)
Campinas, Sao Paulo, Brazil	17.9	Anaruma Filho <i>et al.</i> (2003)
Halifax, Nova Scotia, Canada	17	Embil <i>et al.</i> (1988)
France (South west)	15 (13/89) Midi-Pyrenees 4.8 (8/166) Toulouse	Caucanas <i>et al.</i> (1988)
Japan	14	Yoshida <i>et al.</i> (1999)
Irbid, Jordan	10.9	Abo-Shehada <i>et al.</i> (1992)
The Hague, The Netherlands	11	Buijs <i>et al.</i> (1994)
Rotterdam, The Netherlands	6	
Turkana District, Kenya	7.5 (17/228)	Kenny <i>et al.</i> (1995)
Canberra, Australia	7.5	Nicholas <i>et al.</i> (1986)
Lima, Peru	7.3	Lescano <i>et al.</i> (1998)
USA	7.3–4.6	Herrmann <i>et al.</i> (1985)
Havana, Cuba	5.2 (8/156)	Montalvo <i>et al.</i> (1994)
Gwang-do, Korea	5.1	Park <i>et al.</i> (2002)
Switzerland (Basel, Jura, Zurich)	5.1 (765)	Sturchler <i>et al.</i> (1986)
Switzerland	2.7	Jacquier <i>et al.</i> (1991)
Marche, Italy	1.6	Habluetzel <i>et al.</i> (2003)

significant morbidity in humans, and may not generate significantly elevated humoral antibody responses. Currently, attempts at developing ultra-sensitive antibody detection assays to detect humoral antibody responses to low larval burdens can result in reduced assay specificity. Glickman and Schantz (1981) proposed that the clinical and pathological manifestations of VLM and OLM are related to the larval burden of the host: the larval burden in OLM cases being less than in VLM cases which can harbour up to 300 larvae per gram of liver tissue (Beaver, 1966). Anti-*Toxocara* antibody

titres in VLM cases are consistently higher than those of OLM but lower than those of concurrent VLM and OLM.

At low infective doses, the antigenic mass is insufficient to stimulate a protective immune response, which would result in larval death, and larvae survive for prolonged periods of time (Glickman and Schantz, 1981). Such individuals probably remain asymptomatic unless a larva causes ocular damage. At higher infective doses, the immune response entraps larvae in organs such as the liver and lungs giving rise to clinical

signs relatively soon after infection (Smith, 1993). Larvae may overwhelm the filtering effect of the liver and associated immune response, which can lead to concurrent VLM and OLM, occasionally with associated myocarditis and/or neurological symptoms.

Glickman and Schantz (1981) suggest that the critical infectious dose at which VLM is more likely to develop than OLM lies between 100 and 200 larvae, but this assumes that infection stimulates a similar quality of immune response in each individual infected. As the antigenic mass is too small to stimulate a protective response in individuals infected with small numbers of larvae (Glickman and Schantz, 1981), the time period during which larvae can migrate throughout the soft tissues of the body producing and depositing *T. canis* excretory-secretory antigen (TES) would be extended.

The amount of TES produced by an individual larva in culture is between 9 pg and 8 ng of protein per day (de Savigny, 1975; Badely *et al.*, 1987; Smith, 1993), which, if produced *in vivo* daily by 100 larvae (the upper limit for OLM), would generate up to 800 ng protein daily, or 0.3 mg TES annually. For VLM, at least twice the amount of TES would be produced daily, until preferential killing at higher infecting doses were to occur. A consequence of larval degeneration following immune killing is the release of previously covert, somatic antigens, which could prove useful in following the efficacy of chemotherapy.

Serodiagnosis – Choice of Antigens

Table 7.3 lists the assays and antigens used in studies of infection prevalence and for human diagnosis. Prior to the use of TES as a diagnostic antigen, most surveys of human infection were based on tests using water-soluble, somatic antigens derived from *T. canis* embryonated eggs, larvae or adults. Many of these somatic antigens lacked sensitivity and possessed a high degree of cross-reactivity with *Ascaris* (Girdwood *et al.*, 1978; Smith *et al.*, 1982, 1983a,b) and other ascarid antigens.

In the mid-1970s, two advances enabled the development of more sensitive assays for human toxocariasis. First, the ability to culture *T. canis* second-stage larvae for prolonged periods of time *in vitro* and to collect TES (de Savigny, 1975) and

secondly, the introduction of ELISAs. Both helped rectify the lack of sensitivity and specificity associated with many of the earlier serodiagnostic techniques and permitted the development of standardized assays with considerable improvement in their specificity, sensitivity and reproducibility of detection. Using TES, both antibody to larvae and TES, and circulating antigens could be detected readily in experimentally infected animals (e.g. Hogarth-Scott, 1966; de Savigny and Tizzard, 1977; Matsumura and Endo, 1982; Smith *et al.*, 1982; Sugane and Oshima, 1983; Matsumura *et al.*, 1984; Robertson *et al.*, 1988). TES are the first mosaic of antigens to which an antibody response is detectable following infection (Smith *et al.*, 1982) and currently provide us with the most specific target antigen for serodiagnosis in humans (de Savigny and Tizzard, 1977; de Savigny *et al.*, 1979; Smith *et al.*, 1980; van Knapen *et al.*, 1983; Glickman *et al.*, 1985; Magnaval *et al.*, 2001).

Glickman *et al.* (1985) compared the sensitivity and specificity of a *T. canis* embryonated egg antigen and TES for OLM serodiagnosis by ELISA and found that, while both were similarly sensitive, TES was better able to discriminate between sera from patients with OLM and sera from patients with retinoblastoma (some presentations of OLM being the differential diagnosis for retinoblastoma). While pre-absorption of test sera with *Ascaris suum* embryonated egg antigen was essential to prevent false positive results with the embryonated egg antigen, it was not so critical for TES.

Serodiagnostic Assays – Choice of Assay Formats

Currently, TES is the most commonly used antigen in antibody detection assays, although soluble somatic L2, soluble embryonated egg extract, soluble adult antigen and recombinant *Toxocara* antigens are also used. The search for a robust, reliable, rapid, sensitive and specific test is also exemplified in the assay formats tested. These include radio-iodination, Western (immuno)blot (WB), indirect antibody competition ELISA (IACE), latex agglutination, radio-allergosorbent test (RAST), indirect and direct fluorescent antibody tests (IFAT, DFAT, CNBr-IFAT), haemagglutination, *in vitro* L2 fluorescence, sandwich ELISA, counter immunoelectrophoresis (CIEP),

Table 7.3. Tests and antigens employed in prevalence studies and/or diagnosis.

Assay	Antigen	Sera from	Reference
All isotypes ELISA, IgG ELISA, ELISA (Bordier Affinity Products kit)	TES	Argentina	Radman <i>et al.</i> (2000); Alonso <i>et al.</i> (2000)
All isotypes ELISA	TES	Australia	Nicholas <i>et al.</i> (1986)
IgG ELISA, IgG IACE, WB	TES	Brazil	Matos <i>et al.</i> (1997); Nunes <i>et al.</i> (1997, 1999); Moreira-Silva <i>et al.</i> (1998); Anaruma Filho <i>et al.</i> (2002, 2003)
IgG-ELISA	TES	Canada	Embil <i>et al.</i> (1988)
All isotypes ELISA, IgE ELISA; IgG WB	TES	France	Glickman <i>et al.</i> (1987); Caucanas <i>et al.</i> (1988); Magnaval <i>et al.</i> (1991, 1992)
IgG WB, IgE <i>Toxocara</i> RAST (CAP [®] , Pharmacia Diagnostics AB)	TES	France	Magnaval <i>et al.</i> (2002)
	AWA		
IgG and IgM ELISA (Melotest <i>Toxocara</i> Antibody kit)	TES	India	Mirdha and Khokat (2002)
IgG ELISA (LMD Lab., Inc. kit, CA, USA)	TES	Indonesia	Chomel <i>et al.</i> (1993); Uga <i>et al.</i> (1996)
IgG ELISA	TES	Ireland	Taylor <i>et al.</i> (1988); Holland <i>et al.</i> (1995)
IgG, IgE, IgM, IgA ELISA	TES	Ireland, Scotland, France	Smith <i>et al.</i> (1988)
IgG ELISA, IgE RAST, All isotypes ELISA	TEE, TES	Italy	Brunello <i>et al.</i> (1983); Habluetzel <i>et al.</i> (2003)
<i>Toxocara</i> CHEK kit; ELISA, DGFT, CIE, WB, IFAT	TES, TEE	Japan	Akao <i>et al.</i> (1997); Yoshida <i>et al.</i> (1999)
DDGT, ELISA	<i>T. cati</i> & <i>T. canis</i> ; AWA	Japan (?)	Nagakura <i>et al.</i> (1990)
IgG ELISA Kit (Pharmacia Diagnostics AB)	TES	Jordan	Abo-Shehada <i>et al.</i> (1992)
All isotypes ELISA	TES	Kenya	Kenny <i>et al.</i> (1995)
IgE ELISA, IgG ELISA, IgG immunoblot	TES	Korea	Park <i>et al.</i> (2000, 2002)
IgG ELISA	TES	La Reunion	Magnaval <i>et al.</i> (1994)

IgG ELISA, IgG CELISA (Cellabs kit)	TES	Malaysia	Hakim <i>et al.</i> (1992, 1993, 1997); Patrick <i>et al.</i> (2001)
IgG WB	RTES30	Malaysia, Japan	Yamasaki <i>et al.</i> (1998)
IgG ELISA	TEE	Nepal	Rai <i>et al.</i> (1996)
IgG ELISA	TES	Peru	Lescano <i>et al.</i> (1998)
ELISA-IgG Test kit; IgG and IgM ELISA, ELISA NOVUM kit, IgG ELISA, ToxocaraCHEK kit	TES	Slovak Republic	Havasiova <i>et al.</i> (1993); Dubinsky <i>et al.</i> (2000)
All isotypes and IgG ELISA	TES	Switzerland	Speiser and Gottstein (1984)
IgG ELISA kit (Biokema-Affinity Products kit)	TES	Switzerland, Italy	Jacquier <i>et al.</i> (1991)
IgG-ELISA	TES	The Netherlands	Buijs <i>et al.</i> (1994)
All isotypes IFAT; skin test	DE, L2; intact L2	UK	Bisseru and Woodruff (1968); Woodruff (1970)
All isotypes ELISA; IgG ELISA	TES	UK	de Savigny <i>et al.</i> (1979); Gillespie <i>et al.</i> (1993)
All isotypes CIE, IFAT, PRIST	AWA	UK, Scotland	Girdwood <i>et al.</i> (1978)
IgG ELISA	TEE, TES	USA	Glickman <i>et al.</i> (1979, 1985) and Ellis <i>et al.</i> (1986)
IgG ELISA	TEE	USA	Felberg <i>et al.</i> (1981), Herrmann <i>et al.</i> (1985) and Worley <i>et al.</i> (1984)
IgG, IgM, IgA ELISA, Protein A WB	TES	Venezuela	Lynch <i>et al.</i> (1988a,b)

AWA, Water-soluble extract of adult worm antigens; CIE, counter immunoelectrophoresis; DE, *T. canis* decoated eggs; DDGT, double diffusion in gel (Ouchterlony) test; EEE, embryonated egg antigen; IACE, indirect antibody competition ELISA; IFAT, indirect fluorescent antibody test; L2, *T. canis* infective larvae; PRIST, paper radioimmunosorbent test; RAST, radioallergosorbent test; RTES30, recombinant TES-30; TEE, water-soluble extract of *T. canis* embryonated eggs; TES, *T. canis* excretory–secretory antigen; WB, Western (immuno-)blot.

double gel diffusion test (DGD^T), paper radio-immunosorbent test (PRIST) and a rapid test (ToxocaraCHEK kit), but the indirect TES ELISA for antibody detection is currently the most common format (Table 7.3). Other assays that have been used to confirm clinical diagnosis include intradermal sensitivity, complement fixation, bentonite flocculation, larval precipitation and capillary tube precipitation (Mirdha and Khokar, 2002). Table 7.4 lists the commercially available serological tests for human toxocariasis. All but two are based on ELISAs. Of the non-ELISA tests, one is a WB kit and the other a rapid test based on immunogold detection and visualization. All commercial tests employ TES, which increases ELISA specificity significantly (Jacquier *et al.*, 1991).

Rationale for TES Serodiagnosis – Role of Endemic Polyparasitism

The emphasis placed on *Toxocara* serodiagnosis differs in different parts of the world. Concurrent parasitic infections of humans are less frequent in temperate, developed regions of the world than in tropical, undeveloped and underdeveloped regions where parasitism can be endemic. Toxocariasis, particularly VLM, is regarded as a disease of childhood, but in endemic areas, other parasitic infections, including ascariasis, trichuriasis, hookworm and filariasis occur more frequently and with greater sequelae. In these situations, toxocariasis may have less clinical relevance resulting in fewer serodiagnostic tests being

Table 7.4. Some commercial *Toxocara* enzyme immunoassay kits.

Kit	Company/Manufacturer	Specificity (%)	Sensitivity (%)
ToxocaraCHEK (3 min rapid test)	E.Y. Laboratories, Inc., USA	96	100
NOVUM <i>Toxocara canis</i> IgG/IgM ELISA	NOVUM Diagnostica, GMBH, Germany	80	96.7
ELISA PU	Parasitological Institut SAS, Germany	82.8	96.7
<i>Toxocara</i> Western Blot-G Kit (WB-1gG)	LDBIO Diagnostic, France	Not stated	Not stated
<i>Toxocara canis</i> ELISA kit	Bordier Affinity Products SA, Switzerland	Not stated	> 90
<i>Toxocara</i> Microwell Serum ELISA (Cat. No: 8206-3)	Diagnostic Automation, Inc., USA	87.5	93.3
ELISA <i>Toxocara</i> Ab Cypress Diagnostic (IgG)	Cypress Diagnostics, Belgium	98	86
NovaTec IgG ELISA	GMBH Immunodiagnostica, Germany	97	93
DRG <i>Toxocara canis</i> IgG (EIA-3865)	DRG International, Inc., USA	Not stated	Not stated
EIA <i>Toxocara canis</i> IgG	TEST-LINE s.r.o., Clinical Diagnostics, Czech Republic	Not stated	Not stated
<i>Toxocara</i> antibody IgG by ELISA	ARUP Laboratories, Inc., USA	Not stated	Not stated
<i>Toxocara</i> CELISA kit (cat. No: Z1KT2)	TCS Biosciences Ltd., UK	Not stated	Not stated
RIDASCREEN [®] <i>Toxocara</i> IgG test (cat. no: K7421)	R-Biopharm Clinical Diagnostic, Germany	Not stated	Not stated
<i>Toxocara</i> IgG CELISA kit (cat. no: KT3)	Cellabs Pty Ltd, Australia	Not stated	Not stated
Melotest <i>Toxocara</i> Antibody	Melotec Biotechnology, SA, Barcelona, Spain	Not stated	Not stated

performed. By extrapolation, specific antibody cross-reactions, inherent in using native TES as the diagnostic antigen, are less likely to be encountered and identified.

Serodiagnostic tests are often developed to address specific clinical and epidemiological issues in defined populations and their usefulness for broader studies can be constrained. Toxocariasis has been incorrectly seen as a disease of temperate climates, with much emphasis placed on effective serodiagnosis prior to treatment. Most *Toxocara* serodiagnostic tests were developed for such scenarios and while the sera used in their development would include those from individuals with a variety of parasitic infections, they probably would not include sufficient samples from case- and age-matched controls from areas where parasitic, and particularly ascariid, infections are hyperendemic. Cases of parasitism in temperate climates frequently involve single exposures with single species infections: polyparasitism, and its serological consequences, are uncommon. Furthermore, often reports of increased specificity and sensitivity are quoted in the context of increased differences between sera from cases and controls resident in temperate climates, rather than the ability to determine accurately the significance of antibody reactive to *Toxocara*, in the presence of a plethora of cross-reacting antibodies to other parasites/infectious diseases/antigens.

While TES should be an effective serodiagnostic antigen in temperate countries where few, if any, cross-reacting antibodies to it occur, *Toxocara* serodiagnosis in tropical countries will be fraught with concerns about the presence of cross-reacting antibodies from other commonplace parasitic infections, notably among the Ascaroidea of medical importance, particularly, ascariasis and the ascarid zoonoses, etc., in test sera. In Malaysia, and numerous other countries, clinical requests for *Toxocara* serology are usually infrequent, often due to lack of awareness and suspicion of the infection or for differential diagnosis. This situation results in smaller-scale production of locally produced, commercial *Toxocara* tests (as compared with other 'more common' parasite serology tests), which inadvertently results in higher cost and prices. This situation is aggravated by the low specificity of these tests when employed on serum samples from individuals exposed to other parasitic infections (Lynch *et al.*, 1988b; Rahmah *et al.*, 2005).

A major problem with *Toxocara* serodiagnosis in tropical countries is cross-reactivity with antibodies to soil-transmitted helminth (STH) infections such as *Ascaris lumbricoides*, *Trichuris trichiura*, hookworm and *Strongyloides stercoralis*. Although the use of TES has enabled greater assay specificity, it may not be sufficiently specific for use in countries where STH infections are prevalent (Lynch *et al.*, 1988; Rahmah *et al.*, 2005). Thus, commercially available ELISAs can have limited usefulness for *Toxocara* diagnosis in tropical countries. The tests may only prove useful for differential diagnosis, i.e. when the results are negative and the diagnosis of toxocariasis can be excluded, thus avoiding unnecessary treatment. However, when test results are positive, unless the titre is significantly higher in cases than in controls, and accompanying symptoms associated with *Toxocara* infection are present, test results may prove inconclusive.

Cypress *et al.* (1977) reported that ascarid antigens can not only cross-react with antibodies to other members of the family Ascarididae, but also with various proteins found in human sera, including C-reactive protein, rheumatoid factor and heterophile antibody. Solid-phase adsorption of test sera with extracts of a wide variety of non-homologous parasites revealed the existence of significant cross-reactivity in ELISA using TES (Lynch *et al.*, 1988), and these authors suggested that sera should be pre-absorbed with non-homologous parasite extracts to increase TES specificity prior to toxocariasis testing in tropical populations. Pre-absorption of test sera with *A. lumbricoides* antigens reduces cross-reactions (Cypress and Glickman, 1978; Chieffi *et al.*, 1990; Lescano *et al.*, 1998; Anaruma Filho *et al.*, 2003; Campos Junior *et al.*, 2003). Immunoblot analysis indicated that a ~55–66 kDa antigen complex was responsible for the cross-reactivity between *T. canis* and *A. suum* (Nunes *et al.*, 1999) while Lynch *et al.* (1988) identified an 81 kDa antigen as being responsible for the strong cross-reactivity between *T. canis* and *Ascaris* extracts.

Quality of TES

Variations in serodiagnostic assay sensitivity and specificity using TES probably occur between published studies worldwide because of differences in TES preparation (see below). For instance, failure to ensure that dead larvae are removed regularly

from cultures by Bärmannization will lead to TES contamination with soluble somatic antigens from dead or degenerating larvae.

Sufficient quality control and quality assurance (internal and external) should be in place to ensure strict reproducibility among batches of TES, so that the accumulation of dead or degenerating larvae in cultures, which occurs more frequently with older cultures, does not occur. With sufficient quality assurance and quality control, TES-based ELISA can be standardized for the serodiagnosis of human toxocariasis. Some limitations of TES ELISA for serodiagnosis and monitoring treatment of human toxocariasis are presented in Table 7.5.

TES is composed of a restricted set of discernible molecules, which exhibit an overlapping but not identical antigenic repertoire to those epitopes expressed at the larval surface (Sugane and Oshima, 1983; Maizels *et al.*, 1984). TES is composed of five major antigenic molecules as determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of relative molecular masses 32, 55, 70, 120 and 400 kDa (Maizels *et al.*, 1987; Maizels and Robertson, 1991). TES are heavily glycosylated proteins, possessing 40% carbohydrate by weight, with *N*-acetylgalactosamine, the immunodominant sugar of human A blood group antigen, contributing 58% of the total carbohydrate (Meghji and Maizels, 1986). The biology of TES is reviewed in Chapter 1 (this volume).

Kennedy *et al.* (1987) compared species-specific and common epitopes on the secreted and surface antigens of *T. cati* and *T. canis* infective larvae. While SDS-PAGE profiles of surface antigens of the two species showed few similarities, profiles of *T. cati* and *T. canis* TES showed

considerable homology. In addition, serum from infected animals and a human VLM patient exhibited complete cross-reactivity. Of a panel of eight monoclonal antibodies tested, one (Tcn-2) did not react with *T. cati* surface, somatic or TES antigens. Kennedy *et al.* (1987) concluded that the serological response in humans to *T. canis* is, by current serological methods, indistinguishable in specificity from that induced by *T. cati* infection, and that Tcn-2, which appeared to be *T. canis*-specific, could be used to permit discrimination.

Speiser and Gottstein (1984) tested two batches of TES, prepared independently in two different Swiss (Zurich and Basel) laboratories. SDS-PAGE and WB revealed at least ten different antigenic components common to the two TES preparations, but qualitative and quantitative differences between each TES were observed, one having a more complex composition than the other. Despite these differences, in round-robin testing, an accordance of serodiagnosis was obtained in 80% of 25 sera from patients with suspected *Toxocara* infection tested independently in two different ELISA systems in the two different laboratories, using the two TES batches. Using the 'Basel' TES and ELISA, specificity was 93% (46 of 3396 sera from patients with parasitologically proven extra-intestinal helminthic infections). Cross-reactions occurred mainly with sera from patients infected with filariae (five of 13 sera with very high filaria ELISA titres) and the reproducibility (intra- and inter-test variation) of two of the ELISA systems tested, using their corresponding TES, varied from 5 to 15%.

Our experiences indicate that antibodies from humans with *T. trichiura* cross-react more frequently with TES than antibodies from humans with *A. lumbricoides*. Eight of 11 sera (90%) from

Table 7.5. Limitations of TES ELISA and TES WB for serodiagnosis and monitoring treatment of human toxocariasis.

- Sensitivity and specificity are dictated by the manufacturer's choice of the target antigens and the quality control and quality assurance in place
- TES ELISA and TES WB offer the best compromise to date with native proteins
- Paired serum samples may be required for the best serodiagnostic outcomes
- Negative serum results do not rule out infection and/or disease, particularly for OLM
- Asymptomatic or equivocal symptoms: positive results must be treated with caution, especially in regions of endemic polyparasitism
- Treatment monitoring may be possible with paired serum samples, but results should be interpreted with caution. Serology does not offer short-term proof of eradication

Malaysians with *T. trichiura* infections were cross-reactive, as compared with nine of 15 (60%) sera from *A. lumbricoides*-infected patients (N. Rahmah, unpublished observations). The results reported by Hakim *et al.* (1992) also allude to this observation.

TES contains epitopes reactive with human anti-A and anti-B agglutinins (Smith *et al.*, 1983c) and their removal is recommended for improved test specificity in *Toxocara* serodiagnosis (Smith *et al.*, 1983c). However, Glickman and Schantz (1985) showed that sera with high isohaemagglutinin titres were not reactive when tested by IgG TES ELISA. Similarly, Magnaval *et al.* (1991) demonstrated that isohaemagglutinins did not affect the results of their *Toxocara*-specific IgG WB (IgG TES WB).

Confirmation beyond ELISA, WB and Dipsticks

Magnaval *et al.* (1991) demonstrated that a positive TES ELISA can be confirmed by WB, which was as sensitive as TES ELISA, and specific, particularly when low molecular mass (M_r) bands from 24 to 35 kDa were present. Positive reactions in IgG TES WB were confirmed by the presence of IgG antibody binding to four low M_r bands (24, 28, [fused bands of 24 and 28], 30 and 35 kDa) and three high M_r bands (132, 147 and 200 kDa). The low M_r banding pattern appeared to be specific for toxocariasis, but reactivity with the high M_r bands can also be found in sera from individuals with various helminthic diseases (Park *et al.*, 2000). A commercial IgG TES WB kit is available in Europe (*Toxocara* Western Blot IgG, LDBIO Diagnostics, Lyon, France).

In the rapid ToxocaraCHEK kit (EY Laboratories, Inc., Hong Kong), TES is immobilized on the nitrocellulose membrane of the test device and antibodies to TES bind to the immobilized antigen. Bound antibody is detected following the addition of a protein A-colloidal gold conjugate, which binds to form a red-coloured spot on the membrane where the test sample was applied (Akao *et al.*, 1997; Dubinsky *et al.*, 2000). ToxocaraCHEK was reported to show high correlation with ELISA, immunoblot and double gel diffusion tests (Akao *et al.*, 1997; Dubinsky *et al.*, 2000). Although ToxocaraCHEK has lower specificity than ELISA, being rapid, producing a result in

3 min, with no requirement for additional equipment, and is suitable as a screening test (Dubinsky *et al.*, 2000). Some limitations of TES WB for serodiagnosis and monitoring treatment of human toxocariasis are presented in Table 7.5.

Recombinant TES Antigens

Limitations due to the presence of cross-reactive antigens in TES and in regularly producing sufficient quantities of a standardized product have led researchers to investigate the possibility of using recombinant TES antigens for serodiagnosis. Recombinant immunogenic proteins must share important antigenic and immunogenic structures with natural protein antigens produced during infection, otherwise outcomes can be disappointing, particularly if the recombinant protein differs significantly from the native antigen. Recombinant antigens can provide a basis for serological tests with increased sensitivity and specificity compared with tests using native TES.

Two recombinant TES proteins have potential value for the serodiagnosis of human toxocariasis. TES-30 is a recombinant protein from *T. canis* second-stage larvae, corresponding to the 30 kDa antigen from TES. Sequence analysis of cDNA revealed an open reading frame encoding a ~24 kDa protein with similarity to regions corresponding to epidermal growth factor-like and lectin-like domains of the core proteins of vertebrate chondroitin sulfate proteoglycans (Yamasaki *et al.*, 1998). When used as antigen in WB, TES-30 reacted specifically with sera from toxocariasis patients, but not with sera from patients with *Brugia malayi* infection, dirofilariasis or ascariasis. Cross-reactions with anisakiasis sera occurred, but were ablated when these sera were pre-absorbed with *Anisakis* antigen (Yamasaki *et al.*, 1998). TES-30 appears sensitive and specific (Yamasaki *et al.*, 1998, 2000; Coelho *et al.*, 2003) with no cross-reactions reported with sera from individuals with *Ascaris* and hookworm, and only minimal cross-reactions with sera from individuals with gnathostomiasis, *Paragonimus miyazakii* and spirometrias. Yamasaki *et al.* (2000) examined 153 human serum samples from patients infected with 20 different helminths, including 11 cases of toxocariasis by ELISA. Cross-reactions were observed in 79 (55.6%) and 61 (43.0%) of 142 cases, respectively, when native TES was coated on to

ELISA plates at concentrations of 0.5 and 0.125 µg/ml. In contrast, cross-reactions were observed in 19 (13.4%) of 142 cases when TES-30 was coated at 0.5 µg/ml, but, when used at 0.125 µg/ml, cross-reactivity decreased to 2.1% (three of 142 cases).

The second recombinant antigen is expressed from the *Tc-muc-1* gene encoding TES-120, which reacted with all (8/8) toxocariasis sera tested but did not react with sera from patients with various helminthiasis and protozoan infections (0/45) (Fong *et al.*, 2003). Thus, TES-30 and TES-120 recombinant antigens should be evaluated further in order to confirm their potential as diagnostic antigens. Maizels *et al.* review the biology of recombinant TES proteins in Chapter 1 (this volume).

An ELISA based on solid phase polysiloxane/polyvinyl alcohol (POS/PVA) beads was reported to provide better results than a microplate ELISA when TES-30 was employed as antigen. Greater differences in mean absorbances between positive and negative sera were seen using POS/PVA beads (Coelho *et al.*, 2003) and this approach should also be tested using TES-coated beads.

Role for Minor Antibody Isotypes in Serodiagnosis

In infected rabbits, TES is the first mosaic of antigens to which an antibody response is detectable following infection, and could prove useful as an antigen for detecting recent infection (Smith *et al.*, 1982). Anti-*Toxocara* IgG antibody is usually the isotype targeted in serodiagnostic tests because of its abundance in serum and its increased avidity and affinity, due to the anamnestic response. The IgG isotype is a good reporter of infection/disease; however, other isotypes/IgG subclasses may also throw light on disease progression and possibly on treatment outcomes and can prove helpful in the diagnosis of toxocariasis (Smith, 1993; Magnaval, 2001).

One approach to determining the diagnostic potential of various antibody isotypes/IgG subclasses is to test numerous sera from serologically proven toxocariasis cases to identify benefits such as increased specificity to TES and reduced cross-reactivity, manifest as increased differences between titres of cases and controls. Although more difficult, another approach is to follow the natural history of

infection/disease in human hosts to determine the contributions of antibody isotypes/IgG subclasses over time, using sequential serum samples.

Smith (1993) reported that *T. canis* infection in humans stimulated the production of IgM, IgG and IgE antibodies to TES. TES-positive sera were tested for the presence of isotype-specific antibodies and serum selection was based on individuals with either classical presentations of VLM and OLM or non-classical presentations of toxocariasis. Sera from patients with CT were also tested. Where possible, repeat samples were obtained at varying time intervals, from seropositive patients. Sera containing high levels of non-*Toxocara* IgE or IgM antibodies including 33 samples from asthmatic patients with asthma of a non-parasitic aetiology and 13 from patients with atopic dermatitis, all with IgE levels >1000 IU, and sera from 12 patients with a positive rheumatoid factor were used as immunoglobulin-positive controls (Oliver *et al.*, 1986), but none was cross-reactive in the assays used.

The possible interference of IgG antibody in the detection of IgM and IgE antibody was assessed using the following procedures: (i) development of an antibody isotype-specific capture system, the reaction being completed following the addition of liquid-phase TES, a monoclonal antibody (Tcn-2; Maizels *et al.*, 1987), which was biotinylated, and streptavidin-conjugated enzyme; (ii) depletion of IgG from the test sample by adsorption on to *Staphylococcus aureus* (Cowan strain 1, NCTC 8530) and centrifugation; and (iii) increasing the amount of plate-bound TES (Smith, 1988). All procedures increased the sensitivity of detection, and procedure (iii) was found to be the most convenient and reproducible.

Sera from 27 Scottish patients, six of whom met criteria for VLM, nine who met criteria for OLM and 12 who did not meet the classical criteria for either VLM or OLM according to their clinical summaries, were tested for isotype-specific responses to TES, using a TES ELISA (Smith, 1993). Varying levels of IgG, IgE and IgM antibodies were present in these sera indicating that these major antibody isotypes are produced by individuals with either VLM or OLM. IgG was the predominant isotype in 52% of these cases, and either IgM or IgE titres were higher than IgG titres in 26% of cases. Some sera which initially had IgE titres below a diagnostic cut-off titre of 1:50, when retested at lower dilutions had titres

between 1:10 and 1:40. In one sample, IgM antibody (<1:50) was not detected (Smith, 1993).

Smith (1993) also reported on the analysis of 67 sera from patients with CT that demonstrated levels of total (heavy and light chain-specific anti-human antibody) antibody to TES similar to those of the VLM/OLM group. However, the IgE antibody responses of the former differed markedly from those of the VLM/OLM group, being less marked in the CT group. Comparison of total antibody titres of both the VLM/OLM and CT populations revealed that their distribution varied little between the two populations. Whereas the VLM/OLM populations exhibited a normal distribution, the distribution of CT titres was slightly negatively skewed, with 51% falling within the 1:50–1:100 serum dilution range. Over half (61%) of CT-positive sera exhibited IgE titres below 1:50 compared with 30% of the VLM/OLM-positive sera. Conversely, whereas 14% of the VLM/OLM group had IgE anti-TES antibody titres of 1:200 or above, only 1.5% of the CT group were positive at these higher titres. No statistical differences were observed for IgM responses between these two groups.

Interestingly, analyses of sera from individuals where repeat samples were available, and which were collected over a period of 2–3 years after their first presentation, showed that IgG, IgE and IgM isotypes were detectable at the majority of sampling points at varying titres, indicating that while class switching and affinity maturation of the immune response occurred, the IgM response is not transient in human toxocariasis.

Thus, antibody isotypes can vary in different presentations of human toxocariasis (VLM, OLM and CT) and over time in infected individuals. Where polyparasitism is not an issue, increasing TES coating concentration in TES ELISAs, in order to reduce interference from the major (IgG) antibody isotype, is advantageous when determining minor (IgM, IgE) antibody isotypes.

Antibody Isotypes and Serodiagnosis – Which Isotype/IgG Subclass?

IgE

Elevated IgE is a hallmark of tissue migratory parasites (Smith and Kennedy, 1993). An increase

in IgE specific for TES, measured by radioimmunoassay (Genchi *et al.*, 1988) and ELISA (Magna-val *et al.*, 1992; Smith, 1993, Smith and Kennedy, 1993) was reported in patients with clinical signs suggestive of *Toxocara* infection. Brunello *et al.* (1983) compared a radioallergosorbent IgE assay employing somatic larval antigen and an IgG TES ELISA and reported good qualitative agreement between IgG and IgE in terms of detecting larva-specific antibodies. The detection of larva-specific IgE could, therefore, represent an alternative approach to the clinical diagnosis of human toxocariasis. Magnaval *et al.* (1992) determined that the sensitivity and specificity of an IgE TES ELISA was moderate and insufficient for confirming a diagnosis of toxocariasis when used alone, but would complement an IgG TES ELISA. Of interest was the significant decrease in specific IgE antibodies in a diethyl carbamazine-treated group compared with specific IgG levels, which did not vary significantly in either pre- or post-treatment samples. Thus, an IgE TES ELISA could prove more valuable than an IgG TES ELISA for post-treatment follow-up assessment. At the Laboratoire de Parasitologie, Centre Hospitalier Universitaire Purpan, Toulouse, France, the WB procedure remains the first-line assay for serodiagnosis of human toxocariasis. A serum sample found to be negative in the WB is tested by IgE TES ELISA. Sera containing greater than 500 *Toxocara* units/l of IgE are classified as toxocariasis cases (Magnaval *et al.*, 1992).

IgM

Smith (1993) demonstrated that the IgM response can be long lasting in human toxocariasis, but few published reports identify the usefulness of specific IgM for *Toxocara* serodiagnosis. A case report described nephrotic syndrome in a 7-year-old boy who had *Toxocara*-specific IgM. Treatment with corticosteroids resulted in remission of renal symptoms as well as a decrease in IgM titre (Shetty and Aviles, 1999). Another report described a case of VLM, with elevated *Toxocara* specific IgG and IgM titres. Following treatment, serology for *T. canis* revealed decreased IgM levels and elevated IgG levels (Arango, 1998). Matsu-mura *et al.* (1998) stated that the absorption of test sera with protein A could prove useful for detecting IgM antibodies to TES.

IgG subclasses

Obwallner *et al.* (1998) examined IgE reactivity by TES WB, IgG subclasses (IgG1–4) reactivity by TES ELISA and the formation of IgE/anti-IgE immune complexes in serum samples from persons with symptomatic (VLM, OLM) and asymptomatic infection. IgE TES WB reactivity was marginally higher in symptomatic (35%) than in asymptomatic (24%) persons, but without statistical significance. TES-specific IgG subclass reactivity was similar in both symptomatic and asymptomatic (IgG1 > IgG2 > IgG4 > IgG3) persons and IgG1, -2 and -4 showed significant differences between patients with signs and symptoms of VLM and asymptomatic persons ($P < 0.001$) but not between patients with signs and symptoms of OLM and asymptomatic persons. Significantly elevated levels of IgE/anti-IgE immune complexes were detected in sera of both VLM and OLM individuals ($P < 0.001$).

Detection of *Toxocara*-specific IgG4 can increase the specificity of *Toxocara* serodiagnosis (Wiechinger, 1998; Rahmah *et al.*, 2005). Comparison of an IgG TES ELISA and an IgG4 TES ELISA showed that the sensitivity of the IgG TES ELISA was 97.1% while that of the IgG4 TES ELISA was 45.7% and the specificity was 36.0 and 78.6%, respectively (Rahmah *et al.*, 2005). The authors suggested that an IgG4 immunoassay would also prove useful for the secondary screening of recombinant antigen clones in an effort to develop improved serological tests for toxocariasis (Rahmah *et al.*, 2005).

IgG antibody avidity

Determining antibody avidity can prove useful in the diagnosis of infectious diseases. Their uses include distinguishing between reactivation, reinfection or primary infection, and such assays can improve serodiagnosis (Gutierrez and Martoto, 1996). Neoantigens from parasites that are killed following treatment, or reactivated, can induce a primary-type immune response, producing a low-avidity antibody response, although increased avidity to previously recognized antigens, due to an increased secondary response, can also occur (Mechain *et al.*, 2000).

Hubner *et al.* (2001) developed an IgG avidity test for diagnosing acute toxocariasis. The index of avidity was calculated as the ratio of IgG values in sera treated with urea and the value of IgG in non-treated sera, multiplied by 100. An index up to 40 was considered low avidity indicative of a recently acquired infection, 36–40 was borderline and more than 40 was considered high avidity indicative of past infection. In a group of 1376 patients, only 5.09% of sera had low-avidity antibody, indicative of a recently acquired infection.

Thus, investigations into IgM, IgE and IgG subclass reactivity, together with investigation into antibody avidity, could help improve *Toxocara* serodiagnosis. Furthermore, increasing TES coating concentration in order to reduce interference from the major (IgG) antibody isotype can prove advantageous when determining minor (IgM, IgE) antibody isotypes and probably IgG subclasses.

Serology and Ocular Larva Migrans

Serodiagnosis of OLM, using the IgG TES ELISA, is more challenging than serodiagnosis of VLM and CT since serum antibody levels are usually low or undetectable (Glickman *et al.*, 1986), and eosinophilia is often absent. When OLM is suspected, aqueous or vitreous fluids offer the best diagnostic options. Biglan *et al.* (1979) used a *T. canis* embryonated egg antigen to demonstrate the presence of anti-*Toxocara* antibodies in both vitreous fluid and serum in five patients with a clinical diagnosis of OLM. In all but one case, antibody titres were substantially higher in vitreous fluid than in serum (Felberg *et al.*, 1981). In another OLM case report, the antibody ELISA titre of vitreous fluid was 1:1024, compared with 1:64 in the serum sample (Biglan *et al.*, 1979). TES can discriminate more readily between sera from OLM cases and retinoblastoma cases than *T. canis* embryonated egg antigen, and preabsorption of sera with *A. suum* embryonated egg antigen, essential for preventing false positive reactions with *T. canis* embryonated egg antigen, was not so critical for TES ELISA (Glickman *et al.*, 1985). Pollard *et al.* (1979) and Shields *et al.* (1979) recommended that ocular fluids should be diluted to 1:8 prior to testing,

compared with a dilution of 1:32 for sera from suspected VLM cases, yet with this potential for increased sensitivity, ~10% of patients with characteristic clinical features of OLM have negative ELISA findings.

In OLM, the higher titres of anti-TES antibody in ocular fluids than in serum (Brasseur *et al.*, 1984; Magnaval, 2001) are suggestive of local antibody production (Felberg *et al.*, 1981; Benitez del Castillo *et al.*, 1995) and Schneider *et al.* (2000) reported a case of OLM with an optic disc granuloma that was TES ELISA negative when the patient's serum was tested. Clinical diagnosis was confirmed by TES ELISA using aqueous humour, which contained anti-TES antibody.

Genchi *et al.* (1986) compared TES ELISA for larva-specific IgG and TES RAST for larva-specific IgE on 12 sera from patients with a clinical diagnosis of OLM. The IgE assay had greater sensitivity and they recommended that both IgG and IgE antibody levels should be determined in cases of OLM. Similarly, Golab and Dzenski (1993) reported that an IgE TES ELISA improved the immunodiagnosis of toxocariasis, and recommend that it should be used as a complementary serodiagnostic method.

According to Magnaval *et al.* (2002), an IgG TES WB coupled with an IgE TES ELISA is a convenient procedure for the immunodiagnosis of OLM, provided that testing is simultaneous on both serum and aqueous fluid. The IgG TES WB is more sensitive than the IgE TES ELISA, when low M_r bands (24–35 kDa) are present (Magnaval *et al.*, 1991). The IgE TES ELISA was 67.9% sensitive and 76.2% specific when the cut-off value was 5 *Toxocara* units (TU), and, according to the authors, although the IgE TES ELISA was positive using sera from individuals with non-toxocaral helminthic infections, the IgG TES WB is *Toxocara* specific only when the high M_r bands are absent (Magnaval *et al.*, 1992). Further evidence of the usefulness of the IgE TES ELISA and the IgG TES WB were presented in a study of five OLM cases, where both IgG TES WB and IgE TES ELISA were positive in all cases (Park *et al.*, 2000).

Petithory *et al.* (1990) reported that the differential diagnosis between retinoblastoma and OLM using gel precipitation methods (electrocyneresis, Ouchterlony double diffusion, immunoelectrophoresis) is more effective than using TES ELISA. Using TES from *T. canis* and *T. cati* larvae

to detect antibodies in endo-ocular fluids, Petithory *et al.* (1990) demonstrated positive reactions in all eight cases of OLM, and no false positive reactions from 11 control sera.

Importantly, many ELISA-based commercial kits do not provide information on the usefulness of their test for diagnosing OLM, particularly whether serum and ocular fluid can be used, the initial dilution for ocular fluid, cut-off titres/optical densities for ocular fluids and test interpretation.

Supporting Investigations

A single seropositive result has limited diagnostic significance, since current immunodiagnostic tests are not capable of distinguishing between current and past infection. Only patients with clinical signs and symptoms consistent with active *Toxocara* infection are candidates for therapy (Magnaval *et al.*, 2001). Immunological testing should therefore be accompanied by blood eosinophil count as a minimum, and where possible, total IgE level in serum. A finding of both a peripheral eosinophilia and a positive serologic test, in the absence of any evidence of helminth infection or other positive helminth serology, is highly suggestive of active toxocariasis. Awareness of the signs and symptoms identified in Table 7.1 is a prerequisite.

The diagnosis is less certain in individuals without eosinophilia but who present with one or more clinical signs of covert toxocariasis. In such individuals, a concentration of serum total IgE > 500 IU/ml can support other evidence of recent *Toxocara* infection. The detection of eosinophil cationic protein, released by activated eosinophils, could also be helpful (Magnaval *et al.*, 2001).

For OLM, in the majority of instances, the clinical appearance will be the diagnostic criterion, with a positive serology substantiating the diagnosis. In some patients, opaque media radiological imaging can be beneficial (Sabrosa and de Souza, 2001) and ultrasonography can reveal a highly reflective, peripheral mass, vitreous bands or membranes and traction retinal detachment (Wan *et al.*, 1991). Ultrasound biomicroscopy, computerized tomography scan and magnetic resonance imaging studies may also be helpful (Schantz and Glickman, 1978; Parke and Shaver, 1995; Tran *et al.*, 1999).

Future Directions

Toxocara antibody titres can remain positive in the absence of disease and eosinophilia can take more than 2 years to decline to normal values (Ree *et al.*, 1984). In addition, current laboratory investigations are not useful for measuring treatment success (Gill *et al.*, 1978). In many temperate countries, TES-based ELISAs and WBs may provide sufficient support for clinical suspicion, but the proven TES cross-reactivity with antibodies from common helminth infections of humans, among others, reduce the usefulness of TES-based serodiagnosis in regions where polyparasitism is endemic. Few cross-reactions with TES have been identified where ocular fluids are used to support the diagnosis of OLM, and TES ELISA and TES WB may still have a role to play using these fluids.

Similarly, there is little information available on the usefulness of serology for detecting anti-TES antibody in cerebrospinal fluid (CSF) in suspected cases of neurological toxocariasis (Gould *et al.*, 1985; Hill *et al.*, 1985; Russeger and Schmutzhard, 1989; Smith, 1991) and further investigations are required in this area. Here, round-robin testing would prove useful.

In order to improve diagnosis, treatment strategies and patient management, further research is required to develop or improve assays that can determine the course of disease and the efficacy of treatment and to develop parasite viability tests for TES-seropositive individuals. Here, IgG4 TES ELISAs may prove more effective than IgG TES ELISAs. Serological studies into human lymphatic filariasis have identified that specific IgG4 is a good marker of active infection (Ottesen *et al.*, 1985; Kwan-Lim *et al.*, 1990) and the role of *Toxocara*-specific IgG4 antibody responses for serodiagnosis should be investigated further. This will require better communication between clinicians and diagnostic laboratories so that time-course serostudies can be performed and investigations into antibody and antigen kinetics before, during and after treatment can be undertaken and evaluated.

A role for IgE antibody detection, particularly for post-treatment follow-up, has already been identified (Magnaval *et al.*, 1992). Similarly, decreased IgM antibody levels and elevated IgG antibody levels have also been reported following

treatment (Arango, 1998). Smith (1993) reported that IgM antibody was not transient in human toxocariasis. The sensitivity and specificity of a *Toxocara*-specific IgM antibody approach to serodiagnosis is also worthy of further investigation. Such an approach, coupled with antibody avidity studies, could determine whether infection was acquired recently. IgG antibody avidity studies (Hubner *et al.*, 2001) indicate that recently acquired infection can be distinguished from chronic/past infection, and this interesting observation deserves further exploration.

A major advantage of using TES WB is the ability to identify antigens deemed important in serodiagnosis and their discrimination from other, cross-reactive antigens in TES. The lower M_r antigens in TES (24, 28, 30 and 35 kDa) appear to predict exposure to *Toxocara* better than the higher M_r antigens (132, 147 and 200 kDa) (Magnaval *et al.*, 1991), as reactivity with the high M_r bands occurs using sera from individuals with various helminthic diseases (Park *et al.*, 2000). Analysis of some ascaridid cross-reactions by immunoblot revealed that antigens > 50 kDa were cross-reactive: Nunes *et al.* (1999) identified a ~55–66 kDa cross-reactive antigen complex, while Lynch *et al.* (1988) identified that an 81 kDa antigen was responsible for the strong cross-reactivity between *T. canis* and *Ascaris* extracts. Further TES WB testing should be undertaken with sera from individuals from areas of endemic polyparasitism to determine whether the low M_r antigens, as opposed to the higher M_r antigens, are, indeed, diagnostic for human toxocariasis.

Based on the above information, a purified native TES product containing antigens of <50 kDa should prove equally effective and less cross-reactive for *Toxocara* serodiagnosis in a variety of assay formats including ELISAs, WB dot blots and rapid assays. Simple protein sieving technology such as gel filtration and ultrafiltration would deliver the required product. Again, the focus for testing a purified native TES product would, undoubtedly, be sera from individuals from regions of endemic polyparasitism, given the problems identified with using TES in these regions. In dot blot or rapid assay format, such a product would also be useful for screening, and may enable a lower dilution of serum/diagnostic fluid to be used because of reduced cross-

reactivity/increased specificity. Furthermore, the usefulness of minor antibody isotypes and IgG subclasses should also be tested with a purified TES reagent.

Recombinant antigens should offer further solutions for *Toxocara* serodiagnosis by providing increased sensitivity and specificity, compared with native TES. Important candidates include those corresponding to the lower M_r antigens in TES (24, 28, 30 and 35 kDa) such as TES-30. Recombinant antigens must share important antigenic and immunogenic structures with native TES, otherwise they cannot provide these much required solutions. Although much of the antibody reactivity in human toxocariasis is directed against carbohydrate epitopes (Smith, 1993), the use of eukaryotic expression systems should address this issue.

The detection of circulating *Toxocara* antigen has been reported (Gillespie *et al.*, 1993; Robertson *et al.*, 1988; Dzbenski *et al.*, 1999) and remains an option for serodiagnosis, although capture antibody specificity and the requirement to detect uncomplexed circulating *Toxocara* antigen limit its application. Immune complex formation, in the presence of high-avidity anti-*Toxocara* antibodies, also reduces test sensitivity, but immune complex dissociation prior to performing antigen detection can relieve this problem. Reports indicating that TES contains carbohydrate epitopes suggest the possibility of heat dissociation prior to antigen testing. However, since L2 are normally tissue associated and larval burdens can be low, it may prove difficult to develop high-sensitivity, serum antigen detection tests. The possibility of developing an antigen detection test for ocular fluids (and CSF) should not be overlooked, given the frequently higher antibody titres found in these fluids in OLM cases.

Currently, the best serodiagnostic options are using the IgG TES ELISA as a screening test (Tables 7.3 and 7.4), with confirmation by IgE TES ELISA and WB (Magnaval *et al.*, 2001; Table 7.3). Increased specificity can be achieved by using an IgG4 TES ELISA (Rahmah *et al.*, 2005) and IgG4 WB may also be useful as it may provide further discrimination following IgG TES ELISA screening. Better insight into the significance of minor antibody isotypes can be provided by increasing TES coating concentration, although its usefulness may be outweighed in regions where polyparasitism is endemic. While

TES can be used in temperate countries, species-specific recombinant antigens, or cocktails thereof, are necessary for use in tropical countries.

The use of PCR for detecting *Toxocara* DNA in human biopsy material has not been reported, but it has been used to detect *Toxocara* DNA in experimental murine liver biopsy material (Rai *et al.*, 1997). rDNA and mitochondrial markers can offer good insight into species identity and have been shown to be useful for epidemiological investigations and the diagnosis of *Toxocara* infection (Zhu *et al.*, 2001; Chapter 2, this volume).

An area which lags behind in the field of *Toxocara* serology is that of determining the contribution to human toxocariasis from *T. cati*. A *T. cati*-specific, recombinant, serodiagnostic antigen is feasible, since antibody cross-reactivity between *T. canis* and *T. cati* is not complete. Sakai *et al.* (1998) reported a case of OLM where antibody reactivity to *T. canis* TES ELISA was borderline, but was more reactive (< 0.35 absorbance units versus ~0.55 absorbance units) with *T. cati* adult worm antigen.

Human Toxocariasis – Beyond *T. canis*

While the debate surrounding the choice of antigens and assay formats for this enigmatic parasite continues, and also whether *T. cati* is a significant cause of human toxocariasis, reports of a new species of *Toxocara*, *Toxocara malaysiensis* (Gibbons *et al.*, 2001; Zhu *et al.*, 1998), in cats have appeared in the literature (see Chapter 2, this volume). Of the ascaridid nematodes examined from 50 cats, 26% were identified as *T. malaysiensis* and 16% as *T. cati* (Sani *et al.*, 2004). Whether this species contributes to the burden of human toxocariasis is not known currently, but as *T. cati* has been implicated, then so might *T. malaysiensis*. *T. malaysiensis* larval migration can be studied in suitable animal models, which may throw light on larval migration patterns in humans, and the pathologies and disease states that it might generate. As for *T. cati*, should adult *T. malaysiensis* infections of humans occur, and if *T. malaysiensis* completes its larval development in humans, would it cause less pathology than *T. canis* in humans? For these reasons, the development of diagnostic antigens that can detect *T. malaysiensis*

infection is desirable. This adds further mystery and impetus to the debate on clinical presentations, epidemiology and serodiagnosis, which can be addressed using multidisciplinary approaches and the tools currently available to us.

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8 Management and Treatment Options for Human Toxocariasis

Jean-François Magnaval¹ and Lawrence T. Glickman²

¹Department of Parasitology, Rangueil University Hospital, Toulouse, France

²Department of Veterinary Pathobiology, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana, USA

Introduction

Human toxocariasis is a zoonosis caused by infective larvae of *Toxocara canis* (Beaver, 1956) or *Toxocara cati* (Nagakura *et al.*, 1990). These ascarids are commonly found in the tissues (larvae) and intestinal tract (adult worms) of dogs and cats, respectively. Infection results from ingestion of embryonated eggs in soil (Glickman and Schantz, 1981) or on contaminated fomites (Vazquez Tsuji *et al.*, 1997). Live larvae can be ingested with raw or undercooked meat, giblets or offal (Nagakura *et al.*, 1989; Stürchler *et al.*, 1990; Fan *et al.*, 2004; Taira *et al.*, 2004).

Toxocara infection results in a wide variety of syndromes in humans, although most infections are probably subclinical. Visceral larva migrans (VLM) was first described in 1952, in children with an enlarged liver and hypereosinophilia (Beaver *et al.*, 1952). The typical VLM patient is a child between the ages of 2 and 7 years with a history of geophagia and exposure to puppies in the home. The clinical signs of VLM are usually associated with hepatic and pulmonary larval migration and include abdominal pain, decreased appetite, restlessness, fever, coughing, wheezing, asthma and hepatomegaly (Ehrard and Kernbaum, 1979). Infection is usually characterized by marked and chronic eosinophilia ($>2.0 \times 10^9$ cells/l), leukocytosis and hypergam-

maglobulinaemia. In industrialized countries, VLM is relatively uncommon, and a review of the literature from 1952 to 1979 found only 970 reports (Ehrard and Kernbaum, 1979).

Other forms of toxocaral disease were identified in the 1980s by two case-control studies conducted in adults in France (Glickman *et al.*, 1987) and in children in Ireland (Taylor *et al.*, 1988). In French adults, toxocariasis was characterized clinically by weakness, pruritus, rash, difficulty in breathing and abdominal pain. Significant laboratory findings included mild peripheral eosinophilia and increased total serum IgE. This syndrome was termed 'common toxocariasis' in adults (Magnaval *et al.*, 1994a). In Ireland, the most frequent clinical findings in children infected with *Toxocara* larvae included fever, anorexia, headache, abdominal pain, nausea, vomiting, lethargy, sleep and behaviour disorders, pharyngitis, pneumonia, coughing, wheezing, limb pains, cervical adenitis and hepatomegaly. Twenty-seven per cent of patients had high anti-*Toxocara* antibody titres, but a normal eosinophil count. This form of the disease in children was coined 'covert toxocariasis' (Taylor *et al.*, 1988).

Toxocariasis has also been associated with allergy-related syndromes including angioedema (Magnaval and Baixench, 1993), chronic urticaria (Wolfrom *et al.*, 1996), prurigo (Humbert *et al.*, 2000) and reactive arthritis (Bethel, 1981).

Ocular toxocariasis (see Chapter 9, this volume) typically occurs unilaterally in children and young adults. The most common symptom is visual loss with onset over a period of days to weeks. In some individuals these signs may wax and wane over a period of years, often related to migration of larvae in the retina and granuloma formation. Many ocular infections are subclinical and detected during a routine eye examination. Ocular toxocariasis apparently is an endemic disease in some areas of the USA (Maetz *et al.*, 1987), and the prevalence was estimated at 6.6 cases per 100,000 persons in Ireland (Good *et al.*, 2004).

Toxocara larvae readily migrate to the brain of experimentally infected laboratory animals (see Chapter 5, this volume). However, a review of the literature in English from 1950 to the present, found only 29 cases of neurological toxocariasis in humans (Moreira-Silva *et al.*, 2004). *Toxocara* infection of the central nervous system (CNS) elicits non-specific neurological signs such as seizures and headache, thus leading to an underdiagnosis of this condition (Magnaval *et al.*, 1997).

Diagnostic Methods for Toxocariasis

A definitive diagnosis of toxocariasis is often a significant challenge for the clinician, since the clinical picture of this helminthiasis is quite non-specific (see Chapter 7, this volume). For example, the symptoms can mimic those found with haematological malignancies, infections with other helminthic parasites and non-infectious conditions, including allergies and asthma. At this stage in the diagnostic process, a careful history regarding occupational and household chemical exposures, drug exposures, asthma, eczema or rhinitis, travel to tropical areas and country of origin, contact with domestic animals particularly puppies, consumption of raw or undercooked meats, and pica, specifically geophagia, should be obtained.

Human toxocariasis is most often a benign, asymptomatic and self-limiting disease, as long as re-infection does not occur. Residual anti-*Toxocara* antibodies have no pathological significance but can persist for years. Anti-*Toxocara* antibodies measured by ELISA were found to persist for up to 2.8 years in infected adults in Switzerland (Jeanneret, 1991), while anti-*Toxocara* antibodies detected by Western blot (WB) can persist for over

5 years (Rubinsky-Elefant, 2004). Seroprevalence surveys in Western countries found that 2–5% of apparently healthy adults from urban areas had a positive anti-*Toxocara* antibody titre compared with 14.2–37% of adults in rural areas (Magnaval *et al.*, 1994a). In tropical countries, the seroprevalence of toxocaral infection was much higher, namely 63.2% in children and teenagers in Bali (Chomel *et al.*, 1993), 86% in Saint Lucia, West Indies, among children (Thompson *et al.*, 1986) and 92.8% in adults in La Réunion Island (Magnaval *et al.*, 1994b). Since the presence of anti-*Toxocara* antibodies alone does not distinguish between current and past infections, it should be accompanied by other laboratory tests for blood eosinophil count and total serum IgE.

Chronic eosinophilia is generally considered a reliable indicator of active helminthiasis. However, the differential diagnosis of this sign is consistent with (in decreasing frequency): common allergies, hypersensitivity to drugs and chemicals (especially β -lactam antibiotics and cholesterol-lowering agents), helminthiasis, non-allergic conditions including neoplasia (e.g. hepatic and pulmonary carcinomas, Hodgkin's disease), dermatologic diseases (e.g. bullous pemphigoid), digestive diseases (e.g. Crohn's and Whipple's disease), vasculitis (e.g. Churg–Strauss syndrome, polyarteritis nodosa) and hypereosinophilic syndrome (HES).

Following a history and clinical examination, biological investigations should include non-specific tests for erythrocyte sedimentation rate and C-reactive protein, and measurement of serum immunoglobulins IgG and IgE. Since the first report in 1968 (Johansson *et al.*, 1968) of elevated total serum IgE in Ethiopian preschool children with ascariasis, elevated total serum IgE concentrations have also been found in patients with anisakiasis, cystic echinococcosis, filariasis, schistosomiasis, strongyloidiasis and toxocariasis. The finding of a substantial (\geq fourfold the upper normal value) increase of serum IgE is therefore a valuable indication of helminth infection, especially when associated with blood eosinophilia. Non-allergic causes of eosinophilia, such as carcinomas and vasculitis, are usually not associated with an elevated level of total IgE, except in one form of HES (Roufosse *et al.*, 2004).

Repeated stool examinations, including the Baermann method for detection of *Strongyloides stercoralis* larvae, should be performed to rule out other parasitic infections. Negative stool exams in

the presence of eosinophilia and an elevated serum IgE are indications for specific immunodiagnostic tests for parasites including anisakiasis, ascariasis, strongyloidiasis, trichinellosis and toxocariasis. Serological tests for cystic or alveolar echinococcosis may be indicated in endemic areas for this parasite, as are tests for tropical helminthic diseases including filariases and schistosomiasis for immigrants from endemic areas and persons with a travel history (MagnaVal, 1998b). In patients presenting with severe hepatomegaly, lymphadenopathy and/or splenomegaly, a bone marrow examination after aspiration or biopsy is indicated for the diagnosis of possible haematological malignancy.

Non-atopic patients who exhibit allergic signs, especially chronic urticaria together with high titres of anti-*Toxocara* IgG antibodies, and elevated total serum IgE, but without eosinophilia, represent a diagnostic challenge. Depending on whether these patients have active toxocaral infection, the therapeutic options will differ. There are at least three causes of such a clinical and laboratory picture. These are: (i) clinical syndrome, the aetiology of which is not determined, associated with a self-cured toxocariasis; (ii) clinical disease due to self-cured but repeated light toxocaral infections leading to the presence of circulating IgE/anti-IgE complexes (Obwaller *et al.*, 1998), a situation associated with allergic syndromes such as chronic urticaria (Kikuchi and Kaplan, 2001); and (iii) active, but covert toxocariasis, in which the lack of eosinophilia does not exclude an extravascular eosinophilia, since eosinophils may preferentially accumulate in certain tissues (Rytonaa, 1960). Only the third option will require specific anti-*Toxocara* drug therapy.

Medical imaging can be used to detect and localize granulomatous lesions due to migrating *Toxocara* larvae in tissues and to support a tentative diagnosis of toxocariasis, especially in patients exhibiting clinical signs of VLM. Abdominal ultrasound (US) demonstrated multiple hypoechoic areas in the liver of 14 children presenting with hepatomegaly, eosinophilia and positive *Toxocara* serology (Baldissarro *et al.*, 1999; Gonzalez *et al.*, 2000). Using computed tomography (CT), hepatic lesions may appear as low-density areas (Dupas *et al.*, 1986; Ishibashi *et al.*, 1992; Hartleb and Januszewski, 2001).

The diagnosis of ocular toxocariasis is particularly challenging, since unlike the signs associated with the peripheral forms of toxocaral

disease, eosinophilia is uncommon or mild even in patients with severe ocular manifestations (Glickman and Schantz, 1981; Altcheh *et al.*, 2003). Furthermore, serum anti-*Toxocara* antibodies may not be detected either by ELISA (Glickman *et al.*, 1986) or by the more sensitive WB (MagnaVal *et al.*, 2002). Anti-*Toxocara* antibodies when found in the aqueous or vitreous fluid of patients with clinical signs of ocular toxocariasis, however, should be considered diagnostic for ocular toxocariasis. The anti-*Toxocara* antibody titre in these fluids has been found to be higher than that found in serum obtained from patients with ocular toxocariasis (Brasseur *et al.*, 1984; Bertelmann *et al.*, 2003). Imaging techniques are especially helpful and, when they reveal an ocular granuloma, argue against the need for puncture of the anterior chamber to obtain aqueous fluid for immunodiagnosis. Ultrasound of 11 patients with ocular toxocariasis revealed a highly reflective peripheral mass, vitreous band or membrane and traction retinal detachment (Wan *et al.*, 1991). These results are consistent with those found by CT or magnetic resonance imaging (MRI) (Templeton and Rao, 1987; Mafee *et al.*, 1989). A related imaging method, ultrasound biomicroscopy (USB), appeared to be more accurate. In the eyes of 15 patients with clinical and laboratory diagnosis of peripheral vitreoretinal toxocariasis, the following abnormalities were observed using USB: vitreal membranes (13 cases), *Toxocara* granuloma (11 cases), pseudocysts (eight cases), thickening of the ciliary body (six cases), cystic formation (two cases), peripheral retinal detachment (two cases), rectification of the iris root (one case) and posterior synechiae (one case) (Cella *et al.*, 2004).

Neurological syndromes observed during *Toxocara* infection of the CNS are generally non-specific and peripheral eosinophilia is often lacking. Medical imaging is especially helpful to investigate these patients further. MRI can detect granulomas located cortically or subcortically, and these may appear as hyper-intense foci on proton-density and T₂-weighted images (Ruttinger and Hadidi, 1991; Ota *et al.*, 1994; Xinou *et al.*, 2003). When associated with eosinophilia in the cerebrospinal fluid (CSF), such images are consistent with a toxocaral infection. The finding of *Toxocara* larvae in CSF, in brain tissue or in the meninges and/or a positive anti-*Toxocara* antibody titre in CSF represents a further decisive argument (reviewed by Moreira-Siva *et al.*, 2004).

Rationale for Treatment with Anthelmintic Drugs

Limitations of experimental studies

There is scarce information about the drug susceptibility of *Toxocara* larvae in paratenic hosts. Most experiments have been done with experimentally infected *T. canis* mice and no drug susceptibility tests have been conducted with live larvae *in vitro*.

When comparing the results of experimental drug efficacy studies, there is always a concern about the relative size of the inoculum used. For example, in one study, rodents whose weight was about 35 g were given from 500 to 2000 embryonated eggs, corresponding to a dose of about 1–4 million eggs for a human adult. An analogous situation would only be encountered with children having a history of geophagia or pica that results in repeated infections with large numbers of *Toxocara* larvae. In most other clinical forms of toxocaral disease, such as covert or ocular toxocariasis, the larval inoculum is likely to be much smaller. In one experiment done in 1959, a human volunteer was given about 100 *T. canis* embryonated eggs *per os*. His blood eosinophil count increased to 13.5×10^9 cells/l on day 30 post-infection; 4.5 months post-infection it was 6.15×10^9 cells/l, and was accompanied by a persistent cough (Chaudhuri and Saha, 1959). This syndrome is similar to that observed in people with common or covert toxocariasis.

Testing anthelmintic activity in *Toxocara canis*-infected animals

Most anthelmintic drug studies were conducted during the 1970s to the mid-1980s (Dafalla, 1972; Nicholas and Stewart, 1979; Holt *et al.*, 1981; Abo-Shebada and Herbert, 1984; Abdel-Hameed, 1984; Delgado *et al.*, 1989; Fok and Kassai, 1998), and there have been very few studies conducted since then (Hrčková and Velebný, 2001) (see Chapter 12, this volume). The efficacy of drugs has been generally assessed following infection and treatment of mice using larval recovery from artificially digested tissues as the outcome measure, and by comparing the findings

with untreated and infected control animals. Some drugs including thiabendazole (TBZ) had a negligible larvicidal effect, but produced marked inhibition of larval migration through the tissues (Abdel-Hameed, 1984). Levamisole, ivermectin, albendazole (ABZ) and fenbendazole (FBZ) have been associated with larval retention in the liver followed by migration of very few larvae to muscles and brain of treated mice (Abo-Shebada and Herbert, 1984). Most larvae retained in the liver subsequently died and were not recoverable by day 35 post-treatment. However, these findings are likely to have minimal application to the treatment of humans, since treatment of patients with VLM or ocular toxocariasis would typically be instituted long after most larval migration ceases.

Interesting results were obtained in a study conducted by Fok and Kassai (1998) in which mice infected once with *T. canis* larvae were treated on days 2, 14, 81, 87 or 123 post-infection using several drug regimens. The larvicidal potential of ABZ, FBZ, flubendazole (FUBZ), oxbendazole (OBZ) and ivermectin was assessed. Reductions of 98.8 or 100% in group mean larval counts were recorded after a 30-day course of FBZ at 750 mg/kg b/w daily, or ABZ at 220 mg/kg b/w daily, respectively. Efficacy rates of 88.2 or 81.1% were achieved by a 20-day course of FUBZ at 700 mg/kg b/w daily or OBZ at 750 mg/kg b/w daily, respectively. Ivermectin, when given at various doses, showed only moderate larvicidal potential. The blood–brain barrier was found to be permeable to most drugs evaluated, but the daily doses used in this study were far greater than those currently recommended for human therapy.

When mebendazole (MBZ) was tested in experimentally infected mice, a 3-day regimen of 100 mg/kg b/w daily, given from days 1 to 3 post-infection, resulted in a 43% decrease in whole-body larval recovery (Bardon *et al.*, 1995). Diethylcarbamazine (DEC) given intraperitoneally at 25 μ g/kg b/w for 3 days post-infection elicited a 84.7% reduction in larval recovery (Dafalla, 1972).

Anthelmintic therapy in humans

Though numerous anthelmintic drugs have been tested for efficacy against *T. canis* in animals, few have been licensed for use in humans, and randomized studies have rarely been conducted.

Benzimidazole derivatives

These compounds bind selectively to parasite β -tubulin and prevent microtubule formation (Martin *et al.*, 1997).

Albendazole

ABZ (methyl-5-propylthio-1*H*-benzimidazol-2-ylcarbamate) is poorly absorbed from the gastrointestinal tract, and should be taken with fat (Dayan, 2003). A controlled randomized study in which ABZ was given at 10 mg/kg b/w daily for 5 days found a clinical success rate of 47% (Stürchler *et al.*, 1989). Sixty per cent of patients complained about minor side effects. Despite these mixed results, a review of the literature found numerous anecdotal reports (Bhatia and Sarin, 1994; Varga and Auer, 1998; Abe *et al.*, 2002; Inoue *et al.*, 2002; Bachmeyer *et al.*, 2003) indicating that ABZ was a commonly used drug for the treatment of toxocariasis. This is probably related to the fact that its use is associated with few significant adverse reactions and it is widely available in most countries.

A large, randomized, controlled study of ABZ using DEC as reference drug was conducted from 1998 to 2004 in the Department of Parasitology of Toulouse University Hospital. The ABZ treatment arm comprised 42 patients and the DEC arm 44 patients, all diagnosed by WB using *T. canis* excretory–secretory antigens (MagnaVal *et al.*, 1991). The design of the study was similar to that previously described for the assessment of the efficacy of DEC and MBZ (MagnaVal, 1995). The treatment groups were not statistically different with respect to demographic characteristics (age and sex ratio) and epidemiological features (type of residence, risk factors for toxocariasis), body weight, duration of the disease prior to first consultation, clinical impact of the disease (using a scoring system; MagnaVal, 1995) and presence of atopy as determined by the detection of specific IgE against common inhalant allergens and laboratory parameters (blood eosinophil count, serum total IgE titre and specific anti-*Toxocara* IgE).

A longer course of ABZ was used than in a study by Stürchler *et al.* (1989). In the treatment of neurocysticercosis with ABZ, good results were obtained with regimens of 15 mg/kg b/w daily for 15 days (Garcia *et al.*, 1997). Moreover, in

1998, two large outbreaks of trichinellosis involving 448 persons occurred in the Toulouse area of France. According to the instructions from the French Ministry of Health, these patients were treated with a combination of ABZ (13 mg/kg b/w daily for 10 days) and corticosteroids. This regimen was found to be safe and efficient (Leclerc *et al.*, 1999). As a consequence, ABZ was given in the toxocariasis study at 10–13 mg/kg b/w in two divided doses daily for 15 days, but no corticosteroids were added. For the DEC arm, the therapy started at 25 mg daily ($\frac{1}{4}$ tablet) and the dose progressively increased in an attempt to avoid adverse reactions due to parasite lysis. The full dosage was 3–4 mg/kg b/w in three divided doses daily for 21 days. No anti-histamine drugs were used.

Preliminary results at 4–6 weeks post-treatment showed that both DEC and ABZ elicited a significant decrease in the clinical score and blood eosinophil count, but these differences were not statistically different between the two treatments. More information on changes in specific anti-*Toxocara* IgE is described below. The rate of side effects was similar in both groups: 15 patients (35.7%) in the ABZ group complained of mild asthenia and/or nausea, while 21 (47.7%) in the DEC group reported neurological disturbances (dizziness, headache), gastric pain, and/or an increase in allergic signs.

The rate of unsuccessful treatments consisting of patients who did not exhibit clinical improvement together with a $\leq 30\%$ decrease in their eosinophil count, was significantly greater in the ABZ group (11 patients (26.2%) versus three (6.8%) in the DEC arm (χ^2 : 5.92; $P = 0.014$).

These failures in ABZ therapy might have been due to slow and erratic drug dissolution and absorption *in vivo*, a problem previously noted in the treatment of neurocysticercosis (Jung *et al.*, 1998). In healthy volunteers, it was demonstrated that the blood concentration of the metabolite ABZ sulphoxide varied sixfold 1 h after a 600 mg ABZ dose, and this variability was still observed from 1 to 4.5 at 24 h post-treatment (Sarin *et al.*, 2004).

Mebendazole

MBZ (methyl-5-benzoyl-1*H*-benzimidazol-2-ylcarbamate) is practically insoluble in water and therefore should be taken with a fatty meal

(Dayan, 2003). Large variations however, in the plasma concentrations of the active metabolites of MBZ have been observed in patients treated for hydatid disease or for toxocariasis (Luder *et al.*, 1986; Magnaval *et al.*, 1989).

Various drug regimens were used in three different controlled randomized trials. MBZ was given at either 25 mg/kg b/w daily for 7 days (Magnaval and Charlet, 1987) or at 20–25 mg/kg b/w daily for 3 weeks (Magnaval, 1995). A discontinuous regimen, namely 10–15 mg/kg b/w daily for 3 consecutive days in a week for 6 weeks, was compared with a placebo, to assess efficacy against dormant *Toxocara* larvae in tissues (Magnaval and Charlet, 1992). In this study, MBZ efficacy was found to be similar to that of placebo. The use of MBZ continuously at a higher daily dosage yielded better results, namely a 57% cure rate for clinical manifestations (Magnaval and Charlet, 1987) or a 70% reduction in the clinical score (Magnaval, 1995). Side effects including weakness, dizziness, nausea and abdominal and gastric pain were mild. The incidence of adverse effects ranged from 9.6% (Magnaval and Charlet, 1987) to 17% (Magnaval, 1995).

Thiabendazole

TBZ (2-thiazol-4yl-*IH* benzimidazole) has poor solubility and should be given with a fatty meal.

The efficacy of TBZ was assessed in three controlled, randomized trials. TBZ was given *per os* daily from 25 mg/kg b/w (Magnaval and Charlet, 1987) to 50 mg/kg b/w (Bass *et al.*, 1987; Stürchler *et al.*, 1989) for 3 days (Bass *et al.*, 1987), 4 days (Bass *et al.*, 1987), 5 days (Stürchler *et al.*, 1989) or 7 days (Magnaval and Charlet, 1987). The cure rate for clinical manifestations ranged from 50% (Magnaval and Charlet, 1987) to 53% (Stürchler *et al.*, 1989). Moderate side effects were observed in 50% (Magnaval and Charlet, 1987) to 60% (Stürchler *et al.*, 1989) of patients and included dizziness, nausea or vomiting. Such side effects have been previously reported with the use of TBZ (Parfit and The RPSGB, 1997). However, more severe adverse reactions, including cholestasis (Rex *et al.*, 1983), cholestatic hepatitis (Eland *et al.*, 1998) or ductopenia (Manivel *et al.*, 1987; Skandrani *et al.*, 1997), have been reported with a 2 or 3 day course of TBZ at 25 mg/kg b/w daily for treatment of strongyloidiasis. Due to only moderate efficacy

together with a relatively high rate of side effects, some of which may be serious, the use of TBZ for treating toxocariasis cannot be recommended.

Diethylcarbamazine

DEC (diethyl-4-methylpiperazine-1-carboxamide) is a highly water-soluble compound and has been the mainstay for filariases chemotherapy since 1949. In the presence of specific antibodies, it enhances both the adherence and cytotoxicity of neutrophils and eosinophils to microfilariae after altering their surface layer (Piessens and Beldekas, 1979). DEC also activates platelets that release free radicals; this action is antibody independent and triggered by a filarial excretory antigen (Cesbron *et al.*, 1987). Moreover, DEC interferes with arachidonic acid metabolism resulting in the production of prostaglandin E2 (PGE2), PGE12 and thromboxane in both the filarial parasites and the host (Martin *et al.*, 1997). A direct anthelmintic effect has also been demonstrated *in vitro* on *Wuchereria bancrofti* microfilariae characterized by morphological alterations such as loss of the microfilarial sheath and lysis of the cytoplasm together with the destruction of organelles and the formation of vacuoles (Peixoto *et al.*, 2004).

In a controlled, randomized study versus MBZ, DEC, when given according to the regimen described above, resulted in a 70% decrease in the clinical score (Magnaval, 1995). Twenty-eight per cent of patients reported minor side effects including increased weakness, dizziness, nausea, vomiting or abdominal pain. These disturbances were dose dependent and waned when the daily dosage was tapered. In 10% of subjects, a Mazzotti-like reaction (itching, urticaria and/or oedema) was observed, suggestive of accelerated larval lysis. One patient experienced a major adverse reaction (severe gastric pain) and had to stop treatment. A consideration in the use of DEC is antagonism by corticosteroids that partially inhibit DEC's mechanism of action (Maizels and Denham, 1992). Therefore, DEC and corticosteroids must be given sequentially.

Ivermectin

Ivermectin is primarily a veterinary drug that became available in the 1980s for the chemotherapy of some human helminthiasis. This macrocyclic lactone compound has dramatically

improved the outcome for patients with onchocerciasis (Boussinesq *et al.*, 1997), the cause of river blindness (Addiss *et al.*, 1997; Shenoy *et al.*, 1998). Ivermectin has also been registered in the European Union and in the USA for treating strongyloidiasis (Marti *et al.*, 1996). Physicians may therefore be tempted to use ivermectin for the treatment of toxocariasis, particularly because it can be given in a single 12 mg dose and does not usually elicit side effects. However, no controlled study has been conducted to evaluate its efficacy for toxocariasis. Ivermectin was tested on 17 consecutive patients with common toxocariasis and resulted in a 40% reduction in clinical manifestations, but no significant decrease in blood eosinophil count (MagnaVal, 1998a). Thus, ivermectin should not be used for the treatment of ocular toxocariasis until the question of its efficacy is answered by a controlled study.

Patients Eligible for Therapy

Whether a patient with toxocariasis should be treated depends on the clinical presentation or syndrome. All children and adults with acute VLM should be treated. Patients presenting with common toxocariasis (Glickman *et al.*, 1987; Magnaval 1994a) or covert toxocariasis (Taylor *et al.*, 1988) that have blood eosinophilia should not necessarily be treated, since persons with these forms of the disease typically recover spontaneously. Anthelmintic treatment need not be started immediately and need only be considered for patients who remain symptomatic following measures to prevent re-infection (see below). Asymptomatic subjects presenting with chronic eosinophilia do not require any specific therapy, but rather prophylaxis.

Since ocular toxocariasis is an uncommon and often severe disease, no controlled therapeutic trials have been published. Based on results of anecdotal case reports or cases series (Dinning *et al.*, 1988; Gillespie *et al.*, 1993; Saint-Blancat *et al.*, 1997) and our own experience (Glickman and Magnaval, 1993), corticosteroids are recommended for initial therapy of ocular toxocariasis. These drugs reduce the inflammatory process caused by local release of excretory-secretory antigens from larvae. If use of oral and/or topical corticosteroids is ineffective, the addition of a

specific anthelmintic drug should be considered. It is not known, however, whether the benzimidazole derivatives or their active metabolites penetrate into the human eye. However, ABZ given together with corticosteroids was found to be effective (Dietrich *et al.*, 1998; Barisani-Asenbauer *et al.*, 2001).

In mice infected with *T. canis*, DEC accumulated in the brain and in the aqueous fluid (Hawking, 1979). Further circumstantial evidence of the efficacy of DEC for toxocariasis is based on experience gained with treatment of human onchocerciasis (Dadzie *et al.*, 1987). Most data suggest that DEC could be used for treating ocular toxocariasis. However, DEC should not be given concomitantly with corticosteroids. In a collaborative multi-centre study including 19 subjects, eight patients with ocular toxocariasis were treated with corticosteroids followed by DEC (3–4 mg/kg b/w daily for 21 days), with good to excellent results. In contrast, when both drugs were given simultaneously to three patients, two did not exhibit any improvement after 2 months. An important deterioration occurred in the third subject who presented with chorioretinitis and hyalitis, leading to enucleation of the eye (Glickman and Magnaval, 1993; Magnaval, unpublished data).

Medical or surgical methods such as cryopexy or vitrectomy may help to restore vision in patients with ocular damage due to larval migration and granuloma formation, but these will not be discussed further.

Drug therapy for neurological toxocariasis has consisted primarily of corticosteroids (Robinson *et al.*, 2002), or of the combination of corticosteroids and DEC (Komiya *et al.*, 1995), MBZ (Duprez *et al.*, 1996) or TBZ (Kumar and Kimm, 1994). In some patients with CNS signs, DEC alone (Ruttinger and Hadidi, 1991) or TBZ alone (Russegger and Schmutzhard, 1989) has been used with equivocal results.

Post-treatment Follow-up

The evaluation of treatment efficacy relies primarily on the clinical response, but some signs may not resolve completely until many months following cessation of treatment. The appropriate time interval for evaluation of treatment efficacy is

critical. This was evident in a study of the use of ABZ (Stürchler *et al.*, 1989) in which a twofold increase in clinical signs was noticed between follow-up exams performed at the 2nd and 6th week post-treatment. In contrast, a clinical improvement observed after a year or more could have been related to treatment or simply corresponded with the natural course of the disease (Wolfson *et al.*, 1996; Rubinsky-Elefant, 2004). Based on personal observations, we suggest for the follow-up of VLM or covert toxocariasis that clinical exams be performed between the 4th and the 6th week post-treatment.

The use of a scoring system to quantify the clinical severity as described elsewhere (Magnaval *et al.*, 1992b; Magnaval, 1995) is helpful for assessing treatment efficacy. Abnormal patterns detected by medical imaging techniques including hypoechoic lesions on US, low-density areas on CT in the liver or T₂-weighted images by MRI in the brain usually resolve within 1 to 2 months following treatment.

Among non-specific laboratory tests, a high rate of decrease of blood eosinophilia seems to have good prognostic value (Magnaval, 1995).

The detection of specific anti-*Toxocara* IgG antibodies by ELISA appeared not to be useful for monitoring therapy. When ELISA IgG titres were compared between treated and untreated children with VLM, the kinetics of specific anti-*Toxocara* IgG did not differ (Bass *et al.*, 1987). In another study, 23 Brazilian patients with VLM were treated with TBZ and a follow-up exam was performed 22–116 months later (Rubinsky-Elefant, 2004). Only ten subjects were found to have a significant decrease in ELISA anti-*Toxocara* IgG titres, and the decline in IgG titres was consistent with predicted normal clearance rate (Jeanneret, 1991). WB, when used to detect specific IgG, was no more useful. In the unpublished study comparing the efficacy of ABZ with DEC (see above), only one subject in each group was negative at the post-treatment consultation. In the Brazilian study, WB was evaluated at the same time as IgG ELISA, and a lower-intensity banding pattern was observed in 12 of 23 patients. Conversely, specific anti-*Toxocara* IgE antibody titres determined by ELISA (sIgE) seemed to correlate better with the clinical outcome. If elevated prior to therapy, the mean sIgE level significantly decreased (Magnaval *et al.*, 1992), and in eight

of nine Brazilian patients the sIgE decreased by two or more dilutions (Rubinsky-Elefant, 2004). DEC-treated atopic subjects had a significant reduction of IgE following treatment compared with non-atopic patients (Magnaval, unpublished data).

Prophylaxis for Toxocariasis

Regardless of the clinical form of toxocariasis encountered or the chemotherapy regimen used, measures should be taken to prevent re-infection. The patient or surrogates should be questioned carefully to identify personal risk factors for *Toxocara* infection and to identify likely sources of *Toxocara* eggs in the environment. Risk factors for infection include behaviours such as geophagia and poor personal hygiene. Any roundworm-infected dogs or cats in the patient's environment should be treated by a veterinarian (see Chapters 16 and 17, this volume), and any contaminated soil removed or the area closed so it is not accessible to small children. Household gardens should be fenced to eliminate contamination by dogs or cats. Similarly, smaller gardens and sand boxes should be covered by appropriate materials. Vegetables or fruits gathered in possibly contaminated gardens should be thoroughly washed before eating. Raw or undercooked meat that could harbour *Toxocara* larvae should be avoided. Parents and children should receive counselling for geophagia. Personal hygiene including hand-washing is important, especially when handling foods and dogs.

Puppies should be treated for roundworms starting about 3 weeks of age (Harvey *et al.*, 1991) and the treatment repeated every 2 weeks until 12 weeks of age (Soulsby, 1987) in order to avoid environmental contamination with *T. canis* eggs. Adult dogs should be dewormed or have a faecal exam twice a year, except for bitches which should also be treated before and 1 month after whelping. Cats should be similarly treated or tested for roundworms in the first few weeks of life. Adult outdoor cats can be re-infected by preying upon paratenic hosts and should therefore be retreated or tested two or three times a year unless they are kept strictly indoors (see Chapters 16 and 17, this volume).

Table 8.1. Recommended treatments for toxocariasis.

Syndrome	Drug of choice	Recommended dose	Major side effects	Minor side effects	Availability	Cost	Strength of evidence for efficacy	Alternative	Adjunctive therapy
VLM	DEC	3–4 mg/kg b/w daily for 21 days	Burst of allergy signs; gastric pain; vomiting	Dizziness; nausea	USA; Western Europe; filariasis-endemic countries	Very low	Weak	MBZ	Corticosteroids
Common/covert toxocariasis	DEC	See above	See above	See above	See above	See above	Strong	ABZ	None
	MBZ ^a	25 mg/kg b/w daily for 21 days	None	Dizziness; nausea; abdominal or gastric pain	Worldwide	Low	Strong	ABZ	None
Ocular toxocariasis	Corticosteroids (prednisone)	1 mg/kg b/w daily for 1 month	See <i>Martindale</i> [®] (Parfitt and The RPSGB 1997)	See <i>Martindale</i> [®] (Parfitt and The RPSGB 1997)	Worldwide	Moderate	Moderate	None	Surgery and other non-medical interventions ^b
	DEC ABZ	See above 400 mg (children) 800 mg (adults) b.i.d. for 10–14 days	See above None	See above Mild weakness; nausea	See above Worldwide	See above Moderate	Weak Weak	ABZ	See above See above

DEC, diethyl-carbamazine; MBZ, mebendazole; ABZ, albendazole.

^aIf DEC not available, or if occurrence of major side effects.

^bCryopexy, photocoagulation.

Conclusion

Corticosteroids are indicated for the treatment of acute inflammatory manifestations of both VLM and ocular toxocariasis. There is currently no information available on the efficacy of the non-steroidal anti-inflammatory drugs for *Toxocara*. For many years, a commonly held belief was that anthelmintic therapy of toxocariasis was unsatisfactory, especially for VLM. This was based on the high larval inoculum size that causes this form of toxocariasis, the lack of effective prophylaxis for high-risk children with geophagia that results in repeated infections, as well as equivocal results of drug tests in rodent models. However, results from controlled and randomized human drug trials suggest effective therapy is possible for both the common and covert forms of toxocariasis.

The appropriate drug for treating toxocariasis depends on several factors including what is licensed and available for use in a physician's country as well as a physician's previous experience with treating toxocariasis (see Table 8.1). DEC, if available, is probably more effective than ABZ for the treatment of toxocariasis. However, the drug has been associated with a high rate of neurological side effects and should preferentially be used by well-experienced physicians. MBZ is available in many countries and would appear to be a good alternative to DEC, e.g. if the occurrence of major DEC-related side effects is feared. ABZ, in spite of a recent report supporting its efficacy (Despommier, 2003), should not be considered as the drug of choice for reasons discussed previously. However, ABZ is widely available, has proved to be safe and could therefore be used to treat lightly infected persons with toxocariasis.

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9 Ocular Toxocariasis

Mervyn R.H. Taylor

Departments of Paediatrics and Zoology, Trinity College, Dublin, Ireland

Overview

Since 1950 it has been recognized that the larvae of *Toxocara canis* may invade the eye and cause blindness. Despite the importance of visual loss, the data available regarding ocular toxocariasis in both animals and humans are remarkably patchy and some appear contradictory. The apparent contradictions may be due to species differences. The condition is usually painless and visual loss is the most common presenting complaint. Serum antibody levels are not diagnostic. Intraocular antibodies appear more promising as a diagnostic aid, but standardization of the tests and more experience in their application is required. At present, diagnosis is usually made on clinical findings, especially ophthalmoscopic appearance. Treatment largely depends on the use of steroids to control inflammation. The value of anthelmintics and anti-larval treatment is unproven. Vitrectomy may be of value in appropriate cases. Laser coagulation can be beneficial in eliminating choroidal neovascularization. The site of the lesion plays a major part in the end result of infection. Peripheral lesions may leave only a small visual defect provided the vitreous haze has cleared. Central lesions are likely to result in a considerable permanent defect. Preventive measures for humans have been recommended on a common sense approach. These measures are likely to prove difficult to implement and data are not available regarding their effectiveness. Ocular toxocariasis is a fascinating area for research

with many questions to be answered and potentially considerable benefits to be achieved for those at risk.

General Background

The world human population for 2003 is estimated at above 6395 million (Population Division, International Programs Centre, 2004). The worldwide total of 45 million blind humans includes 1.4 million children (World Health Organisation, 1997, 2000). An estimated 500,000 children become blind each year. In developing countries, 60% of them are thought to die within a year of becoming blind. The prevalence ranges from 60 per million population in affluent societies to 600 per million in the poorest communities. The reasons for this difference include the prevalence of potentially blinding conditions in poorer countries (such as vitamin A deficiency, cerebral malaria, measles, congenital rubella, ophthalmia neonatorum and harmful traditional eye remedies), in addition to a lack of facilities and skilled clinical staff to provide treatment. In Latin America and some eastern European countries, retinopathy of prematurity is becoming an important cause of blindness (Gilbert *et al.*, 1997; Kocur *et al.*, 2001). In affluent countries unavoidable causes such as congenital anomalies, central nervous system disorders and hereditary retinal dystrophies form the largest group.

Any loss of vision in a child is a regrettable as in most cases the child has a lifetime of defective vision ahead. For the same reason blindness in one or both eyes is a disaster. Visual defects can affect opportunities for education, employment and earning potential. Because of the large number of blind children worldwide, attention has been focused on the level of vision in the better eye and a level of 6/60 or less in the better eye is commonly used as a measure of blindness. Such an approach ignores conditions which are usually unilateral such as ocular toxocariasis. A report of visual impairment in children aged 0–16 years in Liverpool (UK) found 199 children aged (18.1 per 10,000 population) with corrected vision sufficiently abnormal to interfere with development or to have continuing educational implications. In cases where distance acuity could be measured and was relevant, this equated to acuity of 6/18 in the better eye (Rogers, 1996). Ocular toxocariasis did not appear in the list of diagnoses and the author in discussion with five or six other ophthalmologists in the Liverpool area could only recall three cases of ocular toxocariasis between them over a period of 20 years (Good *et al.*, 2004).

The data available for human ocular toxocariasis are not reflected in the animal world. There are few data available regarding the natural occurrence of ocular toxocariasis in animals. Such studies would be difficult to perform and would almost certainly be affected by the increased liability to predation of animals with visual defects. Data relating to ocular invasion in animals come almost exclusively from laboratory research studies in which known numbers of ova have been fed to the subject animals. It is unlikely that such studies reflect natural infection although ‘trickle’ administration of small numbers of ova may be closer to natural infection than studies in which the ova are given as a single bolus. Such an argument may not apply to animals that are carnivores and may, from time to time, ingest a heavily infected animal.

On the one hand we have data from carefully controlled animal studies in a laboratory environment and on the other, data from humans of naturally occurring infection in a normal environment. There are no studies of experimental ocular toxocariasis in humans. Very few early studies exist of the result of giving *Toxocara* ova to humans (Chaudhuri and Saha, 1959). Very little information was obtained from these studies and no eye disease was reported. Ethical principles

make it unlikely that such studies in humans will be repeated or undertaken in any form.

The information regarding human ocular toxocariasis is patchy. While the clinical appearance of the condition is now well recognized, treatment and prevention remain unsatisfactory and much remains to be learned about the prevalence and natural history of the disease.

Systemic toxocaral infection is relatively common in humans with seroprevalance rates varying from 3.6 to 92.6% in different countries (Magnaval *et al.*, 1994; Holland *et al.*, 1995). Ocular infection appears to be much less common than systemic infection. There are numerous reports of single cases of ocular toxocariasis, fewer reports of groups of cases and only two reports of the prevalence of the condition in humans. There is no report of its prevalence in any wild species of animal although ocular toxocariasis has been reported in a group of New Zealand sheep dogs (Hughes *et al.*, 1987).

Only a single estimate of the prevalence of ocular infection in humans, one case per 1000 population in Alabama, USA, had been published up to 2004 (Maetz *et al.*, 1987). To Western European clinicians this estimate seems rather high in comparison with their clinical experience, particularly the experience of Rogers and his colleagues as noted above, who could only recall three cases over a period of 20 years. A second and much lower estimate was produced by Good *et al.* from Ireland in 2004.

Ocular toxocariasis can be produced, it is generally accepted, by the migration of a single larva to the eye. It may occur at the same time as symptomatic systemic infection but in most cases, ocular disease may only be discovered apparently unrelated to systemic infection and a prior or concurrent asymptomatic infection may be presumed.

Many aspects of the epidemiology of ocular toxocariasis are unclear and the behaviour of the larvae in paratenic hosts varies from species to species.

Historical Background in Humans

The works of Wilder (1950) and Nichols (1956) are of key importance in ocular toxocariasis. Wilder recognized that the disease affecting a number of

eyes which had been removed from children because of a presumed diagnosis of retinoblastoma (a malignant tumour of the retina) was caused by a nematode.

In humans, ocular toxocariasis is a disease which is just over 50 years old in terms of its recognition, but nematode infection of the eye was reported in 1771 (Jones *et al.*, 1938) and later by von Nordmann (1832) who found two dead *Filaria* in a lens. The histological changes of ocular toxocariasis were described by Wilder in 1950. She showed that 24 of the 46 eyes she examined contained nematode larvae and not a tumour. All 24 of the nematode-containing eyes came from children. In one case the visualization of the larval fragments required the examination of thousands of sections from the eye. B.G. Chitwood identified the larvae in nine of the eyes as third-stage hookworm larvae, and said that *Ancylostoma* sp., *Necator* sp. and *Uncinaria* sp. were also possibilities. Wilder, however, did not rule out the possibility of other nematodes as causative agents in endophthalmitis. Nichols (1956) later identified the larvae as *T. canis* in sections from four out of five of the eyes examined by Wilder. In the fifth eye, he was not able to make a positive identification of the larva. Nichols' record of identification is given in a single paragraph in the discussion section of his paper. Nichols' paper reported detailed morphological descriptions of the larvae of *T. canis* and *Toxocara cati* in the mouse, in order to facilitate reliable diagnosis in necropsy and biopsy specimens.

Pathological Features

The larva of *T. canis* initially evokes an eosinophilic response and becomes surrounded by eosinophils producing an eosinophilic abscess (Shields, 1984). Later there is a focal granulomatous reaction comprising eosinophils, lymphocytes, epithelioid cells and giant cells. Older lesions are characterized by epithelioid cells and dense fibrous tissue. The size of the inflammatory reaction greatly exceeds the size of the larva it envelopes. In long-standing lesions there may be no larval remnants, either due to the destruction of the larva or because it has migrated to a new site leaving behind antigen which has resulted in continuing inflammation.

Lyness *et al.* (1987) have traced the presumed track of a *T. canis* larva from the histological findings in an enucleated eye from a 12-year-old boy (Fig. 9.1). This emphasizes the mobility of the larva within the eye.

Wilder (1950) found that in almost all the human cases she examined, the anterior segment was comparatively free from inflammation. Cataract was an occasional complication and sometimes the posterior capsule was perforated. Retinal and vitreous haemorrhages were often present. An eosinophilic abscess was the characteristic lesion. The abscesses were surrounded by epithelioid cells, occasional giant cells and inflammatory tissue infiltrated by eosinophils, lymphocytes and plasma cells, which were frequently

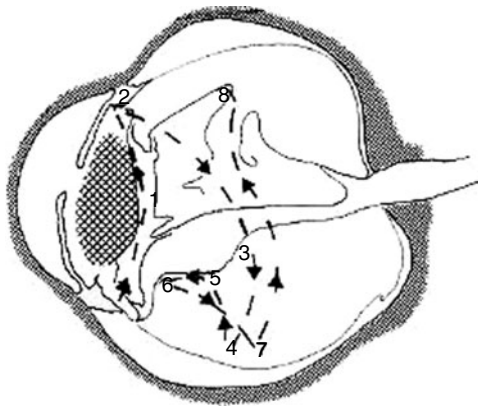


Fig. 9.1. The presumed track of a *Toxocara canis* larva from the histological findings in an enucleated eye from a 12-year-old boy (Lyness *et al.*, 1987). Reproduced with permission from the BMJ Publishing group.

multinucleated. The proportion of each cell present seemed to depend on the age of the lesion and the stage of disintegration of the larvae. The eosinophilic abscesses were distributed on the underside of the retina, in retinal folds and in the vitreous membrane. They also marked the sites of entrance of the larvae from the choroidal vessels. Ashton (1960) identified the fact that *T. canis* infection of the eye can occur as a single retinal granuloma.

In mice, Ghafoor *et al.* (1984) found grey fluffy masses in five out of 54 eyes from 27 animals infected with *T. canis*. Larvae were found mainly at the posterior pole but five were in the peripheral retina and one in the vitreous. On histological examination they noted evidence of occlusive retinal disease. This occurred between the 6th and 13th day after infection in the form of superficial and deep haemorrhages, in some cases extending into the subretinal space. Microinfarcts were found commonly throughout the time of the experiment (63 days). Haemorrhagic pseudocysts were found in the retina and the vitreous. Retinal vasculitis was more frequent in the later stages of the experiment. In only one case was there evidence of an inflammatory reaction in the presence of a larva. Non-granulomatous inflammatory cell infiltrates (lymphocytes and plasma cells) were found in the anterior uvea and the pars plana, succeeding a polymorphonuclear infiltrate, which occurred in the first 13 days. The vitreous was

commonly involved in the inflammation. The infiltrates were mainly lymphocytic and haemorrhagic. Macrophages in the vitreous contained melanin and red cell degradation products. A granulomatous response was rare and apart from one orbital lesion, occurred in the retina in ten eyes removed later than the 6th day of the experiment. Eosinophils were not observed at any stage of inflammation. Optic nerve involvement was relatively uncommon. Details of the findings in the 54 eyes from 27 mice are given in Table 9.1. These histological changes were found in eyes between 6 and 63 days after ingestion of 1500 infective eggs.

In gerbils infected with *T. canis*, 95% developed choroidal haemorrhage, 55% retinal haemorrhages, 45% white-centred retinal haemorrhages and 5% vitreous haemorrhages. Exudative lesions occurred in 70% of gerbils, vasculitis in 24% and larvae were seen in the eyes of 80%. No case of granulomatous lesions or retinal detachment was found (Takayanagi *et al.*, 1999).

These reports underline the differences between species in this infection. Part of the difference between the laboratory animal and the human studies may be due to the fact that the laboratory studies were made early in the disease (up to 63 days in the case of the mice and 158 days in the case of the gerbils) whereas in the case of the humans the condition may have been present for several years.

Table 9.1. Frequency of histopathological features in 54 mouse eyes examined between days 6 and 63 after infection via intragastric intubation (after Ghafoor *et al.*, 1984).

	Number of eyes showing feature	% of eyes
Iridocyclitis	20	37
Pars planitis	29	53
Vitreous haemorrhage	28	52
Vitreous infiltrate	37	69
Optic nerve papillitis	7	13
Retinal superficial haemorrhage	9	17
Retinal deep haemorrhage	6	11
Subretinal haemorrhage	8	15
Microinfarcts	27	50
Vasculitis	15	28
Subretinal infiltrate	11	20
Retinal granuloma	10	19
Choroid granuloma	0	0
Orbit granuloma	1	2

Table 9.2. Original complaint and clinical findings in 43 child patients seen in the children's tumour clinic with ocular toxocariasis as recorded by Brown (1970).

	No.	%
Original complaint		
Strabismus	19	44
Failing vision	5	12
Leukocoria (white eye)	3	7
Found at routine examination	3	7
Blind eye	1	2
Fixed pupil	1	2
Red eye	1	2
Clinical findings		
Solid retinal tumour	23	53
Posterior pole	18	42
Periphery	5	12
Vitreous mass or haze (no fundus seen)	12	28
Retinal detachment	10	23
Cataract	2	5
Chorioretinitis	1	2
Iris heterochromia	1	2
Microphthalmos	1	2

Clinical Features

Brown (1970) recorded the clinical features reported in the 245 published human cases that he reviewed. He also recorded the findings in 43 patients with ocular toxocariasis seen in the children's tumour clinic as well as the pathological diagnoses in ten patients who had enucleations. Summaries are given in Tables 9.2 and 9.3. Both Gillespie *et al.* (1993) and Yokoi *et al.* (2003) emphasize that milder disease is more common than is suggested by reviews of the literature. This is not altogether surprising as attention was first drawn to the condition by Wilder's 1950 report of disease so severe that enucleation had been undertaken. Yokoi *et al.* (2003) found that, of 36 cases, visual

acuity was maintained at over 20/20 in 50% and less than 20/200 in 14% of cases. Peripheral lesions (as opposed to posterior pole lesions) had worse outcomes as regards visual outcome, frequency of ocular complications and effectiveness of vitreous surgery in their series.

Clinical presentation varies according to the anatomic site of involvement (Brown 1970): peripheral retina and vitreous, posterior pole endophthalmitis, optic nerve and anterior segment. Involvement of the peripheral retina and the vitreous are the most common either separately or together. A hazy, ill-defined, white lesion may be seen in the posterior pole or in the periphery. Varying degrees of vitritis may be present. A peripheral raised white mass is usually seen as the inflammation resolves. Typically this is as-

Table 9.3. Pathological diagnoses in ten of the 43 child patients who had enucleations (Brown, 1970).

<i>T. canis</i> larvae found	0
No larvae found, eosinophilic granuloma	5
Congenital abnormalities	2
Coats' disease	1
Retinoblastoma	1
Toxoplasmosis	1

sociated with retinal folds extending towards the macula. On occasion, the granuloma presents posteriorly as an intraretinal or subretinal mass. Endophthalmitis usually presents with a quiet external eye with little pain but severe vitreous inflammation accompanied by a mild anterior chamber reaction and often a secondary cataract. Optic papillitis can also occur, usually because of invasion of the nerve by a larva or as an inflammatory response to the organism at another ocular site.

Gillespie *et al.* (1993) reported the findings in 33 patients with ocular toxocariasis and all with positive *Toxocara* serology. Of the 33 patients, 26 (79%) complained of visual loss and eight (24%) complained of eye pain, underlining the fact that ocular toxocariasis is usually painless. A retinal abnormality was present in 17 patients. Uveitis was found in 20 patients and endophthalmitis in nine patients. Five patients were seropositive with inactive ocular disease. Two patients had pars planitis.

Age of Onset and Male/Female Ratios in Humans

Ideally, age in this condition should be recorded as the time of onset of eye infection but this is not possible. The next best estimate would be age at first symptom, but ocular toxocariasis can be asymptomatic and so a group of asymptomatic patients would go uncounted. It is usually assumed that age at presentation is the age recorded in published reports, but in some papers exactly what the age recorded represents is not defined. Wilder (1950) merely states the age of the children. As she was dealing with enucleated eyes it

seems fair to assume that the age is the age at enucleation and that, as in most cases the diagnosis was retinoblastoma, enucleation took place fairly soon after presentation, but we have no idea of the time interval between infection of the eye and presentation to the doctor. The majority of Wilder's eyes containing larvae were from children aged 3–5 years. Schantz *et al.* (1979) give the age at diagnosis, while Perkins (1966) records age at onset. It is also important to consider whether age was a criterion in selecting the cases. This appears not to be so in the studies of Wilder (1950), Duguid (1961), Brown (1970), Gillespie *et al.* (1993) and Schantz *et al.* (1979, 1980), while Perkins (1966) restricted his study to uveitis starting before 16 years of age and Dean Hart and Raistrick (1977) to those over 20 years of age. The patients of Gillespie *et al.* (1993) were selected by being seropositive by *Toxocara* ELISA so any seronegative cases will have been excluded. The age of ocular toxocariasis patients is stated to be older than that of visceral larva migrans (VLM) patients, but this seems to relate to age of presentation. Good *et al.* (2004), taking the age at which an eye abnormality was first noted, recorded an average age of 6.5 years with a range of 2–14 years in schoolchildren. It is of note that, in an earlier study of Irish schoolchildren, Holland *et al.* (1995) found that 20.8% of 4-year-old children, 26.1% of 5-year-olds and 29.2% of 6-year-olds were *Toxocara* seropositive (at a titre of 1:50 or above) suggesting widespread infection by *Toxocara* before 4 years of age in this population. It can be seen from Table 9.4a that the age reported for ocular toxocariasis patients ranges from 2 to 70 years. In the past, probably as a result of Wilder's 1950 study of children's eyes, ocular toxocariasis has

Table 9.4a. Age of patients with toxocaral eye disease according to various authors.

Reference and Location	Age range (years)	Mean age (years)	No. cases
Wilder (1950) USA	3–13	NA	24
Duguid (1961) UK	2–7	4.6	6
Perkins (1966) UK	3–13	8.6	15
Brown (1970) 12 countries	2–31	7.5	61
Dean Hart and Raistrick (1977) UK	20–50	34.4	8
Schantz <i>et al.</i> (1979) USA	2–19	8.6	17
Schantz <i>et al.</i> (1980) USA	4–17	8.9	24
Gillespie <i>et al.</i> (1993) UK	NA	15.9	33
Poulson <i>et al.</i> (2001) UK	4–70	2.6	24
Good <i>et al.</i> (2004) Ireland	2–14	6.5	11

NA, not available.

Table 9.4b. Age of patients with toxocaral eye disease from Yoshida *et al.* (1999).

Age group (years)	Number in group	% of total
0–9	1	2.6
10–19	3	7.9
20–29	3	7.9
30–39	17	44.7
40–49	11	28.9
49–50	2	5.2
60–69	1	2.6

been viewed as a disease of children rather than adults. However, the work of Poulson *et al.* (2001) and Yoshida *et al.* (1999) emphasizes that this is a disease of both adults and children (Tables 9.4a and b). It is not clear whether the new cases identified in adults represent recent invasion of the eye or a long-standing (but unrecognized) invasion dating back to childhood. An intensive longitudinal study of a large group of children would be needed to decide whether indeed the age of ocular infection is significantly later than that of systemic infection. It appears that in mice and gerbils, invasion of the eye occurs as part of the systemic invasion, but whether or not it also occurs later has not been determined.

Only Wilder (1950) recorded ocular toxocariasis more commonly in females than males. In other studies, the male: female ratio varies from 1.1 to 2.5. The highest ratio was found by Dean Hart and Raistrick (1977) who studied only patients over 20 years of age (Table 9.5). Males are in general more susceptible to infection than females and this susceptibility may be responsible for the sex difference, but on the other hand the more active and exploratory lifestyle of boys may

be an important factor. Ehrhard and Kernbaum (1979) in their review of 430 case reports of ocular toxocariasis concluded that there was a mild male predominance with a male:female ratio of 1.31, but that this difference disappears in adult patients.

Bilateral Eye Disease

Brown (1970) recorded possible bilateral lesions in six of the 245 human cases (including both ocular and systemic toxocariasis) whose reports he reviewed, while Ehrhard and Kernbaum (1979) recorded suspected bilateral disease in 12 out of the 430 case reports of ocular toxocariasis that they reviewed. Neither Gillespie *et al.* (1993) nor Poulson *et al.* (2001) reported bilateral cases in 33 and 24 ocular cases, respectively. Yoshida *et al.* (1999) recorded only unilateral lesions in 38 ocular patients. It can be concluded from these studies that, in humans, bilateral ocular toxocariasis is uncommon.

In gerbils, Takayanagi *et al.* (1999) reported frequent bilateral changes after infection with *T. canis*. Choroidal haemorrhage was observed in

Table 9.5. Male:female ratios in ocular toxocariasis.

Reference	Male	Female	Male:female ratio
Wilder (1950)	10	14	0.7
Duguid (1961)	4	2	2.0
Perkins (1966)	8	7	1.1
Brown (1970)	52	39	1.3
Dean Hart and Raistrick (1977)	6	2	3.0
Schantz <i>et al.</i> (1979)	11	6	1.8
Schantz <i>et al.</i> (1980)	16	8	2.0
Gillespie <i>et al.</i> (1993)	22	11	2.0
Good <i>et al.</i> (2004)	6	5	1.2
Yoshida <i>et al.</i> (1999)	27	11	2.5
Total	162	105	1.54

30 eyes from 19 gerbils and white-centred retinal haemorrhage in 12 eyes from nine gerbils. In mice, white-centred retinal haemorrhage was observed in nine eyes from eight animals. Ghafoor *et al.* (1984) in their study reported that 'in the majority of animals the disease was unilateral' without recording the number of bilateral lesions in the 54 eyes examined. From their data it appears that larvae were found bilaterally in four mice and granulomata bilaterally in one.

Eye Disease in Dogs

The definitive host of *T. canis* is the dog, yet relatively little work has been published on toxocaral eye disease in this host. Intraocular nematodiasis in dogs is regarded as rare although aberrant localization of *Dirofilaria immitis* has been reported (Ott 1974). Hughes *et al.* (1987) studied the eyes of 1448 working New Zealand sheep dogs by ophthalmoscopic examination in the course of routine veterinary practice and found that 39% had varying degrees of multifocal retinal disease whereas only 6% of 125 New Zealand dogs raised in an urban environment had similar disease. Both eyes from 70 of the working sheep dogs were examined histologically. Forty-seven of the 70 had ocular inflammatory disease. Ten other dogs had non-inflammatory disease and 13 dogs had normal eyes. The diseased eyes were categorized into three groups (based on morphology). The authors suggested that these changes represented the progressive effects of parasitism which started in young dogs. Four dogs under 3 years of age had migrating nematode larvae which were morphologically identified as *Toxocara*. No other agent than *T. canis* was found to account for the lesions observed. *T. canis* is common in New Zealand dogs with a prevalence of 38% (Okoshi and Usui, 1968). The authors concluded that toxocaral ocular larva migrans (OLM) may be the cause of a highly prevalent, potentially blinding syndrome of working sheep dogs in New Zealand. The authors also studied 125 pet dogs living in an urban environment and 70 pet dogs living in a rural environment. Only 6% of the urban dogs had similar lesions to the working sheep dogs while the figure for the rural pet dogs was 43%.

New Zealand working sheep dogs are kennelled in close proximity in an environment that is

often heavily contaminated with faeces. The dogs are usually fed on the ground or on the floor and heavy exposure to *T. canis* eggs is likely. Usually dogs kept on New Zealand sheep farms for reasons other than working sheep are also exposed to this contaminated environment.

These figures suggest that environmental factors are important in the pathogenesis of this disease. The implications of these findings and suggestions, if applicable to humans, are considerable.

Laboratory Studies

Among the experimental animals which have been used to study ocular toxocariasis are mice (Olson *et al.*, 1970; Ghafoor *et al.*, 1984; Good, 1998), rabbits (Brown, 1971), guinea pigs, rats, hamsters, gerbils, pigs and primates (see Chapter 6, this volume). From the researcher's point of view, gerbils have the advantage that they are particularly susceptible to ocular invasion and the pathology resembles that found in humans. Throughout the studies of experimental toxocariasis runs the difficulty of comparing the results of published work as the hosts, parasitic loads, modes of administration (trickle or bolus) and routes of administration vary from study to study. In addition, the numbers of animals in each group may be small because of ethical or licensing considerations, thus reducing the power of the study to identify significant differences between the groups. As a result the findings of these studies, while of great interest and undoubted benefit, may not reflect the natural course of toxocaral infection in humans or indeed in other species.

Gerbils

The Mongolian gerbil (*Meriones unguiculatus*) has been put forward as a model for human ocular toxocariasis (Takayanagi *et al.*, 1999) (see Chapter 6, this volume). These rodents seem particularly susceptible to ocular invasion, much more so than mice and humans. In this respect they are not a good model for humans but the high frequency of ocular invasion in Mongolian gerbils combined with the availability of an ophthalmoscopic method of examining the eyes allows detailed observation of the progression of disease. The ophthalmoscopic approach allows the study to

progress while observations are made without necessitating the death of a proportion of the gerbils at intervals for histological examination of the eyes. This has been necessary in the past when studying mice and other animals. It has not been possible to apply the ophthalmoscopic method to BALB/c mice as their fundi are albinotic and larvae and exudative lesions cannot be detected. In addition, their corneae easily became dried out and cloudy making fundal observation difficult. The reason for the Mongolian gerbil's susceptibility to ocular invasion by *T. canis* larvae is not known but the major factors are likely to be immunological and anatomical. It may be that Mongolian gerbils are little exposed to toxocaral infection in their natural habitat and a poor immunological response to the organism is no handicap to their survival in their natural habitat. The information obtained from gerbils should only be applied to human disease with extreme caution, but has considerable potential to extend our knowledge of this condition.

Relation of Eye Disease to *Toxocara* ELISA titres

Up to 1970 only 245 reports of ocular toxocariasis had been published in 45 papers. These had been summarized by Brown (1970). In 1979 Pollard *et al.* reported the use of a *Toxocara* ELISA prepared from *Toxocara* eggs containing second-stage larvae to detect serum antibodies. This test was used in 41 patients with *Toxocara* eye disease diagnosed by fundoscopy. Thirty-seven of the 41 patients tested positive. The patients had four different presentations:

1. Diffuse nematode endophthalmitis which was often associated with retinal detachment.
2. Focal posterior retinal granuloma.
3. Peripheral inflammatory mass usually appearing as unilateral pars planitis but sometimes associated with retinal detachment.
4. A combination of the above three presentations often with a retinal fold from the peripheral mass to the optic nerve or vitreous bands.

Sixty-five control patients were tested and 47 (72%) had no detectable antibody while the re-

mainder had titres of 1:2 to 1:8 in 14 (21%) patients, 1:16 to 1:64 in three (5%) patients and 1:128 in one patient. In the ocular toxocariasis patients, two (5%) had no detectable antibody, four (10%) had titres of 1:2 to 1:8 and 35 (85%) had titres of $\geq 1:16$ of whom two had titres of $\geq 1:512$. The same paper reported the case of a 4-year-old girl with a *Toxocara* titre of 1:16 who had an enucleation-proved retinoblastoma underlining the fact that a retinoblastoma does not protect against toxocaral infection and that a positive *Toxocara* titre does not exclude a diagnosis of retinoblastoma.

Further work has since been carried out on patients with low or negative toxocaral serology. Schantz *et al.* (1979) reported a series of 17 ocular toxocariasis patients of whom six (35%) had negative serology by *Toxocara* ELISA though the method used was not exactly the same as that used by Pollard *et al.* (1979).

More recent studies which have involved measuring toxocaral antibodies in aqueous humour have reported cases in which the aqueous humour antibody titre was positive although the serology titre was negative (Magnaev *et al.*, 2002). Yoshida *et al.* (1999) found that 50% of 22 patients with uveitis of unknown origin had vitreous fluid positive for *Toxocara* antibodies. Yokoi *et al.* (2003) analysed 36 cases of ocular toxocariasis attending a uveitis clinic in Japan. All except two of the cases were adults. All cases were unilateral. Samples for toxocaral antibody titres were taken from serum, aqueous humour and vitreous humour. Thirty-three pairs of serum and intraocular fluid samples were available for analysis. In 12 cases, the intraocular fluid was aqueous humour and in 21 cases vitreous humour. Fifteen cases were antibody positive in both serum and intraocular fluid. Ten cases were positive only in serum and eight cases were positive only in intraocular fluid. It is apparent that measuring *Toxocara* antibody titres in intraocular fluid may be helpful in diagnosing ocular toxocariasis. However, basic problems underlying all *Toxocara* antibody measurements remain: Are the tests standardized so that results from different authors can be compared? What are the 'normal ranges' for humans never infected by *Toxocara*, and: (i) for those infected with *Toxocara* but with no invasion of the eye; and (ii) for those with (a) recent eye invasion and (b) eye invasion in the distant past?

Prevalence of Toxocaral Eye Disease in Humans

It is surprising that despite the damage which can be caused by ocular invasion in humans little effort has been made to establish the human prevalence of the condition. While a number of authors had commented that ocular toxocariasis was uncommon (Shields, 1984; Gillespie *et al.*, 1993), Yokoi *et al.* (2003) found that ocular toxocariasis cases comprised 3.2% of all 1301 cases attending a uveitis clinic in Japan. Prior to 2004 only a single abstract had been published on the subject of the prevalence of human ocular toxocariasis.

Maetz *et al.* (1987) reported (in an abstract only) a prevalence of 1 per 1000 population. They undertook a survey of ophthalmologists and optometrists in Alabama. They were asked to report the number of patients seen per year and the best approximation of the number of cases of ocular toxoplasmosis and toxocariasis seen within the previous 5 years. They were also asked to take part in a 1 year prospective study. The response rates for the retrospective and prospective studies were 52 and 40.7%, respectively. The authors concluded that the prevalence of ocular toxocariasis was 1 per 1000 population and were concerned that this might be an underestimate. However, this result did not fit well with the generally accepted concept of this being an uncommon disease and if such prevalence were widespread outside Alabama, then many more case reports and series reports of ocular toxocariasis would be expected. Rogers stated that in discussion with five to six ophthalmologists on Merseyside (Liverpool, UK), whose hospital practices served a population of about 200,000 children, they could only recall three cases of ocular toxocariasis between them in the previous 20 years (Good *et al.*, 2004). Brown (1970) reviewed 245 reported cases of ocular toxocariasis from 12 countries plus Reunion Island (including the USA, Europe and Australia). Gillespie *et al.* (1993) reported a further 33 cases from the UK. These reports support the suggestion that in general ocular toxocariasis is not common, but of course, this does not preclude a higher prevalence in specific areas.

Clemett *et al.* (1987) examined the fundi of 102 hydatid control officers (28.4% of whom were *Toxocara* seropositive) and found no evidence of

ocular toxocariasis. Holland *et al.* (1995) found no toxocaral eye disease in a study of 2129 Irish schoolchildren, 31% of whom were seropositive. Good *et al.* (2004) reported a questionnaire study of 121,156 Irish schoolchildren and concluded that the prevalence of consultant ophthalmologist-diagnosed ocular toxocariasis was 6.6 cases per 100,000 persons when only cases regarded as definite by the consultant were included. This rose to 9.7 cases per 100,000 when both definite and strongly suspected cases were included.

These two studies are the only reported estimates of the prevalence of ocular toxocariasis to date. As the study of Maetz *et al.* (1987) has only been reported in abstract form, it is not possible to examine that study in detail. The results of Good *et al.* (2004) are more in keeping with what might be expected from the general trend of reports in the literature, but it is likely that not only are there wide prevalence differences between countries but also inter-county differences within countries. Good *et al.* (2004) noted that seven of 11 cases they reported occurred in the warmer and moister south-western counties of Ireland. While similar factors may be important in the difference between the Alabama and Irish results it would seem more likely that such differences in environment would be reflected in a difference in the prevalence of toxocaral infection in general rather than a variation in distribution of the parasite within the body. No information is available on the prevalence of *Toxocara* seropositivity in the Alabama population.

Route of Entry to the Eye

The possible routes for invasion of the eye are through blood vessels, the optic nerve and through the soft tissues and cerebrospinal fluid. For entry to occur via the cornea or anterior sclera, the larva would have to be deposited on the front of the eye. It seems unlikely that this would happen except by deposition of infected saliva or in droplets coughed up by an infected animal or in the case of humans by contaminated hands. These events are unlikely to occur frequently. When they occur it would be expected that infection would involve the anterior of the eye but this is not a common finding. Brown (1970) recorded three reports of corneal larvae in his review of 245 reported cases. The histological

changes in ocular toxocariasis occur mainly in the posterior part of the eye leaving the anterior compartment comparatively free of disease (Wilder, 1950; Molk, 1983; Ghafoor *et al.*, 1984; Dinning *et al.*, 1988). *Toxocara* larvae have the ability to burrow through soft tissues and also migrate in blood vessels. They are likely to be capable of escaping from a blood vessel that has become too narrow to allow their passage by burrowing through the vessel wall. Studies in Mongolian gerbils have noted an association with retinal haemorrhage suggesting blood vessel involvement in eye invasion but it is not possible to say whether this is due to larvae exiting from blood vessels or damaging retinal capillaries as they arrive in the eye (Takayanagi *et al.*, 1999). Macroscopic haemorrhages have also been reported in the lungs of mice in the early stages of *T. canis* infection (Biseru, 1969; Medvedova *et al.*, 1994). Ghafoor *et al.*, (1984) identified larvae within the lumen of retinal vessels on two occasions in their examination of 54 eyes from 27 mice. The majority of the larvae they identified within the eye were lying within the retinal substance or were in the subretinal space.

It has also been suggested that invasion may occur via the optic nerve (Watzke *et al.*, 1984; Fenoy *et al.*, 2001; Hayashi *et al.*, 2003) but Ghafoor *et al.* (1984) concluded that the route of entry was vascular in their study of infected mice. Hayashi *et al.* (2003) in their study of 17 gerbils found a larva in the optic nerve of a gerbil 6 days after direct intracranial inoculation of 300 second-stage *T. canis* larvae. Two larvae were found in the optic chiasma and one in the retina of another gerbil, 9 days after a similar inoculation. There were no inflammatory changes around the larvae but eosinophil infiltrations were found beneath the sheath of the optic nerve. The authors concluded that *T. canis* larvae migrate from the brain to the retina of gerbils through the optic nerve. However, it would be wise to view their conclusion with caution. The route of inoculation is unusual and unlikely to be common in the wild. It is not possible to determine that the larva found in the optic nerve was on its way to the retina. Out of 300 larvae inoculated, only one larva was found in the optic nerve and only two were found in the optic chiasma. The suggestion has also been made that invasion may occur from the brain to the cerebrospinal fluid and then to the choroid (Fenoy *et al.*, 2001). Invasion via the blood vessels also seems to be a likely route. Ghafoor *et al.* (1984) concluded from their data that

the sites of entry were mainly the pars plana, the optic disc and the retinal blood vessels, but not the choroidal blood vessels. Whether the route of invasion varies from species to species or indeed from strain to strain is not known and it is possible that entry by all routes may occur in varying frequency in all species.

Ocular Invasion

In laboratory animal studies, ocular invasion occurs in the early stages of infection although in the small number of pig studies undertaken, ocular invasion has not been found. While a single larva can produce ocular disease, multiple larvae per eye have been reported in mice (Olson *et al.*, 1970; Ghafoor *et al.*, 1984; Fenoy *et al.*, 2000). It has been suggested that in humans too, eye invasion takes place in the early stages of the infection. It has also been suggested that ocular infection in humans may be the consequence of rather slow larval migration following a prior infection at another site. This hypothesis has been put forward to explain the reported older mean age of ocular toxocariasis patients when compared with toxocaral VLM patients. Because ocular toxocariasis is usually painless and unilateral, children and adults may present late in the course of the disease. It is almost certain that in most cases the date of infection is far earlier than the date of presentation or diagnosis. This of itself would lead to these patients belonging to an older age group than the symptomatic VLM group.

Olson (Olson, 1976; Olson *et al.*, 1970) found that, after a single dose of 3000–5280 *T. canis* eggs per mouse, most of the larvae which invaded the ocular chamber had arrived by the 3rd day post infection and the number tended to increase over the following 4 months. Similar early eye invasion has been reported by Prokopic and Figallova (1982), Maruyama *et al.* (1994) and Fenoy *et al.* (2000). Fenoy *et al.* (2000) also reported that when doses of 200 and 100 embryonated eggs were used, larvae arrived in the eye later than when 1000 eggs were given. When 50 eggs were given, no eye invasion was observed. However, studies using doses of six, 12 and 25 eggs resulted in a high risk of ocular infection (Fenoy *et al.*, 2000). Further studies are required to clarify these findings.

T. canis* and *T. cati

It has been suggested that *T. cati* may also cause eye disease. Nichols (1956) did not find *T. cati* larvae in any of the five eyes which he examined from Wilder's series (Wilder 1950). Halldorsson and Bjornsson (1980) studied Icelandic schoolchildren legally diagnosed as blind and recorded no case of ocular toxocariasis. In order to prevent hydatid disease, dog ownership, except for working dogs, had been banned in Iceland for 40 years prior to the study but cat ownership had been unrestricted. These two studies suggest that *T. cati* eye disease is uncommon in humans relative to that caused by *T. canis*, if indeed it occurs at all. Schantz *et al.* (1980) found no association between ocular toxocariasis and household cats or pets other than dogs. Petithory *et al.* (1993) reported positive ELISA tests for *T. cati* in the vitreous of six out of nine patients with ocular larva migrans, all nine of whom also had positive vitreous ELISA titres for *T. canis*, raising the possibility of *T. cati* as a cause of eye disease in humans.

Eberhard and Alfano (1998) reported four cases of adult *T. cati* infection in children under 7 years of age in the USA (Pennsylvania, Connecticut, Missouri and Kansas). None of the children had signs of OLM or VLM. In two cases, *Toxocara* titres were measured in serum and in both the result was negative. The authors suggest that the worms had been ingested as immature worms passed by infected cats and not through the ingestion of infective eggs. However, Sprent (1956) documented the development of adult *T. cati* worms in the intestinal tract of cats without undergoing liver-lung migration.

In mice, *T. canis* and *T. cati* are distributed differently in the body after experimental infection (Good, 1998). Larvae are predominantly distributed in the brains of *T. canis*-infected mice, the majority being in the cerebellum and telencephalon while in *T. cati* infection, the larvae are predominantly in muscle. In *T. cati* infection, the histological signs of infection disappear by 120 days after the initial inoculation. It is thought that the distribution of larvae may vary depending on the strain of mouse studied. Good (1998) noted a reduction in the number of larvae recovered from the brains of *T. canis*-infected mice when repeated small doses of *Toxocara* ova were given, suggesting that such a mode of exposure resulted

in some degree of immunoprotection, but noted that increased liver entrapment did not appear to be the protective mechanism. This is in contrast to the findings of Parsons and Grieve (1990) who found that in *T. canis* infections as few as 25 eggs were capable of sensitizing a murine liver to trap larvae when challenged with a further infection.

In contrast to *T. canis* infections, Good (1998) found no difference between the numbers of larvae recovered from mice bolus infected and repeatedly infected with *T. cati*.

Recent work in gerbils comparing the ocular effects of *T. cati* and *T. canis* infection has confirmed that both species can cause eye lesions in these mammals (Akao *et al.*, 2000). Eye lesions were less prevalent in *T. cati*-infected gerbils than those infected with *T. canis*. Ophthalmic lesions did not reappear in the eyes of *T. cati*-infected gerbils but repeatedly appeared in those infected with *T. canis*. However, there have been no reports of *T. cati* larvae being recovered from the eyes of mice or humans.

Differential Diagnosis

The major differential diagnosis of ocular toxocariasis in humans has been retinoblastoma which was the reason for enucleation of eyes because of the fear of involvement of the other eye. Shields (1984) recorded the final diagnosis in 136 children with possible retinoblastoma. Forty-four per cent had a final diagnosis of retinoblastoma and 56% had 'pseudoretinoblastoma'. Of the 76 children with a 'pseudoretinoblastoma' diagnosis, 26% had ocular toxocariasis, 20% persistent hyperplastic primary vitreous, 16% Coats' disease, 8% coloboma, 7% cataract, 5% retinal detachment, 4% retrolental fibroplasia (retinopathy of prematurity), 4% vitreous haemorrhage and 10% miscellaneous diagnoses. Brown's data on the final diagnoses of ten children who had enucleations are given in Table 9.3.

Risk Factors and Associated Features in Humans

Shields (1984) in his review of ocular toxocariasis reported that the relationship of ocular toxocariasis to VLM was unclear. Occasionally a clear cut

history of VLM was found, in others the VLM and OLM occurred simultaneously while in other cases no history of VLM could be elicited. Work emphasizing the occurrence of covert toxocariasis has helped clarify the fact that toxocaral infection may not be obvious (Taylor *et al.*, 1988) and prior or concurrent systemic infection may easily be missed. Brown (1970) recorded VLM syndrome in five out of 245 cases of ocular toxocariasis, Gillespie *et al.* (1993) recorded systemic illness in three out of 33 ocular toxocariasis patients all with positive toxocara serology, while Poulson *et al.* (2001) recorded no symptoms of systemic toxocariasis in 24 patients with ocular toxocariasis.

Good *et al.* (2004) compared children with ocular toxocariasis with two groups of controls: (i) children from the same county; and (ii) children from the same school. Those from the same school would have been more likely to share a similar environment than those drawn from the same county. Using the county-matched controls, dog ownership at any time just failed to reach significance while dog ownership in the previous 2 years was significantly associated with ocular toxocariasis, as were a history of convulsion and of geophagia (earth-eating). In the school-matched controls, only a history of convulsion and geophagia were significantly associated with ocular toxocariasis.

Schantz *et al.* (1980) reviewed risk factors for ocular toxocariasis and found the strongest association was with pet dogs of any age in the home within 1 year of onset of the eye disease. A pup in the home within 1 year of onset, pet dogs in the home some time before the onset of the eye disease and exposure to dogs other than in the home were all significantly associated with ocular toxocariasis. The presence of cats or other pets in the home was not significantly related to the condition. Schantz noted that only one of the 24 cases which he recorded did not have exposure to household dogs and in this case there was a history of geophagia.

Yoshida *et al.* (1999) recorded that there was a history of close contact with dogs and/or cats in 61% of his 38 cases and 26% of cases had a habit of eating raw meat and bovine or chicken liver. They also drew attention to Japanese reports of toxocaral infection after eating raw snails, meat and liver.

The report of Good *et al.* (2004) is the first report of a statistical association between ocular toxocariasis and convulsion in humans. Lyness

et al. (1987) reported a case of histologically proven ocular toxocariasis in a 12-year-old boy with a past history of febrile convulsions at 6 years of age. Gillespie *et al.* (1993) reported one case of anterior uveitis in an 18-year-old patient with cough, bronchospasm and convulsions. Yokoi *et al.* (2003) reported that two patients had convulsions on tapering their corticosteroid treatment (one of whom had a cystic lesion in the temporal lobe of the brain). It is not clear whether the convulsions were related to the corticosteroid treatment or to the toxocariasis or, in one case, to the cystic brain lesion. *T. canis* larvae have been found in the brain of a child aged 2.5 years (Hill *et al.*, 1985). The larvae were found in the pons, right frontal lobe and in the white matter of the cerebellum. There was no history of epilepsy. Behaviour disorders have been reported in children with toxocaral infection (Dent and Carrera, 1953; Huntley *et al.*, 1965). *Toxocara* seropositivity has been found more frequently in children with seizures than in controls (Arpino and Curatolo, 1988) and it has been associated with epilepsy (Woodruff *et al.*, 1966; Glickman *et al.*, 1979; Arpino *et al.*, 1990) but in none of these latter four reports is ocular toxocariasis recorded. Glickman *et al.* (1979) suggested that as pica and behavioural abnormality were significantly more common in the epilepsy group, the more common seropositivity in this group was more likely to be a consequence of the behaviour rather than the cause of it. Arpino *et al.* (1990) found pica more commonly in their control group than in the epilepsy group. Significant abnormality in behaviour has been recorded in *T. canis*-infected laboratory mice. (Hay *et al.*, 1986; Cox and Holland, 1998; Holland and Cox, 2001). Hayashi *et al.* (2003) reported severe convulsions, followed by death with neurological symptoms 2 weeks later, in six out of 14 gerbils inoculated intracranially with 300 *T. canis* larvae. Whether the convulsions were due to the larvae, residual culture medium or to the physical operation of intracranial inoculation is not clear.

The association of convulsion and ocular toxocariasis suggests both eye and brain involvement by larvae or else that those susceptible to convulsion are also susceptible to toxocaral infection or are particularly liable to brain and eye spread when infection occurs. It is postulated that 'febrile convulsions', which are common, occurring in about 3.6% of children, are related

to brain maturation as they generally occur between 6 months and 6 years of age. Seven of the 11 cases reported by Good *et al.* (2004) were within the febrile convulsions age range at the onset of eye symptoms. It may be that the abnormality which produces susceptibility to 'febrile convulsions' may also produce susceptibility to brain and eye invasion. Alternatively, the convulsions related to toxocaral eye disease may be the result of brain invasion and the eye invasion is merely an extension of the brain involvement.

Treatment

The treatment used for ocular toxocariasis may include laser photocoagulation in addition to drug therapy (see also Chapter 8, this volume). Generally steroids are used to damp down inflammatory reactions and anthelmintics may be given in an attempt to kill the larvae. A combination of both forms of drug treatment may be used.

The 'gold standard' method of assessing the effectiveness of a treatment is the double blind placebo controlled trial, used in a situation where the diagnosis has been confirmed by uniform agreed criteria in all the cases under study. Trials of treatment fulfilling these requirements are difficult to find in ocular toxocariasis. Many reported treatment studies in this condition do not fulfil these requirements. This is understandable given the ethical difficulty of withholding a potentially effective treatment. The use of an 'open' study in which the observer is aware of the type of treatment given (whether the test treatment, a currently accepted treatment or a placebo) leaves the results susceptible to observer bias. Because of the relatively small number of cases seen by clinicians it may be difficult to gather together a large group of human patients and so the numbers studied may be small. Such studies may not have the statistical power to show whether the trial treatment is truly beneficial. Studies are commonly found in which there is no control group and the progress of the patients is reported but these studies do not distinguish between benefit due to the treatment and the natural course of the disease. Such studies are of limited value.

The treatment of ocular toxocariasis is usually said to be 'unsatisfactory', meaning that it does not return the eye to the normal state and

in the case of drug treatment there is often little evidence that it is effective.

It is generally felt that the inflammation in the eye is due not only to the physical presence of a larva or its remnants but also to a reaction to antigen shed from the larva, which may remain even though the larva has moved on or died. Steroids are regarded by most ophthalmologists as a mainstay in the management of inflammation due to ocular invasion by *Toxocara* larvae. They may be given orally or, less commonly, by intra-orbital injection.

Vitrectomy has been used in the management of vitreous opacity with reported success. In this procedure, the opaque vitreous is removed and replaced with a saline solution by way of a cannula inserted through the pars plana. Surgery has been used in conjunction with vitrectomy to remove both the subretinal and epiretinal parts of the granuloma (Werner *et al.*, 1999). Surgery may be necessary where traction bands have caused retinal detachment. The main benefit of laser coagulation is to eliminate choroidal neovascularization.

Gillespie *et al.* (1993) recorded the treatment given to 15 patients with ocular toxocariasis. Topical steroids were used in two patients, systemic steroids in seven (in five cases in combination with anthelmintic medication). Three received no treatment as the condition was inactive. Seven patients were given anthelmintic medication. Five received thiabendazole, one received albendazole and one was given diethylcarbamazine.

Albendazole and steroids were given to five patients with clinically diagnosed ocular toxocariasis in seven eyes by Barisani-Asenbauer *et al.* (2001). Visual acuity improved in all patients with a mean change from 20/40 to 20/20. The authors concluded that albendazole in combination with steroids is a useful regimen to treat OLM. There was no control group. The observation period ranged from 3 days to 24 months. There is no indication as to which drug produced the effect or even whether the effect might merely be the normal course of the disease.

Albendazole and thiabendazole have been used by Altchek *et al.* (2003) in children with toxocariasis, of whom 14 were reported to have ocular toxocariasis, but without a control group it is not possible to attribute efficacy to the treatments.

Mebendazole (Park *et al.*, 2000) and diethylcarbamazine (Magnaval *et al.*, 2001) have also been

used in ocular toxocariasis. It has, however, been suggested that as a local cellular response may occur following the use of diethylcarbamazine it might be wise to avoid the use of this drug when the eye is involved. Magnaval *et al.* (1992) reported the use of mebendazole in a double blind placebo controlled study in minor forms of toxocariasis and concluded that mebendazole showed moderate efficacy in comparison with placebo. Hřčková and Velebny (2001) have noted that the benzimidazole carbamates (mebendazole, albendazole and fenbendazole) have a very low bioavailability due to their extremely low solubility in water, even though they are rapidly absorbed from the small intestine. They noted that if liposome-incorporated albendazole and fenbendazole were co-administered with liposome-incorporated glucan then improved efficacy of action was recorded. Fenbendazole given in this way was more effective in muscles and albendazole was more effective in the brain. Liposomes with incorporated benzimidazole carbamate anthelmintics provide sustained release reservoirs when given subcutaneously and can considerably improve drug efficiency. However, the improvement obtained did not result in the elimination of all *Toxocara* larvae but in a reduction in the numbers found in the tissues of the mice studied. While this is a promising line of investigation, the aim must be the elimination of all larvae as even a single larva may cause blindness.

Central lesions are likely to result in a considerable permanent defect. Yokoi *et al.* (2003) reported that of 41 cases of ocular toxocariasis, the final visual acuity was 20/20 in 50% of cases and less than 20/200 in 14% of cases. Fifteen cases underwent vitreous surgery and good visual acuity was maintained or poor visual acuity improved in ten cases. The site of the lesion plays a major part in the end result of infection.

Peripheral lesions may only leave a small visual defect, provided the vitreous haze has cleared.

Prevention

No studies exist to show the efficacy of the forms of prevention which have been recommended. It is generally accepted that ocular toxocariasis occurs following a systemic infection, however minor. The Centers for Disease Control (Atlanta, USA) in association with the American Associ-

ation of Veterinary Parasitologists (Centres for Disease Control and Prevention, 2002) has produced a fact sheet on toxocariasis which includes recommendations on prevention (see also Chapter 17, this volume). The recommendations made have been based on an attempt to prevent the initial systemic infection. A variety of recommendations have been made by various authors, which include the following:

- Avoid contact with dogs, especially young dogs.
- Worm bitches during pregnancy and after whelping.
- Worm dogs regularly especially young dogs and puppies.
- Prevent children from eating earth.
- Prevent children from playing on areas soiled with animal faeces.
- Bury or bag pet faeces.
- Wash hands before eating and after contact with dogs.

While these recommendations appear logical, they are likely to prove difficult to implement effectively in practice. The association of toxocaral infection and contact with young dogs is probably due to the excretion of *Toxocara* ova by young dogs and puppies, with consequent soil contamination, rather than due to direct contact with the animals themselves.

To be effective, worming of dogs and puppies would have to be universal as a few unwormed dogs will continue to contaminate the locality with ova (see Chapter 16, this volume). Even if all pet and working dogs were wormed, the problem of wild hosts, such as foxes, stray dogs and paratenic hosts, would remain a reservoir for the parasite.

Anyone who has looked after small children for a few days will appreciate the extreme difficulty of preventing them putting all sorts of things, including earth, in their mouths. Handwashing may be enforced but to be truly effective it should be enforced meticulously, which is unlikely to happen.

The size of the problem is underlined by the seropositivity figures from Ireland (31% of school-children seropositive; Holland *et al.*, 1995) and Reunion (92.8% of subjects over 15 years old seropositive; Magnaval *et al.*, 1994). These figures imply that to be effective in these countries any preventative measures would have to be applied across the whole population and cannot be applied just to a restricted subset of the population.

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10 Toxocariasis and the Skin

Renaud Piarroux¹, Béatrice Gavignet¹, Stéphanie Hierso² and
Philippe Humbert²

¹Department of Mycology, University Hospital Jean Minjoz, Besançon cedex, France

²Department of Dermatology, University Hospital Saint Jacques, Besançon cedex,
France

Introduction

Toxocariasis is a worldwide zoonotic parasitic disease due to human infestation with L2 larvae of *Toxocara canis* and *Toxocara cati*, two parasites, the adult stages of which are found in the canid and felid intestines, respectively. This disease is one of the most frequent zoonotic helminthiases in temperate countries (Schantz, 1989) and its incidence is increasing. Indeed, in industrialized countries, *T. canis* is found in 7–52% of adult dogs and in 49% of puppies, but higher prevalences are encountered in dogs from developing countries (Glickman *et al.*, 1985). Four clinical syndromes of human toxocariasis have been described: (i) visceral larva migrans (VLM); (ii) ocular larva migrans (OLM); (iii) covert toxocariasis; and (iv) common toxocariasis. More rarely, cases of toxocariasis have been found associated with systemic conditions, involving skin manifestations. Some cases of Reiter's syndrome, hypodermatitis and mastocytosis have also been reported (Humbert, 1995).

In this chapter, we will first consider skin disorders associated with toxocariasis clinical syndromes, particularly with VLM and common toxocariasis. Furthermore, we will consider the relationship between toxocariasis and cutaneous symptoms or diseases such as pruritus, eczema, hypodermatitis, vasculitis and chronic urticaria.

Finally, we will discuss the suspected role of *Toxocara* infection in the occurrence of other skin disorders and its consequence in terms of treatment. The different skin disorders associated with toxocariasis are documented in Tables 10.1 and 10.2.

VLM and Skin

Beaver initially described VLM in three children presenting with hypereosinophilia (Beaver *et al.*, 1952). Following this first description, numerous papers have been published resulting in a more complete description of the clinical and biological spectrum of the disease whose main symptoms include abdominal pain, decreased appetite, restlessness, fever, coughing, wheezing, asthma and hepatomegaly. In order to understand better the clinical features of VLM, Ehrhard and Kernbaum (1979) analysed 350 cases of toxocariasis corresponding to 122 papers. The disease mainly occurred in children (82%) and was more frequent in males (60%). Skin symptoms such as transient rash, urticaria and hypodermic nodules were frequently reported in these VLM cases, reaching 23% in children and 29% in adults. Other cutaneous symptoms such as pruritus or skin disorders such as eczema were not reported in this review. Indeed, the relationship between pruritus, eczema

Table 10.1. Studies concerning skin disorders associated with toxocariasis.

Studies	Skin symptoms	Reference
57 adults suffering from VLM 265 children suffering from VLM	Rash + urticaria + hypodermic nodules (29.3%) Rash + urticaria + hypodermic nodules (22.9%)	Ehrhard and Kernbaum (1979)
41 children suffering from covert toxocariasis	Rash (7%) versus controls (12%, $n = 129$)	Taylor <i>et al.</i> (1988)
13 adults and 7 children suffering from VLM ($n = 17$) or OLM ($n = 3$)	Urticaria (20%)	Scaglia <i>et al.</i> (1989)
47 patients suffering from evolutionary ($n = 32$) or cicatricial toxocariasis ($n = 15$), Department of Dermatology	Pruritus or prurigo (27.5%) Chronic urticaria (25.5%) Acquired eczema (21%) Atopic eczema (4.2%) Palmo-plantar dermatosis (4.2%) Miscellaneous dermatologic lesions (17.6%)	Buchet (1993)
138 adults suffering from common toxocariasis	Urticaria (7.2%) Chronic pruritus (4.4%) Eczema (3.6%)	Magnaval <i>et al.</i> (1994)
65 patients suffering from evolutionary or cicatricial toxocariasis, Department of Dermatology	Chronic urticaria (23%) Pruritus and prurigo (20%) Exacerbation of atopic eczema (9%) Palmo-plantar dermatosis (6%) Miscellaneous dermatologic lesions (20%)	Humbert <i>et al.</i> (1995b)

65% positive anti- <i>Toxocara</i> serology in a chronic urticaria group ($n = 51$) versus 21% in a control group ($n = 81$), Department of Dermatology	Chronic urticaria	Wolfrom <i>et al.</i> (1995; 1996) Wolfrom <i>et al.</i> (1996)
38.1% positive anti- <i>Toxocara</i> serology in a prurigo group ($n = 21$) versus 12.7% in a control group ($n = 236$), Department of Dermatology	Prurigo	Humbert <i>et al.</i> (2000)
19.5% positive anti- <i>Toxocara</i> serology in an urticaria group ($n = 128$) versus 12.7% in a control group ($n = 236$), Department of Dermatology	Urticaria: chronic urticaria ($n = 111$), acute urticaria, Quincke's oedema, cold urticaria or pressure urticaria	Humbert <i>et al.</i> (2000)
18.6% positive anti- <i>Toxocara</i> serology in an eczema group ($n = 72$) versus 12.7% in a control group ($n = 236$), Department of Dermatology	Eczema (miscellaneous eczema or dyshidrosis)	Humbert <i>et al.</i> (2000)
15.4% positive anti- <i>Toxocara</i> serology in a pruritus group ($n = 52$) versus 12.7% in a control group ($n = 236$), Department of Dermatology	Pruritus	Humbert <i>et al.</i> (2000)
8.3% positive anti- <i>Toxocara</i> serology in an atopic dermatitis group ($n = 108$) versus 12.7% in a control group ($n = 236$), Department of Dermatology	Atopic dermatitis	Humbert <i>et al.</i> (2000)
29% positive anti- <i>Toxocara</i> serology in a chronic urticaria group	Chronic urticaria	Demirci <i>et al.</i> (2003)

VLM, visceral larva migrans; OLM, ocular larva migrans.

Table 10.2. Case reports of skin disorders associated with toxocariasis.

Case report	Reference
A case of systemic granulomatous vasculitis associated with VLM in a 2-year-old child (transient oedema of the face)	Brill <i>et al.</i> (1953)
A case of subcutaneous lesions associated with <i>Toxocara canis</i> encephalomyelitis in a 32-year-old woman	Brain and Allan (1964)
A case of a severe evolving toxocariasis in a newborn baby presenting a significant pruritus with cutaneous excoriation	Nelson <i>et al.</i> (1966)
A case of a migrating panniculitis (subcutaneous nodules) in a man with evolving toxocariasis	Rook and Staughton (1972)
A case of Henoch–Schönlein purpura associated with <i>T. canis</i> infection	Pawlowska-Kamieniak <i>et al.</i> (1998)
A case of Henoch–Schönlein purpura associated with <i>T. canis</i> infection in a 17-year-old boy (palpable purpuric rash)	Hamidou <i>et al.</i> (1999)
A case of Ofuji's disease (eosinophilic folliculitis) associated with toxocariasis in a 51-year-old Caucasian woman.	Gesierich <i>et al.</i> (unpublished observations)

VLM, visceral larva migrans.

and toxocariasis was observed later, when the use of more sensitive serological tools allowed the detection of antibodies in patients presenting with milder forms of toxocariasis. From a biological point of view, all cases published from 1952 to 1979 presented with eosinophilia higher than 400/mm³, 76% of them being higher than 5000/mm³. This raised eosinophilia is probably due to the fact that, at this period, diagnosis of toxocariasis was considered only in patients presenting with hypereosinophilia, with the notable exception of ocular toxocariasis in which identification of the parasite in the eye allowed the diagnosis even when other clinical or biological signs were lacking.

Covert Toxocariasis, Common Toxocariasis and Skin

With the help of more sensitive and specific diagnostic tools, new clinical forms of the disease were described during the 1980s. In Ireland, Taylor *et al.* (1987) performed a case-control study comparing 14 children with raised *Toxocara* ELISA titres with 34 negative controls. The most frequent clinical findings in children infected with *Toxocara* were fever, anorexia, headache, abdominal pain, nausea, vomiting, lethargy, sleep and behaviour disorders, pharyngitis, pneumonia, coughing, wheezing, limb pains, cervical lymphadenitis and hepatomegaly. Blood eosinophils were elevated

in only seven of the 14 patients. The combination of abdominal pain, headache and cough was even more significantly associated with a high titre ($P < 0.05$) than were individual clinical features. This form of the disease in children was called 'covert toxocariasis'. Although not mentioned in this description of covert toxocariasis, skin symptoms were not totally lacking in Irish patients. Indeed, in a study published the following year to investigate the real spectrum of covert toxocariasis in more detail, the same group showed that rashes were noted in 7% having high levels of anti-*Toxocara* antibodies (Taylor *et al.*, 1988). However, the frequency of skin symptoms was not found to be significantly different from controls. Actually, skin symptoms were also found in 12% of the 129 controls, and 4% of patients presenting with low levels of antibodies also presented with rash. It should be noted that in this study, involving a majority of children under 15 years of age, urticaria and eczema were excluded and nothing was known about any possible relationship between seroprevalence of *Toxocara* and urticaria or eczema.

Glickman *et al.* (1987) published a case-control study, carried out in 37 adults in France, with the help of sensitive diagnostic assays. Their objective was to search for other manifestations of toxocariasis. In this study, the diagnosis of VLM was based on an increased specific antibody titre to *T. canis* as detected by ELISA and was confirmed by Western blotting. The authors demonstrated that this disease was quite common in

adults and was clinically characterized by weakness and breathing difficulties, abdominal pain, but also cutaneous signs such as rash and pruritus. Biologically, patients showed allergic manifestations including eosinophilia and increased serum IgE. This syndrome, found in adults with symptomatology which was less severe than VLM, has been described as 'common toxocariasis' (Magnaval *et al.*, 1994).

Other studies reported skin disorders associated with toxocariasis, mainly in cases of common toxocariasis and VLM. Four cases of urticaria have been described in 17 patients with VLM (children aged between 1 and 8 years, and adults) (Scaglia *et al.*, 1989). Between February 1989 and May 1994, Humbert *et al.* (1995b) reported 65 cases of evolutionary or cicatricial toxocariasis associated with cutaneous lesions. The cases of evolutionary toxocariasis were diagnosed by a positive *T. canis* serology (ELISA IgG *Toxocara* excretory-secretory antigens (TES) $\geq 50\%$ and/or ELISA IgE TES ≥ 30 U/l and/or a positive *Toxocara* Western blot) associated with the presence of more than $500/\text{mm}^3$ eosinophilia in the blood and/or a count of total IgE more than 500 kIU/l. For the cicatricial toxocariasis, a count of eosinophilic cells in the blood less than $500/\text{mm}^3$ and a total IgE titre less than 500 kIU/l were found in association with a positive *Toxocara* serology (ELISA IgG TES $> 30\%$ and ELISA IgE TES > 10 U/l and/or a positive Western blot). Twenty-three per cent of the cutaneous signs were chronic urticarias, 20% pruritus and prurigos, 21.5% acquired eczemas, 9% exacerbations of atopic eczema, 6% palmo-plantar dermatosis and 20% miscellaneous dermatologic lesions. A higher number of these patients were from rural areas.

Toxocariasis and Vasculitis

Cases of vasculitis and systemic disorders involving the skin, related to toxocariasis are extremely rare in the medical literature. One case of systemic granulomatous vasculitis associated with evolutionary toxocariasis has been described by Brill *et al.* (1953). These authors reported the case of a 2-year-old child presenting with fever, hepatomegaly, abdominal pain and neurological disorders leading to coma and death. The child presented with no rash but had an episode of

transient oedema of the face. Blood count showed hypereosinophilia leading to suspicion of toxocariasis diagnosis. Autopsy confirmed diagnosis of toxocariasis showing the existence of many necrotic and granulomatous sources in the liver, kidneys, heart and lungs. Granulomas were made up of histiocytes, giant cells and many eosinophilic cells centred by one venular necrosis. In one of these lesions, an L2 larva was detected. For these authors, the granulomatous reaction observed was related to a response of cellular oversensitiveness of parasitic origin. However, the presence of the whole larva is not essential for the development of a granuloma. In contrast, Parsons showed that TES antigens could be the cause of these granulomas (Parsons *et al.*, 1986). Histologically, they are the result of an acute inflammatory reaction, initially made of an eosinophilic polynuclear, neutrophil and monocyte aggregate. Then, the infiltrate becomes granulomatous with the presence of granulocytes and lymphocytic infiltrate (Kayes and Oaks, 1976).

More recently, Hamidou *et al.* (1999) reported a case of Henoch-Schönlein purpura (HSP) in the onset of *T. canis* infection. A 17-year-old adolescent boy was admitted with a 1-week history of abdominal pain, fever and arthritis affecting wrists, elbows, shoulders and ankles. He had a temperature of 39°C and a palpable purpuric rash on the lower limbs. He had no history of pica but had daily domestic contact with a puppy. His white blood cell count was $15,500/\text{mm}^3$, neutrophil count $11,000/\text{mm}^3$ and eosinophil count $1500/\text{mm}^3$. C-reactive protein was 126 mg/dl and erythrocyte sedimentation rate was 54 mm/h. Gamma-glutamyltransferase level was 200 IU (normal < 40 IU). Urinalysis revealed microhaematuria with moderate proteinuria. Blood cultures, stool examinations for parasites and routine cultures were negative. Serology for human immunodeficiency virus, Epstein-Barr virus, cytomegalovirus, adenovirus, parvovirus B19, rickettsia, hepatitis virus A, B and C, distomatosis, ascariasis and trichinosis were negative. *Toxocara* ELISA was positive for IgG and anti-*Toxocara* IgE (5 *Toxocara* U/l). Western blotting on blood identified a full pattern with seven specific bands (24, 28, 30, 35, 132, 147 and 200 kDa) for TES. Skin biopsy showed a thickening and perivascular granulocyte infiltrate of the superficial dermis. Piroxicam provided no significant improvement for the arthritis. The association of

close contact with a puppy, eosinophilia, leukocytosis, anti-*Toxocara* IgG, full pattern Western blot and anti-*Toxocara* IgE suggested recent toxocaral disease. However, the authors did not find any larvae within the skin biopsy. Finally, a hypersensitivity vasculitis to *T. canis* was suggested. The clinical and biological course was spontaneously favourable in 15 days without antiparasitic treatment (anthelmintic therapy was not given in order to avoid a possible aggravation of the immune vasculitis process in case of acute larval lysis). Another case report of HSP (rheumatoid purpura) associated with a toxocariasis infection was published in 1998 (Pawlowska-Kamieniak *et al.*, 1998).

Reiter's Syndrome and Toxocariasis

The most complete clinical expression of reactive arthritis is Reiter's syndrome in which cutaneous lesions are common. It occurs as a result of a variety of infections, which are essentially genital (*Chlamydia trachomatis*, *Ureaplasma urealyticum*) or gastro-intestinal (*Shigella*, *Salmonella*, *Campylobacter*, *Yersinia*) in subjects with a particular genetic predisposition characterized by the presence of the HLA-B27 antigen (Delcambre *et al.*, 1983). Doury thought that *T. canis* could be incriminated in some cases of inflammatory arthropathy, in particular Reiter's syndrome, due to an indirect immunological mechanism (the parasite is present in the organism, but at a distance from the lesion) (Doury, 1990). Clinical signs are the mono-, oligo- or poly-arthropathies of the inflammatory type. The clinical expression of the arthropathies of parasitic rheumatism are most likely to be related to the genetic background, in particular associated with the presence of the histocompatibility HLA-B27 antigen; the parasite is likely to trigger immunological mechanisms. The synovial hypereosinophilia is inconsistent but very evocative. The synovial liquid is sterile; it does not contain any larvae. In parasitic rheumatoid arthritis, there is neither deformation nor articular destruction. The diagnosis of parasitic rheumatism is significant, in particular for two reasons: it is a completely, quickly and definitively curable inflammatory rheumatism. Doury observed that antiparasitic treatment is effective during these rheumatologic reactionary parasitic demonstrations in contrast to the non-steroidal anti-inflammatory drugs.

Isolated Skin Symptoms and Toxocariasis

The frequency of cutaneous signs associated with VLM and common toxocariasis has led some physicians to address the following question: can cutaneous symptoms be the only clinical manifestations of toxocariasis? Indeed, a positive serological test can be the only biological finding in patients presenting with pure cutaneous symptoms and several studies, including some case-control studies have been carried out in order to seek an association between the presence of antibodies to *Toxocara* and pruritus sine materia, eczema and chronic urticaria (Buijs *et al.*, 1994; Wolfrom *et al.*, 1995; Humbert *et al.*, 2000).

Pruritus and Toxocariasis

For Afifi *et al.* (2004), toxocariasis was the most frequent systemic aetiology of the pruritus sine materia. Indeed, the relationship between toxocariasis and pruritus has been suspected for some time. In 1966, Nelson reported the case of a severe evolving toxocariasis in a newborn baby presenting a significant pruritus with cutaneous excoriation (Nelson *et al.*, 1966). Thiabendazole proved to be effective. In 1972, Rook and Staughton (1972) reported the case of a man whose dermatological demonstrations had begun with a severe pruritus of the palms of the hands and the planter surface of the feet, followed by a thoracic attack.

In the cases reported by Buchet *et al.* (1994) 13 of the 47 patients presented with a generalized pruritus accompanied by lesions of scraping or a nodular prurigo (Buchet, 1993). Among them, 11 had an evolving toxocariasis. An 11-month average course before the first consultation in dermatology revealed the chronic pattern of the pruritus. Semiologically, the lesions began at the thoracic level in seven cases and at the extremities in three cases. Eleven of the 13 patients presented with an attack on the upper limbs. Figure 10.1 shows a patient who suffered from a typical prurigo related to toxocariasis. This patient was cured after antiparasitic treatment.

Humbert *et al.* (2000) observed a significantly greater proportion of subjects with IgG antibodies



Fig. 10.1. Typical prurigo related to toxocariasis in a patient cured after antiparasitic treatment.

against *Toxocara* in the prurigo group ($n = 21$) than in the control group ($n = 236$), 38.1 and 12.7%, respectively. Considering patients suffering from pruritus, no statistically significant difference between the control group ($n = 236$) and the pruritus group ($n = 52$) was observed. Nevertheless, the authors suggested a strong significant association between *Toxocara* ELISA positivity and prurigo. They suggested that, in some cases, non-specific pruritic papules of prurigo might be considered as a systemic skin reaction to parasitic antigen stimulus, e.g. toxocariasis. Indeed, in their personal experience, anthelmintic treatment of patients with prurigo and toxocariasis led to a complete clearing in 80% of cases. The authors concluded that patients from a region with a high prevalence for this parasitic infection and suffering from prurigo must be checked using a blood test for *Toxocara* infection.

Eczema and Toxocariasis

In the medical literature, eczema is rarely reported in toxocariasis patients. In 1994, Buchet *et al.* reported ten cases of acquired eczema among 47 patients presenting with cutaneous demonstrations and a positive *T. canis* serology (Buchet *et al.*, 1994). No other aetiology was found for the ten cases of eczema. In eight cases out of ten, the patients had an evolving toxocariasis. In certain cases, eczema had developed over a 4–6 month period before the first consultation in dermatology. In seven cases out of ten, the lesions prevailed on the limbs. In addition, there were two cases of severe atopic eczema associated with toxocariasis (one evolving and the other cicatricial). These cases developed over several years. Clinical improvement was obtained after treatment with

thiabendazole (two tablets per day over 5 days). The authors concluded that it is useful to carry out *Toxocara* serology, in particular in the presence of risk factors (pets untreated for parasites) in cases of severe atopic eczema, knowing that prevention of re-infection and anthelmintic treatment are likely to improve the evolution of eczema.

In his study published in 2000, Humbert did not observe any statistically significant difference in *Toxocara* infection between the control group and the eczema group (Humbert *et al.*, 2000). The proportion of subjects with IgG antibodies against *Toxocara* in the eczema group ($n = 72$) was 18.6 versus 12.7% in the control group ($n = 236$).

Panniculitis and Toxocariasis

The relationship between panniculitis and toxocariasis has been explored rarely in medical litera-

ture. Two cases have, however, been published in which the role of *Toxocara* has been suspected due to the finding of a larval fragment in one case, and improvement after treatment in the other case.

In 1972, Rook and Staughton related one case of migrating panniculitis in a man with evolving toxocariasis (Rook and Staughton, 1972). This patient presented with subcutaneous nodules on the abdomen, thorax and limbs. The authors observed lengthened nodules progressing from the surface to the depth of the tegument and disappearing spontaneously without suppuration within 3 weeks. Several signs supported *Toxocara* as the aetiological agent; significant hypereosinophilia, a positive *Toxocara* skin test and positive *Toxocara* serology by immunofluorescence. Histologically, a dermis perivascular infiltrate was found at the level of two lesions. It was rich in eosinophilia and contained a fragment of a parasitic larva that could not be identified.



Fig. 10.2. Urticaria associated with active toxocariasis.

Other authors reported a case of subcutaneous lesions associated with a *T. canis* encephalomyelitis (Brain and Allan, 1964). A 32-year-old woman complained of headache and convulsions, starting about 10 months before. In addition she had circular, firm, subcutaneous lesions in the right kidney and on the plantar surface of the right foot. These lesions disappeared spontaneously after about a week, and then reappeared a few months later at the same site. These subcutaneous lesions gradually enlarged for about a week. The larger (2 cm diameter) was on the right foot; the smaller (1 cm) was in the right loin. Both were circular, slightly raised, firm and slightly fluctuant. Both cutaneous lesions were excised. They had the appearance of a granuloma, containing lymphocytes, fibroblasts, plasma cells and eosinophils, the last in large numbers. Areas of necrosis were present. No larvae were found. The toxocaral skin test was positive. After treatment by diethylcarbamazine, the subcutaneous lesions disappeared.

Chronic Urticaria and Toxocariasis

Among the skin disorders being reported in patients presenting positive serology for toxocariasis, urticarial manifestations have been reported more commonly by a series of authors (Magnaval *et al.*, 1983; Scaglia *et al.*, 1989; Buchet *et al.*, 1994; Wolf from *et al.*, 1996; Oteifa *et al.*, 1998; Humbert *et al.*, 2000). These urticarial manifestations were more often chronic than acute. Figure 10.2 shows a patient who suffered from urticaria associated with active toxocariasis.

Chronic urticaria has been defined by Degos as an outbreak of oedematous, projecting, well-delimited, rounded papules, measuring from a few millimetres to several centimetres and with a pruriginous and fleeting evolution (Degos, 1976). Urticaria is common and it is estimated that approximately 15–20% of the population present with an urticaria episode at least once during their lifetime. However, in most cases, these are acute episodes and their cause can be identified. On the contrary, the chronic form, which corresponds to lesions persisting for more than 6 weeks or 3 months, depending on the authors, is less frequently encountered, its prevalence being less than 5% in the whole population (Bouyer *et al.*,

1993). Its cause is rarely identified and 70–90% of cases remain unexplained (Champion *et al.*, 1969; Champion, 1988; Cribier and Noacco, 2003). Finally, relapses are frequent, and definitive cure of patients can be assessed only after a prolonged follow-up (Cribier and Noacco, 2003).

Nevertheless, several studies have shown a positive correlation between chronic urticaria and toxocariasis. In a case-control study, Wolf from *et al.* found 65% positive anti-*Toxocara* serology among 51 cases of chronic urticaria compared with 21% in a control group (risk factor 6.9). Moreover, patients having chronic urticaria with positive *Toxocara* serology were more often exposed to pets than patients having chronic urticaria with negative serology (84 versus 50%, $P < 0.001$) (Wolf from *et al.*, 1995, 1996). In most cases, chronic urticaria evolves favourably with treatment: of the 33 cases of chronic urticaria with antibodies to *T. canis*, 14 were treated with thiabendazole or ivermectin. After a 1 year follow-up, four were cured of their urticaria (29%), four significantly improved (29%) and six (43%) had no benefit. No improvement occurred in the 19 untreated patients (Wolf from *et al.*, 1996).

These findings have been confirmed by two other case-control studies. The first one was performed by Humbert who observed a significantly greater proportion of subjects with IgG antibodies against *Toxocara* in the urticaria group and in the prurigo group than in the control group (Humbert *et al.*, 2000). The authors found 19.5% positive anti-*Toxocara* serology among 128 cases of chronic urticaria and 38.1% positive anti-*Toxocara* serology among 21 cases of prurigo, compared with 12.7% in a control group ($n = 236$). The other study was performed by Demirci and colleagues, who found a 29% seroprevalence of toxocariasis in patients presenting with chronic urticaria (Demirci *et al.*, 2003). Based upon these findings, some authors considered that cases of chronic urticaria may be directly due to toxocariasis. Magnaval concluded that a proportion of *Toxocara*-infected patients with a favourable genetic background may present with clinical features associated with atopy, such as urticaria, asthma, rhinitis and high levels of anti-*Toxocara* IgE (Magnaval, 1987).

Numerous hypotheses have been proposed to explain the persistence of urticaria in patients infected with *Toxocara*. Urticaria may become

chronic due to iterative infections with *Toxocara*. Even in the absence of re-infection, chronic clinical features may be due to the persistence of living *Toxocara* larvae in human tissues (Wilder, 1950; Raistrick and Hart, 1975). Ashton demonstrated that living larvae may survive for more than 6 years (Ashton, 1960). Beaver (1969) observed that larvae switch to diapause and are surrounded by a capsule which appears to limit the effect of the immune system of the host (Beaver, 1969). Berezantsev assumed that *T. canis* larvae may interfere with the immune response of the host, in particular with the excretion of substances forming a capsule functioning like a semi-permeable membrane ensuring the nutrition and vital processes of the parasite (Berezantsev, 1974). Smith *et al.* (1981) and Rockey *et al.* (1983) provided new data in favour of this hypothesis, showing that larvae may excrete a surface substance which protects them from the immune response of the host. Even after their death, larvae seem to be able to provoke an immune response from the host (Byers and Kimura, 1974; Ehrhard and Kernbaum, 1979). Some hypersensitivity reactions may be caused by the liberation of somatic, semi-necrotic and antigenic products belonging to the dead larva (Ehrhard and Kernbaum, 1979). This hypothesis may be an explanation for those cases of cicatricial toxocariasis, in which larvae are dead, encysted in tissues, without either hypereosinophilia or an increase in IgE.

However, the relationship between chronic urticaria and infectious conditions, including toxocariasis, is still questioned by some authors. In a study including patients with chronic urticaria, toxocariasis seropositivity was not significantly higher compared with healthy controls ($P > 0.05$) (Demirci *et al.*, 2003). In a recently published review by Cribier and Noacco, the authors explained that chronic urticaria is an often capricious disease evolving over more than 6 weeks and that only a prolonged follow-up after anthelmintic treatment can provoke an effective cure (Cribier and Noacco, 2003). The authors concluded that there is a lack of controlled studies exploring the efficacy of anthelmintic treatment on the course of chronic urticaria.

At this stage of the review, it should be obvious that there are difficulties in establishing a clear causal link between *Toxocara* infection and the occurrence of skin disorders. Indeed, even if stud-

ies have shown a significant statistical association between the presence of anti-*Toxocara* antibodies and occurrence of skin symptoms, this statistical association may have numerous explanations and does not necessarily demonstrate unequivocally the role of *Toxocara* as one of the main aetiological agents of these disorders. First, the diagnosis of the disease is only an indirect diagnosis based upon serological findings. Humans act as paratenic hosts for *Toxocara*; therefore if no adult worms are present, worms and eggs cannot be found in stools. Moreover, larval stages are located in the tissues, and are only exceptionally detected, even in authentic VLM. Second, both the sensitivity and specificity of serological diagnosis of toxocariasis are variable. Furthermore, the detection of specific antibodies against *Toxocara* cannot differentiate between patients presenting with a recent or past infection. Crucially, the thresholds defined for ELISA tests, which is the more commonly used method to detect anti-*Toxocara* antibodies, have been chosen to fit a diagnosis for VLM. These thresholds are often too high to detect lower levels of antibodies, which are encountered in OLM and in some cases of covert toxocariasis. Using immunoblotting, we are able to demonstrate that sera with antibody titres lower than the threshold recommended by manufacturers often present a complete Western blot pattern, showing all the specific bands for toxocariasis (unpublished data). Consequently, it may be assumed that seroprevalence of toxocariasis is underestimated and that some association between skin disorders and the presence of low levels of *Toxocara* antibodies may not be detected. Third, even if a significant relationship between occurrence of skin disorders and detection of anti-*Toxocara* serology is described in the literature, this does not necessarily imply that *Toxocara* is the cause of skin disorders. Indeed, both findings may be the consequence of a third condition which can explain the higher level of *Toxocara* infection as well as the occurrence of skin disorders. These conditions or risk factors may include pet ownership, atopy and/or a genetic predisposition to *Toxocara* infection and urticaria. Finally, the lack of parasitological proof demonstrating that patients suffering from skin disorders have been recently infected by *Toxocara* may be compounded by the fact that treatment for toxocariasis is not always efficient.

Treatment of Skin Disorders associated with Toxocariasis

The requirement for treatment of toxocariasis depends upon the type and severity of clinical features. Patients presenting with VLM associated with cutaneous signs should be treated either symptomatically or with specific anthelmintic drugs (benzimidazole derivatives such as albendazole, 10 mg/kg b/w daily for 5 days or diethylcarbamazine 3–4 mg/kg b/w daily for 21 days starting at 25 mg daily with a progressive increase). Patients presenting with common toxocariasis or with isolated cutaneous symptoms may improve with specific drugs. However, since these clinical forms of the disease are usually self-limiting, use of anthelmintic drugs should be considered only when patients remain symptomatic despite the removal of risk factors for re-infection with *Toxocara* eggs (Humbert *et al.*, 1995a; Degouy *et al.*, 2001). Prevention of re-infection requires a preliminary investigation in order to identify the source of *Toxocara* eggs by studying possible environmental and personal risk factors for the patient. Personal hygiene should be improved. Salads and

vegetables collected on the soil should be thoroughly washed. Moreover, because consumption of raw or undercooked meat can be a source of infection, these foods should be avoided. Pets have to be de-wormed by adequate treatment given at 2 or 3 weeks of age and every 2 weeks until 12 weeks of age, then twice a year over their lifetime (Chabasse *et al.*, 1982; Soulsby, 1987; Harvey *et al.*, 1991; Magnaval *et al.*, 1994).

To conclude, although data on the relationship between toxocariasis and skin symptoms are still lacking, it is more and more probable that this parasite is playing a role in some cases of pruritus, prurigo, chronic urticaria and maybe eczema. Consequently, toxocariasis must be considered along with bacterial, parasitic or viral causes every time a physician suspects skin manifestations may be due to an underlying infectious condition. In the absence of other aetiology, the physician should consider *Toxocara* diagnosis and try to support it by serological testing including immunoblotting. Unexplained skin disorders associated with positive serology should lead the physician to at least recommend pet deworming and improvements in general hygiene and, if symptoms persist, to prescribe anthelmintic drugs.

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11 Inflammatory and Immunological Responses to *Toxocara canis*

Stephen G. Kayes

*Department of Cell Biology and Neuroscience, College of Medicine,
University of South Alabama, Alabama, USA*

Introduction

A member of the superfamily Ascaroidea, *Toxocara canis* is the enigmatic and ubiquitous, cosmopolitan roundworm of dogs. When humans share their personal habitats with dogs, the dogs in return share their most prized parasites with humans. But perhaps no host-parasite relationship has evolved so insidiously as has the one involving *T. canis* and humans. It is an enigma how adult dogs can develop an immunity to this parasite which eliminates the adult worms from the intestine, while humans mount a significant immune response to the immature worms involving virtually every conceivable weapon in the immune armamentarium but cannot kill or eliminate this organism. As a consequence of this evolutionary failure, *T. canis* may be one of the most under-diagnosed parasitic infections in the developed countries of the world.

In the canine host, the life cycle of *T. canis* is quite similar to that of *Ascaris lumbricoides* in humans. Eggs hatch in the small intestine where they liberate a single second-stage larva (L2), which then invades the intestinal wall seeking entrance into the vasculature or lymphatic system (via the lacteals). The vasculature will take most of the larvae to the liver and from there they pass to the heart with the venous return and finally on to the lungs. When the calibre of the worms

approximates the calibre of vessels in the septal walls, the larvae break out into the alveolar air-spaces. After spending approximately a week in the lungs, the larvae moult to the third stage, migrate up the trachea and are swallowed. This leads to a passage through the gastric acid bath (to which the larvae are completely resistant) and arrival in the small intestine where this migratory odyssey began. Following another moult to the fourth stage, the worms mate and begin producing prodigious numbers of eggs that pass on to the soil contained in the faecal stream. To this point, the life cycle of *T. canis* is no different than that of *Ascaris* (Schantz and Glickman, 1981; Parsons, 1987; Magnaval *et al.*, 2001).

However, *Toxocara* has acquired some adaptive tricks which increase the probability of the worms infecting its definitive host. First, in female dogs, a significant cohort of larvae, while migrating as just described, become arrested in the visceral tissues and do not complete the liver-to-lung-to-small intestine migratory route. Instead they wait for the dog to become pregnant and then reactivate. The reactivated larvae then migrate to the uterus and infect the pups during the third trimester of pregnancy. In the embryonic pups, they migrate to the liver and again become dormant to await parturition. Upon birth of the pups, the worms reactivate and migrate to the lungs and development ensues as before. Additional cohorts of worms invade the mammaries and suckling pups

can become infected following ingestion of infective larvae contained in the mother's milk. If the puppies become heavily infected, they may pass advanced-stage larvae in their faeces, which the mother may ingest during the act of nest cleaning or maintenance. The mother then becomes more heavily infected. The result of the parasite having multiple routes of infection is to ensure the build-up of high numbers of egg-producing female worms which can contaminate the local environment with infective *Toxocara* eggs to an incredible level. Immunologically, the canine host is exposed to antigens associated with the fluids contained within the eggs, antigens associated with L2, L3, L4 and adult stages of the parasite. In contrast, non-canid hosts are only exposed to egg and L2 antigens. Additionally, different organs are exposed to different antigens and thus antigen presentation may differ in different parts of the body.

To appreciate fully all of the complexities of the disease process that are attributable to *T. canis*, it is necessary to appreciate not only the histopathological response to the parasite but also the immuno-inflammatory processes that are operative as well. It is the purpose of the remainder of this chapter to link these physiological responses to the parasite and its biology.

***T. canis* Infection in the Canine Host**

The most significant aspect of canine toxocariasis is the ability of the parasite to infect the next generation of puppies during gestation. This occurs when larvae previously arrested in their development in the pregnant bitch reactivate, cross the placenta and invade the fetal pups. Some of these reactivated larvae also invade the mammary glands and infect suckling puppies but transmammary infection only contributes about 2% of the larvae infecting newborn puppies (Burke and Roberson, 1985). If there are subsequent pregnancies, additional larvae will infect the next litter as well. Little or nothing is known about what retards large numbers of L2 larvae and prevents them from completing their development and becoming adults in the intestine but there are several interesting observations that suggest immune mechanisms may be at play in the adult dog.

What makes canine toxocariasis so fascinating is that the majority of dogs become resistant to

adult worms between 3 and 6 months of age as reflected by a dramatic drop in faecal egg output and/or the finding of few if any adult worms in the gastrointestinal (GI) tract at necropsy. Greve coined the term 'age resistance' to describe this phenomenon (Fernando *et al.*, 1973). Fernando and colleagues characterized the canine response to the establishment of a superinfection administered orally (infective ova) to naturally infected mongrel puppies and attributed the effects noted to the presence of complement-fixing (e.g. IgG) antibodies. All puppies in these studies harboured fully patent *T. canis* worms in their GI tracts. While superinfection with 100,000 embryonated ova was invariably fatal to the puppies, smaller infections with between 25,000 and 50,000 ova led to the elimination of the intestinal worm burden in just over half of the puppies. In those pups which retained their adult worms, egg production dropped to almost zero within 21 days of the superinfection but egg output did return to high levels about a week later (Fernando, 1968). The basis for this observation is not clear. It was further observed that a month after the superinfection challenge was administered, second-stage larvae appeared to be 'trapped' in the liver and lungs and it was suggested that these were now 'arrested' L2 larvae. It was concluded that superinfection of naturally infected puppies led to: (i) the elimination of the primary infection by a mechanism that was similar to, if not identical with, the so-called self-cure phenomenon described in sheep infected with *Haemonchus contortus* (Stewart, 1950); and (ii) the immune mechanism elicited by the primary (i.e. sensitizing) infection prevented the challenge larvae from further development beyond the L2 stage. It was further suggested that the developmental arrest was antibody-mediated because L2 larvae, but not L4 larvae, developed circumoral precipitates and died following incubation in immune dog serum but survived in normal serum (Fernando *et al.*, 1973).

Canine antibody responses against *T. canis* have been extensively studied by Matsumura and colleagues. Using ELISAs employing four different stage-specific antigens it was observed that in naturally infected puppies, the absorbance values of the assay were inversely correlated with the adult worm burden and that as resistance to the intestinal worms increased with age, the ELISA absorbance value increased significantly (Matsumura and Endo, 1982). Interestingly, the

ELISA absorbance value was greater when unfertilized ova were found in the faeces (possibly suggesting that male worms can modulate the antibody response) (Matsumura and Endo, 1982). In the prototypical humoral immune response to a peptide antigen, IgM is the first isotype produced and levels are detectable 5–7 days after antigen encounter (Abbas *et al.*, 1997). IgM titres usually decline to background levels as IgG titres rise but in canine toxocariasis high IgM levels persist for months. In naturally infected puppies, IgM levels increase steadily for the first 3 months of life and persist well into adulthood even though there is no correlation between IgM titres and adult worm burdens (Matsumura *et al.*, 1983b), nor with IgA levels, which are often associated with enteric mucosal-surface immunity (Matsumura *et al.*, 1983a). The IgM levels were found, however, to correlate with the levels of circulating *Toxocara* antigens, which also persist into adulthood (Matsumura *et al.*, 1983b).

Immune Responses in the Non-canid Host

The granulomatous reaction of tuberculosis has long been considered the *sine qua non* of cell-mediated immunity and this immunity is transferable to a naive animal with T lymphocytes but not immune serum (Abbas *et al.*, 1997). Beaver *et al.* (1952) and others (Zyngier, 1974; Kayes and Oaks, 1978) noted florid granulomatous reactions encapsulating viable second-stage larvae. By abrogating various correlates of cell-mediated immunity, it has been shown that *T. canis* elicits a T cell-dependent response that includes peripheral blood eosinophilia and the granulomatous encapsulation of migrating larvae (Kayes and Oaks, 1980).

In the paratenic host, *T. canis* L2 larvae neither moult, grow nor replicate (Faust *et al.*, 1968; Parsons, 1987). Because experimental infections typically are initiated by gastric intubation of a specified number of eggs, it is therefore possible to study the immune response of the paratenic host to ever increasing sizes of the primary infection and this has been exploited by many laboratories. The constant finding of all these studies employing varying infection sizes is that the magnitude of immune response is directly proportional to the number of eggs intubated into the paratenic host.

Antigen-specific and non-specific lymphoproliferative responses, antibody titres, eosinophilia and splenomegaly have all been shown to increase with increasing numbers of larvae (Kayes *et al.*, 1985). Trapping or arresting of challenge infection larvae in the liver is also proportional to the size of the primary infection (Sugane and Oshima, 1983; Parsons and Grieve, 1990). These studies would seem to suggest that the larva migrans observed in paratenic hosts is similar if not identical to that observed in superinfected puppies (Fernando, 1968; Fernando *et al.*, 1973). Also common to virtually all experiments with increasing infection sizes up to 20 eggs/g body weight (mouse) is the observation that there exists a plateau for each response and with very low infections it may not be possible to see any response until more time passes (Kayes *et al.*, 1985). The fact that there is a plateau to most responses also raises questions about the interpretation of the significance of responses obtained from animals, especially mice, given thousands of eggs in a single bolus. These results may reflect little more than the response of an overwhelmed and/or failing immune system.

Antigen-specific T lymphocytes are not uniformly distributed throughout the paratenic host and probably not uniformly distributed in the definitive host either. Comparison of T-cell populations and their responses from the lung (recovered by bronchoalveolar lavage (BAL)) with responses of T cells isolated from the spleens of the same animal have identified several significant differences. In the spleen, the ratio of CD4- to CD8-positive T cells varies over a very narrow range (between 1.6 and 2.0) while in the lungs of mice infected with 250 ova, the corresponding ratio was 7.3 to 15.8 over the first 6 weeks of infection (Kayes, 1997). Another significant finding was that the lung when compared with the spleen from the same animals contained a much greater proportion of antigen-specific T cells (Kayes *et al.*, 1987) and as a consequence of this latter observation it was determined that T cells recovered from the lungs by BAL were able to transfer *T. canis* antigen-specific granulomatous hypersensitivity to naive recipient mice while immune spleen cells and/or immune serum were not (Kayes *et al.*, 1988). As these studies were performed in a single strain of inbred mouse (i.e. CBA/J) it is not clear whether spleen cells of other strains comparably infected would have successfully transferred granulomatous hypersensitivity.

A major drawback to studying the immunoregulation of granuloma formation around *T. canis* larvae is the well-documented inability to assign a time frame to a particular lesion. The most that can be said is that the lesion is not older than the number of days since the initiation of the infection. This is because the larvae are able to leave the confines of a fibrotically encapsulated granuloma, migrate elsewhere and begin the granulomatous process all over again. What causes *T. canis* larvae to do this is not known at this time. It is not uncommon to cut serial sections through an entire granuloma and not find any evidence of the larva which served as the nidus for the eosinophil-rich inflammatory reaction to form. This may reflect the so-called activation of arrested larvae and while little or nothing is known about the signal to re-activate, it has been speculated that, at least in the dog, pregnancy-related hormones may be the trigger (Parsons, 1987).

To overcome the empty granuloma conundrum, and achieve a synchronous population of granulomatous reactions, plastic beads coated with *T. canis* antigens can be embolized via the tail vein into the lungs of mice. The lungs can be removed, fixed, sectioned and stained 6 or more days after the embolization. The granulomatous reactions are highly amenable to morphometric analysis and it is possible to identify the various cells making up the lesion by direct microscopic examination of stained histological sections. Such studies have been carried out in mice with schistosomiasis (von Lichtenberg *et al.*, 1971; Dunsford *et al.*, 1974; Carrick and Boros, 1980) and toxocariasis (Kayes *et al.*, 1988). In the lungs of normal mice, *T. canis* antigen-coated beads elicit a foreign body reaction which is not much greater in diameter than the bead itself. This histopathological reaction consists of a layer or two of tightly adherent fibroblasts surrounding the bead. In contrast, when the antigen-coated beads are embolized into *T. canis*-infected mice they elicit large, florid, eosinophil-rich granulomatous reactions that resemble the granulomas seen around viable larvae in liver or muscle (Kayes *et al.*, 1988).

One of the more interesting observations to come out of these *in vivo* bead granuloma studies and one which may have a bearing on whether *T. canis* is a contributor to an allergic or asthmatic phenotype concerns the reaction around beads coated with methylated bovine serum albumin

(MBSA). MBSA is an antigen totally unrelated to *T. canis* and was embolized in mice, both infected with the parasite and immunized with MBSA. The experiment involved three groups of mice immunized with MBSA, two of which were simultaneously infected with *T. canis*. One of these two *T. canis* infections used only 10 eggs (small infection) while the other used 250 eggs (large infection). Two additional groups of mice received either the small or the large *T. canis* infection, but no MBSA. After an appropriate time all mice were challenged with MBSA-coated beads and their lungs removed for histological examination 6 days after MBSA-bead embolization. MBSA-coated beads embolized in mice immunized with MBSA elicited a large florid granuloma containing epithelioid macrophages and Langerhans foreign body giant cells (consistent with a tuberculosis-like, cell-mediated hypersensitive granulomatous reaction). As expected, MBSA-coated beads embolized in mice infected with 10 *T. canis* eggs and not immunized with MBSA elicited the minimal foreign body reaction. In sharp contrast and very unexpectedly, MBSA-coated beads embolized in mice that were both infected with 250 *T. canis* ova and immunized with MBSA or embolized into mice infected with 250 ova only elicited large florid eosinophil-rich granulomas in their lungs at necropsy. Thus, if there were high numbers of eosinophils circulating in the peripheral blood (as a consequence of the *T. canis* infection), eosinophils appeared to be the predominant cells contributing to the granulomatous reaction. The fact that MBSA-coated beads elicited an eosinophilic reaction in the lungs of mice that had only been infected with *T. canis* suggests that a non-specific antigen could trigger a *T. canis* reaction and it was this observation of a non-specific allergic (i.e. eosinophilic) inflammatory response that first suggested that infective *T. canis* larvae could prime the immune system for an allergic response to an irrelevant antigen such as occurs in asthma (Kayes, 1986).

Toxocara infection in humans may take any of at least three different clinical presentations. These include classical visceral larva migrans (VLM), ocular larva migrans (OLM) and a third form called occult or clinically inapparent larva migrans (Glickman and Magnaval, 1993; Magnaval *et al.*, 2001). Often, but not always, VLM is the only one of the three presentations that is symptomatic (other than loss of sight when

it occurs in OLM). OLM is thought to represent infection with only a few infective eggs and is the display of the unfortunate circumstance where the eye is invaded to the detriment of the patient's sight (see Chapter 9, this volume). If the patient had been infected with a greater number of eggs, current dogma would suggest that the induced inflammatory and/or immunological response would have prevented any larvae from getting to the eye, possibly because the larvae would become encapsulated in a fibrotic granuloma. But, if the patient ingests an extremely large number of eggs, it is likely that there would be a combined presentation of VLM and OLM. Certainly, the idea of an immunological threshold below which there is not enough antigen to drive the immune system into action is consistent with such an explanation and is consistent with findings in mice that were infected with only five infective eggs. These mice had significantly detectable eosinophilia and splenomegaly by day 14 post-infection (p.i.). However, antigen-specific antibody responses did not become measurable until 28 days p.i. This was in contrast to larger infections where significant eosinophilia and antibody titres were seen as early as 14 days p.i. (Kayes *et al.*, 1985). Clinical support for this conceptual framework comes from the work of Obwaller *et al.* (1998). This study examined serological variables in four groups of patients: symptomatic VLM and OLM patients were compared with allergy patients (high IgE titres) and/or patients infected with helminthiasis other than toxocariasis (i.e. seronegative for *T. canis*). Analysis of IgG subclass specificity against *Toxocara* excretory-secretory (TES) antigens indicated that IgG1 was the predominant species followed by -G2, -G4 and -G3 in all three (*T. canis*-positive) patient groups. Within the *T. canis*-seropositive patients, IgG1, -G2 and -G4 levels were significantly higher in the VLM patients than in the asymptomatic patients while only IgG1 titres were significantly higher in OLM patients compared with asymptomatic patients. It can be argued that these findings are consistent with the idea that the antigenic load in OLM is not strong enough to stimulate IgG2, -G3 or -G4 titres (or alternatively not enough time has passed but this does not seem likely). More interesting yet was the finding that VLM and OLM patients could both be distinguished from the asymptomatic patients by the amount of IgE-IgG anti-IgE circulating immune complexes. IgG anti-IgE is an autoantibody. The

fact that IgE production following infection with *T. canis* is dependent on the Th2 cytokine IL-4 led Obwaller *et al.* to suggest that immediate hypersensitivity may be important in the pathogenesis of infection (Obwaller *et al.*, 1998), especially because both human mast cells and eosinophils have Fc_ε receptors (Dombrowicz and Capron, 2001).

The Inflammatory Reaction in Larva Migrans

It is well established that larva migrans occurs in both the canid definitive host and in the paratenic host (Parsons, 1987). However, it is not at all clear that the inflammatory and/or immune responses in these disparate hosts are the same. This is because immunological and genetic tools available for murine experiments are not as available for canine studies. However, it seems reasonable to assume that the migrating L2 larvae are essentially behaving the same in both types of hosts. This means that once the larvae exit the vasculature and enter the tissues, the antigens that the host is both exposed and responds to are those collectively referred to as TES antigens.

First isolated by de Savigny (1975), these *Toxocara*-specific antigens have been extensively characterized (Maizels *et al.*, 1984) (also see Chapter 1, this volume). The TES family consists of five major molecules (for reviews, see Maizels *et al.*, 1993), all of which are heavily glycosylated (i.e. are glycoproteins) and having molecular weights of 32, 55, 70, 120 and 400 kDa as determined by gel electrophoresis. The potential significance of the heavy glycosylation will be discussed below. In particular, the antigens produced by the oesophageal glands and secretory apparatus (Page *et al.*, 1992a,b) seem to attract the most attention from the host's immune system and it is the products of the worm's secretory system that may be dispersed over the entire surface of the larva giving rise to the epicuticle and creating a disposable and insulating barrier between the parasite and its host (Page *et al.*, 1992b). By electron microscopy, the epicuticle appears as a dense granular material that is shed from the surface of the larva when it comes under attack by host inflammatory cells and/or antibody (Badley *et al.*, 1987).

Shortly after infective larvae escape from their eggshells, they burrow into the wall of the

small intestine and gain entrance into the circulation (Glickman and Schantz, 1981; Parsons, 1987; Magnaval *et al.*, 2001). Larvae 'break out' of capillaries that can no longer accommodate the diameter of the fleeing nematodes. This event is accompanied by a small amount of vascular leakage but the capillaries apparently heal quickly and tissue histiocytes clean up the spill almost immediately (Kayes, unpublished observation). Another reason for considering the number of infective eggs administered to an experimental animal is that a moderate to small number of worms synchronously breaking out of capillaries is manageable, while thousands of larvae can lead to the destruction of organ-specific vascular beds and frank haemorrhage. Meanwhile, the larvae are now completely within the extracellular matrix (ECM) of whatever organ the circulation has taken them to and the fibres and ground substance are closely applied to the larval surface, especially the epicuticle, the main source of antigens that will lead to the establishment of the Th2 immune response. All these events occur within the first 24 h of an oral infection (Kayes and Oaks, 1976).

Once larvae are free of their eggshells, both inflammatory and immune cells begin encountering *T. canis*-specific antigens and the dance of the mediators is afoot. Whether or not larvae contained within the vasculature interact specifically with any of the selectins or integrin adhesion molecules on the luminal surface of the endothelium is not currently known. However, as soon as some of the tissue resident cells encounter the newly released TES antigens, early response mediators such as tumour necrosis factor alpha (TNF- α) and interleukin 8 (IL-8) will begin diffusing through the ECM until they encounter specific receptors on the adluminal surface of the endothelium of the microvascular capillaries. Ligand-receptor interactions on the adluminal surface of endothelial cells lead to upregulation of leukocyte-specific adhesion molecule genes and shortly thereafter, the adhesion molecules are expressed on the luminal surface of the capillaries. TNF and IL-8 are considered to be neutrophil-associated mediators (Rampart *et al.*, 1989; Kunkel *et al.*, 1997; Lukacs and Tekkanat, 2000) and in fact the first cells to contribute to the inflammatory infiltrate into the lung of mice infected with *T. canis* are neutrophils (S.G. Kayes, unpublished observation). This early inflammatory infiltrate reflects the innate immune response. However, an eosino-

phil-selective set of adhesion molecules consisting of VCAM-1 and ELAM-1 (vascular cell adhesion molecule 1 and endothelial leukocyte adhesion molecule 1, respectively) will begin to be expressed on the endothelium near the end of the first week of infection. This set of adhesion molecules binds VLA-4 expressed on eosinophils and ensures that a preponderance of eosinophils will be attracted to the site of tissue migration in response to the Th2 cytokines (Weller *et al.*, 1991; Weller, 1992a,b; Resnick and Weller, 1993).

The Th2 response is generally accepted as being the responsible mechanism leading to two of the most striking clinical features of larva migrans, namely the spike in eosinophilia and the IgE hyperglobulinemia (Del Prete *et al.*, 1991a,b, 1994; Grieve *et al.*, 1993). Originally described in mice, Th1 and Th2 CD4⁺ T cells are distinguishable by the primary cytokines they secrete. In response to specific antigen stimulation, Th1 cells secrete IL-2 (T-cell growth factor) and interferon (IFN)- γ (macrophage activator), which collectively prime for a cytotoxic cellular response. In contrast, the Th2 response results in the production of IL-4 (helps B cells switch from an IgM response to IgG, IgA and, more importantly, IgE), IL-5 (an eosinophilopoietic cytokine) and IL-13 (which is associated with the hyperreactivity of the asthmatic lung and upregulating the expression of VCAM-1 by pulmonary microvascular endothelial cells) (Bochner *et al.*, 1995; Schnyder *et al.*, 1996; Ying *et al.*, 1997).

Given the magnitude of the eosinophilia and IgE responses that are routinely encountered in larva migrans, one would think that these responses could control or eliminate immature stages of the parasite. However, study after study has found that eosinophils with or without antibody are unable to kill second-stage larvae (Badley *et al.*, 1987; Parsons *et al.*, 1993; Jones *et al.*, 1994; Dent *et al.*, 1997, 1999; Ovington and Behm, 1997; Takamoto *et al.*, 1997). This is in part because the larvae are constantly sloughing off their epicuticle taking the attached inflammatory attack cells with it (Badley *et al.*, 1987) and in part because mouse eosinophils (in which the majority of the studies have been performed) express Fc receptors only for aggregated IgG while lacking Fc receptors for IgG, -A and -E (Jones *et al.*, 1994). Nude mice congenitally lack a functional thymus and hence lack T cells of all kinds including CD4 and CD8 T lymphocytes. Because these mice

have no Th2 cells and therefore do not make IL-5, an eosinophil response following *T. canis* infection was not expected in these mice. However, following infection with *T. canis* there was a moderate eosinophilia and this finding led to the search for alternative sources of IL-5 (Kusama *et al.*, 1995). To date, several alternative sources of IL-5 have been identified besides Th2 cells and these sources include tissue-resident basophils (i.e. mast cells) (Phillips *et al.*, 2003), airway epithelium (Salvi *et al.*, 1999) and even eosinophils themselves (Lamkhiioued *et al.*, 1995). Takamoto *et al.* (1995) even identified a population of non-CD4 and non-CD8 T cells, which in nude mice apparently produce IL-5 leading to eosinophilia following *T. canis* infection.

Asthma is another condition in which Th2 cytokines are increased, especially IL-13 (Grunig *et al.*, 1998; Wills-Karp *et al.*, 1998; Li *et al.*, 1999). Just as is seen in VLM, eosinophil and IgE parameters are greatly increased in asthma (see Chapter 4, this volume). Asthma is an inflammatory disease of the lower airways and, as such, the asthmatic lung is characterized as exhibiting airway hyper-responsiveness (AHR), excessive mucous secretion from hyperplastic goblet cells, airway remodelling – manifested as an increased amount of smooth muscle in the walls of the airways and blood vessels accompanied by the deposition of new collagen fibres within the basement membranes – and varying amounts of pulmonary oedema (Busse and Lemanske, 2001). The inflammatory component of bronchial asthma presents as eosinophil-rich exudates that frequently ‘cuff’ small- and medium-calibre blood vessels and bronchioles. Eosinophils also cross the respiratory epithelium and are seen in the airway lumen admixed with swirls of mucous. AHR and mucous secretion have been shown to be IL-13 dependent (Doucet *et al.*, 1998; Wills-Karp *et al.*, 1998; Corry, 1999).

In efforts to understand the pathogenesis of asthma, investigators have turned to a mouse model in which mice are sensitized by intraperitoneal injections of ovalbumin (OVA) and after several weeks, challenged by inhalation of OVA. There has been much work in this model attempting to define the role of the eosinophil in ‘mouse asthma’ but just as in VLM, eosinophil function remains elusive. To quote Marsha Wills-Karp on a role for eosinophils in asthma:

A role for eosinophils is plausible given their ability to secrete both cytotoxic (eosinophil peroxidase)

and bronchoactive (leukotrienes) mediators. Eosinophils can also present antigens and secrete Th2 cytokines perhaps amplifying or perpetuating Th2 inflammatory processes at sites of disease. Despite this promising repertoire of immunoregulatory and effector properties, pulmonary eosinophilia has often been separable from disease pathophysiology in mouse models of allergic asthma.

Wills-Karp and Karp, 2004

Even though there is no compelling set of observations indicating what exactly it is that eosinophils do in parasitic infections other than discriminate the infected from the uninfected animals, the question remains as to whether eosinophils do anything in either VLM (or any other invasive helminthic infection) or in asthma. Because both toxocariasis and asthma are characterized by Th2 cytokines it is reasonable to ask how much these two entities have in common. Yoshikawa *et al.* (1996) reported that rats infected with *T. canis* exhibited a lung that upon pathological and physiological examination resembled late-phase asthma. Histologically, these lungs had large numbers of eosinophils in the airways (confirmed by BAL). Additionally, eosinophils were found in inflammatory infiltrates forming ‘cuffs’ around airways and blood vessels. Evidence of pulmonary oedema formation was also noted. Consistent with these changes that resemble late-phase asthma, the response of lungs from infected rats as compared with uninfected rats challenged with acetylcholine required 18 times less drug (i.e. consistent with airway hyperresponsiveness) and, at the same time, the infected lungs exhibited a dynamic compliance that was 56% of control (i.e. infected lungs were stiffer than corresponding controls reflecting the onset of fibrosis). More recently, Hall *et al.* (1998) using an *in vivo* challenge of IL-5 knockout mice previously sensitized with *Brugia malayi* microfilariae concluded that in this model of an asthma-like component often seen in tropical pulmonary eosinophilia, IL-5 and/or eosinophils were mechanistically linked to the development of AHR based on the observation that tracheal rings from wild-type mice required less carbachol to stimulate smooth muscle contraction than rings from the IL-5^{-/-} mice. Other studies have consistently failed to show a mechanism by which eosinophils *per se* are responsible for AHR especially in the OVA mouse model of AHR (Kips *et al.*, 2000; Eum *et al.*, 2003). Lastly, it is important to understand that the mouse

eosinophil and human eosinophil have major differences. For example, mouse eosinophils, in contrast to human eosinophils, do not degranulate in response to cell-surface ligands (Persson and Erjefalt, 1999; Malm-Erjefalt *et al.*, 2001). Mouse eosinophils in contrast to human eosinophils do not express the low-affinity IgE receptor (CD23) (Jones *et al.*, 1994), nor do they express FcR α (Dombrowicz and Capron, 2001).

From the preceding discussions it is clear that there is much in common in rodent toxocariasis and rodent asthma but, as already stated, it is difficult to dissect eosinophil function cleanly from these two enigmatic model systems. One way to approach this conundrum is to use transgenic animal technology to produce mice that congenitally lack eosinophils. Recently, two laboratories have achieved this feat using two different molecular strategies. Lee *et al.* placed the *Diphtheria* toxin A chain under the control of the eosinophil peroxidase promoter (Lee *et al.*, 2004), while Humbles *et al.* deleted a GATA-1 binding site within the GATA-1 promoter sequence (Humbles *et al.*, 2004) to produce their eosinophil knockout mice. After verifying that the mice completely lacked eosinophils, both laboratories then used the OVA asthma model to evaluate the role of eosinophils in this paradigm. The GATA-1-targeted mice still exhibited AHR and exaggerated mucous secretion but there was an apparent amelioration of the smooth muscle mass increase. Surprisingly, transgenic mice made by targeting the peroxidase promoter did not develop AHR and exhibited less mucous hyperplasia than wild-type mice. Thus, even this state-of-the-art technique to ablate eosinophils failed to resolve the question of what eosinophils do in this asthma model. It should be noted that the GATA-1 mice were generated in a strain of mice different from the peroxidase mice (BALB/c vs C57BL/6 mice, respectively). As pointed out by Wills-Karp and Karp, IL-5-deficient mice produced on a BALB/c background are protected from developing AHR whereas similar mice produced on the B/6 background are not (Wills-Karp and Karp, 2004).

In summary, a variety of cells can produce IL-5, which invariably leads to eosinophilia, but the eosinophilia does not control *T. canis* infection. Interestingly, eosinophils have been shown to interfere significantly in other helminth infections in mouse models including *Nippostrongylus brasiliensis*

(Bozza *et al.*, 1997; Ovington and Behm, 1997; Dent *et al.*, 1999), *Strongyloides ratti* and *Heligmosomoides polygyrus* (Ovington and Behm, 1997). In contrast, eosinophils have no effect on either a primary or challenge infection of mice infected with *Trichinella spiralis* (Herndon and Kayes, 1992).

Eosinophils and Alternatively Activated Macrophages

The inflammatory response begins when the presence of an invasive pathogen is detected by cells whose primary role is to perform surveillance for such invaders. Because many of the potential pathogens are well known (i.e. they are the 'usual suspects'), these watchdog cells have on their surface receptors for recognition of common patterns or motifs such as the well-characterized Toll receptors, which recognize the endotoxin or lipopolysaccharide of Gram-negative bacteria. Engagement of these receptors leads to the elaboration of early response mediators or chemokines including TNF- α and IL-8 (Chensue *et al.*, 1994; Bittleman *et al.*, 1996; Proost *et al.*, 1996; Schluger and Rom, 1997). These molecules induce the expression of adhesion molecules on the luminal surface of the endothelial cells lining the capillaries perfusing the vicinity of the pathogen, which then coordinates the accumulation of specific types of inflammatory cells at this site. Once attached to the capillary wall, these cells diapedese into the extracellular matrix and then follow chemotactic gradients towards the pathogen focus. Thus, elaboration of TNF- α and IL-8 leads to an inflammatory infiltrate rich in neutrophils. In the past, inflammation was considered acute if it occurred in 24 h or less and consisted of (polymorphonuclear) neutrophils. Subacute inflammation was more than 24 h old and was a mixture of polymorphs and mononuclear cells (macrophages and lymphocytes), and chronic inflammation was seen after 48 h and consisted almost entirely of mononuclear cells. Helminth infections such as toxocariasis attract eosinophils during the subacute phase of inflammation that can and does persist well into the chronic phase. The Th2 signature cytokine, IL-5, and/or eotaxin(s) are responsible for converting the neutrophil-rich inflammatory infiltrate to one dominated by eosinophils. Ultimately, inflammation must re-

solve either by healing (return to normal), fibrotic scarring or encapsulation (i.e. in a granulomatous reaction). Several studies have linked transforming growth factor- β secreted by eosinophils with the subsequent stimulation of collagen secretion by tissue-resident fibroblasts (Ohno *et al.*, 1996; Minshall *et al.*, 1997). This may be relevant to the development of fibrotically encapsulated granulomas in VLM and the stiffening of the asthmatic lung, which is reflected in the measurement of dynamic compliance as noted above (Yoshikawa *et al.*, 1996).

Two major concerns in the understanding of the immunoregulation of helminth inflammation (and all inflammation, for that matter) are how does it get started and how does it end? The answer to the latter question in particular, is extremely important because a lingering chronic infection requires a significant caloric commitment on behalf of the host and it is in its best interest to either resolve the infection quickly, or come up with a less energy-intensive solution. Put another way, it may be in the host's best interest to adopt an anti-inflammatory response in order to minimize the amount of tissue damage that an all-out cell-mediated response can engender. Because most pathogens are either bacterial or viral, the prevailing immune response is usually a type 1 response characterized by the elaboration of IFN- γ and IL-2. Macrophages that encounter bacterial products such as lipopolysaccharide in the presence of IFN- γ go on to produce antimicrobial or microbiocidal products such as nitric oxide while at the same time ramping up their antigen-presenting capabilities (Noël *et al.*, 2004). In the case of tissue-invasive helminths such as *Toxocara*, where the prevailing cytokine in inflamed tissue is the Th2 signature cytokine, IL-4 macrophages of a different kind have been identified. Termed alternatively activated macrophages (Stein *et al.*, 1992) these cells express F4/80 (a macrophage surface marker molecule) and 10% of the mRNAs isolated from F4/80-positive cells were found to have encoded the YM-1 protein (Nair *et al.*, 2003). YM-1 is a member of the chitinase 18 family and in mice is expressed in alveolar macrophages, splenic neutrophils and bone marrow macrophages (Nio *et al.*, 2004).

In 1992, Fuhrman *et al.* reported that the *B. malayi* microfilarial antigen recognized by a monoclonal antibody produced some 8 years earlier had been successfully cloned and

sequenced. Comparative computer sequence analysis indicated significant homology with a chitinase enzyme. Levels of the functional enzyme in the host's bloodstream increased as the microfilariae matured and injection of the monoclonal antibody partially cleared the microfilariae. Use of the purified recombinant chitinase to immunize jirds induced partial protection against a *B. malayi* challenge infection but did not exhibit any effect on adult worms (Wang *et al.*, 1997). Fuhrman *et al.* also identified additional chitinases from a closely related filariid, *Brugia pahangi*, based on sequence homologies and noted that there were significant differences in the glycosylation patterns of each of the five chitinases now identified (Fuhrman *et al.*, 1995; Arnold *et al.*, 1996). Another similar chitinase activity has also been described from *Acanthocheilonema viteae* microfilariae (Adam *et al.*, 1996).

Peritoneal exudate cells (PECs; primarily macrophages) isolated from mice harbouring implanted *B. malayi* adult worms exert an antiproliferative effect on a cloned T-cell line stimulated with its cognate antigen. Implantation of a comparable number of dead worms did not induce antiproliferative PECs (Allen *et al.*, 1996). These results were subsequently extended to show that excretory-secretory (ES) products from just a single live *B. malayi* worm maintained in culture and then injected daily into mice for 2 weeks could stimulate development of PECs with comparable antiproliferative activity. ES material from two non-related nematodes, *N. brasiliensis* and *T. canis*, were equally efficacious at inducing suppressive PECs (Allen and MacDonald, 1998). Collectively, these results led to the suggestion that dead or dying worms *in vivo* led to the induction of a pro-inflammatory reaction while live worms (or the injection of their ES products on a daily basis) would lead to a skewing of the host response to the Th2 phenotype. The Th2 phenotype is now thought to consist of the signature cytokines (IL-4, -5 and -13) and the alternatively activated macrophages which exhibited the antiproliferative activity. It was further suggested that the antiproliferative activity was consistent with down-regulation of the inflammatory reaction to the implanted worms (Allen and Loke, 2001).

Subsequent studies to characterize the phenotype of alternatively activated macrophages found that 9% of the clones derived from a

cDNA library constructed from PECs expressed mRNA which encoded the protein YM-1 (also known as ECF/L; see below) (Falcone *et al.*, 2001). A well-characterized protein thought to be contained in *Brugia*-secreted ES antigens is a homologue of the human cytokine macrophage inhibition factor (*B. malayi* MIF) (Pastrana *et al.*, 1998). When this protein was injected into mice as a substitute for implanting worms as a method of generating alternatively activated macrophages, the macrophages that were recovered were found to contain a marked increase in the amount of YM-1 mRNA being expressed and attracted eosinophils *in vivo* (Falcone *et al.*, 2001). To determine whether the ability to stimulate alternatively activated macrophages, and by extension elicit a Th2 immune response, was a property of just parasitic helminths, a side-by-side comparison was made between extracts of *Brugia* and the free-living ascarid *Caenorhabditis elegans* to immunize mice for Th2 cytokine production. *C. elegans* extracts were almost as effective as the *Brugia* extracts. Because it is well known that nematode ES molecules are heavily glycosylated and to determine whether or not the carbohydrate moieties were important in the induction of Th2 responses, the extracts of both *Brugia* and *Caenorhabditis* were treated with sodium periodate (which breaks the sugar rings while leaving the protein backbones intact) and then used to immunize mice. Destruction of the glycans resulted in lymph node cells that in the presence of antigen could not produce the Th2 cytokine IL-4. These results led to the suggestion that the Th2 response typically observed in (invasive) helminthic infection are stereotypic (because both parasitic and non-parasitic worms could elicit them) and that pathogen pattern recognition motif receptors may recognize these periodate-sensitive helminth glycans (Tawill *et al.*, 2004).

Recently, Zhu *et al.* (2004) reported that a member of the family of chitinase enzymes, acidic mammalian chitinase (AMCase) was greatly elevated in the mouse model of asthma and in the lungs of human patients with asthma. AMCase activity was found to be IL-13 dependent, as IL-13 knockout mice were unable to produce the enzyme in the OVA mouse model of asthma. In experiments using either an antibody against AMCase or an inhibitor of the chitinase enzyme (allosamidin) there was a marked decrease in AMCase activity in the airway as measured in

the BAL fluid. Anti-AMCase treatment also led to a marked reduction of AHR in OVA-sensitized mice and it resulted in a markedly decreased inflammatory response including decreased numbers of lymphocytes and eosinophils. In histochemical studies, AMCase was localized in both alveolar macrophages and airway epithelium and a similar pattern was seen in human lung using *in situ* hybridization.

Owhashi and colleagues, using *T. canis*-infected mice, identified an eosinophil chemotactic factor made by T lymphocytes (ECF/L). Secretion of ECF/L into culture fluid was abolished by treatment with anti-Thy 1 and complement (anti-T lymphocyte) and anti-CD8 and complement, but not by treatment by anti-CD4 and complement. Because ECF/L activity did not support eosinophilopoiesis it was not considered to be the CD8-dependent form of IL-5 described by Takamoto and Sugane (1993). The factor activated marrow-derived but not peritoneal exudate eosinophils to migrate in a Boyden-type chemotactic assay, which suggested that PEC eosinophils were already activated. Thus, ECF/L was chemotactic and able to activate naive eosinophils (Owhashi *et al.*, 1998). ECF/L was subsequently cloned, sequenced and determined to be a member of the chitinase 18 family (Owhashi *et al.*, 2000). The molecule lacks chitinolytic activity due to two amino acid substitutions within the putative active site. In contrast, lung macrophage YM-1 isolated from three strains of mutant mice did have chitinase activity (Guo *et al.*, 2000). Additionally, the levels of YM-1 in these mice were high enough to favour the formation of a very characteristic crystal found in the airways and within alveolar macrophages.

In summary, the role of macrophages and chitinases in VLM is largely unexplored territory. Based on much of the work just described, it is obvious that there are two significant sources of the chitinases. One source is clearly of host origin and is a product of the professional phagocytic cells of the body; namely neutrophils, macrophages and CD8⁺ T cells. These host-derived chitinases have been described under the names of YM-1 and -2, ECF/L and AMCase and all are members of the family 18 chitinases. The other source is the worms themselves where it is thought that the chitinases are required for developmental processes such as larval moulting or eggshell casting. Both have been reported in mice and

humans and both have been implicated in helminth infection and in asthma and allergy. It is of interest that cytokine homologues secreted or elaborated by infective worms can stimulate host cells involved in the immunoinflammatory response to produce one or more mammalian chitinases which subsequently dampen down the inflammatory response by inhibiting cell proliferation by cells such as lymphocytes. The Th2 cytokines are still produced in this milieu. This is mutually beneficial for both host and parasite. For the parasite, it calls off the dogs of war and gives them a survival advantage. From the host perspective, it conserves valuable caloric currency that does not have to be expended against a parasite like *Toxocara*, which has been shown to be resistant to antibody and cellular attack (Badley *et al.*, 1987; Jones *et al.*, 1994). That link is that the chitinase described as ECF/L can attract eosinophils by directed chemotaxis. While this does not shed light on what it is exactly that

eosinophils do in either of these two conditions, which are both reflections of the Th2 immune response, it certainly opens new doors for experimentation. That chitinase is involved in toxocarasis is suggested in the ECF/L studies of Owhashi *et al.* (2000), Allen *et al.* (1996) and Allen and MacDonald (1998) where it was demonstrated that ES products from *Toxocara* elicit peritoneal exudate cells that inhibit T-cell proliferation and Zhu *et al.* (2004) showing that chitinase is important in rodent and human asthma. In closing, we began with a discussion of *Toxocara*, the enigmatic parasite, and end not with an enigma within an enigma but rather with one enigma, *Toxocara* meeting head on, another enigma, the eosinophil, which may be drawn to the honey (ES glycans) released while the parasite migrates through the host's tissues. Alternatively activated macrophages and chitinases somehow inexorably link these two enigmas to one another.

Dedication

This chapter is dedicated to the memory of George L. Freeman, Jr., parasitologist and friend.

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12 Novel Approaches to Immunoprophylaxis for Toxocariasis

Gabriela Hrčková

Parasitological Institute, Slovak Academy of Sciences, Kosice, Slovak Republic

Introduction

One of immunology's greatest practical successes is vaccination. Mass immunization programmes have led to the virtual eradication of several diseases that used to be associated with significant mortality and morbidity. In addition to viral, bacterial and several protozoal infections, where safe and effective vaccines are widely used, perhaps the most progress in this field was achieved in the characterization of protective antigens against taeniid cestodes. Thus, highly effective recombinant vaccines have been developed against *Taenia ovis*, *Taenia saginata* and *Echinococcus granulosus* (Lightowlers *et al.*, 2003a) and several candidate vaccine molecules have also been isolated from gastrointestinal nematodes (Knox *et al.*, 2003).

Much less information is available about *Toxocara* sp. molecules that can mount sufficient protective immunity in definitive canid hosts against challenge infection, probably due to the availability of effective anthelmintics. A major challenge to be faced will be finding a safe, inexpensive and simple method for vaccination of bitches, the result of which should be sustained killing of the reservoir larvae in the tissues or of newly acquired parasites. As toxocariasis is now recognized worldwide as a human infection (Schantz, 1989), much attention has been paid to the investigation of immune defence mechanisms in humans. Clearly, vaccination of humans against toxocariasis on a large scale is unlikely to be warranted, as the disease incidence in indus-

trial countries is low (Maizels *et al.*, 2000). In humans, prophylaxis should therefore be focused on the prevention of ingestion of infective eggs. Once the disease has been diagnosed, elimination of infection (mainly in the chronic stages) is still relevant (Pawlowski, 2001), and research on new approaches to therapy using carriers, adjuvants and immunomodulators is important. Recent data will be summarized in this chapter.

Implications for Vaccine Development

The following major steps were shown to be successful in the development of vaccines against several metazoan parasites; these include: the identification and purification of antigens which induce protective immune response during natural infections, stimulation of appropriate immune response by means of vaccination (containing either single or multiple antigens) which would lead to the rejection of the parasites and the production of protective antigen(s) in recombinant form for large-scale production.

There are some specifics which apply to the development of vaccines against parasitic nematodes. Helminths have developed unique immune evasion strategies, which include sequential antigenic changes during development, immune suppression and shedding of antibody-bound surface coats (Maizels *et al.*, 1993) (see Chapters 1 and 3, this volume). Both the complexity of nematode parasites and their distinct evasion

mechanisms have probably contributed to the limited success of vaccination using partially separated fractions of parasites. This may be due either to the scarcity of the protective antigens within the complex mix of molecules or to the presence of immunomodulatory molecules within the extracts that may suppress protective immune responses (Meeusen, 1996).

Toxocara larvae excrete and/or secrete antigenic substances (*Toxocara* excretory–secretory antigen, TES) in *in vitro* culture medium (De Savigny, 1975), which are used in ELISA and Western blot (WB) analysis with sera of infected hosts. These sera (obtained either from patients with visceral larva migrans, or from experimental animals at the stage of their developmental arrest in the tissue phase (muscles, brain)), revealed several bands among which seven were the most prominent in all sera (MagnaVal *et al.*, 1991; Maizels *et al.*, 2000). Biochemical analysis of ES products of larvae using SDS-PAGE revealed at least 15 bands between 29 and 94 kDa by silver staining and four to six others around 200 kDa by immunoperoxidase staining (Badley *et al.*, 1987b). Similarly, Akao *et al.*, (1983) described 20 different antigens using a silver stain and eight distinct bands with Coomassie blue. Antibody responses to TES appeared as early as 1 week after infection and persisted for the dur-

ation of the study (Bowman *et al.*, 1987). The array of molecules expressed by larvae within the early phase of their somatic migration (up to 7–14 days post-infection (p.i.)) and recognized by the specific IgM and IgG antibodies was not identical to that seen during the late stage of infection. This was demonstrated in our experiments on mice infected with 1000 eggs (Fig. 12.1), as we found that 132 and 200 kDa antigens (TES) were seen by corresponding IgG antibodies from day 7 p.i. and were expressed within the entire experimental period (14 weeks p.i.). These antigens also produced strong bands with positive human sera (MagnaVal *et al.*, 1991). IgG antibodies to more-specific larval antigens with molecular weights (MW) of 26, 28, 35 and 70 kDa appeared on WB from week 3 p.i.

In rabbits infected with *Toxocara canis* infective eggs, Akao *et al.* (1983) reported in their early work that high MW substances reacted mainly with the serum from rabbits after 2 weeks of infection and low MW molecules reacted with the serum after 26 weeks p.i. Using the same experimental model system, Morales *et al.* (2002) showed that the antigen–antibody pattern visible on WB correlated with the stage of infection and that two antigen bands of 35 and 92 kDa were identified from the beginning (from week 2 p.i.) and throughout the course of infection. Such differential expression

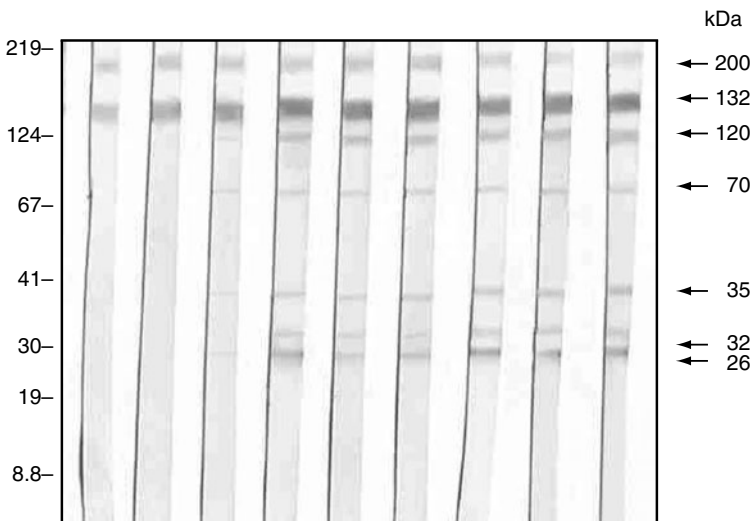


Fig. 12.1. Time course of IgG antibody pattern to *Toxocara canis* excretory–secretory (TES) antigens monitored within 14 weeks p.i. Sera were from mice infected with 1000 eggs and blots correspond to weeks 1, 2, 3, 4, 5, 6, 8, 10 and 14 p.i. Marker, Kaleidoscope prestained standards (Bio-Rad).

of certain antigens is probably related to the induction of a given immunological phenotype by *Toxocara* larvae. Morales *et al.* (2002) proposed that the low molecular weight fractions are more specific to the genus *Toxocara* than those of high MW. Similarly, the stage-specific antigenic variations were described in a related nematode, *Ascaris suum* (Jungersen *et al.*, 2001).

The specific IgM response to *T. canis* TES antigens is evident as early as the first week of infection in parallel with the highest amount of circulating antigen employing the direct ELISA assay. The antigen-specific IgG response appears later, from the second week of infection in mice (Bowman *et al.*, 1987). These authors suggested that distinct antibody specificities are under temporal regulation of certain antigenic epitopes. In agreement with this, the extensive studies conducted on various models infected with taeniid cestodes generated evidence that the host-protective immunity is associated with the early stages of parasites (Lightowlers *et al.*, 2003b). For example, the high expression of protecting antigens in extracts of *T. ovis* unhatched eggs and in ES products of activated oncospheres was demonstrated in the vaccination trials on lambs, which showed approximately 90% protection from an oral challenge infection (Osborn *et al.*, 1981). Therefore, the strategy for *Toxocara* vaccine development could be focused on identification of natural antigens that are presented to the host's immune system during natural or experimental infection mainly/selectively at the early stage of infection.

***Toxocara* Vaccine Candidate Molecules**

In the immunization studies carried out on experimental paratenic hosts, the biological activity of either *Toxocara* antigens (TES), whole larval somatic antigens or infective irradiated eggs has been investigated. Abo-Shehada *et al.* (1991) examined the effects of four *T. canis* antigenic mixtures, given intraperitoneally (i.p.) in either distilled water or physiological saline, against a challenge infection with 1000 infective *T. canis* eggs in mice. The UV-irradiated egg antigen gave the best protection with approximately 40% reduction of larvae and a significant immune response was mounted. Fewer larvae established in the musculature and brain and

more were retained in the liver. Administration of one dose of TES vaccine (8 mg protein) permitted some small degree of protection, but adult female worm antigen and whole second-stage (L2) somatic antigen gave no protection. Similarly, soluble extracts of female or male adults, or from embryonated eggs of *T. canis*, given to mice in two doses without adjuvant, was only slightly protective against infection with 2000 eggs (Concepcion and Barriga, 1985). Irradiation of eggs was shown to retain the antigenic properties of molecules, whereas it abolished or decreased infectivity of *T. canis* eggs containing second-stage larvae. It is known that irradiation can interfere with the synthesis of proteins and the function of enzymes. Regarding the type of irradiation which was used for *Toxocara* vaccine preparation, Kamiya *et al.* (1987) found that eggs irradiated with gamma rays (1 Mrad) lost their infectivity nearly completely as only a few larvae were recovered from the digestive tract of inoculated mice and no visceral larval migration was observed. On the other hand, irradiation of infective *T. canis* larvae with X-rays reduced pathogenicity but did not completely prevent their migration (Barriga and Myser, 1987).

The effect of either soluble or particulate fractions from embryonated *T. canis* eggs was studied in mice and these antigenic fractions were given in either complete Freund's adjuvant (CFA) or *Escherichia coli* lipopolysaccharide (LPS). Whereas injection of soluble extract alone reduced the parasitic load of mice by 37%, the soluble extract with LPS reduced it by 76% and the particulate extract with CFA increased larval number by 60% in comparison with non-immunized mice (Barriga, 1988). The last result indicates that some insoluble parasite antigens might suppress the host's immunity.

In the experimental mouse model, Dvorožňáková *et al.* (2002) recorded moderate protective effects of TES antigens (40.5%) given i.p. in incomplete Freund's adjuvant (IFA) against infection with 1000 infective *T. canis* eggs. They also showed that soluble somatic antigen from L2 larvae decreased the number of larvae (30%) in challenged mice. When mice were immunized with TES antigen in CFA by three subcutaneous injections, larval numbers in infected (challenged) interleukin (IL)-5 transgenic or normal mice decreased by approximately 70% (Sugane *et al.*, 1996). The same high protection towards infection in mice hyperimmunized with TES antigens given in CFA was noted by Nicholas *et al.* (1984). The higher protection achieved with TES anti-

gens in comparison with somatic antigens could be partially associated with *Toxocara* polyprotein allergen (TBA-1), responsible for type-1 hypersensitivity. Kennedy *et al.* (1989) found this antigen in *T. canis* worm body extracts but not in TES, and the IgG response was only transient in nature. Regarding the recorded differences in immunoprotection in the above studies, we assume that the route of vaccine administration together with adjuvant type could partially account for observed variations in protection. Also, in immunization experiments with *Toxocara vitulorum* in mice, soluble antigens from infective larvae or their TES products, but also antigens from the adult parasites given in CFA, induced statistically significant protection against infection (Amerasinghe *et al.*, 1992). Regarding *T. canis* larvae, according to Maizels *et al.* (2000), the primary candidates for vaccine antigens may well be those associated with the surface and the secretions, such as the C-type lectins TES-32 and TES-70. Data from studies with various nematode species indicate that proteolytic enzymes (proteases) and the host's protease inhibitors could be promising molecule candidates for vaccination. Multiple enzyme activities were demonstrated in TES products of *T. canis* by substrate gel electrophoresis, and were associated with proteins of molecular weights of 120 and 32 kDa. The majority of enzyme activity was consistent with that of a serine protease (Robertson *et al.*, 1989).

Taken together, these findings suggest that protective vaccine molecules might be present equally in either mature eggs containing infective larvae, larval TES antigens or could be a part of larval somatic proteins, so-called 'hidden antigens' (Munn, 1997). It is noteworthy that the dose of antigens and adjuvant used are the important factors for inducing efficient immunological memory. Although the characterization of *Toxocara* native molecules and their recombinant analogues is in progress, the effect of no single candidate molecule has yet been elucidated in vaccination trials *in vivo*.

Increased Resistance to Re-infection after Repeated Infections in Paratenic Hosts

Many immunological studies have revealed that a primary infection induces protection against re-infection and that the expression pattern of larval

antigens in challenged mice changed. Lee (1960) reported that partial resistance to *T. canis* re-infection resulted in 20% fewer larvae in mice, 8 weeks after the initial infection with 2000 embryonated eggs, given in a single or in fractionated doses. Kondo *et al.* (1976) found that infection with 500 embryonated eggs divided into five or ten administrations reduced significantly the number of liver larvae resulting from a later challenge with 1000 embryonated eggs. Barriga (1988) proposed that this schedule of immunization produced enough immune resistance to kill many of the liver larvae rather than simply inhibiting their migration. Schumunis and Pacheco (1976) showed that only infection that lasted more than 30 days produced protection. In addition, it seems that one *T. canis* infection appeared to produce immunosuppression, whereas two or three seemed to cause immunoprotection. Similarly in rabbits, repeated infection 45 days later after the first also induced significant protection (Barriga and Carrillo, 1987). Barriga and Omar (1992) studied immunity to *T. vitulorum*, the buffalo-specific parasite, after repeated infections in a rabbit model. First and secondary re-infection increased the expulsion rate of parasites from the gut six or nine times, indicating that the protection to re-infection is induced at the intestinal level by inhibiting hatching and invasion of the gut tissues rather than by killing the parasites. However, some larvae, which successfully penetrated the gut wall, stimulated humoral immunity detectable on WB. Interestingly, the total number of antigens seen by IgG antibodies increased with time and the number of infections, but some disappeared, whereas new antigens appeared in the course of the observations. These authors suggested that four antigens (32–41 kDa MW) may be related to the protection. Similar experiments with *T. vitulorum*-repeated infection on mice confirmed the induction of significant protection against challenge infection (Amerasinghe *et al.*, 1992).

Primary Site for Inducing Protective Immunity

After an oral infection of embryonated eggs, which hatch in the small intestine of paratenic hosts and dogs, the released larvae start to

penetrate the gut wall (Sprent, 1952, 1958). This process of hatching and penetration in a primary infection usually lasts a few hours and, during this period, the local non-specific immune response is stimulated. As mucosal immunity represents the first barrier for larvae, its sensitization with appropriate antigens is expected to prevent/decrease migration to other soft tissues of the host, such as liver and lungs. Abo-Shehada *et al.* (1991) examined the differences in larval migration of *T. canis* through the gut wall in immunized and non-immunized mice. They found that the penetration of larvae was reduced significantly in mice immunized with *T. canis* antigens, the greatest effects being seen after administration of a vaccine containing TES antigens. When the ileum was examined histologically in previously sensitized or vaccinated animals, 5 and 9 h p.i., lymphatic infiltrations were seen around the penetrating larvae, forming early stage granulomata in the vaccinated mice, but not in the control mice, although some larvae were seen migrating away from such reactions (Abo-Shehada *et al.*, 1991). The liver is also recognized as an important site for controlling the migration of *T. canis* larvae (Glickman and Schantz, 1981; Grieve *et al.*, 1993), with peak numbers of larvae being usually found within 2 days following infection (Abo-Shehada and Herbert, 1984). Similar migratory behaviour and the highest accumulation in the liver on day 2 and in the lungs on day 5 p.i. was documented in mice experimentally infected with the closely related *Toxocara cati* (Hřčková *et al.*, 2000). The higher liver trapping of larvae in mice, which had been previously sensitized in a primary infection, was observed after their re-infection (Akao, 1985; Parsons and Grieve, 1990). The result of such increased trapping was the profound reduction of larval counts in the muscle and brain. Granulomatous infiltrates were seen around the larvae in the liver of mice with the primary infection (Parsons *et al.*, 1986) and also after the challenge infection of mice previously vaccinated/infected (Abo-Shehada *et al.*, 1991). Abo-Shehada and colleagues found that, whereas in non-sensitized mice larvae appeared to be viable and migrated out of the liver, in primed mice killing of the trapped larvae occurred. Data imply that stimulation of cytotoxic cellular and humoral immune responses in the liver after sensitization can lead to the damage/rejection of trapped larvae. According to Grieve *et al.* (1993), CD4 cells

but not CD8 cells participate significantly in liver trapping but they represent only one component of this complex effector system.

Opinions therefore vary as to the principal site of induction of protective immunity against larvae in immunized animals. Some authors consider the liver, while others consider the intestinal wall to be of greater importance, but both seem to be important due to the specific migratory behaviour of larvae. Based on the present level of information from vaccination experiments on laboratory models it is difficult to assign the main role to either site. Strong inflammatory reactions around the penetrating larvae in the intestine occurring only in the sensitized and vaccinated mice on one hand, and significant reduction of larvae in the liver of challenged/immunized mice on the other hand, indicate that the induction of protective immunity to *T. canis* infection is a very complex immunological process.

Cellular Immune Responses in Infected Mice

Antibody-dependent cellular cytotoxicity (ADCC), opsonization or complement-fixation antibodies are obvious immune effector mechanisms that could be active against metazoan parasites. ADCC reactions involve stimulation of non-antigen-specific and Fc receptor-bearing cell types (neutrophils, eosinophils and macrophages) which can damage worms in collaboration with various antibody isotypes, cytokines and pharmacologically active and parasite-damaging molecules (Joseph, 1982; Mitchell, 1991). Infection with *T. canis* larvae induces adaptive immunity in their hosts, characterized by high and persisting eosinophilia, tissue mastocytosis, production of cytokines typical for a Th-2-type immune response (IL-4, IL-5, IL-10) and increased IgE antibody titres (Kayes, 1997) (see Chapters 4 and 11, this volume). A result of this type of immune response is the establishment of long-lasting parasitization of larvae in the muscles and brain (arrested stage) with only partial elimination of larvae. Although the adherence of effector cells to the larval surfaces of *T. canis* in the presence or absence of immune serum has been demonstrated *in vitro*, there was no evidence of damage to the worms (Fattah *et al.*, 1986; Lombardi *et al.*, 1990).

Histological observations of eosinophil-rich tissues from experimentally infected mice confirmed that *T. canis* larvae are neither advantaged nor disadvantaged by high eosinophilia (Badley *et al.*, 1987a). A similar conclusion was suggested by Dent *et al.* (1999) who assessed IL-5 transgenic mice with life-long eosinophilia for their resistance to primary infection with *T. canis*. Thus, the role of eosinophils in the resistance to incoming and migrating *Toxocara* larvae *in vivo* is less clear and is relatively minor (Dent *et al.*, 1999; Meeusen and Balic, 2000). Moreover, the overall immunopathology indicates that ADCC directed against the incoming larvae and secreted mixture of antigens in non-sensitized hosts is hardly protective. As immunization with various antigenic preparations or irradiated infective eggs has proved to induce a partial or even high degree of protection to the challenge infection, the very low resistance to primary infection is probably connected with live larvae and some of their products. Many studies on nematode immunity have shown that Th2-type immunity is the prominent feature of the protective immune response in most gastrointestinal nematode infections (Meeusen, 1996). In cases of immune responses to *Toxocara* infection after vaccination trials, information is very limited and determination of the role of Th1- versus Th2-type responses awaits further study.

Humoral Immune Response and Antibody Isotypes

In mice, different cytokines preferentially induce switching to different antibody isotypes: IL-4 induces switching to IgG1 and IgE, and transforming growth factor (TGF)- β induces switching to IgG2b (Th2-type) and IgA. Although inflammatory CD4⁺ cells are poor initiators of antibody responses, they do participate in isotype switching by releasing interferon (IFN)- γ , which preferentially induces switching to IgG3 and IgG2a (Th1 response). In addition, phagocytes are activated by IgG antibodies, especially IgG1 and IgG2 types, for their cytotoxic effects (Janeway and Travers, 1994). Following infection with *Toxocara* larvae, a strong antibody response is mounted, but these antibodies are hardly protective for the host in primary infection. It has been suggested by many authors that larval products stimulate synthesis of ineffective antibodies.

IgG is the dominant antibody isotype in toxocariasis, but has not proved to be protective/cytophilic in primary infections. Higher IgG1 levels were detected in serum of mice infected with *T. canis* than in uninfected controls (Cuellar *et al.*, 2001; Fan *et al.*, 2003). Also, in patients with toxocariasis, IgG1 antibodies to TES antigens were the predominant IgG subclass (Obwaller *et al.*, 1998). The IgG1 subclass is considered in mice to be the second class of anaphylactic antibodies (eosinophil-dependent), able to induce histamine release from the mast cells. On the other hand, no significant difference in titres of IgG3 and IgG2a antibody, indicators of Th1-type responses, was seen between uninfected and infected groups of mice (Fan *et al.*, 2003; Hřčková personal observations).

The preferential stimulation of IgE and IgG1 antibodies in mice following primary infection is in agreement with the findings of other authors, who provided evidence that natural infection with *T. canis* eggs induces predominantly a Th2-type response. However, in challenged and re-infected mice, the high liver trapping of larvae followed by their degradation was, according to Akao (1985), mediated by IgG antibody (of unknown isotype) directed against some particular components of TES antigens as a result of sensitization of a specific pool of B cells. Immunization of mice with either one or two doses of TES antigens in IFA (i.p.) (Dvorožňáková *et al.*, 2000) led to much lower levels of IgG1 and IgG2 antibodies and reduction of larval counts than did infection only. As these subtypes are characteristic of a Th2 response, this could imply that some protection following TES vaccination was associated with a change in the ratio of Th1/Th2-type of antibodies towards a diminished Th2-type as opposed to the effects of live larvae. The effect on Th1-directed antibodies was not investigated.

High levels of non-specific IgE antibodies have been described in several experimental and natural nematode infections. For example, in humans, total serum IgE levels were significantly increased, whereas *Toxocara*-specific IgE was not detected or was at a very low level (Bass *et al.*, 1983; Uhlíkova *et al.*, 1996). A high proportion of elevated IgE levels in *T. canis*-infected mice were found to be antigen-unspecific as they were able to reduce *Trichinella spiralis*-specific IgE titres using an ELISA test, probably due to cross-reactivity (Takamoto *et al.*, 2001). The low levels of antigen-specific IgE in primary *Toxocara* infection in mice

might explain the low cytotoxic capacity of tissue eosinophils to the larvae. Thus, it is possible that either re-infection or immunization could profoundly change the ratio of these antibodies towards the antigen-specific IgE. The increased level of protection to a challenge infection, seen as a reduced number of *T. canis* larvae, was also demonstrated after passive transfer of immune serum or mesenteric lymph node cells from twice-infected mice (Concepcion and Barriga, 1985).

The indirect evidence that immunization can specifically activate this effector arm of Th2-type immunity could be derived from the study of Sugane and Oshima (1984), who observed that in immunized mice, among ES products of *Toxocara* larvae, antigens with low molecular weight were able to induce a much greater eosinophilic response (associated probably with the high levels of antigen-specific IgE antibodies) than did other antigens. Levels of antigen-specific IgE antibodies correlated with the degree of protection. The role of IgE in protective immunity was also demonstrated in other nematode infections: *T. spiralis* (Dessein *et al.*, 1981) and *Nippostrongylus brasiliensis* (Jarrett *et al.*, 1976). A study of the time course of production of nematode-specific versus non-specific IgE showed that, after re-infection, most of the total IgE response was associated with the parasite-specific IgE, implying that parasite-specific B and T cells on re-stimulation with worm antigens produced factors which stimulated parasite-specific responses (Kayes and Oaks, 1980). The main role of 'activated' eosinophils in rejection of nematode *Haemonchus* larvae in immunized sheep was also proposed by Meeusen and Balic (2000). From the present limited information, it is difficult to be certain about the participation of *Toxocara* antigen-specific IgE antibodies in vaccinated and challenged animals. We can only hypothesize that, in the high expulsion rates, believed to be due to a strong inflammation in the gut of mice immunized with *T. canis* egg antigens or TES antigens (described above), the 'activated' eosinophils and specific IgE antibodies produced by memory B cells could also have contributed. Thus, vaccination or even re-infection of paratenic hosts definitively leads to the production of cytotoxic antibodies and can also modulate the proportion of individual isotypes.

So the rationale for *Toxocara* vaccine research would be the identification of natural antigens which can trigger cytotoxic antibodies as a part of the ADCC response leading to either early

expulsion of *Toxocara* larvae from the gut of infected dogs or their rapid killing in the liver, thus preventing their further somatic migration.

T-Cell Subtypes and Cytokine Responses

The Th1 subset of T helper cells produces IL-2 and IFN- γ , which mediate delayed-type hypersensitivity reactions and production of non-reaginic antibodies. Th2 cells produce IL-4, IL-5 and IL-10 cytokines (Janeway and Travers, 1994). Del Prete *et al.* (1991) showed that the T-cell clones from healthy humans challenged with TES antigens *in vitro* exhibited a Th-2 like profile of cytokine production, and all TES-specific Th2 clones had no cytolytic activity. The authors suggested that the presence or absence of IL-4 or IFN- γ at the time of antigen recognition may have remarkable regulatory effects on the *in vitro* development of human CD4⁺ T cells into Th1 or Th2 clones. In contrast, Inuo *et al.* (1995) found that the cultivation of human peripheral blood mononuclear cells (PBMCs) from healthy donors with *T. canis* adult worm antigen resulted in the significant elevation of IL-2, IL-3, IL-4, IL-5 and IFN- γ expression, indicating that this antigen does not provoke obvious preferential Th2 activation but rather both Th-1- and Th-2-type responses. In their study, the proliferative response of PBMCs was greatly enhanced in a dose-dependent manner *in vitro*.

Similar stimulation of the proliferative response of spleen T and B cells from mice vaccinated with two doses of larval TES antigen in IFA along with significantly elevated amounts of IFN- γ cytokine after challenge infection in comparison with non-vaccinated animals was demonstrated by Dvorožňáková *et al.* (2002). Somatic larval antigens used in vaccination had a reduced effect on the production of this cytokine. In addition, the same authors showed that IL-5, the prominent Th2-type cytokine involved in eosinophilia, was also significantly enhanced after immunization with either antigen in comparison with non-vaccinated infected mice, and more after somatic antigens. As mixed stimulation of both Th1- and Th2-type responses was seen in all these studies, we could hypothesize that each of these antigenic mixtures could contain separate groups of

molecules with opposite biological activity, inducing either Th1 or Th2. Also the other possibility exists that the protective antigen(s) presented in these vaccines triggered the concomitant production of both Th1- and Th2-type cytokines, the response which is characteristic for the Th0 subset of CD4 T cells (Street and Mosmann, 1991; Cameron *et al.*, 2003). These findings give rise to the question of which type of host-protective immune mechanism should be stimulated with the potential vaccine against challenge *Toxocara* infections, i.e. whether it should be exclusively Th1-type or mixed Th1/Th2?

To answer this, the biological activity of single protein molecule(s), native and recombinant, should be examined *in vitro* and *in vivo*. In this respect, several antigens as vaccine candidates from other ascarid nematodes have been characterized and their protective effects examined. For example, in immunization trials using antigens derived from larval stages of the related nematode *A. suum*, the protective 16 kDa antigen was identified. Mice vaccinated with the recombinant antigen generated high specific IgG and IgE antibody responses and evoked a mixed (both Th1 and Th2) type of immune response characterized by elevated IFN- γ and IL-10 (Tsuji *et al.*, 2004). So it seems that, at least in case of this experimental system, activation of mucosal-associated immunity (Th2) and the antigen-specific antibody-mediated cytotoxic immunity (Th1) could be more effective than one type alone. The rationale for stimulation of both types of CD4 plus helper cells in protection for *Toxocara* infection could be explained in the light of interesting findings discovered by cellular immunologists. Daynes *et al.* (1990) have established that T cells in mucosal lymph nodes have a greater propensity to produce Th2-type cytokines, whereas peripheral lymph node T cells produce exclusively Th1-type cytokines after polyclonal or antigen specific stimulation. In addition, it was found that antigen-specific memory T cells (Th1 and Th2) follow a different migratory pathway, which is independent of the site of immunization (Premier *et al.*, 1996), so whereas proliferative memory T cells would migrate mainly into peripheral nodes, the Th2 helper cells migrate to mucosal lymph nodes, where they initiate Th2-type responses. Consequently, vaccine components (antigens and adjuvant) able to induce synergistically strong Th1 and Th2 response would ensure larval rejection by the mucosal immunity in

the gut and kill those larvae which migrated out to the liver.

The complexity of host-parasite interactions indicates that a better understanding of the rejection process could lead to a novel approach for preparation of *Toxocara* vaccines.

A new approach for the identification of separated natural (stage-specific) protective antigens employs antibody-secreting cell (ASC) probes (Meeusen and Piedrafita, 2003). It was based on the observation that B cells are rapidly recruited to the site of infection with a pathogen and to the local draining lymph node before they migrate into target tissue and differentiate into ASCs. It was therefore assumed that antibodies secreted by parasite-activated B cells are likely to recognize antigens important in the rejection of the parasite by the host. ASCs from immune animals, harvested during stimulation by parasites from lymph nodes draining the site of infection, recognized a much limited and different group of antigens than did serum antibodies. In addition, different antibody isotypes were induced in different tissue compartments such as liver, lung or GI tract. Restricted antibody profiles identified antigens which were unique to the susceptible larval stages of the parasites. This approach was successfully applied in the identification of protective (larval-specific) antigens of the gut nematode *Haemonchus contortus* (Bowles *et al.*, 1995).

Effect of Adjuvants for *Toxocara* Antigens on Th1/Th2 Responses

In the longer term, it would be preferable to have parasite vaccines based on non-infectious parasite molecules that can be produced in commercial quantities by recombinant or synthetic means. Compared with attenuated live vaccines, subunit vaccines are safer as they contain only a small number of defined antigens but are often poorly immunogenic. This limitation could be balanced by combination with specific adjuvants. By definition, adjuvants are any material which can enhance the immune response when administered with antigen. Adjuvants can be broadly divided into two classes, based on their principal mechanism of action: vaccine delivery systems and 'immunostimulatory adjuvants'. This topic has received much attention in the last decade and

has been reviewed by several authors (Aucouturier *et al.*, 2001; Singh and O'Hagan, 2003). Generally, vaccine delivery systems are particulate, e.g. emulsions, microparticles, immunostimulating complexes, QuilA, liposomes, and their function is to target antigens into antigen-presenting cells (APC) and/or slow down release of antigen into the body. In contrast, immunomodulatory adjuvants often represent pathogen-associated molecular patterns (PAMPs), e.g. LPS, which can activate cells of the innate immune system. As yet, no PAMPs have been identified for any of the veterinary or human helminth parasites (Meeusen and Piedrafita, 2003).

In all published experimental vaccination studies with various preparations of *Toxocara* antigens mentioned above, the classical adjuvants used were CFA (oil with inactivated mycobacteria), IFA (an oil alone) or LPS as an immunomodulatory substance. In general, the highest degree of protection to re-infection (approximately 70%) was observed in mice immunized with TES larval antigens when vaccine contained CFA (Nicholas *et al.*, 1984; Sugane *et al.*, 1996) in contrast to a study in which the insoluble egg fraction in CFA increased parasite burden in immunized mice (Barriga, 1988). TES antigen given in IFA (Dvorožňáková *et al.*, 2002) was less potent and reduced the number of *T. canis* larvae by 40.5%. On the basis of information discussed in previous parts of this chapter, differences in the observed level of protection are associated with adjuvant type and particulate type of immune response evoked by either CFA or IFA. In the work of Yip *et al.* (1999), the effect of both adjuvants with different protein antigens on Th1/Th2-type immunity was examined in a total of six mouse strains. Interestingly, both adjuvants induced pools of antigen-specific memory T cells of comparable specificity, but CFA induced a Th1-type immune response, and cytokines and IFA stimulated a Th2-type response. From limited information from *Toxocara* immunization experiments in mice and rabbits using CFA and IFA it is difficult to draw a conclusion as to which type of adjuvant-guided induction of Th response (Th1 or Th2) the potential anti-*Toxocara* vaccine should generate.

It seems that the combination of antigens and adjuvants which would result in stimulation of a mixed-type Th immune response could form the rational basis for immunoprophylaxis of toxocariasis. It was mentioned previously that

Th2 cells and cytokines are a prominent feature of the protective immune response in many GI nematode infections. As the GI tract is the first niche for *Toxocara* larvae, activation of Th2-type responses also seems to be important for inducing anti-*Toxocara* resistance in successful vaccination procedures. For example, in the case of the gut nematode *H. contortus*, immunization with ES antigen in aluminium adjuvant Al(OH)₃, which is a strong inducer of the Th2-type response, produced significant protection against challenge infection and a reduced egg output of 89% (Vervelde *et al.*, 2003).

It should be also taken into consideration that the immune response induced with a particular antigen may vary with the adjuvants used, as was demonstrated, for example, in the work of Geldhof *et al.* (2004). They found that intramuscular immunization of calves with ES antigen with cysteine proteinase activity from the nematode *Ostertagia ostertagi*, given in QuilA adjuvant (Th1-type inducer) reduced egg output and worm counts significantly, but the same antigen in Al(OH)₃-based vaccine yielded no protection. Therefore, further investigations are necessary to elucidate the biological roles played by native or recombinant proteins alone in combinations with an adjuvant of choice in the prevention of toxocariasis.

Immunostimulatory Adjuvants

Immunostimulatory adjuvants need not be administered together with the antigen to enhance immunity. They are generally given to induce a non-antigen-specific enhancement of the immune system and, in combination with antigen, these adjuvants can potentiate antigen-specific immune responses. For example, immunization with LPS-based vaccine, containing soluble antigens from *T. canis* infective eggs, had a similar protective effect (76%) in challenged mice (Barriga, 1988) to larval TES antigens administered with CFA (Nicholas *et al.*, 1984). There are other substances with proven immunostimulatory activity that could be used as the anti-*Toxocara* vaccine component, such as glucan and muramyl dipeptide (MDP). MDP is an essential structure of Gram-positive bacteria and its synthetic analogues were shown to enhance non-specific immunity. In mice infected with 2500 *T. canis* eggs, administration of

MDP restored and significantly stimulated cellular immunity, elevated antibody production and reduced the counts of migrating *T. canis* larvae in the mice by 30% (Dvorožňáková *et al.*, 1999). β -Glucans, the polysaccharides found in cell walls of fungi and yeast, are able to activate the innate immune system (reviewed by Bohn and BeMiller, 1995) and were shown to possess anti-tumour as well as anti-infective activities (see review by Kogan, 2000).

The effect of soluble glucans on several experimental helminth infections has been studied in our laboratory for several years. Thus, in mice infected with 2500 embryonated *T. canis* eggs, two intramuscular doses of glucan (0.5 mg/kg b/w) resulted in partial restoration of blastogenesis in T and B lymphocytes suppressed by infection. Moreover, a significant reduction of larval numbers in brains and muscles was observed (Šoltýs *et al.*, 1996). However, data are lacking on the effect of MDP or glucan as components of anti-*Toxocara* vaccine on the degree of protection and immunity in paratenic hosts.

New Approaches to the Control of Toxocariasis

Human infections are usually classified into four major clinical forms, namely: systemic, characterized as visceral larva migrans syndrome (VLM), compartmentalized (ocular and neurological toxocariasis), covert and asymptomatic (Pawlowski, 2001). Manifestations of disease and clinical course are closely associated with the number of infective eggs and localization of larvae and depend upon the immunological responses of the host. As human toxocariasis is a chronic infection, which often lasts several years, and re-activation of circulation of dormant larvae may occur, effective treatment is the choice for control of human disease (see Chapter 8, this volume).

Among anthelmintic drugs recommended for treating human toxocariasis, a few are potentially efficient: benzimidazoles (albendazole, fenbendazole and mebendazole) and diethylcarbamazine (DEC). Therapy with DEC is accepted as one of the most effective (WHO, 1995); however, this drug can elicit or boost severe allergic reactions in patients. Benzimidazoles, given for several weeks, usually do not cause serious side effects; however, their efficacy at the recom-

mended dosage of 15 mg/kg b/w was shown to be weak (Stürcheler *et al.*, 1989).

Authors have suggested that one explanation for limited efficacy might be the presence of low plasma concentrations due to an inadequate dose. After oral administration, benzimidazole carbamates are rapidly absorbed from the lumen of the small intestine and are extensively metabolized in the liver. In addition, their bioavailability for the tissues is very low because of extremely low water solubility (0.2 μ g/ml at pH 7.4) due to their lipophilic properties (Gottschall *et al.*, 1990). As a consequence, continuous therapeutic plasma levels of benzimidazoles are not possible to achieve after the recommended daily oral administration of free drugs (Bogan and Marriner, 1980).

Our approach to more effective treatment of larval *T. canis* infections in humans and dogs involves carriers of drugs, such as microscopic vesicles (liposomes, niosomes) of various composition and surface modifications, studied in mice experimentally infected with *T. canis* and *T. cati*. Liposomes are formed as concentrically arranged phospholipid bilayers in which lipophilic drugs can be incorporated. In multilamellar vesicles, bilayers are separated by water compartments, in which the water-soluble compounds can be entrapped (Bangham, 1968). Administration of immunomodulator β -glucan in liposomes enables its specific delivery to the cells of the mononuclear phagocytic system (MPS), the result of which is a very efficient activation of cellular and humoral immune responses.

Our experiments aimed to study the effects of different kinds of liposome carriers and glucan on the efficacy of two benzimidazoles – albendazole (ABZ) and fenbendazole (FBZ) – as suitable drugs for liposome incorporation (see review by Hrčková and Velebný, 2001). Because of their biocompatibility, liposomes are suitable for virtually every route of administration, so we tested the effect of subcutaneous and oral administration of liposomized drugs and in combination with intramuscularly given liposomized glucan. Treatment with FBZ (in total 80 mg/kg) and liposomized glucan, both subcutaneous (s.c.), was most effective during the acute stage of infection (days 3–4 p.i.), and nearly completely inhibited migration of larvae into muscles (96.7% larval reduction) and brain (100% larval reduction). Interestingly, ABZ in combination with liposomized glucan was significantly less effective in this stage of infection (Velebný *et al.*, 1996).

However, the majority of clinical human cases are diagnosed for toxocariasis when the disease occurs in the chronic period (from week 3 p.i.), which in experimental animals corresponds to the localization of the larvae in muscles and brains. We and others have demonstrated experimentally that treatment in the later stage of disease is unlikely to kill all migrating or dormant larvae (Delgado *et al.*, 1989). Subcutaneous administration of ABZ and FBZ incorporated into multilamellar liposomes alone resulted in about two- to threefold lower larval numbers compared with therapy with free drugs. Efficacy was enhanced when liposomized glucan was co-administered (Velebný *et al.*, 1997, 2000a).

These data indicate that liposomal carriers can markedly prolong the plasma levels of lipophilic drugs, whereas liposomized glucan has a synergistic effect and can significantly potentiate therapy. The basis of this enhancement with glucan was probably due to non-specific activation of various cell types and stimulation of cytokine production, mainly IL-1 (Sherwood *et al.*, 1986). Consequently, this probably leads to more effective larvicidal host immune responses, as we have shown that treatment with only two doses of liposomized glucan decreased larval counts (Velebný *et al.*, 2000b). Due to the natural origin of liposome components, any route of liposome administration is plausible and safe for their main function as drug carriers (Fielding, 1991). After

s.c. administration, liposomes are drained via the lymphatic system into the circulation (Hirano and Hunt, 1985). Orally administered liposomes are usually broken down by digestive enzymes and gut surfactants, but the entrapped lipophilic drugs inside liposomes may aid their incorporation into lipid micelles and result in an enhanced uptake into the systemic circulation (New, 1994).

The main problem affecting all particulate drug delivery systems is non-specific uptake by phagocytic cells of the MPS, but circulating cells may act as the second circulating reservoirs of drugs. New liposome formulations, so-called sterically stabilized liposomes ('stealth' liposomes), have the ability to evade the rapid uptake by the cells of MPS due to a surface coating of lipid derivatives of poly(ethylene glycol). Such liposomes were detected in the circulation after more than a week compared with several hours in the case of conventional liposomes (Woodle and Lasic, 1992). We have prepared stealth liposomes with either ABZ or FBZ and evaluated their efficacy after s.c. and oral administration during the chronic stage of infection (end of week 4 p.i.). Moreover, the effect of combined treatment with liposomized glucan was evaluated. The efficacy of FBZ and ABZ (total dose 250 mg/kg) incorporated into stabilized liposomes (stab.lip.) after s.c. administration is shown in Table 1. Whereas treatment with stab.lip.FBZ was more effective on muscle larvae (73.6%), therapy with stab.lip.ABZ showed

Table 12.1. Recovery of *Toxocara canis* in the muscles and brain of mice and efficacy of treatment (%) with albendazole and fenbendazole entrapped in poly(ethylene glycol)-stabilized liposomes and with liposomized glucan after oral administration from day 29 p.i.

Number of <i>T. canis</i> larvae and efficacy (%) of preparations					
Group number	Drug preparation	Muscles		Brain	
		Mean ± SE	Efficacy (%)	Mean ± SE	Efficacy (%)
1	Stab.lip. FBZ	81.8 ± 11.4	73.6	78.0 ± 9.9	45.8
2	Stab.lip.ABZ	98.6 ± 10.0	68.2	17.4 ± 2.8§	88.0
3	Stab.lip.FBZ + lip.glucan	26.2 ± 2.5§*	91.5	60.3 ± 6.9	58.1
4	Stab.lip.ABZ + lip.glucan	93.1 ± 9.9	70.0	11.2 ± 2.2§*	92.2
5	Lip.glucan	286.0 ± 16.4	7.8	123.7 ± 10.9	14.1
6	Control	310.2 ± 13.1		144.0 ± 7.1	

Stab.lip.FBZ (fenbendazole) or stab.lip.ABZ (albendazole).
§Significant difference between groups 1–2 and 3–4 ($P < 0.01$), ANOVA.
*Significant difference between groups 1–3 and 2–4 ($P < 0.01$), ANOVA.
From Velebný *et al.* (2000b)

very high efficacy on larvae in the brain (88.0%). Again, potentiation of the larvicidal effect of both drug formulations was achieved after co-administration of two doses of liposomized glucan. From the practical point of view, the oral route of drug administration is preferred to s.c. for the treatment of patients. Therefore we performed a study in which BALB/c mice with chronic *T. canis* infection were treated orally with stab.lip.ABZ alone or in combination with glucan. ABZ was given in ten doses (two per day) in a total dose of 350 mg/kg b/w from day 29 p.i. and efficacy was evaluated 4 weeks after termination of therapy (Fig. 12.2). For free ABZ we found a 29.6% reduction of larval counts in the brain and only 7.4% in the muscles. The efficacy was significantly ($P < 0.01$) elevated when ABZ was incorporated in stabilized liposomes (49.5% brain, 24.5% muscles) and even more when glucan was co-administered (61.8% brain, 31.2% muscles). These data clearly indicate that stabilized liposomes provide sustained drug-release reservoirs for

benzimidazole carbamates, enhance their bio-availability and therefore could be proposed as effective drug formulations for oral treatment of toxocariasis.

In addition, the drug effect was evaluated using histological sections of brains after immuno-histochemical staining of larval TES using IgG antibody from immunized rabbits. During somatic migration in the host, larvae release a large amount of their antigenic products and shed their surface coat (Smith *et al.*, 1981). This was observed in the tissues, in the absence of larvae or their remains, for example in the lungs (Fig. 12.3) or brain (Fig. 12.4). In brains from untreated mice, no damage to the larvae was observed (Fig. 12.5) and TES products were dispersed in large areas, mainly white matter of the corpus callosum. Our observations might imply that more ABZ sulphoxide given in liposome formulations crossed the blood-brain barrier, and in agreement with this, 4 weeks after the end of therapy with stab.lip.ABZ a high proportion of larvae appeared dead or

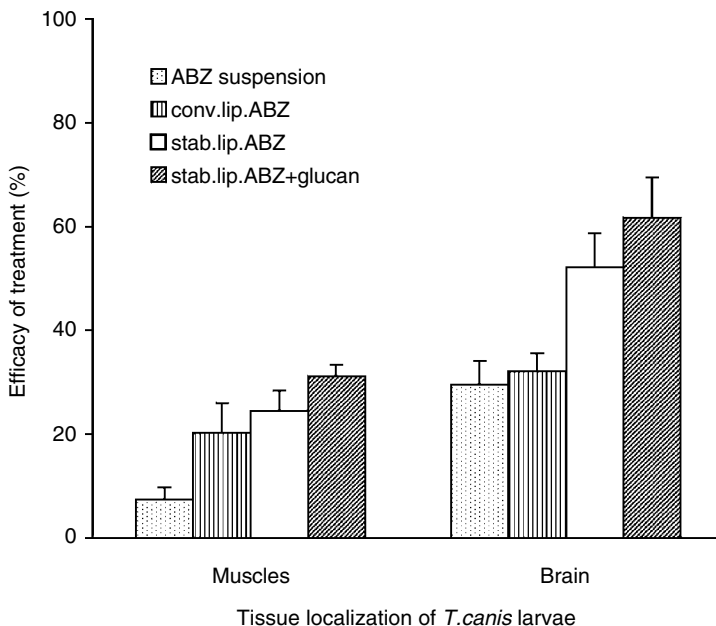


Fig. 12.2. Efficacy of ten doses (total 350 mg/kg) of albendazole (ABZ) in suspension, albendazole in conventional liposomes (conv.lip.ABZ), albendazole incorporated into sterically stabilized liposomes (stab.lip.ABZ) administered alone and in combination with liposomized glucan. Days of drug administration: 29–33 p.i. The reduction (%) in *Toxocara canis* larval numbers in muscles and brains was evaluated 4 weeks after termination of therapy.

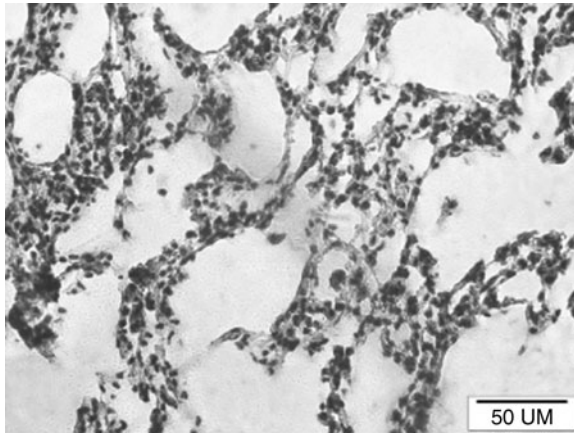


Fig. 12.3. Immunohistochemical localization of excretory–secretory antigens of *Toxocara canis* (TES) in a section of lungs from mice 4 weeks after infection. Larvae were not seen in the lungs during this period. Unpublished. For a colour version of Figs 12.3–12.6 see <http://www.glac.ac.uk/ibls/DEEB/mukimages/>

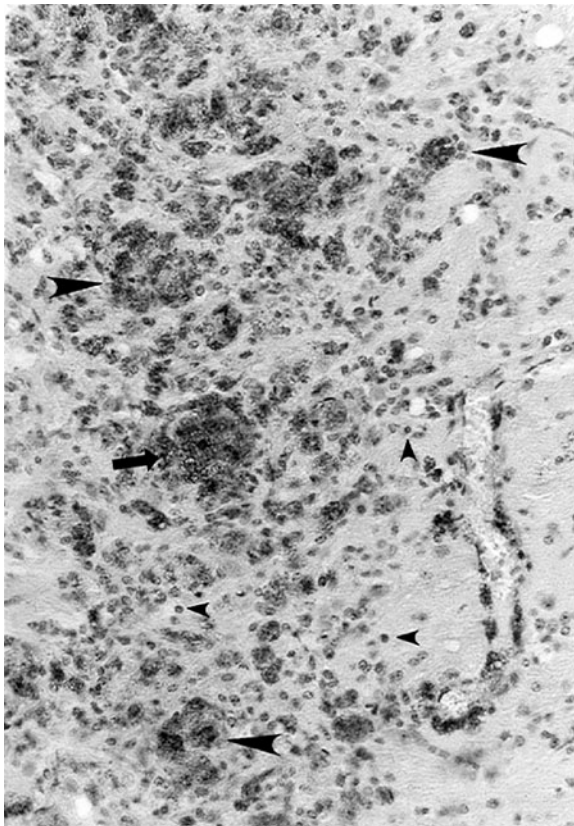


Fig. 12.4. Section of brain from mouse infected with 1000 *Toxocara canis* eggs 6 weeks post-infection. Note (large arrows) TES deposited mostly in the white matter of brain and around the migratory path in the absence of larvae. Glial cells are indicated by small arrows. Magnification x400. Unpublished.

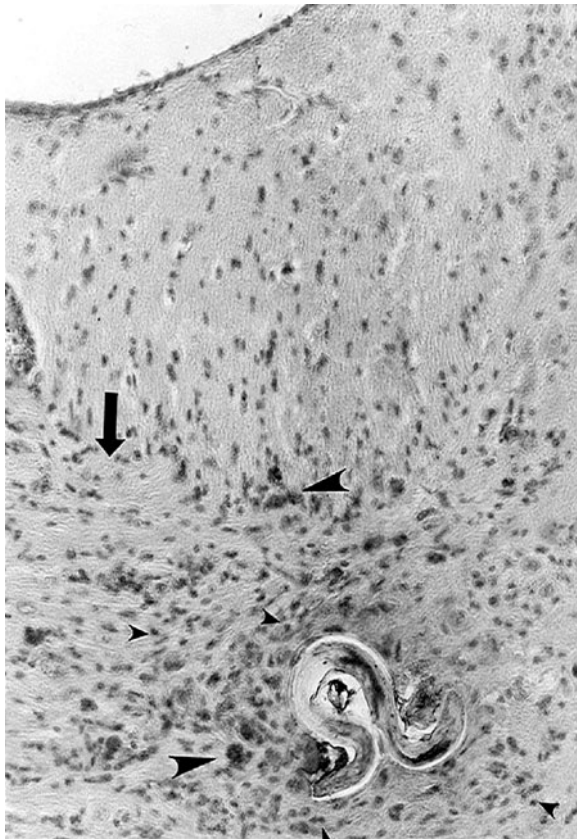


Fig. 12.5. Section of brain from infected untreated mouse showing immunohistochemical localization of *Toxocara canis* larva, surrounded by a large amount of TES (large arrows). Small arrows show glial cells. Magnification x225. Unpublished.

damaged in the brains of mice (Fig. 12.6). In helminths, benzimidazole carbamates inhibit tubulin polymerization in dividing cells and therefore their toxicity is high against developing stages of the parasites. However, there is extensive evidence of impairment in non-developing stages of nematodes (Lacey, 1990), indicating that these drugs could interfere with some crucial metabolic processes. Consequently, drug-induced larval death could be assessed indirectly by means of levels of larval secretion and determination of circulating immune complexes (Bardon *et al.*, 1995). Indeed, efficacy of the oral treatment correlated well with this immunological parameter and the levels of immunocomplexes (TES + IgG antibody) decreased proportionally in all treated groups in comparison with the control (Fig. 12.7).

Finally, using the WB technique, we examined levels of TES-specific antibodies in treated groups to see whether ABZ could target synthesis of some TES antigens. Within the period of 10 weeks following therapy, the intensity of bands corresponding to 32 and 70 kDa antigens gradually diminished, decreasing the most in the stab.lip.ABZ-treated group in comparison with the control (Fig. 12.8). It might be plausible to suggest that lower levels of antigens secreted by reduced numbers of worms surviving therapy mounted a lower levels antibody level response. From these preliminary data we can only hypothesize that ABZ interferes with the function of cells secreting these important antigens and that this deleterious effect could be extended when ABZ sulphoxide plasma levels are maintained for longer periods by means of suitable drug carriers. Further studies are required to verify this assumption.

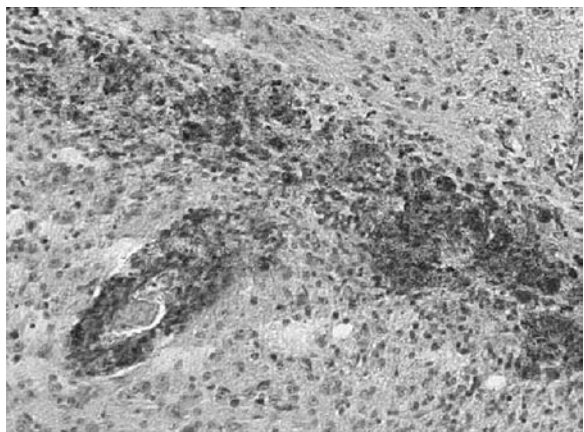


Fig. 12.6. Section of brain from mouse infected with 1000 *Toxocara canis* eggs and treated with stab.lip.ABZ on week 4 after termination of therapy. Focus of TES antigens is visible surrounding what is probably the remains of a dead larva. Magnification x400. Unpublished.

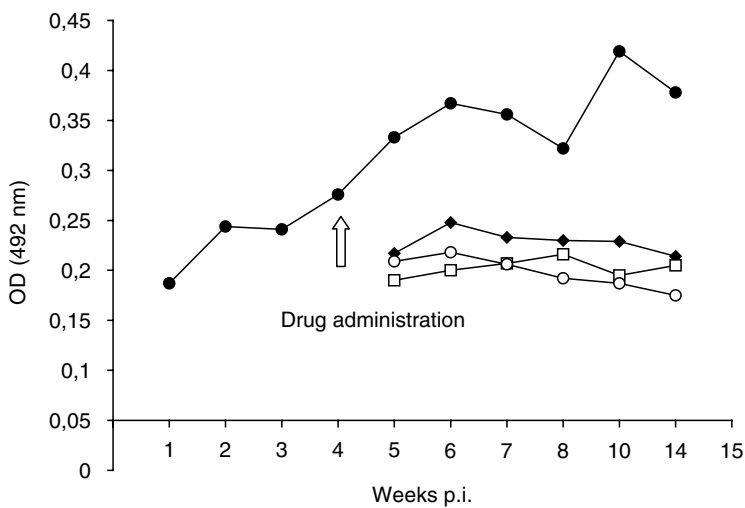


Fig 12.7. Dynamics of circulating immunocomplexes (IgG + antigens) in BALB/c mice infected with 1000 *Toxocara canis* larvae evaluated by ELISA. Titres of immunocomplexes in control untreated mice (●-), treated with albendazole (ABZ) in suspension (◆-), albendazole incorporated into sterically stabilized liposomes (stab.lip.ABZ) administered alone (□-) and in combination with liposomized glucan (○-).

Application of the Mouse Model for Vaccine Development in Dogs

The suitability of a mouse model system for vaccination studies that would be applicable to definitive canid hosts was suggested by Abo-Shehada and Herbert (1984), who showed that

visceral migration in mice is similar to that of the final host and that the somatic phase of *T. canis* migration in mice is similar to that seen in non-pregnant bitches. To date, no *Toxocara* vaccine has been tested on laboratory dogs. Bitches can accumulate somatic larvae over their lives that may be transmitted to their offspring, and adult *T. canis* are also present in the intestine of bitches and

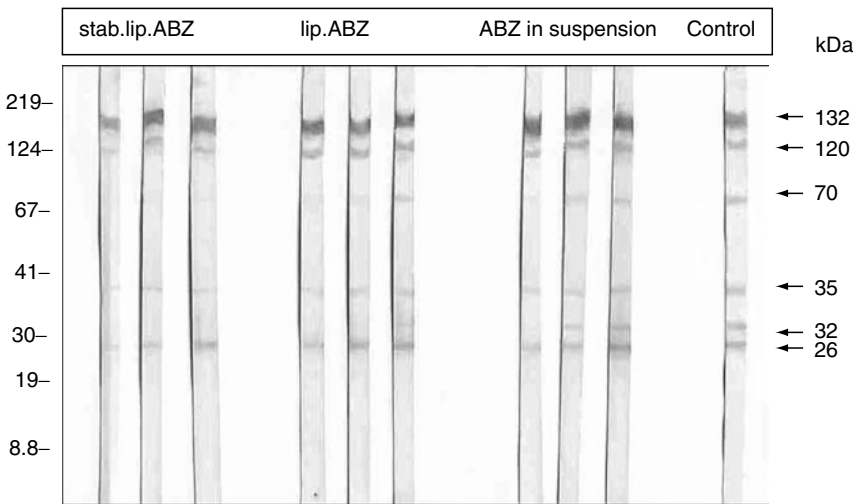


Fig. 12.8. Banding profile of *Toxocara canis* excretory–secretory (TES) antigens after therapy with albenbazole in stabilized liposomes (stab.lip.ABZ), albenbazole in conventional liposomes (lip.ABZ) and albenbazole in suspension. TES antigens were separated by SDS-PAGE, transferred on to PVDF membrane and incubated with sera from treated groups of mice obtained on weeks 1, 2 and 4 after termination of therapy (weeks 5, 6 and 8 p.i.). Marker, Kaleidoscope prestained standards (Bio-Rad).

mature dogs (Lloyd, 1993). Severe intestinal pathology occurs only in heavily infected puppies and no typical VLM is observed in mature dogs. Infected animals are usually recognized after serological examination and egg production (Rubel *et al.*, 2003). Glickman *et al.* (1981) showed that antibody levels in dogs were strictly dose related. This might indicate that re-infection did not switch effective protective immunity in dogs, which is mounted in re-infected or vaccinated mice and rabbits, as described above.

Probably for ethical reasons, the number of experiments on dogs with toxocariasis, which are focused on the study of treatment schedules or anthelmintics, is very limited (Jacobs and Fisher, 1993). Therefore little is known about immune responses following single and multiple infections

and after different infective doses in dogs. If such experiments were conducted, information obtained from the experiments on paratenic hosts could be extrapolated. They might indicate that rather than using the whole TES or somatic antigens, vaccine containing a single TES antigen, preferentially recombinant, and either Th1- and/or Th2-type directing adjuvants, could reveal the protective potential of individual native antigens. Such an approach was successful in vaccination trials on dogs using vaccine containing two different recombinant TES antigens with protease activity of the canine hookworm *Ancylostoma caninum*. Vaccine-mounted protective immunity was characterized by specific IgG2 and IgE antibodies and reduced egg production in challenged dogs (Hotez *et al.*, 2003; Loukas *et al.*, 2004).

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13 Epidemiological Surveillance of *Toxocara* and Toxocariasis

John W. Lewis

School of Biological Sciences, Royal Holloway, University of London, Surrey, UK

Introduction

Many of the new emerging and re-emerging diseases in humans over the past two decades are known to be caused by microparasites and/or macroparasites which originated from animals or products of animal origin, and hence are referred to as zoonotic diseases. A comprehensive review by Cleaveland *et al.* (2001) identified 1415 species of infectious organisms known to be pathogenic to humans and of these 61.6% was zoonotic and 12.3% associated with emerging or re-emerging diseases. A total of 616 pathogens was documented for domestic livestock with 77.3% considered multiple species. For domestic carnivores, the total was 374 pathogens with 90% being classified as multiple species, but no attempt was made to catalogue the number of infectious and infective agents found in wildlife as data on existing diseases of many wild animal species are lacking. This therefore highlights the need to improve surveillance and monitoring systems to further our understanding of the epidemiology of zoonotic infections involving domestic and wild animals.

Recent examples of emerging zoonoses provide evidence of their impact and unpredictability in that they fit a range of epidemiological situations. Some pathogens are largely confined to animal reservoirs with human cases being infrequent or representing dead-end infections (e.g. Nipah virus in Malaysia). Other pathogens are either well es-

tablished in both animals and humans (e.g. bovine tuberculosis) or are mainly confined to animals as the main hosts with occasional outbreaks in humans, occasionally leading to extinction (e.g. monkey pox in the USA). Some pathogens with an animal origin suddenly appear in human populations, for example human immunodeficiency virus (HIV) and severe acute respiratory syndrome (SARS) or gradually adapt to human-to-human transmission as in the case of tuberculosis.

The more classical zoonotic infections such as toxocariasis may still be regarded as emerging but only if the term 'emerging disease' encompasses either a previously unknown agent detected for the first time or a known infectious agent appearing in a new geographic area or in a hitherto unsusceptible species and shows increases in incidence or expansion in a geographic host or vector range (WHO, 2004a). Some of these diseases may further evolve and become effectively transmissible from human to human (Daszak *et al.*, 2000). An emerging zoonosis may thus be an existing infection or disease which appears again and invades new territories. Many diseases, zoonotic or otherwise, which had once been controlled in many parts of the world have started to re-emerge and these include infections or vector-borne diseases, such as cholera, tuberculosis, dengue fever, yellow fever and malaria. It is unlikely that a re-emergence and an increase in transmission of a helminth zoonosis linked with the ascarid nematode *Toxocara* will occur, but

Jensen *et al.* (2001) and Magnaval *et al.* (2004) reported a re-emergence of alveolar echinococcosis following the translocation of Arctic carnivores to more depleted southern reserves.

Changes in pathogen/parasite and/or host ecology are primarily responsible for the majority of emerging diseases and those resulting from evolutionary changes alone are comparatively rare. Ecological changes, largely a consequence of human activity, include changes in agricultural practices, urbanization and globalization. Furthermore, changes in climate may affect the distribution and abundance of vectors and wildlife in those habitats where primarily agricultural production takes place, thereby influencing the survival time of pathogens/parasites outside the host (Schrag and Wiener, 1995). Environmental changes may have influenced disease emergence towards the end of the 20th century because these changes have been rapid, whereas evolution has played a major role in disease emergence over the longer term (Diamond, 2002).

Nevertheless, classical public health and sanitation measures have long served to minimize dissemination and human exposure to many pathogens/parasites which are spread by traditional routes such as soil, water and food or are preventable by immunization or vector control. However, the pathogens/parasites themselves often remain in small pockets of infection and may re-emerge if circumstances allow. These include a breakdown in disease prevention and control measures, especially in developing countries and inner cities of the industrialized world.

***Toxocara* and Toxocariasis**

Toxocariasis was recognized as a zoonotic disease some 50 years ago and was described as the clinical condition of visceral larva migrans (VLM) in children (Beaver *et al.*, 1952). This cosmopolitan parasitic zoonosis occurs sporadically in urban and rural sites as a result of an accidental human infection acquired by swallowing eggs of the ascarid nematode, *Toxocara canis*, found worldwide in dogs, foxes and other canine hosts, or *Toxocara cati* found in cats and other feline hosts (Lewis and Maizels, 1993). Children are at the highest risk of infection because of pica, geo-

phagia and playing on soils contaminated with infected faeces. The extent of such contamination has been assessed in many developed and developing countries by analysis of soil and faecal samples from school playgrounds, public parks, gardens and other recreational areas used by the public where for example in Lima, Peru, 70.6% of parks were contaminated (Castillo *et al.*, 2001). Rodents can also become infected by ingestion of *Toxocara* eggs and, as in the case of humans, are regarded as paratenic or abnormal hosts, in which immature second-stage larvae of *Toxocara* undergo a somatic migration through the organs of the body but fail to reach maturity. In the wild, rodents harbouring these larval stages are preyed upon by dogs, foxes and cats, in which the larvae develop into adult worms in the intestine. The major clinical syndromes of human toxocariasis include ocular larva migrans (OLM) (see Chapter 9, this volume), VLM and covert toxocariasis. OLM is associated with larval invasion of the eye causing occasional blindness, whereas in VLM, a raised *Toxocara* titre is accompanied by an increase in eosinophilia and leukocytosis. Covert toxocariasis is non-specific with recognizable symptoms associated with a raised *Toxocara* titre but not falling into either category of OLM or VLM. Other non-specific forms of toxocariasis are described as neurological, pulmonary, cutaneous and rheumatological. Apart from OLM, which is usually diagnosed by ophthalmology, there is neither clinical nor parasitological evidence of the infection in humans. Toxocariasis can be suspected when eosinophilia occurs, but this symptom can also be associated with other helminth infections and other diseases. Routine diagnosis of toxocariasis therefore relies upon immunological tests using ELISA for the detection of specific anti-*Toxocara* IgG and IgE and a Western blot test for the detection of specific IgG using excretory-secretory antigens of *T. canis* larvae.

Human toxocariasis is traditionally thought to be contracted through ingestion of eggs from contaminated soil, but occupational and recreational contact with dogs is associated with a higher seroprevalence than in the general population. Occupations at special risk are those in close contact with dogs. Wolfe and Wright (2003) found *T. canis* eggs in 25% of the faeces of dogs examined from various sites in the UK and Ireland and proposed that direct contact with dogs may pro-

vide a better explanation of the epidemiology of the disease. However, there is, overall, a large variation in human seroprevalence of toxocariasis from country to country (Taylor, 2001), suggesting that the diagnosis of the disease is dependent upon the quality of antigen used and the relationship between clinical cases and the nature of the serology. Taylor also highlights the need for an improvement in the clinical definition of OLM by ophthalmologists and this was confirmed by Good *et al.* (2004) who showed that an increase in the prevalence of OLM was related to consultant-diagnosed toxocaral eye disease in definite and strongly suspected cases.

Other uncertainties about diagnosis include non-specific cutaneous manifestations of toxocariasis where symptoms such as rashes, urticaria or hypodermal nodules are frequently reported in subjects with VLM or covert toxocariasis. In some patients, cutaneous symptoms are the only clinical manifestations of the disease and Humbert *et al.* (2000) showed that patients suffering from urticaria or prurigo should undoubtedly be tested for *Toxocara* infection (see Chapter 10, this volume). Seroepidemiological studies have also demonstrated that allergic manifestations, including asthma, are more frequent in *Toxocara*-seropositive individuals (Buijs *et al.*, 1997), but no significant association between the presence of *Toxocara* antibodies and asthma has been reported. On the other hand, Pinelli *et al.* (2001) have shown that using murine models the presence of larvae in the lungs could explain persistent pulmonary inflammation accompanied by a Th2 type of immune response (see Chapter 4, this volume).

In temperate climates, human toxocariasis is probably one of the most common zoonotic helminthiases (Schantz, 1989) with some countries more at risk than others based on seroepidemiological studies in schoolchildren, for example, in Ireland (Holland *et al.*, 1995). In the definitive canine host, there are many isolated and recent examples of relatively high prevalences of adult worm infections or eggs in faecal samples from dogs including Minaar *et al.* (2002) in Bloemfontein, South Africa, Borkovcova (2003) in South Moravia, Czech Republic, Barutzki and Schaper (2003) in Germany and Tomczuk (2003) in Lublin, Poland, and from foxes in the UK where up to 61.6% were found to be infected with *T. canis* (Smith *et al.*, 2003). In the majority of these cases little is known about the transmission potential of

Toxocara to humans and particularly the role of *T. cati*, despite high prevalences in cat populations including recent reports by Fromont *et al.* (2001) in France, Heilskov (2002) in Denmark, Labarthe *et al.* (2004) in Rio de Janeiro, Brazil, and Robben *et al.* (2004) in The Netherlands.

Wild carnivores such as the fox (Luty, 2001; Richards and Lewis, 2001) and the wolf (Segovia *et al.*, 2001) as definitive hosts and wild rodents as paratenic hosts (Dubinsky *et al.*, 1995) clearly contribute to the transmission and spread of *Toxocara* in both rural and urban environments. There is, however, a lack of good-quality data on the relative risk of human infection from dogs, cats, foxes and rodents and the degree of environmental contamination in any given country. Estimates of environmental contamination with eggs and infective stages of *Toxocara* are to date not entirely comparable from country to country as flotation and identification (including molecular) techniques need to be standardized to produce reliable epidemiological data. During the course of sampling, many factors influence the results of soil examination such as site selection, the number and volume of samples, depth and type of soil, presentation and methods of egg recovery and season (Mizgajski, 2001). In addition, the degree of mechanical transmission of *Toxocara* eggs by insects (Thyssen *et al.*, 2004) and in vegetation, food, water, waste water and sludge (Paulino *et al.*, 2001) requires further investigation.

An integrated epidemiological approach is therefore needed to detect zones at risk of contracting zoonotic diseases such as toxocariasis in urban and rural environments and to relate any positive clinical findings with environmental, public health, socioeconomic and cultural conditions of the region. To achieve this and to improve surveillance of toxocariasis in zones at risk in different parts of the world, a comprehensive database incorporating details of the definitive, paratenic (and human) hosts and environmental contamination with standardized and current protocols is described in association with SparkleSystems, Inc., Kamloops, British Columbia, Canada.

***Toxocara* Data Resource**

SparkleSystems, in collaboration with the author (JWL), has designed a database for the input and sharing of data associated with *Toxocara* infections

in the definitive canine host, the human and murine paratenic hosts and contamination of the environment with eggs of *Toxocara* (Fig. 13.1). The ‘Sparkle’ data resource is web-enabled, continually updated and uses an innovative generic data

processor for the interpretation and querying of multidisciplinary, multimedia data.

‘Sparkle’ makes the collection, integration, correlation and centralized storage of new data seamless. It has sophisticated searching and shar-

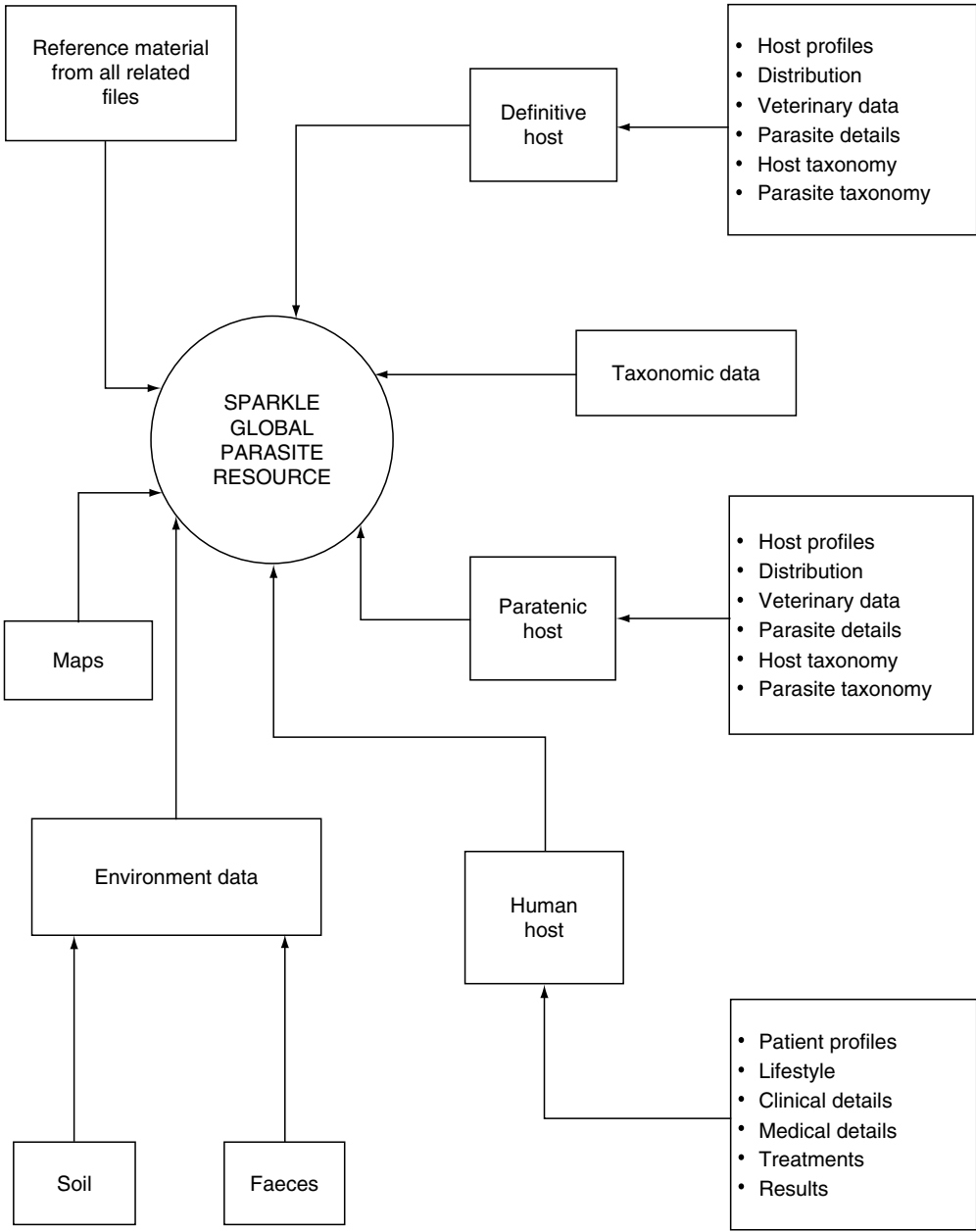


Fig. 13.1. Flow chart of the SparkleSystems global parasite resource based on the design of the *Toxocara* database.

ing facilities providing remote users 'real time' collaboration and also a standard for transferring data viably by crossing language and numerical format barriers. It automatically makes data conversions so that recipients of data see it in their own preferred formats, regardless of the format of the sender. Users have the ability instantly to establish secure private networks from their desktops. It provides multi-user access and is accessible at different points anywhere in the world at any time, but nevertheless provides users with absolute control over their own data. Users will never lose their data, as these are protected from catastrophic loss, and users can see and edit their data from any locality. Within organizations, 'Sparkle' permits a team leader to delegate levels of use and responsibility to different members of a team. Diverse work units can be linked together within a building, between buildings or between HQ and a field team anywhere in the world. It is interoperable, has its own global positioning satellite (GPS) and geographical information systems (GIS) and its own (optional) handheld wireless devices for field and hospital access. 'Sparkle' can make maps on the fly, construct databases on the fly and show a team leader where distant team members are on the map. Security and flexible searchability of data is paramount in the design. The service can be used by individuals, teams, whole institutions or at an enterprise level. All datasets can be merged and searched seamlessly without collision of data, e.g. two databases, one from an institution working on *Toxocara* and one from another institution working on pharmaceuticals can be merged, can be used immediately, and can be searched in concert immediately.

'Sparkle' is designed with a powerful management core to which different types of 'user systems' can be attached through standard interfaces. This provides flexibility of use. Forms are provided to enter, store, search and retrieve data for all parasites and in the case of *Toxocara*, this involves multiple hosts and a complex life cycle, and in all cases details of the recorder, researcher with dates are included.

Definitive Host

Details listed for the definitive canine/feline host (dog, fox, cat) include host taxonomy, geographical distribution and host profiles such as body weight,

body length, breed/strain, age, sex, reproductive status, location (urban, rural), year, month and season (Figs. 13.2 and 13.3). This is followed by the input of parasite details, e.g. species of *Toxocara* plus any concurrent infections with other parasite species. Parasite numbers are recorded together with size, sex, fecundity plus, where necessary, molecular characterization using techniques such as PCR/RFLP/AFLP, microsatellites and others (see Chapter 2, this volume). Forms for the treatment of infected, definitive hosts provide a list of anthelmintics, dose and method of drug administration and efficacy (Fig. 13.4 and see Chapter 16, this volume). Worm numbers and worm measurements and changes in serology are also recorded.

Human Host

The format here is more extensive than in the definitive host due to the need to acquire clinical data. Host (patient) profiles are listed to include patient ID, age range, sex, ethnicity, location and occupation, financial and mental status and lifestyle. An entire list of risk factors is shown ranging from pica and pet ownership to raw meat consumption. Diagnostic features of the database (Figs 13.5 and 13.6) include a range of immunological tests used (ELISA, Western blot and others) together with the source and quality of the antigen and antibody isotypes (with total and specific values), data types (titre, cut-off, optical density) and cell-mediated responses (Th-1, Th-2). The blood picture requires information on total white blood cells, eosinophils, leucocytes, neutrophils, platelets and plasma details (see Chapter 7, this volume).

Clinical data relating to disease include a selection of symptoms in each of the disease types, i.e. OLM, VLM, covert toxocariasis and other non-specific forms such as pulmonary, cutaneous, neurological and rheumatological.

Data for treatment are similar to the format used for the definitive host except more treatment regimes are included to cater for the various disease syndromes; for example, treatment for OLM may involve corticosteroids as mandatory plus anthelmintics compared with anthelmintics plus prophylaxis in VLM and covert toxocariasis. The nature, dose and timescale of drug administration are included together with the measurement of larval numbers, serological changes (and eosinophilia), laser photocoagulation (in the case of retinal

SparkleSystems: TOXOCARIASIS

Definitive Host

Type Dog

ID DTR-01

New

Find

Save

Delete

Close

Host 1 of 26

Details 1

Details 2

Details 3

Probes

Infections

Treatment

Bookmarks

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Glossary

Definitive

Human

Paratenic

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Search

Bibliography

Search Biblio.

Sites

Taxonomy

Supplements

Host

Host Animal Dog z

Scientific Name Canis familiaris

Common Names Hond (Dut); Le Chien (m) La Chienne (f) (Fr); Hund (Ger); Cane (It); Cachorro (Port); Perro (m) Perra (f) (Span); z

Strain or Breed Street Dog z

Host ID DTR-01 z Auto ID DH-15 Documents

Date Recorded 05 Jul 2002 Recorder sh z Sue Hughes

Research Date 1990 y Jan m 1 d Researcher dtr z Dr. David Richards

User ID sparkle Contact jwl z Prof. John Lewis

Research Location Royal Holloway z Royal Holloway
Egham, Surrey, England, United Kingdom
Region: Europe
Setting: Urban

D:\DemoMarch2004\DemoToxotestData.mdb

Fig. 13.2. Screen format for data input for the definitive host, the dog: common and scientific names are entered on the main form and selected from a pick list and any number of foreign or vernacular names can be added at any time. Buttons on the left take the user to other parts of the SparkleSystems parasite resource and this can be accessed at any time with the click of a mouse: the sub-form on the bottom half of the screen contains metadata which has been entered by a recorder/researcher with date and location.

SparkleSystems: TOXOCARIASIS

Definitive Host

Type Dog

ID DTR-01

New

Find

Save

Delete

Close

Host 1 of 26

Details 1

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Details 3

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Host Profile

Sex ☒ Male ☐ Female ☐ Unknown

Maturity Pubescent z

☐ Pregnant ☐ Lactating ☐ Suckling

Body Weight 12.8 kg

Body Length cm

Lens Weight 0.0 mg

Age Range 13-26 weeks

Actual Age 26 weeks

Living/Dead Dead

Status Wild animal z
Urban pet
Suburban pet
Rural pet
Street (stray)
Farm animal
Feral animal
Wild animal
Police dog
Racing dog
Hunting dog
Other

Fig. 13.3. Screen format for data input for the definitive host, the dog, with reference to size, age, sex and status, all selected by a pick list on the right of the screen.

SparkleSystems: TOXOCARIASIS

Definitive Host Type Fox ID DTR-F14

Host 26 of 26

Details 1 | Details 2 | Details 3 | Probes | Infections | Treatment

Bookmarks

Memo

Glossary

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Environment

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Supplements

Treatment ID: Test 1 Auto ID Tmt-3 Treatment 1 of 2

Date Started: 2001 y Apr m 6 d

Treatment: z

Anthelmintic: Melarsomine (dropdown menu open showing: Melarsomine, Ivermectin, Thiabendazole, Fenbendazole, Mebendazole, Oxfendazole, Oxendazole)

Dose: mg/kg: 2 Method: z intramuscular Frequency/per: 1 / Day Duration: 2 Days

Results

Documents

First Previous Next Last New Find Save Delete Close

Fig. 13.4. Screen format for data input for the definitive host, in this case the fox; this record is surrounded by a black frame and demonstrates a treatment regime; navigation buttons at the bottom of the frame allow the user to move through individual treatment records; pick lists are available for the name of the drug, method, frequency and duration of administration.

SparkleSystems: TOXOCARIASIS

Human Host ID Huw-024

Host 42 of 45

Details 1 | Details 2 | Details 3 | Probes | Infections | Treatment

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Supplements

Antibody Isotypes

Isotypes

OK

Diagnostic Test 2: Quantitative

Sample ID: Huw024G Auto ID: Quant-125

Test For: IgG (dropdown menu open showing: IgA, IgE, IgG, IgM)

Type of Test: ELISA Source: z Quality: z

Total Value: 800.00 Titre (1:X) Cutoff

Specific: z Notes: z

Documents

First Previous Next Last New Find Save Delete Close

Fig. 13.5. Screen format for data input for the human host; the test records are surrounded by a black frame and the user can record multiple tests for one person; the user can choose between serum and aqueous fluid, select from a list of test types, source and quality and give results in titres or international units; the isotypes in the subform (on the left of screen) can be accessed by clicking the 'Test for' field with a Z (zoom) button.

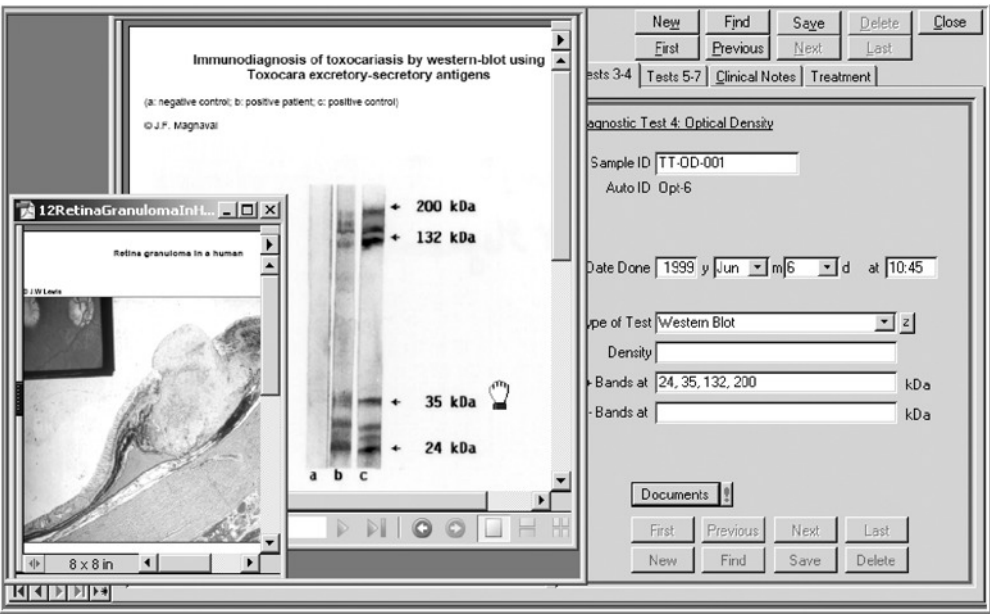


Fig. 13.6. Screen format for the human host to show the recording of test data for immunodiagnosis; this can be appended to any number of relevant images (retinal granuloma) or digital data.

detachment only) and drug efficacy. The patient profile also takes into account concurrent infections with other parasites (Fig. 13.7) (see Chapter 8, this volume).

Paratenic Host

Human hosts are in the same category as rodents and rabbits, which act as paratenic hosts, but with humans, the *Toxocara* life cycle is usually at a dead end, as canine or feline definitive hosts are only likely to feed upon these small mammals to generate transmission. The format for the paratenic host is therefore similar to that already described for the human host, except that data for paratenic rodent hosts are needed from laboratory mouse models (Figs 13.8 and 13.9) and also from wild hosts (see Chapter 6, this volume). In experimental infections in mice and other models (rat, gerbil, rabbit and pig), data on behavioural changes such as activity, social behaviour, novelty and anxiety responses and learning and memory plus clinical data on pathological changes and allergic reactions are listed (Fig. 13.9). In addition, mice shown

to harbour *Toxocara* larvae in the somatic tissues are also likely to be infected with a range of other parasites particularly from the wild (Cox, 1987; Lewis, 1987) and hence a comprehensive list of these parasites as evidence of concurrent infections and their sites of infection is included (Fig. 13.8).

Environmental Contamination

Apart from recording geographical location, year, month and season, the major components include selecting a range of habitat types (in urban and/or rural environments) from parks, playgrounds and sandpits to kennels, open pasture and woodlands (Figs. 13.10 and 13.11) (see Chapter 14, this volume). Data on temperature, ultraviolet light, rainfall, snowfall and sewage are also recorded. Evidence of faecal contamination either by dogs, cats and/or foxes is necessary as well as size, texture and depth of soil samples examined. For extraction of eggs, standardized flotation media using sodium nitrate or magnesium sulphate (Fig. 13.12) should be used if possible using a

SparkleSystems: TOXOCARIASIS

Human Host ID **Huw-024** [New] [Find] [Save] [Delete] [Close]

Host 42 of 45 [First] [Previous] [Next] [Last]

Details 1 | Details 2 | Details 3 | Details 4 | Details 5 | Probes | Tests 1-2 | Tests 3-4 | Tests 5-7 | Clinical Notes | Treatment

Bookmarks | Memo | Glossary | Definitive | Human | Paratenic | Environment | Search | Bibliography | Search Biblio. | Sites | Taxonomy | Supplements

Parasites Present *Toxocara canis*

Date of Birth: 1992 May 12
 Sex: ☐ Male ☒ Female ☐ Unknown
 Maturity: Child z
☐ Pregnant ☐ Lactating
 Body Weight: 21.0 kg
 Body Height: cm
 Age Range: 0-5 years
 Actual Age: 5 Years
 Living/Dead: Living
 Ethnicity:
 Religion:
 [First] [Previous] [Next] [Last]

Parasites Present
 Select items within this field
 Use the single arrow to select/deselect items from the list.
 Use the double arrow to select/deselect the entire list.
 Use + or - to expand or contract the list.

Genus

- + Ascaris
- + Brachylaima
- + Capillaria
- + Cryptocotyle
- + Dipylidium
- + Echinococcus
- + Macracanthorhynchus
- + Prosthynchus
- + Strongyloides
- + Taenia
- + Toxascaris
- **Toxocara**
 - sp.
 - spp.
 - cati
 - malaysiensis
- + Trichuris
- + Uncinaria

[Edit Main List] [Edit Sub List]

Selected Items
Toxocara canis

Fig. 13.7. Screen format for the human host; the subform (on the right of screen) opens when the 'Parasites Present' button is pressed, revealing a parasite list, and the process can be repeated as many times as necessary; the user can select a genus or genus/species combination.

SparkleSystems: TOXOCARIASIS

Paratenic Host Type **Mouse** ID **MH test 23** [New] [Find] [Save] [Delete] [Close]

Host 1 of 1 [First] [Previous] [Next] [Last]

Details 1 | Details 2 | Details 3 | Probes | Infections | Tests 1-2 | Tests 3-4 | Tests 5-7 | Clinical Notes

Bookmarks | Memo | Glossary | Definitive | Human | Paratenic | Environment | Search | Bibliography | Search Biblio. | Sites | Taxonomy | Supplements

Larval Species Present *Toxocara leonina, Toxocara canis*

Details of *Toxocara canis* Infection ID
 Worm Burden: ☐ Low ☒ Medium ☐
 Actual Dose:
 Number Recovered:
 Prevalence:
 Organ/Tissue: Brain (Cerebellum), Brain (Pons)
 [First] [Previous] [Next] [Last] [New]
 Recorded by: hm z 20 Jan 2004 Researcher: Prof. Hanna Mizgajski
 [First] [Previous] [Next] [Last]

Select Organ/Tissue
 Select items within this field
 Use the single arrow to select/deselect items from the list.
 Use the double arrow to select/deselect the entire list.
 Use + or - to expand or contract the list.

Organ/Tissue

- + Brain
- + Diaphragm
- + Eye
- + Heart
- + Intestine
 - Intestine (Section 1)
 - Intestine (Section 10)
 - Intestine (Section 2)
 - **Intestine (Section 3)**
 - Intestine (Section 4)
 - Intestine (Section 5)
 - Intestine (Section 6)
 - Intestine (Section 7)
 - Intestine (Section 8)
 - Intestine (Section 9)
- + Kidney
- + Liver
- + Lung
- + Mesenteries

[Edit Main List] [Edit Sub List]

Selected Items
 Brain (Cerebellum)
 Brain (Pons)
 Kidney
 Liver

Fig. 13.8. Screen format for the paratenic (mouse) host which in the case of *Toxocara* will be larvae (Larval Species Present); the subform (on the right of screen) allows the user to select organs and tissues; if the intestine is selected to record other parasite species a variety of intestinal sections can be selected for parasite distribution.

s: TOXOCARIASIS

ostType MouseID MH test 23

NewFindSaveDeleteClose

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Details 1Details 2Details 3ProbesInfectionsTests 1-2Tests 3-4Tests 5-7Clinical Notes

Clinical Notes

Pathology/Allergy

Ocular

Cerebral

Liver

Lung (Asthma)

Peribronchiolitis

Perivascular Infiltrate

Hypertrophy of Goblet Cells

Alveolitis

Other

Enlarged

Yes

1

3

2

4

Behavioural Changes

Activity (Immobility)

Social Behaviour (Aggression, Flight)

Novelty/Anxiety Responses

Learning and Memory

Fits and Spasms

Other

Sluggish

Timid

Shivering

Zoom Control

Please edit the text as you wish in the box below:

Within two days the mouse became totally lethargic, and spent most of its time curled up in the corner of the cage shivering

Accept Text and Exit

Reset Text to Original

Clear Text on Form

Clear T and E

Fig. 13.9. Screen format for the paratenic (mouse) host; in addition to recording pathological/allergic and behavioural changes, Z (zoom) buttons open a free text box (zoom control towards the right of the screen), which allows the user to add text.

Environmental Data

Site CoP-003Location Lubuski Park

NewFindSaveDeleteClose

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Sites

Taxonomy

Supplements

Site

Details

Probes

Faeces

Soil

Site ID

CoP-003

Auto ID

Site-7

Documents

Habitat

Park

Degree of Shading

Less than 50%

General Comments

Date Recorded

05 Jul 2002

Recorder

sh

Sue Hughes

Collection Date

2001 y

Sep m

d

Researcher

hm

Prof. Hanna Mizgajski

User ID

spikle

Contact

hm

Prof. Hanna Mizgajski

Research Location

Lubuski Park

Lubuski Park

Dept of Parasitology CHU

Toulouse

France

Lubuski Park

Poznan

Poland

Malaria

Malaria, Egypt

North Africa

NSW

New South Wales, Australia

Australasia

Royal Holloway

Egham

Surrey, England, United King

Europe

Scottish Parasitology Diagnostic

Glasgow

Scotland, United Kingdom

Site 54

Greentown

British Columbia, Canada

North America, NW

Spikle Research Lab

Calgary

Alberta, Canada

North America, NW

Fig. 13.10. Screen format for environmental contamination to show the selection list for research locations and the nature of the collecting site.

The screenshot displays the 'Environmental Data' software interface. The main window has a title bar 'Environmental Data' and a menu bar with 'File', 'Edit', 'View', 'Data', and 'Help'. Below the menu bar, there are tabs for 'Site', 'Details', 'Probes', 'Faeces', and 'Soil'. The 'Site' tab is active, showing 'Site CoP-003' and 'Location Lubuski Park'. On the left, there are buttons for 'Bookmarks', 'Memo', 'Glossary', 'Definitive', 'Human', 'Paratonic', 'Environment', 'Search', 'Bibliography', 'Search Biblio.', 'Sites', 'Taxonomy', and 'Supplements'. The main area shows a 'Sample Location' table with 'Lubuski Park' and 'Poland'. Below this is a 'Time' section with fields for 'Year' (2001), 'Month' (September), 'Season' (Summer), and 'Notes'. On the right, a 'Locations' subform is open, showing a list of locations sorted by 'LocationName'. The selected location is 'Lubuski Park, Poland'. The subform contains fields for 'Identifier' (Lubuski Park), 'Country' (Poland), 'Province/State...', 'County...', 'Town or City' (Poznan), 'Address' (City Centre), 'Post Code', 'Coordinates', 'Setting' (Urban), 'Region' (Europe), and 'Notes'. At the bottom of the subform, it says 'Location 5 of 12' and has buttons for 'First', 'Previous', 'Next', 'Last', 'New', and 'Save'.

Fig. 13.11. Screen format for environmental contamination to show a subform (on the right), which allows details about one site to be entered once. A list of sites is generated so that when the user selects one site all details are available for that particular record.

McMaster technique, although the database provides for other appropriate options, for example, the need to identify the egg to species level using morphological features and molecular methodology, i.e. *T. canis*, *T. cati*, *Toxocara vitulorum*, *Toxascaris leonina*, *Ascaris suum* or other parasite eggs which may be present (Fig. 13.12).

Linked Documents and Database Searching

Within the data resource, any number of documents may be linked to any record, whether of immunodiagnosis or retinal pathology in the human host (Fig. 13.6), to the egg of *T. canis* from an environmental site (Fig. 13.13) or to adult worms in the intestine of the definitive fox host (Fig. 13.14). It is possible to link text files, photographs, drawings, video clips, sound files, spreadsheets, digital files and any other databases such as the reference and taxonomy databases (Fig. 13.1). Linked documents containing images

of retinal damage can be readily accessed to assist ophthalmologists in the specific diagnosis of OLM. Linked documents that are stored once, can be used repeatedly in different places and are classified by title, author, abstract, file type and relevance so that when a search is being made it is possible to stipulate, for example, all photographs by a named author with the word *Toxocara* in the title. A user can also build a complex query, for example in searching for symptoms in patients (male and in the age range 16–20 years) with OLM (Fig. 13.15).

Conclusions

Ecological, environmental and social changes associated with human demographics, globalization and climate change have created an era of new and re-emerging diseases. The majority of these result from either vector-borne or zoonotic infections/infective agents that have crossed the species barrier from animals to humans (Woolhouse,

Environmental Data

Site CoP-003Location Lubuski Park

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SiteDetailsProbesFaecesSoil

Soil Samples

Auto ID Soil-12

Sample ID Cop003-LP02

Sample Date 1999 y Feb m 21 d

Notes

Texture Sand z

Type of Wash 5% NaOH z

Depth 0-3 z cm

Flotation Media Sodium Nitrate z

Technique Willis z

Sample Size 40 g

No. of Samples 32

Sample Mass 1280 g

All EggsToxocara Eggs

No. of Samples with eggs

Prevalence 3.1%

Total No. of eggs 1

Mean No. Eggs per g of Soil 0.0008

Details of Eggs

SpeciesEggs FoundPrevalence

Toxocara canis1

Toxocara cati0

Toxocara spp.0

Toxascaris leonina0

FirstPreviousNextLastNewFindSaveDeleteDocuments

Fig. 13.12. Screen format for environmental contamination is contained within the black frame and this form allows multiple entries for one site; the user can enter information about soil samples, i.e. soil texture, depth, number and methods of collection, flotation and media; the program calculates total sample mass and prevalence (%); details of eggs in samples can be recorded for any number of species.

2002). High-profile events such as the outbreak of SARS in 2003 and the transmission of avian influenza to humans in Asia and the West Nile virus across the USA have highlighted the capacity of animal-borne diseases to occur unexpectedly and in new locations. These epidemics are likely to raise the profile of less-well-known zoonotic infections such as toxocariasis and toxoplasmosis, and the use of computerized databases, mapping software and GIS for data processing and analysis are essential tools to strengthen disease surveillance. Molecular diagnostic methods have also enhanced the capability of veterinary and human health communities for rapid recognition and response with the view to preventing and controlling these zoonoses.

The Centers for Disease Control and Prevention, (CDC) in Atlanta, Georgia, USA, have already developed a national reporting system called Arbonet to monitor the spread of West Nile virus throughout the country, together with collating and reporting surveillance data from humans, mosquitoes, birds, mammals and sentinel

chicken flocks (CDC, 2003). But Arbonet does not connect with other surveillance systems and many other zoonoses remain unmonitored. However, surveillance of infections in wildlife is especially problematic, few diseases are notifiable and the collection and sharing of data, not always using standardized protocols, are often fragmented.

There is also still much to be learned about the natural ecology and epidemiology of infectious and infective diseases and for developing methods for predicting their emergence, re-emergence and spread.

For diseases of public health concern, the World Health Organisation (WHO) launched the Global Outbreak Alert and Response Network (GOARN) in 2000 to gather epidemic intelligence from informal sources. This was followed up (WHO, 2004b) when WHO in conjunction with the Food and Agriculture Organisation (FAO) and World Organisation for Animal Health (OIE) embarked on the development of a Global Early Warning System (GLEWS) to share the results of disease tracking systems and to

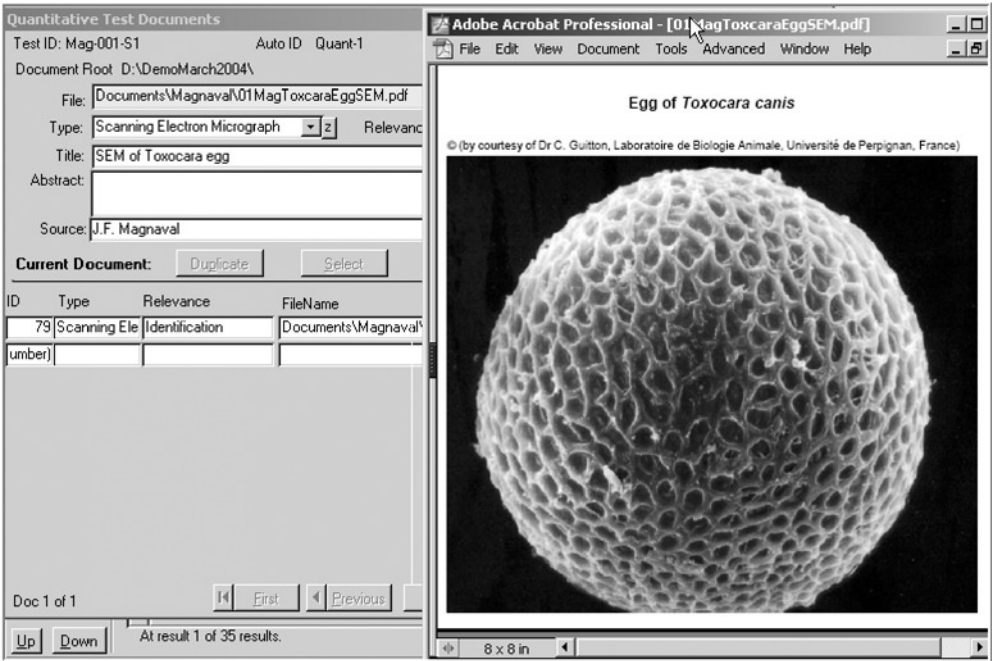


Fig. 13.13. Screen format to show that documents within the database can be linked to any record, e.g. the egg of *Toxocara canis*.

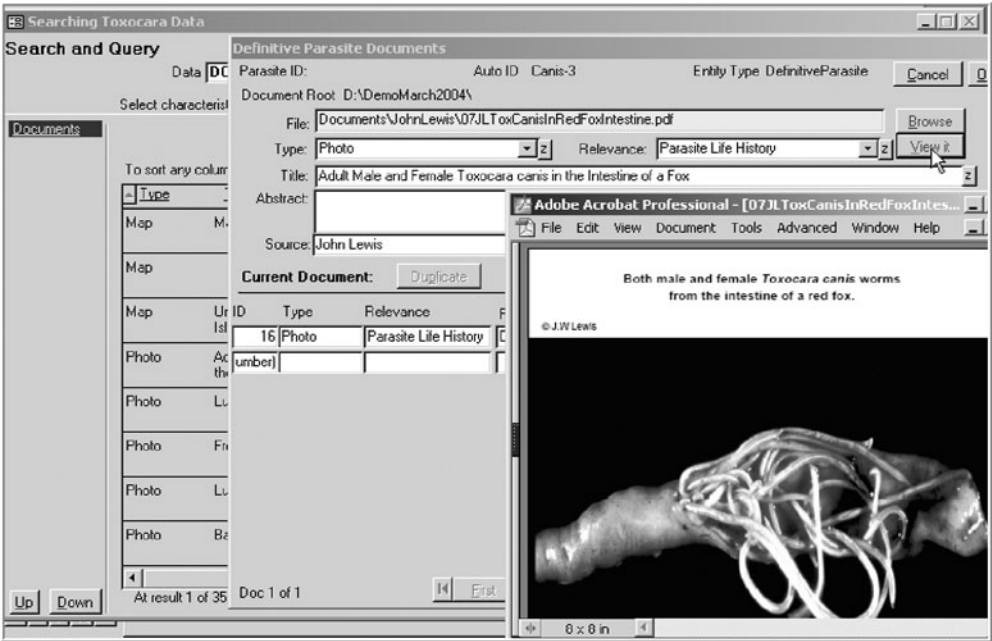


Fig. 13.14. Screen format to show that documents within the database can be linked to any record, e.g. adult worms of *Toxocara canis* from the intestine of the red fox.

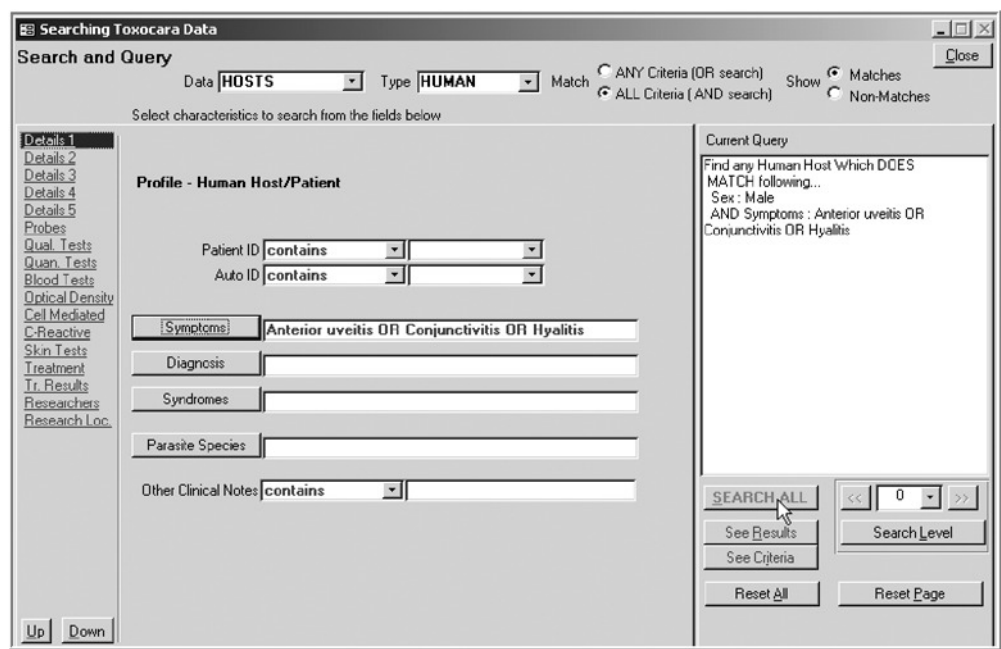


Fig. 13.15. Screen format for searching the database where users can build a complex query using ‘AND’ and ‘OR’ options; in the current query (on the right of the screen) human male patients are searched for the symptoms ‘anterior uveitis’ or ‘conjunctivitis’ or ‘hyalitis’; further parameters can be added as necessary; pressing ‘Search All’ will then produce a results list from which any record can be viewed.

establish a coordinated response to animal health emergencies (OIE, 2004). Such a system could provide the focus for receiving and sharing data on zoonotic infections from non-traditional sources worldwide, including a SparkleSystems web-based global resource on *Toxocara* and toxocarasis. Apart from improving methodology in the diagnosis and treatment of OLM, VLM, covert and other non-specific forms of human toxocarasis, the ‘Sparkle’ data resource addresses questions raised about the role of foxes, cats and rodents in the transmission and spread of *Toxocara* in potentially endemic sites in both the urban and rural environment. Such a centralized resource will considerably enhance our understanding of the epidemiology of human and canine/feline toxocarasis and will provide for the first time an

opportunity for correlating descriptive, experimental and theoretical epidemiological aspects of this disease in zones and countries at risk.

Acknowledgements

Grateful and sincere thanks are extended to Bob and Sue Hughes of SparkleSystems, Inc. (www.sparklesystems.com) for their invaluable input and permission to use the screen dumps as figures in this chapter and to the following for their kind support and constructive comments during the development of the ‘Sparkle’ database, viz: Soledad Fenoy, Celia Holland, Gabriela Hreckova, Christian Kapel, Jean-Francois Magnaval, Hanna Mizgajska, Elena Pinelli and Huw Smith.

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14 Exposure and Environmental Contamination

Hanna Mizgajska-Wiktor¹ and Shoji Uga²

¹Department of Biology and Environmental Protection,
Eugeniusz Piasecki University, School of Physical Education, Poznan, Poland

²Department of Medical Technology, Faculty of Health Sciences,
Kobe University School of Medicine, Sumaku, Kobe, Japan

Introduction

Humans act as paratenic hosts for *Toxocara* spp. and therefore do not spread *Toxocara* eggs, which are a source of infection. Human toxocariasis depends on accompanying definitive hosts, which contaminate the environment with the eggs. The intensity of *Toxocara* infection among dogs, cats, foxes and other *Carnivora*, and the influence of environmental factors on eggs deposited in soil as well as hygiene and sanitation constitute the risk of human exposure to *Toxocara* infection. These risks can, however, be reduced through preventive measures.

Egg Survival

Toxocara spp., like other geohelminths, have eggs with thick and complex shell layers, which protect them from the influence of environmental factors (Fig. 14.1). Under favourable conditions, that is, out of direct sunlight and under appropriate temperature, humidity and oxygen conditions, eggs of *Toxocara* spp. can survive in the soil for several years, although during the first 6 months following deposition from the definitive host, most eggs perish (Dunsmore *et al.*, 1984; Lloyd, 1993). Eggs

can survive on the soil surface throughout the winter even when air temperatures fall to -29°C , but only if they are under snow cover where it is relatively warmer (-0.2°C). Similarly, *Toxocara* eggs at 3 cm depth in a sandpit have been reported to survive even in midsummer, when the sand surface reaches 56°C at maximum (air temperatures, higher than 30°C), but sand temperatures at 3 cm depth reach only 37°C . Therefore, it is thought that the survival of *Toxocara* eggs in sandpits except at the surface is not influenced by air temperature (Uga and Kataoka, 1995). On the other hand, rapid freezing to -40°C and consequent heating in a water bath to 40°C kills eggs quickly, while exposure to sunlight under natural conditions kills them at 37°C (Levine, 1968).

During aerobic waste treatment at 50°C , *Toxocara* spp. eggs perish after 2 h, while it takes 2 days at 45°C . Anaerobic waste treatment kills *Toxocara* spp. eggs at temperatures in excess of 45°C , while lower temperatures only hinder their growth (Reimers *et al.*, 1981). However, *Toxocara canis* eggs can survive for up to 1 year under compost (Pegg and Donald, 1978). In addition to high temperatures, ultrasound also kills *Toxocara* spp. eggs effectively; at 49 kHz and 26 W it takes 9 min to kill *Toxocara* embryos, and only 6 min at 64 kHz and 74 W. It should be noted, however, that *Ascaris* sp. eggs could survive under

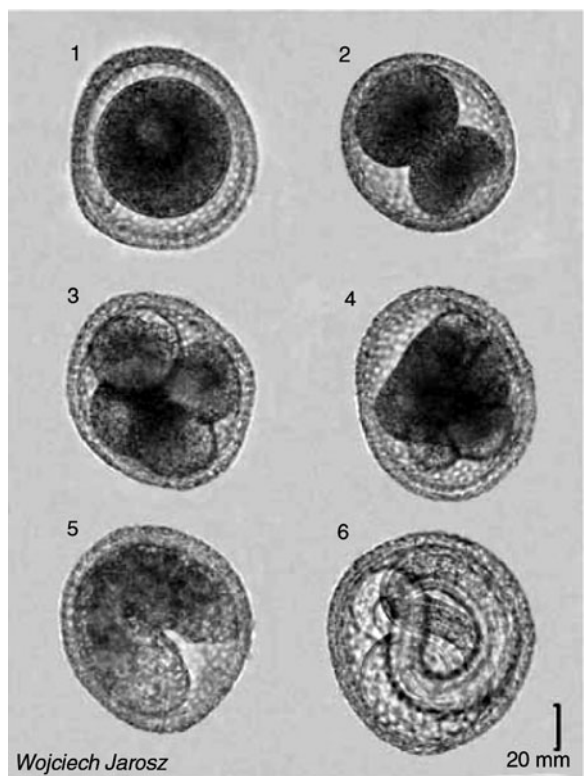


Fig. 14.1. The egg of *Toxocara canis*. 1: one-cell stage, 2: two-cell stage, 3: four-cell stage, 4: early morula stage, 5: blastula stage, 6: larva stage. (Photomicrograph prepared by W. Jarosz.)

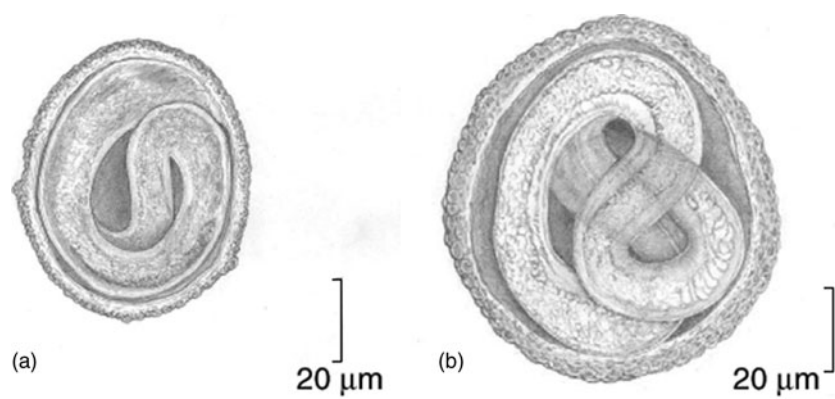


Fig. 14.2. *Toxocara cati* (a) and *T. canis* (b) eggs (Drawing prepared by A. Wiktor).

such conditions (Reimers *et al.*, 1981). Meanwhile, UV radiation is highly detrimental to *T. canis* eggs, as is exposure to continuous darkness (Cuellar del Hoyo *et al.*, 1986).

Toxocara spp. eggs are resistant to many disinfectant solutions and can survive, for example, for 8 days in 40% formalin, 4% lysol and saturated sodium chloride solution, and up to 1 month in

10% formalin and 10% hydrochloric acid (Levine, 1968).

Soil organisms, especially fungi, play an important role in geohelminth egg destruction. For example, there are more than 90 ovicidal fungal species as well as various bacteria with ovicidal properties (Lysek, 1966) that threaten *Ascaris* sp., and it is thought that similar is true for *Toxocara* eggs. Four fungal species have been studied with regard to *Toxocara* spp., of which *Fusarium pallidoreseum* and *Paecilomyces lilacinus* (Thom) are strongly ovicidal, *Paecilomyces marquandii* is relatively ovicidal and *Mucor hiemalis* is weakly ovicidal (Basualdo *et al.*, 2000; Ciarmela *et al.*, 2002).

It should be stressed that *T. canis* eggs have been studied most extensively with regard to *Toxocara* spp. egg survival. The properties of *Toxocara cati* eggs are most probably similar, even though Liberkind (according to Levine, 1968) noted their reduced resistance to environmental exposure and O’Lorcain (1995a) demonstrated that in laboratory conditions they are more resistant to freezing than *T. canis*. In the latter experiment, 68% and 22% of *T. cati* and *T. canis* eggs, respectively, survived for 1 month at temperatures of -7 to -20°C . On the other hand, when the survival of embryonated *T. canis* eggs kept at -20°C for 24 h was examined, no viable eggs, regardless of the presence or absence of cryoprotective agents, were detected (S. Uga, unpublished data).

It is not known what proportion of deposited *Toxocara* spp. eggs develop into the infective form (see Fig. 14.1). For geohelminths it has been established that no more than 5% of deposited *Ascaris suum* eggs survive a period of 17 months in the climatic conditions of Central Europe (Mizgajska, 1993). The survival period of *Toxocara* spp.

eggs, which are less resistant to adverse conditions than *Ascaris* eggs (Reimers *et al.*, 1981), is probably shorter.

Eggs in the Soil Profile

Storey and Philips (1985) were the first to provide experimental data on the penetration of geohelminth eggs in soil. They demonstrated that *Ascaris* sp. eggs deposited on the soil surface subsequently penetrated it and were dispersed by rainwater. Mizgajska (2001) compared the penetrative ability of *T. canis*, *T. cati* and *A. suum* eggs in loose sandy soil under natural conditions. After 1 year, eggs of all three species were found only up to a depth of 3 cm (Table 14.1). Earthworms, which carry living *Toxocara* spp. eggs, cast them from deeper layers to the surface and maintain them in the superficial layer of soil for up to at least 1 year after contamination (Mizgajska, 1997). It is worth noting that the earthworm, a paratenic host of *Toxocara* spp., is the main food source of many small mammals, which are important reservoirs of toxocariasis (Pahari and Sasmal, 1991; Dubinsky *et al.*, 1995). In addition to the earthworm, the cockroach (*Periplaneta fuliginosa*) is also thought to be an important mechanical vector, as cockroaches consume *T. canis* eggs and then excrete them in their faeces over the next 2 days (Takahashi *et al.*, 1990).

Rainfall plays an important role in horizontally distributing *Toxocara* spp. eggs throughout the soil. *Toxocara* spp. eggs in a park were shown to be significantly dispersed after heavy rainfall (precipitation the previous day was 30 mm or more); however, this phenomenon was not observed in a sandpit unaffected by heavy rain (S. Uga, unpublished data).

Table 14.1. Distribution of *Toxocara canis*, *T. cati* and *Ascaris suum* eggs in the soil profile of loose sandy soil 1 year after experimental contamination with 35,000 eggs. (Mizgajska, 1998.)

Depth (cm)	<i>T. canis</i>		<i>T. cati</i>		<i>A. suum</i>	
	Total	Alive	Total	Alive	Total	Alive
0–1	93.8%	88.3%	99.6%	67.1%	93.8%	90.1%
1–2	5.7%	82.6%	0.4%	0	6.2%	91.0%
2–3	0.5%	100.0%	0	0	0	0
3–6	0	0	0	0	0	0
Eggs detected	<i>n</i> = 407	88.0%	<i>n</i> = 232	66.8%	<i>n</i> = 371	90.0%

Soil Contamination

Prevalence of eggs in soil

Human toxocariasis is a consequence of soil contamination with dog, cat and/or fox faeces that contain eggs of *Toxocara* spp. (Richards and Lewis, 1993). The long-term survival of *Toxocara* spp. outside their hosts (up to several years), coupled with the high reproduction of these nematodes (adult females lay about 200,000 eggs daily), is responsible for the significant contamination of soil with infective eggs (Barriga, 1988). Eggs are found in soil worldwide, in public and private places such as backyards, country yards, playgrounds, parks, sandpits, gardens, fields, lakeside beaches, pavements and streets, as well as in kindergartens surrounded by fences (Table 14.2). The degree of soil contamination with eggs was shown to be largely dependent on the number of pets frequenting the area, and the prevalence of *Toxocara* spp. eggs was much higher in city centres than in suburban or rural areas (Glickman and Schantz, 1981; Uga *et al.*, 1989; Mizgajska, 2000). In Poland, eggs were most easily recovered from backyard soil samples (Table 14.3) (Mizgajska, 2001) and in Japan from sandpits (Uga *et al.*, 1996a). However, soil examination methods are not standardized, and therefore it is difficult to compare specific examination results.

Objectivity of methods

In the course of sampling and laboratory analyses many factors influence the soil examination results. These include the sampling site, number and volume of samples, depth of sampling, season of examination, recovery methods, type of soil examined, preservation of samples (time and conditions) and laboratory capability (Mizgajska, 2001).

Sampling site selection

Site selection could follow a specific schedule (Fig. 14.3) or depend on, for example, exposure to sunshine, land configuration, ground structure and cleanliness. In addition, samples might be examined separately or mixed to represent a single area,

that is, a sandpit or backyard, from which small samples (for example, 40 g) might be examined.

Number and volume of samples

The number and volume of collected samples is important when presenting results as a percentage of positive samples and as soil egg concentration.

Depth of sampling

The influence of soil sampling depth on the frequency of geohelminth egg detection was determined between 1980 and 1982 during the course of a study on soil contamination in recreational areas (Mizgajska, 1998). Two hundred soil samples were collected from two layers 0–5 and 6–10 cm deep, respectively, at each site. Geohelminth egg occurrence (*Ascaris* sp., *Trichuris* sp. and *Toxocara* spp.) was four times higher in the upper layer than in the lower layer. Similar results were obtained by Uga (1993) during a study on sandpit contamination; *Toxocara* spp. eggs were seven times more abundant in the first 0–3 cm than at depths of 15–18 and 35–38 cm, respectively.

Sampling season

The degree of soil contamination with *Toxocara* spp. eggs was studied in Poznan, Poland, during different seasons (Mizgajska, 1998). The first finding was that soil contamination with ova, in backyards, was much higher in the spring than in the autumn. This was attributed to the reproductive period of the definitive hosts, the activity of soil organisms and the physical destruction of eggs by high temperatures and sunshine during the summer. Later observations focused on changes in the level of soil contamination throughout the year. Each month, identical volumes and numbers of soil samples were taken from the same sites in six backyards and tested for prevalence of *Toxocara* spp. eggs. Eggs were present in all areas examined with an average prevalence of 16.7–47.7%. Three peaks were observed throughout the year, in December, June and August. The lowest soil contamination was observed in July followed by May (Fig. 14.4) (Mizgajska-Wiktor, 2004). In Japan, the embryonation rates of *Toxocara* spp. eggs recovered from sandpits were highest in au-

Table 14.2. Worldwide soil contamination with *Toxocara* spp. eggs from 1990 to 2003.

Locality	Sources	Samples examined	Positive samples (%)	Reference
Dublin, Ireland	Parks, gardens	53	6	Holland <i>et al.</i> , 1991
	Public playgrounds	228	15	O'Lorain, 1994
London, UK	Parks, gardens	520	6.30	Gillespie <i>et al.</i> , 1991
Praha, Czech Republic	Sewage treatment plants	3	12–47 eggs/100 g sludge	Horak, 1992
Utrecht, Netherlands	Parks, sand-boxes	108	7	Jansen <i>et al.</i> , 1993
Basrah, Iraq	Public places, schools	180	12.50	Mahdi and Ali, 1993
Tokushima, Japan	Sandpits	46	29 sandpits	Shimizu, 1993
Tenerife, Spain	Parks, gardens	54	37	Toledo-Seco <i>et al.</i> , 1994
Havana, Cuba	Residential areas	45	42	Dumenigo and Galvez, 1995
Surabaya, Indonesia	Parks	223	17	Uga <i>et al.</i> , 1995
Kuala Lumpur, Malaysia	Parks, slum areas	89	1	Uga <i>et al.</i> , 1996b
Reykjavik, Iceland	Playground	32	13	Skirnisson and Smaradottir, 1996
Lublin region, Poland	Backyards, playground, sandpits, streets	273	36	Gundlach <i>et al.</i> , 1996
Mexico City, Mexico	Public parks, home gardens	281	12.50	Vasquez <i>et al.</i> , 1996
Osaka, Japan	Sandpits	40 sandpits	30 sandpits	Abe and Yasukawa, 1997
Songkhla, Thailand	Rural villages	102	19	Uga <i>et al.</i> , 1997
Poznan region, Poland (city and villages)	Backyards, beaches, gardens, parks, playgrounds, squares	534	10	Mizgajska, 1997
Cairo, Egypt	Sports clubs, public parks	600	30	Oteifa and Moustafa, 1997
Warnemunde, Germany	Beach sand	126 (seaside resort)	2	Schottler, 1997
Petaling Jaya, Serdang, Malaysia	Public parks, playgrounds	46	55 (urban) 46 (suburb)	Loh and Israif, 1998
Sao Paulo, Brazil	Public parks	120	17.5	Santarem <i>et al.</i> , 1998
Wroclaw, Poland	Backyards	100	6	Mizgajska, 1999

Continued

Table 14.2. (Continued) Worldwide soil contamination with *Toxocara* spp. eggs from 1990 to 2003.

Locality	Sources	Samples examined	Positive samples (%)	Reference
Campo Grande, Brazil	Public parks	74	15 parks	de Araujo <i>et al.</i> , 1999
Central Italy	Public playgrounds	22	12 playgrounds	Giacometti <i>et al.</i> , 1999
Cracow region, Poland (city and villages)	Backyards, recreation areas, streets	160	23	Mizgajska, 2000
Ancona, Italy	Playgrounds	22	14 playgrounds	Giacometti <i>et al.</i> , 2000
Aracatuba, Brazil	Schools' sandboxes	535	0	Nunes <i>et al.</i> , 2000
Kathmandu, Nepal	Street in the city	122	23	Rai <i>et al.</i> , 2000
Ankara, Turkey	Public parks	170	30.6	Oge and Oge, 2000
Buenos Aires, Argentina	Squares and public places	242	13.2	Fonrouge <i>et al.</i> , 2000
Santiago, Chile	Public squares and parks	288 faecal samples	13.5	Castillo <i>et al.</i> , 2000
Elblag, Poland	Backyards, playgrounds	72	14	Jarosz, 2001
Easter, Spain	Public parks	644	1.2	Ruiz de Ybanez <i>et al.</i> , 2001
Sorocaba, Brazil	Public squares	30 squares	16 squares	Coelho <i>et al.</i> , 2001
Resistencia, Argentina	Public park, kindergarten sandpits, housing estates	475	1.3	Alonso <i>et al.</i> , 2001
Marche region, Italy	Farms, parks	6 parks	3 parks	Habluetzel <i>et al.</i> , 2003
Melbourne, Australia	Parks	108	1	Carden <i>et al.</i> , 2003

Table 14.3. Prevalence of *Toxocara* spp. eggs in soil samples in Poland in the 1990s (Mizgajska 2001).

Area examined	Samples examined	Positive samples		Eggs/100 g of soil
		No.	%	
Backyards	549	95	17.3	6.4
Parks	284	24	8.5	4.9
Streets and roads	84	5	6.0	6.0
Playgrounds and sport fields	270	4	1.5	3.8
Sandpits	144	6	4.2	6.3
Lake beaches	175	5	2.9	5.0
TOTAL	1501	139	9.3	6.0

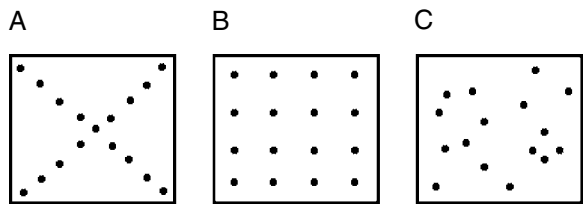


Fig. 14.3. Patterns of sampling. (A) cross-sectional, (B) even, (C) taking into consideration diversity of the environment (Mizgajska, 1998).

tumn (September to November) and lowest in winter (December to February) (Uga, 1993).

Preservation of samples

When soil samples are stored, the eggs undergo quantitative and qualitative changes, the significance of which depends on storage time, soil bioactivity (the occurrence of fungi, bacteria or mites), temperature, humidity and the chemical environment. According to O’Lorcain (1995b), the optimum laboratory condition for *T. canis* egg development is in 0.4% formalin solution at 30°C.

Methods of recovery

There are many methods for recovering *Toxocara* spp. eggs from soil. Flotation, flotation-sedimentation and sieving techniques are the most frequently used (Horn *et al.*, 1990). The efficiency of these techniques in experimental conditions was estimated as over 40% depending on the washing and flotation fluid applied as well as the

soil type (Dada, 1979; Nunes *et al.*, 1994; Smith, 1998). On the basis of years of experimentation, Mizgajska–Wiktor (2005) suggests the modified flotation method of Quinn *et al.* (1980) with the use of saturated sodium nitrate (NaNO₃) as the most useful and efficient for *Toxocara* egg detection (its efficiency rate varies from approximately 65 to 82.5%), while Uga *et al.* (1989) recommend sucrose solution with a specific gravity of 1.20. When selecting a method for *Toxocara* spp. egg recovery, it is important to choose the method that shows the least false-negative results. For this purpose, the recovery rate of the chosen method as well as the sample sizes that can be examined need to be considered.

Laboratory skills

Examination results are largely dependent on the skills of the researcher, notably his/her power of observation during microscopic examinations. Geohelminth eggs (*T. canis*, *T. cati* and *Ascaris* sp.) usually look very much alike, and can only be

distinguished by an experienced researcher (Fig. 14.2).

Type of Soil and Egg Prevalence

Although some physical soil properties (humidity, oxygenation and compactness) influence the survival of eggs, no direct correlation has been found between soil texture (percentage of gravel, sand, clay and silt) and the prevalence of *Toxocara* spp. eggs. This suggests that, apart from soil characteristics, there are other factors that influence the degree of soil contamination with geohelminth eggs; for example, the intensity of contamination or action of invertebrates (Mizgajska, 1997).

T. canis and *T. cati* Egg Discrimination

The infective eggs of *T. canis* and *T. cati* are similar both in size and morphology (Fig. 14.2). Although, egg size is usually determined as the minimum and maximum diameter, or as the average diameter, their reported sizes vary according to the author. According to Stefański (1968), *T. canis* eggs are almost spherical and 66–85 by 64–77 μm , while *T. cati* are spherical and 66–77 μm . Thienpont *et al.* (1979) on the other hand reported that *T. canis* and *T. cati* eggs are 75–90 and 65–75 μm in diameter, respectively, while both have a characteristically thick and folded eggshell. Averbeck *et al.* (1995) found that *T. canis* eggs are subspherical to ellipsoid and measure on average 80 by 90 μm . By examining eggs isolated from the uteri of adult female nematodes, Mizgajska and Rejmenciak (1997) found that both *T. canis* and *T. cati* eggs differ in size and fall within a different size range. In most cases (75%), they found *T. canis* eggs to be 78–88 by 69–74 μm , while *T. cati* eggs were commonly (91%) 59–74 by 54–69 μm . The maximum and minimum lengths of the *T. canis* and *T. cati* eggs were 98 and 78 μm and 74 and 59 μm , respectively. Uga *et al.* (2000) reported that the major and minor axes of *T. canis* eggs are 71.6–91.2 by 63.4–79.0 μm , while those of *T. cati* eggs are 63.7–88.1 by 53.3–73.3 μm .

An attempt was made by Mizgajska and Rejmenciak (1997) to determine the morpho-

logical differences between *T. canis* and *T. cati* eggs using light microscopy. They found that both eggs vary in shape from spherical to oval and with regard to their eggshells, those of *T. canis* are thicker, less transparent and with visible semicircular cavities on the surface (Fig. 14.2). Uga *et al.* (1989) reported that the contamination ratio of *T. canis* and *T. cati* in sandpits in Japan was 1:3; this was determined by measurements of egg size. But despite the differences mentioned above, discrimination between *T. canis* and *T. cati* eggs is not easy. Thus, many authors, when studying the degree of soil contamination with *Toxocara* spp. eggs, make no distinction between species (for example, Collins and Moore, 1982; Duwel, 1984; according to Barriga, 1988). Uga *et al.* (1989, 2000) rightly point out that the most accurate method of egg discrimination is scanning electron microscopy as it allows precise visualization of the structure of the different eggshell layers, which are characteristic for each species, but this technique is impractical for the routine study of soil contamination worldwide. Mizgajska and Jarosz (2000) determined *Toxocara* eggs based (as described above) on the size of eggs and transparency of the shell layers. During this study, in some eggs only generic features were revealed; therefore genetic confirmation of the microscopic observations was undertaken. The polymerase chain reaction technique, using the specific genetic markers constructed by Jacobs *et al.* (1997) for identification of *T. canis* and *T. cati*, was applied. This method appeared to be simple, sensitive and efficient – it was possible to identify the species of *Toxocara* eggs isolated from the soil by flotation technique, irrespective of their number, the stage of development of the embryo and without using anti-inhibitors (Fogt *et al.*, unpublished observations).

Exposure

Infective *Toxocara* spp. eggs in soil are a major source of human toxocariasis, although there are also other sources of infection. Beer *et al.* (1999) confirmed *Toxocara* infection among children as a result of involuntary ingestion of water in urban non-flowing reservoirs. Nakagura *et al.* (1989) also described toxocariasis among Japanese individuals who had eaten raw chicken livers from chickens kept in farmyards with dogs. Furthermore, in

Switzerland, *Toxocara* antibodies were found in children who had eaten rabbit offal or undercooked meat (Struchler *et al.*, 1990). Since it takes at least 2–3 weeks for deposited eggs to develop into the larval stage (Fig. 14.1), infection through direct contact with dogs or cats seems unlikely; however, Wolf and Wright (2003) found infective *T. canis* eggs in dog hair and consequently suggest otherwise. It has also been demonstrated that vegetables such as carrots or radishes, if not washed properly, can also be a source of toxocariasis (Vazquez *et al.*, 1997).

Toxocara spp. eggs are found in soil all over the world (Barriga, 1988; Glickman, 1993; Mizgajska, 1998). In the absence of objective methodology, studies of *Toxocara* spp. prevalence in natural environments with regard to the countries or regions with the highest risk of exposure are difficult. *Toxocara* spp. eggs are found in soil samples in both developing and developed countries, and contamination is high, for example, in Germany (87% positivity), Japan (42%), Norway (39%) and the UK (66%) (Glickman, 1993; Mizgajska, 1998). Many authors believe that the degree of soil contamination with *Toxocara* spp. eggs is greater in rural than urban areas (for example, Abo-Shehadeh, 1989; Conde Garcia *et al.*, 1989; Holland *et al.*, 1995). An extensive study conducted in Poland in the 1990s, during which 1184 and 590 soil samples were collected from six cities and seven villages, respectively, indicated a similar distribution of soil contamination with *Toxocara* spp. eggs (14 and 12% positivity in rural and urban areas, respectively). Mizgajska (2001) considered that the most contaminated areas are backyards and gardens where 6.9 eggs/100 g soil have been found. In the centre of Poznan city, Poland, where soil contamination with *Toxocara* spp. eggs is high (38–53% of positive soil samples), the prevalence of toxocariasis among children (7.9% seropositivity) was more than three times higher than the average for the Poznan region (2.4% seropositivity) (Mizgajska, 1997; Luzna-Lyskov, 2000; Paulowski and Mizgajska, 2002). Moreover, analysis of these results revealed that the exposure of children (0–15 years) to *Toxocara* spp. was in proportion to the level of contamination in this area (Fig. 14.5) (Mizgajska *et al.*, 2002).

Although *T. canis* and *T. cati* can cause human toxocariasis, it is often accepted that dogs are the major disseminators of human-infective eggs for the following reasons: dogs are more often in direct

contact with people than cats, *T. canis* appears to be more prevalent in paratenic hosts and cats usually select sandy soil for defecation and tend to bury their faeces, thus preventing egg transmission (Overgaaauw, 1997a). Furthermore, studies conducted in Iceland showed that the role of cats in causing toxocariasis in people is insignificant. *Toxocara* spp. eggs were detected in sandpits in Iceland (9% of samples), and as pet dogs are banned here, cats were identified as the source; however, *Toxocara* antibodies were not found either in adults or children (Woodruff *et al.*, 1982; Skirnisson and Smaradottir, 1996). Despite this, many authors claim that the role of cats in *Toxocara* egg contamination and in causing human infections is underestimated (Uga *et al.*, 1995, 1996a; Overgaaauw, 1997b; Mizgajska, 1998). Twenty-four-hours-a-day observations of sandpits in the city of Kobe, Japan, as well as morphological examinations of the eggs recovered from soil in urban areas, indicated that in places where children play, soil contamination with *T. cati* eggs is usually much higher than *T. canis* contamination (Luzna, 1996; Uga *et al.*, 1996a; Mizgajska, 2001). In Poznan, of 3472 eggs recovered during a year-long study of backyards, 89.9% were recognized as *T. cati* and the remainder as *T. canis* (Fig. 14.6) (Mizgajska-Wiktor, 2004).

There is, therefore, no conclusive answer as to which species, *T. canis* or *T. cati*, has a bigger share in global soil contamination with infective eggs. In addition, it is not known which of these nematodes invades human tissue more often, as the immunological methods generally used to detect the invasion of humans do not allow for such identification.

Countermeasures

Soil contamination with *Toxocara* spp. eggs has been reported all over the world (Table 14.2), but active countermeasures to curtail the transmission of *Toxocara* from pets to humans have not been suggested. In early studies of toxocariasis, washing hands after playing in sand (Emehelu and Fakae, 1986; Schantz and Stehr-Green, 1988), eliminating intestinal parasites from pets, (Schantz and Stehr-Green, 1988; Schantz, 1989), fencing off sandpits, (Duwel, 1984; Snow *et al.*, 1987), educating pet owners (Worley *et al.*, 1984) and reduction of roaming dogs (Barriga, 1988)

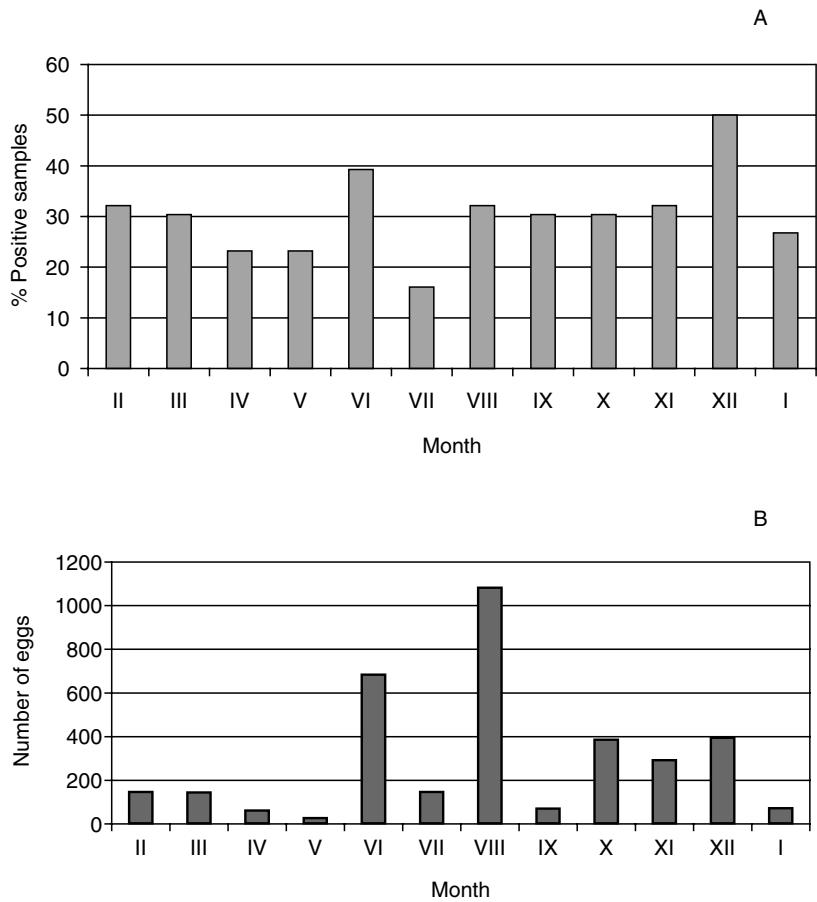


Fig. 14.4. Degree of soil contamination in city backyards throughout the year. (A) Prevalence; (B) number of eggs recovered each month. (Mizgajska-Wiktor, 2004)

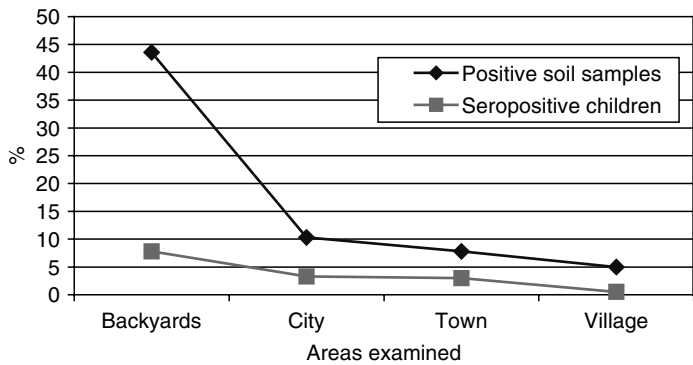


Fig. 14.5. Relationship between the degree of soil contamination with *Toxocara* spp. eggs and the prevalence of toxocariasis among children in Poznan region, Poland (Mizgajska *et al.*, 2002).

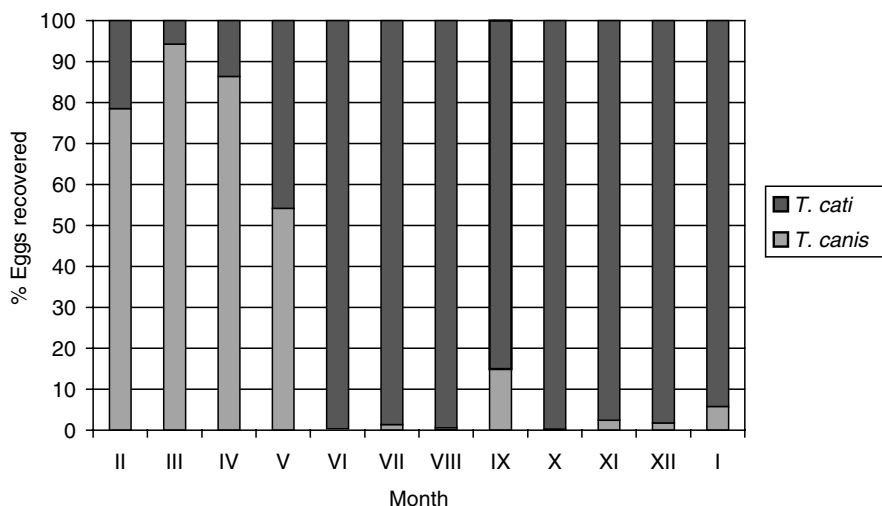


Fig. 14.6. The proportion of *Toxocara canis* and *T. cati* eggs in soil samples from city backyards (Poznan, Poland) (Mizgajski-Wiktor, 2004).

were all proposed as prophylactic measures. Evidence of the effect of these measures is, however, not available. Schantz and Stehr-Green (1988) proposed that not allowing children to play in areas contaminated with eggs was unrealistic as most play areas are contaminated. Contrary to this, many trials against egg contamination in sandpits in public parks in Japan have been conducted, and countermeasures consisting of two courses of action were suggested, cleaning contaminated sandpits and maintaining sandpits, once they were treated.

Methods for cleaning contaminated sandpits

The typical method for cleaning contaminated sandpits is to replace the contaminated sand. However, this method is not effective, because one to nine new faecal deposits per sandpit were found daily, with eggs reappearing in the sand 6–9 weeks after replacement (Uga and Kataoka, 1995). Furthermore, the costs of providing clean sand and disposing of contaminated sand (treated as industrial waste) is expensive. Commercially available bactericidal ceramics, which contain silver ions, were developed for disinfecting sand in sandpits. These ceramics dissolve very slowly in

water, releasing silver ions and disinfecting the microorganisms in sandpits. The lethal effect on *T. canis* eggs was confirmed *in vitro*, but a field survey is yet to be conducted. Special machines that make use of dry and wet heat have also been developed for sterilizing sand (Fig. 14.7), and one local government in Japan succeeded in keeping *Toxocara* spp. egg contamination at a low level with repeated dry heat sterilization.

Methods for maintaining the cleanliness of treated sandpits

Various methods for maintaining treated sandpits have been evaluated. Duwel (1984) and Snow *et al.* (1987) reported the usefulness of fencing off playgrounds, but in Japan, the effectiveness of placing fences (90 cm high with a 1 m wide gate) around sandpits has not been confirmed. In addition, mothers often dislike the idea of their children playing in fenced-off sandpits. No live *Toxocara* spp. eggs have been detected in sandpits built under highways or in those that are roofed. But, such sandpits are often not suitable as play areas because the sand is often too dry to allow children to build sandcastles and tunnels. The prohibition of pets is often enforced in parks, but this method is not effective in Japan because the cause of animal

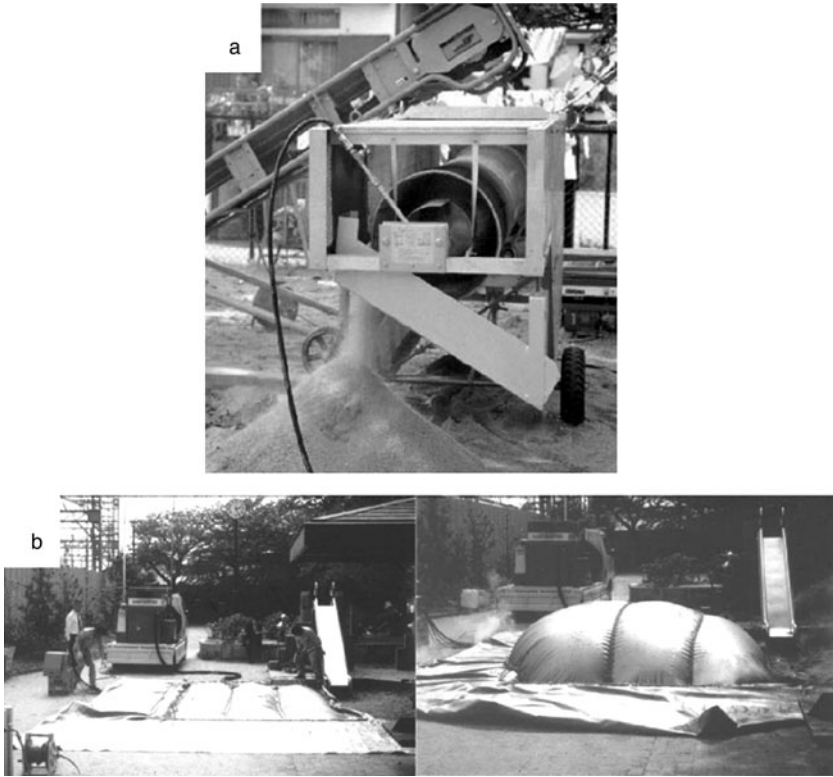


Fig. 14.7. (a) A heat sterilizer used to treat contaminated sand. Just after treatment the temperature increases to above 100°C, therefore killing not only the *Toxocara* spp. eggs but also any bacteria present. The contaminants mixed with sand can be removed by filtration during this procedure. (b) A wet sterilizer placed on a car. Steam is introduced below a sheet placed on the sandpit. It is possible for one individual to operate this machine, but stones, woodchips, and pieces of broken glass within the sandpit cannot be removed by this procedure.

faecal contamination is reportedly cats (Uga *et al.*, 1996a). A trial by kindergarten teachers in Japan, which involved covering sandpits with vinyl sheets at night (80% of defecation occurs between 06:00 and 18:00) proved ineffective, because no teachers were able to continue this for the 3 month study period. A questionnaire survey revealed that most teachers found this procedure too time-consuming. Repellents which deter cats were effective in reducing faecal contamination to a third of the original level; however, application is required every 2 months. Recently, the authors evaluated the effects of a machine that sprays water at cats and dogs when they enter a sandpit. This system utilizes thermosensors, which are used for automatic doors. The system was evaluated for 3 months using a camcorder, thus revealing its success; no

cats defecated in the sandpits under this system. However, it can only be used in limited places such as kindergartens, private gardens, or elementary schools because it is unsociable for people to be showered with water when visiting sandpits.

Conclusions

1. Soil contamination with the eggs of *Toxocara* spp. occurs worldwide, irrespective of socioeconomic and developmental status.
2. There is a clear connection between the degree of soil contamination with *Toxocara* spp. eggs and the prevalence of toxocariasis among people.

3. The method of recovery of *Toxocara* spp. eggs in soil should be standardized.
4. Species recognition of *Toxocara* eggs isolated from soil during the environmental studies will allow better prevention.
5. Destroying the eggs of *Toxocara* spp. is difficult because of their resistance to adverse conditions, easy spreading and longevity. Liquidation of the eggs in soil is now possible but only in limited areas such as sandpits or soil in gardens. There are still no methods which could be used extensively in semi-natural areas.
6. Effective control of toxocariasis should include: prevention of contamination of public places with pet faeces, systematic disinfestations

of definitive hosts and development of educational programmes aimed at increasing public awareness of potential zoonotic threats.

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15 Baylisascariasis: an Emerging and Potentially Fatal Form of Larval Migrants

Corey S. Watts, Jennifer L. Liang and Peter M. Schantz
*Division of Parasitic Diseases, National Center for Infectious Diseases,
Centers for Disease Control and Prevention, Atlanta, Georgia, USA*

Introduction

In continental USA, when patients present with eosinophilic meningitis with no recent history of travel to tropical areas, alert physicians consider *Toxocara* spp. infection high among the list of differential diagnoses. However, in recent years a new helminth agent of severe neurological larva migrans was identified: *Baylisascaris procyonis*. This common intestinal ascarid of the raccoon, *Procyon lotor*, was identified as the causative agent in young patients with severe and ultimately fatal eosinophilic meningoencephalitis (Huff *et al.*, 1984; Fox *et al.*, 1985). Subsequently, with greater awareness and availability of diagnostic services, an increasing number of cases have been attributed to this nematode parasite of a mammalian host that has emerged as a common urban and suburban pest (Prange *et al.*, 2003).

Life cycle

Baylisascaris spp. are classified as Phylum Nematoda, Order Ascaridida, Superfamily Ascaridoidea. These adult worms can reach a size of 20–22 cm long in the female and 9–11 cm long in the male (Kazacos, 2001). The raccoon is the natural definitive host of

B. procyonis. Infection in the raccoon is generally subclinical and adult worms reside in the small intestine. Raccoons usually acquire infection through ingestion of paratenic hosts (rodents, birds, rabbits), but may also ingest embryonated eggs during feeding and grooming activities (see Fig. 15.1). Infected raccoons can shed millions of eggs in their faeces daily, as adult female *B. procyonis* worms are extremely prolific (115,000–179,000 eggs per worm/day) (Kazacos, 2001). These eggs may remain viable for years in moist soil, and are extremely resistant to environmental degradation and attempted decontamination. With adequate temperature, moisture and aeration, *B. procyonis* eggs can reach infectivity (second-stage larva) in 11–14 days (Kazacos, 2001).

As with *Toxocara* spp., transmission of *B. procyonis* to humans occurs directly through ingestion of embryonated eggs from the soil or from contaminated hands or toys. Eggs of *B. procyonis* are sticky and tend to adhere to surfaces and objects (Gavin *et al.*, 2002; Murray and Kazacos, 2004). When ingested, the eggs hatch and larvae are released. After penetrating the gut wall, the larvae migrate through the liver and lungs. They then enter the vascular system and are disseminated into various tissues (viscera, muscles, eyes, central nervous system (CNS)). This aggressive and widespread migration can lead to clinical visceral larval

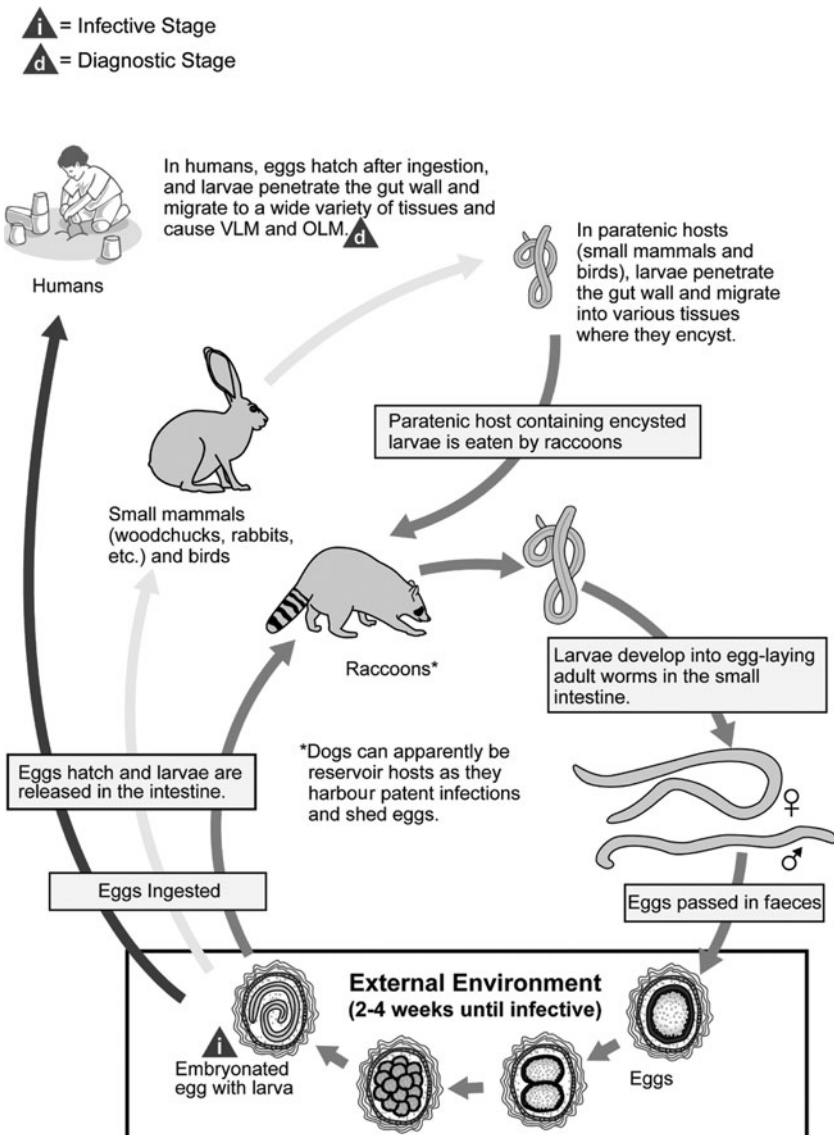


Fig. 15.1. *Baylisascaris procyonis* completes its life cycle in raccoons (*Procyon lotor*), with humans acquiring the infection as accidental hosts. Following ingestion by many different hosts (over 50 species), eggs hatch and larvae penetrate the gut wall and migrate into various tissues, where they encyst. When raccoons eat these hosts, the larvae develop into egg-laying adult worms in the small intestine and eggs are eliminated in raccoon faeces. Humans, particularly children, become accidentally infected when they ingest infective eggs in the environment. After ingestion, the eggs hatch and larvae penetrate the gut wall and migrate to a wide variety of tissues (liver, heart, lungs, brain, eyes), and cause visceral (VLM) and ocular (OLM) larva migrans syndromes, similar to toxocariasis. Tissue damage and the signs and symptoms of baylisascariasis are often severe because of the size of *Baylisascaris* larvae, their tendency to wander widely and the fact that they do not readily die.

migrans (VLM), ocular larval migrans (OLM) or neural larval migrans (NLM) in a variety of paratenic hosts including dogs, rabbits, birds and humans (Kazacos, 2001).

B. procyonis is endemic in North American raccoons with prevalences as high as 68–82% in raccoon populations in the Midwest, Northeast and West Coast (Fig. 15.2). Recent surveys in Europe have detected the parasite in imported raccoons; however, the host species is almost exclusively limited to North America (Gey, 1998). In recent decades, raccoons have readily adapted to human environments, and are increasingly found in urban and suburban areas throughout the USA (Prange *et al.*, 2003). Their peculiar defecation habits may contribute to areas of high *B. procyonis* egg concentrations in the soil. ‘Latrines’, the preferred communal sites where raccoons defecate, can be found frequently at the base of trees, raised flat surfaces such as large logs, old tree stumps, wooden decks, patios and rooftops (Kazacos, 2001; Page *et al.*, 1999). Patent *Baylisascaris* infections in raccoons are typically more common in autumn than at other times of the year (Kidder *et al.*, 1989).

A sporadic yet significant finding is that dogs may act as either paratenic hosts (harbouring larval forms in tissues) or adult hosts of *Baylisascaris*. Adult

worm infections have been demonstrated in dogs from Iowa, Minnesota and Prince Edward Island (Greve and O’Brien, 1989; Averbeck *et al.*, 1995; Conboy, 1996). These dogs were presumably infected by consuming paratenic hosts such as small mammals or by inadvertently ingesting eggs in host faeces. In each case, positive identification was made of *Baylisascaris* eggs in the canine faeces as well as adult worms in the intestinal tract (Bowman, 2000). However, none of the documented human baylisascariasis cases to date has been associated epidemiologically with dogs.

Pathogenesis

Pathogenesis of *Baylisascaris* is similar to that of *Toxocara* spp., but the resulting disease is usually more severe. *B. procyonis* migration is more aggressive than *Toxocara* spp., often involving the CNS within 2–4 weeks of ingestion. Furthermore, unlike *Toxocara* spp., *B. procyonis* larvae increase in size while migrating (Kazacos, 2001). The combination of these factors ensures that severe neurological disease is likely with sufficient oral inoculum. Neurological disease is further complicated by delayed encapsulation and prolonged



Fig. 15.2. Geographic occurrence of *Baylisascaris procyonis* infection in raccoons in the USA. Shaded states indicate positive reports of intestinal *Baylisascaris procyonis* in raccoons according to published reports cited in Kazacos (2001). Data is approximate and limited by the absence or limitations of published surveys in some states.

migration of *B. procyonis* larvae within the CNS as compared with other tissues (Kazacos, 1997). In addition, the migrating larvae secrete and excrete toxic products, eliciting a marked peripheral and cerebrospinal fluid (CSF) eosinophilic inflammatory response. This delayed and relatively ineffective host inflammatory response is neurotoxic, and probably contributes to the severe encephalitis and neuroretinitis that often occurs in baylisascariasis (Moertel *et al.*, 2001).

Clinical Manifestations

The clinical manifestations of human baylisascariasis range from mild to severe. As in toxocariasis, the rapidity of progression and severity of clinical disease are dependent upon dose and migration patterns of the larvae, especially the number of larvae entering the CNS or eye (Kazacos and Boyce, 1989; Kazacos, 2000, 2001). The most severe manifestation of baylisascariasis is acute, fulminant NLM which is usually seen in individuals who have apparently ingested large numbers of *B. procyonis* eggs (Kazacos, 2000). Clinical symptoms in such cases include sudden lethargy, loss of muscle coordination, decreased head control, torticollis, nystagmus, stupor progressing to coma, ataxia and death. Those with lighter infections usually only suffer milder clinical disease (e.g. lethargy, irritability) or may remain asymptomatic (Gavin *et al.*, 2002). Larvae can also migrate throughout the body resulting in fever, hepatomegaly and respiratory disease.

OLM without other clinical manifestation may be a sequela to ingestion of smaller numbers of larvae. Baylisascariasis has been implicated in diffuse unilateral subacute neuroretinitis (DUSN) due to larval migration damage and related inflammation (Goldberg *et al.*, 1993). OLM may also be seen in conjunction with NLM as a result of larval dissemination associated with heavy infection (Park *et al.*, 2000; Rowley *et al.*, 2000; Kazacos, 2001).

History

The first confirmed cases of baylisascariasis in humans were described in 1984 and 1985 in two young boys: a 10-month-old from Pennsylvania

and an 18-month-old from Illinois (Huff *et al.*, 1984; Fox *et al.*, 1985). These boys presented with a rapidly progressive and ultimately fatal eosinophilic meningoencephalitis (Kazacos *et al.*, 2002). An earlier unconfirmed case of NLM was documented in 1975 in an 18-month-old girl from Missouri, who presented with clinical signs and laboratory findings consistent with baylisascariasis, such as acute hemiplegia, CSF and peripheral eosinophilia and elevated iso-haemagglutinins (Anderson *et al.*, 1975). Existing technology at that time was not able to differentiate this infection from other larval migrans syndromes; therefore the diagnosis was presumptive based upon these clinical signs and laboratory findings (Kazacos *et al.*, 2002).

Baylisascariasis is becoming increasingly recognized for its role as a cause of severe and fatal NLM in young patients. Since 1984, at least 12 cases of *B. procyonis* have been diagnosed and reported in the USA in Pennsylvania, Illinois, California, New York, Oregon, Michigan and Minnesota (Huff *et al.*, 1984; Fox *et al.*, 1985; Goldberg *et al.*, 1993; Cunningham *et al.*, 1994; Gavin *et al.*, 2000; Park *et al.*, 2000; Rowley *et al.*, 2000; Moertel *et al.*, 2001; Kazacos *et al.*, 2002) (see Table 15.1).

These cases were reported by authors who confirmed the diagnosis of *B. procyonis* infection based upon one or more of the following criteria: histological evidence of *B. procyonis* larvae from brain biopsy or autopsy, serological evidence, pathological evidence or epidemiological evidence. All cases with confirmed *B. procyonis* infections were in males ranging in age from 10 months to 29 years. This gender predominance is considered more likely a function of the play-habits of boys rather than an inherent increased susceptibility to infection (Gavin, 2002). Ten of the 12 cases were children aged 9 months to 6 years; eight children were less than 2 years of age. As with toxocariasis, children are at a higher risk of acquiring infection due to behavioural characteristics such as geophagia (pica) and poor hygiene. A history of geophagia and/or pica was documented in six cases (Huff *et al.*, 1984; Fox *et al.*, 1985; Gavin *et al.*, 2000; Park *et al.*, 2000; Kazacos *et al.*, 2002). Of the six with geophagia, four were reported developmentally disabled, one with Down's syndrome, and another with Klinefelter syndrome (Fox *et al.*, 1985; Moertel *et al.*, 2001; Gavin *et al.*, 2002; Kazacos *et al.*, 2002).

Table 15.1. Summary of known or presumptive human *Baylisascaris* clinical infections in the USA (1975–2002).

Year*	Age	Sex	Location	Clinical Manifestation	Risk Factors	Outcome	Reference
1980	18 mo	F	Missouri	Eosinophilic meningoencephalitis	Pica	Non-fatal	Anderson <i>et al.</i> (1975)
1980	10 mo	M	Pennsylvania	Eosinophilic meningoencephalitis	Geophagia	Fatal	Huff <i>et al.</i> (1984)
1984	18 mo	M	Illinois	Eosinophilic meningoencephalitis	Geophagia, Down's syndrome	Fatal	Fox <i>et al.</i> (1985)
1990	29 yr	M	California	Diffuse unilateral subacute retinitis		Non-fatal	Goldberg <i>et al.</i> (1993)
1990	13 mo	M	New York	Eosinophilic meningoencephalitis		Non-fatal, severe neurological deficits	Cunningham <i>et al.</i> (1994)
1986	21 yr	M	Oregon	CNS disease	Geophagia, pica, developmentally disabled	Non-fatal	Cunningham <i>et al.</i> (1994)
1994	12 yr	M	Washington	Diffuse unilateral subacute retinitis		Non-fatal	C.G. Wells, unpublished correspondence (1994)
1998	10 yr	M	Massachusetts	Eosinophilic cardiac pseudotumor	Hyperkinetic syndrome	Fatal	Boschetti and Kasznicka (1995)
1998	11 mo	M	California	Eosinophilic encephalitis	Geophagia	Non-fatal, severe neurological deficits, seizures	Park <i>et al.</i> (2000)
1998	13 mo	M	California	Eosinophilic meningoencephalitis, retinitis		Non-fatal, severe residual neurological deficits, seizures	Rowley <i>et al.</i> (2000)
1996	6 yr	M	Illinois	Progressive encephalopathy, diffuse unilateral subacute retinitis	Geophagia, pica, developmental delay	Non-fatal, residual neurological deficits, seizures	Gavin <i>et al.</i> (2002)
2000	2.5 yr	M	Illinois	Progressive encephalopathy	Geophagia, pica	Non-fatal, severe neurological deficits, cortical blindness	Kazacos <i>et al.</i> (2002)
1999	13 mo	M	Minnesota	Eosinophilic meningoencephalitis		Fatal	Moertel <i>et al.</i> (2001)
1999	19 mo	M	Minnesota	Eosinophilic meningoencephalitis	Klinefelter syndrome	Fatal	Moertel <i>et al.</i> (2001)
2002	11 mo	M	California	Eosinophilic meningoencephalitis		Non-fatal	Murray and Kazacos (2004)
2000	17 yr	M	California	Eosinophilic meningoencephalitis	Geophagia, severe developmental disabilities	Fatal	Kazacos <i>et al.</i> (2002)

* Year of diagnosis reported when made available in literature.

The duration, clinical manifestations and progression of the infection have been highly variable and apparently dependent upon dose and migration patterns of the larvae, especially the number of larvae entering the CNS or the eye (Kazacos and Boyce, 1989; Kazacos, 2001). Clinically, 11 individuals developed progressive CNS disease; eight had evidence of eosinophilic meningoencephalitis (Huff *et al.*, 1984; Fox *et al.*, 1985; Cunningham *et al.*, 1994; Rowley *et al.*, 2000; Moertel *et al.*, 2001; Kazacos *et al.*, 2002). Two were reported to also have developed ocular disease, retinitis in one individual and DUSN in the other (Rowley *et al.*, 2000; Gavin *et al.*, 2002). One individual did not exhibit any signs of CNS disease, but was diagnosed with DUSN (Goldberg *et al.*, 1993). Five of the 12 reported cases were fatal (Huff *et al.*, 1984; Fox *et al.*, 1985; Moertel *et al.*, 2001; Kazacos *et al.*, 2002). Of the other seven cases, five developed severe neurological or residual deficits (Goldberg *et al.*, 1993; Cunningham *et al.*, 1994; Park *et al.*, 2000; Rowley *et al.*, 2002; Gavin *et al.*, 2002; Kazacos *et al.*, 2002).

In addition to the 12 confirmed cases, there have been four reported presumptive cases of *B. procyonis* infection. These presumptive cases were never confirmed based upon strict diagnostic criteria, but, retrospectively, the evidence is supportive for *B. procyonis* infection. The first case was reported in 1975 in an 18-month-old female from Minnesota with a history of pica (Anderson *et al.*, 1975). The second case was in a 21-year-old male from Oregon with CNS disease (Cunningham *et al.*, 1994). The patient was seropositive for *B. procyonis* and had characteristic lesions from a brain biopsy. He lived at an institution for the developmentally disabled, and had a history of pica and geophagia (Cunningham *et al.*, 1994). In 1994, a 12-year-old male from Washington developed DUSN (Wells, 1994, personal communication). The family history documented having seven raccoons with some raised from cubs (Wells, 1994, personal communication). Finally, *B. procyonis* was suspected in a 10-year-old male from Massachusetts who died a sudden, unexpected death in 1995 (Boschetti and Kaszinica, 1995). The autopsy finding was an eosinophilic cardiac pseudotumour induced by VLM (Boschetti and Kaszinica, 1995). The morphometry of the larval remains were strongly suggestive of *B. procyonis*.

Diagnosis

One of the difficulties of diagnosing baylisascariasis is the lack of pathognomonic signs or symptoms (Gavin and Shulman, 2003). Progressive encephalopathy with or without retinitis, peripheral and CSF eosinophilia and radiographic evidence of diffuse white matter disease are together strongly suggestive of *B. procyonis*; however, other potential agents of larval migrans, such as *T. canis*, must be ruled out (Kazacos *et al.*, 2002). Unfortunately, the diagnosis of baylisascariasis is often late as it is usually only considered in the presence of eosinophilic meningoencephalitis, a stage of clinical disease in which major neurological damage has already been sustained. Currently, standard confirmation of a diagnosis of *Baylisascaris* infection is accomplished with a brain biopsy (Bonn, 2002). If present, larvae may be easily recognized in histopathological study by their distinctive morphology (lateral alae, triangular excretory columns) and size (60–70 μm in diameter). However, the procedure is invasive and the biopsy sample has a low probability of yielding a nematode larva (Bonn, 2002). More commonly, diagnoses may be made with CSF or serum samples by detecting *Baylisascaris* antibodies with larval excretory–secretory antigens (ELISA or IFA) (Gavin and Shulman, 2003). Immunofluorescence assays using frozen sections of *B. procyonis* third-stage larvae, or enzyme immunoassays using excretory–secretory antigens are sensitive and specific (do not give cross-reactions with sera of patients with *Toxocara* infections) (Kazacos, 2001). Elevations in serum isohaemagglutinins, resulting from the cross-reaction between larval glycoproteins and human blood group antigens may also support diagnosis of baylisascariasis (Gavin and Shulman, 2003). As in toxocariasis, larval stages do not reach the intestine and develop to the adult worm in humans; therefore demonstration of the eggs or larvae in faeces is highly unlikely. Totally non-invasive diagnostic imaging modalities such as computed tomography scan or magnetic resonance imaging may reveal lesions ranging from cerebellar and periventricular deep white matter abnormalities to global atrophy (Cunningham *et al.*, 1994; Moertel *et al.*, 2001). Pathological changes visualized by neuroimaging, however, lag behind severe clinical manifestations (Gavin *et al.*, 2002).

Treatment

The prognosis of *B. procyonis* NLM is grave with or without treatment (Kazacos, 2000, 2001). Albendazole, mebendazole, thiabendazole, levamisole and ivermectin have been unsuccessful in resolving NLM, but may help halt progression of the disease (Anon., 2002). The majority of damage to the CNS probably occurs before the onset of symptoms and diagnosis of the helminth (Kazacos, 2001; Kazacos *et al.*, 2002). As a result, survivors of the disease have profound neurological complications and are severely incapacitated despite treatment (Roussere *et al.*, 2003). The condition may even worsen as the brain undergoes post-inflammatory atrophy (Roley *et al.*, 2000).

Initiation of empirical anthelmintic treatment in humans within 1–3 days of suspected *Baylisascaris* exposure may prevent development or stop the progression of CNS disease by killing larvae before they enter the CNS (Kazacos *et al.*, 2002; Gavin and Shulman, 2003). Albendazole is currently the drug of choice for humans due to its good distribution into the CNS and relatively low side effects (Murray and Kazacos, 2004). Dexamethosone or other anti-inflammatory agents are recommended in combination with the anthelmintics to help control the eosinophilic immune reaction.

In the case of OLM, if an intraretinal larva is observed on ophthalmoscopic examination, the most efficacious treatment is direct laser photocoagulation of the larva (Goldberg *et al.*, 1993). Photocoagulation is a safe and effective means of destroying the larva, preventing further damage to the retina and inflammation caused by larva migration (Raymond *et al.*, 1978; Gass and Braunstein, 1993; Goldberg *et al.*, 1993; Kuchle *et al.*, 1993). Factors that determine visual improvement after treatment are location and extent of intraocular damage and resolution of intraocular inflammation (Kazacos, 2001).

Prevention

Prevention of baylisascariasis is critical as there are no viable treatment options for this disease. In areas where infected raccoons occur, education of the public is the most important measure for preventing human infection. Paediatricians, nurse practitioners, veterinarians and public health

officials should be at the forefront of public education and awareness of the inherent danger with raccoon faeces. The potential use of *Baylisascaris* eggs as an agent of bioterrorism has increased awareness and concern about this zoonotic ascariid (Sorvillo *et al.*, 2002).

Several anthelmintics, such as piperazine citrate (120–240 mg/kg), pyrantel pamoate (6–10 mg/kg) and fenbendazole (50–100 mg/kg \times 3–5 days) have been effective in treating intestinal *B. procyonis* infection in raccoons (Kazacos, 2001). However, good hygiene and strong discouragement of feeding raccoons or keeping them as pets are essential to avoid risk of baylisascariasis (Gavin *et al.*, 2002). Raccoon latrines in the environment should be properly cleaned up and parents should prohibit children from playing in these areas. The importance of hand washing after outdoor play or animal contact should be stressed (Kazacos, 2000, 2001). Children with pica behaviour or geophagia are at high risk and should not be allowed to play unattended in areas where raccoons (or other animals) have defecated. Individuals involved in wildlife rescue and rehabilitation should be made aware of this infection and counselled to observe appropriate precautions when handling raccoons and their cage environments.

Decontamination of areas contaminated with *B. procyonis* eggs is difficult. These eggs are resistant to decontamination with common disinfectants including bleach and can survive in the environment for years (Kazacos, 2001). Use of a 1:1 xylene:ethanol solution can be effective, although often impractical, in decontaminating areas after organic debris has been removed (Kazacos and Boyce, 1989). Heat is the best method of killing the eggs and can be accomplished by flaming the area (including soil) or burning affected material such as straw or wood cages (Kazacos, 2001). Boiling water, steam cleaners or autoclaves are also effective methods of decontaminating areas (Kazacos, 2001). All cages, carriers or areas (regardless of material) occupied by raccoons should be thoroughly cleaned. Other animals should not be housed in cages previously occupied by raccoons as they could acquire the infection, which can lead to profound CNS disease and death. When cleaning raccoon latrines, care should be taken to prevent accidental ingestion of eggs. Personal protection equipment such as disposable coveralls, rubber gloves, washable rubber boots and a particulate

face mask should be used to prevent the inhalation or ingestion of any eggs or faecal fungi stirred up while cleaning (Kazacos and Boyce, 1989). Once clean-up has been completed all disposable equipment should be incinerated, autoclaved or otherwise properly disposed of (Kazacos, 2001).

The appearance of patent *B. procyonis* infections in dogs is a source of increasing concern. Dogs inhabit many households with children and,

unlike raccoons, are indiscriminate defecators. Thus, there would be the potential for widespread contamination of an environment where children play. Monthly heartworm/anthelmintic preventative treatment in pet dogs should prevent infection by this dangerous pathogen as well as *T. canis*. Veterinarians should also perform regular faecal screenings of pet dogs to identify new infections.

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Section 3

***Toxocara* in the veterinary context**

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16 Current and Future Options for the Prevention and Treatment of Canids

Christian Epe

*Institute of Parasitology, Centre for Infectious Diseases,
Hannover School of Veterinary Medicine, Hannover, Germany*

Introduction

The key focus for the control of *Toxocara* and its epidemiology in humans is the contamination of the environment with infective ova. Gravid *Toxocara* females shed resistant ova into the environment; these embryonate and can remain infective, under suitable conditions, for several years. This environmental reservoir is a source of infection for paratenic hosts, including man, and for canine and feline final hosts. To reduce the risk of re-infecting canines and felines, as well as the zoonotic infection of human and other paratenic hosts, the level of environmental contamination must be reduced through the effective use of prevention and control programmes. While cases of human toxocariasis are widely published, medical professionals still face difficulties in diagnosing human infections because there are no pathognomonic signs and serology can be unhelpful (see Chapters 7, 8 and 9, this volume).

The ubiquitous problem of stray dogs and foxes in urban areas emphasizes the need to diagnose and treat infected canines, thus reducing the reservoir of zoonoses including toxocariasis. In many developed countries where human parasitic diseases are rare, canines and felines still harbour significant nematode infections, causing mostly chronic, subclinical infections.

The prevalence of *Toxocara* infections in dogs, determined by examination of faeces for the presence of ova, is presented in Table 16.1. These data have been selected from literature published over the last 27 years, when there was increasing global interest in this canine zoonosis, and probably reflect current infection rates in developed countries of the world. Point prevalences ranging from 0.7 to 82.6% are recorded: the lowest in Germany and the highest in Ireland. While this broad range probably reflects inherent differences in the sensitivity of detection of the method used (e.g. variations in flotation methods and different selection or bias criteria for the sample pool selected) to some degree, these data, obtained from diverse dog populations in many countries, also indicate that *Toxocara* is a significant pathogen of dogs, in both the northern and southern hemispheres of the globe. Consistent with this global contamination of the environment is the increased risk to paratenic hosts, particularly humans.

No direct correlation with prevalence studies in dogs has been documented in the majority of published case reports of human toxocariasis, and seroprevalence studies, performed in endemic or 'poor neighbourhood' areas, are associated with case reports primarily of visceral larva migrans (VLM) or ocular larva migrans (OLM).

For infective *Toxocara* spp. ova in the environment, pica and geophagia are significant risk

Table 16.1. Point prevalences of *Toxocara* spp. infections in dogs determined by stool examination for the presence of ova.

Year	Country	No. of samples examined	Prevalence (%)	Reference
1978	Canada	474	26.6	Malloy and Embil (1978)
1993	Ireland	350	82.6	O’Lorcain (1994)
1996	The Netherlands	272	2.9	Overgauw (1997c)
1999	Argentina	105	9.0–19.0	Rubel <i>et al.</i> (2003)
2000	Hungary	490	24.3–30.1	Fok <i>et al.</i> (1991)
2001	Republic of South Africa	63	21	Minaar <i>et al.</i> (2002)
2002	Brazil	131	19.1	Serra <i>et al.</i> (2003)
2002	Italy	295	33.6	Habluetzel <i>et al.</i> (2003)
2002	USA	130	3.1	Hackett and Lappin (2003)
2003	Japan	1743	4.3	Itoh <i>et al.</i> (2004)
2003	The Netherlands	224	8.5	Le Nobel <i>et al.</i> (2004)
1980–1991	Belgium	2324	17.4	Vanparijs <i>et al.</i> (1991)
1993–1997	Germany	2289	0.7–5.8	Epe <i>et al.</i> (1998)
1997–1998	Poland	445	32	Luty (2001)
1998–2002	Germany	1281	0.8–5.0	Epe <i>et al.</i> (2004)
1999–2002	Germany	8438	22.4	Barutzki and Schaper (2003)

factors for human toxocariasis. However, the recent finding of *Toxocara* spp. ova in dog fur (Wolfe and Wright, 2003) identifies a further potential transmission route to humans. Although these data require corroboration, this limited study, based on the examination of fur from 60 dogs, demonstrated the presence of *Toxocara canis* ova in 25% of samples, 4.2% of which contained embryonated, infectious ova (Wolfe and Wright, 2003).

Confirmation of these data, based on further studies of age, breed and geographic distribution, will necessitate a re-evaluation of the sources of human infection and their attendant risks. Thus, in addition to the presence of infectious ova in the environment, an additional role of the infected dog as a family member should also be considered.

Canine Toxocariasis

Transmission routes in canids

The life cycle of *T. canis* is complex (Parsons, 1987; Overgaaauw, 1997a). Adult worms living in the lumen of the intestinal tract of infected dogs shed large numbers of ova in their faeces and contaminate the environment. There, they can be ingested by final and paratenic hosts. *Toxocara*

eggs are unembryonated when passed into the environment and therefore not infective to final or paratenic hosts. Depending on soil type and climatic conditions, such as temperature and humidity, ova will develop to infectivity within a period of 3 weeks to several months. Infective eggs can survive for more than 1 year under optimal conditions. Multiple studies, worldwide, have demonstrated high rates (10–30%) of soil contamination with *Toxocara* ova in parks, playgrounds, sandpits and other public places (see Chapter 14, this volume). In the canine host, larvae hatch in the intestine and migrate via the blood stream after passing through the liver to all organs; especially in young animals, a tracheal migration occurs via the heart, lungs and trachea and, after swallowing, the larvae mature in the intestinal tract.

Additionally, and typically, in adult dogs with some degree of acquired immunity, larvae undergo somatic migration after leaving the intestinal tract to various tissues and organs where they remain as somatic larvae in a stage of arrested development. This phenomenon also occurs in paratenic hosts. These hypobiotic somatic larvae are released and reactivated in pregnant bitches, where they migrate transplacentally into the organs of the fetus as a vertical infection. In addition to this host-finding strategy, *Toxocara* also infects newborn puppies through lactogenically transmit-

ted larvae. Additionally, infective, somatic larvae in paratenic hosts can infect final hosts when predated upon by dogs. In this instance, larvae develop in most cases directly to adult worms in the intestinal tract without further somatic migration.

Pathogenesis

Larval migration through the liver leads to raised levels of glutamate dehydrogenase and alanine aminotransferase (ALT) (Vossmann and Stoye, 1986). Pneumonia, caused by larvae migrating through the lung, is described within the first days of life. Severe infections cause symptoms beginning in the second week of life, and ascites, anorexia and anaemia and a dilatation of the proximal duodenum have been reported. At necropsy, multiple petechiae, ruptures and perforations of the gut can be seen with parasites penetrating the small intestine wall into the peritoneal cavity. It has been reported that this may be followed by peritonitis or massive blood loss into the peritoneal cavity (Dade and Williams, 1975; Bosse and Stoye, 1981; Vossmann and Stoye, 1986).

Clinical symptoms

Clinical symptoms are dependent on the age of the animal and on the number, location and stage of development of the parasite (Stoye, 1976; Zimmermann *et al.*, 1985; Parsons, 1987). After birth, puppies can suffer acute toxocariasis, with pneumonia due to tracheal migration, and die within 2–3 days. At 2–3 weeks, puppies can show digestive disturbances and emaciation, caused by the presence of mature worms in the stomach and intestine. Diarrhoea, vomiting, coughing, constipation and nasal discharge can be present at clinical examination. Distension of the abdomen ('potbelly') can occur as a result of a heavy worm burden but more probably is due to gas formation caused by dysbacteriosis. Mortality can be a consequence of obstruction of the gall bladder, bile duct, pancreatic duct and rupture of the intestine, but is rare at this stage.

The prevalence of patent *T. canis* infections in adult dogs, at least in most developed countries in Europe and the USA, is low (1–5%) and clinical symptoms are rare. During somatic larval migra-

tion, either symptoms are not recognized by the owner or signs of the clinical disease are not manifested. Larval migration to the eyes (OLM) by *T. canis* larvae is described in rare cases (see Chapter 9, this volume).

Toxocariasis is a public health problem (Schantz, 1989; Overgaauw, 1997b; Lloyd, 1998). Humans act as paratenic hosts whereby *Toxocara* larvae do not develop beyond the second larval stage, but migrate through the soft tissues of the body and survive for a long time. Humans are infected orally following ingestion of infective *Toxocara* ova present in environmental sources, such as contaminated soil (sapro-zoonosis), from unwashed hands or consumption of raw vegetables. Infections can also occur following the ingestion of infective larvae in undercooked foods from paratenic hosts such as chickens, cattle and sheep (Glickman and Schantz, 1981; Stürchler *et al.*, 1990). Vertical transmission in pregnant women, however, does not appear to occur (Taylor, 1993). Human *T. canis* infections are likely to be a hazard for people exposed to contaminated environments, but direct contact with infected dogs was not considered a potential risk factor because recently shed, unembryonated ova are not infective and embryonation of *Toxocara* ova to infectivity requires a minimum of 3 weeks. However, recent findings (Wolfe and Wright, 2003), cited above, raise the need to rethink the concept of direct transmission.

Determining Current Patent Infection and the Benefits of Chemotherapeutic Intervention

Patent *Toxocara* infection in dogs and cats can be tentatively diagnosed from the medical history, particularly the lack of an appropriate anthelmintic schedule, and the clinical symptoms, given that these parameters are known. More commonly, *Toxocara* infection will be diagnosed following coproscopical examination for the presence of the typically dark brown-coloured unembryonated ova, which have thick-pitted shells. Flotation methods (Lindsay and Blagburn, 1995), whereby ova are separated from the bulk of the faecal material, are used commonly. As ova are less dense than the flotation medium, they float to the surface and are skimmed out of the surface film prior to identification by microscopy.

An ELISA, using *Toxocara canis* excretory–secretory (TES) antigens, is described as sensitive for determining whether or not a bitch is carrying somatic larvae (Scheuer, 1987), but, in routine diagnosis, serology is not normally considered to be an option due to its inability to differentiate between current, patent infection and previous infection/exposure.

Obvious benefits of chemotherapeutic intervention include: (i) pathogen removal leading to reduced severity of pathology and curing of disease, particularly in neonates and juvenile hosts; and (ii) prophylaxis, to reduce the likelihood of subsequent infections and to reduce environmental contamination with ova. Frequently, the benefits of a chemotherapeutic intervention have to be explained to the pet owner since cost implications can become primary concerns.

Rationale for Chemotherapeutic Treatment

From the veterinarian's viewpoint, there are two major reasons for controlling *Toxocara* infections in final hosts. The rarer cases of heavy intestinal infections in susceptible hosts should be treated in order to reduce their clinical sequelae, and the risk of infection to pets should be reduced. Furthermore, reducing patent infections in canines and felines reduces the likelihood of human infection. While there are some practical interventions to reducing environmental contamination with infective ova, such as liquid nitrogen treatment or burning of sites of obvious dog fouling, the prevention of initial contamination remains our most important tool (Overgaw, 1997a,b,c). The rationale underpinning the prevention of initial contamination includes chemotherapy to eliminate patent infections in dogs and cats, preventing defecation in public areas and the education of dog and cat owners.

Limitations of Chemotherapeutic Treatment

The differences in the modes of action and the pharmacokinetic properties of registered chemotherapeutic compounds are well recognized and need to be considered in terms of their effi-

cacy. Single treatments can produce different outcomes compared with multiple treatments. For instance, in a study by Hopkins (1991), the efficacy of various anthelmintics (benzimidazoles and pyrantel; Table 16.2) given as a single treatment against natural intestinal *T. canis* infections in dogs was critically evaluated. A 100% efficacy was not achieved with any product: the average percentage reduction per group was between 71.4 and 93.8%, but the variation per animal ranged from 17.5 to 97.6%, indicating the necessity of a multi-day treatment with these compounds, administered as a single formulation.

The study of Mackenstedt *et al.* (1993) investigated the effects of pyrantel pamoate on adult and preadult *T. canis* worms using different incubation periods, different drug concentrations and modes of uptake of pyrantel by *T. canis* worms. The results indicated that adult *T. canis* worms ingest the drug along with their food. The measurement of radioactive drug and the subsequent calculation of the amount of drug ingested after different incubation times revealed that adult worms are capable of limiting their ingestion of pyrantel by stopping oral uptake of food for more than 4 h. This parasite mechanism has to be taken into account when administering pyrantel, particularly when vertical transmission is to be addressed with short-acting formulations. This risk of a drug uptake gap is assumed to be negligible when compounds such as the macrocyclic lactones (Table 16.2), which have a longer efficacy period, are used.

Procedures for Testing Anthelmintic Activity against Adult Worms and Larvae

Some guidance is given regarding the testing of the anthelmintic activity. The World Association for the Advancement of Veterinary Parasitology (WAAVP) published 'Guidelines for evaluating the efficacy of anthelmintics for dogs and cats' (Jacobs *et al.*, 1994) describing controlled or critical test methods against various helminth species, the calculation of efficacy, dose titration and confirmation study designs. For *Toxocara*, either natural or experimental infection is recommended and a mean worm number reduction of > 90% compared with an untreated control

Table 16.2. List of available anthelmintics for treating *Toxocara* spp. infections of final hosts.

Compound class and compound	Trade name ^a	Dosage [mg / kg b / w × days of application]	Application	Safety index	Contra-indications ^b	Comments
Benzimidazoles						
Fenbendazole	Panacur	D/C: 50 × 3 d	P. o.	D: > 10, C: > 3	Not known	
Flubendazole	Flubenol	D/C: 22 × 2–3 d	P. o.	> 7	Not known	
Mebendazole	(Numerous generics)	D/C: Twice daily 20–100 mg/animal × 5 d	P. o.	> 20	Not known	
Tetrahydropyrimidines						
Pyrantel	Drontal ^c , Nemex	D: 5.0–10.0 × 1 C: 20.0 × ?1	P. o.	D: > 10	Not known	
Isothiocyanates						
Nitroscanat	Lopatul	D: 50 × 1	P. o.	D: > 10	Not known	
Macrocyclic lactones						
Ivermectin	Heartgard, Heartgard Chewables, Ivomec	D: 0.006 × 1 (monthly)	P. o.		Not known	With certain Scottish sheepdog breeds (Collie breeds) and others, adverse effects are reported in dosages > 0.006
Milbemycine oxime	Milbemax ^c , Interceptor	D: 0.5–1.0 × 1 C: 2 × 1 (monthly)	P. o.	> 20 ^d	Not known	
Selamectin	Stronghold, Revolution	D/C: 6.0 × 1 (monthly)	Spot-on	> 10 ^d	Not known	
Moxidectin	Advocate ^c	D: 2.5–6.5 × 1 C: 1–2 × 1 (monthly)	Spot-on	> 10 ^d	Not known	
Cyclic octadepsipeptides						
Emodepsid	Profender ^c	C: 3	Spot-on	C: > 5	Not known	Also during lactation and pregnancy

^aAs available; please check in your country of residence. Presence of generic compounds and other/additional trade names.

^bAs far as listed in registration; usual warning comments such as 'Do not use for pups < 6 weeks' or similar, which are requested by law in case of missing documentation of such an application, are not listed, since these comments are not pharmacological contra-indications.

^cCombination product, combination with praziquantel (Cestocide).

^dCollie breeds: > 2.5 for selamectin, 5 for moxidectin; 18 for pups.

P.o., *per os*.

group is defined as effective. Registration studies for marketing approval from authorities nowadays follow international guidelines such as the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Products (VICH) guidelines (<http://vich.eudra.org/>). In addition to the defined standards – the ‘so-called’ Good Clinical Practice (GCP) (VICH GL07; Nov 2000) more detailed requirements have been described (VICH GL19 (Anthelmintics: canine); June 2001) which are obligatory for the pharmaceutical industry. This international harmonization of veterinary regulations has political and economic consequences. Besides having a positive impact on the product approval process, animal welfare also benefits by eliminating the unnecessary duplication of studies, which leads to a reduction in the number of animals required for establishing the safety and effectiveness of veterinary anti-parasite drugs. An additional benefit would be the use of a single set of data to obtain marketing approval of products for the treatment of minor animal species.

When evaluating anthelmintic activity against adult worms, a controlled test of two groups of either naturally or experimentally infected animals can be used, and the anthelmintic of choice is given during the patent period of infection. Worm counts are performed at necropsy (5–14) days following treatment, according to guidelines. To evaluate larvicidal activity, vertically infected pups are treated at 5–7 days of age (Jacobs *et al.*, 1994) or, according to VICH, 8–12-week-old pups are treated 5–7 days after experimental infection. As the development of larvae in naturally infected newborn pups is not synchronous, the *T. canis* population will consist of a mixture of third and fourth larval stages (Sprent, 1958) when treated. It is unlikely that any fifth-stage worms will be present. Worm counts are delayed until the pups can be removed from the bitch without causing undue distress, usually after 3 weeks of age. Egg count reduction tests are normally used in field studies. Here, the dog acts as its own control, and an overall reduction in egg output is used to calculate efficacy (Jacobs *et al.*, 1994). This study type supplements controlled tests and acts as a dose confirmation field study in the registration process.

With these study designs, substance efficacy and control programmes can be evaluated by: (i) measuring the overall reduction in worm

counts at the completion of the control programme, thereby providing an overview of the degree of protection afforded to recipient pups; (ii) measuring the suppression of egg output during the programme to give an indication of benefit in terms of reducing environmental contamination; and (iii) obtaining information on the population dynamics of *T. canis* infection during the programme.

Therapies and Regimes

Chemotherapeutic tools – compounds and properties

The major classes of anthelmintics used for small animals are the (pro-) benzimidazoles (fenbendazole, febantel, flubendazole, mebendazole), the imidazothiazoles (levamisole), the tetrahydropyrimidines (pyrantel) and the macrocyclic lactones (ivermectin, selamectin, milbemycin). Recently data on a complete new class of compounds, the cyclic octadepsipeptides, such as depsipeptide, have become available.

The principal mode of action of benzimidazoles (BZs) is based on the complete reduction of microtubulin polymerization inside the parasite's cells leading to disintegration of the hypodermis, muscle layer and intestine (Lacey, 1988). The formation of gametocytes and gametes is known to be prevented (Hanser *et al.*, 2003). Due to their very low toxicity and their excellent tolerance, an overdosing of most of the marketed formulations is practically impossible (Ungemach, 2002). An exception is their application during pregnancy due to the potential teratogenic and embryotoxic side effect of this group of compounds. However, several studies did not confirm this (Fisher *et al.*, 1993, 1994; Stoye, 1992). The range of parasite species and stages which are affected is dependent on the pharmacokinetics of the single compound, which leads to individual differences within the group of available BZs formulations. The half life of BZ is approximately 10 h.

The tetrahydropyrimidines are represented by pyrantel, which is the only available and marketed compound for companion animals. Pyrantel as a pamoate formulation with low solubility is used as a broad-spectrum anthelmintic for oral use. The pharmaco-dynamic effect is directed at

the m- and n-cholin receptors in parasympathic organs and vegetative ganglions. At higher concentrations, acetylcholinesterase inhibition occurs, which leads to a neuromuscular blockade and parasite death due to spastic paralysis. Although this mechanism can occur in the vertebrate host, there is no apparent effect due to the very low bioavailability of pyrantel pamoate (Ungemach, 2002). The half-life is approximately 4–8 h.

Similar in its mode of action is levamisole, the only representative of the imidazothiazoles (Table 16.2). Due to their good solubility, both oral and injectable formulations are available for companion animals. The therapeutic range of 5–7.5 mg / kg b/w is relatively narrow, and overdosing (> 12 mg / kg) can lead to severe side effects. The half life is approximately 4 h.

The macrocyclic lactones (MLs) are fermentation products of fungi of the genus *Streptomyces* and ivermectin, selamectin, moxidectin and milbemycin oxime are registered for use in companion animals. The mode of action of these very lipophilic molecules is directed against the glutamate- and gamma-aminobutyric acid (GABA)-receptor-mediated chloride channels which are present in a range of nematodes as well as in different ectoparasites, but not cestodes and trematodes, which show a natural resistance to these compounds, due to the lack of GABA- and glutamate-controlled channels. Therefore, the macrocyclic lactones are also called endectocides. The result of this tropism is a hyper-polarization of these channels which results in consecutive paralysis of the parasite. Since the vertebrate blood-brain barrier prevents the penetration of the ML molecule and the glutamate receptor does not exist in the vertebrate host, both the tolerance and safety interval of MLs are very broad. Exceptions include some Scottish sheep-dog breeds which do not prevent ML penetration through the blood-brain barrier and can show similar effects to those seen in the target (parasite) species. Registered for veterinary use in dogs are ivermectin, orally, at a dosage of 0.2 mg / kg b/w, selamectin, as a spot-on application, at a dosage of 6 mg / kg b/w and milbemycin oxime, orally at a dosage of 0.5 mg / kg b/w. Studies indicate that other MLs, such as moxidectin and doramectin, are also able to interrupt vertical infection of *T. canis* from bitch to pups (Schnieder *et al.*, 1996; Bowman *et al.*, 2002). Therefore, in the future more ML group molecules may be regis-

tered for use in dogs and cats, thus enlarging the spectrum of available drugs.

The cyclic octadepsipeptides have been recognized since the 1990s with promising results in terms of efficacy and safety but without a registered product so far on the veterinary market. Their mode of action is a neuro-pharmacological effect on a new heptahelical transmembrane receptor (HC110R) with similarity to human, bovine and rodent latrophilin (Harder *et al.*, 2003). Data from rodent endoparasite studies (Harder and von Samson-Himmelstjerna, 2001) and other studies (Harder and von Samson-Himmelstjerna, 2002) show efficacy against *Toxocara cati* in cats. Since this class of compounds exhibits a nematocidal efficacy, a new anthelmintic for dogs may become available in the future.

Table 16.2 provides an overview of different compounds registered for use with dogs and cats, including treatment application, dosage and regimen. There are numerous recommendations for regular, strategic and anthelmintic treatment, and some of these are discussed in the following sections.

Prophylaxis (Chemoprophylaxis and Vaccination)

The most serious and concentrated source of infection is the nursing bitch with puppies aged between 3 weeks and 6 months (Jacobs *et al.*, 1977). A major aim of long-term prophylactic anthelmintic programmes is to prevent any environmental contamination by suppressing *T. canis* egg output with multiple dosing of pups. Anthelmintic treatment should commence before the age of 3 weeks, since, in vertically infected pups, a shortened prepatent period occurs (Yutuc, 1954). Because lactogenic transmission can occur continuously for up to 5 weeks post-partum, repeated treatments are necessary. Immature adult worms that reach the intestine need at least 2 weeks to mature and start shedding ova; therefore treatment should cover this period, depending on the pharmacokinetics of the compounds used.

Since reinfection of the bitch can occur throughout the suckling period, bitches should always be included in the treatment regime at the same time as the puppies (Jacobs, 1987) for

the first 2–3 months. Control in older dogs can be realized by periodic treatments with anthelmintics whose efficacy can be limited to the intestinal stages, or by treatments prescribed based on the results of periodic diagnostic faecal examinations. Without these measures, environmental contamination with infectious ova, leading to re-infection and infection of paratenic hosts, cannot be addressed but, even with these measures in place, the potential for environmental contamination cannot be excluded, unless a schedule which removes prepatent intestinal worms is used.

The elimination of tissue-dwelling larvae which instigate both vertical, intra-uterine and transmammary transmission would have a significant effect on the parasite population (Kassai, 1995). Deworming of bitches during pregnancy is sometimes advised in anthelmintic schedules, but this advice is questionable. The efficacy of nearly all licensed anthelmintics against somatic larvae in experimental animals and bitches, at various dosages and treatment periods, has been intensively investigated (Bosse and Stoye, 1981; Burke and Roberson, 1983; Lloyd and Soulsby, 1983; Schnieder *et al.*, 1996; Fok and Kassai, 1998). In general, it can be concluded that anthelmintics used at their recommended doses are not effective against inhibited somatic larvae (Epe *et al.*, 1996). The treatment of bitches before mating and 2 weeks before the anticipated whelping date had no useful effect on prenatal transmission (Fisher *et al.*, 1994). Prenatal infection can be substantially reduced by daily treatments with fenbendazole (25 mg/kg b/w) given to the bitch from the 40th day of pregnancy until 2 days post-partum, but this treatment regimen is too expensive for general use (Barriga, 1988), and it appears to be difficult nowadays to explain a daily chemotherapeutic treatment in pregnancy to the owner and to obtain treatment compliance. An alternative and effective treatment for interrupting vertical infection with less-frequent applications of anthelmintic is with use of MLs (Epe *et al.*, 1995; 1996; Schnieder *et al.*, 1996; von Samson-Himmelstjerna *et al.*, 2003) either once, on day 50–55 of pregnancy, or on day 55 of pregnancy and day 5 post-partum. Although no label claim is registered so far for the different MLs (see Table 16.2), this indication may lead to increased compliance by the owner due to the lower frequency of applications.

Toxocara worms should be eliminated in the final host by treatment with an effective and, if possible, larvicidal anthelmintic. Here, the benzimidazoles (pyrantel) and the newer generation MLs (e.g. selamectin, milbemycin, moxidectin) are recommended (Table 16.2).

Following the review by Barriga (1988) on the potential for the immunological control of toxocariasis through the use of vaccines, there has been much focus on TES and its component constituents as the vaccine candidates of choice. Within TES is a group of highly immunogenic mucins (Loukas *et al.*, 2000; Doedens *et al.*, 2001) which coat the parasite's surface and are shed into the host (Badley *et al.*, 1987; see Chapter 1, this volume). Currently, a variety of expressed proteins have been characterized including lectins, superoxide dismutase, resting (dauer)-stage proteins, cysteine proteases and many others (Despommier, 2003) which might prove useful as vaccine candidates. To date, no immunization trials with one or a cocktail of these proteins, in dogs, have been published, although vaccination, as the classical prophylactic measure, remains the subject of many investigations (see Chapter 12, this volume). It appears that a commercially available vaccine, as a ready-to-use tool for the practising veterinarian, is not likely to be on the market within the next few years.

Reducing Environmental Contamination

Reducing environmental contamination is fundamental to the control of toxocariasis in paratenic and final hosts. Since *Toxocara* ova are resistant to adverse environmental conditions and can remain infective for years, a build-up of infective ova can occur in the environment. Thus, the prevention of initial contamination of the environment is a fundamentally important requirement which can be achieved not only by taking measures that include preventing defecation by pets in public areas, hygienic disposal of stools and education of the public, particularly the pet-owning public, but also by eliminating patent infections in dogs and cats with curative and strategic (regular) anthelmintic treatment.

Decreasing environmental contamination can be achieved by the following methods:

- cleaning up faeces from soil and on pavements by dog owners
- preventing access of dogs and cats to public places (especially children's playgrounds)
- strategic anthelmintic treatment of dogs and cats with emphasis on puppies and nursing bitches and kittens and queens (Schantz, 1981)
- restriction of uncontrolled dogs and cats.

Hygiene measures include removing faeces from play areas and the thorough cleaning of kennels with high-pressure steam cleaners and ascarid-effective disinfectants (e.g. cresol formulations). Expelled worms should be destroyed. Dog and cat owners can help to avoid environmental contamination with *Toxocara* eggs. There is a need for effective and responsible pet ownership in all social settings, including advice about deworming schemes, effective anthelmintics (where available) and the requirement to prevent their dogs and cats from defecating in communal areas, particularly those where children congregate and play. Here, veterinarians are the most appropriate avenue for disseminating this information to their clients; thus the control of this zoonosis (and others) with respect to environmental contamination by dogs and cats rests firmly in their hands (Harvey *et al.*, 1991; Overgaauw, 1996).

A more recent approach for parasite control is presented by the Companion Animal Parasite Council (CAPC) (<http://www.capcvet.org/>). Originally established for North America, it is now a World-Wide Web platform containing defined 'guidelines' for controlling parasites in companion animals. It disseminates information on practices and procedures that protect pets from parasitic infections and reduce the risk of transmission of zoonotic parasites, through collaboration among pet owners, veterinarians, physicians and common interest groups. CAPC suggests lifelong preventative treatment to reduce zoonotic infection risk for the dog owner.

Depending upon the pharmacokinetics of the anthelmintic used, treatment is recommended at 4 week intervals. The risk of safety and toxicological side effects is seen as minor. Emphasis is placed on regular treatment and reducing new environmental contamination with roundworm ova (Bowman, 2005). This reflects the approach to interrupting the

parasite's life cycle within the definitive host, since no effective measures can be taken to eliminate infectious stages in the environment. Increasing anthelmintic resistance, as seen in nematode species such as trichostrongyles in small ruminants and cyathostomes in horses (Kaplan, 2004; Wolstenholme *et al.*, 2004), has not been reported for *Toxocara*. One hypothetical explanation lies in the role of the refugium of the parasite. This is known to be important for strongyle infections in small ruminants whereby they escape the selection pressure of each treatment. The larger the refugium is, i.e. the larger the parasite population that escapes chemotherapeutic attack, the lower the selection pressure will be, which leads to a slower development of resistance. The classical example is the dosing of sheep in the dry season in Australia, affecting the majority of the nematodes, since there will be almost no refugium to survive on pasture. Resistant parasites which survive treatment will produce the majority of the next generation nematodes, which is also likely to be resistant. For dogs and cats it can be assumed that frequent treatment will not affect the whole parasite population in a given area, since it is unlikely that all hosts will be treated simultaneously. Therefore, the majority of the *Toxocara* population will escape the effects of treatment remaining in the refugium either as anthelmintic-susceptible or at least heterozygous semi-susceptible.

Conclusions and Future Directions

Unless further advances in vaccine knowledge and development are described, few, if any, alternatives for chemoprophylactic prevention measures appear to be available in the near future. Therefore, strategic control will be the tool for controlling zoonotic risks. In summarizing all aspects of prevention and control, some recommendations are listed below as suggestions for animal owners, veterinarians, physicians and specialists from local and government authorities who have to know and deal with prevention principles.

Chemoprophylaxis

A year-round treatment can be recommended because broad-spectrum anthelmintics with activity against parasites with zoonotic potential are

available. A year-round prevention schedule eliminates the requirement to predict potential transmission seasons and can improve compliance (Bowman, 2003). Pets may travel to regions where transmission is active; dogs and cats may be exposed to, and become infected with, roundworms (and hookworms) throughout the year. Consequently, stages capable of transmitting parasites can be shed into the environment, regardless of season or climate. Adult dogs and cats may develop patent infections leading to environmental contamination.

Supplementary to treatment, a thorough physical examination and complete history are important for diagnosis, treatment and control of most parasites and should be performed at least annually by a qualified veterinarian. Pets should not be fed raw meat but cooked or prepared food and be provided with fresh, potable water. Faecal examinations should be performed two to four times during the first year of life (this could be associated with a vaccine schedule), and one to two times per year in adult pets, depending on patient health and lifestyle factors. This enables the monitoring of compliance with monthly preventive medication, while facilitating diagnosis and treatment of parasites that may not be covered by broad-spectrum preventives for cestode or trematode infection, and includes ectoparasite (e.g. ear mite) control.

Intestinal parasite infections in puppies may cause serious illness or death before diagnosis is possible by faecal examination. Therefore, puppies require more frequent anthelmintic administration than adult animals. They often are serially reinfected during nursing and from the environment, and often already harbour parasite larvae either in migration or arrested development that later mature and commence egg laying.

Puppies and their mothers should be treated with appropriate anthelmintics either on a monthly coverage or when puppies are 2, 4, 6 and 8 weeks of age, and then put on a monthly preventive. Nursing bitches should be treated concurrently with their offspring since they often develop patent infections along with their youngsters, probably due to immunological stress during the nursing period and their close proximity to the patent pups.

Veterinarians should tailor prevention programmes to fit the needs of individual patients. Geographical, seasonal and lifestyle factors can

affect parasite transmission and prevalence. Pet owners should be made aware of lifestyle risk factors such as private or public parks or common recreational spaces visited by pets and children, the close association between household pets and humans and sharing household resting spaces including bedrooms and beds.

Environmental control of parasite transmission

Environmental control is an integral component of parasite prevention and control to minimize environmental stages (eggs, larvae). Ova are long-lived in the environment and responsible for the infection of pets as well as zoonotic transmission. Ascarid eggs are highly resistant to environmental conditions and may persist in the soil for years. Extreme measures are needed for decontamination, including heat (boiling water, steam, propane gun, burning straw, etc.) to kill ova, removal of contaminated substrate (e.g. 10–20 cm/6 inches of soil which should be properly disposed of) and/or entombment of ova under concrete or asphalt. These methods are often not realizable due to cost and organizational issues; therefore, it is most important to prevent initial environmental contamination with parasite stages, for instance through the above-mentioned comprehensive parasite control programme.

Parasitized animals should be treated and monitored by faecal examination to confirm treatment efficacy. At least weekly (preferably daily), faecal cleanup/removal should be conducted by the owner from environments such as private/public gardens, etc. Faeces should be bagged and disposed of according to local government or health care professionals' guidelines. This includes incineration or effective composting. Following anthelmintic treatment, any worms passed should be similarly disposed of. Children's sandboxes should be covered when not in use.

Education of owners, staff and the community at large

Transmission of information about the health risk to pets and people associated with parasitic infections and safe methods of reducing infection is

essential to minimize risk. This can be realized through brochures, posters and veterinary practice staff to convey educational messages to pet owners. When potential zoonotic infections are diagnosed in pets, owners should be advised of their risks and referred to a physician when appropriate. Persons in contact with animals that may transmit zoonotic parasites should be advised of the risks and made aware that risks are increased by pregnancy, underlying illness or immunosuppression. Advice should be given

readily: the veterinarian is a better source of information about zoonoses for pet owners than the physician (Harvey *et al.*, 1991). Precautions to prevent infections and occupationally acquired zoonoses in the veterinary hospital must also be taken. Veterinarians should be encouraged to interact with local physicians and to form liaison groups in order to increase physician awareness and understanding of pet-associated zoonotic infections and the value of preserving the human/animal bond.

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17 Toxocariasis: the Veterinarian's Role in Prevention of Zoonotic Transmission

Peter M. Schantz

Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Introduction

In most countries of the world, zoonotically acquired toxocariasis ranks among the most common helminth infections of humans; even in 'developed' countries where human-adapted geohelminth infections (*Ascaris* and hookworms) are rarely seen because of the comparatively high levels of hygiene and sanitation, infection by *Toxocara* spp., the common intestinal ascarids of dogs and cats, remains a prevalent zoonotically acquired helminth infection of humans (Taylor and Holland, 2001; Gauthier *et al.*, 2003). Because toxocariasis is rarely an officially 'reportable' infection, systematically acquired population-based incidence and prevalence data are not available; however, numerous serological surveys reported from many countries indicate that human exposure to these helminths occurring in pet animals is common. Although rates of infection in humans are generally inversely proportional to socioeconomic levels (Herrman *et al.*, 1985), significant rates of morbidity caused by toxocariasis have been documented in populations enjoying high socioeconomic standards as well as in those enduring impoverished environments. Data on the prevalence in animal hosts and morbidity and socioeconomic burdens imposed on individuals and society by this zoonotic infection of our com-

mon household pets is documented elsewhere in this volume (see Chapter 19, this volume).

Frequent exposure of humans to infection by these nematodes is a consequence of the near universal occurrence and high prevalence of these infections in dogs and cats and the frequency with which these pets are kept in close association with human families (Schantz, 1983). National surveys estimate that approximately 60% of American households currently own dogs, cats or both (AVMA, 2002); the highest rates of dog and cat ownership occur in households of families with young children. The frequency of pet ownership, combined with high rates of *Toxocara* infection in these pets, produces widespread environmental contamination with *Toxocara* eggs, thus serving as a potential source of infection for humans, particularly children whose play habits put them most at risk (Glickman and Schantz, 1982; Schantz, 1989; Schantz and Trello, 1997). Widespread environmental contamination with the infective eggs of *Toxocara* spp., particularly in home environments and outdoor spaces shared by humans and their pet animals, creates a situation of common opportunities for accidental exposure (see Chapter 14, this volume). The reason for the high prevalence of *Toxocara* infection in pups and kittens is that they acquire infections from their dams from which larvae are transferred prenatally and/or via milk. Most pups are infected this way and unless

anthelmintic treatments are administered before 4 weeks of age, the pups will contaminate the environment because female worms become gravid and produce eggs when the pups are 3 weeks of age. Furthermore, these eggs remain infective in most environments for months or even years.

Toxocaral larva migrans, or infection by the common ascarid worms of dogs and cats, is arguably the most common zoonotic infection associated with pet animals in the USA and other industrialized countries (Stehr-Green and Schantz, 1987). We have estimated that, every year in the USA, this infection causes hundreds of cases of unilateral blindness and uncountable numbers of less permanent forms of illness in children (Glickman and Schantz, 1982; Schantz, 1989). The results of numerous serological surveys of children and adults have made it apparent that exposure to *Toxocara* was much more common than most of us were previously aware (Hermann *et al.*, 1985). The principal risk factors for infection were the presence of a household dog, particularly a pup, in the patient's household within 6 months of onset of illness (Schantz *et al.*, 1979; Schantz, 1983). When this is combined with pica behaviour, especially dirt eating, the statistical association becomes very strong. Veterinarians also appear to be at relatively high risk. A serosurvey of veterinarians and other groups believed to be at risk for zoonotic infections (slaughterhouse workers, farmers and zoo employees) revealed that *Toxocara* antibody prevalence was 20 times higher among veterinarians than in the general Austrian population (Nowotny and Deutz, 2000). Another consistent finding is that pet owners have little awareness that their pets might carry illnesses transmissible to people (Schantz *et al.*, 1979; Schantz and Stehr-Green, 1988). Pet owners are generally well informed about rabies and the need to vaccinate their animals; however, their knowledge of other zoonotic risks is usually absent or incorrect (Fontaine and Schantz, 1989). Without such information, pet owners are neither informed nor motivated to take the simple precautions necessary to protect themselves and their families.

Larva migrans syndromes are not reportable in the USA or in other countries, so the actual number of human cases is unknown. However, many human cases continue to be diagnosed. Annually at least 3000–4000 serum specimens from patients with presumptive diagnoses of toxocariasis are sent to the Centres for Disease Control

and Prevention (CDC), state public health laboratories or private laboratories for serodiagnostic confirmation (Schantz *et al.*, 1979; Schantz 1989). A recent national survey of dogs in animal shelters in large cities throughout the country provided uniform national data and indicated that potentially zoonotic helminth parasites were common throughout the USA: almost 36% of dogs nationwide, and 52% of dogs from the southeastern states, harboured helminths capable of causing human disease (Blagburn *et al.*, 1996). Overall prevalence of *Toxocara canis* was 14.5%; however, in the states of the southern USA, the prevalence was 26%. Looking at infection rates in adult dogs, however, provides an underestimation of the potential for environmental contamination. Prevalence of *Toxocara* in young pups is much higher than the population average figures and it is still true that the great majority of pups are born with *Toxocara* infection. By comparison, canine rates of infection with helminth parasites in Australia were significantly lower and this was believed to be associated with the stronger emphasis by Australian veterinarians on pet owner use of broad-spectrum anthelmintics (Bugg *et al.*, 1999; Schantz, 1999).

Some 50 years ago when *Toxocara* worms were first discovered to be a cause of human disease, most of the knowledge of how to prevent it became apparent (Beaver *et al.*, 1952). Its continuing importance as a cause of zoonotic infection is a cause for some embarrassment, especially given the current availability of a variety of safe and effective drugs to eliminate these infections from pets (see Chapter 16, this volume). Veterinarians are ideally placed to limit transmission of toxocariasis and one of our greatest challenges as academic and public health veterinarians is to work more effectively to promote the involvement of our colleagues in practice to prevent this zoonosis.

The Vital Role of Veterinarians

Veterinarians are on the 'front lines' of prevention of transmission of *Toxocara* infection as well as other zoonotic disease agents from pets to people. The veterinarian is usually aware of the characteristics of the pets owned by his/her clients and is looked to by the client as the best source of information concerning those pets; good veterinary practice includes advice about potential zoonotic disease risks of pet

ownership and how to avoid them. Physicians too are concerned about pets as potential sources of infections; however, they are unlikely to be knowledgeable of their patient's pet ownership status until after disease transmission has already occurred, then it is too late for prevention!

We carried out surveys of practising veterinarians in the USA, in 1979 and again in 1989, to assess practices that would lead to prevention of zoonotic toxocariasis and we found that current veterinary practices and recommendations to pet owners were less than ideal to prevent transmission (Kornblatt and Schantz, 1980; Harvey *et al.*, 1991). We found that less than half of surveyed veterinarians recommended the preventive or prophylactic approach to treatment of roundworms. When asked at what age they recommended first diagnostic examination or treatment for intestinal worms, only about a third of veterinarians responded 'at 4 weeks of age or less,' which is the timing necessary to prevent excretion of *Toxocara* eggs most efficiently. Approximately another third of surveyed veterinarians recommended examinations or treatments beginning at 7 or more weeks of age, in which cases environmental contamination might have occurred for at least a month prior to the time the pup was examined. The results of our surveys indicated that veterinarians were ambivalent about discussing the questions of the potential zoonotic hazards of roundworms and hookworms with clients. Somewhat less than a third indicated that they either 'never' discussed this subject or did so 'only when asked'. Thirty-eight per cent discussed it 'when worms were diagnosed in the practice' and the remaining third reported they discussed this with 'new clients' or with 'all clients'. Similar surveys of veterinarian practices have not been reported from other countries.

I can think of several reasons why practitioners were not 'doing the right thing': first, the information about the life cycle of roundworms and hookworms in dogs and the need for early preventive treatments (that all veterinary students learned in parasitology courses) are not reinforced in subsequent training, especially in the clinical and postgraduate years. With time out of school, this information becomes vague and it is understandable that veterinarians are uncomfortable talking about it. Moreover, practising veterinarians do not want to alarm their clients, particularly if this might lead them to give up their pets. This latter concern has been raised to me by

veterinarians on a number of occasions. There may be some basis for that concern, although we have not seen data documenting that fear of disease transmission is a factor associated with keeping, or not keeping, pets.

Veterinarians can Help Prevent Human Disease

Most pet owners do not know that their pets may carry worms capable of infecting people. Therefore, veterinarians can provide an important public service by recommending regular faecal examinations, providing well-timed anthelmintic treatments, counselling clients on potential public health hazards and advising them on any precautionary measures that may be undertaken. Veterinarians are in an ideal position to provide pet owners with this service because of their access to the pet-owning public, their knowledge and training and their role in the human-animal bond (Harvey *et al.*, 1991). Most cases of zoonotic ascarid and hookworm infections can be prevented by practising good personal hygiene, eliminating intestinal parasites from pets through regular deworming and making potentially contaminated environments, such as unprotected sand boxes, off limits to children (Schantz, 1989; Kazacos, 1997). It is also important to clean up pet waste on a regular basis in order to remove potentially infective eggs before they become disseminated in the environment by such factors as physical movement due to rain and insects. Ascarid eggs can develop into infective stage larvae within 2 weeks, depending on temperature and humidity (Bowman *et al.*, 1999). Heavily infected puppies can shed millions of ascarid eggs per day spread throughout the area if the pup is allowed to roam. Once the eggs become infective, they can remain that way in the environment for years (Kazacos, 1997; Bowman *et al.*, 1999).

Preventive Anthelmintic Treatment

To help improve this situation, the American Association of Veterinary Parasitologists (AAVP) and CDC prepared recommendations for veterinarians on treatment of roundworms and

hookworms in dogs and cats. Strategically timed preventive anthelmintic treatments for dogs and cats were recommended in the AAVP/CDC guidelines for veterinarians (CDC/AAVP, 2000). Deworming is most effective for averting morbidity and preventing environmental contamination when aimed at pups and kittens and their dams because they have the highest worm burdens, are most vulnerable to the ill effects of these infections and are the main sources of infective stages (see Chapter 16, this volume). Preventive treatments must be initiated in pups and kittens shortly after birth. Anthelmintic drugs should be given repeatedly to pups at 2, 4, 6 and 8 weeks of age. Because prenatal infection does not occur in kittens, egg excretion begins later than in pups, and in most areas of the USA, rates of acquisition of roundworms and hookworms by cats are comparatively less; therefore, preventive treatments can usually be effectively started at 6 weeks of age and repeated at 8 and 10 weeks. Nursing dams should be treated concurrently because they often develop patent infections about the same time as their offspring. The earliest treatments require providing clients with medications to administer to their pets at home. Thereafter, in areas where heartworm (*Dirofilaria immitis*) infection is enzootic, roundworm and hookworm prevention can be maintained in dogs by using one of the heartworm prevention medications that includes efficacy against these species. Alternatively, control in older dogs and cats can be achieved by periodic treatments with drugs whose efficacy is limited to intestinal nematodes, or by treatments prescribed based on the results of periodic diagnostic stool examinations.

Veterinarians may choose from a great variety of currently available anthelmintic drugs that are safe and effective against roundworms, hookworms and other intestinal helminths of dogs and cats. They include those available in tablet, granule, liquid and other formulations, whose manufacturers recommend single or multiple daily doses, and periodic or continuous administration. Selection of a particular compound should be based on the efficacy for the range of helminth species prevalent in the area and the practitioner's preference. For preventive treatments in very young pups, an anthelmintic approved for, and in formulation feasible for, nursing pups (2–3

weeks of age) should be given. The drug should have a range of efficacy that includes both roundworms and hookworms (unless one or the other of these species is not present in a particular area of practice).

The prophylactic approach to treatment is justified by the frequency with which pups and kittens acquire roundworms and hookworms from their dams and the difficulties in diagnosing these infections in their early stages. Severe morbidity and even death may occur before prenatal or lactogenically acquired roundworms and hookworms become gravid and consequently can be diagnosed by stool examinations (Bowman *et al.*, 1999). Because many pups and kittens are not brought to a veterinarian before 6–8 weeks of age, delaying treatment until that time means that infections will become patent and result in environmental contamination with eggs or larvae. Finally, because young animals acquire new infections continuously from dam's milk and from the environment and many worms are not yet fully mature, faecal examinations are often falsely negative in pups and kittens.

We believe that veterinarians could be playing a direct and much more effective role in preventing transmission of *Toxocara* spp., and other zoonotic helminths, to people, and that this service could be used to strengthen their practices and bolster their public images. The preventive approach to intestinal helminth control can also generate practice income. Surveys in the USA confirm that more than 80% of all dog owners, and about 62% of cat owners visit their veterinarians at least once per year, and that they look to their veterinarians as the major source of information on matters related to their pets (Wise and Yang, 1994). This is one of many opportunities for veterinarians to increase their relationships with clients. What better way for the practitioner than to show concern for the family and to demonstrate that he or she has the knowledge and services to help protect the family's health?

The role of the veterinarian in preventing zoonotic transmission of parasites from pets to people was recently reinforced and expanded by the creation in the USA of the Companion Animal Parasite Council (CAPC) with the goal of improving prevention, treatment and monitoring of parasitic disease, as well as promoting better

pet owner compliance in following veterinary recommendations. The council was formed with the purpose of changing the way veterinary professionals and pet owners approach parasite management, via best practices that better protect pets from parasitic infections while reducing the risk of zoonotic parasite transmission. Veterinarians and pet owners today have an arsenal of safe, effective and affordable products to prevent the most common parasites of companion animals. However, failure to administer these products properly reduces their effectiveness.

The CAPC guidelines (<http://capcvet.org/>) focus on pet owner and staff education, which can directly affect the success of a parasite management and control programme. In addition, the guidelines outline proper diagnostic techniques for veterinary professionals, with an emphasis on properly conducted faecal examinations. The CAPC guidelines recommend annual faecal examinations in adult pets using a centrifugal flotation technique, and encourage multiple examinations for younger animals. Regular faecal examinations greatly increase the ability of veterinarians to provide information-based recommendations.

The CAPC guidelines summary as applied to zoonotic helminths is as follows:

- Administer year-round treatment with broad-spectrum heartworm anthelmintics that have activity against parasites with zoonotic potential (in geographic regions in which heartworm infection is absent; see below).
- Conduct annual physical examination with complete history.
- Feed pets cooked or prepared food (not raw meat) and provide fresh, potable water.
- Conduct faecal examinations two to four times during the first year of life and once or twice per year in adults, depending on patient health and lifestyle factors.
- Administer anthelmintic treatment of puppies at 2, 4, 6 and 8 weeks of age, followed by administration of a monthly preventative.
- Administer biweekly anthelmintic treatment of kittens between 3 and 9 weeks of age, followed by administration of a monthly preventative.
- Treat nursing bitches and queens along with their offspring.
- Tailor parasite prevention programmes to geographical, seasonal and lifestyle factors.

In the absence of optimal year-round heartworm preventive/intestinal parasite combination products, CAPC recommends the following protocol:

- Deworm puppies and kittens at 2, 4, 6 and 8 weeks of age and then again monthly until 6 months of age.
- In kittens, begin biweekly anthelmintic treatment between 3 and 9 weeks of age and then again monthly until 6 months of age.
- Conduct faecal examinations two to four times a year in adult pets, depending on patient health and lifestyle factors, and treat with appropriate parasiticides.

While it has long been recognized that transplacental and transmammary infection of ascarids and hookworms could be prevented through prophylactic treatment of pregnant dogs, no drugs are currently approved in the USA for this use. However, the effectiveness of this approach with different drugs approved for parasite control in dogs has been well documented. Daily treatment of pregnant bitches with fenbendazole from the 40th day of gestation to the 14th day of lactation has been shown to inhibit *T. canis* larvae in tissues, thereby preventing or greatly reducing the incidence of infection in puppies (Duwel and Strasser, 1978). Alternatively, studies have shown that treatment with ivermectin on days 0, 30 and 60 of gestation and 10 days post-whelping reduced the adult *T. canis* worm burden in pups by 100% and prevented the shedding of eggs (Payne and Ridley, 1999). In yet another study, treatment with selamectin at 10 and 40 days both prior to and after parturition was effective in reducing *T. canis* faecal egg counts in both pups and their dams, and adult worms in the pups (Payne-Johnson *et al.*, 2000).

Educating and Counselling Pet Owners

Pet owner education regarding intestinal parasites and their effects on the health of both their pets and family should be an important part of a well-pet examination. Such education should focus on prevention and include the following considerations:

- Local prevalence and types of intestinal helminths that infect dogs and cats, as well as the early signs of illness.

- How dogs and cats become infected and when they are at greatest risk for infection, especially by prenatal and transmammary routes.
- How ascarids and hookworms cause disease in humans, especially in children whose play habits and attraction to pets put them at increased risk.
- How prophylactic treatment of pregnant and nursing animals and their offspring can protect them from infection and from shedding eggs into the environment.
- Regular diagnostic faecal examinations or prophylactic treatment of older pets.
- Prompt collection and disposal of pet waste, especially in areas where children play, to remove eggs from the environment before they can become a problem.
- Keep children away from areas that may be contaminated with pet waste.

Educating clients about the potential health dangers can be done in a way that does not frighten or discourage the potential pet owner from acquiring a pet. Veterinarians have a good

opportunity to boost their public image by playing an important social role and getting involved in this public health issue. Veterinarians sometimes express concern that a client may give up his or her pet after being informed of the risks associated with pet ownership. It is our challenge to make pet owners realize that an appropriate response, which will allow them to enjoy the many benefits of pet ownership and greatly reduce any risk to themselves, is to follow our recommendations for prevention, to be responsible pet owners with regard to the disposal of dog and cat faeces and to provide adequate veterinary care for their pets. Ultimately, clients expect counselling from their veterinarian, and effective communication of the dangers of parasite transmission will benefit clients and veterinary practitioners alike. From an ethical point of view, veterinarians must offer their clients the best available treatments and teach them about measures to help ensure the health of pets, especially in terms of major diseases and zoonoses. Veterinarians are uniquely suited for this role and may be legally responsible for informing the owner about the risks.

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18 *Toxocara vitulorum* in Livestock

Wilma A. Starke-Buzetti

Departamento de Biologia e Zootecnia, Faculdade de Engenharia,
Universidade Estadual Paulista 'Júlio de Mesquita
Filho' (FEIS/UNESP), Campus de Ilha Solteira, Brazil

Introduction

Toxocara (syn. *Neascaris*) *vitulorum* is a worldwide member of the Ascaridoidea, a highly prevalent parasite of cattle and buffaloes in tropical and subtropical regions. It is responsible for high morbidity and mortality rates of 15- to 50-day-old calves. When the infection is not controlled in the field, the prevalence can reach 100% in calves and deaths frequently occur when associated with poor nutrition. The control of this parasite is not easy because the larvae migrate in the host tissues remaining as dormant or hypobiotic parasites and can survive for a long time. Moreover, larval trans-mammary passage to calves occurs after birth and is the most important route of the infection. Only a limited number of studies have been published and one reason for this may be that this parasite is more of a problem of bovine and buffalo calves, particularly from poor tropical countries. However, the water buffalo is considered among the most productive domestic animals in these countries. The world population of water buffaloes is more than 150 million in some 40 countries, ranging from Australia through Indonesia and the Philippines to Pakistan, Indo-China, India, Burma, Iran, Iraq, Turkey, Russia, Egypt and several European countries (Albania, Bulgaria, Greece, Italy, Romania and Yugoslavia). In the New World, there are buffaloes in Brazil, Colombia, Ecuador, Venezuela and the USA (Cockrill, 1981). Buffalo is also a source of quality meat and milk, which, as a

result of its unique conversion capacity, can be produced more cheaply than by cattle and perhaps any other animal. There is an expanding trade in breeding stock and in frozen semen (Cockrill, 1981). The opinion is frequently expressed that water buffaloes are resistant to many of the infections of cattle and other species; however, *T. vitulorum* infection is widespread and worm burdens are invariably much heavier in buffalo calves than in cattle calves. This parasite can be a serious impediment to successful buffalo breeding.

Morphology

T. vitulorum adult is a large roundworm with translucent and soft body surfaces. Female worms measure 150–400 mm in length by 51–70 mm in width and males measure 106–275 mm in length by 25–41 mm in width. The adult worm possesses three well-defined lips, one dorsal and two subventral. The dorsal lip exhibits two large external papillae. Each subventral lip exhibits one large ventro-lateral papilla and two smaller papillae dorso-lateral. Each lip possesses a dentigerous ridge just below the anterior margin of the lip on the inner surface (Fig. 18.1A). The cervical alae are present in *T. vitulorum*, but they are reduced to a short lateral ridge of 0.02 mm wide supported by a V-shaped bar. The tail of the male has a cloaca with two subequal spicules and two rows of single latero-ventral pre-cloacal papillae with one

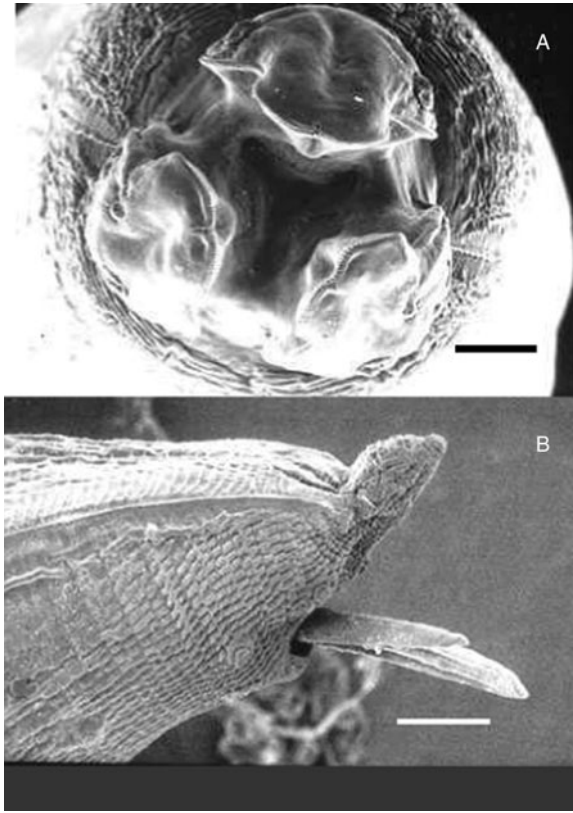


Fig. 18.1. (A) Apical view of the anterior end of *Toxocara vitulorum* (scanning electronic microscope) showing the triradiate oral opening with three (one dorsal and two subventral) labia with dentigerous ridges just below the anterior margin of the lip on the inner surface, apical angled notches, and sensory organs: four labial large papillae (two external papillae on the dorsal lip and one ventro-lateral on each subventral lip). (B) Lateral view showing both spicules and the appendage. Scale bar, 100 μm .

pair of double post-cloacal papillae. The tail is constricted behind the cloaca forming a terminal appendage bearing four semi-paired papillae (Fig. 18.1B).

Warren (1970) described the eggs of *Toxocara* species exhibiting the characteristic pitted, 'honeycomb' or mosaic pattern on the surface of the shell with the scale of the pitting that varies with each species. *T. vitulorum* exhibit the finest pits (Fig. 18.2A).

The dimensions of *T. vitulorum* infective larvae (third-stage, L3) present in the eggs range from 298 to 462 μm long by 10–23 μm maximum diameter (Warren, 1971; Mia *et al.*, 1975; Chauhan and Pande, 1981; Starke-Buzetti *et al.*,

2004). The anterior end is rounded and the larva has a buccal cavity and a vestibular-oesophageal region (Fig. 18.2B).

The larvae of *T. vitulorum* present in the milk are considered to be the L3 stage (Roberts, 1990b) and measure approximately 800–1700 μm long by 30–40 μm maximum diameter with 104–280 μm oesophagus length. The larvae have three distinct lips with a distinct ventriculus located approximately one-quarter along the length of the larvae. The presence of a cuticular lining of the rectum in most *T. vitulorum* in milk is an additional obvious characteristic of this larva (Tongson, 1971; Warren, 1971; Roberts *et al.*, 1990; Starke *et al.*, 1992).

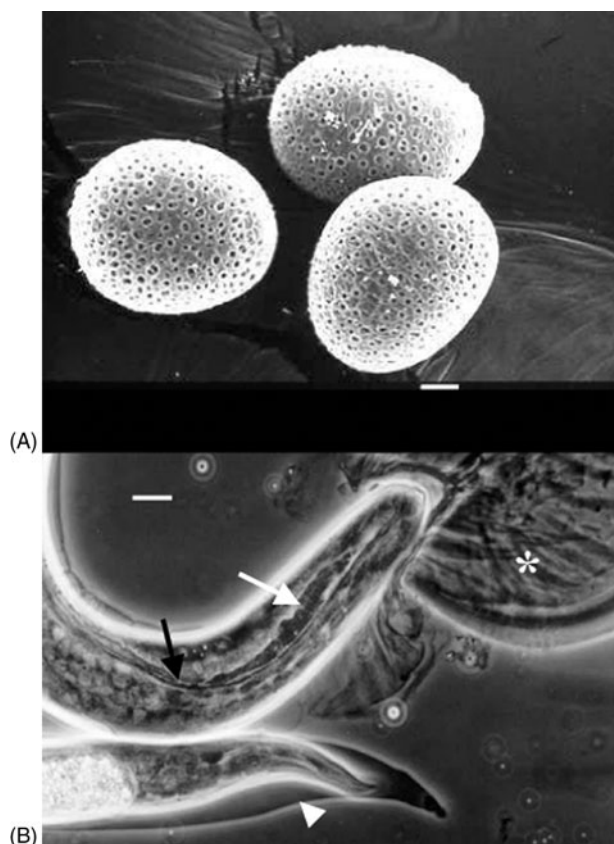


Fig. 18.2. (A) Eggs collected from the female of *Toxocara vitulorum* visualized by scanning electron microscopy. The eggs exhibit the characteristic pitted, 'honeycomb' or mosaic pattern on the surface of the shell. The scale of the pitting varies with the species, but *T. vitulorum* exhibit the finest. (B) *T. vitulorum* larva (L3) of 18 days of cultivation and after artificial hatching on a coverglass. The L3 is covered with a loose L2 cuticle (white arrow head) at the posterior end (larva tail). Note the dorsal glandular cell surrounding the oesophagus (white arrow) and nerve ring (black arrow). Note also the egg shell (white asterisk). Phase-contrast microscopy. Scale bar, 10 μm .

Life Cycle

Larval development inside the egg

Most first stage larvae (L1) develop in 5–11 days and the second (L2) in 10–12 days at 27–29°C (Refuerzo *et al.*, 1952; Chauhan and Pande, 1981). The 17-day-old and slightly older cultures with moulted larvae readily infected a calf, rats and guinea pigs, some of which died of pneumonia a few days after oral administration of eggs, but 5-month-old cultures demonstrated a considerable reduction in infectivity (Refuerzo *et al.*, 1952). Seventy to ninety

percent of eggs obtained from female worms developed to the infective stage in 19–21 days in egg culture at 24–29°C (Starke *et al.*, 1993), but about 95% of eggs from faeces were fertile in culture (Refuerzo *et al.*, 1952; Roberts, 1990a).

Several authors have reported a second larval moult within the egg of ascaridids indicating that the larva had undergone two developmental stages in the egg and that L3 was the infective stage. However, the sheath of the second moult is seen infrequently, suggesting that the larvae lose the cuticle of the first moult when they grow, particularly 30-day-old eggs or older (Bruñaská *et al.*, 1995; Geenen *et al.*, 1999).

infection, had grown by 10–20%, but others had not grown. Over 3–7 weeks the larvae grew by about 10% and no moulting was observed.

8. In pregnant buffalo with natural infection most larvae remained in the liver. In the liver and lungs, larvae grew 500–600 μm 1–8 days before parturition and migrated to the mammary gland around the time of parturition and grew to about 1200 μm and passed into the milk during the first 10 days after parturition.

9. The first appearance of labelled larvae in the mammary gland was shortly before parturition and the numbers increased until 5 days after parturition. By 8 days, 99% of larvae had already passed into the milk of lactating buffalo.

10. The route of migration from liver and lung to the mammary gland was most likely via the bloodstream.

11. The distribution of labelled larvae in pregnant hosts was similar to that in non-pregnant hosts. There was no accumulation of larvae in uterine muscle and there were few in maternal cotyledons.

12. In the absence of re-infection, the reservoir of larvae in the tissues of the maternal host had approximately halved in a year, but a few larvae persisted through two pregnancies (Roberts, 1992).

13. Only a few larvae were found in calves before suckling colostrum, but 93% of *T. vitulorum* was found in the intestines within 6 days of calving, demonstrating the transmammary route of infection in calves.

Routes of *T. vitulorum* infection in calves

Mozgovoi and Shikhov (1971) reported the occurrence of prenatal infection in buffaloes by the presence of *T. vitulorum* larvae in uterine caruncles, in the amniotic fluid, in the small intestine of one fetal calf and in the duodenum of a 3-day-old calf. In contrast, many experiments have failed to prove transplacental infection (Warren, 1971; Mia *et al.*, 1975; Roberts, 1990b). Pregnant cows fed with *T. vitulorum* eggs delivered calves that developed patent infections, but their fetuses and newborn calves without suckling colostrum were negative. Mia *et al.* (1975) considered the dam as an intermediate host for the parasite and did not find any evidence of direct or prenatal infection or of somatic migration in the calves.

Larvae of *T. vitulorum* were recovered in the colostrum/milk from 90% of cattle by day 11 (Warren, 1971). In buffalo, larvae were seen in milk from 2 to 22 days post-partum and disappeared thereafter (Tongson, 1971), 1 to 11 days after parturition (Mia *et al.*, 1975; Gautam *et al.*, 1976b) and 99% within 8 days (Roberts *et al.*, 1990).

Later, Starke *et al.* (1992) reported that 54.8% of buffalo cows naturally infected with *T. vitulorum* were shedding larvae in colostrum/milk during the first 10 days after parturition. One hundred per cent of their calves started to shed eggs from 6 to 29 days after birth: 6.3–26.7% in the first 10 days; 41.3–66.7% by day 20 and 100% by day 30 after birth. Faecal examination of these cows revealed eggs of *T. vitulorum* during the lactation period (Table 18.1).

On the other hand, the direct cycle by oral infection with infective eggs is not clear. Experiments done by infecting cow or buffalo calves with single or multiple doses of infective eggs failed to establish patent infection (Herlich and Porter, 1954; Refuerzo and Albis-Jimenez, 1954; Mia *et al.*, 1975; Akyol, 1993). However, Refuerzo *et al.* (1952) reported that larvae were retrieved from an orally, experimentally infected calf after 14 days of infection. Unfortunately, this calf was killed too early and before establishment of mature worms in the intestines. Later, Roberts (1990b) successfully infected suckling 2–3-week-old buffalo calves by oral administration of larvae hatched *in vitro*, but the life cycle to mature worm in the intestine was not reported.

Occurrence in Paratenic Hosts and Visceral Larva Migrants

Experimental infections of *T. vitulorum* have been undertaken in a wide range of non-normal or paratenic hosts. These include guinea pigs (Refuerzo *et al.*, 1952), mice (Warren, 1971; Chauhan and Pande, 1972; Amerasinghe *et al.*, 1992; Paula *et al.*, 2004), rats (Chauhan *et al.*, 1974), chickens (Chauhan *et al.*, 1974) and rabbits (Barriga and Omar, 1992). Larval invasion has been observed in many organs including the liver, lungs, kidney, heart, pancreas, spleen, hepatic portal vein, abdominal cavity, trachea, oesophagus and mesentery during the first 13 days of infection (Chauhan and Pande, 1972). Larvae were also recovered from skeletal musculature of mice

Table 18.1. Presence of *Toxocara vitulorum* eggs in the faeces of buffalo cows and their calves during the first 30 days post-partum, during the years 1989 and 1990.

Days post-partum	Buffalo cows				Buffalo calves			
	Number examined	Number of positives	%	EPG range	Number of calves	Number examined	%	EPG range
1989								
1–10	15	4	26.7	1–3	15	4	26.7	1–30
11–20	15	7	46.7	1–44	15	10	66.7	1–23,100
21–30	15	7	46.7	1–15	15	15	100	1–60,500
1990								
1–10	16	4	25.9	1–14	16	1	6.3	0–6
11–20	17	2	11.8	1–24	17	7	41.3	1–336
21–30	17	5	29.4	1–22	17	17	100	1–8,108

(Starke *et al.*, 1992)
EPG, eggs per gram of faeces

(Warren, 1971) and rabbits (Omar and Barriga, 1992). The muscle larvae did not develop beyond the infective stage suggesting that they were probably hypobiotic organisms.

There is no evidence yet that infective eggs of *T. vitulorum* can infect humans, but the larvae from the eggs migrate in paratenic hosts, and the possibility of migration in humans has been suggested by Fernando *et al.* (1970). However, the diagnosis of *T. vitulorum* visceral larva migrans in humans is difficult because the antibody ELISA test presently used for diagnosis does not distinguish between infection of *Toxocara canis*, *Toxocara cati* and *T. vitulorum* (Page *et al.*, 1991). *T. vitulorum* antigenic isolation and characterization to develop monoclonal antibodies against epitopes of this particular parasite could contribute to a specific test for diagnosis of human infection in the future.

Geographical Distribution

T. vitulorum in cattle and buffalo is cosmopolitan in tropical and subtropical regions and in regions with a moderate, continental climate. It has been broadly reported from several countries in Africa and Asia where this infection may cause clinical disease with high mortality in buffalo (Over *et al.*, 2004). However, *T. vitulorum* is also present in temperate regions when intensive husbandry procedures provide the conditions for egg development and infection of cows (Thienpont and De Keyser, 1981).

T. vitulorum was also mentioned by Over *et al.* (2004) in cattle and buffaloes in many countries

from Latin America (Brazil, British Guyana, Cuba, Mexico and Peru). In addition, the presence of *T. vitulorum* in Brazil was reported by Silva (1969), Costa *et al.* (1980), Starke *et al.* (1983), Láu (1999), Neves *et al.* (2003) and de Souza *et al.* (2004).

Occurrence and Prevalence

In buffaloes, the eggs of *T. vitulorum* are first detected in the faeces of calves (prepatent period) when they are 11–37 days old. The prepatent period for cow calves was somewhat longer (ranging from 24 to 110 days after birth). The peak egg output normally occurs around 35–62 days after buffalo birth and is followed by a sharp decrease in egg number to zero around 101–120 days. The duration of the peak is about 5.5 ± 2.5 days with $98,000 \pm 63,700$ eggs/g faeces (EPG). The duration of the patent period is around 28–104 days (Roberts, 1990a; Neves *et al.*, 2003; Souza *et al.*, 2004) (see Table 18.2 and Fig. 18.4).

The egg output per female per day at the peak was estimated by Roberts (1990a) to be $110,000 \pm 58,000$ and correlated with the size of the females at autopsy. In Sri Lanka, at least 72% of calves had patent infections and current treatment procedures did not reduce the prevalence (Roberts, 1989b).

Patent toxocariasis occurs in calves aged between 3 and 10 weeks, so if a measured prevalence is to have meaning, it should be restricted to calves in that age range. If the age category selected for a study of prevalence includes animals

Table 18.2. Prepatent, peak and patent periods of *Toxocara vitulorum* infection in cattle and buffalo determined by EPG and by different authors.

	Mean ± SD (range)		
	Prepatent period (days)	Peak (days)	Patent period (days)
Pandey <i>et al.</i> (1990): natural infection in cattle*	41 ± 20 (24–110)	ND	65 ± 30 (14–112)
Roberts (1990a): experimental infection in buffalo**	22.3 ± 1.6	35.7 ± 2.6	35 ± 12
Neves <i>et al.</i> (2003): natural infection in buffalo***	27.6 ± 7.1 (11–37)	49 ± 9.5 (37–62)	78 ± 15.4 (65–104)
de Souza <i>et al.</i> (2004): natural infection in buffalo***	30.3 ± 3.9 (23–36)	44.3 ± 6.6 (35–57)	57.5 ± 20.8 (28–86)

* In Africa (Zimbabwe); ** in Asia (Sri Lanka); *** in Latin America (Brazil).
ND, No data

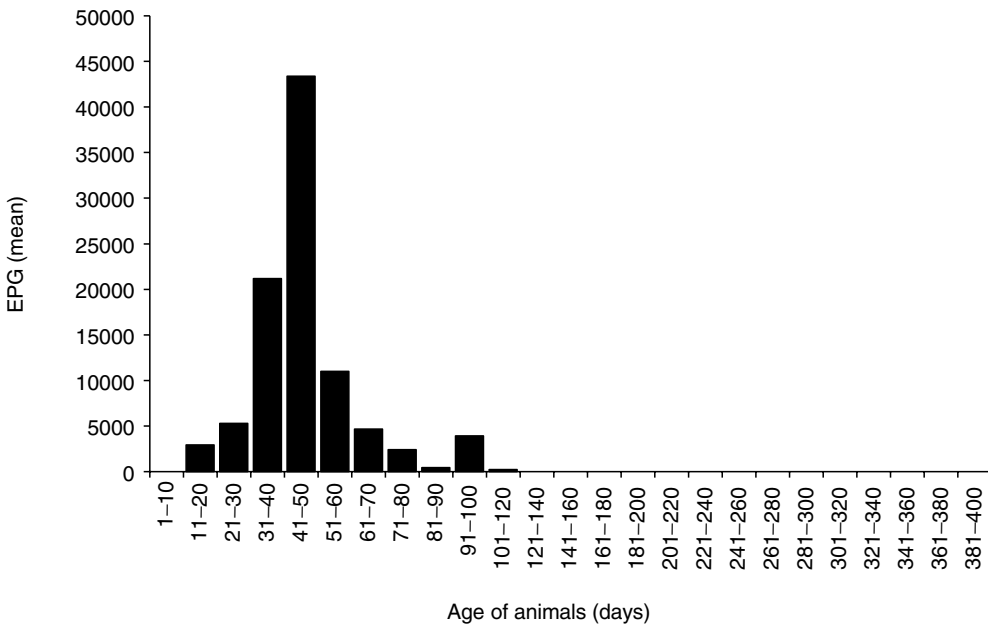


Fig. 18.4. Eggs of *Toxocara vitulorum* per gram of faeces (EPG) from buffalo calves from birth to 400 days of age (Starke *et al.*, 1983).

outside that age range, then the prevalence reported can be underestimated (Roberts, 1993). In agreement with this statement, in Brazil, the prevalence of the infection can be around 100% in calves by 3 months of age (Starke *et al.*, 1983; Barbosa *et al.*, 1992).

In Egypt, Selim and Tawfik (1974) reported different prevalence rates: 70.9, 12.7 and 3.21% of buffalo calves found to be infected during

the first 2 months, 2–12 and 1–2 months of age, respectively. Calves of 4–12 months were negative after repeated examinations and this absence of adult worms was related to a self-curing mechanism. Selim and Tawfik (1974) suggested that the presence of worms in adult animals might be due to the weak immunity developed in some individual older hosts. Buffaloes of 2–4 and 4–6 years old had an infection rate of 6.52 and 11.27%, respectively,

and this high rate was suggested to be due to a decrease in resistance as a result of some stress factor such as pregnancy, lactation, malnutrition or other disease. On the other hand, the rate of infection among buffaloes over 6 years old dropped to 0.05%, and was probably related to age resistance or due to the inhibition of larval development to adult stage.

With respect to the seasonal prevalence of *T. vitulorum*, Chauhan *et al.* (1973) reported that the prevalence of the infection was maximum during summer, when 54.7% of buffalo calves and 37.7% of cattle calves below 1 year of age were infected.

T. vitulorum can infect bison (*Bison* sp.), sheep (*Ovis aries*) or goats (*Capra hircus*); however, according to Roberts (1993), there are controversial data in the literature about the occurrence of *T. vitulorum* in sheep and goats.

Pathogenesis and Clinical Signs

The pathogenesis and clinical signs of *T. vitulorum* infection are related particularly to large numbers of this parasite inside the small intestines. The adult worms are frequently packed into a section of the duodenum and into the first portion of the jejunum.

Calves can die when severely infected and hundreds of worms can be seen in the intestines. Srivastava (1963) counted 318–400 mature adult worms inside the intestine of a single calf. Roberts *et al.* (1990) observed average burdens of 95 ± 65 parasites (range 20–206) in buffalo calves aged 24–28 days. The mucosa of the intestine exhibits small pits as a consequence of the mechanical activity of the worms inside the lumen and a mass of muco-purulent fluid of yellowish grey colour mixed with flakes of mucosa was also reported (Srivastava, 1963). Haemorrhagic enteritis with occasional necrosis, pneumonia, occurrence of peritoneal, pleural and pericardial fluid, intestinal obstruction, intussusception and intestinal perforation can occur as a consequence of a high infection. Intestinal wall perforation at the serosal surface was observed by Das and Singh (1955) and Srivastava (1963). Live adult parasites in the biliary passages and partly attached to the liver capsule were also observed by Srivastava (1963).

The infected intestinal mucosa was associated with infiltration of lymphocytes, eosinophils, fibroblasts and macrophages (Srivastava, 1963). Hyperplasia of mast cells and eosinophils in the mucosa of the duodenum and jejunum, particularly, during the peak of the infection were reported (Neves *et al.*, 2003; Neves and Starke-Buzetti, 2004).

Common signs of infection are: aqueous diarrhoea resembling white scour, inappetance, intermittent colic, tympanism, occasional dysentery, anorexia, constipation, dehydration, steatorrhea, abdominal pain, butyric odour on the breath, loss of weight, loss of plasma proteins into the intestine and loss of glossiness and skin tone with eczema (Das and Singh, 1955; Enyinihi, 1969; Roberts, 1993).

Aktar *et al.* (1982) considered 5000 EPG as a light infection, 5000–10,000 as moderate and above 10,000 as heavy. For Roberts (1990a), a count of 20,000 EPG near the peak output has been suggested as indicative of a pathogenic worm burden. However, the pathogenesis of the infection can be more serious and deaths can frequently occur when associated with poor nutritional status, even with lower EPG.

T. vitulorum infection induces a marked anaemia in addition to a significant leucocytosis with neutrophilia, eosinophilia, basophilia and lymphopenia. Serum alkaline phosphatase, glutamic oxaloacetic and glutamic pyruvic transaminase levels increase probably due to complications caused by the extensive larval migration or due to hepatic toxins produced by metabolism of the larvae or substances formed as result of the decomposition of dead parasites which may be hepatic toxins. Higher values of serum urea and creatinine also seen may be attributed to kidney impairment (El-Albdin *et al.*, 1975). Gupta *et al.* (1976) observed increased levels of histamine in the serum of infected calves, probably indicating the presence of allergenic compounds in the excretory–secretory antigens of the migratory larvae or in the perienteric fluid of the adult worms inside the intestines.

Economic Impact

Infections with gastrointestinal worms often cause outbreaks of diseases and are a continual source of

economic losses. *T. vitulorum* is one of the most dangerous intestinal parasites affecting buffalo calves in tropical and subtropical countries. Although there is enough knowledge on the epidemiology of *T. vitulorum* infection in cattle and buffaloes, very little information on the economic impact of the disease is available.

According to the Food and Agriculture Organization statistics from 1992, the world water buffalo population ranks first in India (53%), second in China (14.8%) and third in Pakistan (12.9%). Water buffaloes from these countries contribute significantly to the national economy, providing up to 71% of milk and 35% of beef production. Brazil has a population about one million, which is estimated to be increasing at a rate of about 8% per year, distributed all over the country, but more concentrated in the north (64.8%) and south (14.3%) (Annual PEC, 2002).

However, *T. vitulorum* seriously affects buffalo calves of less than 3 months of age and this infection is generally recognized as an important limiting factor in calf rearing because of the high morbidity, the attendant loss in production and disturbance in the breeding programme. When toxocarosis is not controlled in the field, the prevalence can approach 100% (Das and Singh, 1955; Starke *et al.*, 1983; Roberts, 1989b) and the mortality rate can be 80% (Das and Singh, 1955). Enyenihi (1969) demonstrated an inverse relationship between the worm burden and appetite and weight gain: heavily infected calves had only half the weight of treated controls at 10 weeks of age. After deworming the heavily infected calves gained weight at the same rate as the controls, but there was no compensatory weight gain. Treated calves had gained 25% more weight than infected calves at 4 months of age.

The high levels of natural infections reported in India and other countries are responsible for poor condition and death among native African and Indian herds, which are frequently reared under conditions of subnutrition. Lack of knowledge among farmers is likely to accelerate the transmission of *T. vitulorum* based upon specific life cycle traits including dormancy of larvae in cows and galactogenic transmission. To protect cattle from *T. vitulorum* infection, farming conditions must be improved in addition to the use of anthelmintics or alternative control strategies including the control of dams (Akyol, 1993).

Epidemiology

In the review of Roberts (1993), four points of the life cycle of *T. vitulorum* were reported as important for the epidemiology of the disease: (i) the long period of survival of eggs in the environment; (ii) the long period of survival of larvae in the tissues of the maternal host; (iii) transmission through the mother; and (iv) the large number of eggs produced over a short period during the early life of the calf.

The vicinity of the aquatic environment of the buffalo, or the yards and dung heaps of an intensive dairy, provide suitable temperature, humidity and oxygen requirements for the development and survival of parasite eggs. Roberts (1989a) reported that some infective eggs of *T. vitulorum* persisted in soil at a 3–5 cm depth for 17 months, finally dying during a prolonged hot, dry period in the villages of Sri Lanka. On the other hand, eggs in a wallow developed intermittently over 16 months as they were flushed with rain water, and eventually died when the wallow dried out. Around the wallows, calf pens and milking areas, where soil was compacted by the activities of adult buffalo and moisture was available, infective eggs were present in the top 1 cm of soil and were transferred to the skin and hair of cows and calves (Roberts, 1989a).

Survival of *T. vitulorum* in the field for more than 2 years has been reported (Thienpont and De Keyser, 1981). The optimum temperature for the development of eggs to infective larvae was in the range of 20–30°C with a period of 2–3 weeks. This condition would be obtained in the pasture during summer (Akyol, 1993). However, the eggs can tolerate low temperatures and develop when the temperature becomes higher. This means that even in the winter eggs buried under snow can survive and begin to develop in spring or summer and the hosts could become infected throughout the year (Akyol, 1993).

Eggs in the pasture can be an important source of infection for cows. The larvae resulting from this infection do not mature until the infection is passed to the calf through the milk of the cow. The life cycle of *T. vitulorum* will be completed when infected cows become pregnant and deliver calves. The life cycle is therefore less

dependent on climate and more dependent on the management of cattle and buffalo. If the larvae are protected in the tissues of the maternal host, seasonal influences do not perturb prevalence levels in calves (Roberts, 1990b). The larvae in the tissues of a cow can persist for up 5 months (Warren, 1971).

Therefore, transmission to female animals is important for determining prevalence. Farmers who allow cows to graze in the pasture and keep cows and their sucklings together in narrow stables may increase the probability of ingestion of eggs by cows in the infected pasture or soil and transmission of infective larvae to the sucklings through milk. Once the infection is introduced in one area, the parasite cannot easily be eliminated from the population of the host animals without chemotherapy (Akyol, 1993). On the other hand, the seasonal calving pattern of buffalo that are born at, or shortly after, the peak of rainfall in the wet season in the humid tropics (Starke *et al.*, 1983; Roberts, 1989a) is favourable for the survival and development of the eggs of *T. vitulorum* in the environment.

Treatment

According to Roberts (1990a), during the patent period calves can excrete about 70×10^6 eggs or more into the environment. Thus the benefit of the ovicidal properties of some anthelmintics against ascarids in reducing environmental contamination is important, particularly for treating the calves once when 10–16 days old

(Roberts, 1989b). However, several regimes have been proposed for treating mature infections, but most are not effective in controlling *T. vitulorum* in calves, dams and in the environment. If an anthelmintic is administered at 25–28 days to treat calves, some animals are already excreting eggs, and calves with a large number of parasites may be suffering morbidity (Roberts, 1989b).

In Brazil, Láu (1980) proposed a programme for parasite control in animals and for the prevention of environmental contamination that consisted of four anthelmintic applications at 15, 30, 60 and 180 days post-birth using mebendazole, oxybenzole, fenbendazole and thiabendazole. However, the economic benefits of this programme were not evaluated.

In Sri Lanka, Roberts (1989a) treated 10-day-old calves with a single dose of an anthelmintic against immature and mature intestinal parasites (pyrantel or oral levamisole) with an efficacy of 97%. According to Roberts (1989a), this procedure could reduce contamination of the environment and also preclude the pathogenic effect of a large number of immature or mature parasites. Also, after eliminating the developing immature worms acquired through the milk, reinfection by the trans-mammary route would be unlikely to recur.

Adult parasites in the intestine of a calf are susceptible to a number of anthelmintics (Table 18.3). Against intestinal immature parasites the efficacy of pyrantel and levamisole was 97%, piperazine 42% and thiabendazole 35%. Against mature parasites, the efficacy of pyrantel was 97%, oral levamisole 83%, cutaneous

Table 18.3. Efficacy of anthelmintics against *Toxocara vitulorum* in calves.

Anthelmintic	Dose rate (mg/kg)	Notes	Efficacy	Reference
Piperazine	15	For 9 days	High (100%)	Das and Singh (1955)
	220			
	200	< 20 days old	Ineffective (100%)	Lee (1955)
Levamisole	6	8–18 days old	Effective (97%)	Roberts (1989a)
	7.5			Láu (1980)
Morantel	10	8–18 days old	Effective (97%)	Roberts (1989a)
Pyrantel	8			Gupta <i>et al.</i> (1983)
Fenbendazole	7.5	8–20 days old	Unreliable	Roberts (1989a)
Febantel	6			Roberts (1989a)
Thiabendazole	66	8–18 days old	(36%)	Roberts (1989a)
Ivermectin	0.2			Gill <i>et al.</i> (1989)

(Data adapted from Roberts, 1993).

levamisole 73%, oxfendazole 89% and piperazine 57% (Roberts, 1989a). Gill *et al.* (1989) observed, based on egg counts, a high efficacy of ivermectin; however, according to Hassnain and Degheidy (1990) it was not effective.

An anthelmintic to kill migratory larvae in the tissues of maternal hosts would prevent calves from acquiring infection from milk. However, little is known about the efficiency of larvicidal drugs regarding *T. vitulorum* in buffaloes. The effects of levamisole, albendazole, fenbendazole and ivermectin (Abo-Shehadeh and Herbert, 1984) and levamisole and ivermectin (Carrilo and Barriga, 1987) were tested only against the larval migratory stage of *T. canis* in mice. Levamisole and ivermectin were considered the most effective, but 38% of larvae remained alive in the liver of the host even after 13 days of treatment. Treatment on days 8–13 after infection had no effect on larval stages suggesting that once the larvae had reached the brain and musculature they were not susceptible to anthelmintic agents. The inhibition of the larval migration by levamisole was transitory and the liver parasites were capable of reaching the brain after treatment was suspended.

When levamisole was injected into pregnant buffalo cows between 7 and 8 months of pregnancy or 21 days before the parturition day, the level of infection of calves was reduced (Simões, 1972; Láu, 1980), but this did not eliminate infection. According to Roberts (1993), the approach of treating pregnant cows with anthelmintic is unlikely to be practicable because of the difficulty of knowing reliably the stage of pregnancy, the harmful side effects from handling and treating animals in late pregnancy and the larger doses of anthelmintic required for a cow. Furthermore, Barriga and Omar (1992) suggested an alternative possibility of controlling this infection by vaccination of the dams to kill the larvae in tissues before they are transferred to the calves.

Humoral Immune Response

T. vitulorum infection can stimulate the immune system of the buffalo. Fernando *et al.* (1987) reported gel-precipitating antibodies against an extract of embryonated *T. vitulorum* eggs in the serum and colostrum of some pregnant buffaloes

and in the serum of their suckled calves. More recently, anti-*T. vitulorum* antibodies against larval excretory–secretory antigen (ES) (Rajapakse *et al.*, 1994), larval soluble extract antigen (Ex) (Starke-Buzetti *et al.*, 2001), and larval ES, Ex antigens and adult perienteric fluid (Pe) (de Souza *et al.*, 2004) were detected in serum and colostrum of buffalo cows and calves naturally infected with *T. vitulorum*. Anti-ES antibody was higher on the first 10 days after parturition and during the perienteric period in the serum of buffalo cows (Rajapakse *et al.*, 1994). Thereafter, de Souza *et al.* (2004) confirmed by ELISA that the antibody levels were highest on the day of parturition in the colostrum, but declined rapidly after the 7th day to reach very low concentrations on days 8–15 for Ex and ES antigens and on days 24–30 for Pe antigen (Fig. 18.5). In both serum and colostrum of buffaloes, the highest levels of activity against *T. vitulorum* were present in the IgG fraction, particularly IgG1 (Fernando *et al.*, 1989; Rajapakse *et al.*, 1994). In the serum of cows, anti-ES, -Ex and -Pe antibodies were detected at high levels from parturition to 300 days after parturition (de Souza *et al.*, 2004). Starke-Buzetti and Ferreira (2004a,b, 2005) characterized Ex, ES and Pe antigens by SDS-PAGE and Western blotting (WB), using immune sera and colostrum of buffaloes naturally infected with *T. vitulorum*. The results showed that the antigens Pe, Ex and ES revealed nine (11, 14, 31, 38, 58, 76, 88, 112 and 165 kDa), 11 (11, 13, 16, 22, 25, 32, 43, 53, 68, 82 and 96 kDa) and eight (19, 48, 56, 64, 90, 110, 150 and 190 kDa) protein bands by SDS-PAGE, respectively. All protein bands were recognized by sera and colostrum sampled from buffalo cows on the day of parturition.

In calves, de Souza *et al.* (2004) determined (Fig. 18.6) that passively acquired anti-ES, -Ex and -Pe antibody concentrations were highest 24 h after birth and remained at high levels until 45 days, the time coinciding with the peak of *T. vitulorum* infection. Rejection of the worms by calves occurred simultaneously with the decline of antibody levels. Circulating antibodies subsequently reached their lowest levels between 76 and 90 days post-partum. Thereafter, anti-*T. vitulorum* antibodies started to rise slowly (possibly a result of acquired immunity) until the animal reached the age of 365 days. By WB, sera of buffalo calves at 1 day of age, after suckling the colostrum and at the beginning of infection, reacted with the

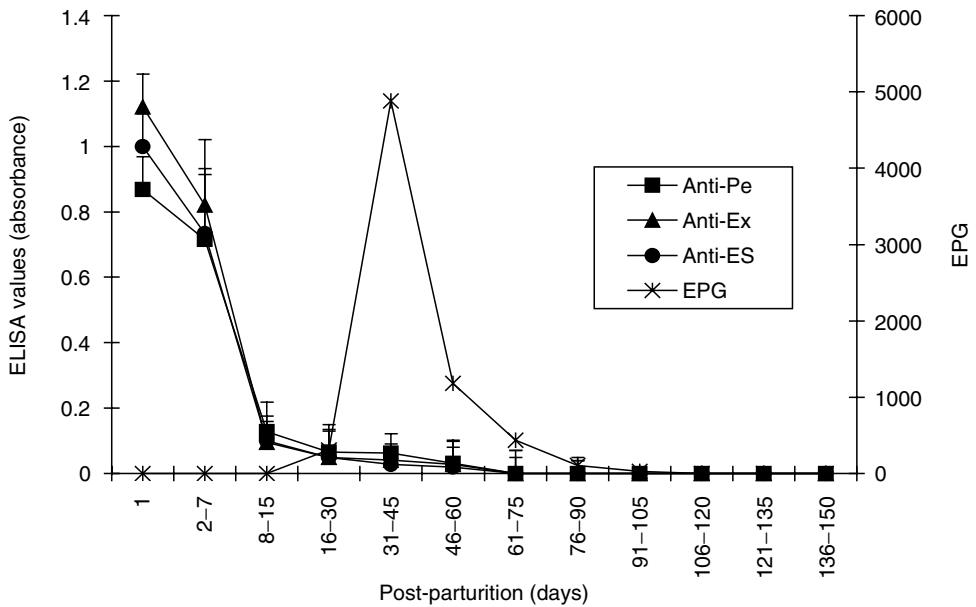


Fig. 18.5. ELISA values of *T. vitulorum* anti-Pe, anti-Ex and anti-ES antibodies in colostrum of buffalo cows during the first 60 days post-parturition compared with the parasitic status of the calves represented by EPG counts. Standard deviation of the mean is also represented (de Souza *et al.*, 2004).

same bands detected by serum and colostrum of the buffalo cows. Only bands of higher molecular weight antigen (76, 88, 112 and 165 kDa for Pe; 68, 82 and 96 for Ex and 190, 110, 150 and 190 for ES) were detected by anti-*T. vitulorum* antibody in the sera of the calves at the peak of the infection, during the decline of EPG counts (rejection of the worms) and during the period of post-rejection. The exception was anti-ES antibodies, which disappeared from the serum of the calves during the period of post-rejection (Starke-Buzetti and Ferreira, 2004a,b, 2005). Thus, antigens of high molecular weights (68–190 kDa) could represent useful larval antigens in reducing larval gut penetration and contribute to the inhibition of larval migration in buffalo cows. This would be particularly useful if inhibition prevented larval migration to the mammary gland of buffalo cows, avoiding or reducing transmission of the parasite through the colostrum.

In mice, Amerasinghe *et al.* (1992) and Paula *et al.* (2004) reported that immunization with *T. vitulorum* Pe, Ex and ES antigens induced strong larval migration inhibition (58–100%), suggesting potential protection against infection.

Barriga and Omar (1992) infected rabbits with *T. vitulorum* eggs and reported that the production of IgG antibody was very strong after the third infection. WB analysis revealed four antigens (32–41 kDa) that may be related to protection.

Cellular Immune Response

T. vitulorum is expelled by calves after the peak of egg output and calves did not shed eggs after more than a year of monthly faecal examination, suggesting some immunological response against intestinal reinfection. Because the drop in egg production was not affected by administration of dexamethasone or by restricting calves to a milk diet, Roberts (1990a) suggested that the drop may be due to an ageing process and reduced fecundity in the parasite. However, this hypothesis does not explain why reinfection is unusual.

Morphological changes and cellular alterations related to *T. vitulorum* expulsion were first reported by Neves *et al.* (2003) and Neves and

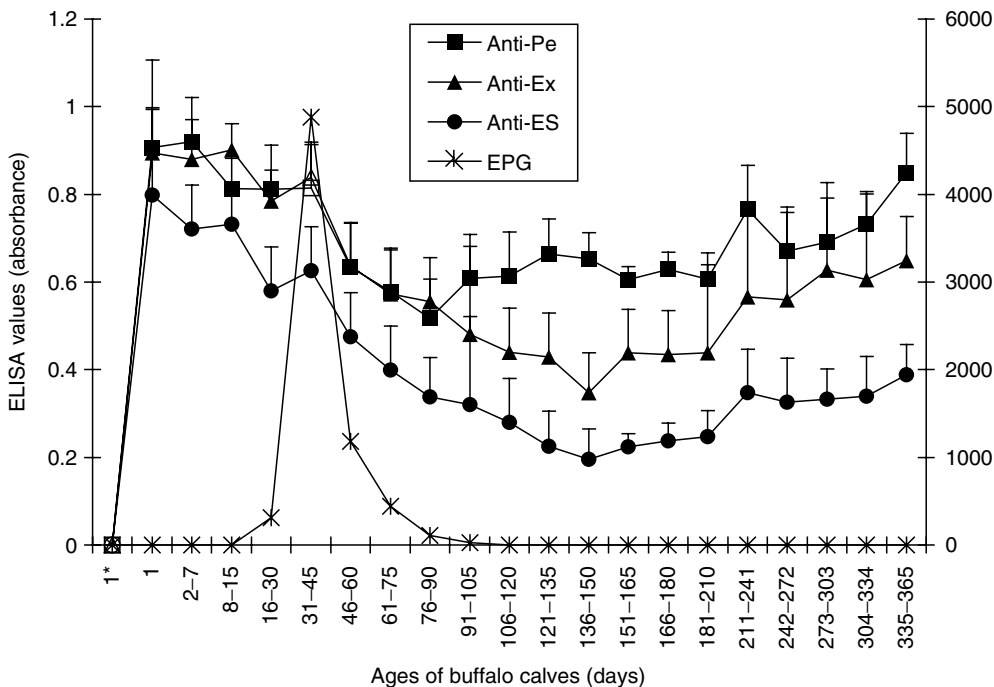


Fig. 18.6. ELISA values of *T. vitulorum* anti-Pe, anti-Ex and anti-ES antibodies in sera of buffalo calves during the period from 1 to 365 days post-birth compared with the parasitic status of the calves represented by EPG counts. Standard deviation of the mean is also represented (de Souza *et al.*, 2004).

Starke-Buzetti (2004). *T. vitulorum* infection elicited hyperplasia of mast cells (Fig. 18.7), eosinophils (Fig. 18.8), goblet cells and intraepithelial lymphocytes in the duodenum and proximal jejunum, during the beginning and at the peak of infection and during rejection of the worms. After rejection of the worms, the numbers of these cells returned to normal levels. Migration of mast cells and eosinophils to intraepithelial layers of the duodenum and jejunum of infected buffalo calves were also reported. One plausible explanation is that these cells may have an important role in the cellular immune response to the rejection of *T. vitulorum* from the intestines of buffalo calves during the course of a primary infection.

Conclusions and Future Directions

T. vitulorum is a pathogenic parasite of large ruminants, particularly in tropical and subtropical countries, and can be a serious parasite for water

buffalo. When the infection is not controlled in the field, the prevalence can reach 100% of the calves and deaths frequently occur when associated with poor nutrition. Control of this parasite is not easy because the larvae migrate in the host tissues, remaining as dormant or hypobiotic parasites, and can survive for a long time. Moreover, larval transmammary passage to calves occurs after birth and is the most important route of infection. On the other hand, the direct cycle by oral infection with infective eggs is not clear. Experiments done by infecting cow or buffalo calves with single or multiple doses of infective eggs failed to establish patent infection.

T. vitulorum can be a serious impediment to successful buffalo breeding, particularly in areas of the world where the animals are reared by farmers living in relatively poor conditions. To protect animals from *T. vitulorum* infection, farming conditions must be improved in addition to the use of anthelmintics or other types of control programme, including the control of dams.

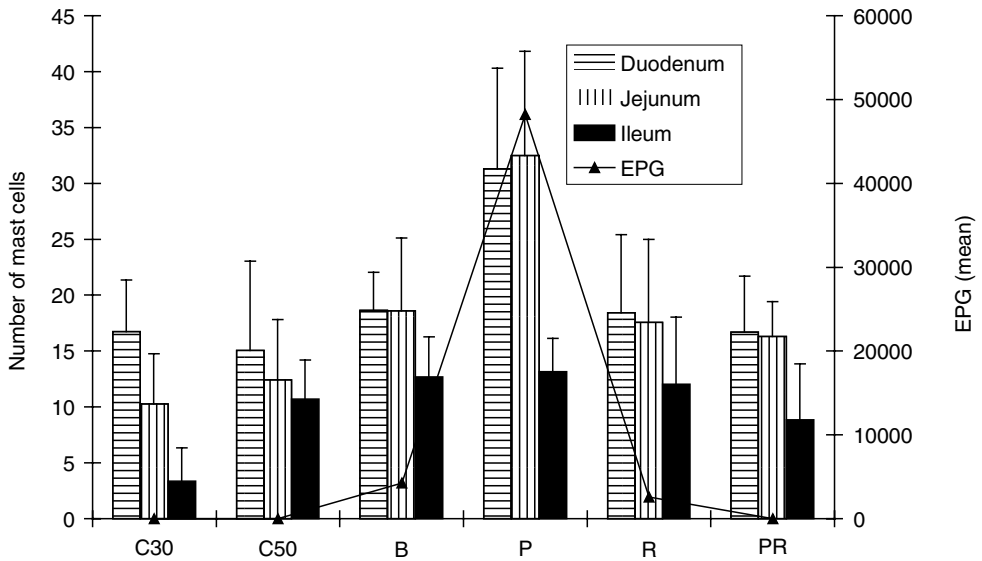


Fig. 18.7. Mean numbers of mast cells in the mucosa of duodenum, jejunum and ileum of buffalo calves uninfected at 30 (C30) and 50 (C50) days after birth with *T. vitulorum* at the beginning (B) and peak (P) of infection and during rejection (R) and post-rejection (PR) of the parasite. The number of cells was compared with EPG counts of the calves (mean \pm SD). From Neves *et al.* (2003).

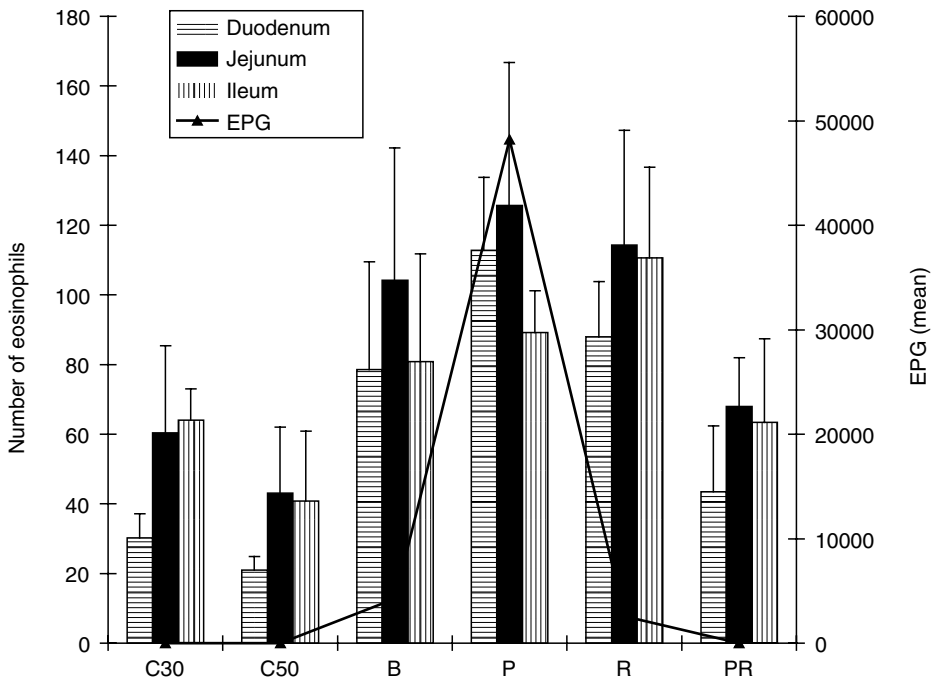


Fig. 18.8. Mean numbers of eosinophils in the mucosa of duodenum, jejunum and ileum of buffalo calves uninfected at 30 (C30) and 50 (C50) days after birth with *T. vitulorum* at the beginning (B) and peak (P) of infection and during rejection (R) and post-rejection (PR) of the parasite. The number of cells was compared with EPG counts of the calves (mean \pm SD). From Neves *et al.* (2003).

An alternative possibility for control could be by vaccination of dams in order to kill larvae in tissues before they are transferred to the calves. Anti-*T. vitulorum* antibodies against larval ES Ex antigens and Pe of *T. vitulorum* adults were detected in serum and colostrum of buffalo cows and serum of calves, indicating that *T. vitulorum* infection can stimulate the immune system of the buffalo. Also, the parasites are expelled by calves after the peak of egg output and calves did not shed eggs after more than a year of monthly faecal examination. An intestinal cellular immune reaction with an increased number of mast cells and eosinophils was detected in the mucosa of infected calves, particularly at the peak of infection, but started to decline after worm expulsion, suggesting that an inflammatory response was elicited by *T. vitulorum* infection. Identification of vaccine molecules and adjuvants whose mucosal adminis-

tration could induce protection against *T. vitulorum* infection in calves could be a promising approach for future studies.

There is no evidence yet that infective eggs of *T. vitulorum* can infect humans, but the larvae from the eggs migrate in paratenic hosts, and the possibility of migration in humans has been suggested. *T. vitulorum* antigenic isolation and molecular characterization to develop monoclonal antibodies against epitopes of this particular parasite could contribute to a specific test for diagnosis of human infection in the future.

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Section 4

Economic impact of the disease

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19 Economic Impact of *Toxocara* spp.

Paul R. Torgerson and Christine M. Budke
Institute of Parasitology, University of Zürich, Switzerland

Human toxocariasis, usually attributed to infection with *Toxocara canis*, is associated with poor health and has been implicated in a variety of disease syndromes. These include specific defined conditions such as ocular larval migrans (OLM) where the condition can be diagnosed and pathological effects quantified. At the other end of the spectrum, there are syndromes suggested because there is an association between seropositivity and non-specific pathological states. This indicates that *Toxocara* may be important in the general aetiology of these diseases, but proof of cause and effect, especially in individual cases, may be elusive.

The global burden of zoonoses, in terms of human morbidity, remains underestimated and unstudied and this is certainly true of toxocariasis. Disease, by definition, will affect the individual's well-being and productivity. It is quantifying the effect in terms of economic or measurable terms of human morbidity that is the challenge. The aim of this review is not to attempt to quantify the economic effects of toxocariasis, but rather to indicate the methods by which such economic effects could be calculated and hence how the burden of disease can be compared with other diseases. Both monetary analyses and non-monetary analyses for the economic evaluation of diseases have been developed. The former is focused on the actual direct financial costs in treating disease and the indirect costs such as loss of income through poor health. In addition, financial analyses can be used to analyse animal diseases in terms of productivity losses as a result of infection and/or cost of treatment. Non-monetary analyses can be measured in a variety of

ways and the preferred method by the World Health Organisation is the use of the disability adjusted life year (DALY) (Murray, 1994), which accounts for both the severity and length of the disease in terms of its effect on incapacitating the individual. Consequently, such analysis 'levels the playing field' when comparing chronic diseases of low morbidity and acute diseases of high morbidity. Whether monetary or non-monetary methods are used, such analyses enable policy makers to prioritize measures to control the disease or encourage research into diseases that present a large burden to society. This review will discuss both monetary and non-monetary methods of measuring disease impact and the benefits of control.

Burden of Disease

The total societal burden of disease caused by *Toxocara* spp. is essentially measured by the sum of its parts. For toxocariasis, the whole spectrum of symptoms attributable to the disease in humans should be included, although these data are highly uncertain. Assuming that there are no economic deficits in animals caused by *Toxocara* spp. in areas that are not endemic for *Toxocara vitulorum*, then the only species in consideration would be humans, and either a monetary or non-monetary analysis can be undertaken. Alternatively, the cost of treating pets for *Toxocara* spp., with added losses due to *T. vitulorum*-related productivity losses in cattle or buffaloes in endemic countries (Omar and Lewis, 1993; see

Chapter 18, this volume) may be considered. It is arguable that if both humans and animals are affected by the disease then only a monetary evaluation of disease burden is applicable, so that the total societal costs of the disease can be calculated and compared with costs of other diseases that drive public health policy (Torgerson, 1997). However, in a recent paper (Budke *et al.*, 2005), cost-benefit analysis on a possible control programme for echinococcosis in a highly endemic region in China demonstrated that it is possible to utilize both monetary analysis and non-monetary analysis. In this case, a proposed control programme was modelled in terms of DALYs saved and financial savings such as improved livestock productivity and lower human treatment costs as the benefits to a control programme.

Morbidity Rates

Unlike many reportable zoonotic diseases, toxocariasis caused by *Toxocara* spp. infection is not officially monitored and the true worldwide prevalence has not been estimated. Small-scale studies have, however, shown the seroprevalence to be alarmingly high in certain populations, even within wealthy industrialized countries such as the USA. One such illustrative study indicated that 50% of Hispanic children living in the city of Bridgeport, located in the US state of Connecticut, were serologically positive for *Toxocara* infections (Sharghi *et al.*, 2001). Due to the worldwide prevalence of *Toxocara* spp. and the often severe clinical signs associated with visceral larval migrans (VLM) and OLM, in addition to clinical signs such as asthma, idiopathic seizure disorders, functional intestinal disorders, prurigo, urticaria and eosinophilic arthritis that have been attributed to covert toxocariasis, the small- and large-scale burden of this disease is well worth considering (Despommier, 2003). Having information on disease burden will aid in the estimation of the economic impact attributable to *Toxocara* infection as well as cost-benefit analysis of a public health intervention strategy.

The first step in measuring the impact of an infectious agent is to assess its frequency of occurrence and whether it is linked to specific symptoms (Merson *et al.*, 2001; Thomas and Weber, 2001). Therefore, a first distinction must be made between the occurrence of infection and the

occurrence of disease (or symptoms) among infected individuals. This in itself may not be straightforward. OLM is a widely recognized although uncommon syndrome and is diagnosed primarily on the basis of clinical criteria during an ophthalmological examination (Despommier, 2003) (see Chapter 9, this volume). The diagnosis of VLM is primarily immunological (see Chapter 7, this volume). However, a large number of clinical conditions and syndromes have been attributed to VLM such as fever, enlargement and necrosis of the liver (Rayes *et al.*, 2001), spleen enlargement, respiratory symptoms, myocarditis (Prunier *et al.*, 2001), nephritis (Shetty and Aviles, 1999) and neurological syndromes (Critchley *et al.*, 1982; Despommier, 2003). More subtle clinical manifestations, as a result of covert toxocariasis, have been associated with asthma (Taylor *et al.*, 1988; Buijs *et al.*, 1994) (see Chapter 4, this volume), functional intestinal disorders (Konate *et al.*, 1996) and skin disorders (Humbert *et al.*, 2000) (see Chapter 10, this volume). These syndromes can also have a variety of other aetiological causes and determining the burden of disease due to toxocariasis would be a significant challenge, particularly since many reports describe a casual association rather than defining cause and effect.

To obtain better estimates of the risk (or cumulative incidence) of infection over a certain period of time or the incidence rate of infection per individual time at risk, a cohort study will need to be conducted where a group of individuals, free of infection at the start of the study, will be followed up for a set period of time. These types of studies are very costly and have not been undertaken for toxocariasis. However, examination of the age-specific incidence rates of human cases can give indicators of the cumulative incidence as individuals can be assumed to be free of infection at birth. All of these measures that rely on serological or other laboratory diagnostic tests will present with some degree of error. These errors must be taken into account to estimate more accurately the real frequency of infection (Basáñez *et al.*, 2004), and it is important not to directly compare or combine, in a single estimate, those estimates obtained using several diagnostic methods and study designs.

Serological prevalence in industrialized countries of apparently healthy adults tends to be between 2 and 5% in urban populations and 14 and 37% in rural populations. In non-industrialized or

tropical countries, seroprevalence rates of between 10 and 90% have been reported (Magnaival *et al.*, 2001). The challenge is then to determine the proportion of the exposed population that has clinical or subclinical morbidity due to toxocariasis. A recent study in Ireland suggested that the prevalence of OLM was approximately 0.0121% (Good *et al.*, 2004) in schoolchildren. This was based on clinical evidence of a defined disease syndrome and is likely to have a reasonable degree of accuracy in that society. However, the attributable risk due to other disease syndromes associated with the clinical/serological manifestations of toxocariasis needs to be calculated and more rigorous studies other than statistical associations are required. The attributable risk, assuming cause and effect, can be estimated from a cross-sectional study because the prevalence of disease in the exposed and unexposed populations are known. This can be illustrated by reference to a simple 2×2 contingency table constructed in an observational study (Table 19.1).

The amount of disease in the population attributable to *Toxocara* can be calculated as the difference in prevalence (or incidence) in the total population $([\alpha + \beta]/n)$ and the prevalence in the unexposed population $(\beta/[\beta + \delta])$. In this case, a prevalence rate of 0.73% for recurrent bronchitis or asthma is attributable to *Toxocara*.

This can then be used to calculate the total number of individuals in the population whose clinical syndromes are due to exposure to *Toxocara*. This figure can be used as a starting point to estimate the burden of disease attributable to *Toxocara*.

Decision Tree Analyses

Information regarding human *Toxocara* infection can be gathered and organized with a decision

tree analysis (Petitti, 2000; Haddix *et al.*, 2003). A decision tree is a type of quantitative analysis defined as a set of tools designed to combine information to arrive at a summary conclusion. Decision analysis may quantitatively compare competing management strategies under conditions of uncertainty, and through economic modelling, may provide a ranking of strategies in terms of costs and benefits associated with their implementation. Decision tree analysis was used to estimate the monetary impact of cystic echinococcosis (CE) in Tunisia in humans and animals (Majorowski *et al.*, 2005). A relatively simple decision tree was used for human economic evaluation for CE as the potential clinical outcomes (space occupying lesion) and associated morbidity are relatively well defined. As more information becomes available on a disease, the decision tree becomes more complicated. This is illustrated in Majorowski *et al.* (2005) by the decision tree for animal production losses where there are a number of possible effects of infection. The sum of all these values would correspond to the average cost per case of the disease of interest. Such an exercise has not been undertaken in evaluating costs of human toxocariasis. Information required would be the attributable incidence or prevalence of the different disease states within a population, the likely outcome and the costs of the disease and treatment. Thus, a probabilistic decision tree can be formulated to model the total costs of human toxocariasis. A starting point could be envisaged as in Fig. 19.1. In this figure, a circle corresponds to a chance node (defined by the probability or incidence rate of the event occurring) and a triangle represents an end node. The number at the top of each branch shows the proportion of each event occurring at that point in the tree. The average cost per case of each event is multiplied

Table 19.1. A 2×2 contingency table constructed for a simple cross-sectional observational study of a disease syndrome associated with seropositivity to *Toxocara*. The data is from Buijs *et al.* (1997), which suggested an association between seropositivity and the presence of recurrent bronchitis or asthma.

	Seropositive subjects	Seronegative subjects	Total
Disease syndrome present	α 17	β 77	$\alpha + \beta$ 94
Disease syndrome absent	γ 97	δ 1180	$\gamma + \delta$ 1277
Total	$\alpha + \gamma$ 114	$\beta + \delta$ 1257	$\alpha + \beta + \gamma + \delta = n$ 1371

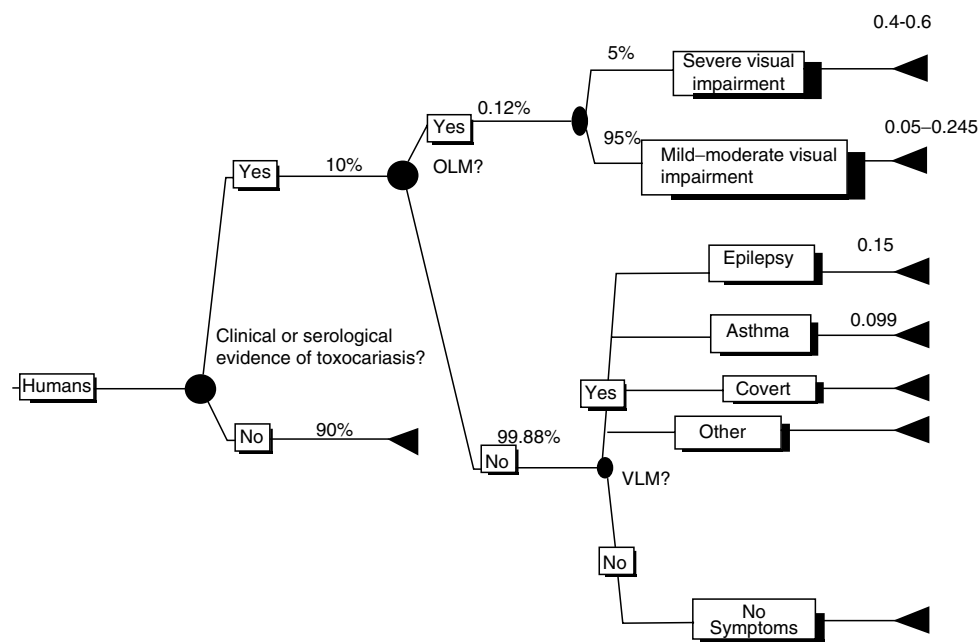


Fig. 19.1. Incomplete decision tree based on the syndromes associated with toxocarasis. Suggested probabilities at some node points are given (e.g. 10% clinical or seroprevalence rate) and weights for DALYs for some end nodes (e.g. 0.15 for epilepsy). For a full economic analysis, more branches may be required along with estimates in terms of probabilities and disability weights.

by the end probability of each branch. In this case, end costs are indicated for some syndromes in terms of DALYs. Carabin *et al.* (2002) used this type of approach to estimate the average cost per case of measles in Canada to be US\$254 in 2000.

Non-monetary Methods of Analysis

Non-financial methods have been developed to estimate human disease burden and are often referred to under the umbrella term of HALYs (health adjusted life years) (Gold *et al.*, 2002). HALYs are summary measures of population health that allow the combined impact of morbidity and death to be considered simultaneously. These methods assess the morbidity of disease states so that comparisons between different diseases can be made. They avoid costing financially the effects of disease, but can be used to prioritize resources to control human disease.

DALYs are the World Health Organization (WHO)’s preferred measure of disease burden and

have been developed for over 100 communicable and non-communicable conditions through the Global Burden of Disease Study in association with WHO (Murray, 1994). However, toxocarasis due to *Toxocara* spp. infection has not yet been evaluated. Parasitic diseases that have been assessed using DALYs on a worldwide scale include malaria, African trypanosomiasis, Chagas disease, schistosomiasis, leishmaniasis, lymphatic filariasis, onchocerciasis, trachoma, ascariasis, trichuriasis infection and hookworm infection (Coulter, 2002). On a smaller scale, DALYs lost due to human alveolar and CE have been calculated for a small region of western Sichuan Province, People’s Republic of China (Budke *et al.*, 2004).

The basic formula for construction of a DALY for a single individual is:

$$-\left[\frac{D C e^{-\beta a}}{(\beta+r)^2}\left[e^{-(\beta+r)(L)}\right.\right. \\ \left.\left.(1+(\beta+r)(L+a))-(1+(\beta+r)a)\right]\right]$$

where D is a disability weight, β is an age-weighting function parameter, C is an age-weighting correction constant, r is a discount rate, a is the age of onset for the condition in question and L is the duration of the disability or the time lost due to mortality (Murray, 1994). Disability weight (D) is defined on a 0 to 1 scale, 0 being perfect health and 1 indicating death, with disability due to a certain disease outcome placed on this scale according to clinical severity. Assignment of disability weights has traditionally taken place using methods such as rating scales, standard gamble, magnitude estimation, time trade-off and personal trade-off. In contrast, disability weights for human echinococcosis were developed for a small-scale study by comparing clinical outcomes with conditions previously assigned disability weights for the Global Burden of Disease Study and then utilizing stochastic processes to model variability in parameter estimations (Budke *et al.*, 2004). In order to construct DALYs for human infection with *Toxocara* spp., disability weights associated with the various clinical outcomes must be determined. Therefore, different disability weights would have to be assigned to the numerous clinical manifestations of larval migrans disease as well as for covert toxocariasis.

The allocation of disability weights (D) to VLM will depend on the organ or organs affected. This makes defining discreet disability weights difficult because of the vast number of possible clinical outcomes due to this affliction. Therefore, disability weights will need to be assigned per organ site or system, e.g. the eyes, liver, lungs and nervous system. In addition, estimated numbers of larva comprising the infection, as well as the age of the human host, will affect clinical outcome and will need to be accounted for in assigning disability. Non-diagnosed cases, especially with regard to covert toxocariasis, will also have to be estimated and allocated appropriate disability weights. In order to illustrate the need to include covert toxocariasis estimates in disease burden estimation, two examples are given. One study, evaluating seroprevalence of *Toxocara* infection in children in Ireland, found that seropositive children were more likely to have one or more clinical signs associated with covert toxocariasis, namely abdominal pain, anorexia, fever, wheezing, limb pain, cervical adenitis and sleep and behavioural disturbances (Taylor *et al.*, 1988). In addition, a study performed in the US state of New York

found 1–12% of 5–15-year-olds tested in New York city were *Toxocara* positive and that positive children performed worse in tests of motor and cognitive function than an age- and gender-controlled group (Marmor *et al.*, 1987). Visual impairment caused by ocular toxocariasis can be weighted according to severity. Blindness is weighted as 0.6 in the global burden of disease study (Murray and Lopez, 1996), but severe bilateral OLM is likely to be extremely rare. Studies (e.g. Yokoi *et al.*, 2003) suggest that ocular toxocariasis has a fair visual outcome; therefore, it would be appropriate to give OLM a weight of 0.05 for mild vision impairment (Stouthard *et al.*, 2000) or 0.245 for moderate impairment of vision (Murray and Lopez, 1996). Other examples of weights that could be given to syndromes associated with toxocariasis include asthma, which has been given a weight of 0.099, and epilepsy with a weight of 0.150.

An age-weighting function parameter (β) is used to capture different social functions at different ages. For example, a young child or an elderly person relies, to a greater extent, on the financial, physical and social support of society than a person of middle age. The constant (C) is used to correct for the differences in the global burden of disease between values obtained using the correction factor and the value that would be obtained using uniform age weights. A discount rate (r), often assigned at 3%, is used to discount the years of healthy life (World Bank, 1993). This is because resources lost now would be worth more in the future due to the possibility of investing these resources and also takes into account inflation. Although discounting is generally better understood for monetary values, it is also used to discount losses of DALYs into the future. Length of time lived with the disability or the length of time lost due to mortality (L), associated with the condition, needs to be estimated for each individual affected. When a condition is considered life-long or fatal, the West Level 26 life expectancy table (Lopez *et al.*, 2000) is most commonly used in order to standardize DALYs lost across populations. Fatal disease is probably very rare as a result of toxocariasis. The West Level 26 life table assumes a life expectancy of 82.5 years for females and 80.0 years for males in order to encompass societies with the longest known lifespans. In order to illustrate this point, since toxocariasis is rarely fatal, the DALY calculation would be based on the length of time that illness persisted (from the

age of diagnosis until the end of all clinical manifestations of disease) and if a lifelong injury resulted, such as loss of visual acuity, disability would be calculated for and applied to the duration of the estimated lifespan. Shorter timespans for the duration of the disability may result from other syndromes. More data are required to define the length of the morbidity effects to define precisely the DALYs lost, although techniques can be used to model uncertainty in the value of L (see below).

The application of DALYs to clinical conditions associated with human *Toxocara* spp. infection will enable health workers to have a more accurate picture of the magnitude of disability that these parasites cause. This, at least in theory, can be a starting point for the development and cost-benefit analysis of control programmes for human toxocariasis. In order to help standardize the evaluation of cost effectiveness, the WHO has developed a rating scale for efficiency of a control programme based on the cost per DALY averted (TDR/Gen 1996). The cut-off for the most cost-effective control programme is under US\$25 per DALY averted, with the next most cost-effective band being less than US\$150 per DALY averted.

Monetary Measure of Disease Burden

Financial estimates of a number of zoonoses, such as toxoplasmosis (Roberts and Fenkel, 1990; Roberts *et al.*, 1994) and echinococcosis (reviewed by Torgerson, 2003) have been made. The economic effects would include a number of potential factors. The most obvious of these is the cost of treatment of patients suffering from toxocariasis. For example, benzimidazoles have been suggested as a treatment for cerebral toxocariasis, but the evaluation of the costs of treatment are confounded by the lack of double-blind case-control studies into treatment efficacy (Vidal *et al.*, 2003; Moreira-Silva *et al.*, 2004). Calculation of potential treatment protocols and costs of such protocols would need to be undertaken in a representative sample of subjects. In addition, costs are likely to vary significantly between countries because of differences in the way health services are organized and differences in technology and wages. This has been illustrated for echinococcosis, where the costs of surgical treatment is considerably higher in the UK, a wealthy indus-

trialized nation, compared with Jordan, which is a middle income developing country (Torgerson and Dowling, 2001; Torgerson *et al.*, 2001).

Treatment costs may only be a small proportion of the financial costs to human patients. The cost of morbidity, which includes loss of earnings due to absenteeism from work, the necessity of taking less well paid work and cost of social security while not working, also needs to be considered. Such studies have yet to be undertaken for human toxocariasis, but recent studies on echinococcosis may demonstrate the way forward in defining these costs. For example, Budke *et al.* (2004) has used the SF-12 v2 Health Survey to investigate the health status of individuals diagnosed with echinococcosis by abdominal ultrasound. Subjects with parasitic lesions had a significantly reduced quality of life compared with matched controls, despite the fact that they were not previously aware they had the condition. Such individuals are more likely to be in a lower income bracket (Budke *et al.*, 2005) than healthy controls, illustrating the potential financial implications of what, to the subject, is an undiagnosed subclinical disease. Such effects are possible with the various syndromes attributable to toxocariasis and would need a careful study design to estimate the potential financial effects in this way. Any long-term deterioration of vision accompanying OLM might be expected to have an effect on the subject's earning capacity, but the low incidence of this disease would make a proper case-control study a challenge in identifying such costs. The implication of *Toxocara canis* in idiopathic seizure disorders (Critchley *et al.*, 1982) would certainly be disabling to an affected subject, but again properly constructed case-control studies would be needed to illustrate this and determine the extent of the economic effect.

As already indicated, mortality rates attributable to toxocariasis are likely to be very low. With non-monetary techniques such as DALYs, a disability weight for death is assigned as 1 for the rest of the life expectancy at the age of death. The financial cost of premature death is more controversial. The capital approach calculates the potential loss of income and can be calculated from salary levels and life expectancy at time of death. Alternatively, the willingness-to-pay approach is based on the extra investment an individual is willing to spend in order to avoid premature death. Whatever method is used, the extremely low mortality rate attributable to toxocariasis

would need to be calculated if mortality costs are to be utilized in the monetary evaluation.

Avoidable Losses

Economic losses or estimates of disease burden have greater validity when considered in the context of preventable losses. With toxocariasis, this in itself presents a challenge. Surveys have demonstrated that there are differences in the seroprevalence and hence exposure and disease rate between different populations such as subjects in industrialized nations and tropical or developing countries. For example, seroprevalence has been reported as approximately 7.5% in Australia and 3.1% in the Canary Islands (Nicholas *et al.*, 1986; Jimenez *et al.*, 1997), while in the tropics, prevalences have ranged from 20.5% in Brazil (Anaruma *et al.*, 2002), 30.4% in Nigeria (Ajayi *et al.*, 2000), 63% in Bali (Chomel *et al.*, 1993) and 86% in St Lucia (Thomson *et al.*, 1986) to 93% in Reunion (Magnaval *et al.*, 1994a). Seroprevalence rates in urban populations in western countries have been reported as between 2 and 5% compared with rural prevalence rates of between 14.2 and 37% (Magnaval *et al.*, 1994b). Likewise, certain risk factors, for example dog ownership, have been linked to increased rates of seroprevalence (Fan *et al.*, 2004). These differences in seroprevalence rates between different communities or between different individuals with different risk factors suggest that the incidence of human toxocariasis could be reduced by providing a detailed knowledge of the source of the infection and by ensuring that the mechanisms of transmission are understood, so that these could be targeted. However, with a high prevalence of infection in some wild animals such as foxes (Richards *et al.*, 1995), and a variety of modes of transmission such as geophagia or consumption of undercooked meat (Magnaval *et al.*, 2001), a proportion of cases would be seen as unavoidable. It is the numbers of cases above this that represent the true societal burden of disease.

Modelling Uncertainty

Whichever measure of disease burden is used, a monetary or a morbidity index such as a DALY, it is essential to model uncertainty. Very few of the

parameters, such as the cost of treatment of visceral toxocariasis or the disability weight associated with OLM, can be estimated accurately.

In the first instance, the point estimate of prevalence or incidence has a degree of uncertainty, which is dependent on the sample size. The population-attributable fraction will also have additional uncertainty due to the efficiency of the diagnostic procedure (few diagnostic tests, for example, have perfect sensitivity and specificity) and the possibility of confounding factors not revealed in the study that could artificially inflate the incidence of disease attributable to toxocariasis. With the advent of modern computers, these uncertainties can be modelled by using Monte-Carlo sampling techniques. The essential methodology behind this is illustrated in economic analyses of echinococcosis in Jordan, China and Tunisia (Torgerson *et al.*, 2001; Budke *et al.*, 2005; Majorowski *et al.*, 2005). These include studies that model uncertainty in financial aspects and estimates of DALYs lost. In a deterministic approach, a single value is assigned to each economic variable and the total economic effect is estimated by summing all of the variables in the model. Monte-Carlo techniques allow the investigator to assign a probability distribution based on the amount of information available. For example, from the study of Buijs *et al.* (1997) the population-attributable prevalence of asthma or bronchial disease has a point estimate of 0.73%. This has uncertainty related to the sample size. One way of modelling the uncertainty is to use a probability distribution for the attributable prevalence rather than a point estimate. This can be modelled, for example, by picking random values of α , β and γ (see Table 19.1) from a multinomial distribution with size n of relative probabilities given by the point estimates. Each set of random values is used to calculate the attributable prevalence and the process is repeated 10,000 times to obtain a distribution of the population-attributable estimate. The outcome of this model is that the 95% confidence intervals of the attributable prevalence are 0.2–1.3% for asthma or bronchial disease. An appropriate probability distribution encompassing these confidence limits can then be included in the economic model. With a larger data set, these uncertainties would narrow. Other estimates may need to be more esoteric, at least until more data are available. The clinical symptomatology associated with covert toxocariasis is

diverse (Taylor, 1993). Therefore, assigning a set of disability weights for this disorder presents a challenge. However, clinical conditions such as pneumonia, abdominal pain, pharyngitis, etc. as described by Taylor (1993) would be expected to be relatively mildly disabling and might suggest a disability weight of up to 0.2. However, because of the arbitrary nature of this assignment, it could be modelled as a uniform distribution between 0 and 0.2, which could be refined as further information became available from, for example, cohort studies. Disability weights for other syndromes, such as epilepsy, can be assigned according to the Global Burden of Disease Study (Murray and Lopez, 1996), with in this case epilepsy being given a weight of 0.15. The challenge with this syndrome is estimating the proportion of epilepsy cases attributable to toxocariasis, and data similar to those reported for asthma or bronchitis would be required. An odds ratio of 2.7 was reported for the development of epilepsy in a recent case-control study (Nicoletti *et al.*, 2002). Although a case-control study on its own cannot give an estimate of the prevalence or incidence of epilepsy in the general population attributable to toxocariasis, if other information is available (such as the prevalence rate of seropositives), then a total estimate can be made. The less information known, or the greater the unreliability of the data, the wider the probability distribution that must be used. In the extreme case when the data are uninformative, a uniform distribution between two theoretical extremes can be used. Thus, rather than putting point estimates into, for example, the decision tree (Fig. 19.1), distributions for the probabilities of each node or disability weights can be used based on the amount of knowledge known for that symptom. Monte-Carlo techniques are then employed to calculate the posterior probability distribution of the total economic loss.

Economics of Animal Health

Canine toxocariasis is widespread. After birth, puppies can suffer from pneumonia associated with tracheal migration within 2–3 days. At 2–3 weeks of age, puppies may show emaciation and digestive disturbances. Mortality is rare, as are clinical manifestations in adult dogs (Overgaauw and van Knappen, 2000). The economic effect of

such problems in companion animals would be minimal although the cost of treatment is arguable. Here, the simplest estimate of treatment costs might be made from the sales records of licensed deworming preparations to the small animal market.

T. canis has been shown to result in migratory lesions in farm animals, at least experimentally, with white spots in the liver described following infection of pigs (Helwich *et al.*, 1999). Such lesions could result in liver condemnation due to ‘milk spots’ in naturally infected pigs and therefore economic losses. The extent to which this could occur following natural infection is not known. Likewise, no significant differences in live-weight gain could be found between experimentally infected pigs and control animals, despite the former receiving 60,000 infective eggs and pathological lesions and viable larvae being recovered on post-mortem examination (Helwich *et al.*, 1999).

T. vitulorum is a parasite of bovids which is highly prevalent in central and north-east Africa, South Asia and parts of Europe, with local prevalence rates in calves of up to 75% recorded (Omar and Barriga, 1991). The parasite is considered to be a major cause of calf mortality and economic losses in many countries and is discussed in greater detail in Chapter 18 (this volume). Therefore, the economic burden of this parasite is likely to be substantial in endemic areas. As an animal health issue, the economic approach is more straightforward. Livestock normally have a market value and the death of such an animal would result in an economic loss equivalent to its market value. Likewise, morbidity leads to production losses and this can be modelled, provided data are available. However, there have been few recent reports of the prevalence of infection in cattle in endemic areas and the productivity effects of infection. Thus, precise estimates of economic effects would be difficult to determine. Even a modest reduction in liveweight gain of infected calves would result in a high total economic burden because of the high prevalences seen in many endemic areas. However, the question must be asked as to how much disease can be prevented as it is only preventable disease that should be costed. In view of maternal transmission of this parasite, prevention of infection could be a challenge. Because of the widespread and often intense infections of calves with

T. vitulorum, the question of human infection must also be considered. However, few studies of human toxocariasis have attempted to differentiate the species of *Toxocara* infecting humans and it is usually assumed to be *T. canis*. A study in Sri Lanka (Iddawela *et al.*, 2003) indeed confirmed that 91% of children seropositive for toxocariasis were infected with *T. canis*. However, some were likely to be infected with *T. vitulorum*. To what extent infection in this small number of individuals results in morbidity is unknown, but it does indicate that *T. vitulorum* may have a burden on human health. An additional threat, at least in theory (Omar and Lewis, 1991), may be that larvae present in the milk of lactating bovids may be consumed by humans resulting in toxocariasis.

Cost Benefits of Control

Before prevention of human toxocariasis can be attempted, the means of human transmission must be better defined. There is a high level of uncertainty as to how much human toxocariasis can be averted. Infection in puppies is ubiquitous because of the mode of transmission, and infection in older dogs is not uncommon. Transmission to humans is not via direct contact with dogs as a period of development of the egg outside the host is required before the egg has embryonated and hence becomes infective. Therefore, controlling contamination of the environment is important and community-based programmes to ensure pets are regularly treated with anthelmintics might reduce the level of environmental contamination (see Chapters 14 and 16, this volume). However, foxes are often highly infected (Richards *et al.*, 1995; Smith *et al.*, 2003) and fox populations are increasing throughout Europe (Harris and Rayner, 1986; Chautan *et al.*, 2000; Gloor *et al.*, 2001). Control of environmental contamination from foxes in theory could be undertaken, as has been attempted by routine anthelmintic baiting of foxes in Zurich to control *Echinococcus multilocularis* infection (Heglin *et al.*, 2003; Deplazes *et al.*, 2004). However, this would be neither inexpensive nor logistically easy. In addition, to control human disease the contribution to human transmission from meat would need to be defined. Human toxocariasis has been associated with the consumption of raw

meat from paratenic hosts such as chickens (Nagakura *et al.*, 1989), lambs (Salem and Schantz, 1992), rabbits (Sturchler *et al.*, 1990) and raw or undercooked calf liver (Baixenench *et al.*, 1992). Prevention of infection of livestock would be difficult, and control through meat inspection unlikely to achieve success although education to ensure meat is adequately cooked may have some effect. Therefore, to define the economics of control and intervention, not only must estimates of the total burden of disease be made, but also the means of transmission to humans must be defined and hence the burden of preventable disease.

In addition, the relationship between levels of environmental contamination and human disease is not known, and it is likely that the relationship is non-linear. Therefore, halving the number of *Toxocara* eggs in the environment through deworming of dogs might not necessarily lead to a halving of human cases. The approach to costing a control programme and possible benefits can be undertaken in a similar way as costing the burden of disease, using Monte-Carlo techniques to model uncertainty. Certainly the costs of a deworming programme could be calculated, but the effects of such a programme on human disease incidence would have a high degree of uncertainty, both in terms of DALYs saved and in monetary gains. However, providing all the information can be gathered, a model can be constructed. With the available data it is likely that the estimates of disease burden in the population, whether financial burden or DALYs, are likely to have a huge degree of uncertainty, as would the benefits of a possible control programme. However, uncertainty can be modelled, and even with limited data to input into a model, sometimes useful information can be generated by using the stochastic techniques already discussed. For example, if the minimum financial burden of preventable disease is higher than the maximum cost estimate of control, then the programme would be financially viable. The corollary to this is that if the minimum costs of control are greater than the maximum costs of the disease, the programme is not financially viable. In terms of DALYs, the economic evaluation would be cost effective in terms of costs per DALY saved. For example, the WHO recommends (TDR/Gen, 1996) that if control costs less than US\$25 per DALY averted then the control programme is among the most highly cost effective. By using

costs per DALY averted, the cost effectiveness can be compared with the cost effectiveness of other public health programmes and such information then used to drive public policy.

Conclusions

There have been few attempts to define the economic costs of toxocariasis. This is confounded by the lack of suitable data on cause and effect and the paucity of data on the frequency of disease in a population directly attributable to toxocariasis. Rather than embarking on an economic analysis of toxocariasis, this chapter has attempted to provide ideas as to how such an economic analysis might be undertaken and has described different measures (such as financial loss or DALYs) that can be used to measure societal burden of disease and how Monte-Carlo techniques can be used to model uncertainty. This technique is particularly useful since many data sources are unreliable due to, *inter alia*, small sample size, non-random sampling techniques or possible confounding factors when associations between disease syndromes and seropositivity are reported. This does not preclude the use of such data, but appropriate probability distributions on the components of an economic model must be exploited when using such data.

A methodological recommendation for assessing the economic burden of toxocariasis would be:

- Assess the diversity of symptomatology and disease associated with *Toxocara* within the study population.
- Estimate the prevalence and attributable prevalence or incidence of each syndrome.
- Build up a decision tree based on this information.
- Decide on financial losses or DALYs for calculating disease burden.
- Assign appropriate financial losses to monetary analysis or disease weights to DALY estimates for each syndrome. This information should be based on appropriate epidemiological studies and added to the decision tree.
- Introduce uncertainty based on sample sizes of attributable prevalence estimates and uncertainty to the severity of the disease syndromes. The degree of uncertainty will reflect the quality of the available data.
- Assign appropriate probability distributions to all uncertain parameters. Probability distributions can be assigned to financial losses or to DALY weights.
- Randomly pick samples from each distribution and sum the results weighted according to the branches of the decision tree. Repeat at least 10,000 times and use the results to draw up a probability distribution of costs associated with toxocariasis.

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