Available online at www.sciencedirect.com

ScienceDirect

Veterinary Parasitology 148 (2007) 31–42

veterinary parasitology

www.elsevier.com/locate/vetpar

Parasitic nematodes—From genomes to control

Makedonka Mitreva [a,*], Dante S. Zarlenga [b], James P. McCarter [a,c], Douglas P. Jasmer [d]

[a] *Genome Sequencing Center, Department of Genetics, Washington University School of Medicine, 4444 Forest Park Boulevard, St. Louis, MO 63108, USA*

[b] *Bovine Functional Genomics Laboratory and Animal Parasitic Diseases Laboratory and U.S. National Parasite Collection, U.S. Department of Agriculture, Beltsville, MD 20705, USA*

[c] *Divergence Inc., 893 North Warson Road, St. Louis, MO 63141, USA*

[d] *Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164-7040, USA*

Abstract

The diseases caused by parasitic nematodes in domestic and companion animals are major factors that decrease production and quality of the agricultural products. Methods available for the control of the parasitic nematode infections are mainly based on chemical treatment, non-chemical management practices, immune modulation and biological control. However, even with integrated pest management that frequently combines these approaches, the effective and long-lasting control strategies are hampered by the persistent exposure of host animals to environmental stages of parasites, the incomplete protective response of the host and acquisition of anthelmintic resistance by an increasing number of parasitic nematodes. Therefore, the challenges to improve control of parasitic nematode infections are multi-fold and no single category of information will meet them all. However, new information, such as nematode genomics, functional genomics and proteomics, can strengthen basic and applied biological research aimed to develop improvements.

In this review we will, summarize existing control strategies of nematode infections and discuss ongoing developments in nematode genomics. Genomics approaches offer a growing and fundamental base of information, which when coupled with downstream functional genomics and proteomics can accelerate progress towards developing more efficient and sustainable control programs.

Keywords: Parasitic nematodes; Expressed sequence tags; Genomics; Genetics; Anthelmintics; vaccines

1. Background

Parasites from the phylum nematoda cause numerous diseases in humans, animals and plants placing major burdens on agricultural production and global health. Infections by these pathogens cause extensive suffering in humans and veterinary animals and major losses in agricultural production due to disease and the cost of implementing control programs (Jasmer et al., 2003). Recent calculations of the aggregate burden of human nematode diseases in disability adjusted life years (DALYs) indicate a tremendous global impact of these pathogens (Hotez et al., 2006). In this review we will discuss genomic information, which is rapidly expanding for nematodes, and its application to improve methods for control of nematode pathogens. While a focus is on parasitic nematodes that infect veterinary animals, the application of genomics has applications in

* Corresponding author. Tel.: +1 314 2862005; fax: +1 314 2861810.

E-mail address: mmitreva@watson.wustl.edu (M. Mitreva).

0304-4017/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2007.05.008

parasitology independent of the host and cited examples therefore sometimes draw upon the human medical and plant pathology literature.

Current methods used to control or reduce the impact of nematode infections (see Section 2) heavily rely on anthelmintics (defined here to be inclusive of plant nematicides). Although short-comings of chemical-based methods are well recognized, the general approach has provided enormous benefits to human health and agricultural production and the use of anthelmintics is likely to remain a major factor in integrated methods of parasite control. Deficiencies of current anthelmintics include: (1) the increasing and now widespread occurrence of nematode strains that have been selected for anthelmintic resistance (Kaminsky, 2003; Roos, 1997); (2) serious occupational exposure and environmental impacts presented by some anthelmintics (Risher et al., 1987; Schneider et al., 2003; Spratt, 1997); (3) the relatively poor efficacy of available anthelmintics against some nematode pathogens of humans (Stepek et al., 2006).

Given the potential benefits, there is a clear need to identify improved anthelmintics that address the short-comings of those currently available. However, the problem of acquired resistance to anthelmintics by nematodes is likely to persist. The integration with anthelmintics of distinct but complementary control methods is likely to have additive benefits while prolonging effective life-spans of each individual method.

Timely research progress on anthelmintic discovery, immunological control, and biocontrol has been impeded by the biological complexity of nematodes and their interactions with the host. Extensive and high quality genomic databases that are emerging for these pathogens provide a welcome infusion of information that should open valuable new avenues for progress. High expectations exist for advances that can be achieved with genome data and major progress will be required to meet these expectations. The accumulating genomic resources for various host species will also accelerate progress that can be made in this research, particularly for dissecting genetic determinants of resistance and breeding of pathogen resistant agricultural species. While not emphasized in this review, genome data can also facilitate development of improved diagnostics and epidemiological information.

2. Control of nematode infections

Extensive experience exists in the control of parasitic nematodes that infect agricultural and companion animals. Methods discussed here (Sayers and Sweeney, 2005; Stear et al., 2006; Waller and Thamsborg, 2004) are diverse and have focused on control rather than eradication due to the host's persistent exposure to long-lived parasites on pasture and the incomplete protective response of the host. This experience offers valuable perspectives on the challenges of nematode pathogens for effective and long-lasting control strategies.

2.1. Chemotherapy

The use of anthelmintics is still the mainstay for nematode control. The successes have been cyclical and directly related to the timely introduction of new drugs as resistance to older drugs has surfaced. Effective drug utilization dates back to the 1960s with development of benzimidazoles (BZ) (Brown et al., 1961), followed by the imidothiazoles-tetrahydropyrimidines in the 1970s, and the production of macrocyclic lactones (avermectins and milbemycin) in the 1980s (Chabala et al., 1980). The first cyclodepsipeptide (Sasaki et al., 1992) was commercially introduced for nematode control in cats in 2006. It is intriguing that with each major drug class, despite distinct modes of action, resistant parasites have begun to appear within just a few years of commercial introduction (Kaplan, 2004). To bypass this, new molecular targets are being sought and tested, such as cysteine proteases (Selzer et al., 1999; Stepek et al., 2005) which seem to attack the parasite cuticle, parasite mitochondrial proteins involved in energy metabolism (Miyadera et al., 2003; Omura et al., 2001), neuropeptides and the nervous system (Geary et al., 2004; McVeigh et al., 2006; Mousley et al., 2005), phosphorylcholine metabolism that plays important roles in nematode development, fertility and survival (Lochnit et al., 2005; Palavalli et al., 2006), and other mechanism-based targets (Geary et al., 2004). Given the refocusing of the pharmaceutical research and development on drugs with known modes of action, target-based anthelmintic discovery is likely to increasingly compete with more traditional *in vivo* screening of compound collections. Genomics plays a key role in the identification, selection, and characterization of potential targets (Section 3).

2.2. Pasture management, biological control and nutrient supplementation

The down-side of anthelmintic efficacy has been a stagnation of research to identify alternative control methods. This has left the organic farmers, and those who experience resistance, with inadequate means of

managing parasite-induced losses. Keeping sufficient parasites in refugia, can maintain the susceptible gene pool, but requires monitoring fecal egg counts to determine the level of pasture contamination and the FAMACHA© method for tracking anemia in small ruminants. Using PCR techniques, one could also decide to drug treat based upon the pathogenicity of the parasite species that populate the pastures (Zarlenga et al., 2001).

Biological control on pasture includes the use of predatory fungi to kill a variety of nematode species and substantially reduce the intensity of infection (Larsen, 1999). Challenges to fungal control have been a requirement for daily administering of fungi to the host and achieving the required fungal density inside the dung. However, a nematode-killing fungus, *Duddingtonia flagrans*, recently discovered in New Zealand (Skipp et al., 2002), was shown to have a trapping efficiency rate of 78% and activity for up to 90 days on pasture, providing a viable alternative to reduce animal mortality from nematode infections (Waghorn et al., 2003).

Well-nourished animals are generally more resistant to the effects of parasite infection. Therefore, nutritional supplementation may reduce the requirement for chemotherapeutic control (Knox et al., 2006; Torres-Acosta et al., 2004). Additional dietary protein (Torres-Acosta et al., 2004), selenium (Au Yeung et al., 2005; Smith et al., 2005), as well as minerals (Islam et al., 2006; Koski and Scott, 2003) may each play a role in countering infections presumably through mechanisms such as enhancing host immunity or maintaining digestive tract integrity. Parasite-inhibiting plants (Niezen et al., 2002) have also been tested for their ability to reduce egg shedding and pasture seeding density.

2.3. Vaccination

In general, nematodes elicit a Th2 type immune response during infection that often does not quickly render the host refractory to re-infection. This is particularly evident in *Ostertagia ostertagi* infections of cattle, where years of host re-exposure to the parasite are required before a meaningful protective response is generated. While progress on vaccines against nematodes continues to move forward, it does so with mixed success. The remarkable protection that can be induced with natural antigens derived from the intestine of *Haemonchus contortus* (Newton and Munn, 1999) is an example of one form of success. Nevertheless, other parasites present greater challenges for identification of good vaccine candidates (e.g. Claerebout et al., 2003; Vercauteren et al., 2004). A major stumbling block, even with successful natural antigens, has been the development of effective synthetic or recombinant vaccines. Other than for the few successes in vaccinating against cestodes (Gauci et al., 2005; Lightowlers, 2004), development of recombinant antigens for control of veterinary parasites has been limited (Zarlenga, 2004). This may, in part, reflect the key roles that co-evolution and adaptation have played in the host–parasite relationship (Zarlenga et al., 2006). Parasites often secrete or excrete products that regulate the local immune environment. Immune modulation of host responses by parasites has taken the form of; (1) sharing interferon gamma epitopes (Grencis and Entwistle, 1997); (2) the production of parasite-derived macrophage migration-inhibitory factors that affect macrophage maturation (Pennock et al., 1998); (3) the secretion of products that bind Toll-like receptors (TLRA-4), thereby down-regulating Th2 responses (Helmby and Grencis, 2003); (4) the release of parasite products linked to immunosuppression in swine (Souza et al., 2002) and cattle (Gomez-Munoz et al., 2004). When parasite adaptation is coupled with the large genetic variability both within and between worm populations and the genetic diversity of the host, the development of long term, unilateral treatments to control parasitic nematodes present a challenge. On the other hand, structural features of nematode antigens that are required to induce immunity are largely unknown. Secondary, tertiary or quaternary structures may comprise many epitopes that induce protection. Therefore, the exact replication of those structures is likely essential for development of efficacious vaccines. Hence, deeper understanding of both the important antigenic characteristics and mechanisms utilized by nematodes to survive host immune responses is indispensable for accomplishing this goal.

2.4. Genetics of host resistance

One alternative approach to controlling parasitic nematode infections is to use the natural diversity of the host genome to reduce parasite transmission. In cows, studies have shown that the number of nematode eggs/gram (EPG) in feces was influenced by host genetics (Leighton et al., 1989) with an estimated heritability of 0.30 (Gasbarre et al., 1990). A small percentage of the herd was responsible for the majority of parasite transmission (Gasbarre et al., 1990), a distribution strongly suggesting that genetic management could reduce overall parasite transmission. Selective breeding

programs have shown that calves could be separated into three types: (i) never demonstrated high EPG values; (ii) showed rises in EPG values only for 2 months post-infection; (iii) maintained high EPG levels throughout the test. These groups followed a normal Hardy–Weinberg distribution through the first generation of breeding suggesting that it may be possible to target the non-responder class of animals in a selective breeding and treatment program. Current efforts now focus on generating quantitative trait loci (QTL) for parasite resistance in cows (Sonstegard and Gasbarre, 2001). Numerous examples show similar correlation between host genetics and nematode EPG values in sheep (McEwan, 1998). For instance putative QTL for parasite resistance have been identified near the telomeric end of chromosome 8 (Crawford et al., 2006), as measured by the number of *Trichostrongylus* spp. adults in the abomasum and small intestine at the end of the second parasite challenge. For hosts that can be genetically manipulated, coupling of genetic-based host resistance with improved methods of vaccination has intriguing potential for immune-based control of parasitic nematodes.

3. Nematode genomics

Challenges to improve control of parasitic nematode infections are multi-fold and no single category of information will meet them all. Nevertheless, nematode genomics offers a growing and fundamental base of information, including both genomic DNA (gDNA) and transcribed sequences (cDNAs), that is strengthening both basic and applied biological research on parasitic nematodes. Large scale genomics facilitates the development, *in silico*, of specific hypotheses that previously could not have been rationalized or effectively conceived.

3.1. Nematode transcriptomics

In comparison to certain protozoan parasites, research progress on nematodes has been slowed by larger genome sizes, tissue complexity, life cycle complexity, and a paucity of genetic and transgenic methods for manipulating genes of interest. Lack of data and tools has limited the scope and quality of hypotheses that can be rationalized for research. Initial progress in improving the capacity for genomics-driven research on parasitic nematodes has come from the generation of large collections of expressed sequence tags (ESTs) representing transcribed sequences. EST projects have benefited from rapidly evolving sequencing chemistry and instrumentation, which has translated into a progressive decrease in sequencing costs. EST approaches have also laid a foundation for current whole genome sequencing projects.

Nematode species with sizable EST collections span nematode clades I–V (Blaxter et al., 1998; http://www.nematode.net; Wylie et al., 2004; http://www.nematodes.org; Parkinson et al., 2004b). Integration of a patchwork of available data from across multiple species will be required for full application of accumulating genomic data to innovative control methods, and meaningful progress has been made towards this goal (Parkinson et al., 2004a; discussed further in Section 3.3).

By the end of 2006 there were ~340,000 ESTs in the dbEST division of GenBank originating from 39 parasitic nematode species. Of these, 34% came from parasitic nematodes of veterinary and laboratory animals (cattle, sheep, pig, dog, rat and mouse). These ESTs have proven valuable for resource and reagent development, genome analysis and functional genomics. For instance, ESTs are one of the most cost-effective routes for gene discovery (discussed below). They provide core resources to design probes for expression microarrays (Section 4.1) and can be translated to supply putative protein sequence information for proteomics methods (Section 4.2). Among numerous bioinformatics uses, ESTs provide reference data for determining alternative splicing (Lee et al., 2006), verifying open reading frames and confirming exon/intron and gene boundaries (Section 3.2).

Resulting databases of ESTs, clustered by sequence (to improve quality and transcript length), provided the first concrete outline of genes (and putative proteins) that underlie the molecular makeup of nematode species. mRNA surveys through generation of ESTs provide an initial assessment of gene expression patterns in parasitic nematodes by life cycle stage and tissue type, information that is not readily obtainable from genomic DNA sequences. cDNA libraries represent mRNA populations at the time of isolation, and cDNA abundance usually correlates with representation of transcripts in the high abundance category of the original biological sample (Audic and Claverie, 1997). Quantitative EST assessments have identified transcripts that are either over- or under-represented by comparison to other transcripts among nematode life-cycle stages or tissues (Geldhof et al., 2005; Jasmer et al., 2004; Maizels et al., 2000a). Over-represented gene products draw attention because their abundance implies biological importance. Comparison

of ESTs from three life stages (infective L3, tissue-arrested or serum-stimulated L3) of the canine hookworm *Ancylostoma caninum* and two life stages (infective L3 and adult) of the canine/feline hookworm *A. ceylanicum*, identified genes up-regulated during the transition to parasitism and tissue penetration/migration, and genes in common for multiple stages (Mitreva et al., 2005). Some of these findings have been further pursued in microarray studies using EST project clones (see Section 4.1). In some cases, ESTs identified expansion and diversity of genes that may reflect evolutionary interactions with the host immune system (e.g. intestinal cathepsin B-like cysteine proteases from adult *H. contortus* (Jasmer et al., 2004), and DNase II-like proteins from immature L1 of *Trichinella spiralis* (Mitreva et al., 2004)).

Large scale nucleotide sequence data, such as EST clusters, can be translated and evaluated with gene ontology programs and provide insight into cellular and metabolic pathways functioning in the parasite (e.g. Yin et al., 2006). Mining of this information can reveal candidate drug targets with characterized pathways that differ from the host having particular interest. Certain nematode protein sequences can be modeled, based on knowledge of structure and function from homologous proteins, to predict drug susceptibility or resistance (Geary et al., 1998; Hussein et al., 2000; Perbandt et al., 2005) or suggest routes to the design of protein inhibitors or activators (Ring et al., 1993). Nematode-specific proteins lacking known functions represent attractive avenues for research, although the utility of the proteins as targets for control requires determination. Because protein structure evolves more slowly than sequence, use of predicted protein fold and secondary structure is an alternative approach to obtain information on putative function (Baker and Sali, 2001). Additional information can come from RNA interference (RNAi) phenotypes of *Caenorhabditis elegans* genes that encode homologs (or better, orthologs) of parasite proteins. Indications that a gene product performs a key function (observable as severe phenotypes) would draw additional interest (Palavalli et al., 2006). This approach applies to protein examples with either known, putative or unknown functions. There is also progress on use of RNAi for functional evaluation of parasite genes (Section 4.3), and interesting potential for application of RNAi in control strategies (Huang et al., 2006). A useful hierarchy of considerations was developed to identify potential control targets based on a matrix of biological data including the mining of parasite EST data (McCarter, 2004).

3.2. Nematode genomes

The first genome project for any multicellular organism was directed at the free-living nematode *C. elegans* (The C. elegans Sequencing Consortium, 1998). *C. elegans* remains the only metazoan for which the sequence of every nucleotide (100,278,047) is finished to high confidence. The impact on the pace of discovery in this field has been breathtaking, further accelerating the already rapid characterization of individual genes, molecular pathways, and developmental mechanisms in this model organism (Bieri et al., 2007). *C. elegans* biologists now routinely integrate information from genomics databases with new findings from genetic and cell biological approaches. Five nematologists have now been awarded the Nobel Prize in Medicine or Physiology, all of whom have studied molecular mechanisms in *C. elegans* (cell death, 2001, and RNA interference, 2006). Within just a few years of the first publication on RNAi (Fire et al., 1998), nearly all ~19,000 genes in *C. elegans* had been tested for transcript knock-down with phenotypes observed for several thousand (Kamath and Ahringer, 2003). *C. elegans* has served as an essential guide for initial analysis of sequences from other nematode species. In turn, parasitic nematode ESTs have also aided *C. elegans* biology, as they help confirm gene predictions and add depth to analyses of protein structure–function studies (e.g. McCarter et al., 2003).

Following the early exploration of parasitic nematode genomes by EST approaches (Parkinson et al., 2004a), parasitic species are now following *C. elegans* toward an era in which the sequences of complete genomes will be available. Genome sequencing data and/or draft assemblies of *H. contortus* (http://www.sanger.ac.uk/Projects/H_contortus/), *Brugia malayi* (http://www.tigr.org/tdb/e2k1/bma1/) and *T. spiralis* (http://genome.wustl.edu) genomes are now on-line (Table 1). In October 2006, NHGRI approved sequencing the genomes of 10 species of parasitic nematodes that primarily infect animals or are of zoonotic significance (Table 1). All are in the order Strongylida (Clade V), inclusive of examples from the superfamilies Ancylostomatoidea, Trichostrongyloidea and Metastrongyloidea (http://www.genome.gov/10002154). The Sanger Institute announced seven additional parasitic nematode genome projects with the majority of species representing human pathogens (Table 1; http://www.sanger.ac.uk/Projects/Helminths/). While a comprehensive genome analysis has not yet been published for any parasitic nematode, the initiation of these projects demarcates a major shift into the genome era for nematodes that infect mammals. Analysis and

Table 1
Animal parasitic nematodes with genome sequencing projects underway or planned (December 2006)

Status	Clade[a]	Species	Primary host	Coverage	Funding	Genome project information
Underway				Available		
	I	*Trichinella spiralis*	Pig to human	35×	NHGRI	http://genome.wustl.edu/genome.cgi?GENOME=Trichinella%20spiralis
	III	*Ascaris suum*	Pig roundworm	1×	NIAID	www.nematode.net
	V	*Ancylostoma caninum*	Dog	1×	NIAID	
	V	*Haemonchus contortus*	Goat and sheep	5×	Welcom Trust	http://www.sanger.ac.uk/Projects/H_contortus/
Planned				Planned		
	V	*Ancylostoma caninum*	Dog	6×	NHGRI	http://www.genome.gov/10002154
	V	*Cooperia oncophora*	Cattle	6×	NHGRI	
	V	*Dictyocaulus viviparous*	Cattle	6×	NHGRI	
	V	*Nematodirus battus*	Sheep and goat	6×	NHGRI	
	V	*Nippostrongylus brasiliensis*	Rat	6×	NHGRI	
	V	*Oesophagostomum dentatum*	Pig roundworm	6×	NHGRI	
	V	*Ostertagia ostertagi*	Cattle	6×	NHGRI	
	V	*Teladorsagia circumcincta*	Sheep	6×	NHGRI	
	V	*Trichostrongylus vitrinus*	Sheep and goat	6×	NHGRI	
	Iva	*Strongyloides ratti*	Rat	8×	Welcom Trust	http://www.sanger.ac.uk/Projects/Helminths/
	I	*Trichuris muris*	Mouse	8×	Welcom Trust	

[a] Phylogeny based on Blaxter et al. (1998).

application of this windfall in information will require both existing and new bioinformatics tools to organize the data. The forthcoming data has the opportunity to shorten the route towards developing more efficient and sustainable control programs.

3.3. Comparative genomics

Nematode comparative genomics describes methods that integrate information from the genomes of multiple species including interspecific analyzes using information from two or more species to pan-phylum comparisons. Current and pending genomic information from multiple nematode species will allow cross-species comparisons that can more successfully resolve structural and functional sequence features than is possible with the genome of a single species. Evolutionary modifications can also be tracked.

3.3.1. Interspecific comparative genomics

Comparisons between parasitic and free-living nematodes have identified genes potentially involved in host–parasite interactions. For example, comparison of transcripts from larval *Toxocara canis* and *C. elegans* (Tetteh et al., 1999) identified parasite-specific genes that may figure into the molecular basis of immune invasion by *T. canis*. Furthermore, these ESTs coupled with protein sequencing have characterized products that may be necessary for the success of the *T. canis* life cycle (Maizels et al., 2000b). Comparisons between potential secreted products in *Nippostrongylus brasiliensis* with homologs from *C. elegans* and more distantly related organisms indicated that secreted proteins may undergo accelerated evolution, because of stronger selective pressure or relaxed functional constraints (Harcus et al., 2004). In addition, comparisons to host pathways can identify distinct nematode features for exploration as promising targets for developing safe treatments. For instance, gene expression in the adult heartworm *Dirofilaria immitis* is consistent with anaerobic energy generation distinct from the aerobic pathway utilized by its mammalian host (Yin et al., 2006). This pathway may be a promising target for development of new macrofilaricides. Although, data from several complete and ongoing nematode genome projects are available, the only extensive comparative study of full genomes to date analyzes *C. elegans* and *C. briggsae* (Stein et al., 2003). For parasitic nematodes, an 83 kb region of the human parasite *B. malayi* has been compared to *C. elegans*, providing evidence for long-range synteny and microsynteny (Guiliano et al., 2002).

3.3.2. Pan-phylum analysis

An extension of interspecific analysis is pan-phylum analysis. For ESTs, the first such comprehensive analysis of the nematode transcriptome was based on over 250,000 ESTs originating from 30 species (28 of these were parasitic), clustered into 93,000 genes (Parkinson et al., 2004a). Further, potential broadly

conserved characteristics were identified. The results also suggested that despite availability of the genomes of two *Caenorhabditis* species the nematode gene space is far from thoroughly sampled. The authors also defined a list of nematode-specific protein families of particular interest as drug and vaccine targets. This data collection was also used to study the codon usage patterns in the nematoda based on over 32 million codons (Mitreva et al., 2006). The difference in codon usage between species resulted from the GC content of coding sequences, which varies in nematodes from 32 to 51%. Furthermore, total genomic GC content, probably the product of directional mutation pressure, appeared to drive codon usage rather than the converse. Analysis of an expanded dataset (over 214,000 polypeptides from 32 species) is underway with the goals of characterizing novel nematode-specific protein domains and identifying gene products that play critical roles in adaptation to parasitism (Mitreva, unpublished).

Another step in pan-phylum analysis is to identify conserved characteristics of gene expression in specific nematode organs or tissues that are potential targets for parasite control. Optimal progress requires integration of both EST and whole genome data. One example is the nematode intestine, which in *H. contortus* has demonstrated application to chemotherapy, parenteral immunization and potentially mucosal immunization (Jasmer et al., 2007; Newton and Munn, 1999). In each case, apical intestinal membrane proteins were the subjects of investigation. Broader potential is illustrated by intestinal cysteine proteases which are targets for control of plant parasitic nematodes (Lilley et al., 1999). Using currently available ESTs from intestinal cDNA libraries and microarray data, intestinal transcripts were compared from a core set of species, *C. elegans*, *H. contortus* and *Ascaris suum* (Mitreva and Jasmer, unpublished), which represent species from nematode clades III and V. Apparent conserved characteristics of gene expression have been identified, which can now be used to assess conservation in other species. Many other parasitic nematodes are too small for effective dissection, including hookworms (Ranjit et al., 2006), *T. spiralis* and *Stronglyoides* spp. as examples. To circumvent this obstacle, the available genome sequence of the clade I nematode, *T. spiralis*, was probed with sequences from apical intestinal membrane proteins apparently conserved among the core species indicated. Prospective *T. spiralis* intestinal genes have been identified including homologs/orthologs of *H. contortus* vaccine antigens (unpublished observations) and can now be tested experimentally for validation. Existing and forthcoming genomic sequences from *A. suum* and *H. contortus* will facilitate extending this analysis to develop a database of conserved nematode intestinal genes. The example discussed can conceivably be applied to other nematode tissues, cells and stages. For example, it is clear from known mechanisms of anthelmintics that the core set of genes expressed by the nematode nervous system would represent a valuable database. In this context, a small set of ESTs has been generated from *A. suum* adult nerve chord and muscle.

Research on nematode genomics research is facilitated through two databases that provide tools for navigating parasitic nematode sequences (Parkinson et al., 2004b; Wylie et al., 2004). In addition, one of these (http://www.nematode.net) offers an extensive mapping of EST clusters to the biochemical pathways of the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto, 2006) and associations to the three organizing principal of Gene Ontology (The Gene Ontology Consortium, 2000) for 11 animal parasitic nematodes. Coupled with statistical tools (e.g. Wu et al., 2006), these databases provide the scientific community with a solid platform for comparative metabolomics and functional genomics in nematodes.

4. Functional genomics and proteomics

Genomics-based approaches can provide useful supporting information in characterizing potential target proteins for vaccines and anthelmintic drugs. Microarrays and proteomic methods can confirm the life-cycle stages and tissues in which messenger RNAs and their corresponding proteins are expressed. Vaccine antigens are believed to be most effective when secreted from glands (Bethony et al., 2005) or expressed on exposed surfaces such as the intestinal lumen (Loukas et al., 2005) where they can come into contact with immune system effector molecules. Antigens can be expressed at multiple stages including in infective larva (L3) and in adults. Expression of anthelmintic target proteins throughout the life cycle is preferable so that all stages could be sensitive to the effects of the interacting compound. For parasitic nematodes of livestock, sensitivity of the adult worms to anthelmintic treatment is a major requirement. Anthelmintic targets must play roles in essential physiological processes in the parasite such that loss-of-function or gain-of-function disruption can result in lethality or paralysis. RNA interference (RNAi) (Fire et al., 1998) is an efficient approach for rapidly determining the phenotypic effects of transcript knockdown in many organisms including the free-living

nematode *C. elegans* and can be used to identify potential loss-of-function anthelmintic targets. There are not yet comparable high throughput approaches to identify new gain-of-function anthelmintic targets such as channels and receptors like those that are the target of many current anthelmintic compounds (Martin, 1997).

4.1. Expression studies based on microarrays

Microarray studies using several hundred to several thousand genes have so far been published for the mammalian parasitic nematode species *A. caninum*, *Trichostrongylus vitrinus*, *Oesophagostomum dentatum*, *Strongyloides ratti*, *B. malayi*, and *A. suum*. Gender-specific gene expression has been described through comparisons of male and female transcripts in animal parasitic *O. dentatum* (Cottee et al., 2006), *T. vitrinus* (Nisbet and Gasser, 2004) and the human filarial nematode *B. malayi* (Li et al., 2005) identifying genes which may play roles in oogenesis, embryogenesis, or spermatogenesis. Changes in gene expression across larval stages have also been described including *S. ratti* free-living L1 versus infective L3 (Thompson et al., 2006) and *A. caninum* L3 prior to and after exposure to host serum (Moser et al., 2005). While infective L3 have morphological similarities to the *C. elegans* dauer stage, a common signature in gene expression was not detected. A study of *A. suum* compared expression in two populations of L4, those in the jejunum which continue to successfully infect and develop versus those that have been swept into the ileum toward eventual expulsion from the host (Morimoto et al., 2003). Microarray studies examining specific tissues of interest such as the intestine or glands isolated by laser-capture micro-dissection have yet to be published.

4.2. Proteomics

LC–MS/MS or 2d-gel followed by LC–MS/MS: Proteomic techniques including two-dimensional gel electrophoresis (2DE), liquid chromatography–tandem mass spectrometry (LC–MS/MS), and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) were used to describe excretory/secretory products (ESPs) of the Strongylid nematode *H. contortus* (Yatsuda et al., 2003). ESPs are of great interest as potential vaccine antigens. 107 proteins were identified including H11, a vaccine antigen previously believed to be absent from the ESPs. A smaller analysis using 1DE and peptide mass fingerprints identified 28 ESPs from *Teladorsagia circumcincta* (Craig et al., 2006). 2DE identified over 200 spots from the proteome of whole adult *A. suum* including several that differed between worms in aerobic versus anaerobic environments (Islam et al., 2004). By coupling protein sequence similarity with signal peptide prediction, 345 *T. spiralis* clusters were identified that had homology with predicted secreted or membrane proteins. The EST clusters supported interpretation of peptide mass fingerprint data obtained from 2DE analysis of muscle larvae ES proteins (Robinson et al., 2005). More recent 2DE electrophoresis of ES proteins was coupled with MALDI-TOF- and LC–MS/MS enabling the most comprehensive identification of peptide spots from *T. spiralis* performed thus far (Robinson et al., 2005). Identities were assigned to 43 out of 52 ES peptide spots analyzed, representing only 13 different proteins indicating that there are multiple protein isoforms present in the ES. The most prominent were serine protease, the 45 kDa antigen, gp43 and 2 unidentified open reading frames (Robinson and Connolly, 2005).

4.3. RNA interference (RNAi)

Following the success of transcript silencing by RNAi in *C. elegans*, a variety of approaches have been attempted to achieve similar effects in mammalian parasitic nematodes including the Strongylid species *Nippostrongylus brasiliensis* (Hussein et al., 2002), *Trichostrongylus colubriformis* (Issa et al., 2005), and *H. contortus* (Geldhof et al., 2006; Kotze and Bagnall, 2006), *O. ostertagi* (Visser et al., 2006) and the filarial species *B. malayi* (Aboobaker and Blaxter, 2003) and *Onchocerca volvulus* (Lustigman et al., 2004). A number of gene targets appear to be particularly susceptible to RNAi including the *N. brasiliensis* secreted acetylcholinesterases (AchEs) whereas other genes showed much more variable responses. While some doubt remains that the machinery required for RNAi is present in all of these species (Geldhof et al., 2006; Knox et al., 2007; Zawadzki et al., 2006), for those where RNAi knockdown has been successfully performed the experiments are not yet routine and reproducible in these parasitic nematodes as they are in *C. elegans*, and methodological advances likely lie ahead.

5. Concluding comments

As is occurring throughout the biological sciences, a decisive move is underway into the genomics era for the study of parasitic nematodes. As technologies improve

and costs continue to decrease, complete genomes are gradually becoming available and will eventually be taken for granted by researchers as being an obvious piece of core knowledge available for any organism under study. While but one type of information about a species, genome data has numerous applications that can advance knowledge of nematode biology on multiple fronts. If exploited, advances driven in part by genomics have the opportunity to lead to innovations that can be broadly integrated into control strategies against nematode pathogens. However, the volume of information pending deposit is well beyond our current capacity to fully exploit; in ten years time it is not unrealistic to envision the availability of complete or draft genomes from 50 to 100 nematode species. There will be a growing need to better organize and provide genomic information in a manner that is accessible to the user community of parasitologists. Adaptation by parasitologists will also be required to effectively utilize genomics in their research programs and to identify the most promising opportunities to apply this knowledge to the development of control strategies. Lastly, the experience with anthelmintic resistance in nematodes of veterinary importance is a likely harbinger of challenges ahead throughout parasitology and infectious disease. Even with genomes in hand, there is a practical limit to the rate at which novel methods of control can be generated and implemented. Therefore, new control methods must be viewed as a precious resource. Equally important to the rapid introduction of new strategies for parasite control will be attention given to their sustainability through the integration of multiple control approaches and resistance management.

Acknowledgement

This work was in part supported by NIH-NIAID research grant AI 46593.

References

Aboobaker, A.A., Blaxter, M.L., 2003. Use of RNA interference to investigate gene function in the human filarial nematode parasite *Brugia malayi*. Mol. Biochem. Parasitol. 129, 41–51.

Au Yeung, K.J., Smith, A., Zhao, A., Madden, K.B., Elfrey, J., Sullivan, C., Levander, O., Urban, J.F., Shea-Donohue, T., 2005. Impact of vitamin E or selenium deficiency on nematode-induced alterations in murine intestinal function. Exp. Parasitol. 109, 201–208.

Audic, S., Claverie, J.M., 1997. The significance of digital gene expression profiles. Genome Res. 7, 986–995.

Baker, D., Sali, A., 2001. Protein structure prediction and structural genomics. Science 294, 93–96.

Bethony, J., Loukas, A., Smout, M., Brooker, S., Mendez, S., Plieskatt, J., Goud, G., Bottazzi, M.E., Zhan, B., Wang, Y., Williamson, A., Lustigman, S., Correa-Oliveira, R., Xiao, S., Hotez, P.J., 2005. Antibodies against a secreted protein from hookworm larvae reduce the intensity of hookworm infection in humans and vaccinated laboratory animals. FASEB J. 19, 1743–1745.

Bieri, T., Blasiar, D., Ozersky, P., Antoshechkin, I., Bastiani, C., Canaran, P., Chan, J., Chen, N., Chen, W.J., Davis, P., Fiedler, T.J., Girard, L., Han, M., Harris, T.W., Kishore, R., Lee, R., McKay, S., Muller, H.-M., Nakamura, C., Petcherski, A., Rangarajan, A., Rogers, A., Schindelman, G., Schwarz, E.M., Spooner, W., Tuli, M.A., Auken, K.V., Wang, D., Wang, X., Williams, G., Durbin, R., Stein, L.D., Sternberg, P.W., Spieth, J., 2007. WormBase: new content and better access. Nucl. Acids Res. 35, D506–D510.

Blaxter, M.L., De Ley, P., Garey, J.R., Liu, L.X., Scheldeman, P., Vierstraete, A., Vanfleteren, J.R., Mackey, L.Y., Dorris, M., Frisse, L.M., Vida, J.T., Thomas, W.K., 1998. A molecular evolutionary framework for the phylum nematoda. Nature 392, 71–75.

Brown, H.D., Matzuk, A.R., Ilves, I.R., Peterson, L.H., Harris, S.A., Sarett, L.H., Egerton, J.R., Yakstis, J.J., Campbell, W.C., Cuckler, A.C., 1961. Antiparasitic drugs. IV. 2-(4′-Thiazolyl)-benzimidazole, a new anthelmintic. J. Am. Chem. Soc. 83, 1764–1765.

Chabala, J.C., Mrozik, H., Tolman, R.L., Eskola, P., Lusi, A., Peterson, L.H., Woods, M.F., Fisher, M.H., Campbell, W.C., Egerton, J.R., Ostlind, D.A., 1980. Ivermectin, a new broad-spectrum antiparasitic agent. J. Med. Chem. 23, 1134–1136.

Claerebout, E., Knox, D.P., Vercruysse, J., 2003. Current research and future prospects in the development of vaccines against gastrointestinal nematodes in cattle. Expert Rev. Vaccines 2, 147–157.

Cottee, P.A., Nisbet, A.J., Abs El-Osta, Y.G., Webster, T.L., Gasser, R.B., 2006. Construction of gender-enriched cDNA archives for adult *Oesophagostomum dentatum* by suppressive-subtractive hybridization and a microarray analysis of expressed sequence tags. Parasitology 132, 691–708.

Craig, H., Wastling, J.M., Knox, D.P., 2006. A preliminary proteomic survey of the in vitro excretory/secretory products of fourth-stage larval and adult *Teladorsagia circumcincta*. Parasitology 132, 535–543.

Crawford, A.M., Paterson, K.A., Dodds, K.G., Diez Tascon, C., Williamson, P.A., Roberts Thomson, M., Bisset, S.A., Beattie, A.E., Greer, G.J., Green, R.S., Wheeler, R., Shaw, R.J., Knowler, K., McEwan, J.C., 2006. Discovery of quantitative trait loci for resistance to parasitic nematode infection in sheep. I. Analysis of outcross pedigrees. BMC Genomics 7, 178.

Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., Mello, C.C., 1998. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature 391, 806–811.

Gasbarre, L.C., Leighton, E.A., Davies, C.J., 1990. Genetic control of immunity to gastrointestinal nematodes of cattle. Vet. Parasitol. 37, 257–272.

Gauci, C., Heath, D., Chow, C., Lightowlers, M.W., 2005. Hydatid disease: vaccinology and development of the EG95 recombinant vaccine. Expert Rev. Vaccines 4, 103–112.

Geary, T.G., Conder, G.A., Bishop, B., 2004. The changing landscape of antiparasitic drug discovery for veterinary medicine. Trends Parasitol. 20, 449–455.

Geary, T.G., Nulf, S.C., Alexander-Bowman, S.J., Mahmoud, B.M., Prichard, R.K., Klein, R.D., 1998. Cloning and characterization of cDNAs encoding beta-tubulin from *Dirofilaria immitis* and *Onchocerca volvulus*. J. Parasitol. 84, 356–360.

Geldhof, P., Murray, L., Couthier, A., Gilleard, J.S., McLauchlan, G., Knox, D.P., Britton, C., 2006. Testing the efficacy of RNA

interference in *Haemonchus contortus*. Int. J. Parasitol. 36, 801–810.

Geldhof, P., Whitton, C., Gregory, W.F., Blaxter, M., Knox, D.P., 2005. Characterisation of the two most abundant genes in the *Haemonchus contortus* expressed sequence tag dataset. Int. J. Parasitol. 35, 513–522.

Gomez-Munoz, M.T., Canals-Caballero, A., Almeria, S., Pasquali, P., Zarlenga, D.S., Gasbarre, L.C., 2004. Inhibition of bovine T lymphocyte responses by extracts of the stomach worm *Ostertagia ostertagi*. Vet. Parasitol. 120, 199–214.

Grencis, R.K., Entwistle, G.M., 1997. Production of an interferon-gamma homologue by an intestinal nematode: functionally significant or interesting artefact? Parasitology 115 (Suppl.), S101–S106.

Guiliano, D.B., Hall, N., Jones, S.J., Clark, L.N., Corton, C.H., Barrell, B.G., Blaxter, M.L., 2002. Conservation of long-range synteny and microsynteny between the genomes of two distantly related nematodes. Genome Biol. 3 RESEARCH0057.

Harcus, Y.M., Parkinson, J., Fernandez, C., Daub, J., Selkirk, M.E., Blaxter, M.L., Maizels, R.M., 2004. Signal sequence analysis of expressed sequence tags from the nematode Nippostrongylus brasiliensis and the evolution of secreted proteins in parasites. Genome Biol. 5, R39.

Helmby, H., Grencis, R.K., 2003. Essential role for TLR4 and MyD88 in the development of chronic intestinal nematode infection. Eur. J. Immunol. 33, 2974–2979.

Hotez, P.J., Molyneux, D.H., Fenwick, A., Ottesen, E., Ehrlich Sachs, S., et al., 2006. Incorporating a rapid-impact package for neglected tropical diseases with programs for HIV/AIDS, tuberculosis, and malaria. PLoS Med. 3, e102 doi.10.1371/journal.pmed.0030102.

Huang, X., Yang, S.-P., Chinwalla, A.T., Hillier, L.W., Minx, P., Mardis, E.R., Wilson, R.K., 2006. Application of a superword array in genome assembly. Nucl. Acids Res. 34, 201–205.

Hussein, A.S., Kichenin, K., Selkzer, P.M., 2002. Suppression of selected acetylcholinesterase expression in *Nippostrongylus brasiliensis* by RNA interference. Mol. Biochem. Parasitol. 122, 91–94.

Hussein, A.S., Smith, A.M., Chacon, M.R., Selkirk, M.E., 2000. Determinants of substrate specificity of a second non-neuronal secreted acetylcholinesterase from the parasitic nematode *Nippostrongylus brasiliensis*. Eur. J. Biochem. 267, 2276–2282.

Islam, M.K., Miyoshi, T., Yamada, M., Alim, M.A., Huang, X., Motobu, M., Tsuji, N., 2006. Fluoride exposure inhibits protein expression and enzyme activity in the lung-stage larvae of *Ascaris suum*. Parasitology 133, 497–508.

Islam, M.K., Miyoshi, T., Yokomizo, Y., Tsuji, N., 2004. The proteome expression patterns in adult *Ascaris suum* under exposure to aerobic/anaerobic environments analyzed by two-dimensional electrophoresis. Parasitol. Res. 93, 96–101.

Issa, Z., Grant, W.N., Stasiuk, S., Shoemaker, C.B., 2005. Development of methods for RNA interference in the sheep gastrointestinal parasite, *Trichostrongylus colubriformis*. Int. J. Parasitol. 35, 935–940.

Jasmer, D.P., Dautova Mitreva, M., McCarter, J.P., 2004. mRNA sequences for *Haemonchus contortus* intestinal cathepsin B-like cysteine proteases display an extreme in abundance and diversity compared with other adult mammalian parasitic nematodes. Mol. Biochem. Parasitol. 137, 297–305.

Jasmer, D.P., Groverse, A., Smant, G., 2003. Parasitic nematode interactions with mammals and plants. Ann. Rev. Phytopathol. 41, 245–270.

Jasmer, D.P., Lahmers, K., Brown, W.C., 2007. Haemonchus contortus intestine: a prominent source of mucosal antigens. Parasite Immunol. 29, 139–151.

Kamath, R.S., Ahringer, J., 2003. Genome-wide RNAi screening in *Caenorhabditis elegans*. Methods 30, 313–321.

Kaminsky, R., 2003. Drug resistance in nematodes: a paper tiger or a real problem? Curr. Opin. Infect. Dis. 16, 559–564.

Kanehisa, M., Goto, S., 2006. KEGG: kyoto encyclopedia of genes and genomes. Nucl. Acids Res. 28, 27–30.

Kaplan, R.M., 2004. Drug resistance in nematodes of veterinary importance: a status report. Trends Parasitol. 20, 477–481.

Knox, D.P., Geldhof, P., Visser, A., Britton, C., 2007. RNA interference in parasitic nematodes of animals: a reality check? Trends Parasitol. 23, 105–107.

Knox, M.R., Torres-Acosta, J.F.J., Aguilar-Caballero, A.J., 2006. Exploiting the effect of dietary supplementation of small ruminants on resilience and resistance against gastrointestinal nematodes. Vet. Parasitol. 139, 385–393.

Koski, K.G., Scott, M.E., 2003. Gastrointestinal nematodes, trace elements and immunity. J. Trace Elem. Exp. Med. 16, 237–251.

Kotze, A.C., Bagnall, N.H., 2006. RNA interference in *Haemonchus contortus*: suppression of beta-tubulin gene expression in L3, L4 and adult worms in vitro. Mol. Biochem. Parasitol. 145, 101–110.

Larsen, M., 1999. Biological control of helminths. Int. J. Parasitol. 29, 139–146.

Lee, Y., Lee, Y., Kim, B., Shin, Y., Nam, S., Kim, P., Kim, N., Chung, W.-H., Kim, J., Lee, S., 2006. ECgene: an alternative splicing database update. Nucl. Acids Res. gkl992 .

Leighton, E.A., Murrell, K.D., Gasbarre, L.C., 1989. Evidence for genetic control of nematode egg-shedding rates in calves. J. Parasitol. 75, 498–504.

Li, B.-W., Rush, A.C., Crosby, S.D., Warren, W.C., Williams, S.A., Mitreva, M., Weil, G.J., 2005. Profiling of gender-regulated gene transcripts in the filarial nematode *Brugia malayi* by cDNA oligonucleotide array analysis. Mol. Biochem. Parasitol. 143, 49–57.

Lightowlers, M.W., 2004. Vaccination for the prevention of cysticercosis. Dev. Biol. 119, 361–368.

Lilley, C.J., Devlin, P., Urwin, P.E., Atkinson, H.J., 1999. Parasitic nematodes, proteinases and transgenic plants. Parasitol. Today 15, 414–417.

Lochnit, G., Bongaarts, R., Geyer, R., 2005. Searching new targets for anthelminthic strategies: Interference with glycosphingolipid biosynthesis and phosphorylcholine metabolism affects development of *Caenorhabditis elegans*. Int. J. Parasitol. 35, 911–923.

Loukas, A., Bethony, J.M., Mendez, S., Fujiwara, R.T., Goud, G.N., Ranjit, N., Zhan, B., Jones, K., Bottazzi, M.E., Hotez, P.J., 2005. Vaccination with recombinant aspartic hemoglobinase reduces parasite load and blood loss after hookworm infection in dogs. PLoS Med. 2, e296.

Lustigman, S., Zhang, J., Liu, J., Oksov, Y., Hashmi, S., 2004. RNA interference targeting cathepsin L and Z-like cysteine proteases of *Onchocerca volvulus* confirmed their essential function during L3 molting. Mol. Biochem. Parasitol. 138, 165–170.

Maizels, R.M., Tetteh, K.K., Loukas, A., 2000a. Toxocara canis: genes expressed by the arrested infective larval stage of a parasitic nematode. Int. J. Parasitol. 30, 495–508.

Maizels, R.M., Tetteh, K.K.A., Loukas, A., 2000b. Toxocara canis: genes expressed by the arrested infective larval stage of a parasitic nematode. Int. J. Parasitol. 30, 495–508.

Martin, R.J., 1997. Modes of action of anthelmintic drugs. Vet. J. 154, 11–34.

McCarter, J., Dautova Mitreva, M., Martin, J., Dante, M., Wylie, T., Rao, U., Pape, D., Bowers, Y., Theising, B., Murphy, C.V., Kloek, A.P., Chiapelli, B.J., Clifton, S.W., Bird, M.D., Waterston, R., 2003. Analysis and functional classification of transcripts from the nematode *Meloidogyne incognita*. Genome Biol. 4 (R26), 19–21.

McCarter, J.P., 2004. Genomic filtering: an approach to discovering novel antiparasitics. Trends Parasitol. 20, 462–468.

McEwan, J.C., 1998. Breeding for ovine host resistance to gastrointestinal parasitism. In: Proceedings from the 28th Seminar of the Society of Sheep and Beef Cattle Veterinarians NZVA. pp. 47–56.

McVeigh, P., Geary, T.G., Marks, N.J., Maule, A.G., 2006. The FLP-side of nematodes. Trends Parasitol. 22, 385–396.

Mitreva, M., Jasmer, D.P., Appleton, J., Martin, J., Dante, M., Wylie, T., Clifton, S.W., Waterston, R.H., McCarter, J.P., 2004. Gene discovery in the adenophorean nematode *Trichinella spiralis*: an analysis of transcription from three life cycle stages. Mol. Biochem. Parasitol. 137, 277–291.

Mitreva, M., McCarter, J.P., Arasu, P., Hawdon, J., Martin, J., Dante, M., Wylie, T., Xu, J., Stajich, J.E., Kapulkin, W., Clifton, S.W., Waterston, R.H., Wilson, R.K., 2005. Investigating hookworm genomes by comparative analysis of two *Ancylostoma* species. BMC Genomics 6, 58.

Mitreva, M., Wendl, M., Martin, J., Wylie, T., Yin, Y., Larson, A., Parkinson, J., Waterston, R., McCarter, J., 2006. Codon usage patterns in nematoda: analysis based on over 25 million codons in thirty-two species. Genome Biol. 7, R75.

Miyadera, H., Shiomi, K., Ui, H., Yamaguchi, Y., Masuma, R., Tomoda, H., Miyoshi, H., Osanai, A., Kita, K., Omura, S., 2003. Atpenins, potent and specific inhibitors of mitochondrial complex II (succinate-ubiquinone oxidoreductase). Proc. Natl. Acad. Sci. U. S. A. 100, 473–477.

Morimoto, M., Zarlenga, D., Beard, H., Alkharouf, N., Matthews, B.F., Urban, J.F.J., 2003. *Ascaris suum*: cDNA microarray analysis of 4th stage larvae (L4) during self-cure from the intestine. Exp. Parasitol. 104, 113–121.

Moser, J.M., Freitas, T., Arasu, P., Gibson, G., 2005. Gene expression profiles associated with the transition to parasitism in *Ancylostoma caninum* larvae. Mol. Biochem. Parasitol. 143, 39–48.

Mousley, A., Maule, A.G., Halton, D.W., Marks, N.J., 2005. Interphyla studies on neuropeptides: the potential for broad-spectrum anthelmintic and/or endectocide discovery. Parasitology 131, S143–S167.

Newton, S.E., Munn, E.A., 1999. The development of vaccines against gastrointestinal nematode parasites, particularly *Haemonchus contortus*. Parasitol. Today 15, 116–122.

Niezen, J.H., Charleston, W.A.G., Robertson, H.A., Shelton, D., Waghorn, G.C., Green, R., 2002. The effect of feeding sulla (*Hedysarum coronarium*) or lucerne (*Medicago sativa*) on lamb parasite burdens and development of immunity to gastrointestinal nematodes. Vet. Parasitol. 105, 229–245.

Nisbet, A.J., Gasser, R.B., 2004. Profiling of gender-specific gene expression for *Trichostrongylus vitrinus* (Nematoda: Strongylida) by microarray analysis of expressed sequence tag libraries constructed by suppressive-subtractive hybridisation. Int. J. Parasitol. 34, 633–643.

Omura, S., Miyadera, H., Ui, H., Shiomi, K., Yamaguchi, Y., Masuma, R., Nagamitsu, T., Takano, D., Sunazuka, T., Harder, A., Kolbl, H., Namikoshi, M., Miyoshi, H., Sakamoto, K., Kita, K., 2001. An anthelmintic compound, nafuredin, shows selective inhibition of complex I in helminth mitochondria. Proc. Natl. Acad. Sci. U. S. A. 98, 60–62.

Palavalli, L.H., Brendza, K.M., Haakenson, W., Cahoon, R.E., McLaird, M., Hicks, L.M., McCarter, J.P., Williams, D.J., Hresko, M.C., Jez, J.M., 2006. Defining the role of phosphomethylethanolamine *N*-methyltransferase from *Caenorhabditis elegans* in phosphocholine biosynthesis by biochemical and kinetic analysis. Biochemistry 45, 6056–6065.

Parkinson, J., Mitreva, M., Whitton, C., Thomson, M., Daub, J., Martin, J., Hall, N., Barrell, B., Waterston, R.H., McCarter, J.P., Blaxter, M., 2004a. A transcriptomic analysis of the phylum Nematoda. Nat. Gen. 36, 1259–1267.

Parkinson, J., Whitton, C., Schmid, R., Thomson, M., Blaxter, M., 2004b. NEMBASE: a resource for parasitic nematode ESTs. Nucl. Acids Res. 32, D427–D430.

Pennock, J.L., Behnke, J.M., Bickle, Q.D., Devaney, E., Grencis, R.K., Isaac, R.E., Joshua, G.W., Selkirk, M.E., Zhang, Y., Meyer, D.J., 1998. Rapid purification and characterization of L-dopachrome-methyl ester tautomerase (macrophage-migration-inhibitory factor) from *Trichinella spiralis*, *Trichuris muris* and *Brugia pahangi*. Biochem. J. 335, 495–498.

Perbandt, M., Hoppner, J., Betzel, C., Walter, R.D., Liebau, E., 2005. Structure of the major cytosolic glutathione *S*-transferase from the parasitic nematode *Onchocerca volvulus*. J. Biol. Chem. 280, 12630–12636.

Ranjit, N., Jones, M.K., Stenzel, D.J., Gasser, R.B., Loukas, A., 2006. A survey of the intestinal transcriptomes of the hookworms, *Necator americanus* and *Ancylostoma caninum*, using tissues isolated by laser microdissection microscopy. Int. J. Parasitol. 36, 701–710.

Ring, C.S., Sun, E., McKerrow, J.H., Lee, G.K., Rosenthal, P.J., Kuntz, I.D., Cohen, F.E., 1993. Structure-based inhibitor design by using protein models for the development of antiparasitic agents. Proc. Natl. Acad. Sci. U. S. A. 90, 3583–3587.

Risher, J.F., Mink, F.L., Stara, J.F., 1987. The toxicologic effects of the carbamate insecticide aldicarb in mammals: a review. Environ. Health Perspect. 72, 267–281.

Robinson, M.W., Connolly, B., 2005. Proteomic analysis of the excretory–secretory proteins of the *Trichinella spiralis* L1 larva, a nematode parasite of skeletal muscle. Proteomics 5, 4525–4532.

Robinson, M.W., Gare, D.C., Connolly, B., 2005. Profiling excretory/secretory proteins of *Trichinella spiralis* muscle larvae by two-dimensional gel electrophoresis and mass spectrometry. Vet. Parasitol. 132, 37–41.

Roos, M.H., 1997. The role of drugs in the control of parasitic nematode infections: must we do without? Parasitology 114 (Suppl.), S137–S144.

Sasaki, T., Takagi, M., Yaguchi, T., Miyadoh, S., Okada, T., Koyama, M., 1992. A new anthelmintic cyclodepsipeptide, PF1022A. J. Antibiot. (Tokyo) 45, 692–697.

Sayers, G., Sweeney, T., 2005. Gastrointestinal nematode infection in sheep—a review of the alternatives to anthelmintics in parasite control. Anim. Health. Res. Rev. 6, 159–171.

Schneider, S.M., Rosskopf, E.N., Leesch, J.G., Chellemi, D.O., Bull, C.T., Mazzola, M., 2003. United States Department of Agriculture–Agricultural Research Service research on alternatives to methyl bromide: pre-plant and post-harvest. Pest Manag. Sci. 59, 814–826.

Selzer, P.M., Pingel, S., Hsieh, I., Ugele, B., Chan, V.J., Engel, J.C., Bogyo, M., Russell, D.G., Sakanari, J.A., McKerrow, J.H., 1999. Cysteine protease inhibitors as chemotherapy: lessons from a parasite target. Proc. Natl. Acad. Sci. U. S. A. 96, 11015–11022.

Skipp, R.A., Chen, L.Y., Yeates, G.W., Glare, R., 2002. Occurrence, morphological characteristics and ribotyping of New Zealand

isolates of *Duddingtonia flagrans*, a candidate for biocontrol of animal parasitic nematodes. NZ J. Agric. Res. 45, 187–196.

Smith, A., Madden, K.B., Yeung, K.J.A., Zhao, A., Elfrey, J., Finkelman, F., Levander, O., Shea-Donohue, T., Urban Jr., J.F., 2005. Deficiencies in selenium and/or vitamin E lower the resistance of mice to *Heligmosomoides polygyrus* infections. J. Nutr. 135, 830–836.

Sonstegard, T.S., Gasbarre, L.C., 2001. Genomic tools to improve parasite resistance. Vet. Parasitol. 101, 387–403.

Souza, V.M.O., Faquim-Mauro, E.L., Macedo, M.S., 2002. Extracts of *Ascaris suum* egg and adult worm share similar immunosuppressive properties. Braz. J. Med. Biol. Res. 35, 81–89.

Spratt, D.M., 1997. Endoparasite control strategies: implications for biodiversity of native fauna. Int. J. Parasitol. 27, 173–180.

Stear, M.J., Doligalska, M., Donskow-Schmelter, K., 2006. Alternatives to anthelmintics for the control of nematodes in livestock. Parasitology 1–13 (Epub ahead of print).

Stein, L.D., Bao, Z., Blasiar, D., Blumenthal, T., Brent, M.R., Chen, N., Chinwalla, A., Clarke, L., Clee, C., Coghlan, A., et al., 2003. The genome sequence of *Caenorhabditis briggsae*: a platform for comparative genomics. PLoS Biol. 1, E45.

Stepek, G., Buttle, D.J., Duce, I.R., Behnke, J.M., 2006. Human gastrointestinal nematode infections: are new control methods required? Int. J. Exp. Pathol. 87, 325–341.

Stepek, G., Buttle, D.J., Duce, I.R., Lowe, A., Behnke, J.M., 2005. Assessment of the anthelmintic effect of natural plant cysteine proteinases against the gastrointestinal nematode, *Heligmosomoides polygyrus*, in vitro. Parasitology 130, 203–211.

Tetteh, K.K., Loukas, A., Tripp, C., Maizels, R.M., 1999. Identification of abundantly expressed novel and conserved genes from the infective larval stage of *Toxocara canis* by an expressed sequence tag strategy. Infect Immunol. 67, 4771–4779.

The C. elegans Sequencing Consortium, 1998. Genome sequence of the nematode *C. elegans*: a platform for investigating biology. Science 282, 2012–2018.

The Gene Ontology Consortium, 2000. Gene ontology: tool for the unification of biology. Nat. Genet. 25, 25–29.

Thompson, F.J., Barker, G.L., Hughes, L., Wilkes, C.P., Coghill, J., Viney, M.E., 2006. A microarray analysis of gene expression in the free-living stages of the parasitic nematode *Strongyloides ratti*. BMC Genomics 7, 157.

Torres-Acosta, J.F.J., Jacobs, D.E., Aguilar-Caballero, A., Sandoval-Castro, C., May-Martinez, M., Cob-Galera, L.A., 2004. The effect of supplementary feeding on the resilience and resistance of browsing Criollo kids against natural gastrointestinal nematode infections during the rainy season in tropical Mexico. Vet. Parasitol. 124, 217–238.

Vercauteren, I., Geldhof, P., Vercruysse, J., Peelaers, I., Van Den Broeck, W., Gevaert, K., Claerebout, E., 2004. Vaccination with an *Ostertagia ostertagi* polyprotein allergen protects calves against homologous challenge infection. Infect. Immunol. 72, 2995–3001.

Visser, A., Geldhof, P., De Maere, V., Knox, D.P., Vercruysse, J., Claerebout, E., 2006. Efficacy and specificity of RNA interference in larval life-stages of *Ostertagia ostertagi*. Parasitology 133, 777–783.

Waghorn, T.S., Leathwick, D.M., Chen, L.Y., Skipp, R.A., 2003. Efficacy of the nematode-trapping fungus *Duddingtonia flagrans* against three species of gastro-intestinal nematodes in laboratory faecal cultures from sheep and goats. Vet. Parasitol. 118, 227–234.

Waller, P.J., Thamsborg, S.M., 2004. Nematode control in 'green' ruminant production systems. Trends Parasitol. 20, 493–497.

Wu, J., Mao, X., Cai, T., Luo, J., Wei, L., 2006. KOBAS server: a web-based platform for automated annotation and pathway identification. Nucl. Acids Res. 34, W720–W724.

Wylie, T., Martin, J., Dante, M., Mitreva, M., Clifton, S.W., Chinwalla, A., Waterston, R.H., Wilson, R.K., McCarter, J.P., 2004. Nematode.net: a tool for navigating sequences from parasitic and free-living Nematodes. Nucl. Acids Res. 32, D423–D426.

Yatsuda, A.P., Krijgsveld, J., Cornelissen, A.W., Heck, A.J., de Vries, E., 2003. Comprehensive analysis of the secreted proteins of the parasite *Haemonchus contortus* reveals extensive sequence variation and differential immune recognition. J. Biol. Chem. 278, 16941–16951.

Yin, Y., Martin, J., McCarter, J.P., Clifton, S.W., Wilson, R.K., Mitreva, M., 2006. Identification and analysis of genes expressed in the adult filarial parasitic nematode *Dirofilaria immitis*. Int. J. Parasitol. 36, 829–839.

Zarlenga, D.S., 2004. Vaccinating against zoonotic parasitic diseases: myth or reality? Anim. Health Res. Rev. 5, 219–222.

Zarlenga, D.S., Chute, M.B., Martin, A., Kapel, C.M., 2001. A single, multiplex PCR for differentiating all species of *Trichinella*. Parasite 8, S24–S26.

Zarlenga, D.S., Rosenthal, B.M., La Rosa, G., Pozio, E., Hoberg, E.P., 2006. Post-miocene expansion, colonization, and host switching drove speciation among extant nematodes of the archaic genus *Trichinella*. Proc. Natl. Acad. Sci. U. S. A. 103, 7354–7359.

Zawadzki, J.L., Presidente, P.J.A., Meeusen, E.N., De Veer, M.J., 2006. RNAi in *Haemonchus contortus*: a potential method for target validation. Trends Parasitol. 22, 495–499.