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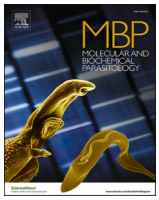

Spirocerca lupi draft genome, vaccine and anthelmintic targets

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ABSTRACT

Spirocerca lupi is a parasitic nematode affecting predominantly domestic dogs. It causes spirocercosis, a disease that is often fatal. The assembled draft genome of *S. lupi* consists of 13,627 predicted protein-coding genes and is approximately 150 Mb in length. Several known anthelmintic gene targets such as for β-Tubulin, glutamate, and GABA receptors as well as known vaccine gene targets such as cysteine protease inhibitor and cytokines were identified in *S. lupi* by comparing orthologs of *C. elegans* anthelmintic gene targets as well as orthologs to known vaccine candidates. New anthelmintic targets were predicted through an inclusion-exclusion strategy and new vaccine targets were predicted through an immunoinformatics approach. New anthelminthic targets include DNA-directed RNA polymerases, chitin synthase, polymerases, and other enzymes. New vaccine targets include cuticle collagens. These gene targets provide a starting platform for new drug identification and vaccine design.

1. Introduction

The parasitic nematode *Spirocerca lupi* causes spirocercosis in domestic dogs. This disease is associated with oesophageal nodular masses which typically lead to neoplastic transformation as well as aortic lesions which occasionally lead to aortic rupture. *S. lupi* is treated with anthelmintics once spirocercosis is diagnosed, which unfortunately in most animals occurs only during the advanced stages of the disease when clinical signs become noticeable [74]. This is problematic as treatment is considered more effective if the diagnosis is made during the early stages of the disease [42].

It has been suggested that *S. lupi* may serve as a model to study the role of nematodes in carcinogenesis [49]. As with other cancer inducing extracellular parasites, such as the Chinese liver fluke (*Clonorchis sinensis*) [82] and the blood fluke *Schistosoma haematobium* [81], genomic data is a valuable resource for understanding the biological processes and divulging new treatment targets in parasites. *Caenorhabditis elegans* has been proposed a model organism in numerous studies. However, the assumption that *C. elegans* would serve as an accurate model for most nematodes was weakened by the genome of *Caenorhabditis briggsae* which showed abundant differences on the genomic level even though the two species are morphologically almost indistinguishable [68,69]. Further genomic sequencing revealed more diversity between the different clades of nematodes (Table 1).

The availability of helminth genome sequences can allow for the discovery of possible new drug and vaccine targets [25,60]. Vaccines provide a promising avenue for helminth treatment, vaccines confer only partial protection, requiring the additional support of anthelmintic treatment. In combination vaccines can become an important component in animal treatment programs especially with continued development of anthelmintic resistance among helminths [11].

Here we present the draft genome of *Spirocerca lupi* from which we identify possible anthelmintic and vaccine targets which can be used as a foundation towards the treatment and prevention of spirocercosis.

2. Materials and methods

2.1. Nematode samples

Nematode samples for DNA extraction were obtained from a dog with spirocercosis that was euthanized at the Pyramid veterinary clinic in Pretoria (Tshwane metropole, Gauteng, South Africa) on 1 December 2017. The dog had an oesophageal nodular mass from which 33 adult *S. lupi* nematodes were removed. The nematodes were stored in 70 % ethanol at 4 °C until DNA extraction could be performed. A second dog died acutely from an aortic aneurysm at Ballito Veterinary Hospital (Ballito, KwaZulu-Natal) on 20 January 2020. Approximately 100 nematodes were removed from an oesophageal nodular mass. The nematode samples were stored in DNA/RNA Shield™ (Zymo Research Corp.) at − 20 °C until RNA extraction could be performed. The NCBI

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Table 1
Comparison between different nematode genomes showing the diversity within and between clades.

Clade	Nematode	Genome size	No. Proteins	GC content	References
I	*Trichnella spiralis*	64 Mb	15 808	27 %	[51]
	Trichuris suis	76–81 Mb	14,470–14,781	43,60 %	[32]
III	*Ascaris suum*	273 Mb	18 542	37,90 %	[31]
	Brugia Malayi	90–95 Mb	11 515	30,50 %	[23]
	Brugia pahangi	85.4 Mb	9 687	28,54 %	[39]
	Loa loa	91.4 Mb	14 907	31 %	[14]
	Dirofilaria immitis	84.2 Mb	10 179	28,30 %	[25]
	Onchocerca volvulus	97 Mb	12,143	29 %	[12]
	Toxocara canis	317 Mb	18 596	40,00 %	[85]
	Spirocerca lupi	150 Mb	13 627	34,67 %	This publication
V	*Haemonchus contortus*	370 Mb	21 799	41,30 %	[38]
	Dictyocaulus viviparus	161 Mb	14 171	34,80 %	[48]
	Caenorhabditis elegans	97 Mb	19 099	36 %	Sequencing consortium, 1998
	Caenorhabditis briggsae	108.4 Mb	22 727	37 %	[68]
	Ancylostoma caninum	347.2 Mb	9 385	43,20 %	[2]

Table 2
Sequencing results obtained from Macrogen Inc. for both the mate-pair and paired-end data. Where Q20 and Q30 depict the percentage of sequences with Phred scores above 20 and 30 respectively.

Data type	Sample	Total read (bp)	Total reads	GC (%)	AT (%)	Q20 (%)	Q30 (%)
Paired end DNA	S_lupi1	11,363,384,356	112,508,756	37.90	62.1	95.13	91.94
Mate pair DNA	S_lupi2	9569,964,120	94,752,120	38.42	61.58	90.62	84.83
Total RNA	S_lupi_RNA_1	4 827 421 452	47 796 252	59.35	40.65	97.88	93.93

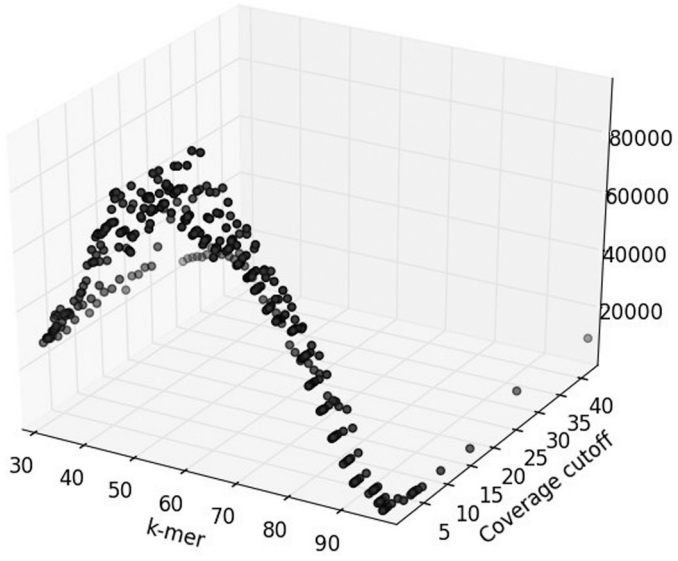

Fig. 1. Range of K-mer values tested with Velvet Optimizer and Velvet.

sample reference is SAMN31399410.

2.2. DNA extraction and quality control

Genomic DNA isolation was performed on a single adult *S. lupi* nematode from the dog euthanized at the Pyramid veterinary clinic in Pretoria (Tshwane metropole, Gauteng, South Africa) using a scaled down version of the method described by Min-Ho Lee and Sudhir Nayak that was originally described for DNA extraction from *C. elegans* [50]. The scaled down method to accommodate a single nematode is described in Rothmann-Meyer et al. [62]. Gel electrophoresis was performed using a 1 % agarose, 1x TAE buffer pH 8.5 (40 mM Tris, 20 mM acetic acid, and 1 mM EDTA) and ethidium bromide (Sigma-Aldrich®) at 0.5 μg/ml of agarose gel. All analyses were performed at 100 V for 30–45 min at constant voltage to ensure adequate separation. Gels were examined under 70 % UV light. NanoDrop ND 1000 Spectrophotometry (Inqaba Biotec) was used to assess the quality of the DNA before shipping to Macrogen Inc. (Seoul, Rep. of Korea). Quant-iT™ PicoGreen™ dsDNA Assay Kit (Invitrogen™) with the method using Victor 3 fluorometry was used by Macrogen Inc. to assess the quality of the DNA before sequencing.

2.3. DNA library preparation and sequencing

The DNA sequencing libraries for *S. lupi* were prepared by Macrogen Inc. (Seoul, Rep. of Korea). The TruSeq® DNA PCR-Free Library Prep kit (Illumina®) was used to prepare a paired-end library and the Nextera® Mate Pair Library preparation kit (Illumina®) was used to prepare a mate pair library for sequencing. The paired-end and mate-pair libraries of *S. lupi* were sequenced by Macrogen Inc. (Seoul, Rep. of Korea) using the Hiseq® 2500 (Illumina®). The sequences generated for the paired-end library were 2 ×100 bp with insert sizes of 350 bases. The sequences generated for the mate-pair library were 2 ×100 bp with insert sizes of approximately 10 kb.

2.4. RNA extraction and quality control

RNA was extracted from a nematode obtained from the dog that died acutely from an aortic aneurysm at Ballito Veterinary Hospital (Ballito, KwaZulu-Natal). All surfaces and homogenization tools were washed with 1 M NaOH followed by absolute ethanol or DEPC-treated water. A drill and grinding stone were used to homogenize 25 mg of nematode tissue in 350 μl Tri-Reagent® (Zymo Research Corp.). The samples were centrifuged, and the supernatant was used for RNA extraction using the Direct-zol RNA Microprep Kit (Zymo Research Corp.) according to the manufacturer's instructions with all samples kept on ice. RNA was eluted in 25 μl of elution buffer supplied with the Direct-zol RNA kit. Extracted RNA was placed on GenTegraRNA (GenTegra® LLC.) and dried using a SpeedVac® for approximately 2 hours. Dried samples were stored at 4 °C before shipping.

Gel electrophoresis was performed using a 1.2 % agarose gel made from 1 x TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA). Ethidium bromide (Sigma-Aldrich®) at 0.5 μg/ml of agarose gel was used to stain the RNA. Electrophoresis was performed at 50 V. The NanoDrop 1000

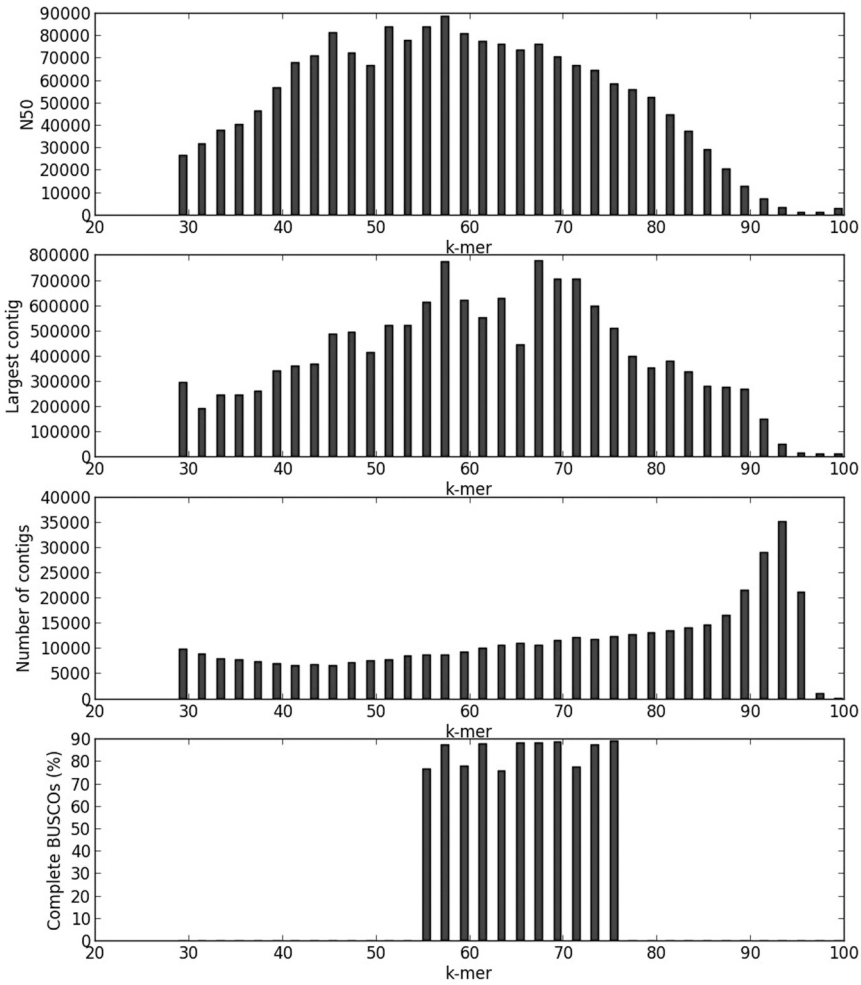

Fig. 2. Results from Velvet optimizer and Velvet with different K-mer values.

Spectrophotometer (Thermo Fisher Scientific) was used to assess the quality of RNA before it was shipped to Macrogen (Europe). Macrogen (Europe) used the 2200 TapeStation to check the quantity and integrity of the RNA.

2.5. RNA library preparation and sequencing

The RNA library was prepared by Macrogen (Europe) using the TruSeq® Stranded Total RNA Library Prep Human/Mouse/Rat kit with Ribo-Zero ribosomal RNA reduction chemistry (Illumina, Inc.). The paired-end library was sequenced using the NGS Illumina platform which utilizes Illumina SBS technology.

Quality control checks were performed on the raw sequence reads with FastQC Version 0.11.9 [3] software that provides a modular set of analyses that were used to interpret the quality of the data. The Trimmomatic Version 0.39 [7] software was used to filter reads that fell below a Phred score of 30 where required.

2.6. Draft genome assembly

Contig sequence assembly was performed with paired-end data using Velvet [83] with Velvet Optimiser [24]. To select the optimal k-mer value a manual script was used to force velvet optimiser to select each k-mer value from 29 to 99 and optimise the cut-off for each k-mer value. Scaffolding was performed with mate-pair data using SSPACE [6]. Assembly completeness was assessed using BUSCO [66] and GenomeQC [45]. The GenBank genome project number is PRJNA892906.

2.7. Protein prediction

Protein coding genes (PCG) were predicted using a combination of ab initio programs such as Augustus [67], Genemark [8] and Snap [37] using the annotation pipeline tool Maker version 2.26 [9] which uses alignments of mRNA, EST's and protein information as evidence to revise the predicted gene structures. Snap was run 6 ways with the data files Ce.hmm and C.elegans.hmm, first double-stranded and then plus and minus separately for each data file. Protein2genome model was also used to improve predictions by using a selection of proteins from various nematode species. Repeat sequences were identified and masked using RepeatMasker version 4.0.9 [71]. Interproscan [61] was used to scan the sequences for matches against the InterPro protein signature databases. The RNA de novo transcripts were assembled using the Trinity platform [26]. The RNA sequences were also mapped to the *S. lupi* genome using HiSat2 [35]. The HiSat2 mappings were used to train Augustus with BRAKER [29]. The trained Augustus model as well as the de novo transcripts from Trinity were included with the Maker 2 platform as RNA sequencing evidence. All gene predictions were imported to the Web Apollo [40] genome annotation and curation tool for manual viewing and editing.

2.8. Comparative analysis

Comparative analysis between *S. lupi* and three other dog parasites, namely *Ancylostoma caninum* (PRJNA72585) (canid hookworm), *Dirofilaria immitis* (PRJEB1797) (dog heartworm), *Toxocara canis* (Roundworm) (PRJNA248777) was performed with Orthovenn2 [78] with an e

Table 3
Final assembly statistics of the draft genome of *Spirocerca lupi* compared to *Toxocara canis*.

	S. lupi	*T. canis*
Status	Draft genome (Scaffold)	Draft genome (Scaffold)
Isolation Source	Domestic dog	Domestic dog
Assembly method	Velvet & SSPACE	SOAPdenovo2 & GapCloser
Sequencing technology	Hiseq 2500 (Illumina)	GA II or HiSeq
Assembled genome size (Mb)	150,351,963	317,115,901
Total scaffold length as percentage of assumed genome size	100.234642	
Scaffold count	5955	22,857
Longest scaffold	1 895 258	1 900 000
Shortest scaffold	500	
Average scaffold	25 248	
% of estimated genome that is useful	93.935356	
Scaffold N50	354 908	375,067
Scaffold N95	14 153	N90: 66,363
L50	130	
NG50	355984	
LG50	129	
DNA GC%	34.67	47.4
DNA at%	56.39	52.6
DNA N%	8.94	5.8
Total coding genes	16 376	18,596
Total BUSCO groups searched	982	982
Complete BUSCOs (C)	874 (89.0 %)	855 (87 %)
Complete and single-copy BUSCOs (S)	865 (88.1 %)	828 (84.3 %)
Complete and duplicated BUSCOs (D)	9 (0.9 %)	27 (2.7 %)
Fragmented BUSCOs (F)	84 (8.6 %)	78 (8.0 %)
Missing BUSCOs (M)	24 (2.4 %)	49 (5.0 %)

value of <1e-10. To identify clusters that excluded orthologs from the dog genome we included *Canis lupus familiaris* (PRJNA615959) in the analysis (WormBase ParaSite version WBPS17, WormBase version WS282, July 2022). KEGG blast was performed with BlastKOALA (Kanehisa *et al.* 2016) and InterPro Scan 5 was used for further protein function classification (Jones *et al.* 2014) of some of the results.

2.9. Anthelmintic targets

Anthelmintic targets were identified through 2 methods that were previously utilized for *D. immitis* [25]. The first method identified orthologs to known targets previously identified in *C. elegans* [27,63]. The second method used an exclusion-inclusion strategy with four strict criteria. Firstly, the gene had to have an ortholog in *C. elegans* that has an RNAi phenotype lethal, embryonic lethal, larval lethal, or adult lethal. Secondly, the gene target had to have an ortholog in the three other parasitic nematodes of dogs namely, *Ancylostoma caninum*, *Dirofilaria immitis*, and *Toxocara canis*. Additionally, the gene had to lack the presence of a paralogue in *S. lupi*. Thirdly, the gene had to have a predicted function as either an enzyme or a receptor according to the chokepoint reaction principles described by Taylor and others [72]. These checkpoints were defined according to the *C. elegans* ortholog data from WormBase. And finally, the gene target had to lack any significant BLAST match (E>10–5) against the predicted proteome of *Canis lupus familiaris*.

2.10. Vaccine targets

Vaccine targets were identified through orthologs of known immune modulators and vaccine candidates. Additionally, an immunoinformatics strategy was followed to identify new vaccine targets using a similar strategy to that used for *Ascaris lumbricoides* [34]. VaxiJen v2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) was used to determine which *S. lupi* proteins are potential antigens. The software uses physicochemical properties to identify potential antigens from protein sequences rather than the sequence alignment approach [20]. The protein subcellular localization was predicted to select for cell membrane proteins using DeepLoc - 2.0 (https://services.healthtech.dtu.dk/service.php?DeepLoc-2.0) [73]. To eliminate potential allergens, AllerTOP v. 2.0 (https://www.ddg-pharmfac.net/AllerTOP/) was used to predict potential non-allergen proteins [16]. NCBI Blastp (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to identify proteins that lacked a significant BLAST match against *Canis lupus familiaris*.

3. Results

3.1. DNA extraction and sequencing

The sequencing results obtained from Macrogen for the paired-end and mate-pair data are shown in Table 2. The paired-end data did not require any pre-assembly editing as a large percentage of the reads were above Phred score 30 and of the same length without any adaptors. These reads were therefore used as is. The mate-pair data required some editing to remove reads with a Phred score below 30.

3.2. Genome assembly

Velvet was used to assemble the *S. lupi* genome with a K-mer of 57 which was chosen as the optimal k-mer using velvet optimiser (Figs. 1 and 2). K-mer 57 displayed the highest N50 as well as the largest contigs with a high complete BUSCO percentage (Fig. 2).

Following the initial contig assembly, SSPACE was utilized to assemble the scaffolds with mate-pair data. Mate pair libraries are much more efficient for genome structure analysis and can increase N50 in scaffolding processes as well as improve contig order [75]. The final draft assembly statistics are shown in Table 3.

3.3. RNA extraction and sequencing

The RNA sequencing results obtained from Macrogen are shown in Table 2. A large percentage of the reads had a Phred quality score above 30 with GC content of 59.35 % and AT content of 40.65 %. RNA sequencing data reduced the final protein count to 13,627 with an average Annotation Edit Distance (AED) of 0.367. With manual annotation and curation, the final gene count was reduced further to 9215 genes.

3.4. Comparative analysis

Comparative analysis revealed that 5381 *S. lupi* genes are shared with *Ancylostoma caninum*, *Dirofilaria immitis*, and *Toxocara canis*. The shared gene clusters are shown in Fig. 3. Full lists of the nematode cluster are available in supplementary tables 7 and 8. Genes unique to *S. lupi* and that did not cluster with any of the three dog nematode parasites are shown in supplementary table 5 with KEGG Blast result and supplementary table 6 with InterPro Scan 5 search results.

4. Discussion

At 150 Mb, the *S. lupi* genome is larger than the *D. immitis* genome but smaller than the *A. caninum* and *T. canis* genomes. Nematode genomes tend to have a wide range of sizes with the median nematode genome size at 122.18 Mb, *S. lupi* genome size is considered average [19]. Of more interest is the genes of *S. lupi* that do not cluster with any of the abovementioned dog nematode parasites. These genes could provide unique insight into the parasitism, migration through the host and cancerous nodular formation that separates *S. lupi* from other canid parasitic nematodes.

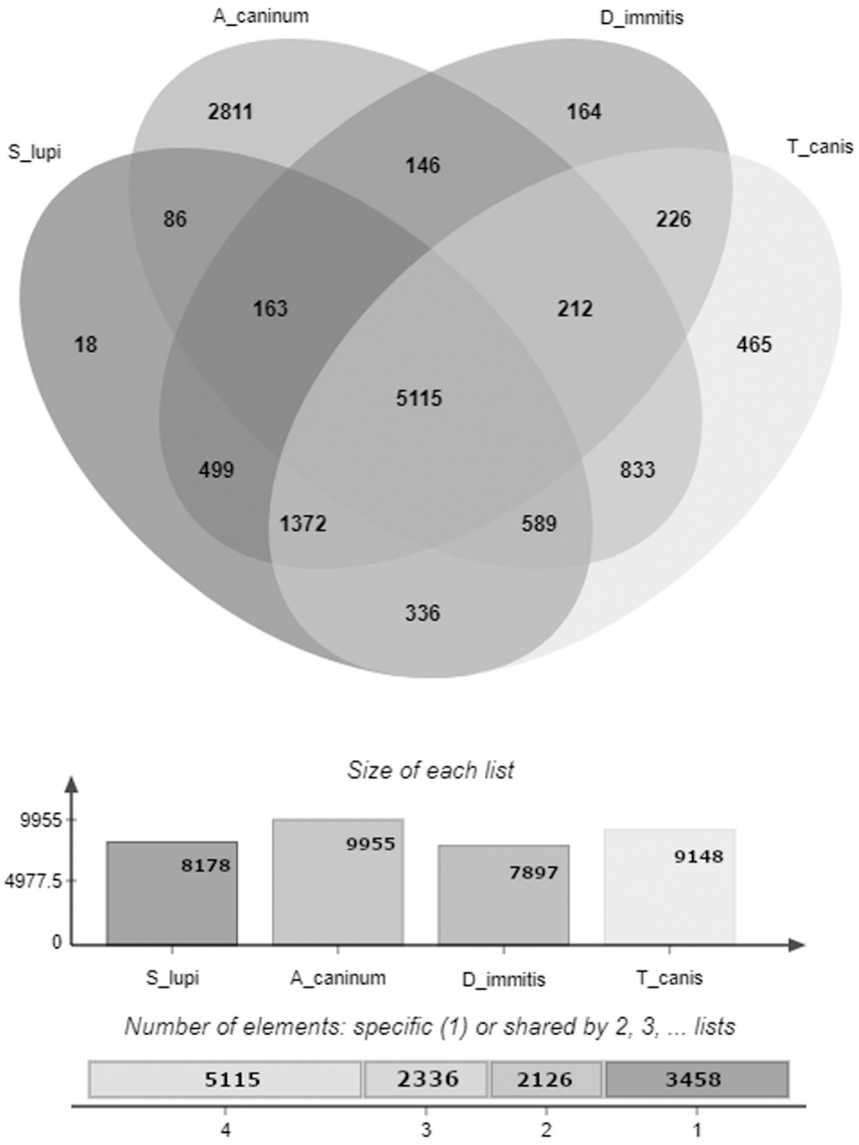

Fig. 3. Venn diagram showing orthologs of the dog nematode parasite gene clusters.

The known anthelmintic targets, identified through the orthology analysis, are only useful when considering anthelmintic resistance (AR) (supplementary table S1). Although AR in intestinal helminths of dogs has not yet been documented in most countries there is evidence to suggest that AR has developed in intestinal nematodes of dogs in some regions of the world [76]. A recent study from Brazil found a dog that was infected with *A. caninum* (hookworms) that had multidrug resistance to pyrantel pamoate with praziquantel and fenbendazole [13]. These gene targets are useful starting points for anthelmintic resistance research although it might be more feasible to look at genome-wide forward-genetic strategies rather than the candidate gene approaches that have been used in the past [18].

The only anthelmintic currently registered for use against *S. lupi* is Advocate® (Bayer Animal Health) which is an imidacloprid / moxidectin formulation [4] but doramectin (a milbemycin) is used off-label as the product is not registered for use in dogs [42]. In a double-blinded, placebo-controlled study of dogs that were infected with naturally occurring oesophageal spirocercosis, Advocate® was found to be less effective for more advanced spirocercosis compared to the antecedent injectable doramectin therapy [64].

Notable new anthelmintic targets (Table 4) obtained through onthology include UDP-galactopyranose mutase (SLUP08465) of which several inhibitors have been identified to potentially combat lymphatic filariasis (caused by a filarial parasite infection) as well as leishmaniasis (caused by a protozoan parasite) [33,56]. The downregulation or inhibition of serine-type endopeptidase activity (SLUP07709) is an important strategy during ivermectin treatment of nematodes to survive antiparasitic treatment [59]. This could potentially be used to combat anthelmintic resistance. Chitin synthase (SLUP04470) is responsible for the synthesis of nematode chitin which is present in the eggshells of nematodes [10]. A study in soybeans showed that host-induced silencing of nematode chitin synthase gene was able to enhance the resistance of the soybeans to both *Heterodera glycines* (soybean cyst nematode) and *Fusarium oxysporum* (an ascomycete fungus) [36]. DNA (apurinic or apyrimidinic site) endonuclease or APN-1 (SLUP06815) is one of two apurinic/apyrimidinic (AP) endonucleases present in *C. elegans* but only APN-1 possesses 3′- to 5′-exonuclease and nucleotide incision repair activities [22]. A study in *C. elegans* showed that UNG-1 (uracil DNA N-glycosylase) and APN-1 are the major enzymes required to repair 5-hydroxymethyluracil DNA damage [57]. Human apurinic/apyrimidinic endonuclease 1 (APE1) was shown to be a valid anticancer drug target [1]. This could be an important target for the cancer-causing *S. lupi*. UDP-glycosyltransferases (SLUP05277) have been implicated in nematode resistance to anthelmintics [17,46]. Cofactor-independent

Table 4
Priority anthelminthic targets through orthology analysis. E values are for BLAST results against Canis lupus familiaris.

S. lupi	E value 1,00E-05	Log10 (E) -5,00	*C. elegans* RNAi	*C. elegans* Molecular function	Gene name
SLUP08465	>1	>1	embryonic lethal, larval lethal, lethal	UGM, UDP-galactopyranose mutase	glf-1
SLUP07709	>1	>1	adult lethal, larval lethal	serine-type endopeptidase activity	bli-5
SLUP04470	5,00E-06	-5,30	lethal, embryonic lethal	Chitin Synthase	chs-2
SLUP06815	>1	>1	embryonic lethal	DNA-(apurinic or apyrimidinic site) endonuclease activity	apn-1
SLUP05277	>1	>1	lethal, larval lethal	glycosyltransferase activity	subs-4
SLUP07344	>1	>1	lethal, embryonic lethal, larval lethal	cofactor-Independent PhosphoGlycerate Mutase homolog	ipgm-1
SLUP05095	>1	>1	lethal	trehalose-phosphatase activity	gob-1

Table 5
Known vaccine and immune modulators.

C. elegans	Gene	S. lupi	Description
WBGene00000534	cpi-2	SLUP04408	Cysteine Protease Inhibitor, cystatin
WBGene00005648, WBGene00005649, WBGene00005647, WBGene00005643	srp-2, srp-6, srp-7, srp-8	SLUP01205, SLUP07777	Serpin domain-containing protein; Serine protease inhibitor
WBGene00006570	tig-2	SLUP04400	TransformIng Growth factor beta, cytokine activity
WBGene00000903	daf-7	SLUP03777	cytokine activity
WBGene00000936	dbl-1	SLUP07310	cytokine activity
WBGene00009108	F25D1.3	SLUP03947	cytokine activity, interleukin 17
WBGene00002915	let-805	SLUP06109	cytokine binding activity and cytokine receptor activity
WBGene00003234	mif-1	SLUP00460	Macrophage migration Inhibitory Factor
WBGene00003235	mif-2	SLUP06253	Macrophage migration Inhibitory Factor
No homolog	SOCS5	SLUP08538	Suppressor of cytokine signaling 5
WBGene00001749, WBGene00001771, WBGene00001773	gst-1, gst-23, gst-25	SLUP01072	Glutathione S-Transferase
WBGene00011474, WBGene00017166	aldo-1, aldo-2	SLUP06279	Fructose-bisphosphate aldolase
WBGene00002250	lap-2	SLUP01670	Leucine Aminopeptidase

phosphoglycerate mutase (SLUP07344) has been proposed as a potential vaccine candidate against visceral leishmaniasis [70]. Additionally, in a recent study, ipglycermide was discovered to be a highly potent macrocyclic chemotype inhibitor of cofactor-independent phosphoglycerate mutase in *C. elegans* (Wiedmann. *In H. contortus* trehalose-6-phosphate phosphatase (SLUP05095) was proposed as a promising vaccine candidate [77]. Perhaps some of these targets could prove useful in the future treatment of spirocercosis. Other targets that were also identified can be found in supplementary table S2. All these targets had at least one ortholog in the three dog nematode parasites namely, *Ancylostoma caninum*, *Dirofilaria immitis*, and *Toxocara canis* which provides room for a potential broad spectrum anthelmintic.

Several known vaccine targets have been identified in *C. elegans*, with orthologs in parasitic nematodes of canids (listed in supplementary table S3). Vaccines are intended to induce immunologic protection in a host against the potential infection from a helminth parasite. This immune stimulation can be achieved through several channels namely vaccination with an attenuated or weakened parasite, the use of native antigens which include excreted/secreted antigens as well as those extracted from the pathogen, and lastly the recombinantly produced antigens [11]. Extracting antigens directly from a parasite can be difficult or even impossible since large quantities of parasites are required. Alternatively, the recombinant or DNA vaccines provide a promising alternative if they can be demonstrated to be safe and not cause any adverse or allergic reactions as happened with the *Ancylostoma* secreted protein (ASP) based vaccine. This vaccine caused a general urticarial reaction (raised, itchy rash) triggered by immunoglobulin E (IgE) against ASP from previous infections [15].

Parasitic nematodes employ several mechanisms to evade host immunity and ensure their survival. This includes host manipulation by releasing molecules into their environment with immunomodulatory properties which are referred to broadly as excretory/secretory (ES) products [21,41]. Two secreted antigens were identified in *B. malayi* namely a cystatin (cysteine protease inhibitor) and a serpin (serine protease inhibitor) [44]. We identified a cysteine protease (SLUP04408) in *S. lupi* as well as two potential serpins (SLUP01205, SLUP07777) (Table 5).

Cytokines are part of ancient metazoan gene families that have been reassigned to the mammalian immune system. Helminths have been found to encode homologues of these cytokines [44]. We identified several potential cytokines (SLUP03777, SLUP07310, SLUP03947, SLUP06109) as well as two macrophage migration inhibition factors (MIF) (SLUP00460, SLUP06253) as were identified in *D. immitis* [25]. In *B. malayi* a protein with a high similarity to the human suppressor of cytokine signalling 5 (SOCS5) was identified [43]. SOCS5 interferes with the IL-4 pathway and Th2 differentiation [65]. A similar protein was found in *D. immitis* [25]. We identified a protein similar to SOCS5 (SLUP08538) of *T. canis* with an ortholog in *A. caninum* (Table 5).

Glutathione S-Transferase, GST, (SLUP01072) has been proposed as a potential vaccine candidate for several parasites [28,30,80]. It is among the 'hidden antigens' and is believed to bind and detoxify free heme in the gut of a parasite thereby detoxifying the heme. A vaccine antigen of GST is intended to block the function of GST by inducing antibodies against it which causes the parasite to be unable to detoxify and thereby be poisoned by excessive heme buildup [84]. Similarly, Fructose-bisphosphate aldolase, which is involved in the glycolysis pathway, has been identified as a potential vaccine candidate [58,79]. We identified an ortholog of *C. elegans* Fructose-bisphosphate aldolase (SLUP06279) in *S. lupi*. Leucine aminopeptidases are metallopeptidases that remove N-terminal residues from proteins and peptides [47]. A chimeric protein of leucine aminopeptidase and cathepsin L1 provided some protection in sheep against *Fasciola hepatica* (common liver fluke) [53]. We identified a leucine aminopeptidase (SLUP01670) with an ortholog in *C. elegans* and three canid parasites.

The new vaccine candidates identified through the immunoinformatics approach are listed in the supplementary table S4. A total of 67 potential vaccine targets were identified. These targets were predicted to be antigens, located on the cell membrane, and non-allergenic. Additionally, the genes had no significant BLAST result when compared to *Canis lupis familiaris* (>1 where no significant similarity was found). Table 6 identifies the top priority targets with a VaxiJen score above 0.9. Some of the targets are potentially cuticle collagen genes (SLUP00269,

Table 6
Potential New vaccine targets.

Protein	VaxiJen v2.0	Deeploc 2.0 Predicted localizations	Deeploc 2.0 Predicted signals	AllerTOP v. 2.0	NCBI Blastp	BLASTp Description
					Canis E-value	
SLUP00850	1.0492	Cell membrane	Signal peptide, Transmembrane domain	NON-ALLERGEN	1,00E-04	Unnamed protein
SLUP00269	1.0072	Cell membrane	Signal peptide, Transmembrane domain	NON-ALLERGEN	>1	Nematode cuticle collagen domain-containing protein
SLUP01393	0.9476	Cell membrane	None	NON-ALLERGEN	>1	Unnamed protein
SLUP02059	0.9469	Cell membrane	Signal peptide, Transmembrane domain	NON-ALLERGEN	>1	Cuticle collagen
SLUP05945	0.9199	Cell membrane, Lysosome/ Vacuole	Signal peptide, Transmembrane domain	NON-ALLERGEN	>1	Hypothetical protein
SLUP05414	0.9125	Cell membrane, Lysosome/ Vacuole	Signal peptide, Transmembrane domain	NON-ALLERGEN	>1	Unnamed protein
SLUP06322	0.9095	Cell membrane	None	NON-ALLERGEN	>1	Hypothetical protein

SLUP02059) as identified through BLAST searches. The cuticle forms part of the external structure of the nematode and is constructed primarily of collagen [54]. Cuticle collagen was previously proposed as a potential drug or vaccine candidate and RNAi studies in the root-knot nematode, *Meloidogyne incognita*, have shown some success with approximately 30 – 38 % (depending on the cuticle collagen gene used) reduction in the number of adult females in tomato plants [5,54]. Another study identified cuticle collagen 14 as a promising candidate for human gnathostomiasis caused by the parasitic nematode *Gnathostoma spinigerum* [52]. These genes may justify further investigation.

5. Conclusion

Whole genome sequencing can be used to advance the development of better diagnostic tests as well as inform vaccine and drug targets to treat spirocercosis. Furthermore, whole genome sequencing would promote advances in understanding several areas of the parasite's biology such as the molecular machinery involved in the life cycle of the parasite, migration through the host, reproduction, immunity and disease including the identity of the cancer-causing agents [55]. The genome therefore provides a useful tool for future molecular studies regarding this parasitic roundworm.

CRediT authorship contribution statement

Pamela Jean de Waal: Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Wiekolize Rothmann-Meyer:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Kershney Naidoo:** Software, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Pamela de Waal reports financial support was provided by the University of Pretoria Faculty of Natural and Agricultural Sciences. Kershney Naidoo reports financial support was provided by University of Pretoria Faculty of Natural and Agricultural Sciences. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.molbiopara.2024.111632.

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