



Glycans of parasitic nematodes – from glycomes to novel diagnostic tools and vaccines

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ABSTRACT

Nematodes, commonly known as roundworms, are among the most prevalent and diverse multicellular organisms on Earth, belonging to the large phylum *Nematoda*. In addition to free-living species, many nematodes are parasitic, infecting plants, animals, and humans. Nematodes possess a wide array of genes responsible for carbohydrate metabolism and glycosylation. The glycosylation processes in parasitic nematodes often result in unique glycan modifications that are not present in their hosts. These distinct glycans can be highly immunogenic to mammalian hosts and play significant immunoregulatory roles during infection. This mini-review article summarises the glycosylation capabilities and characteristics of parasitic nematodes based on glycomic data. It also highlights recent research advances that explore the biological significance of nematode glycans and their potential for diagnostic and vaccine applications.

1. Introduction

Nematodes are multicellular, non-segmented worms that belong to a large phylum *Nematoda*. To date, nearly 26,000 nematode species have been scientifically described, which inhabit diverse environments around the globe. The anatomy of a nematode is relatively simple, consisting of longitudinally oriented muscle cells, a complete digestive track from anterior to posterior, a complex neural network with different types of sensors and neurons, as well as a reproductive system. All the internal organs are firmly packed within a rigid collagenous cuticle that grows and is replaced a few times during worm development via moulting [1]. Nematodes are morphologically and biologically divergent, meaning that they may largely differ in size (from microscopic to meters long), local body shape (e.g., the tail morphology of male worms) and living environment. In addition to a large number of free-living species, which feed on microorganisms such as bacteria, many nematodes are parasites that infect other organisms and use them as hosts to complete their parasitic life cycles and also to propagate. Such hosts include plants, insects, fish, amphibians, reptiles, mammals as well as humans.

In medical and veterinary parasitology, the word ‘helminth’ is frequently used, which refers to parasitic worms in four phyla: *Nematoda*

(roundworms), *Platyhelminthes* (flatworms), *Acanthocephala* (spiny-headed worms), and *Nematophora* (hairworms) [2]. Parasitic nematodes are prominent members of the helminth group, which also include of cestode and trematode parasites (phylum: *Platyhelminthes*), such as tape worms (*Eucestoda*) and blood flukes (*Schistosoma*). In this review, I focus on the parasitic nematodes that take animals and humans as host (Table 1). Human nematode infections are caused primarily by intestinal nematodes including *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms (*Ancylostoma duodenale* and *Necator americanus*), as well as by filarial nematodes, including *Wuchereria bancrofti*, *Brugia malayi* and *Onchocerca volvulus*. These infections are particularly prevalent in regions with poor sanitation and limited access to healthcare, where they constantly pose public health concerns, as reflected in significant losses of disability-adjusted life years (DALYs) [3]. Parasitic nematodes are also widespread in domestic animals, causing various diseases such as ascariasis in pigs (*Ascaris suum*), haemonchosis in sheep and goats (*Haemonchus contortus*), and ostertagiosis in cattle (*Ostertagia ostertagi*). In the livestock and poultry sectors, nematode infections undermine animal health, leading to reduced growth rates, lower milk and meat production, and, in severe cases, animal mortality. Understanding the biology of parasitic nematodes is key to developing effective strategies for controlling their impact on human and animal health.

Abbreviations: α -Gal, Gal α 1,3Gal β 1,4GlcNAc-R; CRP, C-reactive protein; LDN or LacdiNAc, GalNAc β 1,4GlcNAc-R; LDNF or fucosylated LDN, GalNAc β 1,4(Fuc α 1,3)GlcNAc-R; Lewis x, Gal β 1,4(Fuc α 1,3)GlcNAc-R; LN or LacNAc, Gal β 1,4GlcNAc-R; PC, phosphorylcholine; PE, phosphoethanolamine.

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Table 1
A list of parasitic nematodes (alphabetical order) reviewed in this article. Intermediate and definitive hosts of relevant parasites are listed according to three textbooks: Parasitology in Veterinary Medicine (ISBN 978-90-8686-274-0), Veterinary Parasitology (ISBN 978-0-470-67162-7, the 4th edition) and Parasitic Diseases (ISBN 978-0-578-41562-8, the 7th edition). Key studies on nematode glycans are listed under reference (Ref.); N/A, not applicable.

Nematodes	Intermediate Hosts	Definitive Hosts	Ref.
<i>Angiostrongylus cantonensis</i>	Gastropods (snails and slugs)	Rodents, humans	[4]
<i>Acanthocheilonema viteae</i>	Soft tick <i>Ornithodoros tartakovskyi</i>	Rodents	[5–7]
<i>Ascaris suum</i>	N/A	Porcine	[8,9]
<i>Brugia malayi</i>	Mosquitoes	Humans, monkeys, domestic cats, forest carnivores	[10]
<i>Dictyocaulus viviparus</i>	N/A	Cattle, buffalo, deer and camels	[11]
<i>Dirofilaria immitis</i>	Mosquitoes (<i>Aedes</i> , <i>Anopheles</i> , <i>Culex</i>)	Canines, occasionally cat, rarely humans and primates	[12]
<i>Haemonchus contortus</i>	N/A	Sheep, goats, deer	[13–15]
<i>Heligmosomoides polygyrus</i>	N/A	Rodents	[16,17]
<i>Oesophagostomum dentatum</i>	N/A	Porcine	[8,18,19]
<i>Onchocerca ochengi</i>	Blackfly (<i>Simulium</i>)	Cattle	[20]
<i>Onchocerca volvulus</i>	Blackfly (<i>Simulium</i>)	Humans	[5,21]
<i>Ostertagia ostertagi</i>	N/A	Cattle	[22,23]
<i>Parelaphostrongylus tenuis</i>	Gastropods (snails and slugs)	White-tailed deer	[24]
<i>Strongyloides stercoralis</i>	N/A	Dogs, foxes, cats, humans	[25]
<i>Toxocara canis</i>	N/A	Canines, humans	[26,27]
<i>Trichinella spiralis</i>	N/A	Porcine, rats, horses, various mammals, humans	[28,29]
<i>Trichostrongylus colubriformis</i>	N/A	Sheep, goats, cattle, camels, occasionally pigs and humans	[30,31]
<i>Trichuris suis</i>	N/A	Porcine	[32,33]

The glycobiology of parasitic nematodes stands out as a research topic of growing interest, because glycan modifications play crucial roles in their survival and host interactions. It was initially revealed in the 1970s that parasitic nematodes are covered by carbohydrate-rich glycocalyx on luminal surfaces [34,35]. Later investigations demonstrated that glycan modifications also occur on cuticle surface and involve worm-derived antigens [36,37]. Several monoclonal antibodies developed to target native antigens isolated from these parasites exhibited specificity for their carbohydrate-epitopes [16,38,39]. Nematodes are eukaryotic organisms, possessing essential cell organelles such as endoplasmic reticulum (ER) and Golgi apparatus in the cells, in which glycan biosynthesis and glycosylation take place in the presence of glycosyl donor substrates, glycoenzymes and cofactors [40]. Various types of glycans have been described in nematodes, including *N*-glycans, mucin-type *O*-glycans, glycosphingolipids (GSL), glycosaminoglycan (GAG) and chondroitin proteoglycans (CPGs) [41]. In contrast to the anatomical simplicity of nematodes, nematode glycans are rather structurally diverse, presenting many unusual motifs and glycosidic linkages as well as exotic sugar modifications that are absent in mammals [42]. This diversity has been elucidated in a series of studies using gas chromatography (GC), mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR) over the past decades [37,43,44].

This review article recaps important studies on parasitic nematodes' glycans and include recent publications, which not only revealed in greater detail the structural diversity of nematode glycans but also shed light on the biological function of glycans and glycan modifications in the context of parasite-host interactions. Furthermore, it summarises advances in diagnostic tools developed to target carbohydrate-epitopes of native nematode antigens and the promising aspect of developing efficacious vaccines using modern glycoengineering technology.

2. Structural features of nematode glycans

2.1. The informative glycomes

Glycomic studies using advanced analytical techniques, such as MS, have provided increasing structural details of glycan molecules in the past few decades [45]. On one hand, a glycome provides essential information about the structures, epitopes, or motifs present on the organism's glycoconjugates as well as their relative abundances in the glycome, which may imply their roles in a biological context. On the other hand, a complete glycome helps glycobiologists to deduce the underlying glycan biosynthetic pathways and it also indicates the activities of known and putative enzymes, *i.e.*, glycosyltransferases as well

as glycosidases, which are essential for building the structures in the organism. For instance, one can certainly predict that ER and Golgi mannosidase activities are involved in initial steps of *N*-glycan trimming in nematodes, as judged by the observations of abundant oligo- and pauci-mannose type *N*-glycans in almost all *N*-glycomes of different nematode species examined to date [41]. This is in keeping with the general conservation of ER-Golgi glycosylation pathways in *N*-glycan biosynthesis among animals. Moreover, the presence of unusual glycan motifs in nematode glycomes strongly suggests the activity of novel glycoenzymes, for which model species can help identify the corresponding genes.

The free-living nematode *Caenorhabditis elegans* yet serves as a powerful model to explore glycosylation potential and underlying enzymatic basis of nematodes. *C. elegans* was the first animal to have its genome fully sequenced. After its genome was published in 1998 [46], a repertoire of *C. elegans* genes, homologous to the genes involved in mammalian glycosylation, was identified and their biological functions were assessed using RNAi in a few studies [47–49]. Numerous mutant strains with glycogene deficiencies are already available in stock centres, and with the advent of CRISPR-Cas9 technology, custom strains can now be easily generated in-house or through affordable commercial services. As an important resource *C. elegans* mutants have been employed in comparative glycomic studies, led to direct evidence about the *in vivo* role of a gene in glycan biosynthesis and helped to reveal biological impacts of corresponding glycopeptides [50]. Glycomic studies of wild type and mutant *C. elegans* paved the road to understanding glycosylation nematodes including the parasitic species [50, 51].

2.2. Clade-and species-specific features

Nematodes were classified into five clades by Blaxter *et al.*, based on molecular phylogenetic analysis of 18S ribosomal RNA gene sequences [52]. These clades represent major evolutionary lineages within the phylum Nematoda and reflect relationships rather than traditional taxonomy relying on morphological similarity. While subsequent studies have refined this classification [53], its broad five-clade structure is well acknowledged in modern nematology. The clades are labelled **I**, **II**, **III**, **IV**, and **V** and include various parasitic and free-living nematodes (Fig. 1).

Nematodes exhibit distinct *N*-glycosylation patterns compared to mammals. Their *N*-glycomes are typically rich in oligo- and pauci-mannosidic glycans, where the latter are often associated with core fucosylation. Sialic acids, prevalent in mammalian glycans, are however

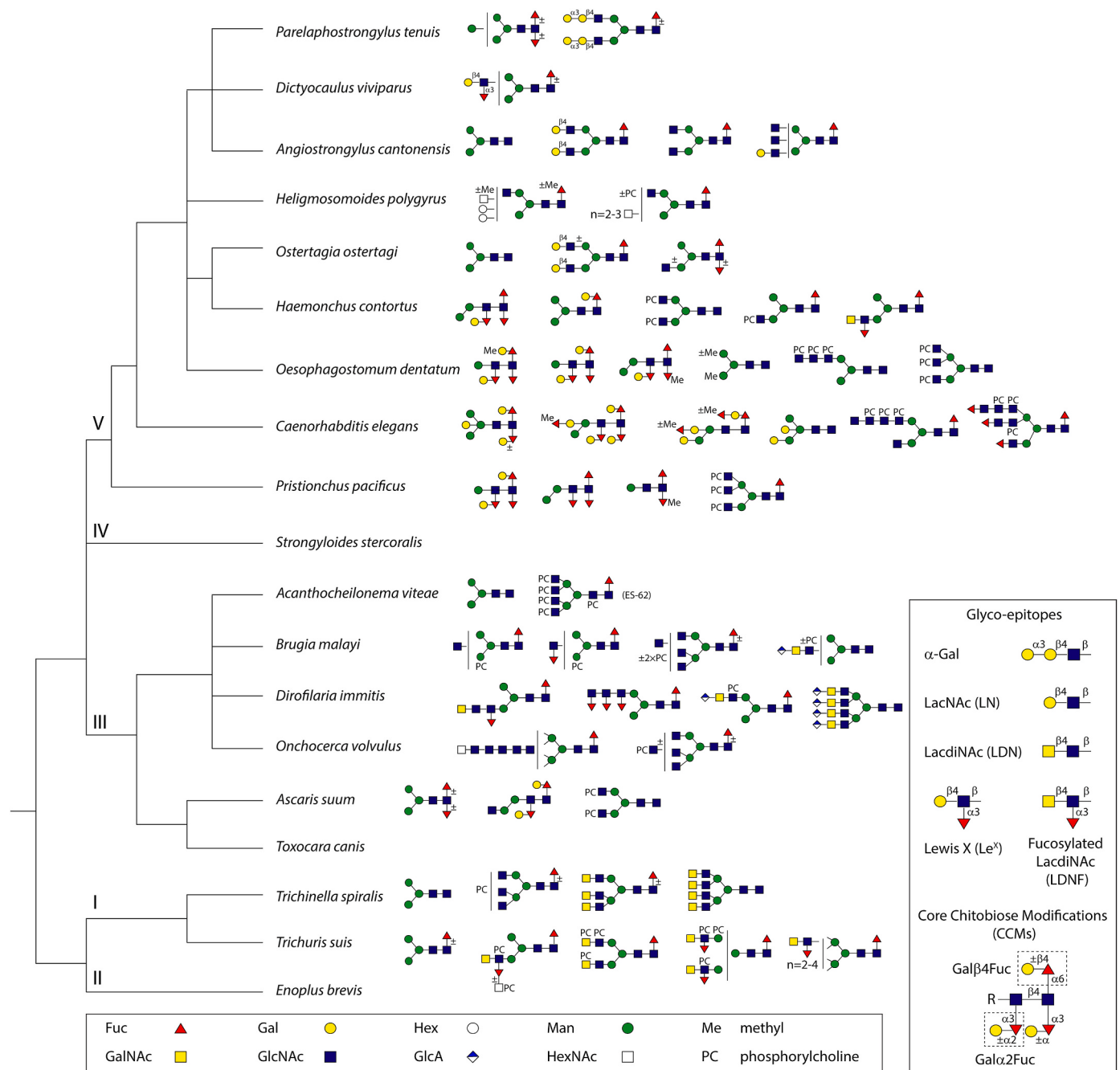


Fig. 1. A summary of parasitic and free-living nematode *N*-glycans. A phylogenetic tree of 19 nematode species, grouped into five Clades (I to V) according to the Blaxter et al. [52], was prepared using the online tool iTOL [54]. Representative *N*-glycans of individual species were selected from relevant glycomic studies (see Table 1) and presented using the format of Symbol Nomenclature for Glycans (SNFG) [55,56]. A legend and selected glyco-epitopes are provided in the boxes.

absent in all nematode glycomes examined to date, possibly due to the lack the necessary sialyltransferases and other components required for sialic acid biosynthesis and incorporation. Similarly, the bisecting *N*-acetylglucosamine (GlcNAc) motif, a feature of mammalian *N*-glycans, is absent because nematodes lack the corresponding GnTIII homolog. Despite these major differences, nematodes can produce multi-antennary complex-type *N*-glycans with features specific to species and their Clades. For instance, structures with up to ten HexNAc residues (Hex₃HexNAc₄₋₁₀Fuc₀₋₁) were found on *N*-glycans of the intracellular parasitic worm *Trichinella spiralis* (Clade I) [28,29]. GC-MS analysis of such glycans, released from *T. spiralis* L1-stage larvae, not only suggested a multi-antennary branching pattern but also discovered non-reducing *N*-acetylgalactosamine (GalNAc) termini, indicative of the LDN (or

LacdiNAc, GalNAc β 1,4GlcNAc-R) disaccharide motif; in addition, PC-modification was found to associate with such glycans [28]. Recent glycomic studies of another Clade I nematode, the porcine parasite *Trichuris suis*, provided more insights into the complex glycans in this Clade. A variety of tri-/tetra-antennary structures carrying PC-modified LDN and LDNF (GalNAc β 1,4(Fuc α 1,3)GlcNAc-R) termini were detected in late eluted HPLC fractions; assayed on microarray, such high molecular weight glycans were recognised by infection-induced IgG and IgM antibodies and were ligands of C-reactive protein (CRP) [32,33].

Complex-type *N*-glycans were also discovered in filarial nematodes (Clade III) approximately 25 years ago, which carry chito-oligomer elongations and PC-substitutions [5]. Recently studies on three filarial parasites (*Dirofilaria immitis*, *B. malayi* and *O. volvulus*) provided more

structural details of such glycans [10,12,21]. Chito-oligomer bearing *N*-glycans of *D. immitis* are not only decorated with PC but also with α 1,3-linked fucoses; removing PC and fucose from such structures led to altered antibody binding specificities, implying their roles in interacting with the host immune system [12]. Notably, chito-oligomer antennae with PC-substitutions were reported on bi-/tri-antennary *N*-glycans of Clade V worms, such as *Oesophagostomum dentatum* and the wild type *C. elegans* [18,57], indicative of a common biosynthetic pathway of such antennae. Interestingly, an anionic sugar glucuronic acid (GlcA) was recently discovered on *N*-glycans and GSLs as a novel non-reducing end in filarial nematodes, a feature not yet observed in other nematode species. Using advanced microarray technology, natural glycans were printed on glass slides and probed with plasma samples from patients diagnosed with different filarial worm infections; all the data demonstrated that filarial nematodes carry immunogenic glycans and glycan motifs, which can induce glycan-specific antibodies in the host during infection [10,12,21].

Core chitobiose modifications (CCMs) of *N*-glycans with multiple fucoses were initially reported in *Haemonchus contortus* (Clade V), a common blood-sucking parasite living in the abomasum of small ruminants [13,14]. The two core GlcNAc residues of *N*-glycans are modified with up to three fucoses, of which one is linked to the OH-3 position of the distal GlcNAc residue and another two are attached to the OH-3 and OH-6 positions of the innermost GlcNAc linked to the protein backbone. Two fucoses of the CCMs can be further galactosylated, forming two distinct Gal-Fuc disaccharide epitopes: Gal β 1,4Fuc-R and Gal α 1,2Fuc-R [15,58]. The former is attached via an α 1,6-linkage to the innermost GlcNAc and it is a ligand of a nematotoxic fungal lectin CCL2 [8], whereas the latter is α 1,3-linked to the distal GlcNAc residue. CCMs seem to be a feature conserved in (at least some of) the Clade V worms, as such modifications are also identified in *C. elegans* [59,60], *Pristionchus pacificus* [61] and *O. dentatum* [18]. *N*-glycans of some species in the Clade III, such as *Ascaris suum* [8] and *Anisakis simplex* (unpublished data), are also modified in this way, but *N*-glycans of filarial worms lack such motifs. Studies using *C. elegans* knockout mutants further elucidated that three core fucosyltransferases (FUT-1, FUT-6 and FUT-8) are responsible for the linkage-specific addition of these fucose residues [62,63].

Non-reducing galactose termini in various linkages are also commonly seen on *N*-glycans of Clade V worms, suggesting α / β -galactosyltransferase activities. In *Parelaphostrongylus tenuis*, also known as meningeal worm as it invades the central nervous system of white-tailed deer, Gal α 1,3Gal β 1,4GlcNAc-R (the α -Gal epitope) has been detected on *N*-glycoproteins and in different tissues of the parasite [24]. β -linked galactose has been found on complex-type *N*-glycans of *Dicτυocaulus viviparus*, *Ostertagia ostertagi*, *Angiostrongylus cantonensis*; the latter two species carry *N*-acetylglucosamine termini (also known as LacNAc or LN epitope, Gal β 1,4GlcNAc-R) [4,22], and its fucosylated form, Gal β 1,4(Fuc α 1,3)GlcNAc-R (known as Lewis x epitope), was found in *D. viviparus* [11]. It is worth mentioning that LN and Lewis x epitopes are commonly found on mammalian glycoconjugates, and their occurrence on helminth glycans, including those of *Schistosoma*, is thought to result from co-evolution with host immune systems [64]. Other motifs carrying α / β -linked galactose have been detected in the wild type and knockout *C. elegans*, such as α 1,2/3/4-linked Gal on core mannose or fucose residues, β 1,4-linked Gal on the bisecting and intersecting positions of oligo-/pauci-mannosidic *N*-glycans [59,65,66], yet to be discovered in other nematode species.

2.3. PC-substitution of glycans

Phosphorylcholine (PC) is a zwitterionic immunogenic determinant found on various helminth and protozoan parasites [67]. Nematode-derived PC-components can be detected using anti-PC antibodies, for instance, the TEPC15 IgA monoclonal antibody that recognises PC-substituted glycoproteins and glycolipids [9,68].

Immunomodulatory properties of PC-bearing molecules have been documented in many publications, particularly by studying the ES-62 glycoprotein secreted by the filarial parasite *Acanthocheilonema viteae* [6]. *In vitro* studies showed that ES-62 downregulates immune responses by inhibiting lymphocyte proliferation, while *in vivo* murine disease models demonstrated its pharmaceutical potential, as ES-62 can efficiently induce anti-inflammatory actions by regulating cytokine secretion [69]. While ES-62 is bound by the acute-phase protein CRP present in sera, complement activation is aborted, which is considered an immune evasion strategy that helps the parasite to survive within the host [70].

Recent glycomic studies provided more structural details of PC-modified *N*-glycans, *O*-glycans and glycolipids, implying that PC-substitution of glycans is common across all examined nematode species. As summarised in Fig. 1, PC in free-living nematodes, such as *C. elegans* and *P. pacificus*, is solely associated with the GlcNAc residues of different classes of *N*-glycans [57,60,61]. Despite the presence of GalNAc residues in wildtype and mutant worms, often attached to the non-reducing termini of glycans, GalNAc is not substituted with PC in these species. This modification pattern is consistent with *C. elegans* *O*-glycans and glycolipids [43,68]. However, such stringent limitation does not seem to exist in parasitic worms, as in addition to GlcNAc, PC-substitutions of both GalNAc and Man are found on complex *N*-glycans of the porcine whipworm *T. suis* [32,33]. Structural analysis of HPLC-purified glycans with MALDI-TOF-MS/MS, in combination with hydrofluoric acid (HF) and glycosidase treatments, revealed novel motifs in the *T. suis* *N*-glycome, including PC-substituted (up to two PC) LDN and LDNF antennae as well as PC-modified HexNAc linked to the antennal fucose [33]. Notably, PC-modified LDN and LDNF epitopes were also found on *N*-glycans of the canine heartworm *Dirofilaria immitis*; removal of PC moieties as well as α 1,3-fucoses from the *D. immitis* glycan antennae led to a significant reduction of glycan recognition to CRP and the complement C1q [12]. Furthermore, PC was also found to modify the trimannosyl core of *N*-glycans as well as antennae in the human filarial nematode *Brugia malayi*, as judged by the presence of glycan compositions Hex₂₋₃HexNAc₂Fuc₀₋₁PC₁ detected after glycosidase digestion [10]. The aforementioned ES-62 secreted by *A. viteae* is heavily PC-substituted on its *N*-glycans [71]; recent glycoproteomic data indicated that a tetra-antennary *N*-glycan can accommodate up to five PC substituents on its GlcNAc residues, including one on the distal GlcNAc of the core [7].

PC substituents are also present on nematode *O*-glycans and glycosphingolipids (GSLs) (Fig. 2). For instance, mucin-type *O*-glycans carrying PC on GlcNAc residues were recently reported to appear at different developmental stages of *C. elegans* [60]. In another study, PC was reported to decorate unusual glycosaminoglycan-like *O*-glycans released from the porcine parasite *O. dentatum* by hydrazinolysis [19]. MS/MS experiments combined with chemical and enzymatic digests provided solid evidence that the terminal HexNAc and core galactose of such glycans are modified with PC. Interestingly, despite similar backbone structures no PC-modification was detected on glycosaminoglycan-like *O*-glycans of *C. elegans* released with the same method.

Early studies on glycolipids of the porcine parasite *Ascaris suum* and the free living *C. elegans* elucidated PC-modified GlcNAc residue of the arthro-series glycosphingolipids, forming a conserved [PC-6]GlcNAc β 1,3Man β 1,4Glc-ceramide structure [68,72]. Elongated structures carrying β 1,4-linked GalNAc and Gal residues are termed as Nz1, Nz2 (component A) and Nz3 [68], some of which were also reported in the human filarial worm *O. volvulus* [44]. In case of *A. suum*, the OH-6 position of β 1,4-mannose can also be substituted with phosphoethanolamine (PE), forming the Gal α 1,3GalNAc β 1,4[PC-6]GlcNAc β 1,3[PE-6]Man β 1,4Glc-ceramide structure (component C) [72]. Interestingly, GSLs of *O. volvulus* and *B. malayi* also carry chito-oligomers with multiple PC-substitutions, and in some *O. volvulus* GSL structures PC-modification is associated with GalNAc residues [10,21].

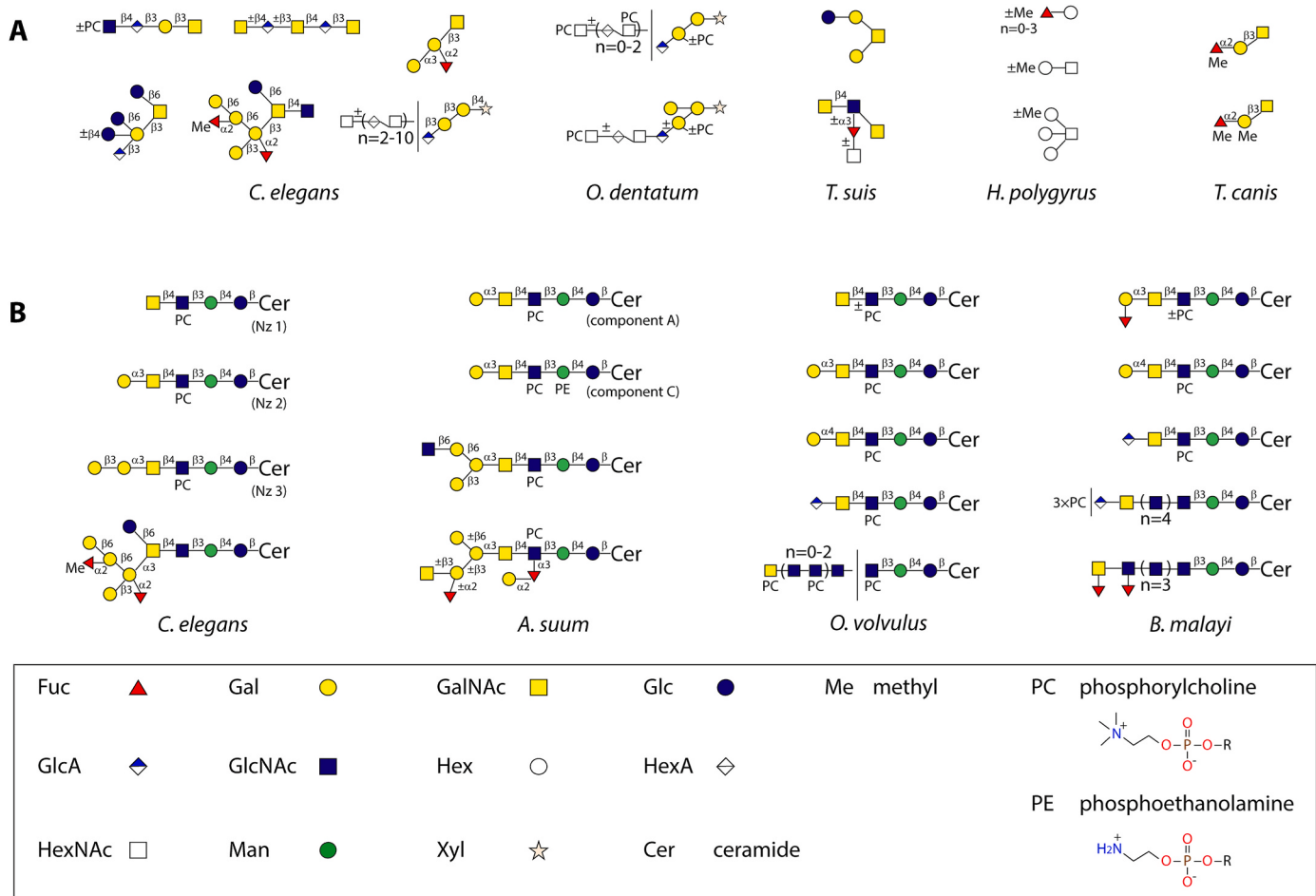


Fig. 2. A summary of nematode *O*-glycans (A) and glycosphingolipids (B). A legend of abbreviations and glycan symbols is provided in the box.

2.4. Methylation of glycans

O-methylation of glycans is a common feature observed in lower organisms, such as bacteria, fungi and green algae as well as some non-vascular plant species [73,74]. In the animal kingdom, methylated glycans are found in nematodes [50], molluscs [75] and starfish species (*Echinodermata*) [76,77]. In case of nematodes, it has been demonstrated in the wild type *C. elegans* glycans carrying methylated fucoses and mannoses are ligands of a fungal lectin, *Laccaria bicolor* Tectonin 2 (Lb-Tec2), which strongly inhibits worm development; therefore, Lb-Tec2 is considered to play an important role in the fungal innate defense against fungiphorous nematodes [78]. Methylated glycans of parasitic nematodes are immunogenic to mammals, which induce glycan-specific antibodies [16,26,38]. Major *O*-glycans released from excretory-secretory (E/S) glycoproteins of *Toxocara canis* are two mucin-type trisaccharides, [Me-2]Fuc α 1,2[Me-4]Gal β 1,3GalNAc-R and [Me-2]Fuc α 1,2Gal β 1,3GalNAc-R, carrying mono-methylated fucose and galactose residues (Fig. 2A) [27]. Synthetic glycans with identical methylation patterns can be bound by monoclonal antibodies raised again *T. canis* E/S products (termed TES); in addition, they are recognised by antisera from *Toxocara*-infected human patients, but such cross-reactivity was not detected in patients infected by other parasitic worms [26]. Methylated glycans were also observed in *Heligmosomoides polygyrus*, a mouse intestinal nematode used as a model to study parasite-induced immunoregulations. Based on LC-MS/MS data, some *N*-glycans of *H. polygyrus* carry methylated core α 1,6-linked fucose, and some *O*-glycan structures are modified with up to three methyl-groups; the anti-Glycan A monoclonal antibody raised against the *H. polygyrus*

E/S products (termed HES) specifically targets methylated *O*-glycans [17].

As judged by glycomic data, there is a tendency that natural methylation occurs preferentially on terminal fucose (6-deoxy-L-galactose) and hexose residues (*i.e.*, galactose and mannose) of nematode glycans. Such methylated glycan motifs widely present on nematode *N*-glycans [18,60,61], *O*-glycans [17,27,43] and glycolipids [68], suggesting that the putative nematode methyltransferase(s) may have a limited enzymatic activity towards a few monosaccharides; none of these enzymes have been identified yet in nematodes.

3. Applications in human and veterinary medicine

3.1. Diagnostic tools

Using anti-parasite antibodies as probes, numerous immunogenic components have been identified from somatic and E/S products of parasitic nematodes. Worm-derived antigens carrying unique carbohydrate epitopes are considered novel biomarkers to aid diagnosis of parasite infections (Table 2). For instance, rapid diagnostic tests developed to monitor bancroftian filariasis in endemic regions use monoclonal antibodies (AD12.1 or Og4C3) that recognise the same carbohydrate epitope of a circulating filarial antigen (Wb-CFA), shed by the adult *Wuchereria bancrofti* parasite. Wb-CFA is a high molecular weight glycoprotein detectable in the capillary blood of infected patients. Cross-reactivity of AD12.1 to proteins of other filarial worms, such as *Brugia* spp., *O. volvulus* and *D. immitis*, indicated a shared antibody epitope [79]. Interestingly, glycan microarray studies revealed

Table 2

A few examples of nematode antigens carrying glycan modifications and their potential applications in human and veterinary medicine. Abbreviations: T, therapeutic agent; V, vaccine antigen; D, diagnostic marker.

Parasites	Antigens	Glycan features	Applications	Ref.
<i>A. viteae</i>	ES-62	PC-glycans	T	[7,71]
<i>H. contortus</i>	H11	core tri-fucosylation; Gal-Fuc	V	[13]
	H-gal-GP	LDNF epitope	V	[81]
<i>O. ochengi</i>	OoGST1	oligo-mannose; core α 1,3-fucose	V	[20]
<i>O. volvulus</i>	OvGST1	oligo-mannose	V	[82]
<i>O. ostertagi</i>	ASP-1	core α 1,3-fucose; LN epitope	V	[22]
<i>T. colubriformis</i>	CarLA	non-typical N- or O-glycans	D	[30]
<i>W. bancrofti</i>	Wb-CFA	terminal β -linked glucuronic acid	D	[80]

that AD12.1 and Og4C3 monoclonal antibodies recognise glycans carrying a terminal β -linked glucuronic acid (GlcA), albeit with varying preferences for the underlying backbone structures. These observations suggested that GlcA is a conserved immunogenic motif among filarial nematodes [80], consistent with recent glycomic data of relevant parasitic worms aforementioned [10,12,21].

Another important carbohydrate larval antigen (CarLA) was identified in the sheep parasite *Trichostrongylus colubriformis* [30]. CarLA is strongly bound by mucosal IgG and IgA antibodies, which are considered to participate the fast rejection of L3 larvae during natural infections. Although the structural details of native CarLA antigen remain unsolved, CarLA carries epitopes shared by infective L3 larvae of other strongylid nematodes. For this property, CARLA® saliva test was developed to monitor sheep and goats' immunity against a panel of intestinal parasitic nematodes, including *H. contortus* and *O. ostertagi* discussed elsewhere in the review article [83]. Additionally, the availability of anti-CarLA monoclonal antibody PAB-1 and single-chain variable fragments (scFv) will permit purification and further structural characterisation of the native CarLA [31], enabling more specific antigen targeting and broadening the application of CarLA in vaccine development.

3.2. Precision glycoengineering of recombinant subunit vaccines

Parasite antigens capable of inducing a protective immunity in the host are considered suitable candidates for vaccine development. Over the past three decades, a repertoire of worm antigens has been isolated and characterised, many of which carry glycan modifications (Table 2). For instance, the native H11 and H-gal-GP antigens, major components of the Barbevax® vaccine, are isolated from adult *H. contortus* using Con A and peanut lectins, which carry tri-fucosylated and LDNF-bearing N-glycans [13,81]. The native ASP-1 antigen (activation-associated secreted protein 1), identified in the E/S products of adult *Ostertagia ostertagi* worms, is another promising vaccine candidate known to carry fucosylated hybrid N-glycans on two N-glycosylation sites [23]. A recent study provided additional glycomic data, suggesting that ASP-1 is modified with N-glycans carrying the LN disaccharide motif on one or both antennae as well as the core α 1,3-fucose immunogenic epitope [22]. Glycan modification of native antigens is crucial for triggering protective immunity via vaccination in the host. Studies demonstrated that deglycosylation of the native H11 antigens led to a complete loss of recognition by antigen-specific IgG antibody [84], and resulted in a significantly reduction of protective immunity in experimentally challenged animals [85].

However, enabling glycan modifications on worm antigens can be a challenging step in developing recombinant subunit vaccines, as it requires suitable expression hosts to glycosylate the recombinant products with the right glycans structures. A few studies have shown that absent or inappropriate glycan post-translational modifications of recombinant nematode glycoproteins correlated with their compromised efficacies in vaccine trials. Recombinant production of worm glycoproteins has been attempted using various of commercial eukaryotic expression systems to allow glycosylation, which include *Pichia pastoris* [86,87], insect cells

[88–90], and the free-living nematode *C. elegans* [84,91,92]. A recent success was achieved by expressing *Ostertagia* ASP-1 using a glyco-engineered tobacco plant, *Nicotiana benthamiana*; the engineered antigens carried identical immunogenic glycan epitopes as the native ASP-1, which was beneficial for inducing a comparable protective efficacy as the native antigen in the host [22]. The same strategy was applied to introduce the LDNF and Lewis X glycan epitopes on recombinant egg antigens of the trematode helminth *Schistosoma mansoni*, and the engineered antigens were capable of inducing Th2-type immune response as the native form [93,94].

With a limited number of successful applications to date, glyco-engineering of vaccine targets not only enhances our understanding of the role of glycosylation in recombinant parasite antigens but also provides a practical approach for producing effective recombinant vaccines. By introducing foreign genes that encode glycosyltransferases or glycosidases with known linkage and substrate specificities, desired glycans or immunogenic epitopes can be introduced to the recombinant antigens during the post-translational modification processes within the cells. However, due to the diversity of glycan profiles of native antigens, designing and developing glycoengineered vaccines may be limited by two major factors: 1, the preknowledge about the precise glycoform of a native antigen; 2, the availability of genes that could be employed to alter the authentic glycosylation pathways of a given expression host or to introduce novel glycan biosynthetic pathways. To overcome such limitations, scientists need to further interrogate the glycobiology of individual parasitic nematodes and characterise parasite-derived antigens, using a combination of state-of-the-art methods such as glyco (proteo)mic approaches using high-resolution mass spectrometry, glycan microarrays and high-throughput screening methods to obtain novel glycoenzymes. A precision glycoengineering strategy tailored for each species is therefore required.

4. Perspectives

The application of next-generation sequencing technologies has accelerated studies on nematode genomes, resulting in an exponential increase in genomic data over the past decade. According to my recent search, genomes of 260 distinct nematode species are publicly available on NCBI (<https://www.ncbi.nlm.nih.gov/datasets/genome/?taxon=6231>), despite poor gene annotations. By contrast, the glycomic investigations of nematodes lag far behind genomic studies and database entries are sparse. Relative detailed glycomic data can be found in less than 20 species in literature, with structural information mostly about N-glycans. The delayed progress in decoding the glycome of parasitic nematodes is partly due to the complexity of their glycans and the shifts in glycan structures at various developmental stages [14,95], which correlate with parasites' intricate life cycles. Additionally, analytical limitations hinder the development of high-throughput glycomic platforms for non-mammalian samples. Detailed structural analysis of nematode glycans often requires multi-dimensional workflows, including HPLC fractionation steps to separate isomeric structures before MS/MS analyses. Enzymatic and chemical digestions are frequently necessary for linkage and anomeric assignments [50]. Future

progress in nematode glycomics will benefit from the integration of improved enrichment techniques, such as the recently developed N-glyco FASP protocol [96], and enhanced glycan release methods, like the application of rice PNGase A [59]. In addition to MALDI-MS/MS-based methods, advanced analytical approaches, including tandem mass spectrometry coupled with glycan-resolving columns (e.g., PGC- LC-MS/MS), can be adapted and optimised for nematode glycome studies. Furthermore, the development of robust bioinformatics tools, including specialised software and databases, will be crucial for accelerating data processing and interpretation.

By far, the glycosylation potential of Clade II and Clade IV nematodes remains poorly understood in contrast to the other Clades as demonstrated in Fig. 1. An early study demonstrated that oxidation of *Strongyloides stercoralis* glycoproteins reduced the seroreactivity of sera from patients with *S. stercoralis* infection, indicated the interplay of parasite glycans with the host immunity [25]. Along with *Strongyloides* spp. that cause diseases in animals and humans, many phytoparasitic nematodes of agricultural importance belong to the Clade IV, such as *Globodera* and *Meloidogyne* spp. Glycomic studies of such species will reveal their glycosylation characteristics and potentially lead to the discovery of novel glycan epitopes that may interact with plant immunity.

Finally yet importantly, nematodes represent a valuable source of novel carbohydrate-active enzymes. As glycans are products of enzymatic processes, such phylum-wide glycomic diversity implicates that nematodes possess complex glycosylation machineries, therefore, it is anticipated that a large set of glycoenzymes conferring different activities is encoded in a nematode's genome. By far, knowledge on the enzymatic basis of glycosylation in parasitic nematodes has been mainly obtained by studying the glycosylation of *C. elegans*. According to the Carbohydrate-Active enZymes database (CAZy: <http://www.cazy.org/>), 400 protein-encoding genes in *C. elegans*, distributed over 77 families, are predicted to associate with the biosynthesis and degradation of carbohydrates, representing at least 2 % of its genome [97]. In contrast, human has 350 CAZy genes in a genome that has roughly the same number of protein-coding genes as a worm. While a large set of glycoenzymes has been predicted by studying worm genomes, only a small number has been biochemically characterised and has their biological significances revealed. Putative PC-transferase and methyltransferases that endue glycans with immunogenic properties remain unknown. Therefore, future research should prioritise investigating the key enzymes responsible for the biosynthesis and modification of glycans in parasitic nematodes, alongside glycomic studies of different species. Such studies will not only unveil the structural diversity and biological roles of these glycoconjugates, but also facilitate the development of innovative carbohydrate-based vaccines against nematode parasites.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author used ChatGPT 4.0 to improve grammatical accuracy. After using this tool, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication. The figures and tables in this review article are manually prepared by the author without using AI-assisted tools.

Declaration of competing interest

I have nothing to declare.

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Data availability

No data was used for the research described in the article.

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