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REVIEW

The relevance of studying insect–nematode interactions for human disease

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ABSTRACT

Vertebrate-parasitic nematodes cause debilitating, chronic infections in millions of people worldwide. The burden of these so-called 'neglected tropical diseases' is often carried by poorer socioeconomic communities in part because research on parasitic nematodes and their vertebrate hosts is challenging and costly. However, complex biological and pathological processes can be modeled in simpler organisms. Here, we consider how insight into the interactions between entomopathogenic nematodes (EPN), their insect hosts and bacterial symbionts may reveal novel treatment targets for parasitic nematode infections. We argue that a combination of approaches that target nematodes, as well as the interaction of pathogens with insect vectors and bacterial symbionts, offer potentially effective, but underexplored opportunities.

KEYWORDS

Vertebrate-parasitic nematodes; entomopathogenic nematodes; inter-species interactions; alternative treatment options; vector control; model organisms

Introduction

Vertebrate-parasitic nematodes (VPNs) infect millions of people worldwide [1,2]. These nematode infections are usually chronic and target a variety of organ systems – from the skin to the gastrointestinal tract [3]. Some of the most deleterious are the filarial nematodes, responsible for debilitating limb edema and renal damage (lymphatic filariasis/elephantiasis), or skin disease and blindness (onchocerciasis) [4,5].

From a public health perspective, the main treatment strategy for filariasis is preventative chemotherapy in the form of Mass Drug Administration (MDA) [6–8]. Mass Drug Administration programs administer antiparasitic medication to all members of a community at risk without first testing individuals for an infection. Mathematical models predicted that these regimens are not sufficient to keep the disease controlled in areas with high prevalence [9]. Adherence to the dosing schedule is one of the challenges associated with MDA as successful elimination requires at least five doses of anti-filarial drugs and one or more doses are often missed [10,11]. Only two combinations of three different drugs are available to treat filariasis and all three target the larval stage of the nematodes only, leaving adult worms unaffected [12,13].

The widespread use of a limited number of anti-filarial medications also carry the risk of drug resistance developing in these nematodes [9,14]. With antimicrobial resistance on the WHO's list of top 10 threats to global health, it is crucial to optimize additional/alternative treatment options [15,16]. The interactions between nematodes, insects, and bacteria – concerning both nematodes responsible for human disease and insect-parasitic nematodes – provide opportunities to explore such alternatives.

Nematode-insect-bacterial interactions

Nematodes capable of infecting and killing insects are known as entomopathogenic nematodes (EPNs) [17–19]. Entomopathogenic nematodes are used as beneficial biological control agents of insect pests, providing an alternative to expensive, broad-spectrum, chemical insecticides [20–22]. Nematodes from the *Heterorhabditis* and *Steinernema* genera are frequently used in biocontrol and are therefore the EPN most commonly studied [23,24]. In addition to acting as definitive hosts to nematodes, insects are often involved in the nematode life cycle as intermediate hosts or vectors [25,26]. Dispersal by an insect vector is a characteristic of many animal and some plant-parasitic nematodes.

Bacteria often play a role in nematode–insect interactions. *Heterorhabditis* and *Steinernema* coevolved with bacteria in the genera *Photorhabdus* and *Xenorhabdus* to become virulent insect pathogens [27,28]. Although axenic nematodes are able to infect and kill an insect host [29,30], bacterial symbionts contribute to killing the host, digesting host tissues, and preventing other micro-organisms from colonizing the carcass [31]. In the case of parasitic nematodes causing human diseases such as filariasis, an intracellular

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endosymbiont, *Wolbachia*, is involved in the nematode's survival and reproduction [5,32]. Furthermore, *Wolbachia* also influences many aspects of insect biology, either as a mutualist or as a pathogen [33,34].

The relative ease with which insects and nematodes can be cultured and manipulated – with or without their bacterial symbionts – make them useful models for observing interspecies relationships [35,36]. Host–parasite interactions such as the insect's immune response to invasion and how the nematode overcomes the immune response can be investigated by studying parasitic nematodes and their insect hosts and vectors [37,38]. Parasitic nematodes and symbiotic bacteria also provide opportunities to study factors influencing mutualism, such as the evolution of biochemical communication between host and symbiont [39,40].

The value of studying the interactions between parasitic nematodes and their hosts extends beyond gaining insight into the particular pest or biocontrol management system. These investigations may also reveal novel treatment strategies for challenging human conditions. For instance, the insect vectors and bacterial symbionts of parasitic nematodes present promising targets for combating these infections in humans. Additionally, the systems that parasitic nematodes use to evade and suppress the human immune response are increasingly well studied [41,42]. This not only enables the nematodes to survive and cause disease within the host, but also influences co-existing infections and noninfectious conditions of the host. The close phylogenetic relatedness among human and insect-infecting nematodes, as well as the presence of orthologous genes involved in virulence and defense, mean the organisms involved in entomopathogenic nematode parasitism (nematode parasite, insect host, bacterial symbiont), can be used as simpler models to study nematode infections in humans.

Insect and bacterial options to manage nematode infections

Target the insects

Filarial nematodes are transmitted to their vertebrate hosts by mosquitoes of different genera [4]. Consequently, transmission can be interrupted by targeting the insect vector. Vector control usually consists of spraying insecticides inside homes and distributing netting material impregnated with long-lasting insecticides [43,44]. Other vector control strategies target the source of mosquitoes, for instance, polystyrene beads that form floating layers on potential breeding sites such as pit latrines and water tanks suffocate mosquito larvae, leading to a drastic decline in the adult mosquito population [45–47]. Combined vector control and MDA suppress the transmission of filariasis more effectively and with less resurgence than MDA alone. A focus on integrated vector management in addition to MDA was therefore included in the strategic plan for 2010–2020 of the Global Programme to Eliminate Lymphatic Filariasis [48].

Effective vector control also impacts diseases that co-exist with filariasis, for instance, malaria and dengue fever, which are transmitted by the same mosquitoes [49]. Unfortunately, wherever chemicals are used, the risk of resistance developing exists and resistance to a number of insecticides have been documented [44,50]. Similarly, the use of polystyrene beads is not foolproof as all the potential mosquito breeding sites in a community have to be identified and treated, its use is limited to smaller bodies of still-standing water, and it is not effective for all mosquito species. Flooding of pits containing these polystyrene beads leads to unsightly pollution and loss of larvicidal function [45].

With an increasing number of insect genomes being sequenced and made available in public databases, together with the development of advanced gene-editing tools, gene modification provides an alternative to traditional chemical or environmental vector control measures [51,52]. Genetically modified mosquitoes are already being released to control mosquito populations responsible for the spread of dengue fever, for example [53,54]. Releasing transgenic organisms is of course not without risks. Modified genes might be transmitted to the wild-type population and changes in the wild-type population could affect the virulence of the vector-borne pathogen. Molecular insight into the interactions between parasites, vectors, and bacterial symbionts is therefore important not only to discover additional treatment targets but also to ensure the safety of existing and developing control measures [55].

Target the bacteria

The nematode species responsible for the majority of filariasis all rely on an intracellular bacterium for their development and reproduction [5,32]. The bacterial symbiont, *Wolbachia*, belongs to the order Rickettsiales – the same order containing *Rickettsia* species associated with tick-bite fever and other spotted fevers. The drugs used to treat rickettsia infections, especially doxycycline, successfully suppress filarial infections [56,57]. Unfortunately, a course of treatment with doxycycline lasts 6–8 weeks and cannot be used in pregnant women or children.

By targeting bacteria and their molecular pathways instead of the eukaryotic pathways of nematodes, drugs with potentially fewer adverse reactions in humans can be developed. The combination of high-throughput assays and bioinformatics tools facilitates the screening of millions of compounds for desirable properties [58]. One such study identified five

compounds with potential fast-acting anti-*Wolbachia* activity [59]. These compounds can now be tested in animal and clinical trials.

Wolbachia bacteria also colonize many insect species and other arthropods, either as mutualists or pathogens [33,34,60]. *Wolbachia* endosymbionts influence the host insect's reproductive fitness and can increase the fertility of infected females or cause sterility in males [61]. Artificial infection of previously uninfected insects can be lethal or reduce the capability to vector certain pathogens [62–64]. The ability of *Wolbachia* to alter insect reproduction earns them a place in vector control and these bacteria are already being investigated for use against malaria, dengue fever, and lymphatic filariasis [65–67]. As in the case of insect vectors and nematode parasites, the molecular mechanisms underlying these interspecific interactions are in need of further investigation [33].

The use of nematodes and insects as mini-host models

Models for nematode infections in humans

Nematode infections in humans are regarded as 'neglected tropical diseases' [8]. Especially poorer socio-economic communities carry the burden of the filarial diseases [2,65,68]. In order to 'Ensure healthy lives and promote well-being for all at all ages', the eradication of neglected tropical diseases forms part of the 2030 Agenda for Sustainable Development [69]. Research into filariasis is, however, hampered by the cost and complexity of studying infections in their vertebrate hosts.

The use of simpler organisms to study complex biological and pathological processes is not new. The free-living nematode, *Caenorhabditis elegans* has since the 1960s been put to use in the investigation of human conditions ranging from neurological degeneration and aging to metabolic diseases and cancer [70,71]. Genes involved in the pathogenicity of medically important fungi, including *Candida* spp. and *Cryptococcus* spp., play similar roles when infecting and killing model invertebrates such as *Drosophila melanogaster* and *C. elegans* [72]. Subsequently, susceptible invertebrates present the opportunity to study fungal virulence mechanisms and even test antifungal treatment without exposing patients to added risks.

Both VPN and EPN suppress the immune responses of their host [38,41]. As VPN and EPN are closely related phylogenetically [73,74], orthologues of genes associated with host immunosuppression can be found in both types of nematodes [30]. Insect-pathogenic *Heterorhabditis bacteriophora* shares ancestral traits with free-living *C. elegans* but is phylogenetically positioned closer to the mammal-parasitic nematodes. *Heterorhabditis bacteriophora*, therefore, represents a 'bridge' species to translate existing knowledge of molecular pathways in *C. elegans* and other EPN, to VPN [73]. Compared to mammalian parasites, EPN culturing requires fewer resources in terms of laboratory equipment and personal protection, as well as host animals. As a result, entomopathogenic nematodes and their insect hosts offer an alternative option to study nematode infections in humans and other mammals.

Models for bacterial infections in humans

Knowledge on interspecies interactions gained from studying EPN systems is not limited to the field of nematode infections. The symbiotic bacteria of EPN represent as important models to study bacteria-host interactions, as nematode–host interactions [17]. Bacteria from the genera *Photorhabdus* and *Xenorhabdus* (the symbionts of *Heterorhabditis* and *Steinernema*, respectively) form part of the Enterobacteriaceae [75]. Other members of this family include the common human pathogens, *Escherichia coli, Salmonella* spp., *Yersinia* spp. and *Proteus* spp. In fact, *Proteus mirabilis* – one of the most common causative agents of urinary tract and hospital-acquired infections [76,77–78] – is the closest phylogenetic relative to *Photorhabdus* and *Xenorhabdus*. Therefore, an understanding of pathogenicity in the entomopathogenic bacteria can contribute to a search for similarities in human pathogens. The discovery of such orthologous virulence pathways could reveal strategies for the prevention and treatment of *P. mirabilis* infection in humans.

Conclusion

Insight into the interactions at play within one multi-species system will benefit the improvement or control of the system in question but could also prove applicable in other settings. The current treatment strategies that only target the nematodes responsible for human infection are unlikely to relieve the burden of chronic, debilitating disease in areas with high prevalence [78]. However, a combination of approaches that also control or manipulate their interactions with insect vectors and bacterial symbionts, has a better chance of being effective and well tolerated.

Insect- and vertebrate-parasitic nematodes both suppress the host immune response, but EPNs are much easier, safer, and cheaper to culture than human pathogens. Although nematode-insect models may not mimic human diseases in every respect, simpler systems do make the application of genetic and

molecular techniques easier in order to dissect pathogen–host interactions [70].

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