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REVIEW

Human strongyloidiasis: identifying knowledge gaps, with emphasis on environmental control

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Abstract: *Strongyloides* is a human parasitic nematode that is poorly understood outside a clinical context. This article identifies gaps within the literature, with particular emphasis on gaps that are hindering environmental control of *Strongyloides*. The prevalence and distribution of *Strongyloides* is unclear. An estimate of 100–370 million people infected worldwide has been proposed; however, inaccuracy of diagnosis, unreliability of prevalence mapping, and the fact that strongyloidiasis remains a neglected disease suggest that the higher figure of more than 300 million cases is likely to be a more accurate estimate. The complexity of *Strongyloides* life cycle means that laboratory cultures cannot be maintained outside of a host. This currently limits the range of laboratory-based research, which is vital to controlling *Strongyloides* through environmental alteration or treatment. Successful clinical treatment with antihelminthic drugs has meant that controlling *Strongyloides* through environmental control, rather than clinical intervention, has been largely overlooked. These control measures may encompass alteration of the soil environment through physical means, such as desiccation or removal of nutrients, or through chemical or biological agents. Repeated antihelminthic treatment of individuals with recurrent strongyloidiasis has not been observed to result in the selection of resistant strains; however, this has not been explicitly demonstrated, and relying on such assumptions in the long-term may prove to be shortsighted. It is ultimately naive to assume that continued administration of antihelminthics will be without any negative long-term effects. In Australia, strongyloidiasis primarily affects Indigenous communities, including communities from arid central Australia. This suggests that the range of *Strongyloides* extends beyond the reported tropical/subtropical boundary. Localized conditions that might result in this extended boundary include accumulation of moisture within housing because of malfunctioning health hardware inside and outside the house and the presence of dog fecal matter inside or outside housing areas.

Keywords: *Strongyloides stercoralis*, strongyloidiasis, environmental control, parasitology, nematode

Introduction

Humans are hosts to two species of the parasitic nematode *Strongyloides*: *Strongyloides stercoralis* and *Strongyloides fuelleborni* (separated into two subspecies, *S.f. fuelleborni* [in Africa] and *S.f. kellyi* [in Papua New Guinea]).[1] Strongyloidiasis is caused by infection by either of these species. The important species in human infection is *S. stercoralis*. Unless otherwise indicated, the remainder of this article will refer to *Strongyloides*, indicating any *Strongyloides* spp. capable of causing strongyloidiasis.

In Australia, a history of successful clinical treatment with antihelminthic drugs[2,3] has meant that controlling *Strongyloides* through environmental control, rather than

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clinical intervention, has been largely overlooked. However, in light of reinfection rates in endemic areas, coupled with concern about the potential for development of antihelminthic resistance, environmental control should be given greater attention, either by altering the soil environment, through physical means such as desiccation or removal of nutrients, or through chemical or biological control.

Strongyloides has a complex life cycle, with early research identifying the unique alternation between the free-living and parasitic stages of *Strongyloides*.[4] Furthermore, in 1905, Looss[5] demonstrated the mode of infection (through the skin) by infecting himself and finding *Strongyloides* in his feces 64 days later, and Fülleborn[6] reported how the parasite moves through the human body to end up in the intestine.[7] Despite this early understanding of the infective nature of *Strongyloides*, and its prevalence, significant gaps in our understanding of *Strongyloides* still exist. These gaps are affecting our ability to control infection rates globally. In this article, we present an overview of our current understanding of *Strongyloides* and summarize the gaps in our knowledge, with a particular emphasis on those gaps that are preventing better environmental control of *Strongyloides*.

Methods

We reviewed the body of literature to identify knowledge gaps that may be hindering progress in the environmental control of *Strongyloides*. Journal indexing services (Google Scholar, PubMed, Ingenta Connect) were queried for publications from the last 25 years that represented the current best practice or best knowledge in terms of treatment, diagnosis, epidemiology, and microbiology of *Strongyloides* and strongyloidiasis. Efforts were directed toward obvious gaps in the literature that represented significant barriers to the understanding of the organisms' survival in the environment, and where research may be directed to address these gaps. The thorough description of these gaps forms the basis of this article. Where appropriate, historical context is provided by older and seminal publications within the field.

Strongyloides' life cycle

The life cycle of *S. stercoralis* incorporates complex host-mediated (homogonic) and free-living environmental (heterogonic) processes. The parasite has the ability to reproduce indefinitely within the host[8] if not treated with antihelminthics.

Human infection occurs when filariform larvae penetrate the skin. These larvae then enter the venous system, where they migrate through the right atrium and ventricle of the heart and then to the lungs and occupy the bronchi and trachea. From this region of the respiratory system, larvae are coughed up and subsequently swallowed. Larvae pass through the digestive system until they reach the small intestine, where they submerge themselves in the intestinal mucosa.[8] Embedded worms undergo further development into predominantly adult females, which are capable of parthenogenic (asexual) reproduction.[9] Adult females drive an autoinfective life cycle, whereby eggs are laid in the gut. These eggs hatch and develop into male and female rhabditiform larvae, and both eggs and larvae are excreted in feces. Rhabditiform larvae then develop into filariform larvae and either repenetrate the gut lining, the skin surrounding the perianal region, or distribute environmentally to a new host.[10]

Because of the complexity of *S. stercoralis*' life cycle,[11] laboratory cultures cannot be maintained outside of a host, and subsequently, only a single heterogonic cycle has been observed.[12] There is no clear consensus backed by empirical evidence demonstrating which factors cause the differentiation of rhabditiform *S. stercoralis* into females, males, or filariform larvae. Early research demonstrated varied effects of temperature on larval development and exposure to fecal dilutions.[13,14] However, more recent research has found that in temperatures below 34°C, larvae molt four times and develop into free-living sexually mature adults, and in temperatures above 34°C, larvae molt twice and develop into infective filariform larvae.[15,16] Chemosensory factors may also influence larval differentiation. Chemosensory and thermosensory neurons are contained in the amphids in nematodes. Research into the function of the amphids has shown several classes of these neurons. The skin-penetrating larvae in *S. stercoralis* have been shown to be thermotaxic, moving upward on a thermal gradient. This is regulated by the paired ALD class neurons.[17] *S. stercoralis* has been demonstrated to be chemotaxic, moving toward the chemical markers present in sweat. Developmental switching (switching between alternative free-living developmental pathways) has been shown to be controlled by the ASF and ASI chemosensory amphidial neurons.[18] Similarly, the development of infective larvae has been shown to be controlled by similar molecular genetic mechanisms such as *Caenorhabditis elegans* (via the AGE-1 region) in *S. stercoralis* via a structural homologue of the AGE-1 region called Ss-AGE-1.[19] Therefore, a chemical agent is likely to be involved in the mediation of differentiation of *S. stercoralis* larvae.

As homogonic *S. stercoralis* primarily exists in tissues, and periodically in feces, it seems likely that a chemical

element present in feces either inhibits or induces larval differentiation. Adult forms and eggs are excreted in feces to either carry out a free-living sexual reproductive cycle or immediately seek new hosts. This requires that eggs either become filariform larvae or produce males to mate with females, and differentiation must occur at an advantageous juncture to provide the highest likelihood of survival. It has been demonstrated that cholesterol and other sterols play a hormonal or signaling role in larval development in the related rhabditiform nematode *C. elegans*.[20,21] It seems plausible that sterols may be a key signaling molecule in the development of *S. stercoralis* larvae and may go so far as to account for differences in host specificity, given the formation of distinctly different fecal sterols in various mammals.[22] Siddiqui et al[23] present evidence of a receptor that they hypothesize supports steroids triggering hyperinfection of *Strongyloides*. This is supported by Wang et al,[24] who suggest that ligand-binding to treat disseminated *Strongyloides* may be pharmacologically possible.

The complexity of *Strongyloides*' life cycle might be a reason for the limited published bioassays assessing the nematode's susceptibility to environmental challenges. Tests rely on *Strongyloides* extracted from feces and are often problematic. *Strongyloides* need to be extracted from feces for each set of bioassays, which presents both ethical and occupational safety problems. The lack of research into maintaining *Strongyloides* in the laboratory affects our ability to assess the *Strongyloides*' susceptibility to environmental control potential, such as desiccation, or to chemical and biological control possibilities.

Prevalence: significantly underestimated?

Strongyloidiasis is widespread within tropical and subtropical areas around the world. On the basis of ratios of prevalence of other helminthes, an estimate of 370 million people infected worldwide has been proposed,[25] making strongyloidiasis a more common infection than malaria, which has an estimated infection level of 219 million cases (uncertainty range, 154–289 million).[26] Other, more conservative estimates suggest a global infection level of 100 million people,[27] although researchers believe this figure is grossly underestimated because of the infection mimicking other illnesses with symptoms such as diarrhea, abdominal pain, septicemia, and vomiting, and is therefore often misdiagnosed.[28,29] The inaccuracy of diagnosis[30] and the unreliability of prevalence mapping,[25] coupled with the fact that *Strongyloides* infection is often not tested for, suggests that the higher figure of more than 300 million cases is likely to be a more accurate estimate. Publications regarding the infection rate and incidence of strongyloidiasis have been described as "patchy" and "virtually nonexistent" and have been highlighted as key gaps in knowledge that would provide insight into how frequently and how rapidly people are reinfected after successful treatment.[16] The prevalence of strongyloidiasis in Australia is equally unknown, with extremely varied estimates ranging from more than 1% to 60%, depending on the community tested and the diagnostic tools used.[31–34] A 20 year retrospective survey of remote communities in Queensland discovered fluctuating prevalence that correlated with both the wet season, where prevalence increased from 12% to 27.5%, and thiabendazole treatment, after which prevalence fell to 7% for approximately 4 years.[33] This work highlighted the effective use of antihelminthics to treat persistent strongyloidiasis and lower reinfection rates, but suggested that without changes to failed infrastructure, eradication may not be possible.

The health consequences of *Strongyloides* infections range from asymptomatic light infections to chronic symptomatic strongyloidiasis and, finally, uncontrolled multiplication of the parasite (hyperinfection) and potentially life-threatening dissemination of larvae to all internal organs among individuals with compromised immune systems.[35] Dissemination and hyperinfection have been replicated with *S. stercoralis* in dogs and have been shown to be a model for the human course of the infection.[36,37] Immunocompromised dogs were shown to be highly susceptible to hyperinfection and disseminated strongyloidiasis. Similarly, hyperinfection has also been induced in gerbils using *S. stercoralis*.[38] Marcos et al[39] suggest that severe strongyloidiasis has a high mortality rate (up to 80%) because the diagnosis is often delayed. This relates to its nonspecific presentation and the host's immunocompromised status. Most immunocompetent individuals who develop strongyloidiasis have asymptomatic chronic infections that result in negligible morbidity. Immunosuppressed individuals are most vulnerable, with mortality rates being highest among these groups.[35,40] with Indigenous Australians suffer high rates of noncommunicable disease,[41,42] which increases the infection risk and worsens their outcomes compared with non-Indigenous Australians.[43]

Detection in clinical and environmental samples

Detection of *Strongyloides* in clinical samples can be classified broadly as either molecular, incorporating either polymerase chain reaction (PCR) or qualitative PCR,[44]

immunological methods[45] or microscopic methods, which include the Koga plate method,[46] the Baerman technique,[47,48] and the Katz thick smear.[49–51]

Microscopic techniques provide conditions that separate intact, living nematodes from clinical samples (primarily feces). These methods all suffer from primarily three main limitations: the identification of *Strongyloides,* using morphological features, can be subjective; working with live *Strongyloides* in an uncontained system is a biosafety hazard; and the larval load in stool varies greatly.[52–54] Identification ambiguities and safety concerns may be mitigated with sufficient training and an appropriate laboratory setup; however, variations in larval load are highly dependent on the parasite's life cycle stage, host health, and treatment status.[52–54] Ultimately, this may produce results in which there are insufficient larvae in the stool to visually confirm infection, resulting in false-negatives. In addition, these techniques can take up to 48 hours for results to be available.

Molecular methods allow for the detection of *Strongyloides* solely on the basis of the presence of target DNA sequences, removing the analyst as a subjective source of bias. Samples (not limited to stool) have their DNA compliment extracted, and primers are added that anneal specifically to target *Strongyloides* sequences. A range of primer sets incorporating both conventional qualitative PCR and probe-based detection systems have been described.[44,55–59] Serological detection methods are available that detect either proteins or antibodies in the blood plasma, using an enzyme-linked immunosorbent assay.[45]

These methods need significant refinement to achieve reliable, intersample quantitation, but molecular methods inherently have several advantages over microscopy-directed diagnostic tools. Once DNA extraction has occurred, samples are entirely noninfective, and samples need not be stored to retain viability of the helminthes present. Samples may be frozen or otherwise heat-killed and should remain PCR-competent, even with low numbers of larvae present, because of the sensitivity of the method. However, the size of the sample processed may lead to false-negatives in samples with low larval loads. Sample processing and detection may occur in a matter of 2–3 hours.

Environmental detection currently remains an understudied area of research, and efforts should be made to better understand the role of environmental reservoirs of *Strongyloides*. Molecular detection methods used in clinical analyses are potentially readily transferable to environmental samples, including soil and animal feces, with little addition or modification to the method; however, the presence of compounds inhibitory to PCR may significantly reduce or halt the progress of the reaction. This is an area that needs to be addressed to allow us to understand and map the distribution of *Strongyloides* in the environment.

Clinical treatment of strongyloidiasis

Strongyloidiasis is commonly treated with antihelminthics such as ivermectin and albendazole. Ivermectin is a broad-spectrum macrocyclic lactone that inhibits the motility of the nematode by increasing the opening of glutamate-gated chloride channels, causing paralysis of pharyngeal pumping.[60,61] Albendazole inhibits the formation of microtubules by selectively binding to β-tubulin.[61] Albendazole is usually prescribed at 400 mg for 3 days and has 38% efficacy.[62] Ivermectin had 83% efficacy when 150–200 μg/kg was administered in a single dose.[60,62] Further work has supported ivermectin's preferential administration, demonstrating an efficacy of 96% when administered at 200 μg/kg that increases to 98% after a follow-up treatment 2 weeks after the initial dose.[63] Alternative antihelminthics thiabendazole, cambendazole, and mebendazole can be used but are significantly less effective than ivermectin.[25,64,65]

Lack of ivermectin resistance

Repeated treatment of individuals with recurrent strongyloidiasis has not been demonstrated to result in the selection of resistant strains. We theorize that this process is inhibited by *Strongyloides*' clonal life cycle within a host. During the host-bound parthenogenic life cycle, all infective individuals are clonally propagated, and as such, the rate of mutation and the rate at which novel genetic information is introduced are low. As a result, only limited adaptation is possible, and so treatment with antihelminthics tends to be successful, even with repeated doses over an extended period of time, with no reported instances of resistance in humans.[66] However, this has not been explicitly demonstrated either in a laboratory setting or through close monitoring of treatment-resistant individuals, and relying on such assumptions in the long-term may prove to be short-sighted.

Furthermore, the repeated administration of ivermectin, and specifically mass drug administrations, may lead to the formation of a resistant population in other parasitic organisms, such as sarcoptic mites (*Sarcoptes scabiei*), which historically have demonstrated the formation of resistance[67–69] and have begun to show resistance to ivermectin treatment.[70] Studies of related soil and veterinary helminthes have raised concerns about the formation of resistance[71–73] and proposed the need for monitoring for resistance[71] or have begun

to suggest the formation of resistance to benzimidazole compounds.[74] It is ultimately naïve to assume that continued administration of antihelminthics will be without any negative long-term effects, particularly without exploring prevention strategies that incorporate environmental control.

Geographical distribution: questioning a strict tropical/subtropical range

Genta[75] reviewed literature reporting the prevalence of *S. stercoralis* among various populations on five continents and found the following risk groups: "residents of and emigrants from any developing country and southern, eastern, and central Europe; travelers and veterans returning from endemic areas; natives and residents of the Appalachian region in the United States and local endemic areas in other countries; and institutionalized persons".[75] Historical studies have also demonstrated that the range of *Strongyloides* is not strictly limited to tropical/subtropical regions, with case reports from urban areas in non-tropical regions.[76,77] Despite the existence of literature indicating that strongyloidiasis is not confined to a tropical distribution, it is often still perceived and treated as such.[78,79]

In Australia, the literature indicates that the primary burden of the disease is borne by Indigenous communities of northern Australia.[33,80] Strongyloidiasis in Australian Indigenous populations has been primarily attributed to individuals and communities who inhabit tropical and subtropical areas of Australia. However, growing evidence suggests that the nematode is more widespread than previously thought within indigenous populations, although further research is required to map infection outside the tropical and subtropical zones.[3] From routine laboratory results and epidemiological surveys, *Strongyloides* is now known to be spread more widely than was previously thought, particularly in Aboriginal communities in arid regions of central Australia,[81–83] although this clinical evidence is not yet supported in the literature. For example, the 8th National Workshop on Strongyloidiasis listed unpublished data of infection rates of between 2%–58% in Australian Indigenous communities, including 32% in one community in 2007 and 15% in another in a 2005 survey.[84] Australia's unique assortment of geographical features, ranging from tropical to arid, presents an opportunity to better understand the climactic limitations of *Strongyloides*' geographic distribution.

Environmental reservoirs

Unlike other diseases such as malaria, *Strongyloides* infection responds readily to chemotherapy.[25,64,65] Possibly as a result of this ease of clinical treatment, tackling environmental reservoirs as a means of controlling *Strongyloides* infection has been overlooked. Soil and feces are assumed to be the environmental reservoirs of *Strongyloides,* and Grove[85] points out that the most effective control measures against human helminthes have been the installation and usage of safe waste disposal systems.[85] Few clinicians have sought an environmental solution to transmission. Durrhiem[86] suggests that a solution to *Strongyloides* transmission might be wearing footwear; however, lack of cultural acceptance of wearing shoes, particularly in a hot climate, might make this simple approach to interruption of transmission not possible. There is a general consensus that enforcing, or even educating for, behaviors that are counter to culturally accepted norms will not be successful.[87,88]

As noted earlier, in Australia, Strongyloidiasis primarily affects Indigenous communities, particularly those remote Australian Indigenous communities in the Northern Territory. There are a number of reasons for this, including malfunctioning health hardware inside the house, malfunctioning health hardware outside the house, the presence of dog fecal matter in or outside housing areas, and the close relationship between dogs and humans.

If the wet areas of a house are not functioning properly, leaking taps and/or poor drainage result in moisture being present for extended periods. The role of failed and poor infrastructure in strongyloidiasis transmission has been previously noted, but not extensively explored as a potential control method.[33] Indigenous houses often have lower levels of working housing infrastructure, such as water and wastewater disposal.[89,90] Inside, this means moisture is retained for extended periods in the bathroom, laundry, and kitchen areas. Outside, prolonged water retention occurs as a result of leaking or malfunctioning rain water tanks and septic systems. This retention of moisture inside and outside the home may mimic other confirmed environmental reservoirs[33,91–93] and provides an environment that could sustain the environmental life cycle stage of *Strongyloides*, possibly for extended periods (although the survival time of *Strongyloides* in the environment has not been quantified).

In Aboriginal communities, dogs and people live in close proximity;[94] this close relationship between dogs and people has been documented.[95,96] High numbers of dogs have been reported in Australian Indigenous communities; for example, Bradbury and Corlette[97] report that more than 50% of homes house three or more dogs, and 10% of homes exceed eight dogs per household. J Driver (Environmental Health Officer, Department of Health, Northern Territory)

and J Kennedy (Child and Family Health Nurse, Department of Health, Northern Territory) (personal communications, November 10, 2013) confirm the presence of dog feces inside homes. Although the presence of dogs and dog feces does not necessarily provide a source of infection for *Strongyloides*, preliminary evidence for infective transfer from dogs to humans has been established.[98]

Another study of environmental reservoirs surveyed garbage collectors in Brazil and concluded that contact with garbage or sewage may be associated with infection with intestinal parasites, with workers surveyed having acquired strongyloidiasis.[99,100]

Controlling *Strongyloides* by addressing the environmental factors that play a role in transmission, in addition to treating the infection once it occurs, should be a priority for researchers.

Is *S. canis* a separate species?

The volume of published material on the existence of a canine-specific *Strongyloides* species is at best scant, with journal indexing services queried (Google Scholar, PubMed, Ingenta Connect) returning fewer than 15 articles mentioning *S. canis*. Similarly, searches of the National Centre for Biotechnology Information contain no submitted sequences from *S. canis*. It is conceivable that a canine adapted species of *Strongyloides* exists and has yet to be thoroughly characterized; however, the distinct possibility exists that canine infections are primarily caused by *S. stercoralis*.[95,101–104] Sequencing of internal transcribed spacer regions 1 and 2, as per Sultana et al,[105] may demonstrate a genetic and possible taxonomic basis for the classification of a canine-specific *Strongyloides* species, but at present, the volume of published material does not support this.

This is a crucial area of research for several reasons. First, if dogs are harboring human *Strongyloides*, their role as a reservoir for human infection needs to be understood, particularly for Indigenous Australian communities, in which dogs play an important cultural role. Second, from a research perspective, laboratory extraction of *Strongyloides* from dog feces carries fewer ethical considerations. However, there are still the associated biohazard risks of dealing with the extracted *Strongyloides* in the laboratory.

Conclusion

At this time, we have limited knowledge about environmental factors that affect *Strongyloides*. The assumption that the disease is restricted to tropical areas is in question, and the reason for its restriction to certain geographical areas is not well understood. On a local scale, we are not sure where in the soil environment the reservoirs that harbor *Strongyloides* exist. Currently used diagnostic methods are unreliable, and emerging, more-reliable techniques are not yet in common use. In many areas, clinicians lack awareness of the infection, so it is not tested for, which combines to result in inaccurate estimates of infection rates. We are not sure of the role that dog feces might play as a reservoir and in human transmission of *Strongyloides*. We do not know what environmental factors might control *Strongyloides* distribution (such as moisture and nutrients), and we do not know of any chemical or biological control agents that might be applicable to its control in the soil. In addition, we do not yet have the ability to maintain a culture of *Strongyloides* in the laboratory for an extended period of time, which hinders laboratory-based experimental research.

For too long we have taken a purely clinical approach to treating *Strongyloides* infection in humans and ignored both the reinfection rates and the potential for development of antihelminthic resistance. There is an urgent need to address these knowledge gaps if we are to approach control of *Strongyloides* through environmental measures, rather than relying solely on clinical intervention.

Disclosure

The authors report no conflicts of interest in this work.

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