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Community-wide mass drug administration for soil-transmitted helminths – risk of drug resistance and mitigation strategies

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Mass drug administration programs for the control of soil-transmitted helminths (STH) in humans most commonly utilize a single class of drugs; the benzimidazoles. Most such programs focus on the treatment of pre-school and school aged children attending schools, although there is increasing interest in the potential utility of community-wide MDA to reduce infection intensity within communities and possibly to interrupt STH transmission. In animals, mass treatment with benzimidazoles leads to the rapid selection of parasites containing resistance-encoding single nucleotide polymorphisms (SNP) and the potential emergence of resistance in parasite species that infect humans is of major potential public health concern. As programs scale up delivery of anthelmintics and consider expanding treated populations, monitoring of drug efficacy and the potential emergence of anthelmintic resistance with sensitive diagnostic tools is critical to ensure the continued success of STH control programs. In particular, as programs consider the adoption of community-wide deworming, there is concern that such a strategy may increase the risk of drug resistance by limiting the number of untreated individuals which serve as a refugia of unexposed worm populations. We review the literature for evidence of drug resistance in human STH infections and explore risks and mitigation strategies for emergence of drug resistance in the context of community-wide deworming.

KEYWORDS

soil transmitted helminth, mass drug administration (MDA), drug resistance, neglected tropical disease, benzimidazole

Introduction

Soil transmitted helminths (STH) are among the most widespread neglected tropical diseases (NTD), affecting ~1.5 billion people globally (1, 2). STH disproportionately impact the poorest and most vulnerable communities. STH include hookworms (*Necator americanus* and *Ancylostoma duodenale*), *Ascaris lumbricoides* and *Trichuris trichiura*. Infection occurs through ingestion of eggs of *A. lumbricoides* and *T. trichiura* or larval penetration of the skin by hookworm larvae present in contaminated soil (3, 4). Moderate- and heavy-intensity (MHI) hookworm infections are associated with lower hemoglobin levels and anemia, which may be particularly detrimental to pregnant women and young children who often have low baseline iron stores (5–8). Children with STH infections also experience malnutrition and poor cognitive development, further impacting school attendance and performance and future economic productivity (9–11). The current World Health Organization (WHO) strategy is focused on eliminating morbidity through the targeted deworming of at-risk populations with anthelminthic medications, including pre-school-age children (PSAC), school-age children (SAC), and women of reproductive age (WRA).

Large-scale deworming programs have led to a significant decline in the prevalence, intensity, and associated morbidity of STH (12). However, until substantial economic development occurs, including significant improvements in access to clean water, sanitation and hygiene (WASH), reservoirs among untreated adults will continue to lead to contamination and persistence in the environment (ranging from weeks for hookworm to years for *Ascaris*) (13). This leads to rapid re-infection in at-risk populations unless deworming is repeated frequently over time. Evidence from mathematical models and field trials indicate that it may be possible to interrupt the transmission of STH in some geographic settings by adopting a community-wide mass drug administration (MDA) strategy in which all individuals, including adults, are treated (14–16). A transmission-interruption strategy could allow for the discontinuation of long-term deworming programs and substantially reduce the need for drug donation upon which deworming programs are highly dependent (17).

The two most commonly used drugs in existing STH programs (and available as part of drug donation programs) are albendazole and mebendazole. Both of these drugs belong to a single class of benzimidazoles that act by inhibiting tubulin polymerization, leading to worm paralysis and death (18). A 2017 meta-analysis demonstrated that while both drugs were highly efficacious against *A. lumbricoides*, albendazole had higher efficacy against hookworm infections, with a cure rate (CR) of 80% and an egg reduction rate (ERR) of 90%. In contrast, mebendazole appeared most effective against *T. trichiura*, resulting in a CR of 42.1% and an ERR of 66% (19). The continued use of drugs with less than optimal efficacy suggests that the likelihood of resistance emerging in human populations may be high and, while global estimates continue to show that morbidity due to STH is declining, some studies in areas with a long history of deworming suggest a decline in efficacy of these drugs (20, 21). In fact, a 2018 review of this subject suggested that the efficacy of both albendazole and mebendazole for the treatment of hookworm infection may have decreased by as much as 15% during a two-decade period from 1995 until 2015 (22). This same review suggests that reduced efficacy is even more pronounced for the treatment of *T. trichiura*, with efficacy reductions at or near 30%. Whether these potential declines in efficacy are a result of the emergence of drug resistance is not clear (23) but such trends provide circumstantial evidence in support of this possibility.

Anthelminthic drug resistance

In the veterinary world, routine mass treatment of livestock has led to widespread drug resistance, with well documented single nucleotide polymorphisms (SNPs) that have resulted in the entire class of benzimidazole drugs being rendered ineffective. Isothermal loop-mediated isothermal amplification (LAMP) assays, PCR, Sanger sequencing, pyrosequencing and next generation sequencing (NGS) based methods have identified SNPs associated with benzimidazole resistance in a variety of human and veterinary nematodes at one of three codons – 167, 198 and 200 – in the gene encoding the β-tubulin isotype 1 (24, 25) (more appropriately named the β-tubulin A locus to prevent false implications of orthology) (26). (Human examples detailed in Table 1). These studies used nucleic acid derived from a variety of sources, including adult worms (from expulsion studies), dissected or concentrated eggs, or stool samples (Table 2). Some studies have recorded treatment history and collected samples both before and after treatment in order to document a temporal association between treatment and the emergence of putative genotypic determinants of

TABLE 1 Single nucleotide polymorphisms described in the β-tubulin A locus of human STH species.

SNP codon	*N. americanus* (27, 28)	*A. caninum* (29, 30)	*A. lumbricoides* (26)	*T. trichiura* (27, 31)	Amino acid substitution
167	T>A(TTC>TAC)	T>A(TTC>TAC)	T>A(TTC>TAC)	T>A(TTT>TAT)	Phe>Tyr
198	A>C(GAG>GCG)	not described	A>C(GAA>GCA)	A>C(GAA>GCA)	Glu>Ala
200	T>A(TTC>TAC)	T>A(TTC>TAC)	T>A(TTC>TAC)	T>A(TTC>TAC)	Phe>Tyr

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TABLE 2 Review of rates of β-tubulin A locus single nucleotide polymorphisms reported in human STH samples.

Species	Year, Reference	Country	Method	Treatment (number of samples/ eggs/larvae)	Codon 167	Codon 198	Codon 200
A. duodenale	2004 (32)	Tanzania	Sequencing	After Treatment (1)	x	x	S
N. americanus	2013 (27)	Haiti	Pyrosequencing	Before Treatment (84)	S	S	S
				After Treatment (14)	S	S	S
	2013 (27)	Kenya	Pyrosequencing	Before Treatment (86)	S	S	2.3%
				After Treatment (127)	S	S	S
	2013 (27)	Panama	Pyrosequencing	Before Treatment (23)	S	S	S
				After Treatment (59)	S	S	S
	2004 (32)	Tanzania	Sequencing	After Treatment (71)	x	x	S
	2007 (33)	Tanzania	Real-time PCR	After Treatment (38)	S	x	S
	2013 (34)	Haiti	Pyrosequencing	After Treatment (25)	x	x	36%
	2013 (34)	Tanzania	Pyrosequencing	After Treatment (5)	x	x	S
	2016 (25)	Sri Lanka	Smart Amp2	No Treatment History (110)	S	18.2%	S
	2018 (35)	Brazil	PCR-RFLP	No details (552)	x	1.4%	1.1%
	2019 (28)	Ghana	Real-time PCR, Sequencing	Before Treatment (109)	1.7%	29%*	41%
				After Treatment (107)	5%	x	52%
	2020 (36)	Brazil	ARMS-PCR	No details (524)	S	x	x
	2021 (37)	Mozambique	Pyrosequencing	No details (15)	S	17% SNP frequency in 1 sample	S
T. trichiura	2009 (31)	Kenya	Pyrosequencing	No Treatment History (39)	x	x	46%
	2009 (31)	Panama	Pyrosequencing	After Treatment (8)	x	x	100%
	2013 (38)	Uganda	Sequencing	After Treatment (27)	S	S	S
	2013 (27)	Haiti	Pyrosequencing	Before Treatment (65)	S	26.2%	26.2%
				After Treatment (38)	S	36.8%	78.9%
	2013 (27)	Kenya	Pyrosequencing	Before Treatment (40)	S	S	51.3%
				After Treatment (90)	S	S	68.5%
	2013 (27)	Panama	Pyrosequencing	Before Treatment (19)	78.9%	S	S
				After Treatment (49)	16.3%	2.4%	11.9%
	2019 (18)	Honduras	PCR, sequencing	After Treatment (45)	S	S	S
	2021 (37)	Mozambique	Pyrosequencing	No details (15)	S	S	S
A. lumbricoides	2009 (31)	Kenya	Pyrosequencing	No Treatment History (38)	x	x	S
	2009 (31)	Panama	Pyrosequencing	After Treatment (29)	x	x	S
	2009 (31)	Uganda	Pyrosequencing	After Treatment (91)	x	x	S
	2009 (31)	Zanzibar	Pyrosequencing	After Treatment (91)	x	x	S
	2013 (27)	Haiti	Pyrosequencing	Before Treatment (37)	100%	S	S
				After Treatment (5)	100%	S	S
	2013 (27)	Kenya	Pyrosequencing	Before Treatment (22)	77.2%	S	S
				After Treatment (19)	94.8%	S	S
	2013 (27)	Panama	Pyrosequencing	Before Treatment (53)	100%	S	S
				After Treatment (70)	100%	S	S
	2017 (39)	Rwanda	PCR, sequencing	After treatment (144)	S	S	S
	2018 (35)	Brazil	PCR-RFLP	No details (601)	S	S	x
	2019 (40)	Brazil	ARMS-PCR	After Treatment (854)	x	x	0.5%
	2019 (18)	Honduras	PCR, sequencing	After Treatment (40)	S	S	S
	2021 (26)	Ethiopia	Sequencing	After Treatment (29)	S	S	S
	2021 (26)	Tanzania	Sequencing	After Treatment (77)	S	S	S

S refers to detection of only susceptible alleles in the samples tested.
x – SNP not tested.
Before Treatment, After treatment: Studies where SNP was tested before and after treatment with anthelminthic drug.
*No before/after treatment information available.

resistance. In addition to presence or absence, pyrosequencing and NGS also allow for estimates on the proportion of the worm population in a sample with SNPs and would be more useful in longitudinal follow up studies with samples collected before and after administration of treatment.

While monitoring for these known SNPs of importance in the veterinary world is a logical starting place for resistance monitoring efforts, a focus on the presence/absence of veterinary markers may not be sufficient to determine whether resistance is emerging in human parasite populations. Resistance development within human-infecting parasite populations may develop through the convergent evolution of similar SNPs but could just as easily occur as the result of genotypic changes in other positions or on other β-tubulin genes. As such, broad surveillance efforts employing less targeted approaches are required and monitoring should not be limited to those SNPs having known associations with veterinary resistance. Rather, effective surveillance will require the use of tools such as RNA sequencing or amplicon sequencing to broadly surveil transcriptional products for the presence of both identical and novel SNPs and SNP locations.

Definitive evidence of clinically relevant benzimidazole resistance has not been clearly documented in humans. However, in some regions, where continued human transmission occurs despite repeated rounds of targeted deworming, benzimidazole resistance may have emerged among different human STH species (27, 31, 41). In such settings, detection of resistance-encoding SNPs may be indicative of resistance in circulating worm populations and if resistance development mirrors patterns seen in the veterinary community, these SNPs might rapidly and irreversibly become dominant due to drug selection pressure (42). This is of concern, as prolonged MDA campaigns at suboptimal coverage could impact helminth populations and result in expansion of anthelmintic resistant worm populations (41). While it is important to remember that SNPs at the aforementioned loci have not been definitively shown to be relevant for resistance development in human-infecting species, clinically relevant resistance, brought about by intervention-mediated selective pressures, may arise by multiple other mechanisms (43). Additional research using sequencing-based approaches at the species level with a wide geographic representation is urgently needed to identify resistance determinants in the human population (should they exist) or to monitor for their development and emergence (43–45).

While generally seen with only limited or modest prevalence in human-infecting STH species, the presence of SNPs associated with benzimidazole resistance in the veterinary world remains a cause of concern among the STH community. For human hookworm infection, most studies documenting the presence of veterinary resistance markers pertain to *N. americanus* (Table 2). SNPs at codon 167 have been identified in Ghana (28), at codon 198 in Ghana (28), Brazil (35) and Sri Lanka (25) and at codon 200 in Ghana (28), Kenya (27), Haiti (34) and Brazil (35). Looking at samples collected before and after treatment, the study from Ghana showed a very modest (1.7% to 5%), but significant increase in detection among samples collected after treatment with albendazole (28). For *Trichuris* infection, where the efficacy of benzimidazoles is intrinsically lower, a study from Panama showed a decrease in resistant SNPs after treatment at codon 167 (27). However, for codon 198 and 200, studies in Haiti, Kenya and Panama showed an increase in SNP proportion with both heterozygous and homozygous populations seen (27, 31). In human *Ascaris* infections, most resistant SNPs reported have been in codon 167, and these have been reported in Haiti, Kenya and Panama (27). While studies documenting the appearance of such SNPs are important and cautionary, at present, their relevance and relationship to resistance development remains unclear.

Mitigation strategies and considerations

If community-wide deworming is adopted to interrupt transmission of STH, drug pressure within the targeted population would be dramatically increased and the proportion of the population left untreated (the refugia) would be minimized. This would decrease the pool of wild-type parasites in the community (42). As such, strategies to mitigate the potential emergence of drug resistance will be needed (44, 46). In the veterinary community, it has historically been suggested that maintaining an untreated sub-population of as little as 20% of a flock (who would harbor drug susceptible helminth populations) can significantly delay emergence and spread of resistance. Delayed resistance, resulting from these relatively low levels of untreated individuals, has been suggested as a possible reason for the lack of confirmed resistance in the human population, where non-adherence alone oftentimes ensures the existence of such a sub-population of untreated individuals (42). However, while the maintenance of an untreated sub-population may delay resistance, it is also counterproductive to efforts aimed at transmission interruption. Balancing such concerns will be critical for the success of any community-wide intervention program.

In addition to maintaining adequate refugia, there are many other lessons to be learned from the veterinary community. The need for sensitive diagnostic tools able to screen for resistance markers at the point-of-collection will be critical to managing and mitigating risk of widespread resistance development, particularly under an expanded treatment landscape. However, while technologies such as LAMP and recombinase polymerase amplification continue to increase the feasibility of field-friendly testing, adequate tools are difficult to envision until a clear definition of resistance markers in the human-infecting parasite community has been defined. Exploring the use of novel deworming agents, repurposing older drugs, the use of combination drug therapies, and a focus on the avoidance of underdosing will also play important

roles in future efforts to avoid the development of widespread resistance within the human-parasite community. Innovative strategies to reduce environmental contamination will also be critical (23, 47) as will integration of community-directed approaches and engagement to affect behavioral change to improve equitable coverage and adherence with MDA and WASH uptake.

Adequate consideration of animal reservoirs of infection and parasite hybridization events are also likely to prove critical if resistance development is to be delayed/avoided. Evidence of the interbreeding of *Ascaris suum* and *A. lumbricoides* presents a cautionary tale of the need to take a One-Health approach to intervention strategies (48). While zoonotic reservoirs and/or interbreeding parasite populations present significant risk for the development of and lateral transfer of resistance, it is also possible that in some instances animal reservoirs could serve as an untreated sub-population, providing beneficial refugia. Consideration of such factors may prove critical for both the success of future intervention efforts and to avoid the development of resistance in STH species affecting humans.

Author contributions

SA, NP and JW conceptualized the review, MM carried out the literature review, SA and NP drafted the manuscript which was reviewed and finalized by all authors. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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