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Considerations for anthelmintic resistance emergence in hookworm at a single locus

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ABSTRACT

Over 800 million people are infected with hookworms around the world. Hookworms of the genus *Ancylostoma* and *Necator* are examples of nematodes that harbor the ability to enter a host by penetrating the skin, and after entry the infective larvae migrate to the small intestine where they encounter host-specific signals that initiate developmental pathways and culminate in maturation to the adult stage. Currently no vaccine is available for the treatment of hookworm infection. The control strategy is limited to anthelmintic drugs, which run the risk of losing efficacy as resistance grows. Genetic resistance has developed against all classes of anthelmintic drugs against livestock parasites, and recently markers of anthelmintic resistance in human hookworm populations have been reported. As anthelmintic resistance develops in human populations of hookworm, new drugs and novel control methods like vaccines will be required in the future to control hookworm transmission. This review outlines how population genetics and anthelmintic resistance could interact at a single locus to influence current control strategies.

1. Introduction

Hookworm parasites cause one of the world's most infectious diseases, with over 800 million people infected (Hotez et al, 2006, 2014). Heavy hookworm infection can result in debilitating, and in some cases, fatal iron deficiency. This is especially devastating in growing children causing developmental delays and cognitive impairment. Pregnant women, and elderly populations are also at high risk for morbidity (Bethony et al., 2002; Pasricha et al., 2008). Using disability adjusted life years (DALYs) as a measure of disease burden, soil-transmitted nematodes including hookworm cause the loss of more than 4 million DALYs, which is the highest morbidity of any parasitic disease except for malaria (Brooker et al., 2004; Hotez et al., 2004; Lopez & Murray, 1998). Infective hookworm larvae enter the human host through the skin and migrate to the lungs within 7–10 days which will result in coughing that expels hookworm larvae from the lungs and allows the nematodes to be swallowed. From here, larvae will end up in the small intestine where they will mature into adults (Hawdon & Hotez, 1996) (Fig. 1). The principal pathology of hookworm infection then begins inside the intestine as a result of blood loss from feeding adults that ultimately causes iron deficiency. The resulting morbidity can be moderate or severe depending on worm burden, species, patient history and underlying conditions. The current control strategy for hookworm is restricted to anthelmintic drugs to control transmission (Montresor, 2012). Hookworm control is most effective *via* mass drug administration (MDA) which runs the risk of selecting populations of parasites harboring resistance alleles, and thereby losing efficacy as resistance grows (Bethony et al., 2002). A recent report described the presence of genetic markers of resistance to the anthelmintic benzimidazole in human hookworm populations in Ghana (Orr et al., 2019). These markers are located in a gene encoding the isotype-1 β-tubulin which polymerize into microtubules to form the principal component of the cellular cytoskeleton. Common markers of anthelmintic resistance in hookworm species include F167Y, E198A and F200Y (Jimenez Castro et al., 2019; Kitchen et al., 2019; Orr et al., 2019) (Fig. 2). Parasitic nematodes of livestock have developed resistance to all classes of anthelmintic drugs (Kaplan, 2004), suggesting that continued practices of mass administration could yield a similar fate for human hookworm populations also. In fact, one of the genetic markers (F167Y) identified in human hookworm populations in Ghana (Orr et al., 2019) had recently been observed in a naturally occurring strain of the canine hookworm, *Ancylostoma caninum* (Jimenez Castro et al., 2019; Kitchen et al., 2019), providing compelling evidence for how patterns of resistance in veterinary parasites can emerge in related human parasites. The F167Y mutation is the result of a SNP (TTC/Phe→TAC/Tyr) in the isotype-1 β-tubulin gene located at codon 167, and is associated with

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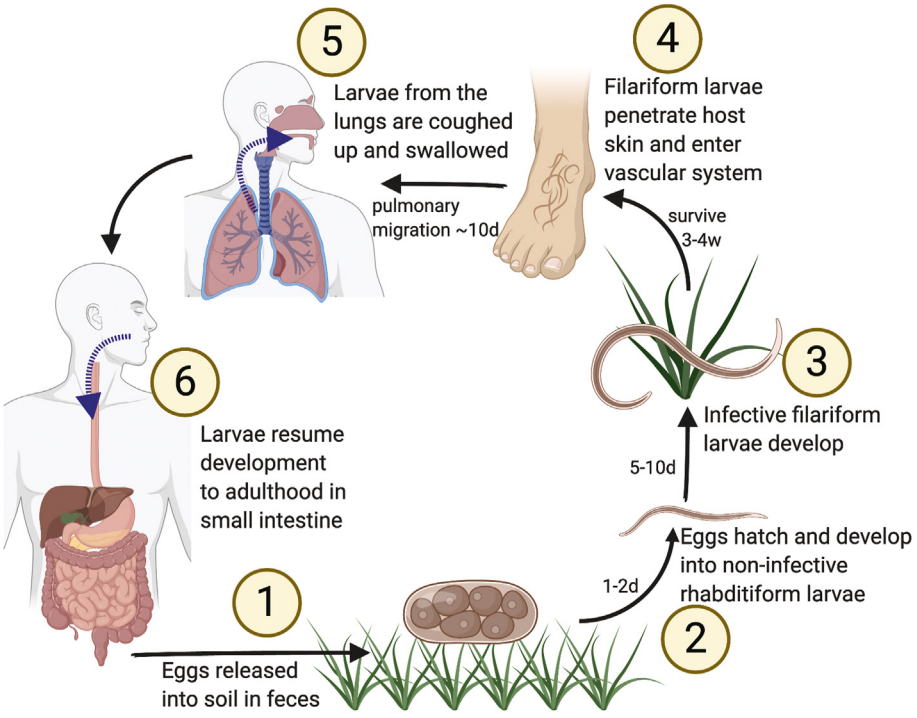

Fig. 1 Summary of the hookworm life-cycle. Hookworm eggs are released in human feces where they hatch and develop in the soil into non-parasitic rhabditiform larvae. The third-stage larva is the infective stage which can be found on blades of grass waiting for the correct host. They can survive at this stage for 3–4 weeks, and when a human host is encountered, will penetrate through the skin, typically at the feet, although it is common for them to enter through the buttocks or abdomen also (Hotez et al., 2004). After host-entry, the larvae will enter the bloodstream and begin a pulmonary migration that takes 7–10 days. Secretions from the lungs that include the larvae will result in coughing that expels larvae from the lungs and results in larvae being swallowed, where they end up in the small intestine and mature into adults. This figure was created with BioRender.com.

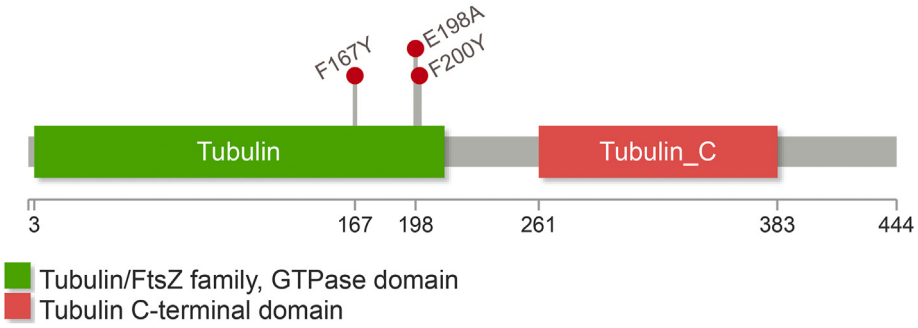

Fig. 2 Structure of the isotype-1 β tubulin protein and location of common variants from hookworm species. Protein domains are from Pfam (Finn et al., 2014), and common hookworm variants (Jimenez Castro et al., 2019; Kitchen et al., 2019; Orr et al., 2019) depicted using lollipops (Jay & Brouwer, 2016).

benzimidazole resistance in parasitic nematodes. Furthermore, F167Y has been shown to confer resistance in *Caenorhabditis elegans* by using CRISPR/Cas9 to engineer the corresponding mutation in the orthologous *C. elegans* gene, *ben-1* (Kitchen et al., 2019).

2. Modelling the emergence of resistance

Building on from these data, this review explores possible patterns of anthelmintic emergence at a single locus and models the resulting effect on current control practices. All experiments described herein are based on a previously described deterministic model which describes the worm burden distribution for cohorts of the population using a negative binomial with a fixed aggregation parameter (Coffeng et al., 2017). The model is based on empirical data collected from a community-intervention trial conducted in Tamil Nadu, India (Sarkar et al., 2017). Here, the model examines two treatment age groups: Group 1 (pre-school aged children (pre-SAC), SAC, and adults); Group 2 (pre--SAC and SAC). Group 1 represents the target for MDA which is most effective in terms of hookworm control on account of worm burden increasing with age specifically for hookworm (Bethony et al., 2002). To determine mean egg production from worm burden, the following term is used:

$$\lambda n e^{-\gamma n}$$

where λ is the maximum mean number of eggs per gram of feces per female worm ($\lambda = 200$), γ is the density-dependent fecundity ($\gamma = 0.02$) and n is the number of females in a host (Truscott et al., 2016). All output data from the model is parsed using in house bash and Perl scripts, and all plots generated in R ver. 3.6.1.

To examine genetic resistance, it was assumed that resistance arose in response to a single anthelmintic at a biallelic locus represented by three possible genotypes: homozygous susceptible (*ss*), homozygous resistant (*rr*) and a heterozygote (*rs*) (Fig. 3A–B). Parsing the model into Group 1 and Group 2 highlights the efficacy of MDA with Group 1 and reveals significantly less variation once resistance frequency predominates. To arrive at these genotypes, we consider the frequency of mutation to be a function of the population size (N) and consider a neutral mutation rate (μ). Altering μ from low (Fig. 3C) to high (Fig. 3D) demonstrates how polymorphic populations increase anthelmintic resistance and significantly weaken MDA. Polymorphism has been shown to be more likely when the difference in resistance conferred by alternate alleles is significant (Antonovics & Thrall, 1994), which may be reasonable in the case of isotype-1 β-tubulin mutations that exhibit significant heterogeneity and effect variation (Hahnel et al., 2018; Kwa et al., 1995). The

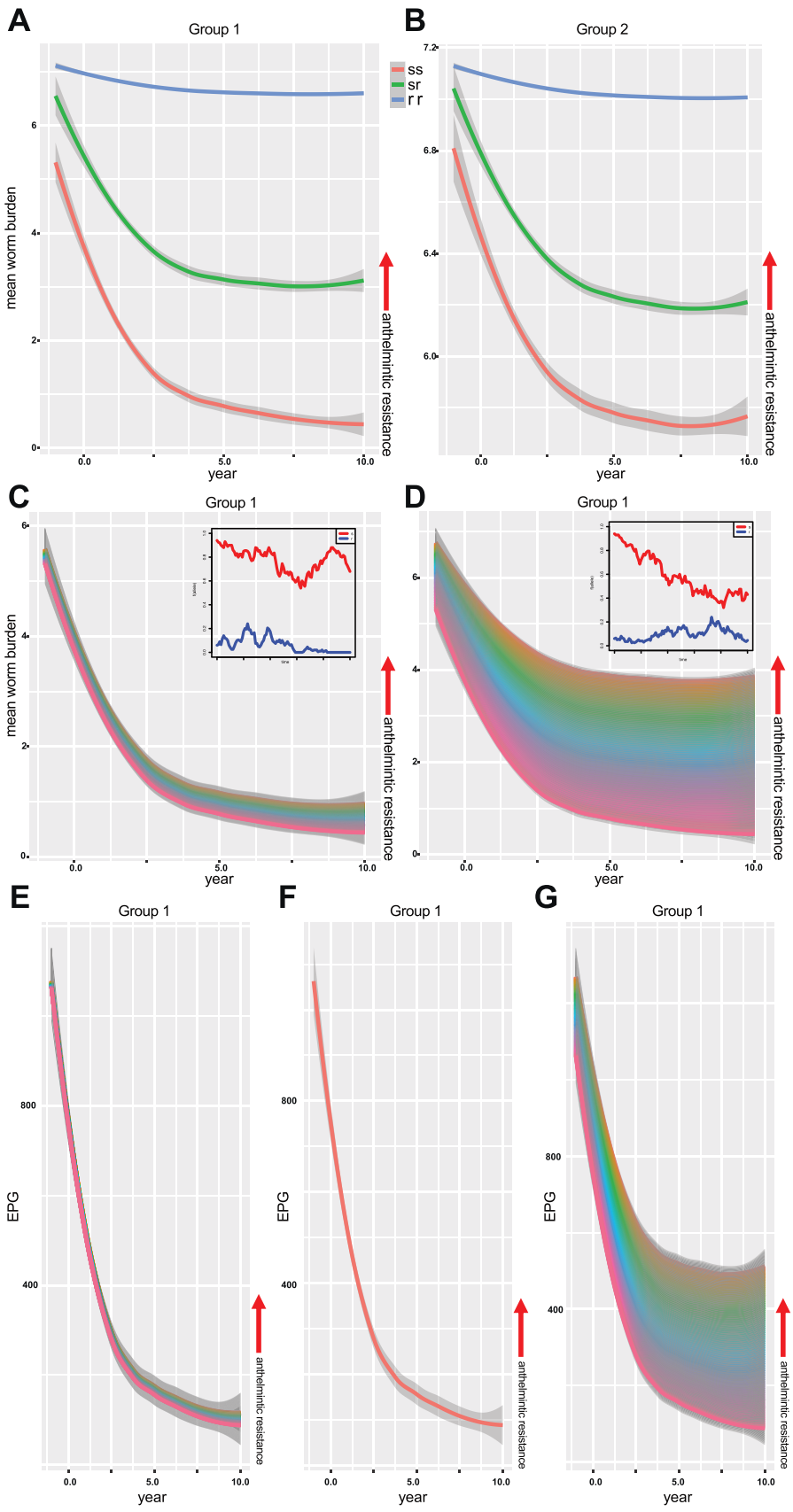

Fig. 3 Changes in mean worm burden during an annual single drug treatment regimen over a 10-year time span in sensitive (*ss*), resistant (*rr*) and heterozygous genotypes (*rs*) for pre-SAC, SAC, and adults (Group 1) (A) and pre-SAC and SAC groups (Group 2) (B). C–D: Effect of lower mutation rate (C) and increased mutation rate on mean worm burden and anthelmintic resistance (D). Insets depict frequency for resistance (*r*) and sensitive (*s*) alleles. E–G: Modelling genotype fitness for *rr* (E), *ss* (F), and *rs* (G) as a function of eggs per gram (EPG) to infer emerging anthelmintic resistance.

F167Y mutation was found to be fixed in the population of a naturally occurring strain of canine hookworm that was described recently (Kitchen et al., 2019). The fixation probability (Kimura, 1962) in this case is dependent on the selective advantage of the resistance allele, where *s* is the fitness benefit or selective advantage, the fixation probability (*p*) is then equal to:

$$p = \frac{1 - e^{-2s}}{1 - e^{-4N_e s}}$$

Within-host competition can have substantial effects on the emergence of resistance, wherein high-transmission groups can interrupt the emergence of resistance in contrast to low-transmission populations (Bushman et al., 2018), and similarly host-type (i.e. dog *versus* human) will alter resistance emergence rates. However, by including minimal fitness benefits into the model we can observe large effects on allele frequency and MDA efficacy (Fig. 3E–G). When *rr* (Fig. 3E) or *ss* (Fig. 3F) confers minimal fitness benefit there is a significant effect on the emergence of resistance in the population over the 10-year forecast (Fig. 3E). When *rs* confers a minimal fitness benefit (Fig. 3G), a more dramatic effect is revealed resulting in resistance rapidly emerging across the population, and significantly altering eggs per gram (EPG) as well as preventive chemotherapy efficacy, suggesting that heterozygote fitness is a key determinant in emerging resistance. Recent work using *C. elegans* has described quantitative differences in fitness for β-tubulin alleles which contribute to anthelmintic resistance (Dilks et al., 2020), and therefore, consideration of allelic fitness during surveillance should be a guiding factor when designing mass treatment campaigns.

Declaration of competing interests

The author has no competing interests to declare.

References

Antonovics, J., Thrall, P.H., 1994. The cost of resistance and the maintenance of genetic polymorphism in host-pathogen systems. Proc. Royal Soc. London. Series B: Biol. Sci. 257, 105–110. https://doi.org/10.1098/rspb.1994.0101.

Bethony, J., Chen, J., Lin, S., Xiao, S., Zhan, B., Li, S., et al., 2002. Emerging patterns of hookworm infection: Influence of aging on the intensity of *Necator* infection in Hainan Province, People's Republic of China. Clin. Infect. Dis. 35, 1336–1344. https://doi.org/10.1086/344268.

Brooker, S., Bethony, J., Hotez, P.J., 2004. Human hookworm infection in the 21st Century. Adv. Parasitol. 58, 197–288. https://doi.org/10.1016/S0065-308X(04)58004-1.

Bushman, M., Antia, R., Udhayakumar, V., de Roode, J.C., 2018. Within-host competition can delay evolution of drug resistance in malaria. PLoS Biol. 16, e2005712 https://doi.org/10.1371/journal.pbio.2005712.

Coffeng, L.E., Truscott, J.E., Farrell, S.H., Turner, H.C., Sarkar, R., Kang, G., et al., 2017. Comparison and validation of two mathematical models for the impact of mass drug administration on *Ascaris lumbricoides* and hookworm infection. Epidemics 18, 38–47. https://doi.org/10.1016/j.epidem.2017.02.001.

Dilks, C.M., Hahnel, S.R., Sheng, Q., Long, L., McGrath, P.T., Andersen, E.C., 2020. Quantitative benzimidazole resistance and fitness effects of parasitic nematode beta-tubulin alleles. Int. J. Parasitol. Drugs Drug Resist. 14, 28–36. https://doi.org/10.1016/j.ijpddr.2020.08.003.

Finn, R.D., Bateman, A., Clements, J., Coggill, P., Eberhardt, R.Y., Eddy, S.R., et al., 2014. Pfam: The protein families database. Nucleic Acids Res. 42, D222–D230. https://doi.org/10.1093/nar/gkt1223.

Hahnel, S.R., Zdraljevic, S., Rodriguez, B.C., Zhao, Y., McGrath, P.T., Andersen, E.C., 2018. Extreme allelic heterogeneity at a *Caenorhabditis elegans* beta-tubulin locus explains natural resistance to benzimidazoles. PLoS Pathog. 14, e1007226 https://doi.org/10.1371/journal.ppat.1007226.

Hawdon, J.M., Hotez, P.J., 1996. Hookworm: Developmental biology of the infectious process. Curr. Opin. Genet. Dev. 6, 618–623. https://doi.org/10.1016/s0959-437x(96)80092-x.

Hotez, P.J., Alvarado, M., Basáñez, M.-G., Bolliger, I., Bourne, R., Boussinesq, M., et al., 2014. The global burden of disease study 2010: Interpretation and implications for the neglected tropical diseases. PLoS Negl. Trop. Dis. 8, e2865 https://doi.org/10.1371/journal.pntd.0002865.

Hotez, P.J., Brooker, S., Bethony, J.M., Bottazzi, M.E., Loukas, A., Xiao, S., 2004. Hookworm infection. N. Engl. J. Med. 351, 799–807. https://doi.org/10.1056/NEJMra032492.

Hotez, P.J., Bundy, D.A.P., Beegle, K., Brooker, S., Drake, L., de Silva, N., et al., 2006. Helminth infections: Soil-transmitted helminth infections and schistosomiasis. In: Jamison, D.T., Breman, J.G., Measham, A.R., Alleyne, G., Claeson, M., Evans, D.B., Jha, P., Mills, A., Musgrove, P. (Eds.), Disease control priorities in developing countries. World Bank, Washington (DC).

Jay, J.J., Brouwer, C., 2016. Lollipops in the clinic: Information dense mutation plots for precision medicine. PLoS One 11, e0160519. https://doi.org/10.1371/journal.pone.0160519.

Jimenez Castro, P.D., Howell, S.B., Schaefer, J.J., Avramenko, R.W., Gilleard, J.S., Kaplan, R.M., 2019. Multiple drug resistance in the canine hookworm *Ancylostoma caninum*: An emerging threat? Parasit. Vectors 12, 576. https://doi.org/10.1186/s13071-019-3828-6.

Kaplan, R.M., 2004. Drug resistance in nematodes of veterinary importance: A status report. Trends Parasitol. 20, 477–481. https://doi.org/10.1016/j.pt.2004.08.001.

Kimura, M., 1962. On the probability of fixation of mutant genes in a population. Genetics 47, 713–719.

Kitchen, S., Ratnappan, R., Han, S., Leasure, C., Grill, E., Iqbal, Z., et al., 2019. Isolation and characterization of a naturally occurring multidrug-resistant strain of the canine hookworm, *Ancylostoma caninum*. Int. J. Parasitol. 49, 397–406. https://doi.org/10.1016/j.ijpara.2018.12.004.

Kwa, M.S.G., Veenstra, J.G., Van Dijk, M., Roos, M.H., 1995. β-tubulin genes from the parasitic nematode *Haemonchus contortus* modulate drug resistance in *Caenorhabditis elegans*. J. Mol. Biol. 246, 500–510. https://doi.org/10.1006/jmbi.1994.0102.

Lopez, A.D., Murray, C.C.J.L., 1998. The global burden of disease, 1990–2020. Nat. Med. 4, 1241–1243. https://doi.org/10.1038/3218.

Montresor, A., 2012. WHO. Eliminating soil-transmitted helminthiases as a public health problem in children. World Health Organization, Geneva. http://www.who.int/intestinal_worms/resources/9789241503129/en/. (Accessed 16 December 2020).

Orr, A.R., Quagraine, J.E., Suwondo, P., George, S., Harrison, L.M., Dornas, F.P., et al., 2019. Genetic markers of benzimidazole resistance among human hookworms (*Necator americanus*) in Kintampo North Municipality, Ghana. Am. J. Trop. Med. Hyg. 100, 351–356. https://doi.org/10.4269/ajtmh.18-0727.

Pasricha, S.-R., Caruana, S.R., Phuc, T.O., Casey, G.J., Jolley, D., Kingsland, S., et al., 2008. Anaemia, iron deficiency, meat consumption and hookworm infection in women of reproductive age in Northwest Vietnam. Am. J. Trop. Med. Hyg. 78, 375–381.

Sarkar, R., Rose, A., Mohan, V.R., Ajjampur, S.S.R., Veluswamy, V., Srinivasan, R., et al., 2017. Study design and baseline results of an open-label cluster randomized community-intervention trial to assess the effectiveness of a modified mass deworming program in reducing hookworm infection in a tribal population in southern India. Contemp. Clin. Trials Commun. 5, 49–55. https://doi.org/10.1016/j.conctc.2016.12.002.

Truscott, J.E., Turner, H.C., Farrell, S.H., Anderson, R.M., 2016. Chapter 3. Soil-transmitted helminths: Mathematical models of transmission, the impact of mass drug administration and transmission elimination criteria. Adv. Parasitol. 94, 133–198. https://doi.org/10.1016/bs.apar.2016.08.002.