

Hookworm Vaccines

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Hookworm infection caused by the soil-transmitted nematodes *Necator americanus* and *Ancylostoma duodenale* is one of the most common parasitic infections worldwide. Although not directly responsible for substantial mortality, it causes significant morbidity in the form of chronic anemia and protein malnutrition. Current global control efforts based on periodic mass anthelmintic administration are unsustainable, and new control strategies must be developed. This review describes progress in the development of vaccines against hookworm infection, including the preclinical and initial clinical testing of the *N. americanus* Ancylostoma Secreted Protein–2 Hookworm Vaccine. Plans call for eventual development of a vaccine that will combine at least 2 hookworm antigens—one targeting the larval stage of the life cycle and another targeting the adult worm living in the gastrointestinal tract.

Human hookworm infection is caused by the soil-transmitted nematode helminths *Necator americanus* and *Ancylostoma duodenale* and is one of the most important parasitic infections worldwide, with up to 740 million persons currently infected [1]; most of these infected people live in impoverished rural areas of the developing world. Hookworm infection does not directly account for substantial mortality; instead, its public health impact comes from the chronic anemia and protein malnutrition caused by severe infection, which can result in impaired physical and intellectual development in children and poor outcomes for pregnant women and their newborns. Current global control efforts rely on the repeated mass administration of anthelmintic drugs, particularly to children, although significant concern regarding the sustainability of this strategy has prompted the search for new approaches to disease control, including the development of a hookworm vaccine [2].

LIFE CYCLE

Hookworm infection occurs when third-stage infective filariform larvae (L3) penetrate exposed skin (figure 1). Larvae subsequently migrate into subcutaneous venules and lymphatics

to gain access to the host's circulation; enter the pulmonary capillaries, where they penetrate into the alveolae; ascend the bronchial tree; traverse the epiglottis; and are swallowed into the gastrointestinal tract, where they develop into adult worms 5–9 weeks after skin penetration.

Adult *Necator* and *Ancylostoma* hookworms parasitize the proximal small intestine, where they live for ~5 years or ~1 year, respectively. Hookworms attach to the intestinal mucosa and secrete enzymes that enable them to invade submucosal tissues and ingest villous tissue and blood. Hemoglobinas within the hookworm digestive canal enable degradation of human hemoglobin for use as an essential nutrient source [2].

After mating in the intestinal tract, female adult worms produce thousands of eggs per day, which are expelled with the feces. Eggs hatch in warm, moist soil, giving rise to rhabditiform larvae, which undergo several molts before developing into motile L3. The motile L3 then seek higher ground to increase the chance of contact with human skin and to complete the life cycle.

EPIDEMIOLOGY AND BURDEN OF DISEASE

Hookworm infection is widespread throughout the tropics and subtropics (figure 2). *N. americanus* is the most prevalent hookworm, being found throughout sub-Saharan Africa, tropical regions of the Americas, south China, and Southeast Asia, whereas *A. duodenale* is more focally endemic in parts of India, China, Africa, and a few regions of the Americas. The prevalence of hookworm infection increases markedly between the ages of 6 and 10 years and then plateaus during adolescence

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Life Cycle of *Necator americanus*

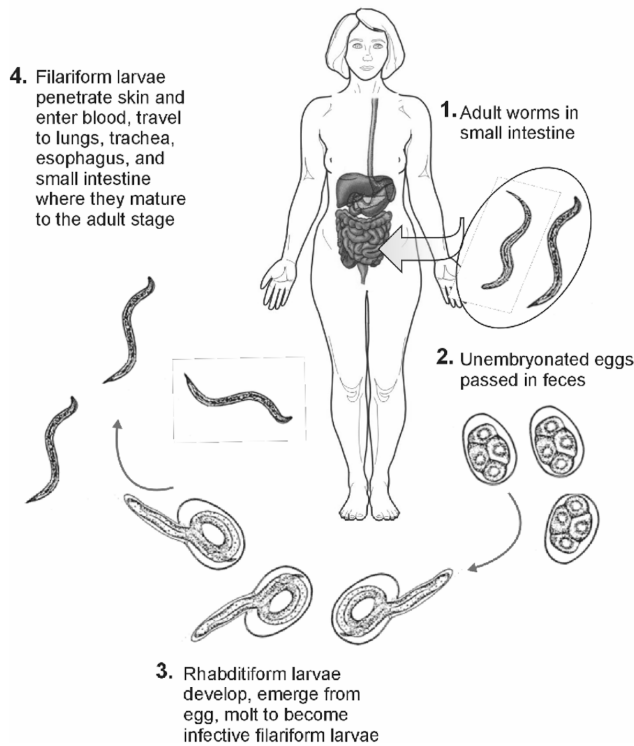


Figure 1. Life cycle of the hookworm *Necator americanus* (reprinted with permission from Nappi and Vass [3]).

and adulthood. In contrast, mean intensity of infection—measured as the number of eggs per gram of feces—increases progressively with age, possibly because of cumulative exposure over time and physiological changes associated with aging [5]. Furthermore, the distribution of infections in communities of endemicity is overdispersed, with a minority of individuals harboring a majority of the worms [6–8]. The reasons for this are unknown, although both genetics and common exposure are possibilities.

Hookworm infection causes more disability than death. Although difficult to ascertain, it is estimated that, worldwide, ~65,000 deaths annually can be directly attributed to infection [9]. However, when the significant long-term consequences of hookworm-associated malnutrition and anemia are incorporated into global disease burden estimates, hookworm infection may account for the loss of up to 22 million disability-adjusted life-years annually [10].

The pathology of hookworm infection is caused by intestinal blood and protein loss, which is proportional to worm burden; however, no numerical threshold exists that is associated with disease, because this is dependent on the host's underlying nutritional status. Clinical disease occurs when infection-associated blood loss exceeds host nutritional re-

serves, resulting in iron deficiency anemia. Because children and women of reproductive age have reduced iron reserves, both are at particular risk. In children, anemia and protein malnutrition resulting from chronic intestinal parasitism can cause impairment in physical, intellectual, and cognitive development [11, 12]. Moreover, severe iron deficiency anemia arising from hookworm disease during pregnancy can result in adverse consequences for the mother, her unborn fetus, and the neonate [13, 14].

DISEASE CONTROL STRATEGIES

Current control efforts focus on the use of anthelmintic chemotherapy to reduce morbidity in areas of endemicity. Annual or semi-annual mass administration of mebendazole or albendazole (2 members of the benzimidazole class of drugs) to school-aged children reduces the intensity of infection to levels not associated with disease. Benefits of regular deworming of school-aged children include improved iron stores, physical development and fitness, cognitive performance, and school attendance [15, 16]. Pregnant women, if treated once or twice during pregnancy, also achieve significant reductions in the incidence of maternal anemia and delivery of low birth-weight babies, as well as improved infant survival at 6 months after delivery [17].

However, periodic mass anthelmintic administration does not interrupt hookworm transmission or eliminate the parasite from areas of endemicity because of the variable efficacy of benzimidazole drugs against hookworm [18], rapid reinfection after mass treatment [19], and diminished efficacy of benzimidazole drugs with repeated use [20]. Worryingly, resistance to benzimidazoles following ubiquitous use has been observed in veterinary medicine [21, 22]. The paucity of new anthelmintic drugs under development has prompted research into alternative control methods, such as an effective vaccine that provides long-term protection and that could potentially interrupt transmission and prevent hookworm disease. Because *N. americanus* is the predominant hookworm worldwide, vaccine development has thus far focused entirely on this species.

RATIONALE FOR A HOOKWORM VACCINE

Several factors suggest that the natural immune response to hookworm infection is inadequate and does not confer protective immunity [23]. First, hookworms can survive in humans for years [24, 25], suggesting that they evade the immune system. Interference with and modulation of the host immune response occur from the very first events of infection, enabling hookworms to direct the immune response in a way that is amenable to parasite survival [26]. In addition, the rapid reinfection that often occurs following anthelmintic treatment and the observation that both the prevalence and intensity of

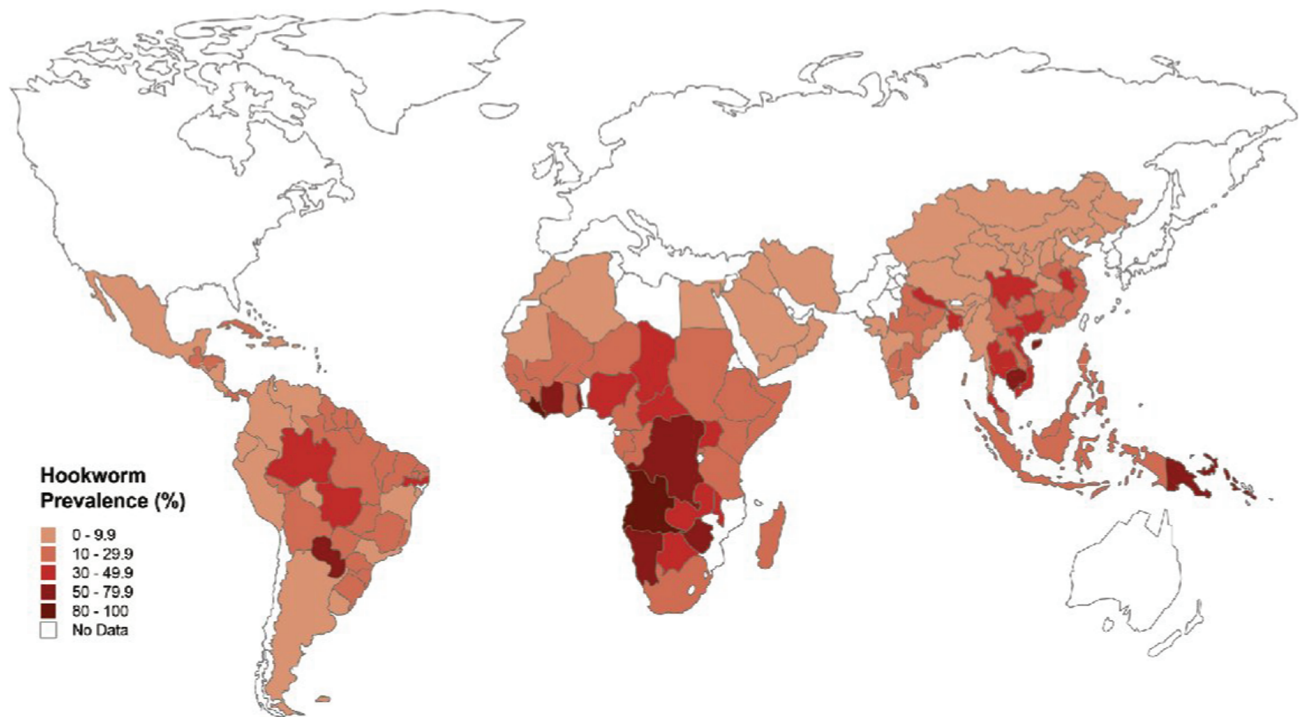


Figure 2. Prevalence of hookworm infection worldwide. Illustration: Margaret Shear, Public Library of Science [1, 4].

infection increase with age imply that previous infection does not confer protective immunity [5, 27–29].

The failure of individuals living in areas of endemicity to develop protective immunity despite frequent exposure suggests that successful vaccine development will be more challenging than the case was for existing viral and bacterial vaccines. However, as early as the 1930s, a vaccine containing live *A. caninum* larvae protected laboratory dogs against challenge infections (reviewed in Loukas et al. [2] and Hotez et al. [30]). Although sterilizing immunity was not achieved, vaccinated canines did not develop anemia, despite receiving challenges of several thousand L3. A vaccine consisting of irradiation-attenuated L3 (irL3) was later developed and also resulted in significant reductions in hookworm burden after challenge infection [31–33], prompting the production of a commercial canine hookworm vaccine consisting of live *A. caninum* irL3, which was marketed in the United States during the 1970s [32, 34]. Although it provided high levels of protection against disease due to *A. caninum*, the vaccine was discontinued 3 years after licensure because of high production costs, challenging storage requirements (at a temperature of 8°–10°C), a short shelf-life, and lack of sterilizing immunity.

The protection obtained by vaccination with live L3 and irL3 is primarily antibody mediated, as demonstrated by the protection of nonvaccinated dogs by passive transfer of serum samples obtained from dogs immunized with either *A. caninum* L3 or irL3 [31, 35, 36]. Recently, it was shown that the pro-

tection induced by the *A. caninum* irL3 vaccine is associated with Th2-type responses against specific antigens secreted by invading L3 [37].

The overall goal of the hookworm development program is to produce a vaccine that would reduce the likelihood of vaccinated individuals developing severe hookworm infections and, thus, reduce blood and nutrient loss to a level not associated with clinical disease. Therefore, sterilizing immunity is not required for a vaccine that would still have a significant impact on reducing the burden of disease.

ANTIGEN DISCOVERY AND SELECTION

Because of the inherent difficulties in producing and using an irL3 vaccine for humans, focus has shifted to identifying antigens that are essential for hookworm development and survival in the human host and developing these as subunit vaccines. On the basis of the prior work done on L3 vaccines, identification of larval antigens was the first target of hookworm vaccine antigen discovery. However, because the canine L3 and irL3 vaccines are unable to induce complete protection against hookworm infection, it is unlikely that a vaccine based solely on ≥ 1 larval antigen would suffice for a human vaccine. In this event, some L3 would still reach the gastrointestinal tract and develop into blood-feeding adult hookworms. Therefore, the approach has been to develop vaccines that would target both the larval and adult stages of the hookworm life cycle

(table 1). Work has proceeded to identify specific antigens produced by L3 that might be determinants of the protection afforded by the whole larvae canine vaccines. In addition, a parallel antigen discovery program has been initiated to identify macromolecules that are required by adult hookworms to use human blood as a food source and that are essential to worm survival (reviewed in Hotez et al. [38]). A hookworm vaccine containing at least 2 components, one targeting the larval stage and the other targeting the adult stage of the hookworm life cycle, is the eventual goal (figure 3).

Larval antigens. Incubating hookworm L3 in vitro with serum to mimic the milieu encountered during invasion of host tissues prompts the release of 3 main products: a metalloprotease [39, 40] and 2 proteins that are members of the pathogenesis-related protein superfamily, *Ancylostoma* Secreted Protein (ASP)–1 and ASP-2 [41–44]. Although the function of the ASPs is unknown, their crystallographic structure on radiograph suggests possible roles in proteolysis and host immunomodulation [43]. First described in *A. caninum*, these proteins have also been isolated from the secreted products of *N. americanus* [43–45].

The 3 major products secreted by activated L3 have all been successfully produced as recombinant proteins in a variety of expression systems. Two surface proteins, *Ac-SAA-1* and *Ac-16*, have also shown promise as potential vaccine antigens [46, 47]. *Ac-16*, although expressed during all stages of hookworm development, is an immunodominant larval surface protein that has been shown to reduce egg counts and blood loss in vaccinated dogs [46].

N. americanus ASP-2 (*Na-ASP-2*) was chosen as the most promising potential larval component of a vaccine targeting this hookworm and was advanced into clinical development on the basis of several pieces of evidence (reviewed in Loukas et al. [2]), including studies in dogs vaccinated with *A. caninum* irL3 that demonstrated that ASP-2 is the predominant antigen to which the protective antibody response is directed [37]. In addition, when recombinant *A. caninum* or *Ancylostoma ceylanicum* ASP-2 were used to vaccinate dogs or hamsters, re-

spectively, high levels of antigen-specific IgG were elicited, as were high levels of protection after challenge with live L3, in terms of reduction in adult worm burdens, fecal egg counts, and host blood loss, when compared with control animals [48–50]. Anti-ASP-2 IgG from vaccinated animals also inhibited the in vitro migration of larvae through tissue [48, 51]. Finally, studies of populations living in areas of Brazil and China where hookworm infection is endemic demonstrated that anti-ASP-2 antibodies are associated with a reduced risk of acquiring severe hookworm infection [48].

It is postulated that vaccines targeting the larval stage of the hookworm life cycle work by eliciting IgG that inhibit larval invasion or attenuate larval development, thereby reducing the number of invading L3 that mature into adult worms (which inhabit the host's gastrointestinal tract), resulting in reduced host worm burdens, fecal egg counts, and intestinal blood loss [2].

Adult hookworm antigens. After ingestion by an adult hookworm that has attached to the intestinal mucosa of an infected host, erythrocytes are ruptured in the worm's digestive tract, releasing free hemoglobin that is degraded by a proteolytic cascade [52]. Several of the proteins responsible for this degradation, which is essential to the worm's nutrition and survival, have been identified. Vaccination with such antigens induces antibodies that can be ingested with a blood meal and then interfere with the parasite's digestive pathway, thus impairing worm survival and fecundity.

Antigen discovery efforts have focused on 3 candidates: 2 are hemoglobinsases that line the parasite digestive tract [52], and the third is a glutathione S-transferase that detoxifies heme [53]. The aspartic protease-hemoglobinase APR-1 and the cysteine protease-hemoglobinase CP-2 have both shown promise as protective antigens in laboratory dogs in terms of reduced host blood loss and fecal egg counts and, in the case of APR-1, reduced worm burden [54, 55]. Efforts are now underway to develop at least 1 of these antigens as a recombinant protein for testing in clinical trials and eventual combination with a larval-stage antigen.

Table 1. Candidate hookworm vaccine antigens.

Vaccine antigen	Size, kDa	Stage of infection	Function	Possible mechanism	Intended effect	Stage of development
<i>Na-ASP-2</i>	21.3	Larval	Possible chemotaxin mimic	Neutralizing antibody	Attenuates larval migration through tissue	Phase 1
<i>Na-APR-1</i>	47.9	Adult	Aspartic protease-hemoglobinase	Neutralizing antibody	Blocks hemoglobinsases lining parasite digestive tract	Preclinical
<i>Na-GST-1</i>	23.7	Adult	Glutathione S-transferase	Neutralizing antibody	Blocks detoxification of host heme	Preclinical
<i>Na-CP-2</i>	36.3	Adult	Cysteine protease-hemoglobinase	Neutralizing antibody	Blocks hemoglobinsases lining parasite digestive tract	Antigen discovery
<i>Ac-16</i>	16.0	Larval and adult	Immunodominant surface antigen	Neutralizing antibody	Attenuates larval migration through tissue	Preclinical
<i>Ac-SAA-1</i>	16.0	Larval and adult	Immunodominant surface antigen	Neutralizing antibody	Attenuates larval migration through tissue	Antigen discovery

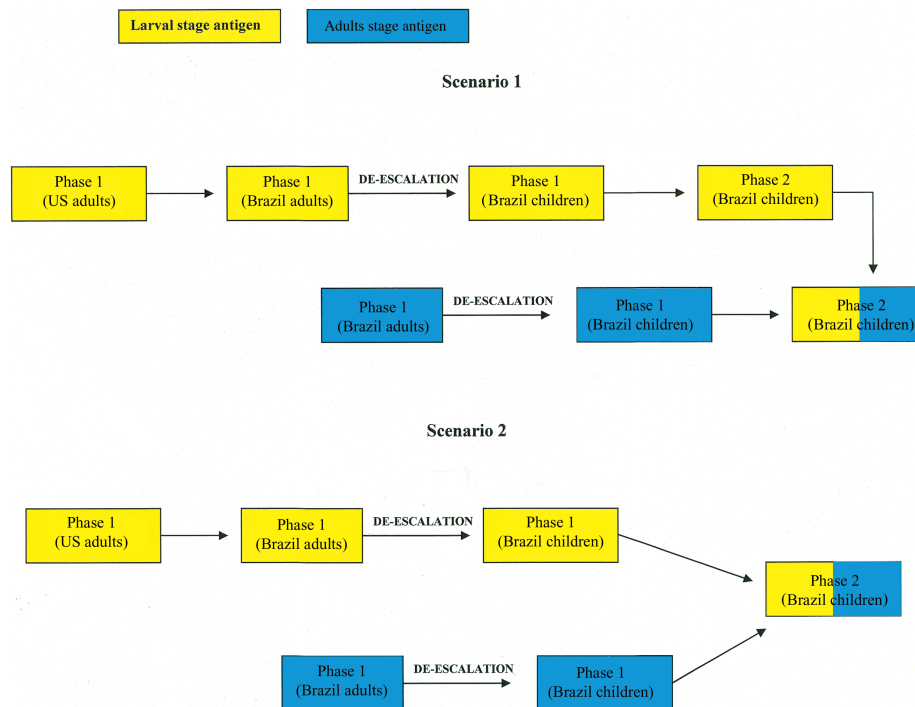


Figure 3. Possible scenarios for the clinical development of a hookworm vaccine with antigens from the larval and adult stages of *Necator americanus* infection. The clinical trials leading to a phase 2 study could be performed following completion of a proof-of-concept (phase 2) study of *N. americanus* Ancylostoma Secreted Protein-2, or the 2 antigens could be studied together in a phase 2 trial.

DEVELOPMENT OF NA-ASP-2

Na-ASP-2 has been successfully expressed in the yeast *Pichia pastoris* and purified by a combination of ion-exchange and gel-filtration chromatography [45]. Recombinant *Na*-ASP-2 is a 21.3-kDa protein containing an N-terminal 6-amino acid vector tag (EAEAEAF) to facilitate expression and increase yield. Antibodies from laboratory animals vaccinated with recombinant ASP-2 recognize the native protein from crude antigen extracts of L3 in immunoprecipitation experiments and inhibit larval migration in vitro [45]. Moreover, radiographic crystallographic analysis revealed that the recombinant protein contains the same pathogenesis-related-1 domain as native ASP-2 [43]. Taken together, these data suggest that recombinant *Na*-ASP-2 is correctly folded and displays conformational epitopes in a manner similar to that of the native molecule.

Alhydrogel (Biosector) was chosen as the adjuvant because of its excellent safety profile and evidence that *Na*-ASP-2 adsorbed to it was immunogenic in laboratory animals [45, 51]. Following manufacture and lot release, an “Investigational New Drug” application was submitted to the US Food and Drug Administration in December 2004.

A phase 1 study evaluating the safety and immunogenicity of *Na*-ASP-2/Alhydrogel in healthy adults without evidence of hookworm infection and living in the United States was conducted from 2005 through 2006. Thirty-six adults were enrolled

in this randomized, double-blind, placebo-controlled study of 3 different concentrations of *Na*-ASP-2 (10, 50, and 100 μ g) adjuvanted with 1.5 mg of Alhydrogel. Participants were injected intramuscularly on days 1, 56, and 112 and were observed until 6 months after the final vaccination. The vaccine was safe and well-tolerated, with the most frequently observed adverse events being injection site reactions, including mild to moderate pain, swelling, erythema, and pruritus (G. Simon, personal communication).

In addition, vaccination induced significant anti-*Na*-ASP-2 IgG and cellular immune responses. On the basis of the encouraging results of this study, a phase 1 trial of the *Na*-ASP-2 vaccine that involves healthy adult volunteers with documented evidence of previous hookworm infection has been initiated in an area in Brazil where hookworm infection is endemic. In this study, the same dose concentrations and vaccination schedule will be used as those used in the initial phase 1 trial conducted in the United States. A second phase 1 study involving adults is necessary, because neither the safety nor the immunogenicity of the vaccine in individuals with long-term exposure to hookworm can be confidently extrapolated from studies performed in unexposed populations.

Provided that there are no significant safety concerns after testing the *Na*-ASP-2 vaccine in hookworm-exposed adults, clinical testing may proceed into either adult or pediatric pop-

ulations in areas of endemicity for a proof-of-principle study. The study design of the proof-of-principle phase 2 trial will consist of assessing the rate and intensity of hookworm infection among vaccinated persons, compared with those among persons administered a comparator vaccine. Because the *Na*-ASP-2 vaccine targets only the larval stage and not adult hookworms that already exist in the gastrointestinal tract, a critical element of the study design of the phase 2 trial will be treatment of infected individuals with an anthelmintic, to eliminate adult worms prior to vaccination.

Vaccine efficacy will be assessed by quantitative fecal egg counts—an indirect measure of worm burden—with the primary end point being mean egg count at a given time after vaccination that is chosen on the basis of expected rates of reinfection derived from prior epidemiological studies. If a hookworm vaccine were to provide sterilizing immunity (i.e., complete protection against infection), the presence of eggs in the feces would be a suitable end point for an efficacy trial. However, a useful hookworm vaccine may be nonsterilizing but still reduce the number of viable L3 that reach the gastrointestinal tract and subsequently develop into adult worms, therefore making egg counts an appropriate efficacy end point. There is a well-documented correlation between egg counts and host worm burden and between egg counts and host blood loss as measured by quantifying fecal heme [56]. Fecal egg counts, fecal heme, and hematological parameters will, therefore, be important end points for assessing the biologic activity of the *Na*-ASP-2 vaccine in a proof-of-principle study [57].

DEPLOYMENT OF A HOOKWORM VACCINE

An eventual hookworm vaccine will most likely be integrated into existing control programs that currently target children living in areas of high transmission, although because of the burden of disease in pregnant women and older individuals, these populations may also be targeted. Discussion with the World Health Organization has been initiated to explore the feasibility of integrating vaccination into established anthelmintic administration programs, thereby ensuring that any newly licensed hookworm vaccine is rapidly made available.

However, the licensure, industrial-scale manufacture, and distribution of a hookworm vaccine will not be easily achieved, because most individuals at risk for infection live in rural areas and are among the world's poorest, resulting in the absence of a viable commercial market [4–59]. To ensure global access to a vaccine despite these barriers, partnerships are being developed with governments and vaccine manufacturers in middle-income countries where hookworm infection is endemic [58, 59]. For example, Brazil has committed to industrial-scale manufacture and sponsorship of phase 3 efficacy studies should proof-of-principle be demonstrated in phase 2 testing. Transfer of manufacturing technology for the *Na*-ASP-2 vaccine to Bra-

zil is already underway. Despite the enormous challenges in developing, testing, and distributing a vaccine for hookworm, the significant public health benefit warrants continuation of current efforts.

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References

1. de Silva NR, Brooker S, Hotez PJ, Montresor A, Engels D, Savioli L. Soil-transmitted helminth infections: updating the global picture. *Trends Parasitol* **2003**; 19:547–51.
2. Loukas A, Bethony J, Brooker S, Hotez P. Hookworm vaccines: past, present, and future. *Lancet Infect Dis* **2006**; 6:733–41.
3. Nappi AJ, Vass E. Parasites of medical importance. Georgetown: Landes Bioscience, **2002**; 7:80.
4. Hotez PJ, Bethony J, Bottazzi ME, Brooker S, Buss P. Hookworm: “the great infection of mankind.” *PLoS Med* **2005**; 2:e67.
5. Bethony J, Chen J, Lin S, et al. 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* **2002**; 35:1336–44.
6. Palmer DR, Bundy DA. Epidemiology of human hookworm and *Ascaris lumbricoides* infestations in rural Gambia. *East Afr Med J* **1995**; 72: 527–30.
7. Naish S, McCarthy J, Williams GM. Prevalence, intensity and risk factors for soil-transmitted helminth infection in a South Indian fishing village. *Acta Trop* **2004**; 91:177–87.
8. Anderson RM, May RM. Helminth infections of humans: mathematical models, population dynamics, and control. *Adv Parasitol* **1985**; 24: 1–101.
9. World Health Organization. Prevention and control of schistosomiasis and soil-transmitted helminthiases. Vol. Technical Report Series 912. Geneva: World Health Organization, **2002**.
10. Chan MS. The global burden of intestinal nematode infections—fifty years on. *Parasitol Today* **1997**; 13:438–43.
11. Smillie WG, Spencer CR. Mental retardation in school children infested with hookworms. *J Educ Psychol* **1926**; 17:314–21.
12. Sakti H, Nokes C, Hertanto WS, et al. Evidence for an association between hookworm infection and cognitive function in Indonesian school children. *Trop Med Int Health* **1999**; 4:322–34.
13. Guyatt HL, Brooker S, Peshu N, Shulman CE. Hookworm and anaemia prevalence. *Lancet* **2000**; 356:2101.
14. Navitsky RC, Dreyfuss ML, Shrestha J, Khatri SK, Stoltzfus RJ, Albonico M. *Ancylostoma duodenale* is responsible for hookworm infections among pregnant women in the rural plains of Nepal. *J Parasitol* **1998**; 84:647–51.
15. Stephenson LS, Latham MC, Kurz KM, Kinoti SN, Brigham H. Treatment with a single dose of albendazole improves growth of Kenyan schoolchildren with hookworm, *Trichuris trichiura*, and *Ascaris lumbricoides* infections. *Am J Trop Med Hyg* **1989**; 41:78–87.
16. Stephenson LS, Latham MC, Adams EJ, Kinoti SN, Pertet A. Physical fitness, growth and appetite of Kenyan school boys with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* infections are improved four months after a single dose of albendazole. *J Nutr* **1993**; 123:1036–46.
17. Christian P, Khatri SK, West KP Jr. Antenatal anthelmintic treatment, birthweight, and infant survival in rural Nepal. *Lancet* **2004**; 364:981–3.
18. Bennett A, Guyatt H. Reducing intestinal nematode infection: efficacy of albendazole and mebendazole. *Parasitol Today* **2000**; 16:71–4.
19. Albonico M, Smith PG, Ercole E, et al. Rate of reinfection with in-

- testinal nematodes after treatment of children with mebendazole or albendazole in a highly endemic area. *Trans R Soc Trop Med Hyg* **1995**; 89:538–41.
20. Albonico M, Bickle Q, Ramsan M, Montresor A, Savioli L, Taylor M. Efficacy of mebendazole and levamisole alone or in combination against intestinal nematode infections after repeated targeted mebendazole treatment in Zanzibar. *Bull World Health Organ* **2003**; 81: 343–52.
 21. Conder GA, Campbell WC. Chemotherapy of nematode infections of veterinary importance, with special reference to drug resistance. *Adv Parasitol* **1995**; 35:1–84.
 22. Geerts S, Gryseels B. Drug resistance in human helminths: current situation and lessons from livestock. *Clin Microbiol Rev* **2000**; 13: 207–22.
 23. Quinnell RJ, Bethony J, Pritchard DI. The immunoepidemiology of human hookworm infection. *Parasite Immunol* **2004**; 26:443–54.
 24. Beaver PC. Light, long-lasting *Necator* infection in a volunteer. *Am J Trop Med Hyg* **1988**; 39:369–72.
 25. Hoagland KE, Schad GA. *Necator americanus* and *Ancylostoma duodenale*: life history parameters and epidemiological implications of two sympatric hookworms of humans. *Exp Parasitol* **1978**; 44:36–49.
 26. Maizels RM, Balic A, Gomez-Escobar N, Nair M, Taylor MD, Allen JE. Helminth parasites—masters of regulation. *Immunol Rev* **2004**; 201:89–116.
 27. Gandhi NS, Jizhang C, Khoshnood K, et al. Epidemiology of *Necator americanus* hookworm infections in Xiulongkan Village, Hainan Province, China: high prevalence and intensity among middle-aged and elderly residents. *J Parasitol* **2001**; 87:739–43.
 28. Fleming FM, Brooker S, Geiger SM, et al. Synergistic associations between hookworm and other helminth species in a rural community in Brazil. *Trop Med Int Health* **2006**; 11:56–64.
 29. Liu Y, Shen Y, Zhang T. Survey of the relationship between hookworm disease and socioeconomic factors [in Chinese]. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi* **1999**; 17:399.
 30. Hotez PJ, Hawdon JM, Cappello M, et al. Molecular approaches to vaccinating against hookworm disease. *Pediatr Res* **1996**; 40:515–21.
 31. Otto G. Further observations on the immunity induced in dogs by repeated infection with the hookworm *Ancylostoma caninum*. *Am J Hyg* **1941**; 33:39–57.
 32. Miller TA. Vaccination against the canine hookworm diseases. *Adv Parasitol* **1971**; 9:153–83.
 33. Otto G, Kerr KB. The immunization of dogs against hookworm *Ancylostoma caninum* by subcutaneous injection of graded doses of larvae. *Am J Hyg* **1939**; 29:25–45.
 34. Miller TA. Industrial development and field use of the canine hookworm vaccine. *Adv Parasitol* **1978**; 16:333–42.
 35. Cort W, Otto GF. Immunity in hookworm disease. *Rev Gastroenterol* **1940**; 7:2–14.
 36. Miller TA. Transfer of immunity to *Ancylostoma caninum* infection in pups by serum and lymphoid cells. *Immunology* **1967**; 12:231–41.
 37. Fujiwara RT, Loukas A, Mendez S, et al. Vaccination with irradiated *Ancylostoma caninum* third stage larvae induces a Th2 protective response in dogs. *Vaccine* **2006**; 24:501–9.
 38. Hotez PJ, Zhan B, Bethony JM, et al. Progress in the development of a recombinant vaccine for human hookworm disease: the Human Hookworm Vaccine Initiative. *Int J Parasitol* **2003**; 33:1245–58.
 39. Williamson AL, Lustigman S, Oksov Y, et al. *Ancylostoma caninum* MTP-1, an astacin-like metalloprotease secreted by infective hookworm larvae, is involved in tissue migration. *Infect Immun* **2006**; 74:961–7.
 40. Zhan B, Hotez PJ, Wang Y, Hawdon JM. A developmentally regulated metalloprotease secreted by host-stimulated *Ancylostoma caninum* third-stage infective larvae is a member of the astacin family of proteases. *Mol Biochem Parasitol* **2002**; 120:291–6.
 41. Hawdon JM, Jones BF, Hoffman DR, Hotez PJ. Cloning and characterization of *Ancylostoma*-secreted protein: a novel protein associated with the transition to parasitism by infective hookworm larvae. *J Biol Chem* **1996**; 271:6672–8.
 42. Hawdon JM, Narasimhan S, Hotez PJ. *Ancylostoma* secreted protein 2: cloning and characterization of a second member of a family of nematode secreted proteins from *Ancylostoma caninum*. *Mol Biochem Parasitol* **1999**; 99:149–65.
 43. Asojo OA, Goud G, Dhar K, et al. X-ray structure of Na-ASP-2, a pathogenesis-related-1 protein from the nematode parasite, *Necator americanus*, and a vaccine antigen for human hookworm infection. *J Mol Biol* **2005**; 346:801–14.
 44. Asojo OA, Loukas A, Inan M, et al. Crystallization and preliminary X-ray analysis of Na-ASP-1, a multi-domain pathogenesis-related-1 protein from the human hookworm parasite *Necator americanus*. *Acta Crystallogr Sect F Struct Biol Cryst Commun* **2005**; 61 (Pt 4):391–4.
 45. Goud GN, Bottazzi ME, Zhan B, et al. Expression of the *Necator americanus* hookworm larval antigen Na-ASP-2 in *Pichia pastoris* and purification of the recombinant protein for use in human clinical trials. *Vaccine* **2005**; 23:4754–64.
 46. Fujiwara R, Zhan B, Mendez S, et al. Reduction of worm fecundity and canine host blood loss mediates protection against hookworm infection elicited by vaccination with recombinant Ac-16. *Clin Vaccine Immunol* **2007**; 14:281–7.
 47. Zhan B, Wang Y, Liu Y, et al. Ac-SAA-1, an immunodominant 16 kDa surface-associated antigen of infective larvae and adults of *Ancylostoma caninum*. *Int J Parasitol* **2004**; 34:1037–45.
 48. Bethony J, Loukas A, Smout M, et al. Antibodies against a secreted protein from hookworm larvae reduce the intensity of hookworm infection in humans and vaccinated laboratory animals. *FASEB J* **2005**; 19: 1743–5.
 49. Mendez S, Zhan B, Goud G, et al. Effect of combining the larval antigens *Ancylostoma* secreted protein 2 (ASP-2) and metalloprotease 1 (MTP-1) in protecting hamsters against hookworm infection and disease caused by *Ancylostoma ceylanicum*. *Vaccine* **2005**; 23:3123–30.
 50. Goud GN, Zhan B, Ghosh K, et al. Cloning, yeast expression, isolation, and vaccine testing of recombinant *Ancylostoma*-secreted protein (ASP)-1 and ASP-2 from *Ancylostoma ceylanicum*. *J Infect Dis* **2004**; 189:919–29.
 51. Fujiwara RT, Bethony J, Bueno LL, et al. Immunogenicity of the hookworm Na-ASP-2 vaccine candidate: characterization of humoral and cellular responses after vaccination in the sprague dawley rat. *Human Vaccines* **2005**; 1:123–8.
 52. Williamson AL, Lecchi P, Turk BE, et al. A multi-enzyme cascade of hemoglobin proteolysis in the intestine of blood-feeding hookworms. *J Biol Chem* **2004**; 279:35950–7.
 53. Zhan B, Liu S, Perally S, et al. Biochemical characterization and vaccine potential of a heme-binding glutathione transferase from the adult hookworm *Ancylostoma caninum*. *Infect Immun* **2005**; 73:6903–11.
 54. Loukas A, Bethony JM, Mendez S, et al. Vaccination with recombinant aspartic hemoglobinase reduces parasite load and blood loss after hookworm infection in dogs. *PLoS Med* **2005**; 2:e295.
 55. Loukas A, Bethony JM, Williamson AL, et al. Vaccination of dogs with a recombinant cysteine protease from the intestine of canine hookworms diminishes the fecundity and growth of worms. *J Infect Dis* **2004**; 189:1952–61.
 56. Stoltzfus RJ, Chwaya HM, Tielsch JM, Schulze KJ, Albonico M, Savioli L. Epidemiology of iron deficiency anemia in Zanzibari schoolchildren: the importance of hookworms. *Am J Clin Nutr* **1997**; 65:153–9.
 57. Brooker S, Bethony JM, Rodrigues LC, Alexander N, Geiger SM, Hotez PJ. Epidemiologic, immunologic and practical considerations in developing and evaluating a human hookworm vaccine. *Expert Rev Vaccines* **2005**; 4:35–50.
 58. Bottazzi ME, Miles AP, Diemert D, Hotez PJ. An ounce of prevention on a budget: a nonprofit approach to developing vaccines against neglected diseases. *Expert Rev Vaccines* **2006**; 5:189–98.
 59. Hotez PJ. The anti-poverty vaccines. *Vaccine* **2006**; 24: 5787–99.