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Advancing a vaccine to prevent hookworm disease and anemia

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ABSTRACT

A human hookworm vaccine is under development and in clinical trials in Africa and the Americas. The vaccine is comprised of two recombinant proteins encoding *Na*-GST-1 and *Na*-APR-1, respectively, formulated on alum. It elicits neutralizing antibodies that interfere with establishment of the adult hookworm in the gut and the ability of the parasite to feed on blood. The vaccine target product profile is focused on the immunization of children to prevent hookworm infection and anemia caused by *Necator americanus*. It is intended for use in low- and middle-income countries where hookworm is highly endemic and responsible for at least three million disability-adjusted life years. So far, the human hookworm vaccine is being developed in the non-profit sector through the Sabin Vaccine Institute Product Development Partnership (PDP), in collaboration with the HOOKVAC consortium of European and African partners. Ultimately, the vaccine will be incorporated into health systems as part of an elimination strategy for hookworm infection and other neglected tropical diseases, and as a means to reduce global poverty and address Sustainable Development Goals.

Human hookworm infection is a neglected tropical disease caused predominantly by the nematode parasite *Necator americanus* [1]. Recent estimates indicate that approximately 439 million people are infected with hookworm worldwide, with the majority of cases found in the developing regions of South Asia (140 million cases), Sub-Saharan Africa (118 million), Southeast Asia (77 million), East Asia (64.5 million), and the Latin American and Caribbean region (30 million) [2,3]. In these areas, hookworm disease is a major cause of iron-deficiency anemia, a consequence of the adult hookworm's ability to extract blood from the intestinal mucosa and submucosa [4].

The Global Burden of Disease Study 2010 (GBD 2010) estimated that hookworm is responsible for a loss of 3.2 million disability adjusted life years (DALYs), making it one of the leading neglected tropical diseases (along with schistosomiasis and leishmaniasis) in terms of disease burden [2] and a leading cause of anemia in large parts of Africa and Oceania [3,4] (Fig. 1). Hookworm infection occurs when the larval stages penetrate the skin of a human host. The primary adverse effect of infection, anemia, disproportionately occurs in children and pregnant women with lower iron reserves compared to other populations [5,6]. Hookworm is hyperendemic among some pediatric populations in sub-Saharan Africa where in countries such as Sierra Leone or Togo one-third of the population under the age of 20 is infected [7]. Children with chronic hookworm infection experience anemia and cognitive and developmental delays with resultant reductions in school performance, attendance and future wage earnings [8,9]. Approximately 7 million pregnant women in sub-Saharan Africa – almost one third of annual pregnancies in Africa – are also infected, making hookworm disease one of the most common complications of pregnancy in that part of the world [5]. Moreover, many of these individuals – both children and pregnant women – are co-infected with malaria, thereby exacerbating anemia and its sequelae [10].

The primary approach to hookworm control is mass drug administration with a single annual tablet of either of the anthelminthics albendazole (400 mg) or mebendazole (500 mg). Single-dose mebendazole, however, has yielded low cure rates, particularly with repeated use [11–13]. One comprehensive meta-analysis showed no impact of mebendazole treatment on improving anemia

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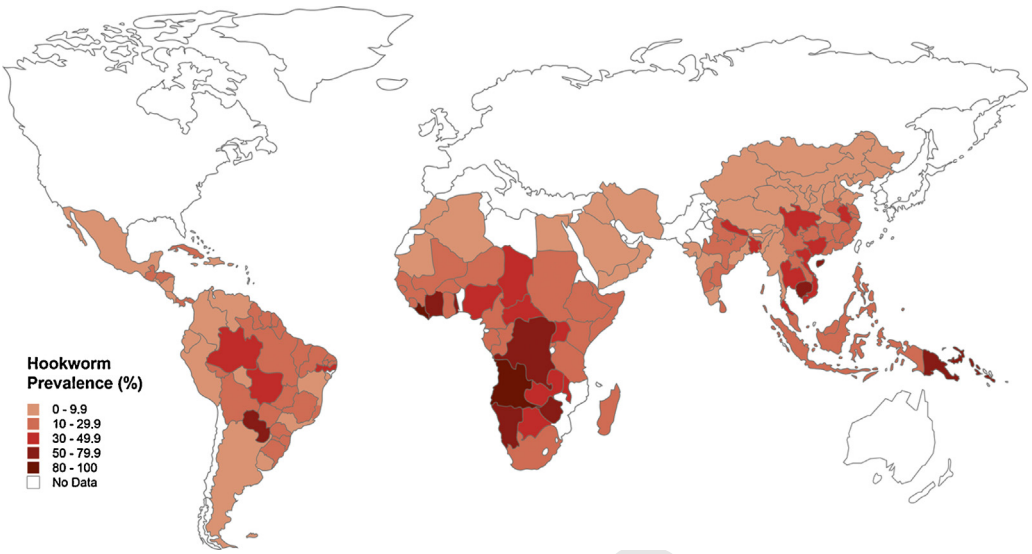

Fig. 1. Distribution of human hookworm infection.
(Reproduced with permission from [5], Brooker et al., *PLoS NTD*).

Table 1
Development status of current vaccine candidates (POC = proof of concept trial).

Candidate name/identifier	Preclinical	Phase I	Phase II	POC	Phase III
Na-GST-1		X			
Na-APR-1		X			
Na-GST-1 and *Na*-APR-1 Co-administered		X			

in hookworm-affected communities [6]. Similarly for albendazole, drug failure has also been reported, though less often [14]. Moreover, children can re-acquire hookworm several months after treatment, especially in areas of high transmission [15,16]. These observations may explain a recent finding from GBD 2013 study that overall global hookworm prevalence has remained essentially unchanged over the last 20 years, while the prevalence of other neglected tropical diseases such as lymphatic filariasis, ascariasis, and trachoma has decreased by 25–33% over the same time period [17]. There is, therefore, a need for new technologies to achieve better control of hookworm infection, particularly if the world is to meet the proposed targets set by the London Declaration for neglected tropical diseases and the follow-up World Health Assembly resolution 66.12 [18]. A safe and effective anti-hookworm vaccine, as a complement to conventional chemotherapy, may provide a cost-effective means of reaching this goal [19] (Table 1).

1. Biological feasibility for vaccine development

As hookworm infection does not typically confer immunity, there is so far no natural immunological correlate of protection on which to base a program for vaccine development. Hookworms are strong immunomodulators from the onset of infection, enabling them to persist in their host for years. Even with chemotherapy, reinfection is the rule. Furthermore, the prevalence and intensity of infection increases with age, adding more evidence that hookworm fails to elicit robust acquired immunity [20]. Biological feasibility for vaccine development was first demonstrated by the relative success of a commercial canine vaccine that consisted of a radiation-attenuated infective larval stage. The vaccine – marketed for dogs in the United States in the 1970s – achieved high levels of protection against infection with *Ancylostoma caninum* and consequent anemia, but was ultimately discontinued due to the high cost of production and complex storage and distribution issues. Before its discontinuation, though, passive antibody transfer studies showed that sera from vaccinated dogs protected non-vaccinated dogs from hookworm challenge [21]. However, clinical trials of hookworm recombinant larval antigens have revealed this approach to be unfeasible because of a high prevalence of IgE antibodies to larval macromolecules such as *Na*-ASP-2 among some hookworm endemic populations [22].

Proteins, especially enzymes, required for adult hookworm blood-feeding may hold promise as an alternative strategy for hookworm vaccine development [23,24] (Fig. 2). This approach was used successfully for a veterinary vaccine against the blood-feeding trichostrongyle, *Haemonchus contortus* that infects sheep and cattle [25]. The enzymes required for hemoglobin digestion and heme detoxification in hookworms have been identified, cloned, expressed and shown to elicit protective antibodies [23,24]. Unlike the larval stage antigens there is no evidence that these enzymes induce IgE antibodies. In the case of *N. americanus*, several enzymes have been identified and developed into recombinant immunogens, including aspartic protease-hemoglobinase, *Na*-APR-1 (a critical enzyme for hemoglobin digestion) and glutathione S-transferase-1 (*Na*-GST-1) (a unique form of the enzyme used for parasite heme detoxification) [23,24]. Both have demonstrated efficacy in immunization/challenge studies in dogs. In the case of *Na*-APR-1, the vaccine induced neutralizing antibodies against multiple heterologous strains of hookworm [26].

2. General approaches to vaccine development for low and middle income country markets

The antigen selection strategy for a human hookworm vaccine is based on several key criteria: (1) efficacy in animal trials, (2) absence of pre-vaccination antigen-specific IgE among endemic populations, (3) feasibility of scaled-up protein manufacturing using low-cost expression systems such as yeast, bacteria or plants and (4) a plausible mechanism of protection [24]. From approximately two-dozen proteins that are putatively involved in the