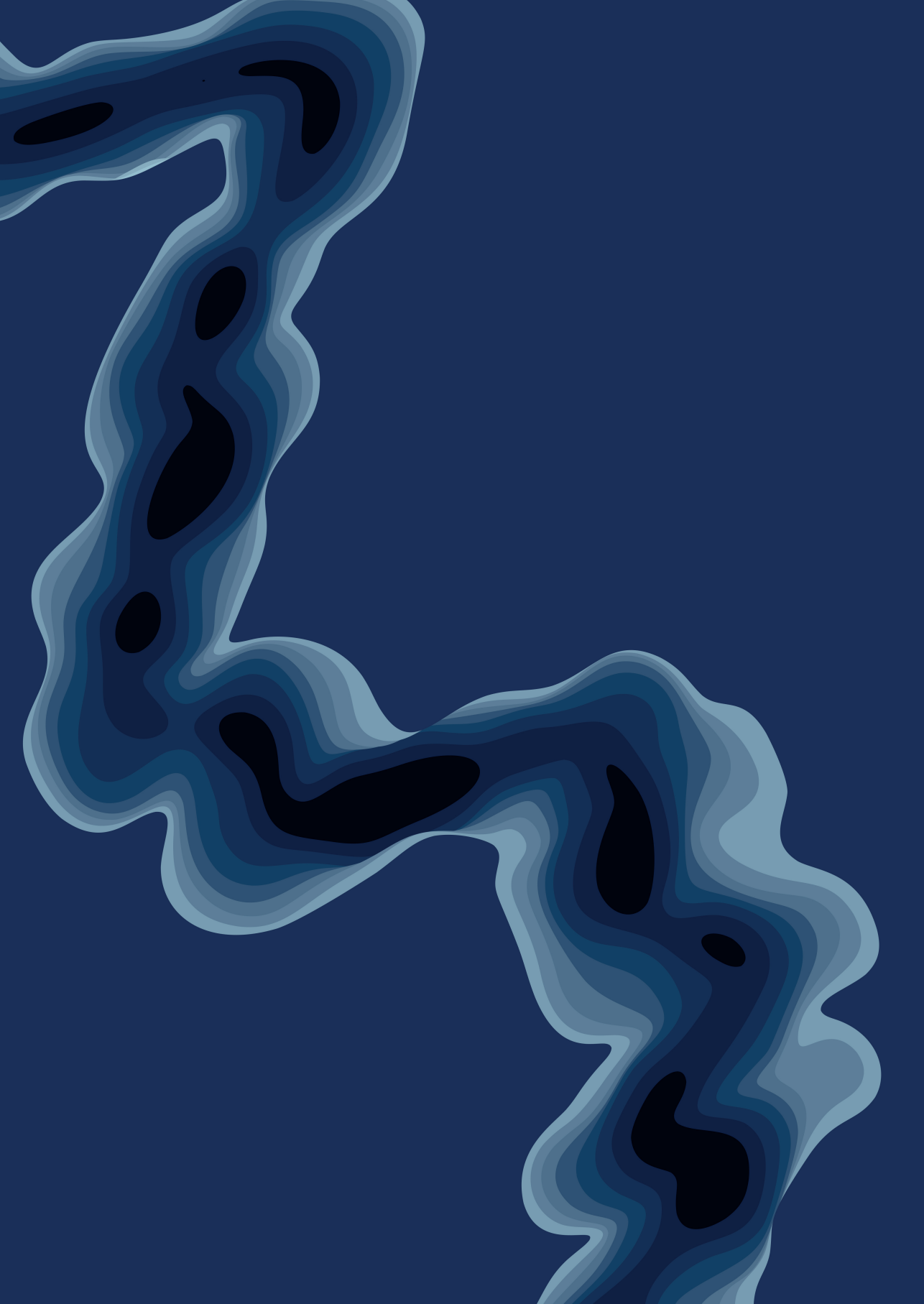
Novel methods to expedite schistosome development

Koopman, J.P.R.



Chapter 1

General Introduction

Schistosomiasis, a parasitic infection with blood flukes of the genus *Schistosoma*, remains highly prevalent, despite extensive control efforts. With more than 150 million people infected with schistosomes worldwide (1), it ranks among the most important neglected tropical diseases (2). Infection occurs through contact with (fresh)water that contains cercariae, the infective larval stage of *Schistosoma* worms, which penetrate the skin, migrate through the lung and end up in and the vessels around the gut (for *Schistosoma mansoni* and *Schistosoma japonicum*) or genito-urinary tract (for *Schistosoma haematobium*). Here they mate and start producing eggs, which are subsequently excreted through faeces or urine into the environment, where they hatch and continue to infect snails, completing the lifecycle. The initial or acute stages of infection are characterised by potential local skin reactions, i.e. cercarial dermatitis, soon after exposure, and acute schistosomiasis syndrome, a transient systemic inflammatory response with flu-like symptoms to the migrating and maturing schistosome (3-8 weeks after infection) (3). Later stages of (chronic) infection are characterised by egg production and egg deposition in tissue triggering a local granulomatous, inflammatory reaction, which are at the core of most severe morbidity observed, such as portal hypertension and haematuria (4).

Schistosomiasis can be diagnosed in various ways (5). The most commonly used method is egg microscopy in stool or urine. The number of eggs is used to determine the infection intensity which ranges from mild, moderate to heavy (6). Other ways to diagnose infection include antigen-based tests, antibody tests, and molecular diagnostics. In particular, the circulating anodic antigen (CAA) test that detects worm-derived CAA in serum or urine is increasingly used in research and clinical settings for diagnosis and treatment evaluation, due to its high sensitivity. For diagnosis of schistosomiasis in returning travellers in non-endemic settings, antibody testing is also suitable, however because antibodies remain detectable for longer periods of time, it is challenging to discriminate active vs previous infection or to monitor treatment effects. Control of schistosomiasis in endemic settings relies on intermittent praziquantel (PZQ) treatment in mass drug administration (MDA) programs (7). However, this approach falls short because reinfection rates are high (8) and praziquantel is ineffective against juvenile worms (9).

ELIMINATING SCHISTOSOMIASIS AS A PUBLIC HEALTH PROBLEM

In its 2020 roadmap, the World Health Organization has set an ambitious target of eliminating schistosomiasis as a public health problem by 2030 through scaling up mass drug administration, WASH interventions, environmental interventions, and behavioural change interventions to reduce transmission and to improve testing (10). Although many national control programs have incorporated various abovementioned measures, decreasing disease burden will be a tremendous challenge and will require sustained effort. Disruptions in schistosomiasis control programs, as seen during the COVID-19 pandemic, can potentially delay achieving elimination of schistosomiasis as a public health problem by up to two years depending on the epidemiological setting (11). This should make us mindful that the commendable progress in reaching disease-control targets that has been achieved in recent years (12) is precarious and new tools for disease control should be pursued.

VACCINES AGAINST SCHISTOSOMIASIS

Unlike for many other infectious diseases, there is currently no licensed vaccine available for schistosomiasis. Modelling studies project that the addition of an efficacious vaccine will increase the likelihood of achieving elimination of schistosomiasis as a public health problem (13). However, the development of vaccines against parasitic infections is complicated for different reasons. Biologically, helminths are complex, multicellular organisms that undergo several developmental stages in humans, yet that have at each stage developed intricate mechanisms to evade immune attack by the human host (14, 15). Moreover, funding into schistosomiasis vaccine research, as for many other neglected tropical diseases, is limited by the lack of commercial interest. Yet despite these hurdles, four vaccine candidates have gone into clinical testing, namely Sh28GST, Sm-14, Sm-TSP2-2 and Sm-p80. All predominantly target *Schistosoma mansoni,* except Sh28GST which targets *Schistosoma haematobium*. An overview of these vaccine candidates and study results is given in **Table 1**. Of these, only Sh28GST has progressed to a phase III study in Senegalese schoolchildren, but unfortunately failed to demonstrate efficacy, despite good immunogenicity (16).

Table 1. Overview of schistosomiasis vaccine candidates in clinical testing.

Vaccine (target)	Adjuvant	Phase I	Phase II	Phase III
rSh28GST	Alhydrogel®	France, 1999 **Safe, immunogenic** (NCT01512277) (17)	Senegal, year unclear, data not published	Senegal 2009-2012 **Safe, immunogenic, but not efficacious** (NCT00870649) (16)
rSm-14	GLA-SE	Brazil, 2011-2014 **Safe, immunogenic** (NCT01154049) (18, 19)	Senegal (2022-now) (NCT05658614)	-
rSm-TSP-2	Alhydrogel® and/or GLA-AF	US 2015-2017, Brazil 2018-2019 **Safe, immunogenic** (NCT02337855; NCT03110757) (20, 21)	Uganda (2019-now) (NCT03910972)	-
rSm-p80	GLA-SE	US (2022-now) (NCT05292391)	Madagascar & Burkina Faso (2023-now) (NCT05762393)	-

NCT numbers refer to clinicaltrials.gov numbers. Colour coding: blue = completed and published, yellow = ongoing

CONTROLLED HUMAN INFECTIONS WITH SCHISTOSOMES

Traditionally, vaccine candidates sequentially move through different testing phases (I, II, to III) that require increasingly large sample sizes and resources. Unfortunately, only few candidates show sufficient efficacy in phase III studies to ultimately become licensed, incurring a substantial waste of resources along the way. To remedy this, controlled human infections can be used where a small number of (healthy) volunteers are intentionally exposed to a pathogen at a fixed time. By doing so, they provide an early estimate of vaccine efficacy in a relatively short period of time that can guide selection of vaccine candidates for larger field studies.

Controlled human infection models (CHIMs) have been established for a wide variety of pathogens, including other helminths (22), but (prior to this work) had not been established for schistosomes. CHIMs are carefully designed with various ethical considerations in mind. It should, for instance, not lead to irreversible harm in participants (23). Although a controlled human infection with schistosomes (CHI-S) would be valuable for schistosomiasis vaccine development, accumulation of eggs and resulting symptomology presents a significant health risk for participants. As such, a mixed-sex CHI-S,

i.e. exposing participants to male and female cercariae with resulting egg production, would not be considered acceptable. A way to avoid these risks, is to expose participants to a single-sex infection with only male or only female cercariae. Single-sex cercariae are produced by exposing a snail to a single miracidium (male or female) (24). After about five weeks, an infected snail will then start shedding cercariae that are either male or female. The sex cannot be distinguished microscopically, and therefore needs to be determined by PCR on the W1 repeat and ITS region. After passing rigorous quality checks including microbial burden of snails, the cercariae can then be administered to participants by pipetting a pre-defined dose onto the skin in mineral water resembling the natural route of infection **(Figure 1)**. After exposure, participants frequently visit the study centre for safety checks and sample collection. In the absence of eggs, infection status is determined by measuring serum circulating anodic antigen (CAA) excreted by juvenile and adult worms. Afterwards, participants are treated with PZQ to cure infection.

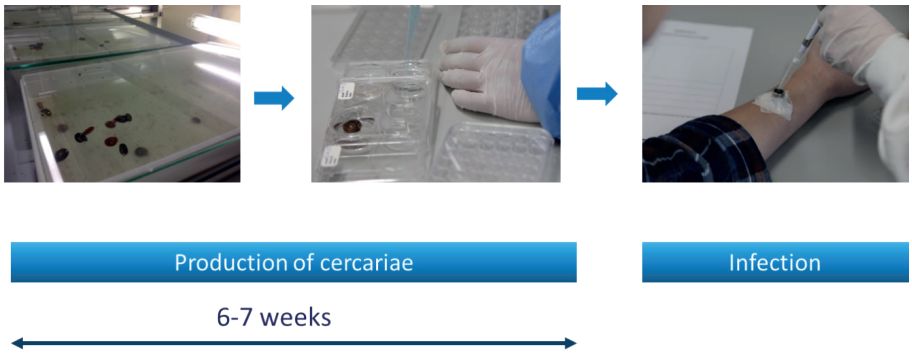


Figure 1. Production of single-sex cercariae for controlled human infection with schistosomes (CHI-S). To produce single-sex cercariae, a single snail is exposed to a single miracidium (mono-miricidial) and housed separately. After five weeks, successfully infected snails start shedding cercariae that are either male or female. The sex is determined by PCR. After quality checks, that include microbial burden assessment, the cercariae are released by our pharmacist. The controlled infection takes place 6-7 weeks after mono-miricidial snail infection by pipetting a pre-defined number of single-sex cercariae in water onto the skin of study participants.

CHALLENGES AND OPPORTUNITIES FOR CHI-S IN SCHISTOSOMIASIS VACCINE DEVELOPMENT

The merits of CHI-S are not limited to vaccine evaluation, but also lie in exploring host-pathogen interactions to help identify potential new vaccine targets and (diagnostic) biomarkers (25). In particular, early stages of infection

have been difficult to study in endemic settings or in returning travellers where timing of exposure is often unclear or diagnosis is only made in later stages of (egg-producing) infection. By design, CHI-S differ substantially from natural infection, because of the single-sex nature, infectious dose, and challenge strain, similar to any other experimental (animal) model. Nevertheless, CHI-S enables us to explore human schistosomiasis in novel ways, for instance in repeat infection studies. A better understanding of immune responses to reinfections may provide leads for new vaccine targets or strategies. Similarly, comparing single-sex male infections and single-sex female infections can help shed light on sex-specific immune responses.

Previously, controlled human infections for tropical diseases were usually performed in non-endemic settings in participants without any prior exposure to the pathogen. However, because of variations in genetics, environmental factors, and (previous) pathogen exposure, responses to vaccines may differ between endemic and non-endemic populations, as seen with malaria, BCG, yellow fever, and rotavirus vaccines (26). Fortunately, there has been a push towards strengthening research capacity to perform controlled human infections in endemic settings (27). To explore establishment of CHI-S in Uganda, where schistosomiasis endemicity is high, a stakeholder's meeting was held in 2017 with regulators, community members, researchers, and policy-makers (28). CHI-S in Uganda was thought to be both feasible and desirable. Several key next steps were formulated before implementation, including a risk assessment for importing laboratory vector snails and schistosome strains from The Netherlands.

To further increase chances of successful vaccine licensure, immunological and methodological aspects of schistosomiasis vaccine development, particularly when transitioning from phase II to phase III, need to be carefully considered. For instance, prior exposure to schistosomes or other infections may affect vaccine efficacy. Moreover, there is ongoing debate on the role of praziquantel (pre-) treatment on protective vaccine responses to the point that praziquantel codelivery was identified as a potential reason for Sh28GST vaccine failure (16). Early identification of these potential interactions can steer study design choices that improve study quality and impact.

SCOPE AND OUTLINE OF THIS THESIS

This thesis explores novel methods to expedite schistosomiasis vaccine development, particularly using controlled human infections with schistosomes. In **chapter 2** and **chapter 3**, we describe the development of single-sex CHIM with *Schistosoma mansoni* using male or female cercariae, respectively. Both were dose-finding studies in which *Schistosoma*-naïve participants were exposed to pre-defined low doses (10, 20 or 30) of cercariae and followed-up over time to assess safety and infectivity.

Given the potential influence of prior exposure to schistosomes, among others, on (vaccine) immune responses, we think it is valuable to transfer the infection model to endemic settings. **Chapter 4** discusses the potential risks related to implementing single-sex controlled human infections in Uganda and aims to provide mitigation strategies to minimise these risks.

Chapter 5 and **chapter 6** focus on the use of CHI-S for vaccine development. In **chapter 5,** we have used the controlled human infection model to investigate whether repeated exposure and treatment leads to protection from reinfection and to identify potential correlates of protection or novel vaccine targets. In **chapter 6**, we present the study protocol for a CHI-S vaccine study. This study will assess the protective efficacy of three immunisations with Sm-p80 + GLA-SE against controlled infection male *Schistosoma mansoni* cercariae in healthy Schistosome-naïve volunteers.

In **chapter 7** and **chapter 8**, we reflect on immunological and methodological aspects of schistosomiasis vaccine development, particularly transitioning from phase II to phase III.

Finally, in the discussion (**chapter 9**) we summarise the main findings of this thesis and discuss these in a broader context.

REFERENCES

1. Diseases GBD, Injuries C. Global incidence, prevalence, years lived with disability (YLDs), disability-adjusted life-years (DALYs), and healthy life expectancy (HALE) for 371 diseases and injuries in 204 countries and territories and 811 subnational locations, 1990-2021: a systematic analysis for the Global Burden of Disease Study 2021. Lancet. 2024;403(10440):2133-61.
2. Hotez PJ, Kamath A. Neglected tropical diseases in sub-saharan Africa: review of their prevalence, distribution, and disease burden. PLoS Negl Trop Dis. 2009;3(8):e412.
3. Ross AG, Vickers D, Olds GR, Shah SM, McManus DP. Katayama syndrome. Lancet Infect Dis. 2007;7(3):218-24.
4. Colley DG, Bustinduy AL, Secor WE, King CH. Human schistosomiasis. Lancet. 2014;383(9936):2253-64.
5. Utzinger J, Becker SL, van Lieshout L, van Dam GJ, Knopp S. New diagnostic tools in schistosomiasis. Clin Microbiol Infect. 2015;21(6):529-42.
6. Wiegand RE, Secor WE, Fleming FM, French MD, King CH, Deol AK, et al. Associations between infection intensity categories and morbidity prevalence in school-age children are much stronger for Schistosoma haematobium than for S. mansoni. PLoS Negl Trop Dis. 2021;15(5):e0009444.
7. Lo NC, Bezerra FSM, Colley DG, Fleming FM, Homeida M, Kabatereine N, et al. Review of 2022 WHO guidelines on the control and elimination of schistosomiasis. Lancet Infect Dis. 2022;22(11):e327-e35.
8. Zacharia A, Mushi V, Makene T. A systematic review and meta-analysis on the rate of human schistosomiasis reinfection. PLoS One. 2020;15(12):e0243224.
9. Wu W, Wang W, Huang YX. New insight into praziquantel against various developmental stages of schistosomes. Parasitol Res. 2011;109(6):1501-7.
10. Kokaliaris C, Garba A, Matuska M, Bronzan RN, Colley DG, Dorkenoo AM, et al. Effect of preventive chemotherapy with praziquantel on schistosomiasis among school-aged children in sub-Saharan Africa: a spatiotemporal modelling study. Lancet Infect Dis. 2022;22(1):136-49.
11. Kura K, Ayabina D, Toor J, Hollingsworth TD, Anderson RM. Disruptions to schistosomiasis programmes due to COVID-19: an analysis of potential impact and mitigation strategies. Trans R Soc Trop Med Hyg. 2021;115(3):236-44.
12. Deol AK, Fleming FM, Calvo-Urbano B, Walker M, Bucumi V, Gnandou I, et al. Schistosomiasis - Assessing Progress toward the 2020 and 2025 Global Goals. N Engl J Med. 2019;381(26):2519-28.
13. Kura K, Truscott JE, Toor J, Anderson RM. Modelling the impact of a Schistosoma mansoni vaccine and mass drug administration to achieve morbidity control and transmission elimination. PLoS Negl Trop Dis. 2019;13(6):e0007349.
14. Hambrook JR, Hanington PC. Immune Evasion Strategies of Schistosomes. Front Immunol. 2020;11:624178.
15. Angeles JMM, Mercado VJP, Rivera PT. Behind Enemy Lines: Immunomodulatory Armamentarium of the Schistosome Parasite. Front Immunol. 2020;11:1018.

16. Riveau G, Schacht AM, Dompnier JP, Deplanque D, Seck M, Waucquier N, et al. Safety and efficacy of the rSh28GST urinary schistosomiasis vaccine: A phase 3 randomized, controlled trial in Senegalese children. PLoS Negl Trop Dis. 2018;12(12):e0006968.

17. Riveau G, Deplanque D, Remoue F, Schacht AM, Vodougnon H, Capron M, et al. Safety and immunogenicity of rSh28GST antigen in humans: phase 1 randomized clinical study of a vaccine candidate against urinary schistosomiasis. PLoS Negl Trop Dis. 2012;6(7):e1704.

18. Santini-Oliveira M, Coler RN, Parra J, Veloso V, Jayashankar L, Pinto PM, et al. Schistosomiasis vaccine candidate Sm14/GLA-SE: Phase 1 safety and immunogenicity clinical trial in healthy, male adults. Vaccine. 2016;34(4):586-94.

19. Santini-Oliveira M, Machado Pinto P, Santos TD, Vilar MM, Grinsztejn B, Veloso V, et al. Development of the Sm14/GLA-SE Schistosomiasis Vaccine Candidate: An Open, Non-Placebo-Controlled, Standardized-Dose Immunization Phase Ib Clinical Trial Targeting Healthy Young Women. Vaccines (Basel). 2022;10(10).

20. Diemert DJ, Correa-Oliveira R, Fraga CG, Talles F, Silva MR, Patel SM, et al. A randomized, controlled Phase 1b trial of the Sm-TSP-2 Vaccine for intestinal schistosomiasis in healthy Brazilian adults living in an endemic area. PLoS Negl Trop Dis. 2023;17(3):e0011236.

21. Keitel WA, Potter GE, Diemert D, Bethony J, El Sahly HM, Kennedy JK, et al. A phase 1 study of the safety, reactogenicity, and immunogenicity of a Schistosoma mansoni vaccine with or without glucopyranosyl lipid A aqueous formulation (GLA-AF) in healthy adults from a non-endemic area. Vaccine. 2019;37(43):6500-9.

22. Roestenberg M, Hoogerwerf M-A, Ferreira DM, Mordmüller B, Yazdanbakhsh M. Experimental infection of human volunteers. The Lancet Infectious Diseases. 2018;18(10):e312-e22.

23. Miller FG, Grady C. The ethical challenge of infection-inducing challenge experiments. Clin Infect Dis. 2001;33(7):1028-33.

24. Janse JJ, Langenberg MCC, Kos-Van Oosterhoud J, Ozir-Fazalalikhan A, Brienen EAT, Winkel BMF, et al. Establishing the Production of Male Schistosoma mansoni Cercariae for a Controlled Human Infection Model. J Infect Dis. 2018;218(7):1142-6.

25. Darton TC, Blohmke CJ, Moorthy VS, Altmann DM, Hayden FG, Clutterbuck EA, et al. Design, recruitment, and microbiological considerations in human challenge studies. Lancet Infect Dis. 2015;15(7):840-51.

26. van Dorst M, Pyuza JJ, Nkurunungi G, Kullaya VI, Smits HH, Hogendoorn PCW, et al. Immunological factors linked to geographical variation in vaccine responses. Nat Rev Immunol. 2024;24(4):250-63.

27. Kapulu M, Manda-Taylor L, Balasingam S, Means G, Ayiro Malungu M, Bejon P, et al. Fourth Controlled Human Infection Model (CHIM) meeting - CHIMs in endemic countries, May 22-23, 2023. Biologicals. 2024;85:101747.

28. Elliott AM, Roestenberg M, Wajja A, Opio C, Angumya F, Adriko M, et al. Ethical and scientific considerations on the establishment of a controlled human infection model for schistosomiasis in Uganda: report of a stakeholders' meeting held in Entebbe, Uganda. AAS Open Res. 2018;1:2.