Novel methods to expedite schistosome development

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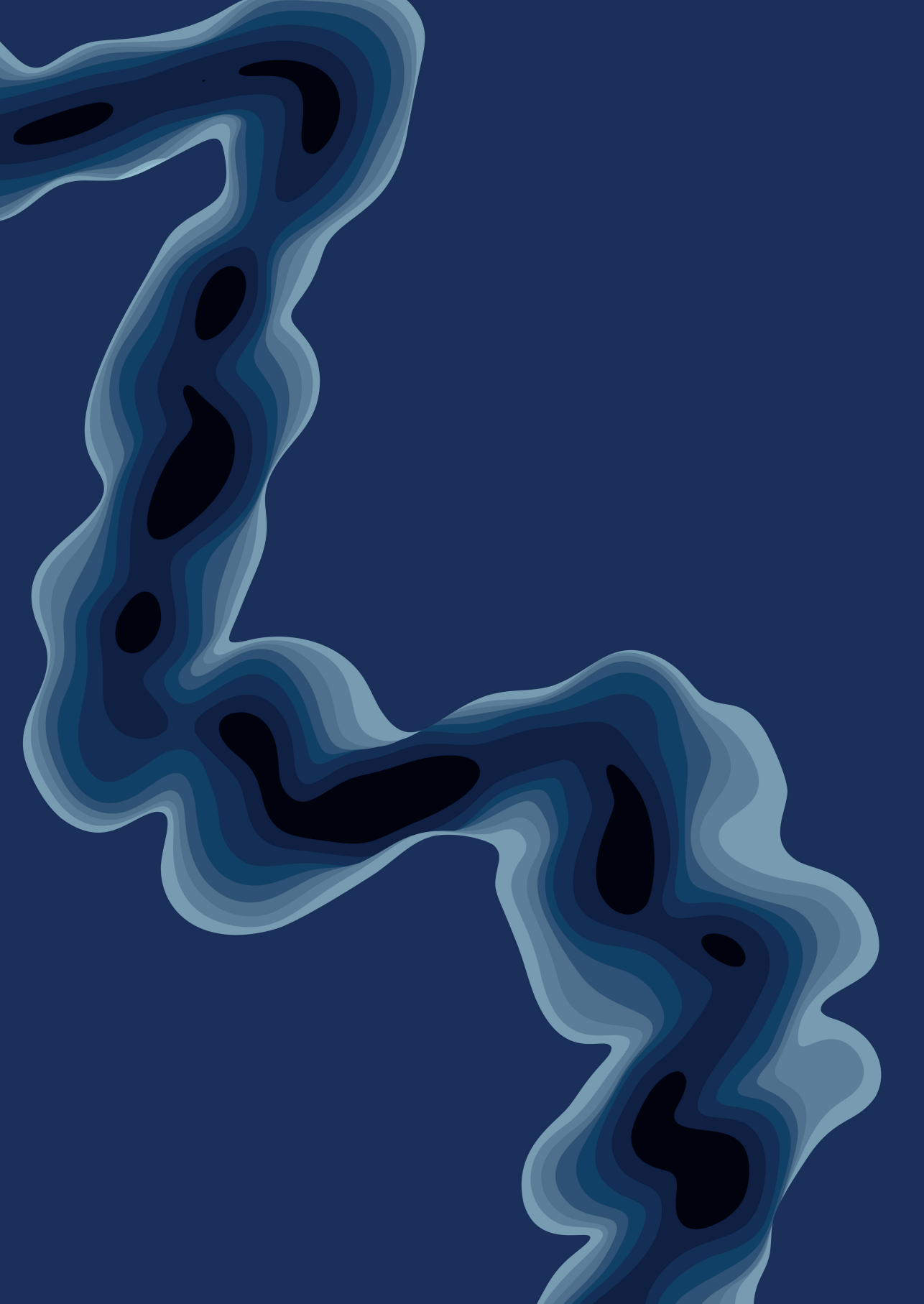

Chapter 8

Methodological considerations for future *Schistosoma* vaccine studies

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BACKGROUND

Schistosomiasis, a parasitic infection with blood flukes of the genus *Schistosoma*, remains highly prevalent with over 150 million infections worldwide (1), despite extensive praziquantel treatment in mass drug administration (MDA) programs of at-risk populations. Praziquantel treatment has significantly contributed to schistosomiasis morbidity reduction, but has its limitations: it is ineffective against juvenile worms and does not prevent reinfection. Moreover, reliance on a single drug raises concerns of drug resistance. A schistosomiasis vaccine is expected to help meet the WHO disease control targets more rapidly, according to modelling studies (2). However, a serious challenge to schistosomiasis vaccine development is the limited available research funding, as for all neglected tropical diseases. Four vaccine candidates are currently in clinical testing, which have been extensively reviewed elsewhere (3), and some of these will soon be evaluated for efficacy. These pivotal studies are costly and require careful planning, not least because conventional vaccine studies incompletely address issues such as praziquantel pre-treatment or do not capture effects on transmission. There is no immunological correlate for protection in schistosomiasis and studies therefore will rely on clinical or infection endpoints. However, whereas the efficacy of vaccines is often measured in relation to the incidence of disease (i.e. clinical endpoint) (4), this is not possible for schistosomiasis, because of the long lag time spanning multiple years between exposure to schistosomes and (chronic) disease manifestations. Consequently, trial endpoints will be focussed primarily on infection incidence. There are several ways to diagnose infection, including egg-based microscopy, antibody-based tests, antigen-based tests, and molecular techniques (5), each with advantages and limitations. For example, egg-based microscopy, currently the gold standard, lacks sensitivity in low infection intensity or prevalence settings. Vaccine efficacy estimates are determined by choices in diagnostic methods and effect measures, and therefore warrant careful consideration. Prior exposure to schistosomes, coinfections, and praziquantel treatment are each likely to affect vaccine immunogenicity and efficacy(6), however the direction and magnitude of these effects are still unclear. As such, heterogeneity in vaccine responses is expected to result from these complex immunological interactions within individuals, and should be considered in schistosomiasis vaccine efficacy studies. Here, we elaborate on the aspects raised above and provide suggestions for design of future studies.

CHOOSING THE MOST SUITABLE SCHISTOSOMIASIS DIAGNOSTIC FOR VACCINE STUDIES

As briefly introduced, microscopic egg detection is widely used for schistosomiasis diagnosis in endemic settings. However, sensitivity of microscopic egg detection in low-intensity infections is suboptimal, risking overestimation of vaccine effects when vaccinated are wrongly classified as protected. PCR detection of eggs has higher sensitivity than microscopy and PCR Ct values have shown good correlation with egg output, making it possible to more reliably assess vaccine effects in low-intensity infections (7). Alternatively, infection can be diagnosed with detection of worm-excreted circulating anodic (CAA) antigen levels in serum. This test has high sensitivity, even in low infection intensity settings, and is a surrogate for worm burden (8).

A schistosomiasis vaccine can have different (biological) mechanisms of action. A schistosomiasis vaccine may kill already present or incoming worms (reduce worm burden) and/or interfere with worm fertility (reduce number of eggs produced or reduce viability of excreted eggs). These effects are also referred to as anti-worm and anti-fecundity effects, respectively. Because egg production is responsible for most severe disease and facilitates onward transmission, egg-based diagnostics are an important potential measure of vaccine effects on transmission. However, it should be noted it only indirectly provides information about the anti-worm effect, which might be the primary mechanism of action of a vaccine. For those vaccines where direct anti-worm effects are expected, we therefore recommend antigen detection as to directly measure the mechanism of action. Ideally for vaccines that are expected to have more than one effect, a combination of diagnostics as co-primary outcomes should be employed to enhance our understanding of the potential impact of vaccines on schistosomiasis infection incidence, transmission and potential burden of disease.

CHOOSING RELEVANT EFFECT MEASURES TO DERIVE VACCINE EFFICACY

Another key component of the vaccine efficacy estimate is the effect measure used (9). Effect measures can be used to 1) compare the incidence of infection after immunisation in the vaccinated and unvaccinated group [risk ratio or rate ratio], 2) compare the average time to recurrent infection between groups [hazard ratio], or 3) compare the (geometric) mean egg output or CAA levels between groups. A phase III rSh28GST schistosomiasis vaccine study in a

highly endemic setting (prevalence 60%) in Senegal used time-to-recurrence as primary outcome, defined as having haematuria in combination with egg detection in urine (10). Notably, infection was assessed routinely only from 82 weeks after administration of the first vaccination (30 weeks after one year booster), by which time 41% of individuals (104 of 249) had recurrent infection. The main finding was that there was no statistically significant difference in time-to-recurrence based on log-rank test, however no hazards ratio and consequently vaccine efficacy was calculated. In some cases, it is possible to look at different pre-defined endpoints and effect measures within one trial, as with the RTS,S malaria vaccine, which used risk ratios for severe malaria, rate ratios for all episodes of clinical malaria, and hazard ratios for first episodes of clinical malaria (11). This is also an attractive approach for schistosomiasis. Through regular active case detection and treatment of cases, for instance every three months, the effect on total number of schistosomiasis episodes can be evaluated (Fig 1A).

Prior-exposure, age, and coinfections are all thought to affect vaccine responses. We will only be able to gain understanding into this interplay if future studies incorporate these within their designs: data on coinfections in individuals can be collected and be explored in pre-defined subgroup analyses, while stratified randomisation may be considered with regards to prior exposure level and age group. These however require a significant increase in study participants to provide sufficient statistical power, incurring further study costs.

USE OF LESS CONVENTIONAL STUDY DESIGNS

As an alternative or addition to more classical phase II clinical trials, novel tools allow for more disruptive trial designs with the potential to inform on vaccine effects. An important development in this space is the recent establishment of a controlled human infection model for schistosomes (CHI-S). In these CHI-S studies, healthy participants are exposed to a low dose of only male cercariae, to avoid egg-associated risks, and followed-up intensively to measure infection by CAA detection. CHI-S studies only require a few participants because everyone is exposed at the same time, and they can be completed relatively quickly (~ one year). Controlled human infection studies are therefore increasingly used as a tool to obtain an early estimate of vaccine efficacy for many different pathogens. Although there are limitations to the model, for instance that it can only assess effects on worm burden, it can help us better understand key issues in schistosomiasis vaccines, particularly when the model

has successfully been set-up in Uganda, where schistosomiasis is endemic (12). It will allow for the exploration of vaccine effects in populations with different levels of prior exposure and coinfections . Moreover, using for instance 2x2 factorial design in which participants are randomised to 1) PZQ treatment and 2) vaccination (**Figure 1B**), the influence of PZQ-codelivery can be investigated before moving to larger field studies.

Up until now, we only considered measuring vaccine effects in studies that individually randomise participants to either vaccine or control arm, which will give an estimate of the direct effect: immunity conferred to vaccinated individuals by vaccination. However, if a vaccine reduces egg output, transmission may also decrease. Hence, future vaccine studies should ideally also evaluate this indirect effect (i.e. reduction in incidence resulting from lower force of infection in community), which can be evaluated in cluster-randomised studies (13). In these studies, a cluster usually consisting of all eligible study participants on school, village, or district level, are randomised to either the vaccine or control arm. It requires a larger sample size that is determined by the number and size of clusters. Previously, Ross et al. performed a cluster-randomised study to investigate the effect of bovine vaccination in combination with other interventions on human schistosomiasis (14). They included 18 pairs of villages and used a 2x3 factorial design consisting of a first randomisation step determining vaccination (or not) of bovines and a second randomisation step for additional intervention (human PZQ prophylaxis, molluscicides, or no additional control measure). In this specific example, decreases in infection incidence in groups that did not receive human PZQ can be ascribed to decreased transmission. As reduction in transmission is an important vaccine goal, we recommend exploring cluster-randomised design studies. Moreover, these studies can incorporate OneHealth elements, such as monitoring of snails, the intermediate hosts, and environmental DNA, a tool for detecting cercariae in freshwater sample (15), allowing us to better understand how *Schistosoma* vaccines impact the epidemiology of schistosomiasis (**Figure 1C**).

CONCLUSION

With several promising schistosomiasis vaccine candidates in early stages of clinical testing, the discussion around the optimal design for a vaccine efficacy study becomes consequential. This includes how infection is measured and how the trial endpoint is defined. Conventional study designs do not appropriately address questions of disease transmission, the influence of PZQ

treatment, coinfections and prior-exposure, and we have therefore suggested alternative study designs, such as a factorial CHI-S study to study effects of PZQ co-delivery and a cluster-randomised study to study transmission. These will hopefully increase the chance of successful licensure and roll-out of a schistosomiasis vaccine in the future.

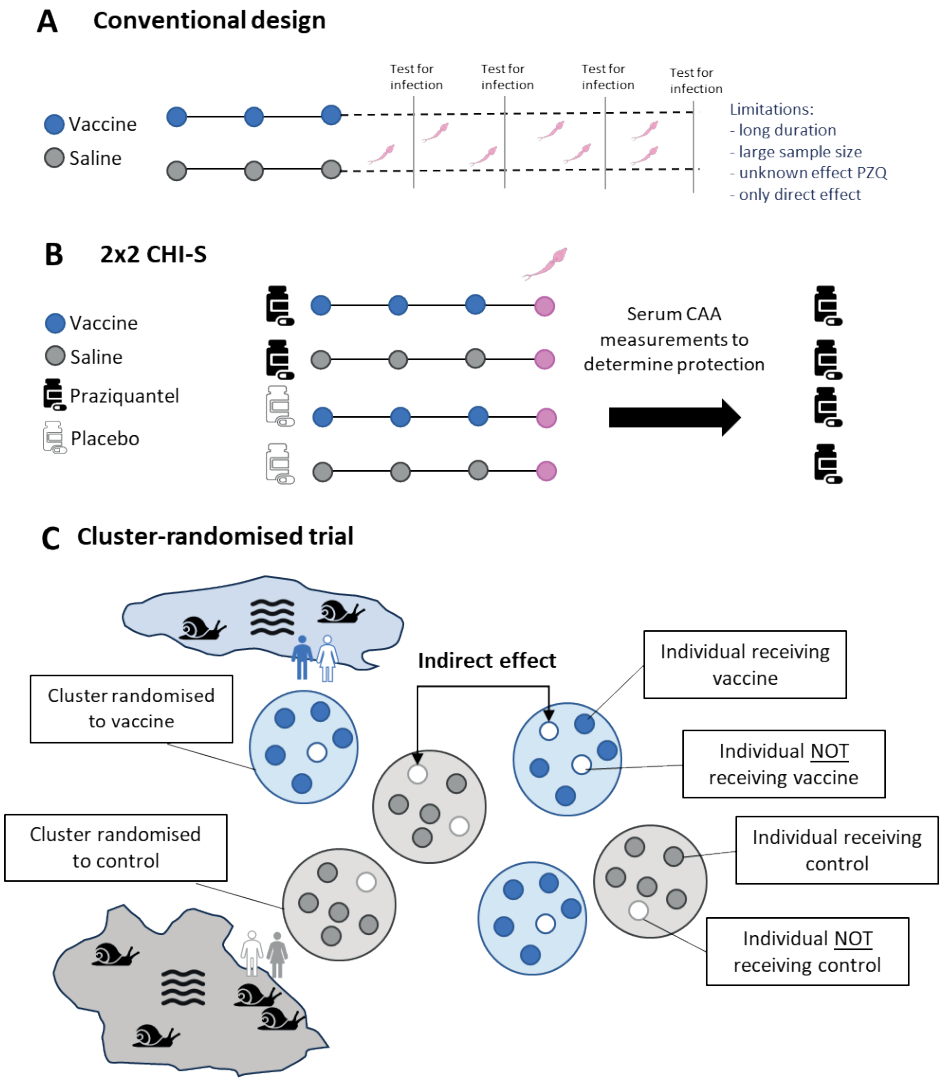

Figure 1. Different study designs for future schistosomiasis vaccine studies.

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