Novel methods to expedite schistosome development

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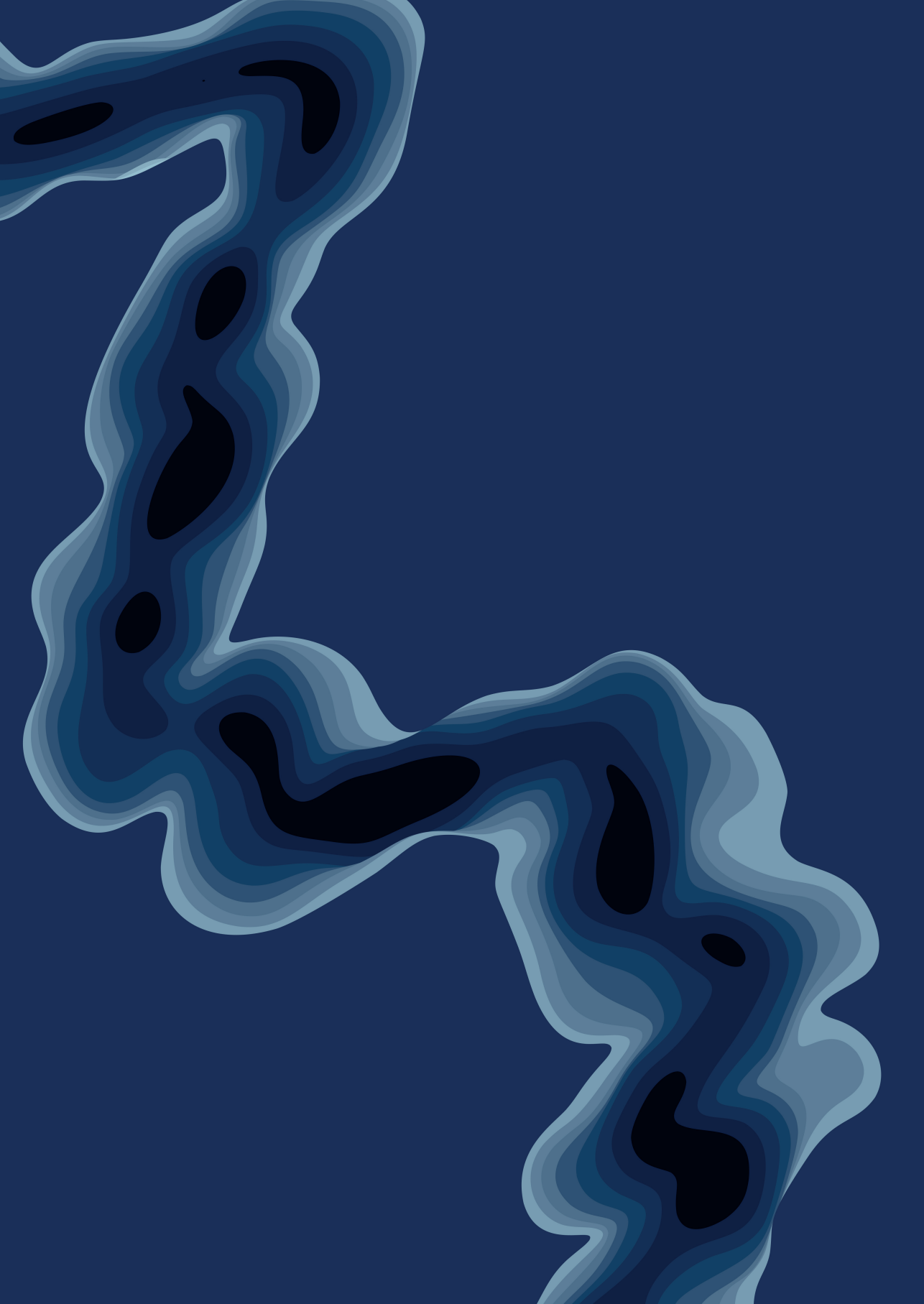

Chapter 9

Summarising discussion and conclusion

To this day, schistosomiasis remains a major cause of morbidity in large parts of the world, particularly in Sub-Sahara Africa (1), despite frequent mass drug administration with praziquantel; water, sanitation and hygiene interventions(2); and health education(3). The development of an efficacious vaccine should be a top research priority to limit further suffering, but the road to eventual vaccine licensure is long and time-consuming, as vaccines against helminths are challenging. In its co-evolution with humans, *Schistosoma* species, much like other parasites, have developed ingenious mechanisms to evade human immune responses. Within the human host, schistosomes undergo critical developmental stages while transiting through skin, blood, and lungs. Yet despite all these developmental changes, the parasite manages to escape immune attack at each life cycle stage (4, 5). Eventually, immunity against schistosomes does develop in chronically-exposed individuals, albeit slow: the prevalence and/or infection intensity decreases with age (6), independent of water-contact (7). In addition to these epidemiological findings, other research has focussed on understanding how some animals, for instance rhesus macaques, are able to self-cure, hoping to find new vaccine targets (8). Perhaps the most promising data in support of vaccination, are immunisation studies with irradiated cercariae in several animal models showing up to 80% reduction in worm burden upon cercarial challenge (9). Together, these epidemiological and animal-model studies have led to the identification of several promising vaccine targets, for which vaccine candidates have been developed. A handful of these schistosomiasis vaccine candidates have now advanced to clinical testing (10), but progress has been slow in part due to a lack of commercial interest.

To speed up schistosomiasis vaccine development, we have established a controlled human infection model (CHIM) for *Schistosoma mansoni*. Controlled human infections with schistosomes (CHI-S) can be used to evaluate vaccine or drug candidates, understand pathogenesis or immune responses, and study infection-derived immunity (11), jointly contributing to vaccine development. Particularly, our understanding of early stages of infection in humans is limited, because timing of exposure is often unknown and infection is only detected at a later, egg-producing stage. In this thesis, we report on the three CHI-S using *Schistosoma mansoni (Sm)* performed to date (**chapter 2, 3, and 5**). In the next paragraphs, we will integrate the findings of the these CHI-S studies and discuss what we have learned so far.

SYMPTOMS AFTER CHI-S

In all controlled human infection studies, safety of participants is paramount and for that reason measures are taken to limit harm (11-13). Given that many of the pathogens for which CHIMs have been developed, have affected human populations for many years, we generally have good knowledge of the symptoms they may cause. For schistosomes, these are: 1) skin reactions immediately after exposure; 2) acute schistosomiasis, a flu-like syndrome; and 3) chronic (egg-producing) schistosomiasis (6, 14). Individual host and/or pathogen factors are believed to influence the risk of severe complications in chronic (egg-producing) schistosomiasis, but the exact mechanisms are not entirely clear. Our single-sex (egg-free) approach, exposing participants to only male or only female cercariae, of the CHI-S model mitigates this risk and is a novelty in the CHIM field. It should be noted, however, that this adaptation does make the CHI-S less comparable to natural infection, and the harm to individuals exposed to a very low number of eggs, resulting from a low cercarial dose and prompt treatment, is likely minimal.

Perhaps the least studied clinical presentation is acute schistosomiasis (AS), previously also called Katayama syndrome, as this is often only recognised in returning travellers (14). AS is a self-limiting illness, that has been attributed to systemic inflammatory responses to the migrating schistosomula and egg deposition. The presentation of AS can be varied, but often includes fever, headache, cough, and urticaria, and a combination thereof. It may also manifest as abdominal pain, diarrhoea, myalgias and neck pain. Estimates on the risk of AS differ substantially between studies (15) and may depend on infectious dose, *Schistosoma* species, duration of water contact and number of exposures. These factors are all effectively controlled for in CHI-S, which provides the opportunity to better understand the development of AS.

Previously, many have attributed AS to onset of egg production, but our findings in single-sex studies clearly show that AS is not limited to egg-producing infection. Moreover, In the small group of participants in the reinfection study who could have potentially developed a mixed-sex, egg-producing infection, we did not see an increase or exacerbation of AS symptoms. Increases in symptoms suggestive of AS were observed three weeks after exposure to male or female cercariae. AS is characterised by diverse symptoms that each by themselves are non-specific and have a high incidence in the general population, making diagnosis challenging. In our initial dose-finding, around half of participants exposed to 20 male or 20 female cercariae developed moderate to severe

systemic symptoms suggestive of AS. This number may have been an overestimation as there was no control group in these studies, and there is considerable overlap between AS symptoms and other intermittent infectious illnesses. Based on the reinfection study that did include a control group, the risk of moderate-severe AS approaches 25-35% after primary exposure to 20 male cercariae. To alleviate symptoms, we have started treating severe AS with prednisolone for at least five days, before tapering off the dose. This strategy has been successful: participants experience improvement immediately after ingestion of the first dose.

Another important finding from the reinfection study is that clinical tolerance to AS symptoms already develops after the initial exposure, with fewer symptoms being reported with subsequent exposures. These results agree with what is commonly described in the field: people in endemic settings appear not to develop acute schistosomiasis except for *S. japonicum* infections, possibly because of early-life exposure to *Schistosoma* antigens (14).

In our studies, we also observe a maculopapular rash at the exposure site in 80% (44/54) of participants, after male or female cercarial exposure. This incidence seems higher than what previous studies have reported in travellers (risk 11-36%) (16), which may be because of recall bias in observational studies, or that the skin reaction has been overlooked as it is only present for a short period of time and is not always pruritic. Similar skin reactions are also reported in Europe after exposure to avian schistosomes in freshwater bodies. These *Schistosoma* species, such as *Trichobilharzia* spp, are not well adapted to the human host and are therefore believed to die in the skin only causing a local, allergic reaction instead of infection. Interestingly, sensitisation to avian schistosomes may lead to worsening of skin reactions upon re-exposure (17). This has also been seen in a controlled human hookworm study where albendazole treatment was given two weeks after exposure as an immunisation method (18) and is associated with protection to hookworm infection. However, repeated exposure did not lead to an increase or decrease in skin reactions in our schistosome reinfection study.

As discussed in **chapter 3,** four participants exposed to 20 female cercariae had a more long-term single sex infection, because of PZQ failure. These participants did not report any symptoms during follow-up, suggesting that more chronic, single-sex infection causes limited harm, in comparison to chronic mixed-sex infections.

INFECTION PARAMETERS AND VALIDITY OF THE CHI-S MODEL

Different diagnostic tools are available for schistosomiasis detection, with each its strengths and weaknesses. In CHI-S, participants have a known time and dose of exposure and are followed up and sampled frequently allowing us to study the kinetics of various diagnostic tests.

In the absence of eggs, we rely on the very sensitive, lab-based UCP-LF circulating anodic antigen (CAA) test to determine infection status for our primary outcome (19). This CAA is excreted by juvenile and adult worms indicating active infection. Although excretion patterns of CAA was thought to differ between single-sex male and single-sex female infections based on animal work and *in vitro* worm cultures (20), the probability of CAA detectable infection, defined as having at least one CAA value ≥1.0 pg/mL, did not differ substantially. Moreover, there is consistency in attack rates of 20 male cercariae between the initial dose-finding study and primary exposures in reinfection and infection controls in the reinfection study, supporting the validity of the male-only model in this *Schistosoma*-naïve population. This is important, because attack rates are used to calculate sample size for future studies and therefore directly impact statistical power. Moreover, unlike for other challenge agents, it is currently not possible to set up a master bank of cercariae (21); for each production we harvest fresh miracidia from hamsters that are used to sustain the parasite lifecycle which, some have argued, might introduce heterogeneity in infectivity.

The studies in this thesis have demonstrated the usefulness of CAA over antibody testing. This has even led to the incorporation of the CAA test in the clinical microbiology lab at LUMC, available to clinicians nationwide. We have convincingly shown that CAA can detect infection and treatment effect, without the downside of antibodies. This is particularly useful in returning travellers to discriminate between active worm infection vs. merely exposure to worms, evidenced by antibody seroconversion in CAA negative participants.

CHI-S have improved our understanding of antibody-based diagnostics. In response to CAA, highly sensitive and specific anti-CAA IgM and IgG antibodies can be detected three to four weeks post-exposure, which can be explored as a novel antibody based diagnostics (22). Antibodies against soluble egg antigen are also present in a subgroup of participants, even in male-only infections, suggesting cross-reactivity of cercarial and egg antigens. However, titres are lower than in (potential) mixed-sex infections as seen in our repeat infection study. Nonetheless, shared cercarial and egg antigens warrant further

examination as these could be ideal vaccine targets that could affect two crucial parasite life stages.

In our studies, we have used PCR on stool to exclude egg production after single-sex exposure as additional quality and safety check. The positive PCR test result led us to discover the accidental exposure to female cercariae in the reinfection study. This triggered a thorough investigation, in which all production-related data were checked and stored cercarial samples retested. Having incorporated strict procedures in line with good manufacturing principles (GMP) and back-up diagnostics allowed us to uncover the error, and demonstrates the value of having such measures in place.

DIFFERENCES IN PRAZIQUANTEL SUSCEPTIBILITY BY CERCARIAL SEX

As mentioned before, treatment of schistosomiasis relies on a single drug, praziquantel of which more than 250 million tablets are distributed yearly (23). Dependency on a single drug raises concerns of drug resistance, which is why recent studies have investigated alternative treatment options, such as artemisinin-based therapies and mefloquine (24-26). Cure rates after praziquantel are ~80% based on Kato-Katz, but are lower when more sensitive antigen tests are used (27). Earlier *in vitro* and animal studies have suggested that unpaired worms may be less susceptible to praziquantel (28), however in humans this has been difficult to corroborate. In our studies, we noticed the following: firstly, a single-dose of 40 kg/mg was not sufficient to clear male-only infections based on CAA values in a substantial number of participants. In order to achieve cure, a higher second dose of 60 mg/kg needed to be administered. This dose has now become the standard dose after CHI-S.

Next, female-only infections are less susceptible to praziquantel treatment, even at 60 mg/kg dose, given 8 and 12 weeks post-exposure or when given three days consecutively. Four (out six) participants had recurring CAA levels, after initial decreases, indicating persistence of the female worms. The underlying reason is not entirely clear, however some explanations have been suggested as discussed in **chapter 3** and include pharmacogenetic variations, immaturity of unpaired worms, location of worms, and intrinsic biological differences in worm biology. We speculate that if the decreased susceptibility of unpaired female worms holds true in the field, it may provide an explanation as to why after repeated treatment with praziquantel the infection intensity is only

temporarily reduced (27), but does not lead to clearance. Moreover, surviving female worms are able to pair with new incoming male worms, and lead to egg-producing infection, as seen in our reinfection study. Decreased treatment susceptibility unfortunately limits the future use of the female CHI-S model, but highlights an important, but overlooked limitation of praziquantel treatment. It further underscore the need for research into new treatment strategies, e.g. combination therapy, and novel therapeutics.

IMMUNE RESPONSES TO CHI-S

Immunology of schistosomiasis is complex and characterised by two distinct phases: the early infection stage and the chronic egg-producing stage (29). Most human data available is limited to the chronic egg-producing stage, as this is when diagnosis is made (30). However, with CHI-S we have now been able to investigate the early infection stage, which are an important target for vaccines. Some animal studies have previously suggested that immune responses to unpaired male or female cercariae differ (31), but we observe similar mixed immune responses consisting of Th1, Th2 and regulatory profiles four weeks post-exposure using flow cytometry. These findings were later confirmed using mass cytometry for high-dimensional profiling (32). Both studies also demonstrated increases in worm-specific IgG1, which has previously been associated with protection in animal studies with irradiated cercariae (9, 33), as well as in endemic populations (8). This raises the question if these responses can be protective. Although we observed boosting in the reinfection study, this did not lead to protection. Apart from the dose that is much higher in animal studies, the difference may be explained by the quality and specificity of the IgG response that may be shaped by the number of cumulative exposures, which is higher in endemic settings. In the reinfection study, we observed that participants accidentally exposed to male-female-male cercariae showed higher eosinophil CCL23, CCL4, and TNFα levels, as well as higher IgG antibody titres against soluble egg antigen, compared to single-sex exposed participants. These findings are consistent with initial responses to egg production in mice (34-36).

Table 1. Summary of CHI-S key findings

Key findings of CHI-S	Supporting data
Symptoms • AS occurs in the absence of eggs • Clinical tolerance to acute schistosomiasis rapidly develops after exposure	• AS is observed after male-only infection • AS symptoms decrease after first exposure in repeated infection study
Treatment • Female-only infections are less susceptible to praziquantel treatment	• Treatment failure after female-only infection
Immune responses • Immune responses to male-only and female-only cercariae are similar • Increases in worm-specific IgG1 responses are not protective • Egg-production is characterised by distinct immune responses	• Both show mixed Th1, Th2, and regulatory profiles • Absence of protection in repeated infections, despite boosting of IgG1 • Increase in eosinophils, CCL23, CCL4, TNFα, and SEA IgG in those exposed to male-female-male cercariae

AS = acute schistosomiasis, SEA= soluble egg antigen

ADAPTATIONS TO THE CHI-S MODEL

As outlined in the previous sections, we have gained many new insights into *Schistosoma* infections by performing these studies in quick succession (summarised in **Table 1**). Along the way, we have been making adjustments to our study procedures based on earlier findings to improve the model. Examples include the adaptive dose design in the female-only study or the higher PZQ dose for treatment in the female-only and reinfection study. Apart from these, there may be a few more modifications to consider for future studies.

With regard to the production of single-sex cercariae, there is need for an additional check to ensure cercariae are of the desired sex, as per protocol. This is achieved in two steps: 1) after the first shed, snails producing the “wrong” cercarial sex are immediately discarded; and 2) at a second shed, PCR on the tentatively selected snail is repeated to confirm the sex of the cercariae. The production of cercariae is a challenging, time-sensitive process with several variables that are difficult to control, such as the take rate of mono-miricidial infection i.e. probability of a snail become infected after exposure to a single miracidium, snail death, and the ratio of female to male secreting snails. Moreover, if this all goes well, the shelf-life of cercariae is only four hours after shedding. Mitigation of some of these risks would be a major step forward for CHI-S and may be brought about in the following ways: further optimisation of cercarial production processes and cryopreservation of cercariae. We collect

all production-related data in a dedicated database, that can be reviewed to examine particular steps in the production process and make the necessary alterations. The ability to freeze and store cercariae, as also done for other challenge inocula, would move the production process closer to GMP principles, where it is common practice to develop a well-characterised master bank (21). Additionally, it would remove the logistical challenge of producing cercariae for fixed-date study visits, as with the reinfection study. It also improves the scalability of CHI-S which, for logistical reasons, is in our setting capped at 24 people per cohort, as well as the transferability of the model.

On the clinical side, we would reduce the frequency of follow-up visits after PZQ treatment at the end of the study to once every four weeks, instead of every two weeks. We observed that CAA that can show a rebound after initial decreases in the first weeks, making the in-between visits of little clinical importance to decide whether to give additional treatment.

TRANSFER OF THE CHI-S MODEL TO ENDEMIC SETTINGS

The benefits of CHIM have been extensively discussed, but it should be noted that CHI studies are complex and require considerable expertise to navigate ethical and logistical challenges. This also applies to CHI-S. The studies described in **chapter 2, 3, and 5** were all conducted in *Schistosoma*-naïve participants in The Netherlands. It is important to note that people living in endemic settings may respond differently to CHI-S, because of prior exposure to schistosomes and PZQ treatment, coinfections and other environmental exposures. The same is to be expected for *Schistosoma* vaccine responses, as seen with Ebola, BCG, and malaria, among others (37). In light of vaccine development, it is therefore especially informative to perform CHI studies in endemic population, who are ultimately the target population for the vaccine. Experiences with controlled human malaria infections in for instance Tanzania and Kenya prove that CHI studies can be successfully performed in endemic settings and high-quality evidence can be obtained on vaccine hypo responsiveness and the influence of prior immunity on controlled human infection (38, 39). For schistosomiasis, we have been working closely together with the MRC/UVRI and LSHTM Uganda Research Unit to facilitate the transfer of the CHI-S model to Uganda, where schistosomiasis is highly endemic and causes considerable morbidity in affected communities. The team in Uganda has extensively engaged with stakeholders to discuss the ethical and scientific considerations surrounding a Ugandan CHI-S and identified key next steps to

move forward, which included the risk assessment in **chapter 4**. In parallel, the Ugandan team completed studies to engage with the target communities from which CHI-S participants might be recruited (40). Using in-depth key informant interviews and group discussions, they observed that communities were willing to take part in future CHI-S studies, but that enough time should be taken for the informed consent process to ensure study procedures, risk, and benefits are well understood. This study convincingly illustrates the value of qualitative research into CHI participation with findings that can be directly integrated in study processes and procedures. Together with the MRC/UVRI and LSHTM Uganda Research Unit, we plan on evaluating the Sm-p80 + GLA-SE vaccine in adults in a non-endemic (Dutch) and endemic (Ugandan) setting using CHI-S. The study protocol for the Leiden study can be found in **chapter 6,** which will also be used as a blue print for the Entebbe (Ugandan) study. Harmonisation of study procedures will enable us to make valid and meaningful comparisons between the study populations.

MAXIMISING SCIENTIFIC BENEFIT OF CHI-S

We focussed here on the main clinical findings of the CHI-S studies, which have already led to many new insights even before the model has been used to test a vaccine (**table 1**) through comparisons between 1) single-sex male vs single-sex female infections, 2) single vs. repeat infection, 3) and, unexpectedly, single-sex vs. mixed-sex potentially egg producing infection. Yet, there are many more insights to be gained from these trials. Because of intensive longitudinal sampling, the dynamics of particular (bio)markers or other outcomes can be assessed over time, as well as the effects of PZQ treatment. Moreover, because of the controlled nature of the studies, these trials are well-suited to explore host-pathogen interaction in the early stages of infection using for instance glycan and/or protein arrays and transcriptomics. While in the first study we performed sputum induction in three participants, that showed a mixed type-1/type-2 inflammatory cytokine profile compared to baseline (41), in the next studies we used nasosorption as a minimally invasive sampling procedure (42), that can be used to investigate pulmonary immune responses. Future exploratory analyses on the sample set is likely to increase the scientific contribution of these studies, but will depend on the following: extensive collaboration, data management and integration, statistical expertise, and data sharing.

To comprehensively understand *Schistosoma* infection, we need input from different research disciplines and involve clinicians, immunologists, biologists, and data specialists to approach it from multiple angles. Also collaboration across research institutes and across borders i.e. internationally, is crucial, especially for neglected tropical diseases, and must aim to build equitable partnerships. The integration of different datasets, for example clinical and immunological or immunological non-endemic and immunological endemic, is necessary to make informative comparisons and is therefore very important, however requires a thorough data management strategy for multi-omics data. The large number of potential outcome variables and repeated measurements make the statistical analyses challenging and requires specific statistical expertise. Lastly, to further increase scientific and societal value, the datasets should be made available to other researchers, in line with Open Science principles. All these fundamental new insights can feed back into vaccine development pipeline hopefully help identify new promising vaccine targets and/or strategies against schistosomiasis.

NEXT STEPS IN *SCHISTOSOMA* VACCINE DEVELOPMENT

Now that some vaccine candidates have shown good tolerability and immunogenicity, further studies in endemic populations are to be initiated. In **chapter 7**, we have discussed key immunological challenges that these vaccine candidates will likely encounter transitioning to phase II and III in endemic settings. In brief, prior exposure to schistosomes, prior or concurrent infections with other pathogens, and praziquantel co-delivery, can all potentially impact vaccine responses. However, whether these augment or antagonise vaccine efficacy is still unclear. Recognising these complexities is essential to designing future *Schistosoma* vaccine trials (**chapter 8**). To help understand these challenging immunological interactions, future studies should at least carefully screen participants for co-infections and previous exposure, and incorporate PZQ, artemisinin and/or albendazole administration at the same time or prior to vaccination. The study outcomes need to also be carefully defined, as there are multiple effect measures, e.g. risk ratio, hazards ratio, mean egg reduction, that can be used to calculate vaccine efficacy (43), but that each refer to different effects. Reducing egg excretion is an important goal of schistosomiasis vaccines, as this will result in reduced onward transmission. Traditional RCTs in which individuals are randomised to either vaccine or control arm, give only an estimate of the direct effect, immunity conferred to vaccinated individuals by vaccination, but do not evaluate indirect effect (i.e.

reduction in incidence resulting from lower force of infection in community). These indirect effects, however, can be evaluated in cluster-randomised studies, that allocate clusters consisting of all eligible study participants on school, village, or district level, are randomised to either the vaccine or control arm (44), and should be considered for future schistosomiasis vaccine studies. To understand how *Schistosoma* vaccines impact the epidemiology of schistosomiasis, it is worth considering adopting a OneHealth approach that includes monitoring of snails, the intermediate hosts, and environmental DNA, a tool for detecting cercariae in freshwater samples.

Also, optimisation of vaccine delivery should be continued. During the COVID-19 pandemic, we witnessed the expedited use of novel vaccine platforms with great success. The use of these platforms, such as mRNA vaccines, should be explored for *Schistosoma* vaccines as well, as currently the vaccine candidates are all subunit vaccines adjuvanted with either GLA-SE or Alhydrogel®.

Lastly, in light of increased reporting on vaccine hesitancy across many parts of the world (45), it is vital to start engaging with communities that either participate in vaccine studies or that will ultimately be provided vaccines upon successful licensure, well ahead of time. The successful roll-out of a vaccine depends strongly on context-specific factors, such as accessibility, timing, and perceived risks, requiring specialist knowledge. The importance of community acceptance and also political will, should not be underestimated. After all, an efficacious vaccine is only of limited value if the uptake is low.

CONCLUDING REMARKS

In this discussion, we have elaborated on the key findings from the CHI-S studies that in short time have contributed a great deal to our understanding of early-stage *Schistosoma* infection. Moreover the male-only CHI-S can now be used to evaluate vaccine candidates. To maximise the use of CHI-S, we have identified steps to optimise the model and enable transfer to endemic settings. Through extensive collaboration and data integration we will be able to explore *Schistosoma* infections in an unprecedented way, that will hopefully lead to fundamental new insights that can feed back into the vaccine development pipeline. We have summarised the challenges vaccine candidates may encounter transitioning to phase II and III studies and have suggested strategies to address these. Altogether, this work (**graphically summarised in figure 1**) hopefully enables us to expedite *Schistosoma* vaccine development,

culminating in successful licensure of an efficacious vaccine to help control this debilitating parasitic disease.

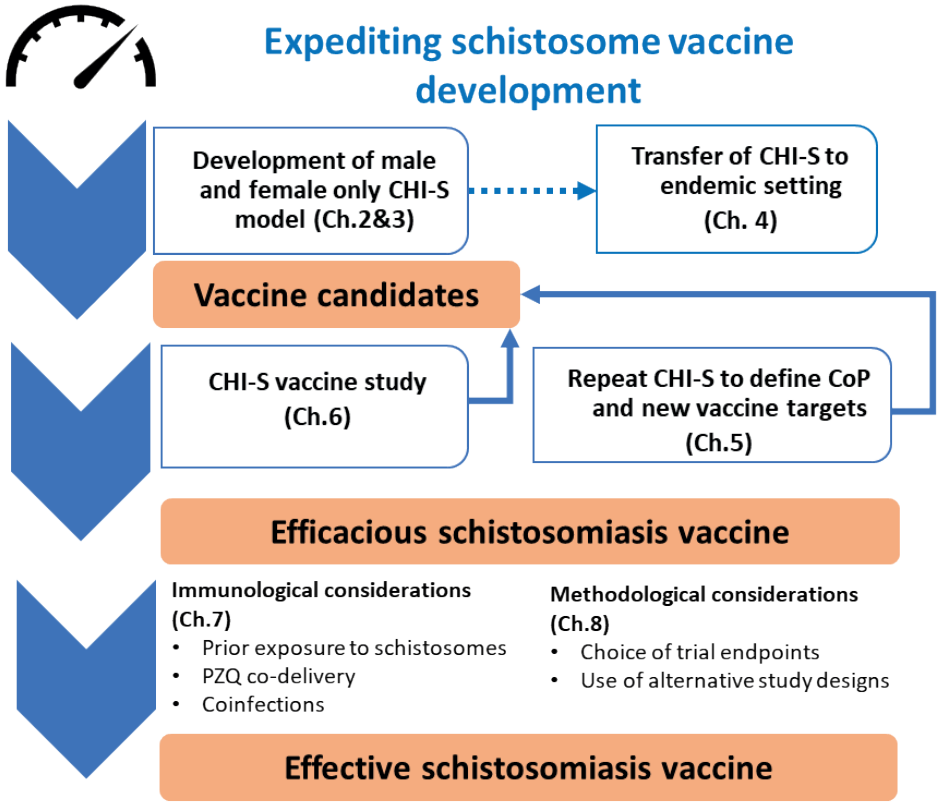

Figure 1. Contributions of this thesis to expediting schistosome vaccine development. CHI-S = controlled human infection with schistosomes; CoP = correlates of protection; PZQ = praziquantel; Ch = chapter.

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