Building bridges: a multidisciplinary approach to controlled human hookworm infection

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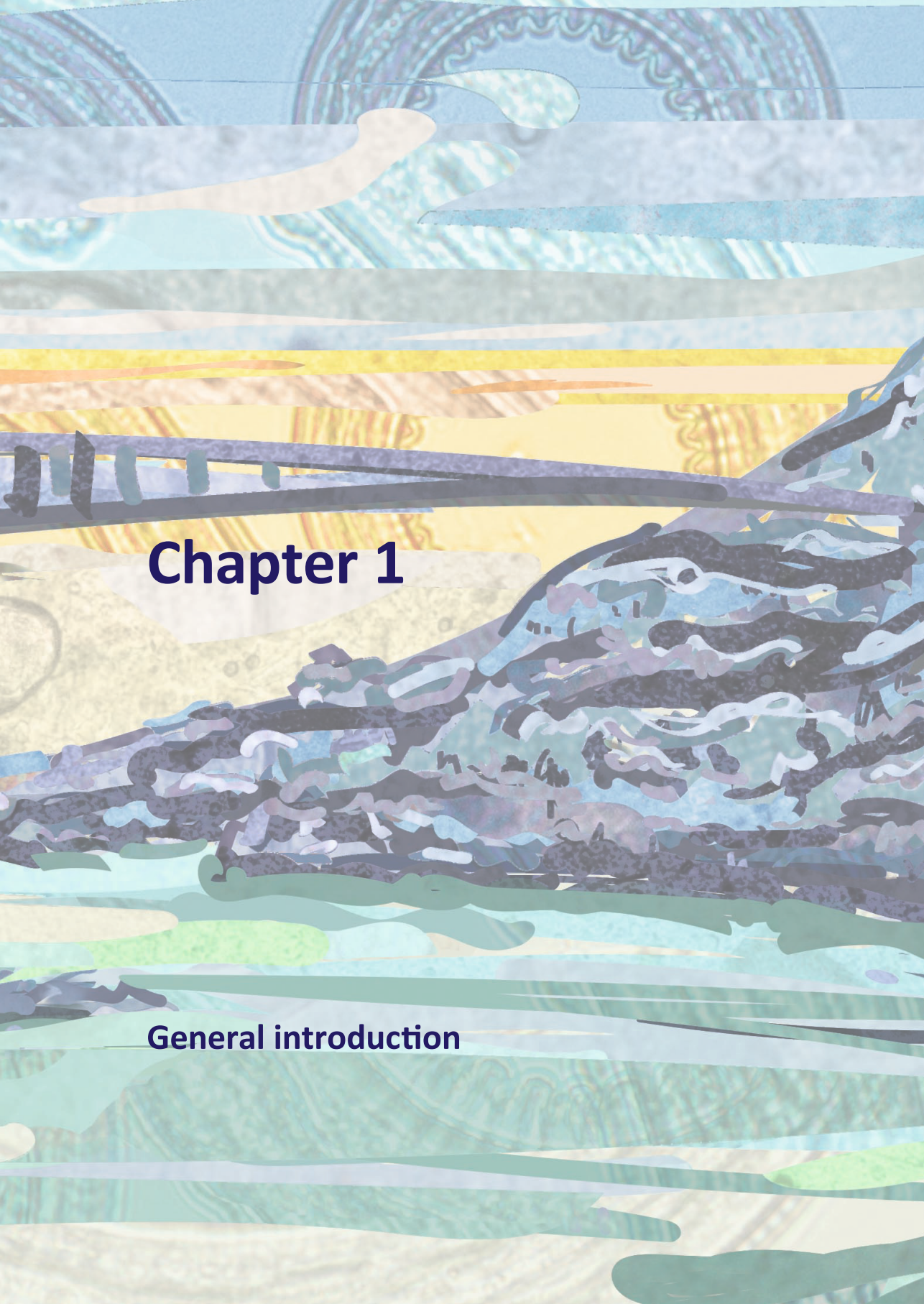

Chapter 1

General introduction

Controlled human infections

The deliberate exposure of humans with an infectious agent dates back to the 18th century, when Jenner inoculated his gardener's son with cow pox to prevent him from developing small pox and thereby laid the foundation for the concept of vaccination. Since then, deliberate infections have led to enormous developments in the preventive field of vaccination, have been used as therapeutic agents, as prove of Koch's postulates and to study pathogenicity factors. Controlled infections are furthermore increasingly applied as an effective and quick method of assessing vaccine- and drug efficacy.[1] Experimental infection of volunteers is also called 'controlled human infection', shortened to CHI. CHI-models are now being used in a range of diseases, including models in bacteria, viruses and parasites and range from colonisation models that generally do not cause symptoms (such as pneumococcus, lactobacillus) to models with overt symptomatology that is even used a primary outcome measure, such as the cholera and typhoid fever models.

Data generated in CHI-studies have led to several major milestones in knowledge of pathogenicity, host-pathogen interactions and vaccine development. CHI-studies in respiratory viruses have generated important knowledge on viral transmission,[2] trials in norovirus identified susceptibility factors of infection related to blood type[3] and CHI-studies have determined the importance of the typhoid toxin in developing typhoid fever.[4] Data from controlled malaria infection studies have played an important role in the development of the first malaria vaccine RTS'S.[5 6] The cholera challenge model demonstrates how CHI-studies into pathogenicity and then into vaccine efficacy can lead to a licensed vaccine. Observations on the development of protection after repeated exposure in controlled infections have first led to the development of an oral live-attenuated cholera vaccine, which in turn has now been licensed based on the convincing data from a human challenge study. [7-9]

Due to their efficiency and potential for quick screening of drug- and vaccine candidates, CHI-studies are particularly valuable for research into pathogens affecting the world's poorest population for which research funding and opportunities are scarce. These include among others schistosomiasis, for which recently a controlled infection model has been developed[10] and soil-transmitted helminths.

The controlled human hookworm infection model

Hookworms are a soil-transmitted helminth affecting around 300 million people worldwide.[11] The chronic blood- and protein loss caused by the attachment and subsequent blood feeding of the hookworm to the intestinal wall results in iron-deficiency anaemia and malnutrition, particularly in children and women of child-bearing age.[12] This significantly impacts children's development, and the resulting impairments have been estimated to generate a loss of productivity of between $7.5 and $138.9 billion annually.[13] As hookworm infection itself is strongly correlated with poor economic circumstances,[14] this reduction in wage-earning potential perpetuates the poverty cycle.

Current hookworm control efforts rely on mass drug administration programs. However, these have not yet succeeded in eliminating hookworm due to high rates of re-infection and the exclusion of adults in treatment programs.[15] In addition, treatment coverage rates still do not meet the standards set by the WHO and fall short of those projected to be necessary to achieve hookworm transmission control.[16 17]

A vaccine would greatly contribute to efforts to control hookworm.[13] Currently two hookworm vaccine candidates are being researched.[18 19] However, development and efficacy testing of vaccines is hampered by a lack of preclinical models, since hookworm has uniquely adapted to the human host and results in animal models cannot be easily translated to humans.[20] This also hinders the understanding of possible mechanisms of protection and human immunological responses against hookworm infections. Field studies in endemic areas can generate more knowledge, however studying immune responses is confounded by co-infections and unknown circumstances of infection intensity and duration. Vaccine field studies would require large sample sizes and impractically long follow-up.[21] The controlled human hookworm infection model (CHHI) could therefore greatly contribute to both effective vaccine-testing and to knowledge of hookworm infection immunology.

The projected hookworm vaccine does not need to induce sterile immunity to be effective, as a reduction in infection intensity will already have a significant clinical effect.[21] Reliable detection of infection is therefore a key issue in developing the CHHI model. Currently the only practical endpoint for a vaccine efficacy trial is egg excretion in faeces, by microscopic techniques or PCR. This however is a highly variable outcome,[22] which reduces study power and necessitates large sample sizes for vaccine efficacy trials. Finding ways to reduce this variation and obtaining egg counts that are comparable to field settings would greatly improve the reliability of the CHHI model. This thesis aims to find improvements to the challenge model and to outcome measures through repeated infection and statistical modelling, and will then apply this model to an immunisation study.

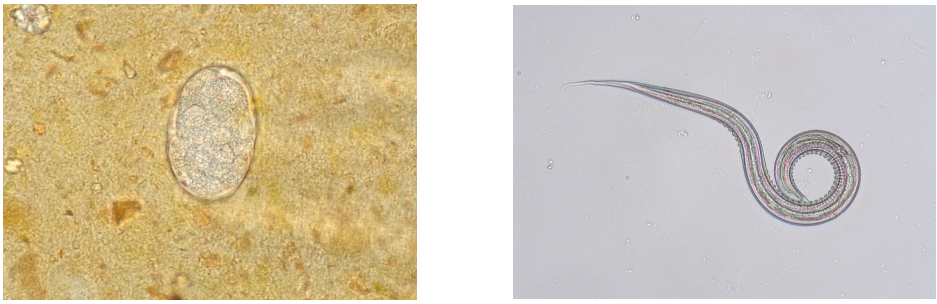

Figure 1 A. Hookworm egg (*Necator americanus*). B. L3 hookwom larvae (*Necator americanus*)

Studying microbiome using controlled human infections

It has been hypothesized that the immunomodulatory effect observed in hookworm infection is mediated through an effect on the gut microbiome.[23] It is therefore of interest to study the changes in gut microbiome after hookworm infection. Field studies have shown an increased richness and species diversity in infected individuals,[24] although clearance of the infection did not show an effect on relative species abundance.[25] However, there are many inconsistencies between results from field studies, due to the presence of confounders such as co-infections, malnutrition and limitations of the usually cross-sectional study designs.[26] Here, studying the microbiome in the controlled setting of the CHHI-model could generate clearer insights into the relation between infection and gut microbiome. In for example pneumococcal colonisation studies the CHI model has already been successfully applied to study susceptibility to colonization in relation to microbiome factors, changes in microbiome following pneumococcal colonization and the relation with viral co-infection.[27] [28] For hookworm, small scale studies with small infectious inoculae[29] [30] have found no major impact on microbiome community structure, although a trend towards an increase in species richness comparable to findings in field studies was described. However, the small infectious doses and limited number of participants reduces generalizability of these results and leaves room for further investigations.

Ethical aspects of controlled human infection studies

Despite the clear practical and scientific arguments for the use of CHI-studies, their conduct is an ongoing source of ethical debate. There have indeed been historical examples of infection studies that breach ethical standards, such as the Tuskegee syphilis study[31] and the Guatemala STD[32] inoculation study. However, these studies are considered unacceptable not because of the deliberate infection itself, but because they breached basic ethical principles, such as informed consent.[32] CHI-studies must adhere to the same guidelines as all other research, although CHI-study specific questions may apply.

The first ethical framework for the evaluation of challenge studies was suggested by Miller and Grady in 2001,[33] posing the questions and considerations that should be taken into account when reviewing a CHI study. The authors conclude that if these questions and considerations are met, CHI-studies may be ethically justifiable. Hope and MacMillan[34] have stated that the central issue regarding challenge studies lies in the risk of harm to participants and argue why in their opinion the concept of deliberate infection itself is no different than other research in healthy volunteers. Bambery et al have expanded the framework suggested by Miller and Grady by incorporating protection of the public, independent ethical review and compensation for harm to the critical questions.[35] When taking into account these criteria and with proper oversight, the authors argue that challenge studies should not only be considered ethically acceptable but may even sometimes be ethically required.[35]

A common factor in current ethical literature is a particular focus on the balance of risks versus benefits, importance of informed consent and the issue of deliberate infection

itself. Another issue that is frequently raised not only regarding CHI-studies, but in all studies involving healthy volunteers, is the motivation of participants and the influence of the financial compensation on the decision to take part.[36] Although this has been the subject of academic debate between researchers and regulatory authorities, the opinions of participants themselves have largely been lacking. This is a gap in knowledge that this thesis will aim to address.

Thesis outline

This thesis starts to provide an overview of controlled human infections in **chapter 2**. We then first focus on the controlled human hookworm infection model.

In **chapter 3** we describe the results of the pilot study to establish the CHHI model in Leiden and show through a prolonged follow-up phase that egg excretion develops a plateau level that can be used as an outcome measure in further studies.

Chapter 4 aims to further improve the CHHI model by investigating the effect of repeated infections on egg output and variability. We show that repeated infection does decrease variability without increasing adverse events and therefore propose an improved challenge model.

Chapter 5 shows another application of the hookworm controlled infection model in the investigation of changes in the gut microbiome after infection. This chapter describes the analysis of gut microbiome composition of the participants in the repeated infection trial from chapter 4 and shows that increased gastro-intestinal symptoms are associated with increased microbiome instability during acute infection which recovers in the immunotolerant phase.

The repeated infection challenge model described from chapter 4 is then implemented in **chapter 6** where it is used as the challenge following immunization using short-term infection. Here, we demonstrate for the first time that protection against hookworm infection can be elicited by repeated short-term infection and describe the importance of the skin in developing an immune response.

In **chapter 7** we investigate motivations and experiences of participants in CHI trials and describe why in our view CHI-participants could be described as 'deliberate decision-makers'.

In the summarizing discussion in **chapter 8** we aim to bring together all research described in this thesis and show how a multidisciplinary approach is needed to enhance improvements in controlled human infection models.

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