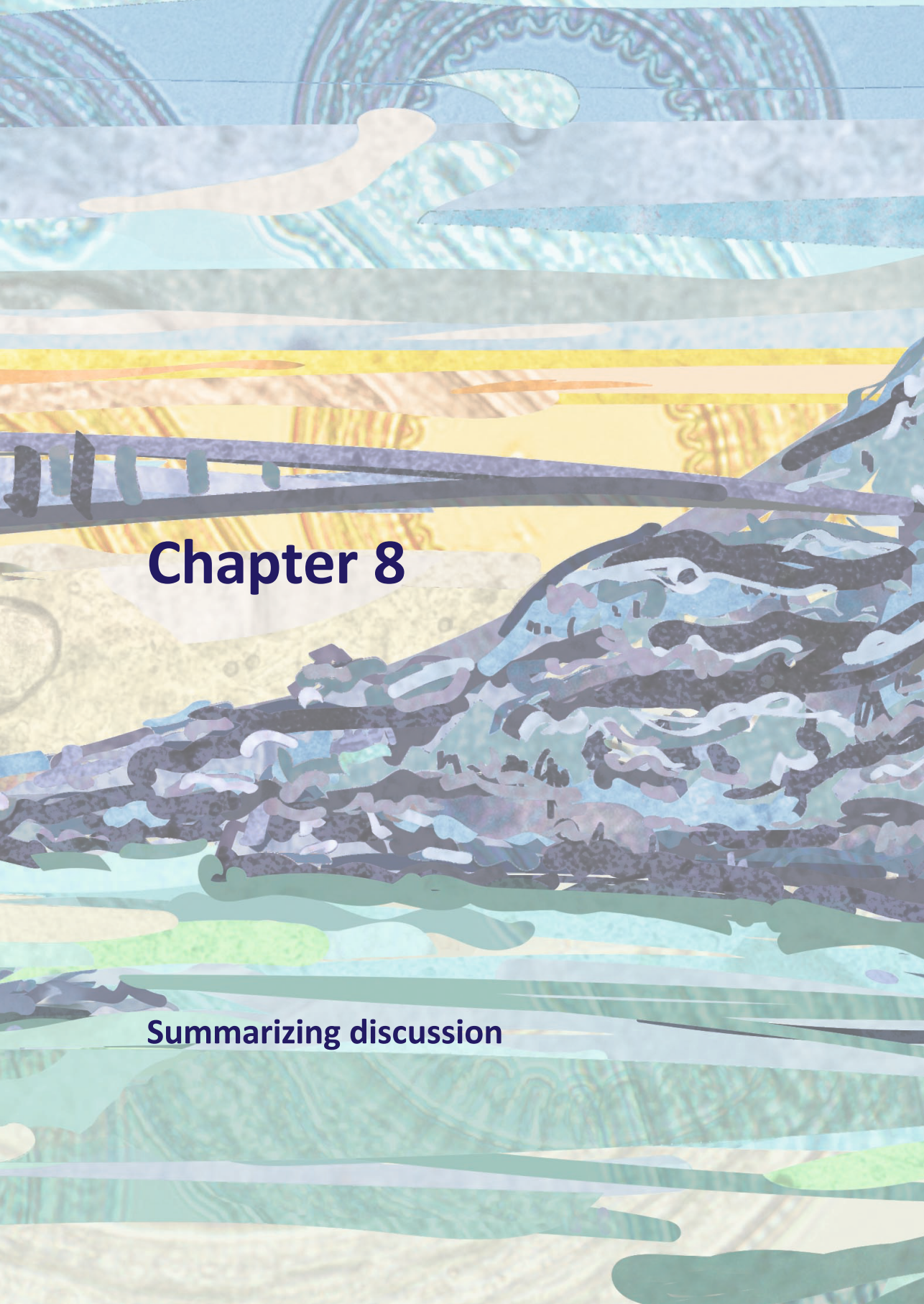
Building bridges: a multidisciplinary approach to controlled human hookworm infection

Hoogerwerf, M.



Chapter 8

Summarizing discussion

Building bridges: connecting disciplines to improve controlled human infections

In this thesis we have approached the controlled human hookworm infection model and the concept of controlled human infection from multiple disciplines and angles.

We started with presenting an overview of controlled human infections. We have developed an improved controlled human hookworm infection challenge model and applied Bayesian statistical modelling to describe the egg output with higher precision. This model has been applied as challenge model in an immunization study investigating the protective efficacy of short-term exposure to hookworm larvae. Additionally, changes in the gut microbiome after controlled human hookworm infection were explored. We furthermore investigated the motivations and experiences of participants and participants' views on ethical questions surrounding controlled infection studies. In this discussion we will summarize findings, merge the different trials together and will argue why it is our ethical responsibility to approach controlled human infection in such a multidisciplinary way.

Controlled human hookworm infection model

In this thesis we have described the results of three controlled human hookworm infection trials. In the first study, the Controlled Human Hookworm Infection Leiden (shortened to CHHIL) described in **chapter 3**, we report a pilot study to establish the hookworm culture in Leiden and investigate long-term kinetics of egg excretion. This pilot has led to the development of a Bayesian statistical model to describe egg excretion. Using this model we described the development of a plateau phase around 12-13 weeks after infection and discovered the use of multiple samples from the plateau phase as a more reliable outcome measure for future trials. We furthermore found that although variability in egg excretion was reduced for samples of the same volunteer on the same day due to the homogenization of faeces, variability between individuals and within individuals on different timepoints was still considerable. We therefore wanted to further reduce variability in egg excretion by introducing a repeated infection. This was investigated in the ReCHHI study (Repeated Controlled Human Hookworm Infection). The effects of one, two or three doses of 50 L3 were described in **chapter 4**. Using the previously established Bayesian statistical model we found that a double infectious dose indeed lowers variability relative to the mean and increased egg load compared to a single dose without aggravating adverse events. A third dose however did not further improve the model. Power calculations based on the statistical modelling showed that the number of samples taken is the most important determinant for study power. The combined results from these two studies leads us to propose a challenge model of 2x50 L3, with a follow-up of 16 weeks after first infection and taking samples from week 12-16 to determine egg excretion in repeated samples. This model was subsequently applied to investigate the protective efficacy of short-term exposure to infective larvae against challenge infection in the ITCHHI trial (Immunisation, Treatment and Controlled

Human Hookworm Infection) in **chapter 6**. Here, we found that those volunteers with severe skin reactions showed significantly lower egg load after challenge, demonstrating a possible relation between the skin phase and the development of protective immunity. Concluding, we have established an improved controlled human hookworm infection model and shown its application in an immunization trial that for the first time showed a protective effect of short-term exposure to infective larvae against hookworm challenge.

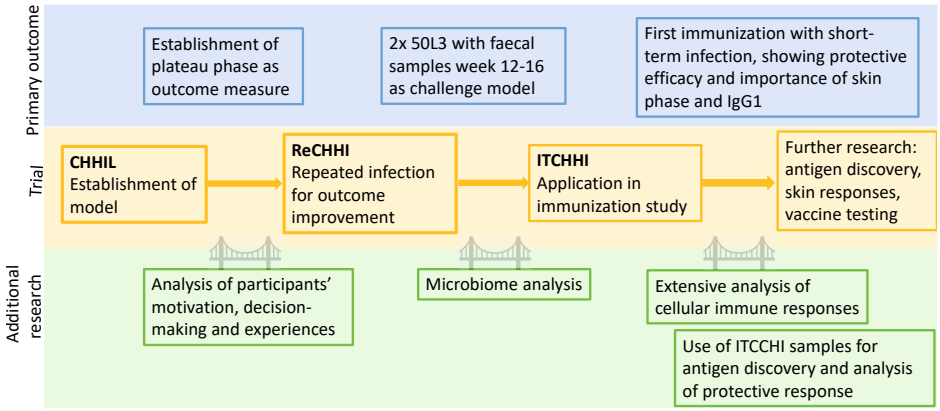


Figure 1. Schematic depiction of controlled human hookworm infections conducted in this thesis with primary research developments resulting from the trial, and examples of additional research conducted.

Multidisciplinary approach

Next to the involvement of advanced Bayesian statistics to improve the controlled human hookworm infection model, we have maximized the scientific output of the studies by taking an interdisciplinary approach. **Chapter 5** describes the analysis of the gut microbiome in relation to the controlled human hookworm challenge. Here, we found that gastro-intestinal symptoms following hookworm infection were associated with increased microbiome instability, which after establishment of the infection stabilized over time. In **chapter 7**, we have explored ethical aspects of controlled human infections. We described how volunteers' motivations for participation are highly varied and that volunteers take many factors into account in their decision to partake. The concept of controlled human infections and critical questions surrounding it, such as restrictions on the right to withdraw and the necessity for quarantining were widely accepted by both participants, students interested in participation and students who would not participate. This led us to conclude that participants in controlled human infection trials are not, as often feared, money-oriented risk-takers, but rather deliberate decision-makers who have made a multi-facetted decision to take part.

Connecting disciplines to improve controlled human infection models

Through this thesis we have aimed to establish improvements to the controlled human hookworm infection model though collaboration with several other disciplines.

Improving outcome measurements in CHI-models

Accuracy in outcome measures is a major challenge in the development of clinical trials. Firstly, the outcome measure needs to be reliable in order to be able to correctly interpret the study results. Secondly, precision in the outcome measure, e.g. the standard deviation, is a crucial component of sample size calculations and therefore affects study power. In any clinical study sample size calculations require a fine balance between including a sufficient number of participants for a meaningful outcome but not exposing an overly large group of participants to the possible harms of the study. Improved precision in outcome measures can reduce sample sizes whilst retaining study power.[1]

In controlled human hookworm infection (CHHI) models, faecal egg output is the main outcome used. Some studies have used endoscopic evaluation of worm establishment in the gut.[2 3] However, this is not a feasible study outcome in larger scale trials because of the burden to the volunteer, high costs and remaining uncertainty about the reliability of this measure. Therefore, examining egg output in the faeces is the best proxy outcome to establish infectious burden, as is done in the field.[4]

In initial controlled human hookworm trials, egg output was significantly lower compared to natural infection and could not be achieved in all donors.[5] This hampers comparability to infections in the field and necessitates large sample sizes in order to establish infection in a sufficient number of participants. In our CHHIL-study, we have shown that it is possible to increase the infectious dose with a tolerable safety profile resulting in the highest egg counts then described in literature. The repeated infection further increased the egg output to a posterior mean at plateau level of around 1500 epg, hereby resembling infection in mild-endemic settings.[6]

Egg excretion is a highly variable outcome, with high sampling errors and dependent on day-to-day variation in egg excretion.[7] In our studies we have used Kato-Katz as measure for egg excretion, as microscopy is a field-applicable technique and mostly used in field studies. Other studies have used culture data as primary outcome.[8] This outcome measure is dependent on culture techniques, egg survival in storage and due to its laborious process cannot be used for multiple sampling. Recent studies are focusing on the use of PCR in field settings. PCR studies are complicated by lack of field-applicability and may suffer from confounding in the outcome measure as the amount of DNA in eggs can differ depending on the cell stage of the egg, which does not reflect the actual egg count but rather the maturity of the eggs. Although some correlation between PCR and egg counts is found this is not repeated in all studies.[9] PCR will probably gain more importance in future as techniques

improve, however PCR similarly suffers from to day-to-day variation in egg excretion, which is considerable.

This high variability hampers the precision of egg counts as outcome measure. Single measures on one day do not give a representative impression of hookworm infection burden. Using 'traditional' statistical methods such as means or medians is possible but has the downside of either missing a lot of variation or being easily skewed due to high variability if using a single measurement of faecal egg load. We have therefore incorporated expertise from advanced Bayesian statistical modelling into the analysis of egg counts. This has led to the development of a statistical model that is able to calculate a posterior estimated mean and give estimates for variability on different levels. This model identified high variability between daily measures in the same volunteer and the development of the plateau phase upon which further studies were based. Application of this model to power calculations further refined the established outcome measure, where it showed that using multiple sequential samples decreased the variability and improves study power. In data with many factors of variability this Bayesian model-based power calculations take into account all sources of variability and uncertainty in the data, thereby generating a more reliable power estimate. The Bayesian model can also generate insight into where most variability in outcomes can be found, targeting possible interventions to reduce variability. This shows that crossing the bridge to involve more elaborate statistical analysis than perhaps is common in this type of trials significantly impacted the reliability and usefulness of this controlled infection model.

Applications in other controlled human infection models

Bayesian modelling has also been applied to controlled human malaria infection, describing the kinetics of parasitemia using qPCR.[10] Power calculations based on this model found that a vaccine targeting the liver stage requires a large sample size compared to an erythrocytic vaccine stage due to the large inter-individual variation in first generation parasites and that needle-based CHMI may result in improved power for hepatic vaccines. Other CHI-models that use quantitative measures of infectious load that may be highly variable, such as viral load in nasal swabs (e.g. influenza, respiratory syncytial virus) or excretion of oocysts in stool (cryptosporidium) may also benefit from a similar approach.

Another approach to improve outcome measures can be done by standardizing the monitoring of clinical symptoms. Some studies use mainly clinical symptoms as outcome of their study as add-on or even instead of microbiologic parameters. In influenza trials, questionnaires have been used to capture participant-reported symptoms.[11] This questionnaire was added to traditional anti-H and anti-N antibody measurements, allowing for better comparison with natural infection and better understanding and tracking of symptom development. This gives a better indication of direct patient benefit of drugs or vaccines in terms of quicker symptom resolution or diminished symptoms than viral shedding or antibody measurements, and therefore results in a clinically more relevant outcome.

An example of the value of the systematic collection of adverse events and the application of statistical techniques for outcome measure improvements is the development of a disease severity score for the *Shigella* and enterotoxic E. Coli (ETEC) CHI-model. Here, available participant data has been gathered for the individual models and through multi-correspondence analysis a disease severity score was developed.[12 13] This disease score has several advantages over the current outcome measures. Most *Shigella* and ETEC CHI-models use stool-based outcome measures. However, these are not field-applicable, suffer from different definitions used in different trials and miss other important disease information. The disease severity scores incorporated objective disease signs, subjective symptoms and stool output. This resulted in an outcome measure that was more in line with the classification of clinical disease and more efficient in capturing disease in participants. Using this new score, the attack rate in the control group was increased. This has the advantage of being able to decrease sample sizes in future studies. There are still several limitations to this score, including a large variation between challenge strains used and these scores which need to be validated in new trials,[14 15] however its development is a good example of the possibilities of combined data analysis.

Adverse events

When performing studies where volunteers can expect little to no personal benefit, as with all CHI-studies, one of the major ethical issues is the possible risk and burden to the volunteers. Adverse event reporting is a major factor in this assessment. Furthermore, as discussed above adverse events may be an important outcome measure of the trial. Understanding which adverse events develop, why and in which volunteers is therefore very important to further improve controlled human infections.

Adverse events in controlled human hookworm studies

In our hookworm studies we have seen a remarkably large variation in the number and severity of skin and abdominal adverse events, both between studies and between individuals. In all trials, there have been volunteers with very few, mild adverse events and others with more severe events. In the ReCHHI study abdominal events were most severe, with three volunteers requiring rescue treatment. No association between infectious dose and abdominal events was found in this study. In the ITCHHI study skin adverse events were most severe, which was found to be related to egg load after challenge. Previous studies had found that exposure to large numbers of larvae at the same site resulted in more severe skin adverse events.[5] We therefore divided the infectious dose over several sites, which was successful in reducing skin adverse events after first exposure. Although there was interindividual variability in severity in our studies, skin adverse events were generally related to repeated exposures and the immunization process. Clear predisposing factors for the abdominal adverse events however were not found. Gastro-intestinal symptoms were not dose-related in our studies. Previous studies with much smaller inocula have described a similar unpredictable pattern in adverse events, although inocula up to 20

larvae do not seem to produce severe adverse events.[5 16 17] In the ReCHHI study we did find a correlation between peak eosinophilia and severity of abdominal adverse events. It can be speculated that this is a reflection of an eosinophil driven enteritis upon establishment of the worms in the gut. Without biopsies it is however not possible to confirm this. In the ITCHHI study the relation between adverse events and eosinophils was less clear although a similar non-significant trend was observed. Identifying factors that predispose volunteers to more severe adverse events would be of great value to improve the risk-benefit balance of the CHHI model. These factors may include a previous history of abdominal symptoms such as irritable bowel syndrome, factors in the gut microbiome or immunological characteristics. In the microbiome study (**chapter 5**) we did find that volunteers with more severe abdominal adverse events had less stable gut microbiome after infection. Whether this instability in microbiome composition was a predisposing factor to, or a consequence of, more severe (eosinophilic) enteritis remains uncertain. More data needs to be gathered to investigate if and which microbiome factors predispose to more severe abdominal adverse events.

If we could better predict which volunteers develop severe adverse events this would greatly improve the safety profile of a CHI-trial. In the controlled human malaria model some research has been performed comparing adverse event profiles using different inoculation methods.[18] However this analysis only looked at immunological factors after infection. No research has currently been done comparing pre-infection parameters to post-infection adverse events. Systematically recording adverse events and recording baseline characteristics together could provide an interesting opportunity to investigate risk factors for the development of severe adverse events. At this moment trial protocols are not developed for this purpose. As very little is known about pre-disposing factors this would also involve the analysis of large amounts of data, necessitating particular expertise in analysing and interpreting large datasets.

Advanced data integration can also be applied to better investigate correlates of protection. We show an example of this in the integrative analysis performed in **chapter 6**. Although the sample size was too small to be used as a predictive tool, the analysis confirmed findings on correlates of protection. In larger data sets, this can be further developed as a prediction tool and may aid in investigating factors associated with adverse events. As this will need to focus on factors that can easily be measured before start of a trial, much more data particularly on easy to assess clinical characteristics is needed for this analysis.

Standardized method of reporting adverse events

As we have argued above a thorough understanding of adverse events is vital for a good assessment of risks and burdens to study participants and could contribute significantly to a better understanding of which volunteers are at risk of developing more severe adverse events. An attempt to review the safety profile of controlled human infections has recently been published, showing serious adverse events are rare and confirming an overall good safety profile.[19] A more in-depth analysis of adverse events however was hampered by the

highly diverse reporting between different studies. Many CHI-studies are relatively small scale and therefore in themselves not powerful enough to identify risk factors for the development of severe adverse events or develop meaningful clinical outcome scales. If adverse event data would be more uniformly documented and reported this would provide highly valuable information and enable conduct of for example meta-analysis of safety outcomes. More standardized reporting would also enable datasets to be combined in integrative analysis for which CHI-studies on their own are usually too small. This is one instance where we may not need to bridge to a different discipline but to other research groups in a similar discipline, harmonizing reports to improve both research quality and participant safety.

Questioning participants and engaging participants in study design

The questionnaire study described in **chapter 8** is one of the first studies to quantitatively study participants' motivations and experiences. Recently Kamuya et al. have described in detail qualitative interviews with participants in malaria controlled human infection trials performed in an endemic setting, providing an important addition to the knowledge of participant experiences in these studies. Although there were differences with the findings in our study, particularly due to the different setting, all studies thus far have described that although the financial compensation is important, participants consider many different aspects before taking part in a study. Volunteers also generally report a satisfactory experience in participating. [20-22]

This knowledge is an important contribution to the ethical debate surrounding controlled human infection. There has been extensive discussion on ethical issues such as social value, acceptable risks and burdens, concepts such as quarantine, the right to withdraw and the influence of payment. [23-25] These are issues that can gain from assessment of participant and public opinion.

The COVID-19 pandemic has greatly increased the voice of the public in the discussion surrounding participation particularly in CHI-trials. The 1DaySooner movement started out as a collective of people willing to participate in COVID-19 challenge studies and is now developing into an important platform of participants speaking out on many aspects of controlled human infections and driving research development in CHI-studies.[26]

When developing controlled human infection models in endemic settings, community engagement is one of the key factors to be taken into account and gains particular attention in set-up of trials. [22 27-29] It is recognized that community acceptance and involvement is vital in the success of these trials. This engagement however is largely lacking in already established CHI-models in the non-endemic areas where most CHI-studies are still conducted. Initiatives such as 1DaySooner aim to mitigate this, but there is still a considerable lack of participant questioning and engagement.

Participant involvement can furthermore aid in the ethical debate surrounding healthy volunteer research. CHI-studies have been ethically scrutinized particularly on the aspects of deliberate infection and perceived breach of the right to withdraw due to quarantine restrictions for transmissible pathogens.[23] Our questionnaire study however found that most participants were happy to agree with these restrictions, as long as it was well explained in the informed consent procedure. Involving participants in trial set-up can also inform researchers about which aspects participants themselves consider to be too high a burden or ethically unacceptable. This can result in a better ethical appraisal of a proposed trial. Routinely adding questionnaires to any participation in a CHI-trial would result in a balanced representation of participants' views that can be used to further improve follow-up studies. An excellent opportunity to build a bridge between the biomedical and social sciences, and society itself.

Maximising scientific output of CHI-studies

The CHI-model has mostly been propagated as a model to test novel drugs and vaccines. However, CHI-trials have much more potential uses than only vaccine- and drug studies, as we have highlighted in **chapter 2** and the studies in this thesis further underscore. The microbiome study presented in **chapter 5** is an example where a more fundamental research question was addressed with samples of a CHHI trial. The ITCHHI study (**chapter 6**) obviously does not immediately lead to a vaccine that is field-applicable, but has generated highly novel insights into the development of protective immunity against hookworm infection, insights which may impact vaccine development in future. Similar more fundamental studies include trials investigating the interplay of influenza infection with pneumococcal nasal carriage[30 31] and the chemo-attenuated malaria model.[32]

CHI-models are not only a vehicle for testing vaccines and drugs, but their controlled nature offers important opportunities to better understand the pathogen-host interactions. These more fundamental questions can be answered either as an add-on to a clinical trial researching a drug or vaccine, or through a separate study. Many clinical CHI-trials have led to multiple publications, one describing the clinical result and others reporting a more fundamental analysis of for example microbiome factors or an extended immunologic analysis. For any clinical trial additional research questions can be formulated. In order to make maximal use of the investments of volunteers' contribution, materials and research funding, already in early planning stages careful consideration must be given to all possible scientific outcomes other than the primary research question. Involvement of researchers from different specialties than the principal investigator aids to find and address these questions. This add-on approach to fundamental questions significantly increases the scientific value of a CHI-study. In fact, it can even be argued that it is our ethical duty as researchers to maximise the output from a given trial to make the maximal use of the investment of the volunteer and specifically look for the fundamental questions that can be answered next to the primary question.

Concluding remarks

In this discussion we have suggested several improvements to be made to CHI-models, including the refining of outcome measures, systematic registration of adverse events, engagement of participants and addressing fundamental questions alongside vaccine- and drug studies.

For these improvements to be implemented a multi-disciplinary approach to CHI is inevitable. In this thesis we have presented the results of collaboration with multiple disciplines which have led to the conclusions presented here. We have demonstrated the added value of advanced statistics in the modelling of hookworm egg excretion and in-depth analysis of all available data to establish correlations. We have also shown the importance of adding an ethical and/or social science approach and have provided an example of how one CHI-trial can be analyzed by several disciplines each bringing up their own novel findings. In addition, we argued for the inclusion of multiple specialties in designing CHI-trials to maximise the output of not only the clinical trial but also fundamental questions that can be answered by a CHI-trial.

Building the bridge

The bridge on the cover of this thesis forms a connection not between two islands but between the two ends of a fjord in Norway. It brings together two pieces of land that, although a connection exists, require a long way to meet. Standing on one coast, it is easy to view the other side as an island without connection. This bridge links the two ends together in a shortcut, greatly reducing travel times along the coast. This is symbolic for the aims in this thesis: scientific disciplines are all connected, we are not islands. However, we cannot always immediately see this connection and may feel like an island. Reaching the same output may then feel like a very long road to travel. Reaching out and bridging the fjord brings the two ends together and allows us to much more rapidly continue our journey to the communal aim: fighting infectious diseases and improving health care for all.

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