Building bridges: a multidisciplinary approach to controlled human hookworm infection

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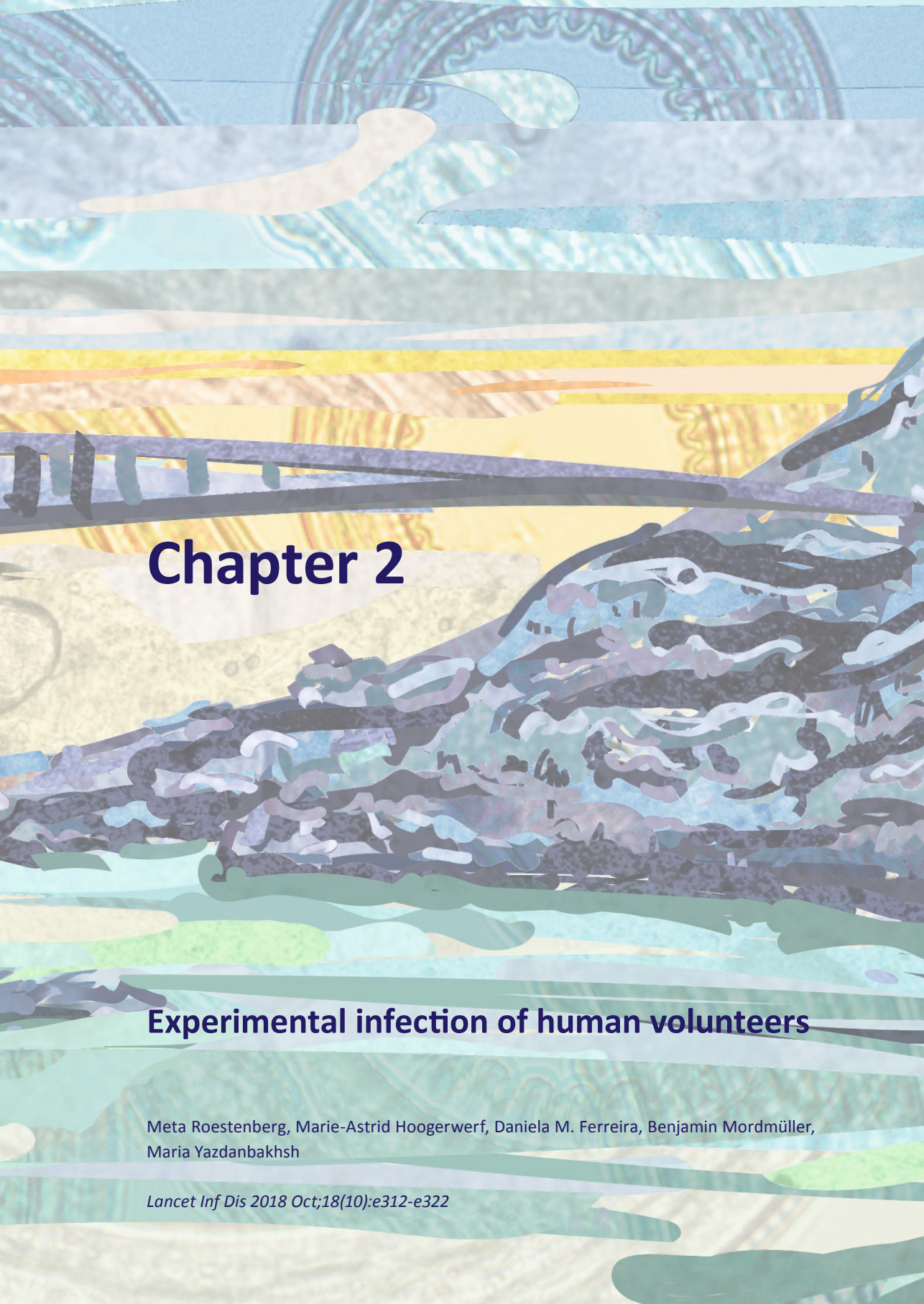

Chapter 2

Experimental infection of human volunteers

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Abstract

Controlled human infection trials (CHI), in which healthy volunteers are experimentally infected, can accelerate the development of novel drugs and vaccines for infectious diseases of global importance. The exploitation of CHI models is expanding exponentially from around 60 studies per decade in the 1970's to more than 120 studies already published in the current decade. Influenza, rhinovirus and malaria are the most frequently practiced CHI models. CHI trials have provided historical landmark data for several registered drugs and vaccines and provided unprecedented mechanistic insight into host-pathogen interaction. Because of their often invasive nature and the use of healthy volunteers, CHI studies contradict the "do not harm" principle and as such demand critical ethical review according to established frameworks which are similar to phase 1 clinical testing. Advances such as the principle of controlled colonisation and the expansion of models to other sites will further broaden the horizon for CHI. Here, we review the use of CHI trials and provide an outlook for this dynamic field in the future.

Introduction

Controlled human infections (CHI), through the transfer of body fluid such as serum,[1] respiratory secretions[2] or faecal filtrates[3] laid the foundation for infectious disease research starting in the 17th century. Unparalleled human experimentation led to the identification of causative organisms (norovirus,[3] influenza,[2] dengue,[4] sarcocystis[5]), not only proving Koch's postulates, but also providing an opportunity to study incubation periods and clinical disease. Important discoveries were made through CHI trials, such as the identification of toxins as causative agents in diarrhoeal disease following instillation of a filtrate of *Vibrio cholera* culture broth in volunteers in 1966.[6] Whilst the ethical circumstances of these initial studies were often questionable, the realization that they provided a core platform for the study of infectious diseases, has resulted in the increased use of CHI models in the past decades. As CHI models have moved into the 20th century, ethical frameworks have been developed and rigorous independent review of risks and benefits of the study are carried out. The aims of the studies involving CHI have shifted from exploratory and descriptive studies to trials that take a central position in the vaccine and drug development pipeline. [7] CHI trials often act as a gatekeeper for proceeding to field efficacy trials, although in exceptional cases, may be accepted as proof-of-efficacy in Phase 3 clinical development.[8] The development of novel CHI models and the exploitation of existing CHI models in the product development pipeline has been propelled recently by the investments of large funders such as the Bill and Melinda Gates Foundation, the Wellcome Trust and the UK Medical Research Council. CHI studies, by allowing preliminary efficacy testing amongst 10-100 participants, are cheaper compared to phase 2 and 3 clinical trials in endemic areas with samples sized ranging from hundreds to 100,000 participants. In addition, CHI studies allow for a large number of candidate products to be tested, increasing the chances of identifying highly potent products and minimizing the risk of late clinical failure and reducing exposure of (vulnerable) populations to inactive interventions (figure 1). The resulting diversity in CHI models is impressive, with considerable heterogeneity in inoculum, endpoint and clinical procedures (table 1). In CHI trials biomarkers, protective responses and mechanisms of disease can be studied more precisely, which ultimately feed back into the product development pipeline to improve the next generation of medicines. Novel technological advances such as –omics tools are applied to identify risk factors such as diet, microbiome, co-infections or genetic background in complex multiparametric analysis. Pathogens are altered by genetic modification in order to identify key virulence genes (NCT03067961) or provide less virulent challenge inocula which will allow for clinically less severe CHI models.[9] In this review, we provide an overview of the active CHI models, discuss their contribution to biomedical science and risk some predictions of what can be expected in this dynamic field in the future.

Table 1. Summary of characteristics per CHI model based on published data. Most commonly used strains are reported, number of volunteers is estimated from publications.

Pathogen	Route	Dose	Strain	End points	Est. # volunteers	In/outpatient/ isolation	Ref
Rhinovirus intranasal		10.000 TCID50	HRV-16, HRV-39	viral replication, clinical symptoms	5760	outpatient	[10]
Influenza virus	intranasal	10^3-10^7 TCID50	*	viral shedding in nasal lavage, clinical symptoms	3540	in patient quarantine	[2]
Plasmodium falciparum	mosquitos, intravenous	5 mosquitos, 3200pfSPZ	NF135.10, NF54	parasitemia	2650	outpatient	[11 12]
ETEC	oral	≥ 5x10^8 CFU	B7A, H10407, E24377A	diarrhoea	1215	outpatient	[13]
Vibrio cholerae	oral	10^5 CFU	El Tor Inaba N16961, O139	diarrhoea	1210	inpatient	[8 14]
***Salmonella* Typhi**	oral	1-5x10^4 CFU	Quailes	fever or bacteraemia	1000	outpatient	[15]
Respiratory syncytial virus	intranasal	4log10 PFU/ml	M37, A2	viral load in nasal lavage, respiratory symptoms	1000	in patient quarantine	[16]
Shigella spp	oral	10 CFU – 10^{10} CFU	*S. flexneri* 2457T, *S. sonnei* 53G	diarrhoea, antibody response	850	inpatient	[17]
Norovirus	oral	48 RT-PCR U	8FIIa, GI.1, GII.4	gastro-enteritis, PCR faeces, ELISA	810	inpatient	[18 19]
Lactobacillus spp	oral, vaginal	oraal 10^9 CFU 1dd, 7.5x10^8 CFU subs	*L. rhamnosis* GR-1, *L. reuteri* RC-14, *L. crispatus* CTV05	clinical UTI	800	outpatient	[20]

Table 1. Summary of characteristics per CHI model based on published data. Most commonly used strains are reported, number of volunteers is estimated from publications. *(continued)*

Pathogen	Route	Dose	Strain	End points	Est. # volunteers	In/outpatient/ isolation	Ref
Streptococcus pneumoniae	intranasal	10^5-10^6 CFU	6B, 23F	colonisation	790	outpatient	[21]
Haemophilus ducreyi	Intraepidermal and intradermal	10-150 CFU	35000HP	pustule formation	550	outpatient	[22]
Dengue virus	subcutaneously	10^3 PFU	DEN2Δ30	viremia, rash, neutropenia	520	outpatient/ inpatient	[4]
Francisella tularensis	aerosol	10^4-10^8 organisms	SCHU S4	systemic symptoms	500	inpatient	[23]
Neisseria lactamica	intranasal	10^4 CFU	Y92-1009	colonisation	310	outpatient	[24]
Plasmodium vivax	mosquitos, intravenous	5 mosquitos, 3200 pFSPZ	wild-type	parasitemia	300	outpatient	[25]
Campylobacter jejuni	oral	10^6-10^9 CFU	initially 81-176, now CG8421	diarrhoea	260	inpatient	[26]
Cryptosporidium spp	oral	10-10^5 oocysts	**	stool oocysts	260	outpatient	[27,28]
Necator americanus	Transdermal	10-50L3 larvae	Papua New Guinea	Eggs in stool	250	outpatient	[29]
***Escherichia coli* (UTI)**	Urethral catheter	10^5-10^6/ml	83792, HU2117	clinical UTI	200	outpatient	[30]
BCG	intradermal	1-4x10^5 CFU	BCG	immune response	140	outpatient	[31]

Table 1. Summary of characteristics per CHI model based on published data. Most commonly used strains are reported, number of volunteers is estimated from publications. *(continued)*

Pathogen	Route	Dose	Strain	End points	Est. # volunteers	In/outpatient/ isolation	Ref
Neisseria gonorrhoea	urethral catheter	$1{,}8x10^3$ Ms11mkC, $1.0x10^5$ FA1090	FA1090, MS11mkC	colonisation	140	outpatient	[32]
Giardia lamblia	oral	$5\text{-}10^4$ trophozoites	GS-M83/85	cysts in stool, antibody response	120	inpatient	[33]
Helicobacter pylori	oral	10^4 CFU	Baylor 100	urea breath test, histology	80	outpatient	[34]
***Salmonella* Paratyphi**	oral	$1\text{-}5x10^3$ CFU	NVGH308 strain	fever or bacteraemia	40	outpatient	[35]
Parvovirus B19	nasal	Up to 5^{10} viral genomes	Wild-type	viremia	12	Inpatient isolation	[36]

ETEC= Enterotoxicogenic Escherichia choli, Salmonella Typhi/Paratyphi = *Salmonella enterica* subsp. *enterica* serovar Typhi

* A/Texas/39/91 (H1N1), A/California/2009 (H1N1), A/Winsonsin/67/2005 (H3N2)

** *C. muris*: RN66, *C. meleagridis*: TU1867, *C. hominis*: Iowa strain, *C. parvum*: Iowa strain

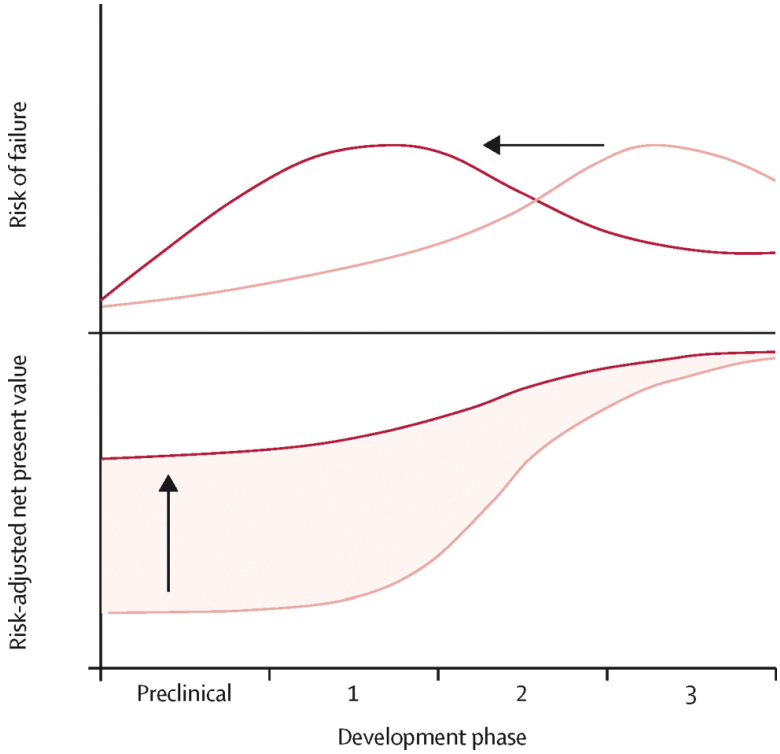

Figure 1. Graphic representation of the risk of failure and the risk-adjusted net present value of a product before (light red) and after (dark red) introduction of a controlled human infection (CHI) model

The CHI model will increase the risk of failure in the early stage of clinical development, but reduce it at a later stage. Because the risk distribution shifts towards higher risks in the early stages of development, the risk-adjusted net present value of the product will increase. As such, the increased initial investments in the CHI models are returned through increased risk-adjusted net present value.

Ethical considerations

The son of Edward Jenner's gardener has become the historic symbol of CHI when he was inoculated with cowpox in 1796. Other famous examples are infection of Macdonalds' children with pertussis, mentally retarded children with hepatitis virus at Willowbrook State school in New York[37] and malaria infections in Nazi Germany.[38] Following these experiments, the Nuremberg Code (1946) and later the Declaration of Helsinki (1964) provided guidelines for the conduct of medical research involving humans including informed consent procedures. CHI studies raise ethical debate because they seemingly breach the "do no harm" principle. However, the purpose of the CHI trial is not to do harm but to ultimately benefit global health.[40] Nonetheless, CHI trials inherently carry a risk for participants. They

can only be performed in treatable or self-limiting diseases where no irreversible pathology occurs.[39] The risk of a serious adverse event (SAE) should be assessed independently from the risk of discomfort. CHI studies may target a certain degree of discomfort (e.g. cholera, malaria, typhoid), but this may not necessarily be serious. In essence, the ethical principles in CHI are similar to those applied in phase I trials where healthy volunteers put themselves at risk without the possibility of deriving direct benefit. [39 40 41] Justification for these trials lies in the potential value of the foreseeable knowledge for society. The degree of risk which is believed acceptable thus depends on the benefits. [34] Formal limits to these risks have not been established and some argue that they should be equal to the risks people would normally take in many areas of life.[40] CHI trials may raise debate on the appropriate compensation of the trial subject, protecting public confidence and the risk of spread of infections, an ethical framework for which is provided by Bambery et al.[42] Using quarantine to minimizing the risk of spread of challenge agents should be carefully considered as it substantially increases discomfort to the trial subjects as well as costs of the trial.

Considering the body of literature on CHI, SAEs seem to be rarely reported. In influenza and malaria CHI, four possibly related SAEs have been reported in an estimated 6000 volunteers. An episode of elevated serum transaminase and dilated cardiomyopathy was recorded in influenza CHI,[43 44] while two cardiac SAEs were reported in a *Plasmodium falciparum* CHI trial.[45 46] The latter episodes might have been myocarditis, an known immunological complication in vaccinology.[47] *P. vivax* CHI experienced a set-back when malaria relapses occurred in two volunteers, due to a previously unrecognized genetic polymorphism that hinders bioactivation of the curative drug primaquine.[48] However, five years of follow-up showed that none had further relapses.[49]

The dynamic scientific context of CHI trials continues to fuel regulatory and ethical discussions. Current ethical debate involves the use of genetically modified organisms (GMOs) and in particular its containment, as well as the use of CHI in populations with increased risks or resource poor environments. For example, pneumococcus colonisation has been performed in elderly and asthmatics (DF, personal comm.), whereas rhinovirus CHI has been performed in mild-to-moderate asthma and COPD patients. [10 50] In this case, the resulting rhinovirus infection was well tolerated despite enhanced respiratory symptoms and secondary bacterial infections requiring increased vigilance.[51]

The transfer of CHI studies to areas where infections are endemic (e.g. malaria, typhoid, pneumococcal disease), will raise specific ethical issues such as cultural acceptance, appropriate remuneration and consent procedures. A recent workshop in Blantyre, Malawi addressed these issues.[52] The ability to study the infection in a population with different disease incidence, co-infections, environmental exposures, nutrition status and immune responses has obvious benefits for the product development pipeline. Needless to say, thorough capacity building of infrastructure, clinical expertise, institutional review boards, pharmacists and ethicists will be needed.

CHI in product development

The contribution of CHI studies to the development of novel vaccines has been championed by the Live Oral Cholera Vaccine CVD 103-HgR study in 197 healthy volunteers.[8] CVD 103-HgR was licenced in several countries since 1993, but only recently in the US.[53] Volunteers were challenged by ingestion of wild-type *V. cholera* and were monitored for the occurrence of moderate or severe diarrhoea. The vaccine showed 90% efficacy, which, together with a good safety profile, led to licensure by the FDA.[8] CHI models have accelerated the development of vaccines or drugs for a number of infections and are increasingly being used as proof in principle by product developers and as gatekeepers for further investment by funders (figure 1). CHI trials are generally small, including between 20-100 volunteers (figure 2B). Influenza, rhinovirus and malaria are the most practiced CHI trials (figure 2A+C).

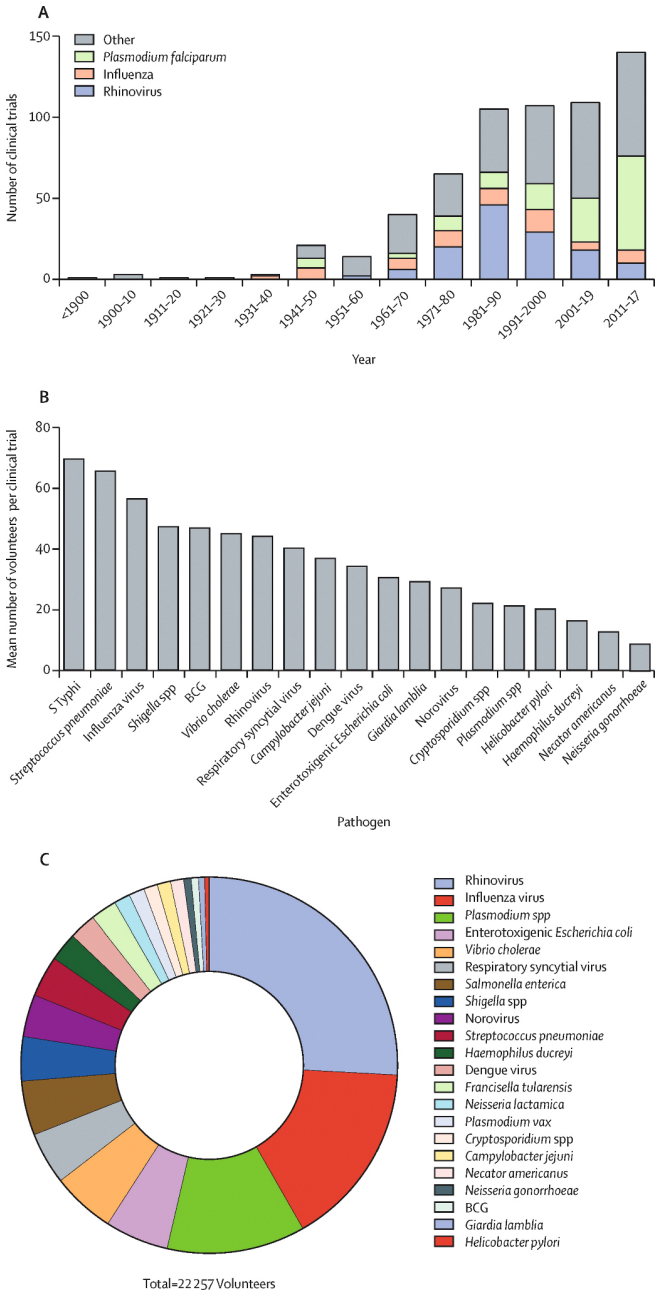

Figure 2. Numbers of volunteers in controlled human infection (CHI) trials
(A) Estimated number of CHI trials reported per decade for rhinovirus (blue), influenza (red), Plasmodium falciparum (green), and other infections (grey). (B) Estimated mean number of volunteers per trial for CHI models with different pathogens. (C) Estimated cumulative number of volunteers previously experimentally infected in a CHI trial per pathogen reported in the literature from 1900. S Typhi=Salmonella enterica serotype Typhi.

An important milestone achieved through CHI has been the licensure of the world's first malaria vaccine based on the subunit circumsporozoite protein (CSP) from *Plasmodium falciparum (Pf)*, which has recently gained EMA approval.[54] Pivotal proof of concept data for this vaccine were generated almost 10 years ago in a series of CHI trials showing cumulatively ~40% protective efficacy after challenge with *Pf* in adults.[55] The CHI results were confirmed in a phase 3 trial, which showed a similar partial (~30%) efficacy in children in Africa.[56] Malaria CHI also proved to be instrumental in identifying candidates with poor efficacy, saving time and efforts by halting their clinical development.[57]

In the field of malaria, CHI has been driven by the development of continuous *in vitro* culture techniques for *P. falciparum* and the rearing of laboratory-infected Anopheles mosquitoes.[58] [59] The salivary-gland parasites were attenuated by radiation and used to inoculate replication-deficient parasites into volunteers. Ground breaking results showed that full protective immunity to *Pf* could be induced by exposing volunteers to the bites of >1000 of these mosquitoes carrying radiation attenuated parasites.[60] The next level in technological advance was the ability to produce aseptic, purified, cryopreserved sporozoites.[61] This work has formed the foundation for the clinical development program of the live attenuated malaria vaccine (PfSPZ Vaccine), which is now given by intravenous injection of radiation-attenuated extracted, purified and cryopreserved sporozoites.[62] In parallel, an even more potent vaccine has been developed based on the exposure of volunteers to live sporozoites under chloroquine prophylaxis (chemoattenuation) resulting in sterile immunity or 100% protection.[63] [64]

The genetic diversity of the malaria parasite poses a major obstacle for vaccine and drug development. The availability of genetically diverse strains of *Pf* for CHI allows for an accelerated assessment of potential cross-strain immunity.[12] [65] In addition, the availability of clinical grade blood stage parasites[66] or purified, vialed and cryopreserved sporozoites for injection (PfSPZ Challenge, Sanaria Inc.)[11] means that the malaria CHI model no longer relies on the production of mosquitoes at the clinical site. This facilitated the transfer of the malaria CHI model to novel sites, an important step to enable phase 2a trials in endemic areas.[67] [68] To increase the array of available strains, controlled production of infected mosquitoes is currently being set up in several centres in Africa.

In malaria drug development, CHI provided the first proof for efficacy of "old" antimalarial drugs such as paludrine[69] but also novel antimalarials such as atovaquone/proguanil[70], ferroquine,[71] artefenomel,[72] griseofulvin,[73] or more recently DSM265.[74] [75] With the advent of molecular methods for detection of parasites as low as 5-20 per mL blood[76] it is possible to carefully dissect parasite growth rates to determine drug and vaccine mechanisms of action. Recently, treatment with piperaquine during CHI blood stage malaria was shown to induce gametocytes, potentially adding a sexual stage *Pf* CHI model to the current portfolio.[77] This may be an important platform to accelerate the development of transmission blocking vaccines, recently identified as a priority in the Malaria Vaccine Technology Roadmap.[78]

The other frequently used model has been controlled influenza infection. Influenza CHI enabled the clinical testing of the first generation of influenza vaccines, which were based on infected and formalin inactivated allantoic fluid.[79] Later, CHI trials led to the first registration of a live attenuated vaccine for influenza A.[80] Immunological analysis showed that pre-inoculation hemagglutinin inhibition titre and particularly neuraminidase inhibition titres in healthy, unvaccinated volunteers, as well as pre-challenge CD4+ T-cell responses (not CD8+ cells) predict clinical outcome after CHI.[81 82] Building upon these immunological findings, a human monoclonal antibody targeting an influenza conserved epitope,[83] a trivalent DNA vaccine[84] and a viral vectored vaccine against conserved influenza antigens[85] were all proven efficacious in influenza CHI, providing hopeful prospects for the development of cross-strain and long-lasting influenza vaccines.

Influenza CHI have also played a central role in the FDA registration of the first influenza antiviral drug amantadine in 1966.[86] Thereafter, studies showed efficacy of the amantadine analogue rimantadine[87] (FDA approved in 1994) and a range of antivirals such as zanamivir, oseltamivir and preamivir.[44, 88-90] Influenza CHI is now applied to study the development of strains resistant to these novel antivirals as people are treated with increasing concentrations of the drug.[91] In terms of respiratory infections, CHI has facilitated the development of drugs for other respiratory infections, such as respiratory syncytial virus.[92]

Impressive progress has been booked in dengue vaccine development. The early down-selection of dengue vaccine candidates is imperative because antibody dependent enhancement of viral replication may pose vaccinees at risk of more severe disease, as was seen in a phase 3 study.[93] Recently, a live attenuated recombinant dengue vaccine (TV0003) showed complete protection in CHI[94] and is now undergoing phase 3 evaluation in Brazil and Thailand (NCT02406729, NCT02332733). Remarkably, the dengue CHI model was developed as a result of the live attenuated dengue vaccine programme, where an insufficiently attenuated dengue strain (rDEN2Δ30) failed as vaccine candidate because it led to viremia and rash, this provided an opportunity for use as CHI.[4]

CHI models have also been instrumental for a number of gastrointestinal infections. For example, the most advanced Norwalk virus vaccine, an intranasal VLP formulation, proved to be efficacious in two separate CHI studies.[18 95] In *Salmonella enterica* serovar Typhi research, CHI allowed for the early benchmarking of novel vaccine candidates against the licenced Ty21a oral live attenuated vaccine.[96] In enterotoxicogenic *E. coli* studies, the therapeutic effect of trimethoprim-sulfamethoxazole was first documented in CHI studies.[97] Multiple prophylactic and therapeutic medicines have been tested in the ETEC CHI model, in which bismuth subsalicylate and an oral colicin E2 treated whole-cell vaccine, showed potential as effective prophylactics. [98 99]

Despite the fact that CHI studies take a central role in the clinical development pipeline, formal statements of regulators are needed to endorse such trials to support the use of

CHI by developers on the path to licensure. Last year, the WHO produced a statement on the regulatory considerations for the use of controlled human infection trials for vaccine development.[100] Such position statements can also highlight the limitation of CHI studies. For example, inoculation routes may differ between natural infections and CHI trials,[2] the trial population may not be similar to the population at risk, challenge strains may differ from natural infections, protective immune mechanism may not be universally applicable and the selection of susceptible adults without pre-existing immunity might reflect intrinsic vulnerability which may not hold true for the whole population. Despite these differences, the results obtained in CHI trials are generally confirmed in phase 2 efficacy trials. To our knowledge, there is no example of a vaccine or drug which failed in CHI and was found efficacious in later phase 2 or 3 field trials.

The predictive value and reproducibility of CHI studies is highly dependent on the quality of the challenge material. The regulatory requirements for the production of this material may vary in different continents. Current regulatory environments have shown that increased control may not always be beneficial to the CHI model, which should preferably remain low cost and be flexible to changes in circulating strains (e.g. influenza) in order to be clinically relevant. Therefore, consistent unifying quality control and assurance measures for challenge material are needed in order to balance safety and costs of production.

Novel CHI models in poverty-related and neglected diseases

Because of the potential to reduce costs and time to registration, CHI models are particularly appealing for the development of products for resource-poor countries where infectious diseases are still responsible for considerable morbidity and mortality. Among the infections that fall in this category, aside from malaria, *Mycobacterium tuberculosis* (MTB) would be obvious. A MTB CHI model could provide a critical platform for the downselection of potential novel antibiotics as well as vaccine candidates. CHI studies with MTB are problematic because diagnosis is not straightforward, there is a potential of spread and treatment is associated with a significant side-effects. As a replacement for MTB infection, intradermal injections with the vaccine bacille Calmette-Guérin (BCG, attenuated *Mycobacterium bovis*) are tested as a surrogate CHI, followed by a punch biopsy 14 days after injection to investigate bacterial persistence and immune parameters.[101] The low recovery of bacteria after CHI limits sensitivity of the model.[102] However, prior vaccination with BCG did result in a decreased recovery rate of BCG after challenge, suggesting that the model is able to reveal protective immune effects.[31] Besides the nature of the pathogen, another important limitation of the model is the dermal inoculation route as opposed to the natural inhalation of mycobacteria and therefore administration of aerosolized BCG or other (attenuated) mycobacterial strains are currently being investigated.[135]

Hookworm infections are one of the most prevalent neglected diseases for which only very limited number of anthelminthic drugs are available.[104] These drugs are widely used and the

concern for the development of resistance is growing. High reinfection rates indicate that vaccines with long-term action are needed to effectively control or eventually eliminate these parasites.[105] A number of vaccine candidates are undergoing early clinical testing.[106] *Necator americanus* hookworm CHI can potentially contribute to go-no-go decisions for these vaccines. As animal models are lacking, hookworm larvae are cultured from faeces of chronically infected donors extensively screened for transmissible diseases such as HIV, HBV and HCV. Hookworm CHI has been performed in ~250 volunteers in order to test whether hookworms, through induction of regulatory responses, can have therapeutic effects on inflammatory diseases such as celiac disease, IBD and allergic rhinosinusitis.[29, 107-109] Standardising the model, through achieving a stable egg output to serve as a reliable quantitative endpoint for vaccine efficacy testing, is the focus of current efforts and will prepare the model for evaluating candidate vaccine efficacy (NCT01940757).

Following the example of hookworm, CHI models are being developed for two other parasitic diseases of global importance: schistosomiasis and cryptosporidium. Because parasites generally have a complex life cycle and may depend on multiple hosts for their maturation and development, the production of challenge material in compliance with all regulatory norms can be a daunting task.

For *Schistosoma mansoni* an important conceptual step to ensure safety of CHI volunteers has been the propagation of single sex cercariae which can infect humans and mature to adult stage without mating. In single sex infections no eggs are produced, circumventing the pathology associated with chronic schistosomiasis caused by egg-induced granuloma formation and fibrosis. A highly sensitive diagnostic test based on circulating anodic antigen was crucial for the development of the model as it allows for accurate quantification of worm loads despite the lack of eggs.[110] The first results of a *Schistosoma mansoni* CHI is expected soon (NCT02755324). The model will be suitable for testing new drugs and currently available vaccines such as Sm14, TSP2 and Smp80.[111] However, anti-fecundity vaccines or drugs that target egg laying, cannot be tested in these single sex infections.

A recent evaluation of the causes of moderate-severe diarrhoea in children <2 years of age revealed Cryptosporidium as being the second or third leading pathogen. It is associated with malnutrition and enteropathy.[112] This is why recent efforts have been put into reviving the pre-existing cryptosporidium CHI model[27] to comply with 21st century regulations and serve the vaccine and drug development pipeline. Unfortunately, cryptosporidium cannot be cultured *in vitro* and is difficult to maintain in animal models. *C. hominis* cysts can only be produced by infection of gnotobiotic neonatal piglets. Considerable investments are now underway to allow purification of this material. However, culture of cysts without the need of animal models would be an important step forward for this model.

Colonisation models

Culture-independent technologies have revealed the diversity of the human microbiome.[113] In an era of increasing antimicrobial resistance, the study of controlled colonisation in healthy volunteers has proven to be instrumental to dissect the dynamics of mucosal carriage of bacteria which precedes invasive bacterial infections. With regards to controlled colonisation, the upper respiratory tract microbiome has been the most studied.

The most frequently used model for colonisation is nasal instillation of *S. pneumoniae*, which leads to a roughly 50% colonization in healthy volunteers and lasts 2-5 weeks as confirmed by nasal washes.[21, 114 115] Interestingly, invasive pneumococcal disease has never been reported in studies of more than 800 inoculations performed so far. Despite the lack of clinical invasive disease, the model successfully predicted the efficacy of the 13-valent conjugate vaccine.[116] This has paved the way for the use of the *S. pneumoniae* colonization model in testing new protein-based vaccines (NCT02116998). The model has also been instrumental in studying natural protection and the dynamics of the nasal microbiome. [117-119] Strain specific immunity was induced by the controlled colonisation procedure, which was illustrated by a second challenge of volunteers with the same pneumococcal strain after 11 months.[120] Analysing the immune responses indicated that high levels of memory B cells and antibodies directed to capsular pneumococcal polysaccharide seem to be key to protection against pneumococcal acquistion.[120 121] Through the use of this model it was also possible to show that asymptomatic upper respiratory viral infections increase the risk of becoming colonized.[123] The effect of the paediatric live attenuated influenza vaccine on pneumococcal carriage is subject of an ongoing controlled colonisation trial in adults (ISRCTN16993271). Nasal mucosa and lung investigations during this co-infection study might provide important insights into how influenza predisposes to secondary pneumococcus infections and lead to better interventions. These studies have also directly assessed for the first time the impact of a viral vaccine on an entirely unrelated human pathogen and have highlighted the need to consider off-target beneficial (or detrimental) effects of vaccines on other important human pathogens. Given the scientific advances and the favourable safety profile of the pneumococcal colonization model, the model has been expanded to explore the susceptibility of at-risk populations including people with asthma (ISRCTN16755478) and the elderly (ISRCTN10948363).

In analogy to colonisation with pathogenic bacteria, it has been possible to deliberately colonize volunteers with non-pathogenic bacteria to investigate the effects on pathogen carriage.[24 30] As an example, intranasal *N. lactamica* colonisation protects from colonisation by *N. meningitides.* The efficacy of this approach was shown to be superior to the commonly used quadrivalent ACWY glycoconjugate vaccine.[24] Similarly, active colonisation of the bladder with non-pathogenic *E. coli* prevents urinary tract infections (UTI) in patients with recurrent UTIs.[124-126] These trials were designed based on studies demonstrating that untreated asymptomatic bacteriuria prevents symptomatic urinary tract infection in young

girls.[127] The concept was further extended to vaginal instillation of lactobacilli in women with recurrent UTI, but results are much less convincing when compared to the *E. coli* colonisation model of the bladder.[20]

More recent efforts include the development of colonization models with Nontypable *Haemophilus influenzae* and *Bordetella pertussis.* The *H. influenzae* model uses a challenge strain genetically modified to be streptomycin resistant which allows the investigators to recover the organism from the nasopharyngeal samples.[128] The pertussis model aims at achieving colonisation in 70% of the exposed volunteers without causing disease. The colonisation period, shedding and exploratory immunology are being assessed during a 17-day inpatient stay and follow-up over 1 year.[129] Very similarly, efforts to develop a Group A *Streptococcus pyogenes* controlled infection model are being undertaken. Because this models is aimed at inducing pharyngitis, it is formally not a model of colonisation but of disease.[130]

Colonisation models in principle do not reflect the pathophysiology of invasive disease, the colonisation phase is increasingly recognised as an important target for vaccination. For example in pneumococcus, colonisation drives transmission primarily amongst children. Protection against colonization, the reservoir of bacteria in the population, interrupts transmission of bacteria and provides herd protection against the disease. Despite their non-invasive design, the risk of invasive disease in these colonisation models cannot be completely averted. Controlled colonisation and infection models share many similarities such as the preparation of challenge material, inoculation routes and ethical considerations including the risk of dissemination of the challenge strain.

Scientific advances

CHI trials offer unprecedented opportunities to study host-pathogen interaction by taking multiple longitudinal samples before, during and after infection. Profiling immune parameters and linking those to the clinical outcome has shown to be extremely valuable in assessing correlates of protection. The availability of these validated correlates of protection will accelerate the development of novel vaccines by providing an easier surrogate endpoint for phase 2 field trials. In addition, they may guide the refinement of vaccine products, for example through rational selection of adjuvants which are known skew the immune response towards the preferred correlate. Also, the comparison of different populations in controlled infection trials may provide insight into population heterogeneity, which may impact the overall efficacy of the product when distributed widely in the target populations. In addition, studying the biology of CHI trials may lead to the discovery of novel vaccine targets. For example, in CHI models for salmonella,[131] shigella,[132] and norovirus[133] antibodies were identified which, if present at baseline, correlated with protection from the challenge. Potentially, these antibodies could provide clues for novel monoclonal antibody-based therapy or lead to the identification of functional antigens. Similarly, a study of peripheral

mononuclear blood cells of volunteers in a rhinovirus CHI study using MHC class II tetramers has led to the identification of specific memory T-cell populations that rapidly respond to infection and target conserved epitopes of the rhinovirus capsid proteins.[134] These epitopes will be subject of further research into their potential use as novel peptide vaccines.

Repeated CHI in the same individuals contributes to understanding the induction of natural immunity. A gradual decrease in the number of people reaching the endpoint is generally a sign of slowly acquired natural immunity, as was seen for shigella,[17] BCG,[31] cholera,[135] norovirus,[136] pneumococcus,[120] and enterotoxigenic E. coli.[137] This was not the case in RSV CHI, where previously protected individuals can again be susceptible after the next inoculation, indicating that naturally acquired immunity in RSV is transient.[138] Dissection of the humoral responses in these subjects revealed a defect in virus-specific IgA memory B-cells.[139] In *H. ducreyi* re-infection, a subgroup of participants maintains consistent susceptibility to clinical disease (pustule formation), whereas others do not. Probably, host factors such as gender, genetic background, immune responses and skin microbiome play a central role.[22 139]

The importance of host factors was also shown convincingly in other models. For example, individuals with an O blood group have increased susceptibility for norovirus while those with a B histo-blood group show a decreased risk of infection.[140] Interestingly, in infection with V. cholera volunteers with O blood group also suffered from more severe symptoms.[141] Because blood group and other related carbohydrate antigens are highly expressed on gut epithelial cells, their involvement in viral or bacterial docking is suspected. Indeed the H type-1 oligosaccharide ligand (a member of the ABH blood group family) was found to be critical for Norwalk virus binding.[140]

In order to fully understand the complex interplay between genetic background, diet, microbiome, co-infections and previous exposure in determining clinical outcome after CHI, comprehensive system biology approaches are required. Recently orthogonal datasets including transcriptomics, immunologic parameters as well as metabolomics signatures to zostavax, a live attenuated vaccine, were integrated and showed reactivation of networks that are tightly coupled with T- and B-cell responses.[142] Interestingly, such network analysis generated novel insights into the endocrine system as well as metabolomics playing a role in vaccine responses. These tools can now be applied to CHI in the context of both vaccine and drug development.

Future challenges and opportunities

The increasing costs for clinical development of novel drugs and vaccines for infectious diseases calls for tools to select those candidates with highest probability of success. The concept of “fast failure”, in which there is an early stop for the development of unsuccessful candidates is extremely important as it will allow reallocation of resources. CHI studies

may be used as model for phase 2 clinical efficacy. As such they may reduce development risk, lower overall costs and increase risk-adjusted net present value. Especially in poverty-related infectious disease research, cost-effective development of novel interventions is imperative. Despite the advantages of CHI in clinical development, these studies also have disadvantages. Particularly the use of a "surrogate" inoculum or volunteers which are much different from the target population poses significant limitations. As such, CHI will not resolve all problems in clinical development but, whenever possible, should be put to use as an accelerating tool.

Because of their often invasive nature and the use of healthy volunteers CHI trials continue to raise ethical debate amongst public, Institutional Review Boards (IRBs) and investigators.[143] The fear of long-term adverse effects such as reactive arthritis or post-infectious irritable bowel syndrome in Shigella or enterotoxicogenic *E. coli* infections are well known examples.[17] Quantitative risk data is needed to facilitate objective risk assessments, which need to be tailored to individual models and research targets. Currently, the lack of standardized reporting of adverse events and in particular serious adverse events as well as inoculation route, dose and timing of events hamper the meta-analysis of available safety data. Similarly, data on possible spread or secondary infections with challenge strain should be made publicly available to indicate the need for quarantine of subjects with gastrointestinal or respiratory infections. Easily accessible standardized safety data on CHI studies will also facilitate the evidence-based establishment or adjustment of CHI regulations and increase the expertise of IRBs in this domain.

A major hurdle in the development of novel CHI models is often the production of challenge inoculum compliant with regulations, which may be difficult, expensive and time consuming. In addition, regulatory requirements may vary across different continents. Public-private partnerships, funders and consortia of CHI researchers should share the responsibility for investing in sustainable, widely available, well-characterized master banks of this material and define the quality control assays that are believed to be essential for volunteers safety. In addition, the open sharing of knowledge and infrastructure would support best practices and provide a knowledge base for CHI model transfer and capacity building.

In conclusion, CHI models are emerging as powerful tools to down select promising new vaccines or drugs on their increasingly complex and expensive path towards licensure. Despite their invaluable contribution to science and product development, the demanding nature of CHI trials and risks involved requires careful risk-benefit assessments in which the safety of participants should be a primary concern at all times.

Search strategy and selection criteria

References were identified in PubMed using the search terms ("experimental infection*" OR "human challenge" OR "challenge study" OR "challenge model" OR "human infection"

OR “infection model” OR “volunteer study” OR “infection in volunteers” OR “volunteer challenge” OR “controlled human infection”), separately combined with each pathogen listed in the table. For each pathogen the Mesh-term was combined with an [All Fields] search of common synonyms. We searched for articles published between Jan 1, 1900 and 1 October 2017. Only articles in English, French or Dutch were reviewed. The references of reviews and key publications were searched to identify any other references. Only studies using pathogens to experimentally infect humans were included. Studies using an attenuated pathogen for the sole purpose of vaccination were not included in the estimation of total volunteer numbers. Articles were the total number of volunteers in the study could not be identified were not included in estimate of total volunteer numbers but were included in the estimation of total studies.

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