Safety, colonisation kinetics, transmissibility, and immune correlates of protection in healthy adults inoculated with *Bordetella pertussis* in England: a single-centre, open-label, phase 1, controlled human infection study

Hans de Graaf, Diane F Gbesemete*, Alison R Hill*, Janeri Fröberg, Muktar M Ibrahim, Adam P Dale, Jay R Laver, Andrew R Gorringe, Saul N Faust, Kent E Kester, Annemarie M Buisman, Guy A M Berbers, Jolanda Brummelman, Cecile A C M van Els, Marc J Eleveld, Dimitri A Diavatopoulos, Robert C Read*

Summary

Background Cyclical epidemics of pertussis, also known as whooping cough, continue to occur despite vaccination, because current vaccines fail to suppress colonisation by, and transmission of, the causative agent *Bordetella pertussis*. We aimed to show the safety of *B pertussis* controlled human infection delivered in an outpatient setting, as well as identify immunological correlates of protection against *B pertussis* colonisation, to guide the future development of pertussis vaccines.

Methods This open-label, phase 1, controlled human infection study was conducted at University Hospital Southampton, Southampton, UK. Healthy volunteers aged 18–55 years who had received a whole-cell pertussis vaccine in childhood were inoculated intranasally with 10^5 colony-forming units of wild-type *B pertussis* strain B1917. Volunteers were monitored as outpatients for 28 days to assess safety, colonisation, and *B pertussis*-specific immunological parameters. Individuals who shared a bedroom with inoculated volunteers were enrolled to measure transmission to close contacts. After approximately 90 days, volunteers who agreed to participate in a rechallenge phase of the study were re-inoculated with the same dose of *B pertussis*. Volunteers received azithromycin 14 days after each inoculation. The primary objective was to assess the safety of delivering this controlled human infection model on an outpatient basis; safety endpoints were the occurrence of possible or confirmed pertussis and unsolicited or serious adverse events. Secondary objectives were the assessment of immunological biomarkers of protection from colonisation and the assessment of transmission from volunteers to close contacts. All volunteers who received the inoculum were included in the safety analysis, and all participants who completed follow-up to day 14 were included in the per-protocol population for colonisation and immunological analysis. This trial is registered at ClinicalTrials.gov, NCT03751514, and is ongoing.

Findings Between Aug 29, 2019 and March 6, 2023, 77 volunteers were screened and 51 were enrolled and inoculated, of whom 50 completed follow-up to day 14 post-inoculation. 20 (40%) of these 50 volunteers became colonised with *B pertussis*. One volunteer developed symptoms suggestive of possible pertussis after initial inoculation; this volunteer tested negative for *B pertussis* throughout the study and all symptoms resolved within 4 days. Adverse events were mostly mild to moderate, with no significant increase in the reporting of any individual symptom by colonised volunteers. All unsolicited adverse events were assessed as either unlikely to be related or unrelated to *B pertussis* infection, with no increase in the frequency of these events seen in colonised volunteers. There were no serious adverse events and no treatment-related deaths. Compared with those who became colonised with *B pertussis*, non-colonised volunteers had significantly higher pre-inoculation serum IgG antibody concentrations against several acellular pertussis vaccine antigens (pertussis toxin [p=0·028], pertactin [p=0·037], and filamentous haemagglutinin [p=0·024]); higher nasal (p=0·022) and serum (p=0·044) IgA antibody concentrations against whole *B pertussis*; higher serum IgA antibody concentrations specific to filamentous haemagglutinin (p=0·024); and higher peripheral T-helper (Th)-22-cell responses to pertussis toxin (p=0·0045) and filamentous haemagglutinin (p=0·029). After inoculation, only colonised volunteers showed seroconversion, IgA and IgG binding to *B pertussis*, and increases in *B pertussis*-specific IgG-secreting memory B-cell frequencies by day 28. Th17 responses to pertussis toxin were increased (p=0·039) and Th22 responses to filamentous haemagglutinin were decreased (p=0·014) in non-colonised volunteers. Of the 13 volunteers who became colonised after initial inoculation and were re-inoculated, only one (8%) became colonised after re-inoculation. Transmission from volunteers to 14 enrolled individuals with whom they shared a bedroom was not detected.

Interpretation Our findings suggest that controlled human infection with *B pertussis* in an outpatient setting is safe. Higher humoral and $CD4^+$ T-cell responses with specificity to acellular pertussis vaccine antigens are associated with

Lancet Microbe 2026; 7: 101313

Published **Online** March 23, 2026
https://doi.org/10.1016/j.lanmic.2025.101313

*These authors contributed equally

Academic Unit of Clinical and Experimental Sciences, Faculty of Medicine and Institute for Life Sciences, University of Southampton, Southampton, UK (H de Graaf PhD, D F Gbesemete PhD, A R Hill PhD, A P Dale PhD, J R Laver PhD, Prof S N Faust FRCPCH, Prof R C Read MD); **NIHR Clinical Research Facility and NIHR Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust, Southampton, UK** (H de Graaf, D F Gbesemete, A R Hill, A P Dale, J R Laver, Prof S N Faust, Prof R C Read); **Radboud Community for Infectious Diseases, Radboud UMC, Nijmegen, Netherlands** (H de Graaf, J Fröberg PhD, M J Eleveld BSc, D A Diavatopoulos PhD); **Laboratory of Medical Immunology, Radboud UMC, Nijmegen, Netherlands** (H de Graaf, J Fröberg, D A Diavatopoulos); **Controlled Human Infection Group, Faculty of Medicine, University of Southampton, Southampton, UK** (D F Gbesemete, A R Hill, M M Ibrahim PhD, A P Dale, J R Laver, Prof R C Read); **UK Health Security Agency, Porton Down, Salisbury, UK** (Prof A R Gorringe PhD); **Coalition for Epidemic Preparedness Innovations (CEPI), Washington, DC, USA** (K E Kester MD); **Centre for Infectious Disease Control,**

protection against colonisation by *B pertussis* after experimental challenge. This technique could enable the testing of potential novel vaccines or formulations for sustained protection against infection and transmission.

Funding EU Horizon 2020 (Innovative Medicines Initiative) and the National Institute for Health and Care Research Southampton Biomedical Research Centre.

Copyright © 2025 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands (A M Buisman PhD, Prof G A M Berbers PhD, J Brummelman PhD, Prof C A C M van Els PhD); Division of Infectious Diseases and Immunology, Department of Biomolecular Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands (Prof C A C M van Els)

Correspondence to: Prof Robert C Read, Academic Unit of Clinical and Experimental Sciences, Faculty of Medicine and Institute for Life Sciences, University of Southampton, Southampton SO16 6YD, UK r.c.read@soton.ac.uk

Introduction

Bordetella pertussis is a highly infectious respiratory pathogen that exclusively infects humans and is transmitted via airborne droplets.[1] Pertussis, or whooping cough, is most severe in neonates and infants, but can occur at any age and has a typical pattern of epidemic peaks every 3–5 years. The incidence of pertussis increased sharply in Europe[2] and the USA[3] during 2023–24, despite high vaccine coverage. Whole-cell pertussis vaccines substantially reduced disease incidence after their implementation in the 1950s, but were replaced by acellular pertussis vaccines in high-income countries between 1992 and 2005.[4] By inference, most individuals in high-income countries who are currently aged 20 years or younger will have been primed in infancy with acellular pertussis vaccines, whereas older adults were either not vaccinated in infancy or were primed with whole-cell pertussis vaccines.

Both epidemiological[5] and preclinical[6] studies provide evidence that acellular vaccines protect less well against *B pertussis* infection than do whole-cell vaccines. This finding is further compounded by the comparatively fast waning of protection induced by acellular pertussis

Research in context

Evidence before this study

Current acellular pertussis vaccines protect against disease in the short term but appear to have little effect on infection with *Bordetella pertussis*. Seroepidemiological studies suggest that asymptomatic infection is common, which could maintain bacterial transmission in the community. Clinical investigation to define the immune characteristics that protect against asymptomatic infection as well as disease would facilitate the development of improved vaccines; however, prospective case ascertainment of asymptomatic infection is practically difficult. Controlled human infection can identify individuals who are relatively protected against experimental infection. We searched PubMed from database inception to Jan 11, 2025, using the Medical Subject Headings terms “*Bordetella pertussis*” AND “wild-type” AND “human challenge trials”, for publications in English that reported controlled human infection with virulent *B pertussis*. One paper was identified, in which our team reported a dose-ranging, controlled human infection study, conducted in an inpatient setting, involving 34 healthy adult volunteers who had no evidence of recent infection or vaccination as determined by anti-pertussis toxin IgG concentrations. An intranasal dose of 10^5 colony-forming units of *B pertussis* resulted in asymptomatic colonisation of 12 (80%) of 15 volunteers. Antibody responses to pertussis toxin were observed in about half of the colonised participants and in none of the participants who were not colonised. No shedding of *B pertussis* was detected in systematically collected environmental samples. A search of Web of Science for publications citing this paper found no further clinical trials reporting human challenge with *B pertussis*.

Added value of this study

This study, which was planned to follow on from our previous controlled human infection study, involved volunteers who were not selected according to anti-pertussis toxin IgG concentration and were monitored as outpatients. 20 (40%) of 50 volunteers were asymptomatically colonised, and those who did not become colonised had higher pre-inoculation serum antibodies against multiple antigens included in the acellular pertussis vaccine and higher nasal and serum IgA binding to *B pertussis*, as well as peripheral IL-22 responses from $CD4^+$ T-helper-22 cells to the vaccine antigens pertussis toxin and filamentous haemagglutinin. Colonised individuals developed immunity to a second challenge conducted 3 months later. The technique described in this study is a safe and convenient method to identify a protected phenotype in human volunteers, and will permit future studies to identify further correlates of protection against infection and the testing of novel vaccines.

Implications of all the available evidence

Our findings show that controlled human infection with *B pertussis* can be safely conducted by outpatient experimental facilities; volunteers become asymptomatically colonised and do not transmit to close contacts. If colonised, individuals develop an immune response and become relatively protected against a second challenge. The protected phenotype has immunological characteristics that include humoral and cellular immune responses against antigens contained in the acellular pertussis vaccine. This finding implies that novel vaccines capable of generating similar and sustained responses could better protect against infection by *B pertussis.* We are aware of at least three other centres that are now actively conducting human controlled infection studies with *B pertussis* with the purpose of improving future pertussis vaccines.

vaccines.[7] Vaccine priming in infancy is a key determinant of lifecourse immunity; humans primed with whole-cell vaccines in infancy express pertussis vaccine antigen-specific T-helper (Th)-1-cell and Th17-cell responses in later life, whereas those primed with acellular vaccines have predominant Th2 cell responses.[8] This imprinting seems to be antigen-specific; epitope mapping has revealed that adult *B pertussis*-specific $CD4^+$ T-cell responses to pertussis antigens that did not arise from the acellular pertussis vaccine exhibit no polarisation as a function of natural exposure, childhood vaccination, or a clinical history of pertussis.[9]

There is strong evidence that the human population is frequently exposed to asymptomatic or undiagnosed *B pertussis* infection. Seroepidemiological evidence supports the existence of an asymptomatic or subclinical reservoir of infection, which would enable the circulation of *B pertussis* during quiescent interepidemic periods. This reservoir will be a key target of future vaccine development, with the aim of reducing the potential for transmission of *B pertussis* to individuals who are most vulnerable. There are substantial knowledge gaps surrounding the nature of protective immunity in the healthy adult population. Controlled human infection is a feasible method to identify immunological determinants of *B pertussis* infection in a population with heterogenous immunity. A previous dose-ranging, controlled infection study was conducted in an inpatient setting among healthy volunteers with anti-pertussis toxin IgG concentrations of less than 20 international units (IU) per mL, reducing the chance that the participants had been infected naturally with *B pertussis* within the past year. This study found that a dose of 10^5 colony-forming units (CFU) induced *B pertussis* colonisation of the nasopharynx within 14 days of inoculation in 12 (80%) of 15 volunteers.[10,11] No symptoms typical of pertussis and no environmental shedding of *B pertussis* were observed.

We aimed to safely translate this controlled human infection model to an outpatient setting and to measure serological and cellular immunological responses to well characterised *B pertussis* antigens before and after challenge.

Methods

Study design and participants

This open-label, phase 1, controlled human infection study was conducted at University Hospital Southampton, Southampton, UK, as part of the PERISCOPE consortium project. The study was conducted in accordance with the provisions of the Declaration of Helsinki (1996) and the International Conference on Harmonization Guidelines for Good Clinical Practice, and was approved by the South Central – Oxford A Research Ethics Committee (17/SC/0006). Healthy volunteers aged 18–55 years, who had been fully vaccinated with a whole-cell pertussis vaccine during childhood but not vaccinated against pertussis in the 5 years before enrolment, were willing to use infection control measures during the study, and did not have regular contact with people vulnerable to severe pertussis, were recruited according to protocol-defined criteria (appendix pp 47–48). Unlike our previous controlled human infection study with *B pertussis*,[10,11] volunteers were not selected on the basis of anti-pertussis toxin IgG concentrations.

See Online for appendix

Challenge volunteers were defined as individuals who were challenged with an intranasal inoculum of *B pertussis*. Contact volunteers were defined as individuals who were intending to share a bedroom with a challenge volunteer on at least one occasion during the study period. Challenge volunteers were excluded from participation if an individual with whom they might potentially share a bedroom was ineligible for the study. Written informed consent was given by all volunteers, and was obtained from both the challenge and the contact volunteer before inoculation of the challenge volunteer.

The study protocol is available in the appendix (pp 2–90). The trial is registered with ClinicalTrials.gov, NCT03751514.

Procedures

Challenge volunteers were inoculated with *B pertussis* strain B1917, which was isolated from a Dutch patient with pertussis and is a fully genotyped representative of current *B pertussis* isolates in Europe.[12] A single-use vial of a working cell bank of B1917, at a concentration of 10^7 CFU/mL, was thawed and diluted in 0·9% NaCl to an inoculum dose of 10^5 CFU in 1 mL under biosafety level 2 conditions. On day 0, 0·5 mL of the inoculum was administered in droplets into each nostril, as described previously.[11] Approximately 90 days after the initial inoculation, challenge volunteers were invited for an optional rechallenge.

Challenge volunteers were followed up for 28 days after both initial inoculation and re-inoculation (figure 1A). At each visit, the volunteers were asked to report any symptoms potentially indicative of early pertussis, including cough, sore throat, nasal congestion, rhinorrhoea, sneezing, and malaise. Additional clinical reviews were arranged if clinically significant symptoms developed between scheduled visits. If early pertussis was suspected, treatment with azithromycin 500 mg once a day for 3 days was commenced. Colonisation was assessed by culture of nasal washes, pernasal swabs, and oropharyngeal swabs. Blood and nasosorption samples were taken at prespecified timepoints to assess immunological parameters.

Contact volunteers were monitored for symptoms of pertussis and assessed for *B pertussis* colonisation by culture of nasal wash samples taken on day 7 and day 14 after the inoculation of their linked challenge volunteer. 2 weeks after inoculation and re-inoculation, all volunteers were given azithromycin to eradicate possible *B pertussis* colonisation.

Colonisation was defined as any positive *B pertussis* culture from nasal or oropharyngeal samples at any timepoint, up to day 14 after challenge. Each swab or nasal wash sample was processed and dilution volumes of 250, 25, and

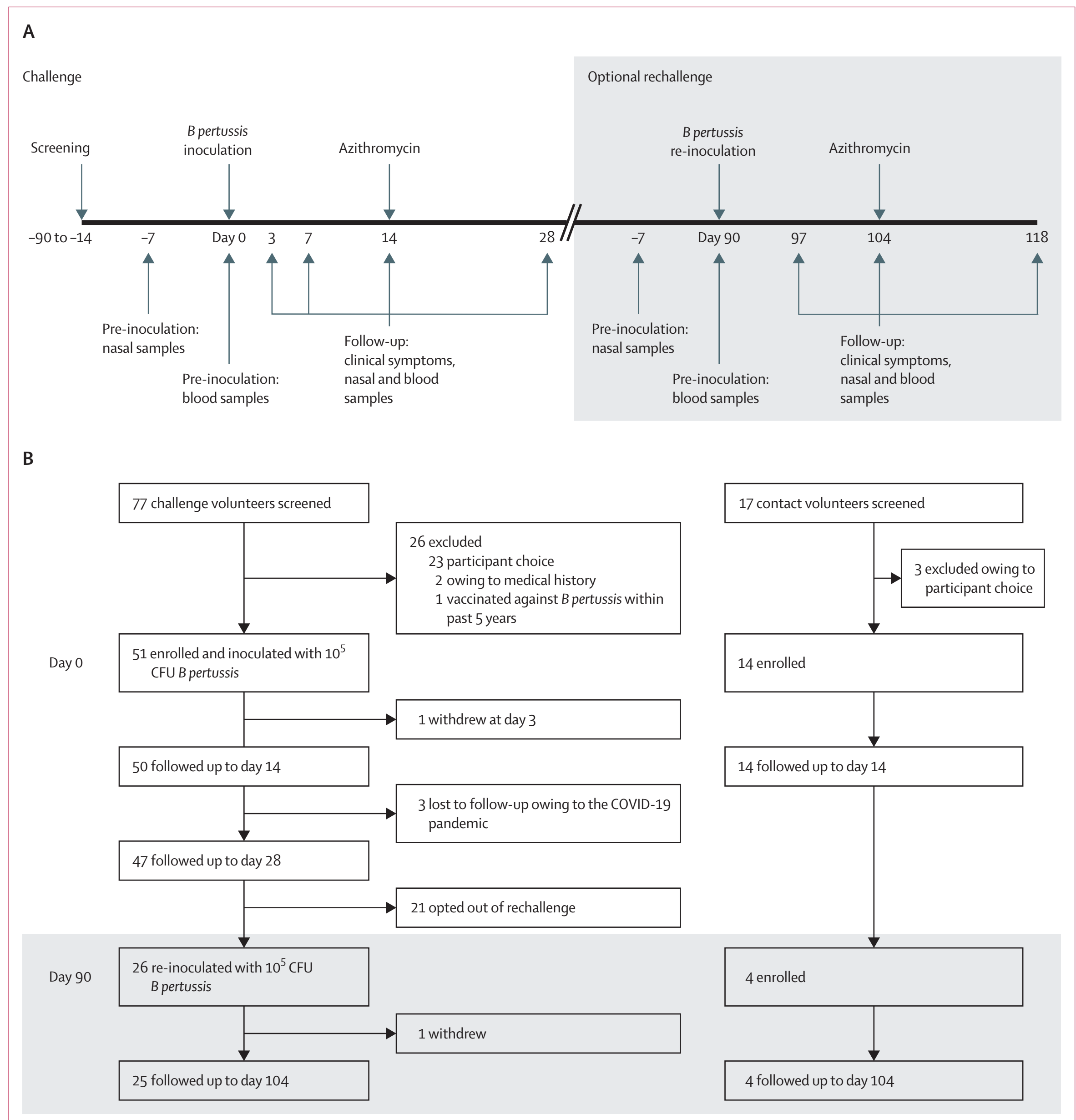

Figure 1: **Study design**
(A) Clinical study design. (B) Trial profile. *B pertussis*=*Bordetella pertussis*. CFU=colony-forming units.

2·5 µL were aliquoted onto charcoal *Bordetella*-selective agar plates (Oxoid, Basingstoke, UK). Culture plates were maintained at 37°C in a humidified aerobic incubator and reviewed for growth of *B pertussis* colonies at day 5 and day 7 of incubation. Probable *B pertussis* colonies were further identified by matrix-assisted laser desorption/ionisation–time-of-flight mass spectrometry (Bruker, Billerica, MA, USA).

The colonisation density at each timepoint was calculated using the bacterial counts from nasal wash sample plates standardised to 1 mL. Bacterial burden was calculated as the area under the curve (AUC) of colonisation density at days 0, 3, 7, and 14. Three culture results were positive but could not be counted for technical reasons. We retrospectively imputed these CFU counts on the basis of quantitative PCR results, as the number of CFU from culture was found to correlate with the number of genome copies (r=0·7062, p<0·0001).

Nasosorption and mucosal lining fluid (MLF) elution methods have been described previously.[13] In short, a nasosorption device (Nasosorption FX·i; Hunt Developments, Midhurst, UK) was inserted into the nose, after

which a finger was gently pressed against the nostril for 60 s. The devices were frozen immediately after collection and stored at –80°C until elution.

A fluorescent bead-based multiplex immunoassay was used to measure the concentrations of *B pertussis*-antigen-specific antibodies—pertussis toxin, pertactin, filamentous haemagglutinin, and fimbriae 2 and fimbriae 3 (fimbriae 2/3)—in serum and MLF.[14] Data were acquired on a validated and calibrated FLEXMAP 3D platform (Luminex, Austin, TX, USA) and analysed with Bio-Plex Manager 6.2 software (Bio-Rad, Hercules, CA, USA).

Antibody responses to whole *B pertussis* bacteria were measured using an antibody deposition assay as described previously,[15] with some modifications (appendix pp 91–92).

Cellular immunological assays

Blood samples were analysed for *B pertussis*-specific memory B cells using an enzyme-linked immunospot assay.[11,16] *B pertussis*-specific Th-cell responses were identified by antigen stimulation assays with a flow cytometric readout on a subset of volunteers (appendix pp 92–95).[17]

Outcomes

The primary objective was to assess the safety of delivering a *B pertussis* controlled human infection model on an outpatient basis. Primary endpoints were the occurrence of possible or confirmed pertussis and unsolicited or serious adverse events between day 0 and day 28 after inoculation with 10^5 CFU *B pertussis*. The assessment of adverse event frequency, severity, and relatedness to the intervention was conducted in line with Good Clinical Practice Guidelines and the clinical study protocols, and was overseen by independent safety monitors. The secondary endpoints for this part of the study were the assessment of any association between colonisation with *B pertussis* and immunological parameters and of transmission from inoculated volunteers to close contacts (appendix p 38).

Statistical analysis

The planned initial sample size was 66 participants (for power calculation, see appendix pp 76, 91), with the intention of conducting an interim analysis and optimising the final sample size. Owing to delays in recruitment during the COVID-19 pandemic, the interim analysis was conducted after the first 50 volunteers had completed the study, as recommended by the trial steering committee. The fraction of non-colonised volunteers was notably higher than anticipated from the previous dose-escalation study, and significant differences in the concentrations of pre-inoculation immunological biomarkers—including serum anti-pertussis toxin and anti-pertactin IgG—were detected between colonised and non-colonised volunteers, therefore achieving the secondary objective. In addition, no safety concerns were reported, so the study was discontinued after consultation with the data and safety monitoring board.

All volunteers who received the inoculum were included in the safety analysis, and all those who completed follow-up to day 14 were included in the per-protocol population for colonisation and immunological analysis. Statistical analyses were conducted using R version 4.1.3. Differences in proportions between two groups were assessed using Fisher's exact test. Shapiro–Wilk normality testing was conducted. Data were summarised by mean (SD), median (range and/or IQR), or geometric mean (95% CI) according to their normality distribution. Parametric data were analysed using a two-sided *t*-test, or a paired *t*-test when groups were related. Non-parametric data were analysed using the two-tailed Wilcoxon signed-rank test (eg, when comparing the colonised *vs* non-colonised groups), or the Kruskal–Wallis test when groups were related (eg, when assessing the change in concentration of antibodies over time). Dunn's post-hoc testing and Benjamini–Hochberg correction for multiple testing were used when assessing more than two groups. Correlation analyses were conducted using Spearman's rho (*r*; eg, when correlating the CFU counts with immunological parameters). Where correlations were significant, data were fitted to a linear regression line with 95% CI for visualisation. All p values were two-tailed and p values smaller than 0·05 were considered statistically significant. Missing data were assumed to be missing completely at random and therefore ignorable under the analysis model.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between Aug 29, 2019 and March 6, 2023, 77 challenge volunteers were screened, of whom 51 were enrolled and inoculated (figure 1B). One volunteer withdrew from the study on day 3, resulting in 50 volunteers who completed follow-up to day 14 after inoculation and were included in the per-protocol analysis. Baseline demographics of the volunteers are shown in table 1. The mean *B pertussis* inoculum doses were 96 049 CFU (SD 27 648) at initial inoculation and 105 877 CFU (SD 33 891) at re-inoculation. The inoculum dose administered was not significantly different between colonised and non-colonised volunteers (p=0·35, two-sided *t*-test; table 1).

20 (40%) of the 50 challenge volunteers were colonised with *B pertussis* by day 14 after inoculation, 18 (90%) of whom were culture-positive by day 7 (figure 2B, C; appendix p 95). All culture-positive volunteers had a positive nasal wash culture, except for one who had a single positive pernasal swab culture only. In 2020, the five-volunteer rolling average colonisation fraction decreased to zero and remained low throughout 2021, before increasing again in 2022. The cumulative colonisation fraction stabilised after the 25th volunteer was challenged, resulting in an overall colonisation fraction of 0·40 (20 [40%] of

	Excluded (n=26)	Enrolled (n=51)	Colonised after primary inoculation (n=20)	Non-colonised after primary inoculation (n=30)	p value*
Age, years					
Median (IQR)	27·0 (22·5–33·5)	27·0 (21·5–37·0)	32·0 (23·5–42·8)	24·0 (21·0–31·8)	0·14
Range	18–52	18–51	18–50	19–51	··
Sex assigned at birth, n (%)					
Male	15 (58%)	23 (45%)	5 (25%)	17 (57%)	··
Female	11 (42%)	28 (55%)	15 (75%)	13 (43%)	0·042†
BMI, kg/m^2					
Median (IQR)	24·4 (21·7–31·2)	25·6 (22·1–31·2)	29·2 (22·7–35·1)	25·2 (21·5–29·0)	0·11
Range	18·7–41·9	18·4–44·4	21·3–44·4	18·4–39·3	··
Anti-pertussis toxin IgG, geometric mean (95% CI), IU/mL	NA	4·0 (2·3–6·9)	1·9 (0·9–4·0)	6·2 (3·0–12·8)	0·028
Female	NA	2·9 (1·4–5·9)	2·4 (1·0–5·7)	3·6 (1·1–11·5)	0·37
Male	NA	5·9 (2·5–13·8)	0·8 (0·2–3·2)	9·3 (3·8–23·0)	0·040
Anti-pertactin IgG, geometric mean (95% CI), IU/mL	NA	12·7 (6·7–24·1)	7·3 (2·4–22·3)	17·6 (8·1–38·2)	0·037
Female	NA	10·5 (4·0–27·6)	11·1 (3·3–37·8)	9·8 (2·0–47·4)	0·42
Male	NA	16·2 (7·2–36·3)	1·7 (0·2–18·6)	27·5 (14·8–51·2)	0·028
Anti-filamentous haemagglutinin IgG, geometric mean (95% CI), IU/mL	NA	18·4 (9·1–37·1)	8·5 (2·5–29·2)	30·1 (13·5–67·3)	0·010
Female	NA	23·0 (6·6–79·5)	51·3 (12·6–208·4)	9·7 (1·3–74·8)	0·32
Male	NA	31·7 (10·7–94·3)	3·5 (0·2–71·7)	53·2 (18·8–150·6)	0·029
Anti-fimbriae 2/3 IgG, geometric mean (95% CI), AU/mL	NA	26·5 (11·5–61·0)	28·2 (7·4–108·0)	25·4 (8·6–75·5)	0·97
Female	NA	16·2 (6·4–41·1)	19·8 (6·6–59·3)	13·1 (2·7–62·5)	0·32
Male	NA	21·5 (7·2–64·0)	0·8 (0·05-12·9)	57·0 (29·4–110·4)	0·065
Inoculum dose, CFU					
Mean (SD)	NA	96 049 (27 648)	91 975 (28 252)	99 589 (27 378)	0·35‡
Range	NA	52 333–170 000	52 333–170 000	57 333–158 333	··

Ethnicity data were not collected or recorded. AU=arbitrary units. CFU=colony forming units. IU=international units. NA=not applicable. *p values compare colonised with non-colonised participants, calculated using the Mann–Whitney test unless otherwise indicated. †Fisher's test. ‡t-test.

Table 1: **Demographic and physical characteristics, serology, and inoculum counts pre-inoculation**

50 volunteers; figure 2A). In colonised volunteers, *B pertussis* density increased from day 3 to day 14, as measured by nasal wash culture (figure 2B). Four (20%) of the 20 colonised volunteers had cleared *B pertussis* before azithromycin was given, and none of the volunteers were colonised at day 28 (ie, 14 days after azithromycin treatment; figure 2B). Among the 26 volunteers who consented to participate in the rechallenge, 14 had been colonised during the primary challenge. Of these 26 rechallenged volunteers, one who was not colonised during the primary challenge withdrew during the follow-up period. After rechallenge, four (16%) of 25 evaluable volunteers were colonised by day 104 (14 days after rechallenge). Of those who had not been colonised during the primary challenge, three (25%) of 12 were colonised after rechallenge. Of those who had been colonised after initial inoculation, only one (8%) of 13 volunteers was recolonised after the rechallenge (figure 2C). This colonisation fraction was significantly lower than after initial inoculation (p=0·045, Fisher's exact test).

B pertussis inoculation was well tolerated and no serious adverse events occurred during follow-up. Early antibiotic treatment was given to one volunteer, who developed symptoms suggestive of possible pertussis after the initial challenge. This volunteer tested negative for *B pertussis* throughout the study and all symptoms resolved within 4 days. Solicited symptoms during the 14-day follow-up period after inoculation were mostly mild, with no significant increase in reporting of any individual symptom by colonised volunteers (table 2). Among colonised volunteers, there was no association between reported symptoms characteristic of pertussis (cough, rhinorrhoea, and nasal congestion) and colonisation density (appendix p 103). Solicited symptoms throughout the challenge period, viral co-infections, clinical parameters outside of the normal range, and other unsolicited adverse events are detailed in the appendix (pp 96–100). All unsolicited adverse events were assessed as either unlikely related or unrelated to *B pertussis* challenge, and no increase in the frequency of these adverse events was seen in colonised volunteers. There were no safety concerns among contact volunteers (appendix pp 101–102).

Of 17 contact volunteers screened, 14 were enrolled into the study, of whom six (43%) shared a bedroom with a challenge volunteer who was colonised (median bacterial burden 2013 CFU [IQR 1143–89 628; range 144–223 020]). No *B pertussis* was detected in any of the samples taken from the 14 contact volunteers.

Pre-inoculation serum IgG concentrations specific to pertussis toxin (p=0·028), pertactin (p=0·037), and filamentous haemagglutinin (p=0·010), and serum IgA concentrations specific to filamentous haemagglutinin

(p=0·024), were significantly higher in non-colonised volunteers than in colonised volunteers (figure 3A, B). Antibody binding to whole *B pertussis* in pre-inoculation serum and MLF was significantly higher in non-colonised volunteers than in colonised volunteers for IgA (serum p=0·044 and MLF p=0·022) but not for IgG (serum p=0·094 and MLF p=0·55); figure 3C). Male volunteers were less frequently colonised than female volunteers (p=0·043;

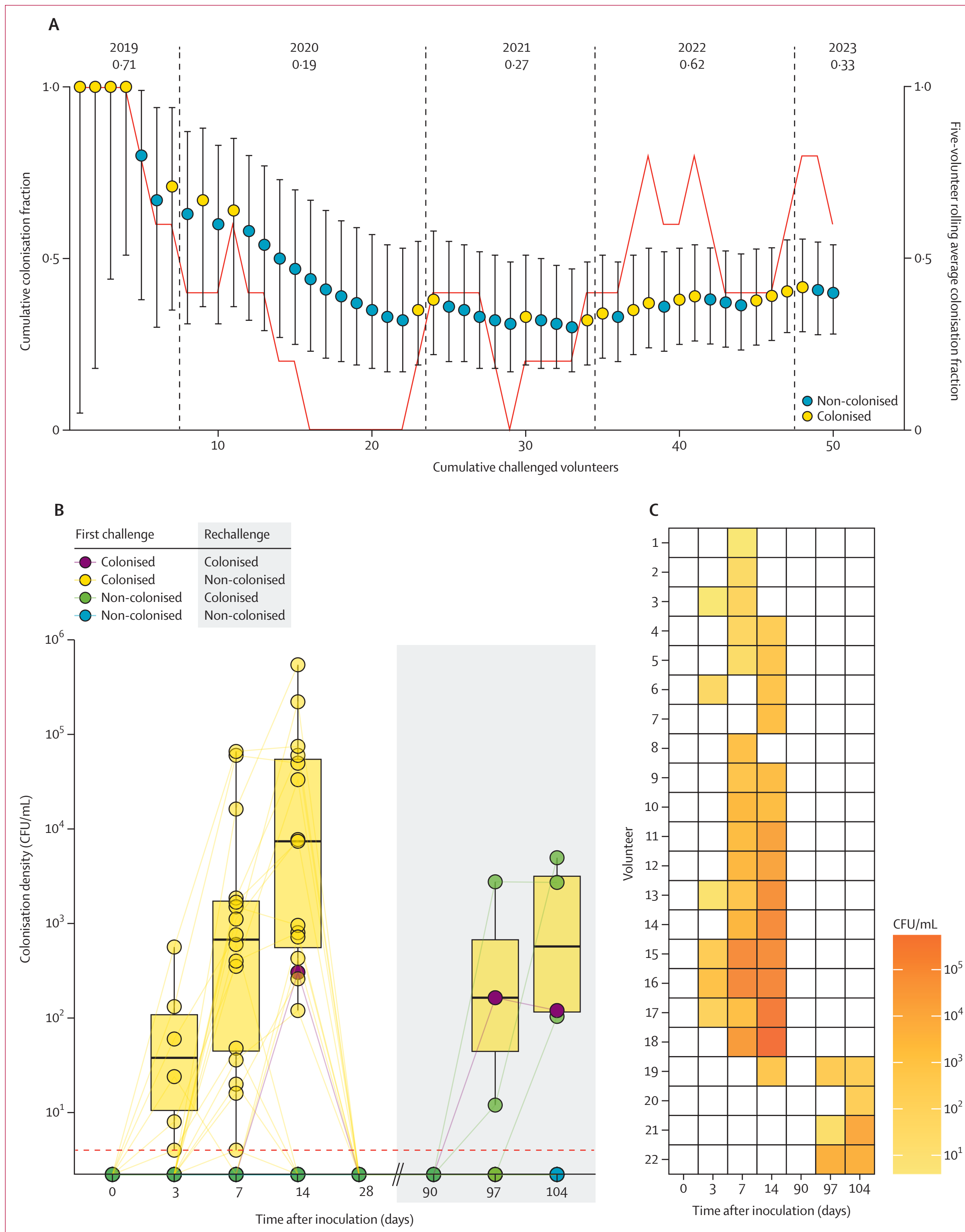

Figure 2: **Colonisation density of *B pertussis* after inoculation**

(A) Left axis: colonisation fraction over time after the primary challenge, presented for the cumulative number of challenged volunteers as the cumulative colonisation fraction (number of colonised volunteers/number of challenged volunteers). Error bars are 95% CI (modified Wald method). Right axis: five-volunteer rolling average of the colonisation fraction (red line), calculated across the cumulative number of challenged volunteers. (B) Colonisation density over time, presented as *B pertussis* colony count (CFU/mL) from culture of nasal wash samples (n=50). Data are presented as individual values for each volunteer (circles), with the colours indicating the colonisation status of the volunteer after the primary challenge and the rechallenge. Purple indicates volunteers colonised at both primary challenge and rechallenge (n=1); orange indicates volunteers colonised at primary challenge and then non-colonised at rechallenge (n=12), not rechallenged (n=6), or non-evaluable at rechallenge (n=1); green indicates volunteers non-colonised at primary challenge and colonised at rechallenge (n=3); and blue indicates volunteers non-colonised at primary challenge and then non-colonised at rechallenge (n=9) or not rechallenged (n=18). Box plots show median (midline) and IQR (box boundaries), and whiskers indicate the minimum and maximum values. The dotted red line represents the lower limit of detection. (C) Colonisation density over time for each colonised volunteer (n=22; excluding the participant who was colonised after primary inoculation but withdrew during the rechallenge phase), presented as *B pertussis* colony count (CFU/mL) from culture of nasal wash samples. Colour intensity reflects colonisation density, with white indicating no detectable colonisation. *B pertussis*=*Bordetella pertussis*. CFU=colony-forming unit.

table 1). Among colonised male volunteers, pre-inoculation IgG concentrations against pertussis toxin, pertactin, and filamentous haemagglutinin were higher than in non-colonised male volunteers, whereas no such differences were observed among female volunteers (appendix p 104).

Among colonised volunteers, bacterial burden inversely correlated with the anti-pertussis toxin IgG concentration in pre-inoculation serum (p=0·029) but not with IgG concentrations specific to the other individual antigens or with IgA concentrations (appendix p 105). Bacterial burden was inversely correlated with IgG binding to whole *B pertussis* in both serum (p=0·042) and MLF (p=0·031; appendix p 105).

After inoculation, the concentrations of all antigen-specific IgG measured (figure 3D) and all antigen-specific IgA measured (figure 3E), as well as the binding of IgG and IgA to whole *B pertussis* (figure 3F), were significantly increased by day 28 in colonised volunteers only. The increase above pre-inoculation titres were sustained until day 90.

Pre-inoculation frequencies of pertussis antigen-specific (pertussis toxin, pertactin, filamentous haemagglutinin, and fimbriae 2/3) IgG-producing memory B cells were not significantly different between colonised and non-colonised volunteers (figure 4A). The pre-inoculation frequency of pertussis toxin-specific memory B cells inversely correlated with bacterial burden in colonised volunteers (p=0·043; figure 4B). After initial inoculation, no changes in *B pertussis*-specific memory B-cell frequencies were detected at early timepoints (day 7 and 14; appendix p 106). However, by day 28, *B pertussis*-specific memory B cells increased in frequency for all *B pertussis* antigens tested in colonised volunteers only (pertussis toxin p=0·013, pertactin p=0·023, filamentous haemagglutinin p=0·0010, and fimbriae 2/3 p=0·0038; figure 4C). By day 90, pertussis toxin-specific (p=0·013) and fimbriae 2/3-specific (p=0·020) memory B-cell frequencies were still significantly higher than pre-inoculation frequencies in colonised volunteers. By contrast, filamentous haemagglutinin-specific (p=0·17) and pertactin-specific (p=0·51) memory B-cell frequencies were no longer significantly increased at day 90 compared with pre-inoculation, with pertactin-specific memory B cells exhibiting a significant reduction in frequency compared with day 28 (p=0·023). No significant changes were detected in memory B-cell frequencies for any of the control conditions (appendix p 107).

Before inoculation, non-colonised volunteers had higher Th22-associated cell responses (ie, expression of IL-22) after stimulation with pertussis toxin (p=0·0045) and filamentous haemagglutinin (p=0·029) than did colonised volunteers. No differences were observed in responses associated with Th1, Th17, and Th2 between colonised and non-colonised volunteers for any of the stimulation conditions tested (appendix p 108).

	Colonised (n=24)*				Non-colonised (n=51)†			
	None	Mild	Moderate	Severe	None	Mild	Moderate	Severe
Cough	14 (58%)	10 (42%)	0	0	39 (76%)	7 (14%)	5 (10%)	0
Rhinorrhoea	17 (71%)	7 (29%)	0	0	31 (61%)	15 (29%)	5 (10%)	0
Nasal congestion	16 (67%)	6 (25%)	2 (8%)	0	33 (65%)	12 (24%)	4 (8%)	2 (4%)
Sneezing	21 (88%)	3 (13%)	0	0	30 (59%)	17 (33%)	4 (8%)	0
Sore throat	18 (75%)	5 (21%)	1 (4%)	0	38 (75%)	9 (18%)	4 (8%)	0
Dyspnoea	24 (100%)	0	0	0	45 (88%)	4 (8%)	1 (2%)	1 (2%)
Malaise	18 (75%)	3 (13%)	3 (13%)	0	43 (84%)	5 (10%)	3 (6%)	0
Tiredness	13 (54%)	6 (25%)	5 (21%)	0	37 (73%)	11 (22%)	3 (6%)	0
Headache	18 (75%)	5 (21%)	0	1 (4%)	41 (80%)	6 (12%)	3 (6%)	1 (2%)
Ear pain	23 (96%)	0	1 (4%)	0	44 (86%)	6 (12%)	1 (2%)	0
Eye pain	23 (96%)	1 (4%)	0	0	49 (96%)	1 (2%)	1 (2%)	0
Epistaxis	23 (96%)	1 (4%)	0	0	45 (88%)	6 (12%)	0	0

Data are n (%). Primary challenge and rechallenge data are included, with volunteers classified by colonisation status at that challenge presented as a percentage of the total group. *20 volunteers were colonised at primary challenge and four at rechallenge. †30 volunteers were non-colonised at primary challenge and 21 at rechallenge.

Table 2: **Solicited adverse events during controlled human infection with *B pertussis***

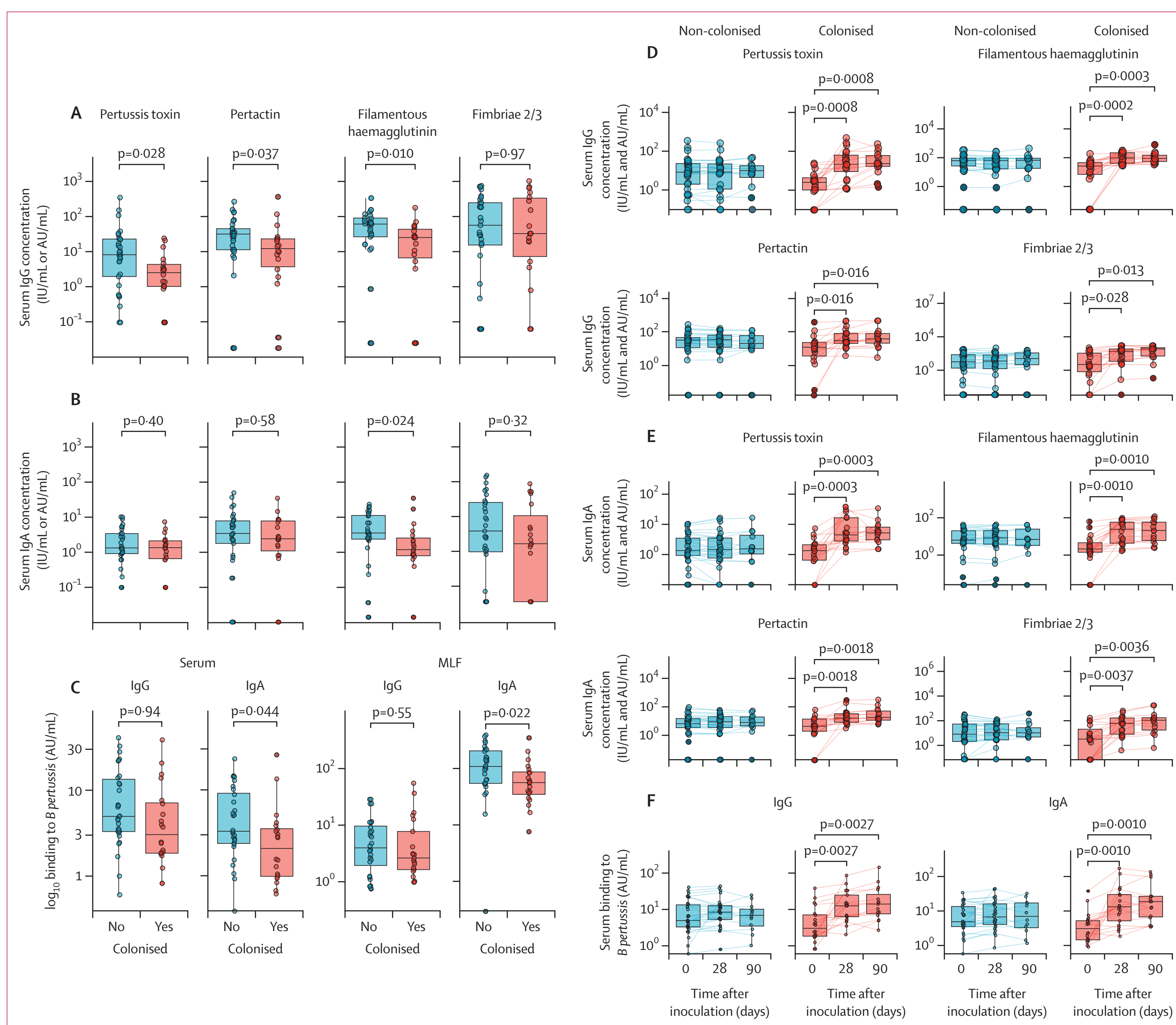

Figure 3: **Antibody profiles in blood and MLF during controlled infection with *B pertussis***
Pre-inoculation concentrations of serum IgG (A), serum IgA (B), and *B pertussis*-binding immunoglobulins in both serum and MLF (C) in volunteers who went on to become colonised (orange) and those who remained non-colonised (blue). Concentrations of serum IgG (D), serum IgA (E), and *B pertussis*-binding immunoglobulins in serum (F) in response to challenge (day 0 and day 28; n=50) and before rechallenge (day 90; n=26) in non-colonised (blue circles) and colonised (orange circles) volunteers. Box plots show median (midline) and IQR (box boundaries), and whiskers indicate the minimum and maximum values. Differences in pre-inoculation serum concentrations between the colonised and non-colonised groups were determined by the Mann–Whitney test (A–C). Comparisons of serological markers between timepoints (D–F) were calculated using Friedman testing with Dunn's post-hoc and Benjamini–Hochberg correction for multiple testing. For (A), (B), (D), and (E), IU/mL is used to report pertussis toxin, pertactin, and filamentous haemagglutinin concentrations; AU/mL is used to report fimbriae 2/3 concentrations. AU=arbitrary units. *B pertussis*=*Bordetella pertussis*. IU=international units. MLF=mucosal lining fluid.

After inoculation, small but significant increases in Th17 responses to pertussis toxin (p=0·039) were observed in the non-colonised group at day 28 compared with pre-inoculation (appendix p 109). Notably, a reduction in Th22 responses to filamentous haemagglutinin was observed in the non-colonised volunteers at day 28 compared with pre-inoculation (p=0·014). Although not reaching the threshold for significance, we observed increased Th22 responses to pertussis toxin at day 28 compared with pre-inoculation in the colonised group (p=0·059), but not in the non-colonised group (p=1·0).

Discussion

This study has shown the safety of an outpatient model of *B pertussis* colonisation, corroborating previously reported safety findings and colonisation dynamics,[11] and shown

that asymptomatic carriers do not transmit to close contacts. In addition, our data identify potential immune determinants of protection against colonisation in healthy adults who had previously received a whole-cell pertussis vaccine in infancy.

We showed that a *B pertussis* controlled human infection study can be conducted in an outpatient setting, which could facilitate future studies such as vaccine-challenge clinical trials. Although minor adverse events did occur, these occurred at similar frequencies and intensities in

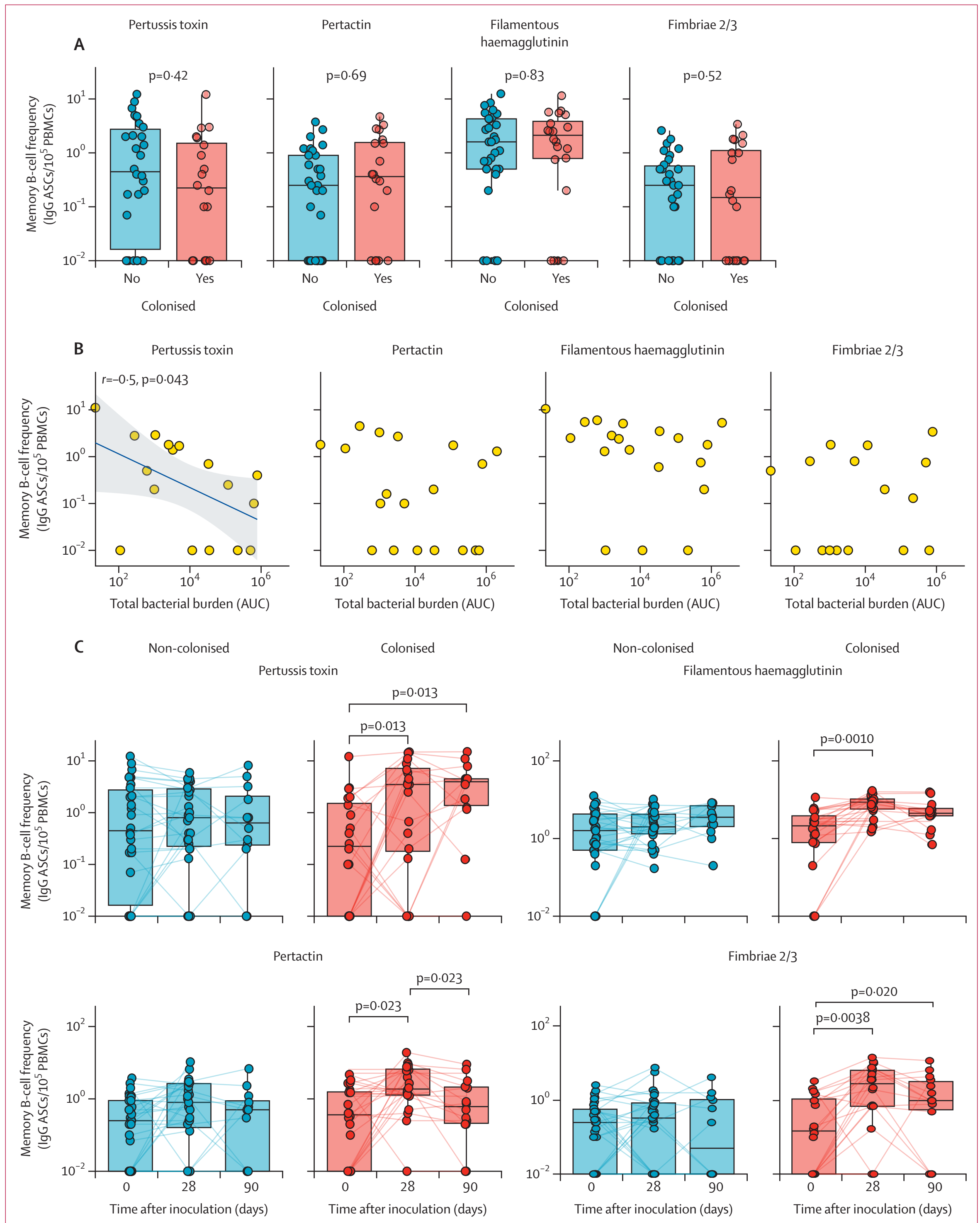

Figure 4: **IgG memory B-cell frequencies during controlled infection with *B pertussis***

(A) Pre-inoculation frequencies of IgG memory B cells specific for *B pertussis* antigens in volunteers who went on to become colonised (orange circles, n=20) and those who were non-colonised (blue circles, n=30). Differences between the colonised and non-colonised groups pre-inoculation were determined by the Mann–Whitney test. (B) Correlation between pre-inoculation memory B-cell frequencies in volunteers who went on to become colonised and their bacterial burden (n=19). Bacterial burden is expressed as the AUC of colonisation density over time, determined by microbiological culture of nasal wash samples obtained at days 3, 7, and 14 after primary inoculation, measured as CFU/mL. Correlations were calculated using a non-parametric Spearman's test. Where correlations were significant ($p \leq 0.05$), data were fitted to a non-linear regression model (blue line) with 95% CI (grey shading). (C) Frequencies of IgG memory B cells specific for *B pertussis* antigens in response to challenge (day 0 and day 28; n=50) and before rechallenge (day 90; n=26) in non-colonised (blue circles) and colonised (orange circles) volunteers. All enzyme-linked immunospot assay data are background control (ie, phosphate buffered saline) subtracted (appendix p 93). Box plots show median (midline) and IQR (box boundaries), and whiskers indicate the minimum and maximum values. Comparisons between timepoints were calculated using Wilcoxon signed-rank testing with Benjamini–Hochberg correction for multiple testing. ASC=antibody secreting cell. AUC=area under the curve. *B pertussis*=*Bordetella pertussis*. PBMC=peripheral blood mononuclear cell.

both colonised and non-colonised volunteers, suggesting that they were unlikely to be a consequence of *B pertussis* infection. The absence of pertussis-like symptoms in colonised volunteers is an intriguing feature of this model, but is reflective of the current consensus that most natural encounters with *B pertussis* are clinically silent. It is possible that symptoms result when the nasopharyngeal bacterial load exceeds a threshold that overcomes mucosal defence; the association of nasopharyngeal genomic bacterial loads with the severity outcomes of hospitalisation and occurrence of complications in children with pertussis supports this notion.[18] No transmission was seen from colonised volunteers to individuals with whom they shared a bedroom, albeit over an observation period of only 2 weeks after inoculation of the challenge volunteer. We speculate that, in the absence of symptoms of pertussis, *B pertussis* cannot be easily transmitted.

The fraction of volunteers who became colonised with *B pertussis* after inoculation changed over the course of the study, and was much lower during the COVID-19 pandemic than before or after. This observation could possibly be explained by a change in pattern of respiratory virus exposure, or by broader effects on the microbiome. Respiratory virus co-infection has been shown to affect colonisation fraction in a pneumococcal challenge model.[19] We speculate that co-infection might lead to cross-protection via activated immune cells, innate immunity, or trained immunity.

Volunteers who were not colonised had higher pre-inoculation serum concentrations of IgA (anti-filamentous haemagglutinin) and IgG (anti-pertussis toxin, anti-pertactin, and anti-filamentous haemagglutinin), and higher binding to *B pertussis* of IgA in serum and MLF, than did volunteers who were colonised. By contrast, no difference in *B pertussis*-specific memory B cells was detected between colonised and non-colonised volunteers pre-inoculation. This disparity could be due to the quiescent state of circulating memory B cells, which do not secrete antibodies at a high rate unless activated.[20] The relative abundance of various pertussis-specific antibodies in individuals who were not colonised could be a consequence of a recent boosting event, such as naturally acquired but clinically silent infection. This explanation would be consistent with the finding that T-cell recognition of antigens that do not arise from acellular pertussis vaccines is common in healthy adults, regardless of their vaccine history or recent documented pertussis episodes.[9] The serum antibody profile shown herein reflects historical seroepidemiological studies in which the concentration of IgG antibodies against pertussis toxin and pertactin in pre-exposure sera correlated with protection against disease.[21] Pre-inoculation serum concentrations of anti-fimbriae 2/3 IgG did not differ between the colonised and the non-colonised groups, which could suggest activity in the clearance of infection and prevention of disease development rather than the prevention of infection.[21] This hypothesis is supported by the increase in serum fimbriae 2/3 concentrations after inoculation.

To our knowledge, the observation that increased concentrations of *B pertussis*-binding IgA in serum and MLF are associated with protection against initial colonisation is new, but is consistent with the established role of IgA in the neutralisation and agglutination of pathogens and with the known efficacy of IgA against *B pertussis*.[22]

Although previous vaccine trials and animal studies have shown a role for Th17 and Th1 cells in the defence against *B pertussis*,[6,23,24] we found no significant differences between colonised and non-colonised volunteers in terms of pre-inoculation Th17 or Th1 responses to *B pertussis* antigens by flow cytometry—albeit in a subset of volunteers only. However, non-colonised volunteers did have higher pre-inoculation frequencies of *B pertussis*-specific Th22 cells in peripheral blood. In addition, after inoculation, Th22 responses to pertussis toxin increased, although not significantly, compared with pre-inoculation in colonised volunteers, suggesting that these responses might also be induced by colonisation. Conversely, in non-colonised volunteers, Th22 responses to filamentous haemagglutinin were reduced after inoculation, which could suggest migration and localisation of pre-existing Th22 cells to the airways. Together, these data are consistent with the potential protective role of IL-22 against extracellular bacteria—ie, the rapid mobilisation of defence against pathogens at mucosal surfaces by supporting epithelial barrier functions including wound healing, and the production of antimicrobial proteins, mucus, and inflammatory mediators.[25]

In colonised volunteers, control of the nasopharyngeal bacterial burden was associated with higher pre-inoculation serum anti-pertussis toxin IgG, higher MLF IgG binding to *B pertussis*, and higher pre-inoculation frequencies of pertussis toxin-specific memory B cells in peripheral blood. This association suggests that these immunological parameters might be important in controlling the

colonisation density of *B pertussis*, rather than preventing the initial colonisation event.

Individuals who were colonised seroconverted to acellular pertussis vaccine antigens and generated *B pertussis*-specific memory B cells, consistent with our previous study that showed plasma cell expansion was positively correlated with CFU count.[26] This finding suggests that these humoral responses are *B pertussis*-specific and are driven by colonisation, supporting induced colonisation as an immunising event, possibly similar to a symptomatic *B pertussis* infection in its capacity to induce IgA and IgG responses.[27,28] Consistent with this immunising effect, we found that colonisation after initial inoculation was associated with protection against colonisation after homologous re-inoculation.

Unexpectedly, we found that relatively more women than men became infected with *B pertussis*, and men who became colonised had lower baseline serum concentrations of IgG against pertussis toxin, pertactin, and fimbriae 2/3 than men who did not become colonised. No such difference was observed between colonised and non-colonised women. This finding could be an artefact of an underpowered cohort, although higher pertussis incidence rates in females than in males have been reported in studies from many countries for all age groups from infancy to older adults, with the excess being most pronounced (65%) in young adults aged 15–44 years.[29]

In contrast to colonised volunteers, we observed no significant *B pertussis*-induced immune responses in non-colonised volunteers after inoculation. We speculate that this might be due to local and innate mucosal immunity, as well as higher antibody titres detected in the non-colonised volunteers at baseline, that might clear the infection before activation and detection of peripheral blood responses. Alternatively, these volunteers might have rapid activation of memory immune responses, which could be detectable at earlier timepoints. Unfortunately, apart from data on memory B cells, we did not collect data at earlier timepoints. However, if the non-colonised volunteers were mounting responses at earlier timepoints, we might have expected to see these responses persist and still be detectable by day 28, because we saw good persistence of responses until day 90 in the colonised volunteers.

A limitation of this study was that measurement of antigen-specific immunity was limited to the antigens included in the acellular pertussis vaccines. However, whole-genome mapping of human *B pertussis*-specific $CD4^+$ T cell responses has shown that healthy adults, regardless of their vaccine history, exhibit reactivity to hundreds of *B pertussis* antigens.[9] Furthermore, animal studies have shown an important role of antigen-specific IL-17 and/or IFNγ-secreting tissue-resident memory T cells in the defence against infection with *B pertussis*,[30] and such cells have been detected in the nasal mucosa of humans who received the whole-cell pertussis vaccine but not those who received the acellular pertussis vaccine.[31] However, this finding could not be corroborated in the current study because nasal biopsies were not taken. Volunteers in this study were primed with a whole-cell pertussis vaccine, and the results might not be translatable to those primed with an acellular pertussis vaccine. Future studies are needed to assess whether the differences in immune-priming found in primates[6] are similar in humans.

In conclusion, our controlled human infection study was safely conducted in an outpatient setting and showed that relative protection against *B pertussis* colonisation is associated with multiple serum and mucosal antibody responses, as well as peripheral Th22 cell responses, with specificity to *B pertussis* antigens associated with the acellular pertussis vaccine. Vaccines that generate sustained immune parameters of this nature could protect against infection by *B pertussis*.

Contributors

HdG, DFG, ARG, APD, JRL, KEK, SNF, AMB, CACMvE, GAM, DAD, and RCR conceptualised and designed the study. HdG and DFG wrote the clinical protocols. HdG, DFG, ARH, JF, APD, JRL, MMI, SNF, and RCR conducted or supported the clinical studies. MMI conducted the microbiological studies to identify *B pertussis*. APD and ARH conducted the memory B cell enzyme-linked immunospot assays. ARH, JB, and JF conducted the multiplex immune assay, MJE conducted the *B pertussis* binding assay, and HdG, DFG, ARH, MMI, JF, and JB collated and analysed data from the microbiology, enzyme-linked immunospot, multiplex immune, and *B pertussis* binding assays. HdG, DFG, ARH, DAD, and RCR verified the underlying data and wrote the paper. All authors critically appraised the paper before submission. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

HdG and DAD received research funding through Radboud University from the Innovative Medicines Initiative (IMI) and Sanofi. DFG received research funding through the University of Southampton and travel expenses from Iliad Biotechnologies. RCR received research funding through the University of Southampton from IMI and Iliad Biotechnologies. CACMvE received research funding through the National Institute of Public Health and the Environment from IMI. SNF acts on behalf of University Hospital Southampton National Health Service Foundation Trust as an investigator and/or consultant for clinical trials and studies of vaccines funded or sponsored by the vaccine manufacturers Pfizer, Sanofi, GlaxoSmithKline, Janssen Pharmaceuticals, Merck, AstraZeneca, Valneva, Moderna, BioNTech, Novavax, MedImmune, Seqirus, MSD, and IMI. No authors received personal financial payment for this work. All other authors declare no competing interests.

Data sharing

All data associated with these studies are present in the paper, associated clinical study protocols, and appendix. Individual de-identified participant data that underlie the results reported in this article, including data dictionaries, will be shared with investigators whose proposed use of the data has been approved by an independent review committee identified for this purpose, and following appropriate institutional and ethical review. Data sharing will be permitted to achieve the aims in the approved proposal. Proposals should be directed to the corresponding author; to gain access, data requesters will need to sign a data access agreement. Data will be available beginning 1 month and ending 24 months after article publication.

Acknowledgments

The PERISCOPE consortium received funding from the IMI 2 Joint Undertaking under grant agreement number 115910. The National Institute for Health and Social Care Research supports the NIHR

Southampton Biomedical Research Centre (NIHR203319). We thank all study participants and research staff at the Southampton NIHR Clinical Research Facility, University Hospital Southampton for the delivery of the clinical component of this study, notably human challenge co-ordinators S Hughes and V Buxton, database manager C Newell, and all investigators, research nurses, and laboratory support staff. We thank all members of the University of Southampton laboratory team, notably J Guy, L Raud, and T Coutinho, members of the PERISCOPE consortium, and Gayatri Amirthalingam of the UK Health Security Agency, who all contributed to the study. We also thank the data safety monitoring board Chair, Stephen Gordon, and the board members (Wei Shen Lim and Patrick Lillie) for their contributions. RCR and SNF are NIHR Senior Investigators.

References

1 Kilgore PE, Salim AM, Zervos MJ, Schmitt HJ. Pertussis: microbiology, disease, treatment, and prevention. *Clin Microbiol Rev* 2016; **29:** 449–86.

2 European Centre for Disease Prevention and Control. Increase of pertussis cases in the EU/EEA. May 8, 2024. https://www.ecdc.europa.eu/sites/default/files/documents/Increase%20in%20pertussis%20cases%20in%20the%20EU-EEA%20-%20May%202024%20FINAL.pdf (accessed Feb 6, 2025).

3 Centers for Disease Control and Prevention. Pertussis: (week 52). Weekly cases of notifiable diseases, United States, U.S. Territories, and Non-U.S. residents week ending December 28, 2024. Jan 2, 2025. https://stacks.cdc.gov/view/cdc/175367 (accessed Feb 6, 2025).

4 Zhang L, Prietsch SOM, Axelsson I, Halperin SA. Acellular vaccines for preventing whooping cough in children. *Cochrane Database Syst Rev* 2014; **2014:** CD001478.

5 Carvalho CFA, Andrews N, Dabrera G, et al. National outbreak of pertussis in England, 2011–2012: a case–control study comparing 3-component and 5-component acellular vaccines with whole-cell pertussis vaccines. *Clin Infect Dis* 2020; **70:** 200–07.

6 Warfel JM, Zimmerman LI, Merkel TJ. Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model. *Proc Natl Acad Sci USA* 2014; **111:** 787–92.

7 Klein NP, Bartlett J, Rowhani-Rahbar A, Fireman B, Baxter R. Waning protection after fifth dose of acellular pertussis vaccine in children. *N Engl J Med* 2012; **367:** 1012–19.

8 Bancroft T, Dillon MBC, da Silva Antunes R, et al. Th1 versus Th2 T cell polarization by whole-cell and acellular childhood pertussis vaccines persists upon re-immunization in adolescence and adulthood. *Cell Immunol* 2016; **304–305:** 35–43.

9 da Silva Antunes R, Garrigan E, Quiambao LG, et al. T cell reactivity to *Bordetella pertussis* is highly diverse regardless of childhood vaccination. *Cell Host Microbe* 2023; **31:** 1404–16.e4.

10 de Graaf H, Gbesemete D, Gorringe AR, et al. Investigating *Bordetella pertussis* colonisation and immunity: protocol for an inpatient controlled human infection model. *BMJ Open* 2017; **7:** e018594.

11 de Graaf H, Ibrahim M, Hill AR, et al. Controlled human infection with *Bordetella pertussis* induces asymptomatic, immunizing colonization. *Clin Infect Dis* 2020; **71:** 403–11.

12 Bart MJ, van der Heide HGJ, Zeddeman A, Heuvelman K, van Gent M, Mooi FR. Complete genome sequences of 11 *Bordetella pertussis* strains representing the pandemic ptxP3 lineage. *Genome Announc* 2015; **3:** e01394–15.

13 Fröberg J, Gillard J, Philipsen R, et al. SARS-CoV-2 mucosal antibody development and persistence and their relation to viral load and COVID-19 symptoms. *Nat Commun* 2021; **12:** 5621.

14 van Gageldonk PGM, van Schaijk FG, van der Klis FR, Berbers GAM. Development and validation of a multiplex immunoassay for the simultaneous determination of serum antibodies to *Bordetella pertussis*, diphtheria and tetanus. *J Immunol Methods* 2008; **335:** 79–89.

15 van Schuppen E, Fröberg J, Venkatasubramanian PB, et al. Prior exposure to *B. pertussis* shapes the mucosal antibody response to acellular pertussis booster vaccination. *Nat Commun* 2022; **13:** 7429.

16 Buisman AM, de Rond CG, Oztürk K, Ten Hulscher HI, van Binnendijk RS. Long-term presence of memory B-cells specific for different vaccine components. *Vaccine* 2009; **28:** 179–86.

17 Corbière V, Lambert EE, Rodesch M, et al. A semi high-throughput whole blood-based flow cytometry assay to detect and monitor *Bordetella pertussis*-specific Th1, Th2 and Th17 responses. *Front Immunol* 2023; **14:** 1101366.

18 Brotons P, de Paz HD, Toledo D, et al. Differences in *Bordetella pertussis* DNA load according to clinical and epidemiological characteristics of patients with whooping cough. *J Infect* 2016; **72:** 460–67.

19 Glennie S, Gritzfeld JF, Pennington SH, et al. Modulation of nasopharyngeal innate defenses by viral coinfection predisposes individuals to experimental pneumococcal carriage. *Mucosal Immunol* 2016; **9:** 56–67.

20 Janeway CA Jr, Travers P, Walport M, Shlomchik MJ. Immunobiology: the immune system in health and disease, 5th edn. New York: Garland Science, 2001.

21 Storsaeter J, Hallander HO, Gustafsson L, Olin P. Levels of anti-pertussis antibodies related to protection after household exposure to *Bordetella pertussis*. *Vaccine* 1998; **16:** 1907–16.

22 Hellwig SM, van Spriel AB, Schellekens JF, Mooi FR, van de Winkel JG. Immunoglobulin A-mediated protection against *Bordetella pertussis* infection. *Infect Immun* 2001; **69:** 4846–50.

23 Ross PJ, Sutton CE, Higgins S, et al. Relative contribution of Th1 and Th17 cells in adaptive immunity to *Bordetella pertussis*: towards the rational design of an improved acellular pertussis vaccine. *PLoS Pathog* 2013; **9:** e1003264.

24 Lambert EE, Corbière V, van Gaans-van den Brink JAM, et al. Uncovering distinct primary vaccination-dependent profiles in human *Bordetella pertussis* specific $CD4^+$ T-cell responses using a novel whole blood assay. *Vaccines (Basel)* 2020; **8:** 225.

25 Zhang K, Chen L, Zhu C, Zhang M, Liang C. Current knowledge of Th22 cell and IL-22 functions in infectious diseases. *Pathogens* 2023; **12:** 176.

26 Diks AM, de Graaf H, Teodosio C, et al. Distinct early cellular kinetics in participants protected against colonization upon *Bordetella pertussis* challenge. *J Clin Invest* 2023; **133:** e163121.

27 Deen JL, Mink CA, Cherry JD, et al. Household contact study of *Bordetella pertussis* infections. *Clin Infect Dis* 1995; **21:** 1211–19.

28 Deville JG, Cherry JD, Christenson PD, et al. Frequency of unrecognized *Bordetella pertussis* infections in adults. *Clin Infect Dis* 1995; **21:** 639–42.

29 Peer V, Schwartz N, Green MS. A multi-country, multi-year, meta-analytic evaluation of the sex differences in age-specific pertussis incidence rates. *PLoS One* 2020; **15:** e0231570.

30 Wilk MM, Misiak A, McManus RM, Allen AC, Lynch MA, Mills KHG. Lung CD4 tissue-resident memory T cells mediate adaptive immunity induced by previous infection of mice with *Bordetella pertussis*. *J Immunol* 2017; **199:** 233–43.

31 McCarthy KN, Hone S, McLoughlin RM, Mills KHG. IL-17 and IFN-γ-producing respiratory tissue-resident memory CD4 T cells persist for decades in adults immunized as children with whole-cell pertussis vaccines. *J Infect Dis* 2024; **230:** e518–23.