

A controlled human infection model of *Streptococcus pyogenes* pharyngitis (CHIVAS-M75): an observational, dose-finding study

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Summary

Background *Streptococcus pyogenes* is a leading cause of infection-related morbidity and mortality. A reinvigorated vaccine development effort calls for new clinically relevant human *S pyogenes* experimental infection models to support proof of concept evaluation of candidate vaccines. We describe the initial Controlled Human Infection for Vaccination Against *S pyogenes* (CHIVAS-M75) study, in which we aimed to identify a dose of *emm75 S pyogenes* that causes acute pharyngitis in at least 60% of volunteers when applied to the pharynx by swab.

Methods This observational, dose-finding study was done in a clinical trials facility in Melbourne (VIC, Australia). Groups of healthy volunteers aged 18–40 years, at low risk of complicated *S pyogenes* disease, and without high type-specific anti-*emm75* IgG antibodies against the challenge strain were challenged and closely monitored as inpatients for up to 6 days, and then as outpatients for 6 months. Antibiotics were started upon diagnosis (clinical signs and symptoms of pharyngitis and a positive rapid molecular test) or after 5 days in those without pharyngitis. Rapid test results were confirmed by standard bacterial culture. After a sentinel participant, cohorts of five and then ten participants were challenged, with protocol-directed dose-escalation or de-escalation for subsequent cohorts. The primary outcome was the proportion of participants at each dose level with pharyngitis by day 5 after challenge. The study is registered with ClinicalTrials.gov, NCT03361163.

Findings Between July 10, 2018, and Sept 23, 2019, 25 healthy adults were challenged with *emm75 S pyogenes* and included in analyses. Pharyngitis was diagnosed in 17 (85%; 95% CI 62–97) of 20 participants at the starting dose level ($1\text{--}3 \times 10^5$ colony-forming units [CFU]/mL). This high proportion prompted dose de-escalation. At the lower dose level ($1\text{--}3 \times 10^4$ CFU/mL), pharyngitis was diagnosed in one of five participants. Immunological, biochemical, and microbiological results supported the clinical picture, with acute symptomatic pharyngitis characterised by pharyngeal colonisation by *S pyogenes* accompanied by significantly elevated C-reactive protein and inflammatory cytokines (eg, interferon- γ and interleukin-6), and modest serological responses to streptolysin O and deoxyribonuclease B. There were no severe (grade 3) or serious adverse events related to challenge.

Interpretation We have established a reliable pharyngitis human infection model with reassuring early safety findings to accelerate development of vaccines and other interventions to control disease due to *S pyogenes*.

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Introduction

Streptococcus pyogenes, the group A *Streptococcus*, is a major cause of infection-related mortality and morbidity across a diverse clinical spectrum, spanning from pharyngitis, impetigo, and cellulitis to severe invasive infections and post-infectious acute post-streptococcal glomerulonephritis, acute rheumatic fever, and rheumatic heart disease.¹ *S pyogenes* causes an immense communicable and non-communicable global burden of disease. Unpredictable outbreaks and an uncontrolled endemic burden in marginalised communities belie the virtual disappearance of scarlet fever and rheumatic fever

from high-income countries.^{2–4} Severe infections are seen in all age groups, but disproportionately affect young children (younger than 1 year), older people (especially those aged 65 years or older), and pregnant women.⁵ Aboriginal and Torres Strait Islander people in Australia and Māori people in New Zealand are affected by a persistent and high burden of *S pyogenes* infections and post-infectious sequelae. Globally, there are more than 30 million prevalent cases of rheumatic heart disease, causing more than 300 000 deaths annually.⁶ With invasive infections such as streptococcal toxic shock syndrome and necrotising fasciitis, at least 500 000 deaths

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Research in context

Evidence before this study

The potential for immunisation to reduce the global burden of *Streptococcus pyogenes* diseases was recognised centuries ago, when its most conspicuous clinical syndromes were described together as scarlet fever. However, *S pyogenes* vaccine development has been frustrated by scientific, regulatory, and commercial obstacles. A renewed global vaccine development effort has prioritised development of new experimental human infection models for vaccine evaluation. We searched PubMed for *S pyogenes* human infection studies published before Sept 15, 2020, with no language restrictions, using combinations of the search terms “streptococcus pyogenes”, “group A streptococcus”, “scarlet fever”, “experimental”, “human infection”, “human challenge”, “protective”, and “vaccine”. Human experiments in the 19th and early 20th century established *S pyogenes* as the cause of scarlet fever. Three human infection studies, including a total of 172 participants, were done in the 1970s. Each was a double-blind, placebo-controlled trial of monovalent M-protein vaccines for protection against pharyngitis in healthy adult volunteers challenged with homologous serotype *S pyogenes* strains applied directly to the pharynx using a swab. More than half of the 84 unvaccinated (control) participants had typical symptoms and signs of *S pyogenes* pharyngitis, such as sore

throat, pharyngeal erythema or exudates, lymphadenopathy, and fever. Vaccine efficacy was as high as 89% (95% CI 23–98) in the first study testing a parenteral M1 vaccine.

Added value of this study

We report the results of a clinical study to establish a new controlled human infection model of *S pyogenes* pharyngitis, developed in accordance with modern Good Manufacturing Practice and Good Clinical Practice principles. We showed that healthy adult volunteers can be challenged safely with *emm75 S pyogenes* to produce a convincing streptococcal pharyngitis clinical syndrome in a high proportion of participants, supported by findings of microbiological, biochemical, and immunological investigations.

Implications of all the available evidence

WHO's 2018 Global Resolution on Rheumatic Fever and Rheumatic Heart Disease listed *S pyogenes* vaccine research as a key priority for prevention and control. The 2018 WHO roadmap for *S pyogenes* vaccine development identified the need for human infection models to accelerate vaccine evaluation. This is the only current *S pyogenes* controlled human infection model, ready to be used as a platform to evaluate new vaccine candidates and therapeutics, and for studying host-pathogen interactions.

are directly attributable to *S pyogenes* each year, making it one of the five leading causes of global infection-related mortality.⁷ At the other end of the spectrum, sore throat is among the most common reasons for seeking primary health care, and widespread inappropriate empirical antibiotic treatment is driven largely by the desire to treat *S pyogenes* pharyngitis and prevent its complications.⁸ Despite this burden of disease, no vaccine is available for prevention.

There is a long history of development of vaccines against *S pyogenes*.⁹ In the early 20th century, experimental vaccines were deployed in institutions and the community to prevent scarlet fever, but interest waned as outbreaks became less frequent and rheumatic fever incidence declined in Europe and the USA, and with the arrival of penicillin.² However, the burden of *S pyogenes* disease has persisted despite its continuing susceptibility to penicillin. Since 2016, reinvigorated global vaccine development efforts have sought to overcome scientific, regulatory, and commercial obstacles that have frustrated previous attempts.^{10,11} The 2018 WHO *S pyogenes* vaccine research and development roadmap called for development of clinically relevant human experimental infection models to support early evaluation of candidate vaccines.¹¹ Controlled human infection models are increasingly contributing to vaccine development.¹² We describe the initial Controlled Human Infection for Vaccination Against *S pyogenes* (CHIVAS-M75) study, with the primary objective of determining a bacterial

inoculum (dose) to cause acute pharyngitis in at least 60% of healthy adult participants when applied by swab to the pharynx.

Methods

Study design and participants

This observational, dose-finding study was based at an inpatient clinical trials facility (Nucleus Network, Melbourne, VIC, Australia). Healthy volunteers aged 18–40 years who were at low risk of complicated *S pyogenes* disease, and met other eligibility criteria (appendix pp 6–8), had screening tests including throat swabs and measurement of pre-existing antibodies against the challenge strain, *emm75 S pyogenes*.¹³ Volunteers meeting eligibility criteria had a transthoracic echocardiogram to exclude subclinical rheumatic heart disease. Participants received AU\$50 (average 2019 exchange rate US\$0.695) per outpatient visit and \$12.50 per h (\$300 per day) during the inpatient admission (2019 national minimum wage was \$740.80 per 38 h week). The CHIVAS-M75 study protocol and challenge strain selection and manufacture have previously been described in detail.^{14,15}

This study was approved by the Alfred Hospital Human Research Ethics Committee (500/17). Written informed consent was obtained from all participants. A safety committee with an independent chair reviewed safety data and details of each pharyngitis diagnosis, meeting to approve study continuation after the first participant attended the initial 1 week outpatient

follow-up visit and before dose escalation or de-escalation.

Procedures

The *emm75 S pyogenes* challenge strain (GenBank CP033621) was collected locally in 2011 from a child with exudative pharyngitis in a previous study and characterised extensively for human challenge use.¹⁵ Manufacture of single-dose vials of *emm75 S pyogenes* followed principles of Good Manufacturing Practice, including quality control testing by an independent laboratory of 10% of vials produced at each of five dose levels ($1-3 \times 10^4$, $1-3 \times 10^5$, $1-3 \times 10^6$, $1-3 \times 10^7$, and $1-3 \times 10^8$ colony-forming units [CFU]/mL).¹⁴

Single-dose vials of *emm75 S pyogenes* in 1 mL animal-free liquid medium were thawed from -80°C before application to the pharynx using a sterile Dacron swab by a standardised procedure.^{14,15} The dose level refers to the concentration (CFU/mL) in a vial. The swabs absorbed approximately 0.1 mL so that the bacterial inoculum (the actual dose) applied to the pharynx was at least 1-log_{10} lower than the total bacteria in each vial.

After screening, participants were admitted on the evening before challenge and confined for up to 6 days until 24 h after starting antibiotic treatment. The *emm75 S pyogenes* strain was applied to the pharynx, following a predetermined dose-escalation scheme (appendix p 2). A de-escalation contingency was introduced if the starting dose unexpectedly caused pharyngitis in a very high proportion of participants (appendix p 2). After a sentinel participant, cohorts of five and then ten participants were challenged, with protocol-directed dose-escalation or de-escalation for subsequent cohorts. Inpatient monitoring included numerical pain rating (0–10 integer Numeric Rating Scale¹⁶ on a visual analogue scale, with 0 meaning no pain, 5 meaning moderate pain, and 10 meaning worst pain possible) four times per day; recording of the duration and severity of solicited and unsolicited symptoms; daily blood cultures; and medical assessment for the diagnosis of pharyngitis every 12 h. Paracetamol for analgesia was administered on request. All participants received intramuscular benzathine penicillin G 900 mg once, and oral rifampicin 300 mg twice a day for eight doses, administered promptly upon the diagnosis of pharyngitis or on day 5 for those without pharyngitis. Contact and droplet precautions were used for CHIVAS-M75 participants, who were physically separated from participants in other studies and from each other. Outpatient visits were at 1 week, 1 month, 3 months, and 6 months after discharge with repeat throat swabs, blood, and urine collection, and echocardiography.

Serious adverse events (defined in the protocol¹⁴) were recorded and followed until resolution or stabilisation from the date of participant consent until completion of the 6-month follow-up period. Non-serious adverse events were recorded until the 1-month outpatient visit.

All adverse events were graded by severity (mild, moderate, or severe) and their association with *S pyogenes* challenge (unrelated, or possibly, probably, definitely related).

To assess host inflammatory responses, C-reactive protein (CRP), inflammatory cytokines, and chemokines were measured in serial serum samples from before challenge until the first outpatient visit (appendix p 9). To assess serological responses, anti-streptolysin O (ASO) and anti-deoxyribonuclease B (ADB) antibody titres were measured before challenge and at each outpatient visit, using a previously described multiplex bead assay.¹⁷

To assess bacterial dynamics after challenge, throat swabs were separately collected for culture and molecular studies at screening and outpatient visits, and twice a day during the confinement period. Bacterial culture followed standard methods with β -haemolytic colonies identified as *S pyogenes* by MALDI-TOF (Bruker MALDI Biotyper, Bremen, Germany) and a semiquantitative plate growth score made for each swab (appendix p 10). A highly sensitive and specific *emm75* quantitative PCR (qPCR) assay was developed for this study, with results reported as cycle threshold values.

Outcomes

The primary outcome was the proportion of participants at each dose level meeting the pharyngitis endpoint by day 5 after challenge, as defined by typical symptoms and signs of streptococcal pharyngitis (sore throat, tonsil swelling, erythema, petechiae, and exudates) supported microbiologically in real time with a molecular point-of-care test (ID NOW Strep A 2, Abbott, Scarborough, ME, USA), and subsequently by throat swab culture (appendix p 3). Secondary objectives were to characterise bacterial dynamics and host responses during experimental infection.

Statistical analysis

The target of causing pharyngitis in at least 60% of 20 participants was set with future randomised trials in mind.¹⁴ Rules for dose escalation and de-escalation were based on CIs for 20 participants at a given dose level. For example, if 12 of 20 participants had pharyngitis, the 95% CI for the true attack rate would be 36–81% so that a Fisher's exact test with 0.05 two-sided significance level would have 90% power to detect a difference between two groups of 23 participants for a vaccine with 80% efficacy.

Post-hoc sensitivity analyses considered modified pharyngitis diagnostic criteria: removing tonsil size, removing cervical lymphadenopathy, requiring diagnosis by 48 h after challenge, and requiring a peak CRP of 20 mg/L or more, or 40 mg/L or more.

Analyses are descriptive and presented as proportions, means with associated 95% CIs, and medians with IQRs. Participant data were recorded in a paper file of source documents, stored securely at the clinical

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See Online for appendix

	Dose 1 (n=20)	Dose 2 (n=5)	All (n=25)
Target range <i>Streptococcus pyogenes</i> challenge dose*	1–3 × 10 ⁵ CFU*	1–3 × 10 ⁴ CFU*	..
Actual <i>S pyogenes</i> challenge dose†	1.72 × 10 ⁵ CFU†	1.62 × 10 ⁴ CFU†	..
Sex			
Female	10 (50%)	2 (40%)	12 (48%)
Male	10 (50%)	3 (60%)	13 (52%)
Age, years	27.5 (5.7; 20.1–38.6)	28.2 (5.8; 20.5–33.1)	27.6 (5.6; 20.1–38.6)
Weight, kg	65.8 (9.8)	65.1 (11.7)	65.6 (10.0)
Body-mass index, kg/m ²	22.4 (2.7)	21.8 (1.7)	22.3 (2.5)

Data are n (%), mean (SD; range), or mean (SD) unless otherwise specified. CFU=colony-forming unit. *Target CFU in 1 mL animal-free liquid medium in single-dose vials. †Actual CFU (mean viable cell count) in ten of 100 frozen vials manufactured for each dose level as determined in an independent quality control laboratory by thawing immediately before serial dilution and spread plating on duplicate horse blood agar plates and incubation overnight at 37°C.

Table 1: Participant demographic characteristics and challenge dose data

trials facility, then transcribed using electronic data capture tools hosted at Murdoch Children's Research Institute (REDCap, Vanderbilt University, Nashville, TN, USA). Data were analysed using GraphPad Prism (version 8.0).

The study is registered with ClinicalTrials.gov, NCT03361163.

Role of the funding source

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

Results

From July 10, 2018, to Sept 23, 2019, 25 healthy adult participants were enrolled and sequentially challenged with *emm75 S pyogenes*, in groups of between one and three, totalling 20 at the 10⁵ CFU/mL dose level and then five at the 10⁴ CFU/mL level (table 1; figure 1). All 25 participants were assessed for the primary pharyngitis endpoint. 103 volunteers were excluded after screening, with the main reasons being withdrawal of consent and not meeting inclusion criteria (appendix p 11). Seven volunteers were excluded due to *S pyogenes* carriage and nine others due to high baseline anti-*emm75* IgG (figure 1).

75 adverse events were recorded in 24 of 25 participants, and 63 (84%) adverse events affecting 23 participants were (possibly, probably, or definitely) related to *S pyogenes* challenge. Of these 63 challenge-related adverse events, six (10%) were of moderate intensity and the remainder were mild. Typical symptoms of streptococcal pharyngitis accounted for 54 (86%) challenge-related adverse events (sore throat, tender lymphadenopathy, sweats, fever, chills, myalgia, arthralgia, headache, abdominal pain, nausea, vomiting, and malaise). There were no severe (grade 3,

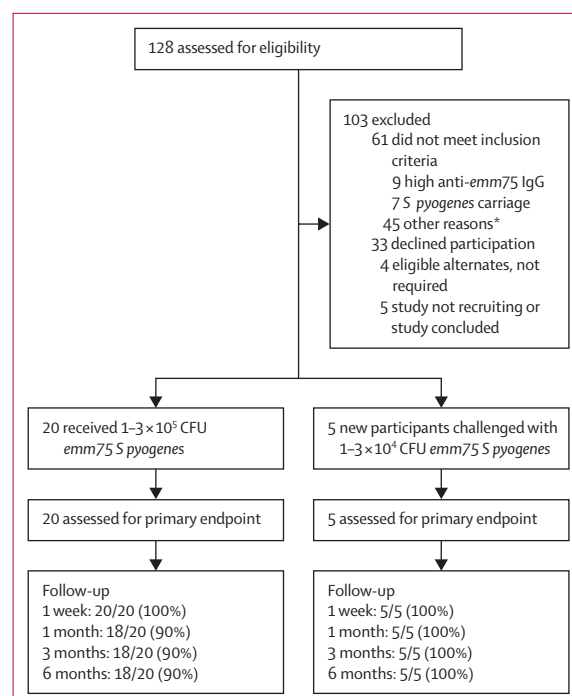


Figure 1: CHIVAS-M75 study overview

CFU=colony-forming units. CHIVAS-M75=Controlled Human Infection for Vaccination Against *Streptococcus pyogenes*. *For other reasons for exclusion see the appendix (p 11).

preventing usual daily activity or requiring complex treatment) or serious adverse events related to challenge and no local or systemic complications of pharyngitis. Two unrelated serious adverse events occurred in the outpatient follow-up period: *Escherichia coli* pyelonephritis and suspected sepsis on return from overseas travel, after a dog bite. Participants with pharyngitis responded clinically to antibiotic treatment with no residual symptoms at the 1-week visit, except for very mild sore throat in two participants that had completely resolved by the 1-month visit, and without subsequent relapse. The challenge strain was eradicated in all participants. No secondary cases of disease caused by *S pyogenes* were reported in study staff or other volunteers.

No clinical episodes were indicative of rheumatic fever or glomerulonephritis, and no signal of subclinical cardiac or renal injury (electrocardiography, echocardiography, urinalysis, urine albumin to creatinine ratio, and serum urea and creatinine) was seen. One participant (075) had *emm77 S pyogenes* isolated from a throat swab at the 6-month visit (ie, not the *emm75* challenge strain). Participant 106 had an abnormal electrocardiogram at the 6-month visit with mild first-degree atrioventricular block and early repolarisation in the anterior leads, varying with heart rate, and intermittent second-degree Mobitz I (Wenckebach) atrioventricular block. Heart block is a feature of rheumatic fever but not in isolation—this participant had no other features of rheumatic fever, rheumatic heart disease, or pharyngitis, and had the

Outcome	Timing from challenge to diagnosis	Peak response to infection		Serology		Signs	Worst symptoms				Paracetamol (total, g)				
		T _{max} (°C)	CRP (mg/L)	ASO†	ADB†		Pharyngitis‡	Tonsil swellings§	Adenopathy	Sorethroat pain score¶		Chills	Headache	Gastro-intestinal	
Cycle threshold value from qPCR*															
1–3×10 ⁵ CFU															
Participant 009	Pharyngitis	24 h	21.98	36.9 (<0.5°C above baseline)	38	0	0	Intense	+1	Yes	7/10	No	Yes	No	2
Participant 068	Pharyngitis	24 h	21.24	37.3 (0.5°C to <1.0°C above baseline)	47	0	+1	Mild	+1	Yes	7/10	Yes	Yes	No	0
Participant 043	Pharyngitis	36 h	20.61	37.0 (<0.5°C above baseline)	86	+1	0	Medium	+2	No	6/10	No	Yes	No	5
Participant 002	Pharyngitis	48 h	22.26	37.5 (1.0°C to <1.5°C above baseline)	36	+1	+2	Mild	+1	Yes	5/10	Yes	No	No	4
Participant 017	Pharyngitis	48 h	22.98	37.3 (0.5°C to <1.0°C above baseline)	71	+1	+2	Mild	+1	Yes	6/10	No	Yes	No	8
Participant 020	Pharyngitis	48 h	19.54	37.3 (0.5°C to <1.0°C above baseline)	25	+1	+1	Intense	0	Yes	7/10	No	Yes	No	3
Participant 025	Pharyngitis	48 h	20.99	37.0 (0.5°C to <1.0°C above baseline)	93	0	+2	Medium	+1	Yes	7/10	No	Yes	No	5
Participant 033	Pharyngitis	48 h	19.59	38.9 (>38.3°C)	47	+1	+1	Mild	+2	Yes	5/10	Yes	Yes	Nausea	8
Participant 059	Pharyngitis	48 h	21.23	38.5 (>38.3°C)	46	0	+1	Mild	0	Yes	6/10	No	No	No	8
Participant 061	Pharyngitis	48 h	21.18	38.0 (1.0°C to <1.5°C above baseline)	131	0	+2	Mild	0	Yes	8/10	Yes	No	Nausea	8
Participant 064	Pharyngitis	48 h	21.14	36.7 (<0.5°C above baseline)	36	+1	+1	Medium	+1	Yes	5/10	No	No	No	2
Participant 075	Pharyngitis	48 h	22.64	37.5 (0.5°C to <1.0°C above baseline)	66	+1	0	Intense	+1	No	3/10	No	Yes	No	0
Participant 071	Pharyngitis	60 h	22.44	37.6 (0.5°C to <1.0°C above baseline)	53	+1	+2	Mild	+1	Yes	5/10	Yes	Yes	No	4
Participant 032	Pharyngitis	60 h	20.61	37.8 (1.0°C to <1.5°C above baseline)	87	0	0	Mild	0	Yes	8/10	Yes	Yes	No	8
Participant 010	Pharyngitis	72 h	20.00	37.5 (1.0°C to <1.5°C above baseline)	12	+1	0	Mild	0	Yes	6/10	No	Yes	Discomfort	0
Participant 055	Pharyngitis	72 h	23.32	37.1 (0.5°C to <1.0°C above baseline)	36	+1	0	Medium	0	Yes	4/10	Yes	Yes	No	0
Participant 026	Pharyngitis	96 h	23.66	37.2 (0.5°C to <1.0°C above baseline)	58	0	+1	Mild	0	Yes	3/10	No	Yes	No	3
Participant 057	Likely pharyngitis	..	21.39	37.1 (0.5°C to <1.0°C above baseline)	25	+1	+2	Medium	0	No	5/10	No	Yes	No	8
Participant 013	No pharyngitis	..	22.98	37.1 (0.5°C to <1.0°C above baseline)	12	+1	0	Mild	0	No	3/10	No	No	No	4
Participant 030	No pharyngitis	..	>35.00	37.0 (<0.5°C above baseline)	<5	+1	0	0	0	No	0/10	No	Yes	No	0
1–3×10 ⁶ CFU															
Participant 081	Pharyngitis	48 h	23.42	37.2 (0.5°C to <1.0°C above baseline)	30	0	+1	Intense	+1	Yes	6/10	No	Yes	No	2
Participant 116	No pharyngitis	..	>35.00	36.8 (<0.5°C above baseline)	<5	0	0	Mild	0	No	0/10	No	No	No	0
Participant 107	No pharyngitis	..	>35.00	36.8 (<0.5°C above baseline)	<5	0	0	Mild	+1	No	1/10	No	No	No	0
Participant 102	No pharyngitis	..	>35.00	37.4 (0.5°C to <1.0°C above baseline)	<5	0	0	Mild	0	Yes	0/10	No	Yes	No	0
Participant 106	No pharyngitis	..	>35.00	36.9 (<0.5°C above baseline)	<5	0	0	Mild	0	No	0/10	No	No	No	0

A DB=anti-deoxyribonuclease B. ASO=anti-streptolysin. CFU=colony-forming unit. CRP=C-reactive protein. qPCR=quantitative PCR. T_{max}=maximum temperature. ULN=upper limit of normal. *Low cycle threshold values indicate high colonisation. †0 indicates that the post-infection titres were not above ULN for age and there was not a two times or greater rise. +1 indicates post-infection titre above ULN for age or two times or greater rise, and +2 indicates post-infection titre above ULN for age and two times or greater rise. ‡Mild indicates mild erythema (hyperaemic vessels), medium indicates moderate intensity erythema with or without palatal petechiae, and intense indicates intense erythema and exudative tonsillitis. §0 indicates no change from baseline, +1 indicates tonsil size score increased by 1 from baseline, and +2 indicates that the score increased by 2 from baseline (see appendix p 3 for scoring system). ¶0 represents no pain, 5 indicates moderate pain, and 10 represents worst pain possible.³⁵

Table 2: Response of healthy adult volunteers to pharyngeal challenge with emm75 Streptococcus pyogenes

ADB=anti-deoxyribonuclease B. ASO=anti-streptolysin. CFU=colony-forming unit. CRP=C-reactive protein. qPCR=quantitative PCR. T_{max}=maximum temperature. ULN=upper limit of normal. *Low cycle threshold values indicate high colonisation. †Indicates that the post-infection titres were not above ULN for age and there was not a two times or greater rise, +1 indicates post-infection titre above ULN for age or two times or greater rise, and +2 indicates post-infection titre above ULN for age and two times or greater rise. ‡Mild indicates mild erythema (hyperaemic vessels), medium indicates moderate intensity erythema with or without palatal petechiae, and intense indicates intense erythema and exudative tonsillitis. §0 indicates no change from baseline, +1 indicates tonsil size score increased by 1 from baseline, and +2 indicates that the score increased by 2 from baseline (see appendix p 3 for scoring system). ¶0 represents no pain, 5 indicates moderate pain, and 10 represents worst pain possible.¹⁵

Table 2: Response of healthy adult volunteers to pharyngeal challenge with emm75 *Streptococcus pyogenes*

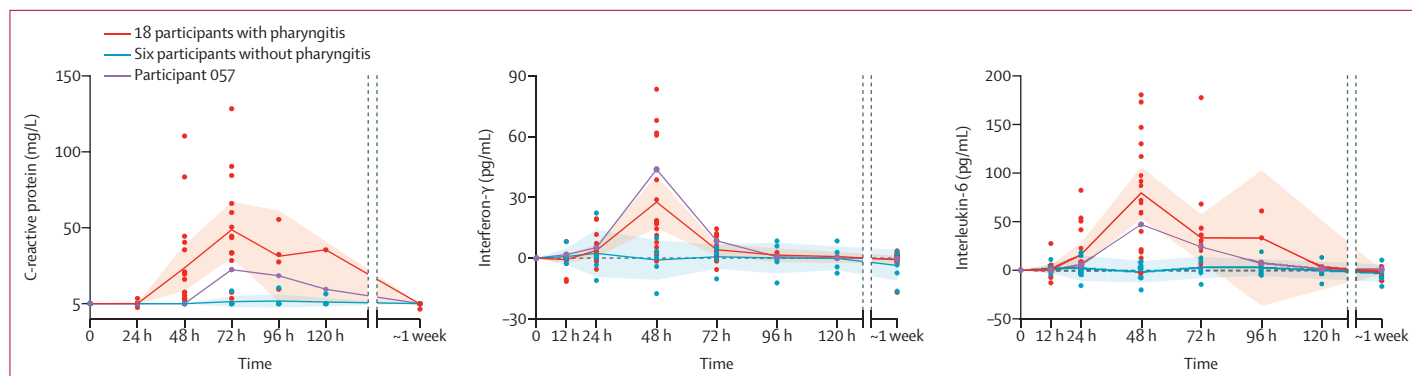


Figure 2: Systemic inflammatory response during experimental human pharyngitis

Serial measurements with mean (lines) and 95% CIs (shading) for serum C-reactive protein (the lower reporting limit for the C-reactive protein assay was <5 mg/L), and deviation from baseline for interferon-γ and interleukin-6 for 18 participants with pharyngitis and six participants without pharyngitis. Results for participant 057 are shown separately.

typical athletic build and low resting heart rate associated with physiological Wenckebach phenomenon. One participant (017) withdrew from the study before the 1-month outpatient visit and declined further visits. Another participant (013) travelled overseas due to a family emergency and could not attend 3-month and 6-month visits, and participant 075 was unable to attend the 1-month visit.

Pharyngitis was diagnosed in 17 (85%; 95% CI 62–97) of 20 participants at the starting dose level ($1\text{--}3 \times 10^5$ CFU/mL) and in one of five participants at the $1\text{--}3 \times 10^4$ CFU/mL level. A protocol deviation affected one participant (057) at the starting dose level: this participant was not diagnosed with pharyngitis despite having clinical features that should have prompted a rapid test to confirm the diagnosis. This test was not done, the participant's clinical syndrome did not progress, and antibiotic treatment was deferred until day 5. Thus, 18 (90%; 95% CI 68–99) of 20 participants might actually have developed pharyngitis at the starting dose level. The aim of causing pharyngitis in at least 12 of 20 participants was sustained in post-hoc sensitivity analyses using modified diagnostic criteria (appendix p 15).

Participants with pharyngitis developed sore throat, pharyngeal erythema, tender anterior cervical lymphadenopathy, and headache (table 2; appendix p 12). For 15 (83%) of 18 participants with definite pharyngitis, the diagnosis was made between 36 h and 72 h after challenge. More severe features such as exudative pharyngitis (four [21%] of 19 participants with definite or likely pharyngitis), maximum temperature of 38.0°C or above (three [16%] participants), and high-grade tonsillar swelling (no participants) were uncommon (appendix p 20). Maximum numerical pain rating scores for participants with definite or likely pharyngitis were higher (mean 5.7 [SD 1.4]; range 3.0–8.0) than for those without pharyngitis (0.7 [1.2]; 0.0–3.0).

Peak CRP for participants with pharyngitis was higher than for those without pharyngitis and varied between 12 mg/L and 131 mg/L, with a median of 47 mg/L

(IQR 36–70; figure 2; appendix pp 16–17). CRP typically peaked 24 h after diagnosis and antibiotic treatment. Inflammatory cytokine and chemokine responses were evident in participants with pharyngitis and most consistent for interferon-γ and interleukin-6. CRP, cytokine, and chemokine concentrations returned to baseline by the 1-week visit.

13 of 25 participants had a two times or greater rise in ASO or ADB antibody titres at the 1-month outpatient visit, including 12 (67%) of 18 participants diagnosed with pharyngitis. Post-challenge titres for ASO or ADB were higher than age-based upper limits of normal (ULNs) in 11 (61%) of 18 participants with pharyngitis, although pre-challenge titres already exceeded the ULN for ASO in seven and ADB in three of these 11 participants (table 2; appendix p 18). Six (33%) of 18 with pharyngitis had both a two times or greater rise and post-challenge ADB titres above the ULN for age. There was no correlation between pharyngitis severity and baseline titres or titre change.

S pyogenes was detected by qPCR from the earliest throat swabs collected 24 h after challenge in all 18 participants diagnosed with pharyngitis, and in 16 (89%) by culture (figure 3; appendix p 19). All isolates at the diagnosis timepoint were confirmed by sequencing as *emm75 S pyogenes*. At the outpatient visit 1 week after discharge, *S pyogenes* was not detected by culture or qPCR in any participant.

Three of the seven participants who were not diagnosed with pharyngitis were colonised by *emm75 S pyogenes* at one or more timepoint (figure 3): Participant 116 had a single positive culture, participant 013 was colonised without other features of pharyngitis, and participant 057 who probably did have pharyngitis but was not diagnosed.

Discussion

In this dose-finding, human infection study, swabbing *emm75 S pyogenes* onto the pharynx induced a convincing acute pharyngitis syndrome in more than 60% of healthy adult volunteers, without challenge-related serious

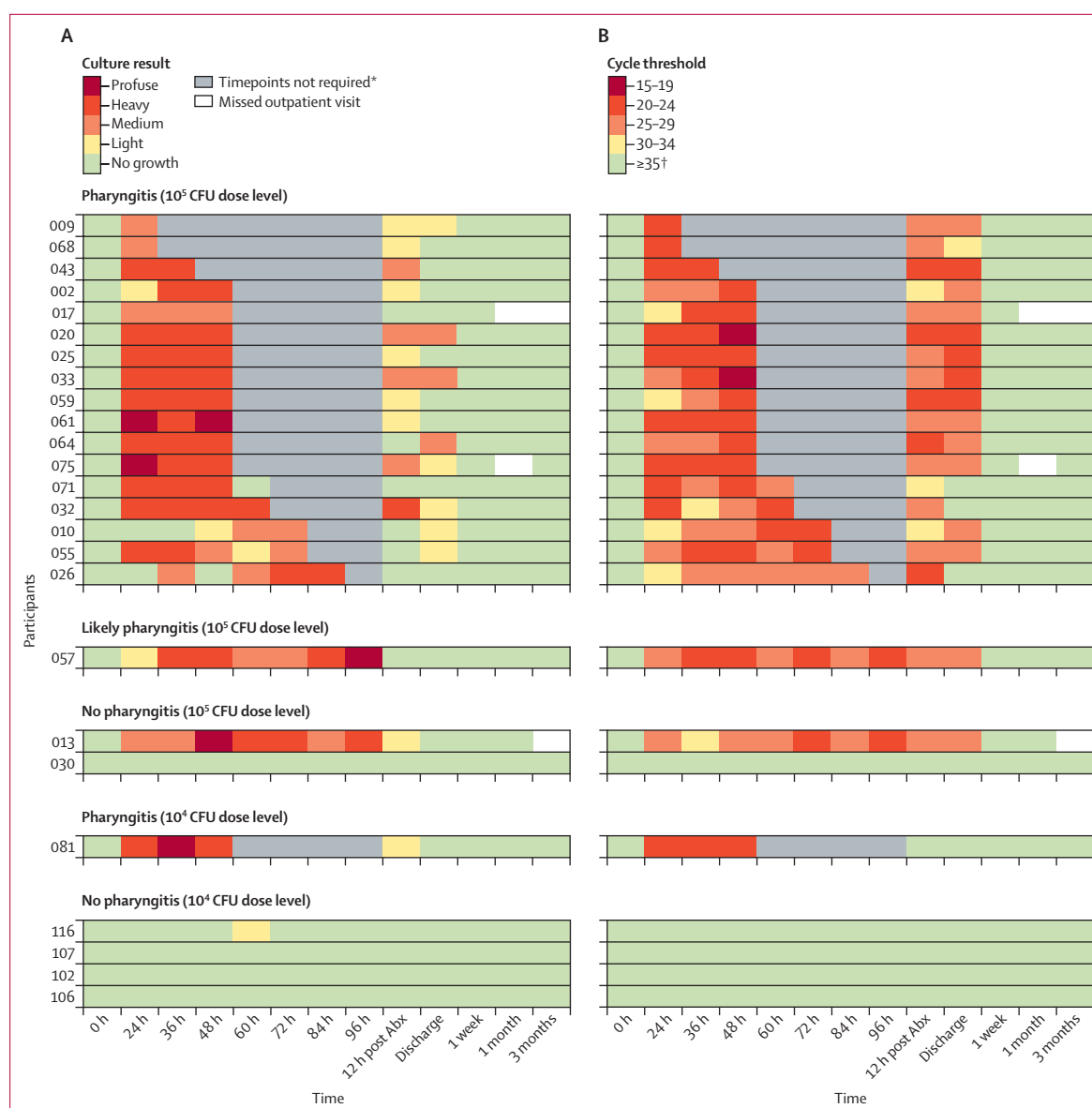


Figure 3: Pharyngeal colonisation by *emm75* *Streptococcus pyogenes* during experimental human pharyngitis

Semiquantitative *S pyogenes* throat swab culture (A) and *emm75* quantitative PCR (B) results from throat swabs collected from 20 participants at the 10^5 CFU dose level and five participants at the 10^4 CFU dose level from challenge (time 0) to discharge, and at outpatient visits approximately 1 week, 1 month, and 3 months afterwards. Abx=antibiotic treatment. CFU=colony-forming units. *Pharyngitis diagnosed, with next swab timepoint 12 h after antibiotics started. †Cycle threshold values of 35 or greater were considered to be a negative reaction.

adverse events. We have established a new controlled human infection model of *S pyogenes* in its only natural host as a platform for evaluating candidate vaccines and novel therapeutics, and for studying host–pathogen interactions.

Participant safety was the guiding principle for our approach to strain selection and protocol development, justified by the reassuring safety findings of this study.^{14,15} We observed a predictable, uncomplicated pharyngitis syndrome, supported by laboratory findings. In the clinic, most of the participants who developed pharyngitis

in our study would be assigned a Centor or McIsaac score of 3 or 4.¹⁸ Exudative pharyngitis and high fever were relatively uncommon, probably due to very early diagnosis, immediate antibiotic treatment, and paracetamol use. As in clinical practice, CRP was elevated in participants with pharyngitis and peak concentrations varied widely.¹⁹ Pharyngitis was characterised by a T-helper-1 cell inflammatory profile, matching previous in-vitro, animal, and human studies.^{20,21}

As previously discussed in detail,¹⁴ there were three *S pyogenes* human infection studies in the 1970s using

similar protocols.^{22–24} Each was a randomised trial of monovalent vaccines for prevention of experimental pharyngitis with homologous strains. Pharyngitis was diagnosed in 44 (52%) of 84 participants in the placebo groups, ranging from 42% to 74% in each study. Vaccine efficacy was as high as 89% in the first study of a parenteral M1 vaccine.²² In our study, 85% of participants were diagnosed with pharyngitis at the starting dose level, 1-log₁₀ lower than in the historical studies (using a similar protocol with different strains),^{22–24} with 4-log₁₀ to 5-log₁₀ fewer bacteria delivered than in non-human primate models using intranasal instillation.^{25,26} With this attack rate in the placebo group, a trial with only 15 participants per group could theoretically show protection conferred by a vaccine with 80% efficacy against pharyngitis, a target listed by WHO-preferred product characteristics for *S pyogenes* vaccines.¹¹ More realistically, future trials will probably follow the successful examples of cholera and typhoid trials in recruiting 25 to 35 participants per group.^{27,28} It is not entirely clear why attack rates were higher in our study than in the historical studies. The most likely explanations relate to intrinsic differences between the strains and differences in their handling from collection through to challenge.

Beyond safety, our study has several strengths, not least that the protocol and single-dose vials add to the model's reproducibility, scalability, and portability. Nonetheless, every model has limitations. First, our pharyngitis model cannot replicate the full strain diversity and clinical spectrum of *S pyogenes* syndromes affecting humans of all ages across all settings. To add generalisability, modifications or extensions might be considered: new strains, a skin infection model, a low-income and middle-income country study, removing the immunological exclusion criterion, and rechallenging participants. However, any changes should increase the model's scientific and strategic usefulness (eg, for vaccine development) without compromising safety. Animal models will remain necessary for studying severe syndromes and interventions with uncertain human safety.^{29–31} Second, *S pyogenes* pharyngitis is not easily defined. There is no objective diagnostic test or specific clinical criteria, and pharyngeal colonisation can be asymptomatic. High fever and exudative pharyngitis might occur more frequently in our model if antibiotic treatment is delayed, as in the 1970s studies. However, the binary pharyngitis outcome will remain most relevant as complications, principally rheumatic fever and heart disease, are not related to pharyngitis severity.¹⁴ As reflected in our study, streptococcal serology using ASO and ADB titres is unreliable and might be attenuated by early antibiotic treatment.³²

The global burden of *S pyogenes* disease is an unmet public health challenge. We have established a clinically relevant controlled human infection model of *S pyogenes* pharyngitis. Our model provides new opportunities to explore *S pyogenes* pathogenesis and the immune response

in its only natural host. Importantly, our model is positioned to make a valuable scientific and strategic contribution to vaccine development. Randomised vaccine-challenge trials could deliver the first human evidence of vaccine protection against *S pyogenes* disease in more than 40 years, building confidence to bridge the gap from preclinical and phase 1 to late-phase field trials.

Contributors

ACS was the chief investigator. JMF and JDL were the study site principal investigators. JO, ACS, PRS, JRC, MFG, JBD, MB, AJP, JSM, MJW, ACC, TS, AG, MP, and CSW designed the study. KIA, LF, HRF, TR-H, MRN, ALW, AG, SJG, CB, MJW, and NJM contributed to work including challenge strain manufacture, assay development, laboratory processing and analysis, reporting echocardiograms, data interpretation, and statistical analysis. JO and ACS drafted the report. All authors critically reviewed and approved the final version. The authors vouch for the integrity and completeness of the data and analyses, and for the fidelity of the study to the protocol. The first and last authors (JO and ACS) had full access to and verified all the data in the study and were responsible for the decision to submit for publication.

Declaration of interests

JBD is the inventor of technologies related to the development of *S pyogenes* vaccines; the University of Tennessee Research Foundation has licensed these technologies to Vaxent, of which JBD is the chief scientific officer and a member. MP and MFG are inventors on patents related to *S pyogenes* vaccines, and Griffith University (Gold Coast, QLD, Australia) has licensed some of these technologies to Olymvox Pharmaceuticals (China). NJM is an inventor on a patent related to *S pyogenes* analytical methods and compositions. MJW has a patent pending related to *S pyogenes* vaccines. All other authors declare no competing interests.

Data sharing

The study protocol is provided in the appendix. Individual participant data will be made available upon requests directed to the corresponding author; after approval of a proposal, data can be shared through a secure online platform.

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