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REVIEW

# Advancing the management and control of typhoid fever: A review of the historical role of human challenge studies

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**Summary** Typhoid infection causes considerable morbidity and mortality worldwide, particularly in settings where lack of clean water and inadequate sanitation facilitate disease spread through faecal–oral transmission. Improved understanding of the pathogenesis, immune control and microbiology of *Salmonella* Typhi infection can help accelerate the development of improved vaccines and diagnostic tests necessary for disease control. *S.* Typhi is a human-restricted pathogen; therefore animal models are limited in their relevance to human infection. During the latter half of the 20th century, induced human infection ("challenge") studies with *S.* Typhi were used effectively to assess quantitatively the human host response to challenge and to measure directly the efficacy of typhoid vaccines in preventing clinical illness. Here, the findings of these historic challenge studies are reviewed, highlighting the pivotal role

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that challenge studies have had in improving our understanding of the host–pathogen interaction, and illustrating issues relevant to modern typhoid challenge model design.


## Introduction

Typhoid fever, caused by systemic infection with *Salmonella* Typhi, affects an estimated 26.9 million people per year, of whom approximately one percent die.[1] Induced, closely monitored human infection ('challenge') studies, in which individuals are deliberately exposed to an infectious agent, arguably provide the most biologically relevant model of infection for human-restricted pathogens including *S.* Typhi.[2–4]

Challenge models of many infectious diseases and pathogens, both enteric (for example, cholera,[5–7] *Shigella*,[8–11] *Escherichia coli*[12–14] and *Campylobacter jejuni*[15]) and non-enteric (for example malaria,[16–19] dengue fever[20,21] and influenza[22–25]) have been developed and successfully used to understand disease pathogenesis and appraise candidate vaccines. Data from these studies has supported phase III trials of promising candidates,[26,27] and in the case of the cholera vaccine CVD 103-HgR, provided sufficient data to allow licensure for use in travellers.[7] Human challenge studies with *S.* Typhi have similarly been used to quantitatively assess the host response to infection and directly measure the efficacy of typhoid vaccines in preventing clinical illness[28–30]; they have also provided unique insights into typhoid pathogenesis.[31,32]

Establishment of a modern typhoid challenge model in the current era of advanced immunological and laboratory techniques could progress markedly our understanding of typhoid host–pathogen interactions. Specifically, challenge studies could provide data relevant to disease transmission and modelling, accelerate the development of improved diagnostic tests, and provide a mechanism for rational selection of early vaccine candidates for further development.

## Historical perspective

Appraising typhoid vaccine efficacy by challenge with virulent *S.* Typhi was first reported by Wright in 1896, who vaccinated two men and then challenged one (who did not subsequently develop illness).[33] In 1904, 14 men ingested a vaccine prepared from the Dorset strain that, despite attempted heat inactivation, remained virulent, with 10 of the participants developing typhoid fever 6–28 days later.[29] This accidental challenge provided the first definitive proof that *S.* Typhi was the causative agent of typhoid fever.[34]

By 1952 efforts to establish a reproducible model of typhoid fever that could be used to assess typhoid vaccines had commenced at the University of Maryland School of Medicine, United States of America.[35] Participants were male volunteers from the Maryland House of Correction, Jessup, Maryland.[36] In 1962 a dedicated Research Ward was built at the penal institution[36,37] and facilities were expanded and improved in 1972. At the time, high risk studies,[38] including challenge studies,[39–41] involving prisoners were common. The Maryland studies gave careful attention to consent and to the principles of the original 1964 Declaration of Helsinki when these were published.[42] Proposed research plans were scrutinised by independent review boards long before it became a compulsory requirement.[28] For study recruitment, inmates were given a brief summary of the research, with interested individuals receiving a second, detailed explanation and written summary of study purpose, procedures, side effects and risks.[43] During the entire period of the challenge study program (1952–1974), only one of 1886 participants requested early withdrawal from study participation.[44] Participant testimony was positive, with participants appreciating the break from routine prison life and the opportunity to contribute to society.[45,46] Ethical concerns about research using vulnerable populations, including prisoners, were raised in the 1970s reflecting a view that prison was an inherently coercive environment and therefore precluded true informed consent. These concerns led to the termination of the typhoid challenge studies in Maryland. Today, the argument has swung back the other way with ethicists arguing that prisoners should not be denied the opportunity to participate in medical research.[47–49]

The first Maryland challenge experiments used wild-type strain Ty2 isolated from an outbreak in Kherson (in modern day Ukraine) in 1918.[50] Two participants ingested $6 \times 10^6$ colony forming units (CFU) of Ty2, but neither developed clinical infection or a serological response.[35] From this inauspicious start, it was hoped that challenge with a more recent isolate given at a higher dose would cause greater infectivity, so further studies using the newly isolated Quailes strain commenced in 1959.[35]

The Quailes strain was a wild-type, Vi-expressing, phage type D-1 strain isolated in 1958 from the gallbladder of Mrs Quailes, a chronic carrier[28]; recent transmission to several family members had confirmed virulence.[35] The Quailes strain was stored at −70 °C in milk, and prior to use in challenge studies, was reconstituted, subcultured for 6 h at 37 °C and harvested before re-suspension in milk ready for ingestion.[28] Challenge with the Quailes strain was successful in inducing clinical typhoid infection, and the pattern of illness seen closely resembled that observed in natural transmission settings.

Between 1959 and1974, six different vaccines (two of which were examined in two different formulations) were investigated in 1886 participants, of whom 762 were challenged with the Quailes strain (Table 1).[43] Investigations into disease pathogenesis and treatment were conducted alongside dose-finding studies and subsequent vaccine studies, providing a wealth of information about typhoid pathogenesis (Table 2).

**Table 1** A summary of findings from previous studies of vaccines with a human challenge model of typhoid fever.

| Vaccine | Finding | Challenge dose | First named author | Year | Reference |
|---|---|---|---|---|---|
| Killed parenteral whole cell vaccine | Following immunisation, one subject was injected with viable *S.* Typhi. Typhoid fever did not develop. | Unknown | Wright | 1896 | 33 |
| Heat treated oral vaccine | 10/13 immunised participants developed *S.* Typhi between 6 and 28 days later, demonstrating Koch's postulates for *S.* Typhi. | Unknown | Tigertt, reported in 1959 | 1904 | 34 |
| Parenteral acetone-killed whole cell vaccines | No protection. | $10^7$ CFU ($ID_{50}$) | Hornick | 1967 | 35 |
| Parenteral acetone-killed whole cell vaccines | 67% efficacy. | $10^5$ CFU ($ID_{25}$) | Hornick | 1967 | 35 |
| Parenteral phenol-heat-inactivated vaccine | No protection | $10^7$ CFU ($ID_{50}$) | Hornick | 1967 | 35 |
| Parenteral phenol-heat-inactivated vaccine | 75% efficacy | $10^5$ CFU ($ID_{25}$) | Hornick | 1967 | 35 |
| Parenteral, denatured Vi polysaccharide | No protection | $10^5$ CFU | Hornick | 1970 | 28 |
| Killed oral vaccine, containing Ty2 organisms inactivated by acetone and freeze drying, in enteric capsules ($100 \times 10^9$ CFU per dose) | 15% efficacy at twice the manufacturer's recommended dose (12 tablets) | $10^5$ CFU | DuPont | 1971 | 32 |
| Freshly harvested attenuated streptomycin-dependent oral vaccine | 66% efficacy | $10^5$ CFU | DuPont | 1970 | 103 |
| Lyophilised attenuated streptomycin-dependent oral vaccine | 19% (non-significant) | $10^5$ CFU | Levine | 1976 | 7 |
| Ty21a grown with exogenous galactose | 87% efficacy | $10^5$ CFU | Gilman | 1977 | 26 |
| Ty21a grown without exogenous galactose | 50% efficacy | $10^5$ CFU | Gilman | 1977 | 26 |

## Insights into the pathophysiology of typhoid fever provided by the early Maryland challenge experiments

### Strain virulence

The majority of wild-type strains, including the Quailes strain, express the virulence (Vi) factor polysaccharide capsule and the H (flagellin) surface antigens. The influence of these antigens on strain pathogenicity was investigated by comparing response to challenge with one of three Vi-expressing wild-type strains (Quailes, Ty2 or Zermatt), a Vi-negative stain (Ty2W) derived from Ty2 or a naturally occurring Vi and H negative strain (O-901) at a dose of $10^7$ CFU.[28] Attack rates with wild-type Vi-positive strains were twice that of Vi-negative strains (51% vs. 26%), confirming the importance of the Vi capsule in virulence.[28] These studies also established unequivocally, that even without expressing Vi, *S.* Typhi could cause typhoid in a quarter of subjects ingesting $10^7$ CFU. Moreover, the clinical severity of resulting illness did not appear to be strain dependent.[28]

The role of the lipopolysaccharide ('O') antigen in the pathogenesis was also investigated extensively.[51,52] Participants challenged intravenously with lipopolysaccharide and later with oral *S.* Typhi showed similar symptomatic responses to both insults; both induced transient anaemia and leucopenia.[51] Participants rendered tolerant to lipopolysaccharide by repeat injection, either prior to or during typhoid infection had no symptom attenuation.[37] Similarly, four participants tolerant to lipospolysaccharide, (thought to have been consequent to repeat exposure from previous intravenous drug use), also demonstrated the fever and toxaemia of typhoid fever.[37] It was concluded that lipopolysaccharide was not the principle mediator of the sustained febrile response in *S.* Typhi infection.

### Infectious dose and clinical illness

Variations in the attack rate and incubation period with different infectious doses were assessed in order to

**Table 2** A summary of findings related to typhoid pathogenesis provided by previous studies using a human challenge model of typhoid fever using *S.* Typhi Quailes strain given in milk.

| Study purpose | Participants | Findings | First author | Year | Reference |
|---|---|---|---|---|---|
| Dose finding studies | 213 males | $ID_{25} = 10^5$ CFU<br>Increasing does increases attack rate and decrease incubation period.<br>Clinical description of typhoid fever. | Hornick | 1970 | 28 |
| Asymptomatic primary bacteraemia description | Case report of 2 participants from vaccine studies | Description of two bacteraemic participants that failed to manifest clinical typhoid fever | Snyder | 1963 | 53 |
| Determine changes in serum complement and properdin levels during acute typhoid fever and subsequent treatment in six participants from vaccine trials. | 6 participants participating in vaccine studies | Complement levels rise and properdin levels fall during acute typhoid fever. | Schubart | 1964 | 62 |
| Determination of histopathological changes in the gut during acute typhoid. | 6 participants in vaccine studies. | Gut biopsies performed on 6 participants with typhoid fever, demonstrating enteritis in acute illness that healed without scarring | Sprinz | 1966 | 31 |
| To examine changes in whole blood amino acids during acute typhoid fever | 17 males participating in vaccine studies. | 9/10 participants who developed typhoid fever had a significant rise in amino acid levels during the incubation period. Illness onset decreased concentration to below baseline. | Feigin | 1968 | 63 |
| Examine effect of parenteral chloramphenicol treatment for typhoid fever. | 24 unvaccinated control participants from vaccine studies. | Parenteral route increased duration of clinical illness. Inferiority to oral route demonstrated. | DuPont | 1970 | 60 |
| Effects of streptomycin pre-treatment | 4 male participants | Allowed typhoid to be induced in one of four participants following challenge with $10^3$ CFU, a dose too low to induce typhoid normally. | Hornick | 1970 | 28 |
| Comparison of response to challenge with different strains of *S.* Typhi | 86 participants given 1 of 5 different strains. | Vi positive strains of *S.* Typhi associated with twice the attack rate of Vi negative strains | Hornick | 1970 | 28 |
| Role of lipopolysaccharide in pathogenesis | Not stated. | Examination and comparison of response to lipopolysaccharide challenge and *S.* Typhi challenge. | Greisman | 1961 | 37,51 |
| | | Participants rendered tolerant to lipopolysaccharide did not have a different clinical course after *S.* Typhi challenge | Hornick | 1970 | |
| Examination of effect of early chloramphenicol therapy in prophylaxis against typhoid fever | 4 participants treated 24 h after challenge, 2 for 7 days and 2 for 28 days. | One participant developed typhoid fever after 7 days of chloramphenicol therapy. All had an immunological response consistent with clinical illness. | Hornick | 1970 | 28 |

Table 2 (*continued*)

| Study purpose | Participants | Findings | First author | Year | Reference |
|---|---|---|---|---|---|
| Rechallenge with homologous strain of *S.* Typhi to determine protection from prior infection | 22 participants challenged up to 12 months prior. 34 control participants in vaccine studies served as comparators. | 23% protection from previous infection | DuPont | 1971 | 32 |

establish a reliable, reproducible model. Between $10^3$ and $10^9$ CFU of *S.* Typhi, suspended in 30—45 ml of milk, gargled and then swallowed by 119 participants.[28,30] The attack rate correlated directly with challenge dose, and incubation period was inversely correlated (Table 3 and Fig. 1). Higher doses were associated with an increased rate of stool excretion of the organism.[28] Using this challenge procedure, the $ID_{50}$ dose was $10^7$ CFU with a median incubation period of 7.5 days, whilst $10^9$ CFU produced a 95% attack rate with a five day median incubation period. The incubation period was highly variable, ranging from 4 to 56 days with the $10^7$ CFU dose.[28] No illness was observed in 13 subjects challenged with $10^3$ CFU.[28]

The clinical illness observed was "typical of that described in naturally occurring typhoid fever".[35,51] Fever, rising in a step-wise fashion over three days, was followed by headache and abdominal pain. Subsequent symptoms often included anorexia, myalgia and fatigue.[28] Illness severity was variable and was not predicted by challenge dose.[28] At one extreme, two of the first 64 participants were bacteraemic but asymptomatic.[53] The first had ingested $10^8$ CFU and was bacteraemic for seven days, with only a mild rise in C-reactive protein (CRP) and a slight headache. The second received $10^9$ CFU and was bacteraemic for one day, but again only experienced a slight rise in CRP. Both participants had moderately high baseline titres to *S.* Typhi O and H antigens.[53] In further challenge studies three additional asymptomatic, afebrile, bacteraemic participants were described, along with five participants that had mild symptoms and no temperature response and six participants who became bacteraemic only after symptoms of up to 17 days duration.[43] This silent' Gram-negative bacteraemia was thought to reflect the organism's ability to hide from the immune system, possibly within the intracellular space, thus limiting activation of the inflammatory response.[28]

**Table 3** The attack rate and incubation period following challenge with different doses of *S.* Typhi Quailes strain given in milk in human challenge studies conducted at the University of Maryland, showing the increase in attack rate and decreasing incubation period with increasing number of organism. Reproduced from Typhoid fever: pathogenesis and immunologic control, R.B Hornick et al., 1970.[28]

| Challenge dose of *S.* Typhi (CFU) | Number of participants challenged | Number diagnosed with typhoid fever (%) | Median incubation period in days (range) |
|---|---|---|---|
| $10^9$ | 42 | 40 (95) | 5 (3—32) |
| $10^8$ | 9 | 8 (89) | |
| $10^7$ | 32 | 16 (50) | 7.5 (4—56) |
| $10^5$ | 116 | 32 (28) | 9 (6—33) |
| $10^3$ | 14 | 0 | |

Disease endpoints definitions in challenge studies are diverse and problematic, particularly in studies of enteric pathogens. A relatively stringent disease endpoint was used in these early challenge studies, requiring an oral temperature of 103 °F (equivalent to 39.4 °C) persisting for 24—36 h.[28] Temperatures were often recorded only once daily during the incubation period, and therefore initial temperature rises (which were more likely to occur in the evening due to diurnal fluctuation) may have been missed.[54] Retrospective analysis of challenge outcomes has demonstrated the marked influence that different diagnostic definitions have on the reported attack rate and illness severity (see Table 4), in part due to the (relatively) small numbers of participants involved.[54]

A less strict definition using a temperature threshold of greater than 100 °F (37.8 °C) for 12 h with a peak of greater than 101 °F (38.3 °C), would have increased reported attack rates from, for example, 26%—41.5% at the $10^5$ CFU dose.[54] Furthermore, with less strict definitions, peak recorded temperature correlated with the log transformed challenge dose and with the number of symptoms and signs, albeit weakly.[54]

Previous military service also influenced observed attack rates. Many participants were U.S. military veterans, in whom vaccination with killed whole-cell typhoid vaccines had been routine since 1911, affecting troops deployed during World War II, the Korean War and the Vietnam War.[55] In a retrospective analysis of participants challenged with $10^5$ CFU, the attack rate in 105 military veterans was 20%, compared to 48% in the 200 participants without military service,[29] representing 58% protective effect from previous military service, presumably consequent to prior vaccination or pathogen exposure.

During these early studies, antibiotic treatment was initiated in participants either diagnosed with typhoid or when clinically indicated.[43] Chloramphenicol, to which the Quailes and other challenge strains were sensitive, was the principle antibiotic used, and its initiation led to defervescence after an average of 3.5 days treatment, and was sometimes associated with the onset of chills and sweats.[28,35] Presumably consequent to the prompt administration of antibiotics, remarkably few participants developed complications.[56] No life-threatening events, deaths or cases of antibiotic resistance occurred.[29] One participant developed

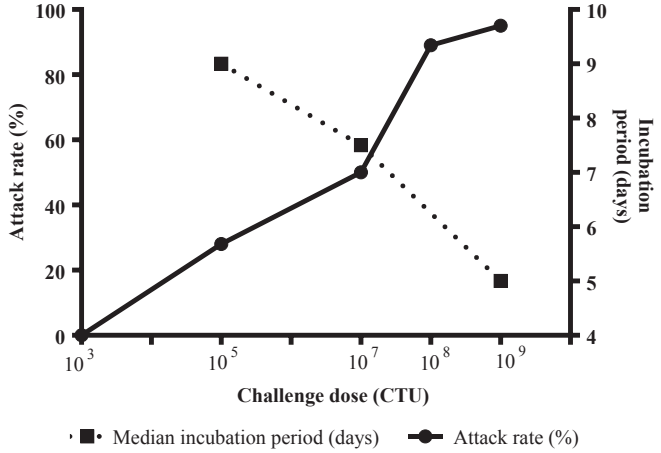


**Figure 1** The attack rate and incubation period following challenge with different doses of S. Typhi Quailes strain given in milk in human challenge studies conducted at the University of Maryland, showing the increase in attack rate and decreasing incubation period with increasing number of organism. Reproduced from Typhoid fever: pathogenesis and immunologic control, R.B Hornick et al., 1970.[28]

mild haemolytic anaemia and another had "several" episodes of temporary confusion,[28] on the background of a previously undisclosed psychiatric history.[43] Chronic carriage of S. Typhi, now recognised to be principally due to sequestration in biofilm associated with gallstones,[57] occurred in one participant with previously undiagnosed gallstones. Carriage was resolved by cholecystectomy; a treatment which was usual practice at that time.[44] Gastrointestinal bleeding is the most feared complication of typhoid fever, the risk of which is now known to increase with treatment delay.[58] One participant experienced bleeding during a relapse of typhoid fever, following an initial, untreated episode.[43] One participant required intravenous fluid replacement for diarrhoea-related dehydration.[43] Two further complications (a pleural effusion and new onset of diabetes) occurred outside the immediate study period and were not thought to be related to challenge.[43]

## Microbiology

Microbiological investigation was limited in the Maryland typhoid challenge studies.[43] Participants challenged with $10^5$ CFU of S. Typhi had at least one blood culture taken during illness (average number of blood cultures per participant, 5.8), mostly during the first 3 days.[43] Overall, bacteraemia was detected in 75% of those with clinical typhoid, peaking in the first couple of days of illness before rapidly declining, even without antibiotic therapy.

Stool samples demonstrated transit of S. Typhi in the immediate post challenge period, with many samples being culture positive during the 48 h after challenge.[28] Early positivity was associated with subsequently developing typhoid fever, with positivity rates of 28% and 30% on days 1 and 2 post challenge, respectively, in those who went on to develop typhoid, compared to 12% and 9% respectively, in those who did not.[43] This association was not absolute however; participants with negative stool cultures often went on to develop typhoid fever and vice versa.[28] Indeed, some participants who remained asymptomatic were noted to excrete S. Typhi in their stools for several weeks.

In those who developed typhoid infection, stool excretion rates fell from day 2 after challenge, reaching a nadir of 6% positivity on day 6, before increasing steadily during the second and third week of incubation, peaking at 61% positivity by day 20.[43] S. Typhi excretion beyond 6 weeks after challenge was extremely rare in both treated and untreated participants. Although excretion rates were significantly higher on each of the 30 days after challenge in those who developed typhoid infection compared to those

**Table 4** The attack rate following challenge with different doses of S. Typhi Quailes strain is variable depending on definition of illness used, reproduced from Glynn et al.[54]

| Challenge dose of S. Typhi (CFU) | Definition of illness[a] | | | |
|---|---|---|---|---|
| | Temperature ≥37.8 °C for 12 h, peak ≥38.3 °C (%) | Antibiotics given for clinical illness | Temperature ≥38.3 °C for 12 h | Temperature ≥39.4 °C for 36 h[b] |
| $10^{8-9}$ | 24/25 (96.0) | 30/34 (88.2) | 24/25 (96.0) | 18/25 (72.0) |
| $10^7$ | 13/27 (48.1) | 13/27 (48.1) | 13/27 (48.1) | 8/27 (29.6) |
| $10^5$ | 83/200 (41.5) | 72/204 (35.3) | 77/200 (38.5) | 52/200 (26.0) |
| $10^3$ | 0/13 (0) | 0/13 (0) | 0/13 (0) | 0/13 (0.0) |

[a] 37.8 °C = 100 °F, 38.3 °C = 101 °F, 39.4 °C = 103 °F, h = hours.
[b] Original definition used.

who did not, the temporal pattern of shedding was comparable between the two groups. Since asymptomatic patients were not treated with antibiotics, it was concluded that this was likely pattern of shedding for naturally occurring infection.[28]

## Disease pathogenesis

Prior to the Maryland challenge studies, the site of S. Typhi invasion was unclear. Participants who gargled and then expectorated milk containing $10^9$ CFU did not develop clinical illness, ruling out the pharynx and tonsils as the site of invasion.[28] It was hypothesised that S. Typhi must be capable of surviving gastric acid exposure, before invading at the intestinal epithelium. Survival of S. Typhi in gastric aspirates for up to 30 min after ingestion was subsequently demonstrated.[28]

Following intestinal invasion, bacterial dissemination to the reticuloendothelial system was thought to occur via a silent primary bacteraemia, followed by a 1—2 weeks incubation period, prior to a symptomatic, secondary bacteraemia. To investigate this hypothesis, four participants were challenged with an $ID_{95}$ dose of S. Typhi and commenced 24 h later on chloramphenicol for either 7 or 28 days. In one of the two participants given a 7-day course, typhoid fever occurred 9 days after finishing antibiotics. This suggested that S. Typhi bacteria must have reached a protected (presumably intracellular) incubation niche within the 24 h prior to antibiotic commencement.[30] In addition, all four participants developed antibody responses identical to those of participants who had not received early antibiotic intervention. These observations indirectly supported the concept of a primary, asymptomatic bacteraemia occurring shortly after organism ingestion.

## Histopathology

Intestinal biopsies were obtained from six participants with typhoid fever following challenge with $1.3 \times 10^9$ CFU S. Typhi.[31] Biopsies were obtained using Crosby-Kugler capsules,[59] prior to challenge, shortly after challenge or as shortly after defervescence as the patient's condition permitted and during the convalescent stage. Biopsy was well tolerated, with no adverse effects reported.[31] Diffuse enteritis affecting the epithelial lining of the villi, the crypt glands and the *tunica propria* occurred prior to the onset of clinical illness and persisted until full recovery. Four of the six participants had specific features of granulomatous enteritis. While there was no relationship seen between the occurrence of enteritis and presence of gastrointestinal symptoms, enteritis severity correlated with overall illness severity.[31] Complete recovery of the gut, without scarring, occurred in all participants including two with mild illness not requiring antibiotics.

## Immunity to typhoid fever

Infection-derived immunity was assessed by re-challenge of 22 participants with blood-culture confirmed typhoid fever within the previous year, with $10^5$ CFU.[32] The attack rate was 23%, marginally less than the 30% observed in typhoid-naïve participants challenged concurrently.[32] Infection-derived immunity was later shown to be significantly less protective than that induced by effective vaccination,[26] suggesting that S. Typhi may be able to modulate and suppress or deflect the immune response in naturally occurring infection.

Antibodies to the surface expressed O, H and Vi antigens of S. Typhi were measured throughout challenge. O and H antibody titres increased soon after ingestion (during the incubation period), while Vi antibody levels did not change significantly (Fig. 2).[60] Baseline H antibody levels appeared moderately predictive of subsequent protection against challenge.[30,61] For example, in studies of an attenuated oral vaccine, unvaccinated controls with a baseline reciprocal H titre of greater than 20 had a 48% attack rate compared to 22% in those who did not.[61] Baseline H antibody titres correlated independently with both participant age and previous military service (Fig. 3). The effect of age on H antibody titre was thought to reflect natural exposure due to the correlation of the reciprocal titre by year of birth with the incidence of naturally occurring typhoid fever in the USA. Participants with previous military service

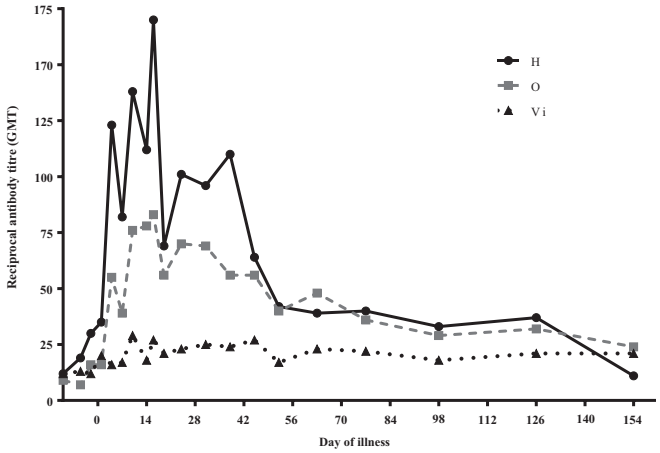


**Figure 2** The kinetics of the total antibody response to the H, O and Vi antigens in participants who developed typhoid fever following challenge with S. Typhi in previous human challenge studies. Reproduced from 'Induced typhoid fever and experimental typhoid vaccines — a study of 1886 participants'.[43]

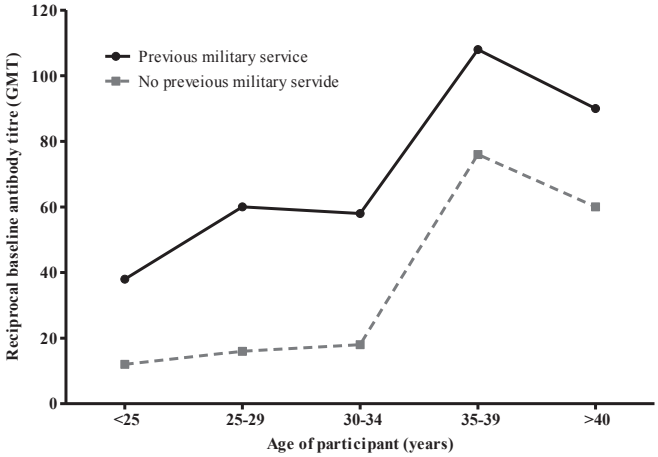


**Figure 3** Variations in baseline antibody levels between participants of different ages, with and without previous military service in previous human challenge studies. Reproduced from 'Induced typhoid fever and experimental typhoid vaccines — a study of 1886 participants'.[43]

had significantly higher H titres without, and were also significantly less likely to develop typhoid.[43] The relative contributions of previous vaccination in the military and natural exposure from deployment are unknown. Although antibodies to the Vi and O antigens were also present at baseline,[62] there was no correlation between baseline antibody levels and subsequent resistance to typhoid fever.[37] In particular, participants with high levels of O antibody from previous lipopolysaccharide challenge were not protected.[37] Clinical illness and relapse (recurrent clinical illness after apparent recovery) occurred at the peak of antibody response.[51,60]

Changes in overall complement levels and properdin (a component of the alternative complement pathway) were examined in 6 participants who underwent challenge.[62] Five of the six participants developed typhoid fever, all of whom were noted to have a rise in total complement,[62] consistent with bacterial infection. Conversely properdin levels fell.[62] This may be due to the role of properdin in stabilising the activating enzymes of the alternative pathway, and/or the direct binding of properdin to damaged or infected mammalian cells.

### Diagnostic tests

With its insidious onset, diverse symptom profile and non-specific signs, typhoid cannot be reliably diagnosed on clinical grounds alone.[58] The challenge studies performed in Maryland provided an opportunity to explore potential diagnostic approaches.

Understanding of the effects of acute infection on metabolism was limited at the time of the early challenge studies, and the carefully controlled nature of these studies provided an ideal opportunity to explore this.[63] The total concentration of whole-blood amino acids following challenge of 17 participants showed that amino acid levels rose during the incubation in nine of the ten participants who subsequently developed typhoid fever, whilst levels in those who did not develop illness stayed the same throughout.[63] With illness onset, whole-blood amino acid levels rapidly fell, falling to below baseline. The potential use of these findings to provide biological markers typhoid disease progression has not been further explored.

*S.* Typhi is excreted in bile, therefore the culture of bile-containing duodenal fluid during acute typhoid infection was appraised in seven challenged participants using string tests.[64] The string test device consisted of a gelatine capsule enclosing a nylon string. After digestion of the gelatin capsule in the stomach, the string uncoiled and passed into the duodenum. Three of the seven participants had *S.* Typhi isolated via this method, two of whom were negative by blood and stool culture at the same time point, suggesting that the device might be a useful adjunct test.[64] The value of duodenal fluid cultures obtained using this method has been corroborated in multiple subsequent field evaluations.[65–67] For example, in a study of 103 children with clinical enteric fever in Chile, a single string test culture was positive in 71%, compared blood culture positivity rate of 61%.[65]

Although not directly appraised in the challenge studies, limitations of the Widal and other serological diagnostic tests, were highlighted by findings from the challenge studies. A fourfold rises in total antibody titres to the O and H antigens, used as the diagnostic threshold for the Widal test, only occurred in 74% and 73% of participants with typhoid[68,69] demonstrating the limited sensitivity of this approach.[70] Variations in baseline antibody titres, as well as increased titres following challenge in participants who did not develop typhoid was also demonstrated,[43] suggesting that a single Widal titre at disease presentation, as is often used in the field,[70] is of limited sensitivity.

## Vaccine appraisal and development using typhoid challenge models

Appraisal of vaccine efficacy using human challenge with *S.* Typhi has existed since the development of the first typhoid vaccines by Wright[33] and independently, by Pfeiffer in 1896.[71] Wright's vaccine was administered to two medical officers, one of whom was then challenged by injection into the flank with "virulent typhoid bacilli".[33,35] Illness did not occur which was taken as evidence of successful

vaccination.[35] These results were used to support the campaign for voluntary vaccination of military personal which became commonplace. In the absence of properly controlled field trials however, true vaccine efficacy remained controversial.[35]

Field trials of killed-whole cell vaccines were conducted in hyper-endemic regions during the 1950s and 1960s. Although vaccine protection was demonstrated, the contribution of chronic, low grade exposure in the enhancement of vaccine efficacy[29] remained unclear. Furthermore, the degree of protection in those from non-endemic areas, not subject to environmental boosting, also remained uncertain. Human challenge studies provided the opportunity for highly controlled vaccine trials to be performed in populations without apparent prior exposure.[29]

## Parenteral vaccines

Two whole cell vaccines were field tested in World Health Organisation (WHO) sponsored trials during the 1950s and 60s[72,73]: vaccine K, killed by acetone inactivation and vaccine L, a heat inactivated and phenol preserved formulation.[35] With a challenge dose of $10^7$ CFU (giving an attack rate of 50% in 30 placebo recipients), vaccine protective efficacy could not be demonstrated; 12/28 (43%) of participants developed typhoid after vaccination with vaccine K, and 13/24 (54%) of those receiving vaccine L.[37] Illness occurred irrespective of pre-vaccination anti-O, -H and -Vi antibody titres, and relapse rates and illness severity were the same in vaccinated and unvaccinated controls.[35,37]

In later studies, the protective efficacy of vaccines K and L and a purified Vi vaccine was investigated using a lower challenge dose of $10^5$ CFU. Seventeen participants were given a single 50 mcg subcutaneous dose of purified Vi vaccine; other vaccinated subjects received 3 subcutaneous doses of K ($n = 43$) or L ($n = 45$) killed whole-cell vaccine.[28] The attack rate in the non-vaccinated controls was 28%, while it was 9%, 7% and 18% in the K, L and Vi groups, respectively. The reduction in attack rate seen in recipients of the K and L vaccines compared to controls was significant (63% and 71% vaccine efficacy), and comparable to that demonstrated in field trials.[30,37] Hence vaccine efficacy appeared to be dependent on the size of the challenge dose, implying that, with the ingestion of a sufficiently high numbers of bacilli, any vaccine-derived immune protection might be overwhelmed.[37] The 25% protective efficacy of Vi vaccine against challenge was not a statistically significant reduction, even at the relatively modest challenge dose used. It was subsequently surmised that the chemical methods utilized in the preparation of this early Vi vaccine would have resulted in a denatured product, perhaps explaining the low efficacy.[74,75]

## Oral typhoid vaccines

Use of parenteral vaccine was limited by high reactogenicity rates and modest efficacy[39,40]; oral vaccination was therefore explored as an alternative.[32] In these studies, the aim of using human challenge models to bridge the gap between theoretical vaccine design and effective end product was finally realised.

The protective efficacy of 'Taboral' (an oral vaccine given as keratine coated tablets each containing $10^{11}$ CFU/dose of acetone-killed Ty2 organisms, given in 6 doses ($n = 35$) or 12 doses ($n = 21$)) was compared to placebo ($n = 34$) using challenge with $10^5$ CFU of *S.* Typhi, 8–10 weeks after vaccination.[32] Six doses were ineffectual (attack rate of 40% ($n = 14$) in vaccinated participants versus 43% ($n = 12$) in controls), but 12 doses provided some protection (attack rate in vaccinated participants of 38% ($n = 13$) versus 54% ($n = 13$) in controls) and decreased overall stool excretion rates from 63% ($n = 15$) to 33% ($n = 7$)[32] but differences did not reach statistical significance. A field trial studying a 3 dose regimen of this vaccine in over 13,000 children in India later confirmed the poor protective efficacy; 60 cases of typhoid fever occurred in the placebo group compared to 44 in the vaccine group,[76] a difference that failed to reach statistical significance.

Between 1970 and 1973, four trials of a streptomycin-dependent live-attenuated oral vaccine in the challenge model were conducted.[61] The trials compared efficacy of freshly harvested versus lyophilised vaccine given in a variable number of doses, with and without concomitant streptomycin, against challenge with $10^5$ CFU *S.* Typhi six weeks later. To neutralise gastric acidity and thereby enhance survival of the live vaccine strain, participants ingested two grams of sodium bicarbonate before ingesting the vaccine contained in 45 ml of milk. Freshly harvested vaccine provided between 66% and 78% protection, and stool excretion decreased from 75% in controls to 17% in vaccinated participants.[61] Lyophilising the live vaccine, considered a prerequisite for commercial manufacture, however negated the protection seen.[61] There was some correlation between rates of seroconversion to the H antigen and subsequent vaccine efficacy; 58% of those receiving fresh vaccine had a fourfold rise in H antibody after vaccination versus 33% in those receiving lyophilised vaccine.

In 1977, the successful trial of another live-attenuated oral vaccine, Ty21a, using the challenge model was published.[26] Ty21a was derived from the wild-type strain Ty2 through non-specific chemical mutagenesis, including inactivation of *galE* (encoding an epimerase), causing toxic accumulation in the bacterial cell of by-products during lipopolysaccharide synthesis. Activity of other Leloir pathway enzymes also diminished, and a separate mutation rendered Ty21a unable to express Vi polysaccharide.

Between five and eight doses of Ty21a (each containing 3-10 $\times$ $10^{10}$ CFU) were given in 45 ml of milk at an interval of three to four days over a four week period. Participants ingested 2 g of sodium bicarbonate in 60 ml of water prior to vaccination. The protective efficacy afforded by vaccine grown with ($n = 100$) and without ($n = 56$) galactose against challenge with $10^5$ CFU *S.* Typhi given 5–9 weeks following completion of the immunization regimen was measured.[26]

Ty21a grown with galactose conferred 87% protective efficacy, significantly decreased excretion of *S.* Typhi after challenge[26] and gave high rates of seroconversion to O antigen. Conversely, Vaccine grown without galactose did not confer significant protection or lead to O antibody seroconversion.[30] Stool excretion of the vaccine strain occurred

in approximately one third of participants for up to 3 days post vaccination, but reversion to wild-type during gastrointestinal passage was not detected in the 958 stool isolates tested.[29] Colonisation of the small intestine by Ty21a was demonstrated in nine participants using nasogastric aspirates obtained six to eight hours after vaccine ingestion. The 87% protective efficacy against challenge was comparable to the efficacy later observed in the first field trial of Ty21a, performed in school children in Alexandria, Egypt. In this trial, children chewed a 1.0 g tablet of sodium bicarbonate, after which they ingested lyophilized vaccine, reconstituted with diluent. Vaccine was given as 3 doses, each containing $10^9$ CFU (one-log fewer CFU than given to adult Maryland volunteers), on alternate days.[77] This regimen conferred 96% protective efficacy over 3 years of follow-up.[77]

Based on robust, long-lived (up to 7 years) efficacy data from multiple field trials that involved the administration of a total of 1.4 million doses[78–80] Ty21a (Vivotif™) went on to become the first and, to-date only, licensed live oral vaccine for the prevention of typhoid infection. In the first three years post licensure, over a million doses of Ty21a were given in the USA alone,[81] and by 2006 150 million doses had been marketed worldwide,[82] providing protection against typhoid fever to vast numbers, a lasting legacy of the Maryland typhoid challenge programme.

## Application of findings from typhoid challenge studies to field settings

The significant advances in understanding and potential for vaccine candidate appraisal using a challenge model of typhoid fever underpines the usefulness of these models. When extrapolating these findings to field settings however it is important to consider the limitations of challenge studies.

The vast majority of typhoid challenge studies have been conducted with the Quailes strain of *S.* Typhi. In a field setting different strains may co-circulate and/or change over time and consequently vaccines must provide cross strain protection. Wild type circulating *S.* Typhi is however clonal in nature and exhibits limited antigenic and genomic variation[83] making it less likely that variations in immune response, pathogenicity or vaccine susceptibility will result from infection with different wild type strains. Recent genetic sequencing has confirmed that the Quailes strain possesses key virulence determinants and has considerable homology with other currently circulating strains.[84]

The number of participants used in vaccine-challenge studies is usually limited and therefore, for adequate power to demonstrate vaccine efficacy, a sufficiently high attack rate is needed, which in turn requires the ingestion of huge numbers of bacteria to produce clinically or microbiologically detectable infection. This may require ingestion of many more bacteria than would be typically encountered in the field. The use of high challenge doses may limit relevance of findings to naturally occurring infection and may overwhelm vaccine protective efficacy with consequent inappropriate rejection of candidate vaccines.[35] The use of relatively modest numbers of participants also means that the observed attack rates in challenge models are sensitive to individual variation in disease susceptibility and to the disease endpoint definitions.[54] Careful participant selection helps negate the effects of individual variation but in turn limit applicability to some populations.[85] Hence, even when efficacy is demonstrated in vaccine-challenge experiments, this may not correspond to protection in target populations exposed to natural infection in the wild.[86]

## The potential of a 21st century challenge model of typhoid fever?

Human challenge with *S.* Typhi has not been conducted since the termination of the University of Maryland programme in 1974. Since this time significant advances have been made in the understanding of, and technologies for, investigating immunobiology, host–pathogen interactions and diagnostic biomarkers. Parallel and equally significant advances have been made in clinical trial methodology and specifically in the bioethical principles underpinning such high profile and potentially high-risk research.

Typhoid fever occurs in the most impoverished and neglected parts of society.[87–89] Costly changes to infrastructure and living standards are occurring and can have a significant effect on typhoid disease burden; but these changes are happening at a discouraging pace and will always be vulnerable to political and civil unrest and neglect.[90] In the interim, safe, effective, novel vaccines that can be used in young children and confer enduring protection, could offer a cost-effective way to diminish the disease burden in endemic areas.

The development of any new vaccine is a long, costly and challenging process, and many candidate vaccines fail on the path to licensure, not least at the later stages in which true population protection is assessed.[91] Limited understanding of typhoid immunobiology, and, in particular, the absence of a correlate of protection that could be used in efficacy trials is problematic for typhoid vaccine development.[91] Similarly, sensitive and specific novel diagnostic tests that are of utility in endemic settings are needed,[92] but their development and validation has been hindered by the lack of an antigen-naïve patient cohort.[68] Accurate data on the microbiological and pathological response to *S.* Typhi ingestion are also needed to inform transmission and vaccine impact modelling to inform decision making for vaccination programmes.

Re-establishing a challenge model of typhoid infection could help overcome these limitations. Promising novel typhoid vaccine candidates are at various stages of development.[93] Safety and immunogenicity of both the live attenuated oral vaccine M01ZH09[94,95] and the live attenuated, Vi expressing strain CVD 909[96–98] has been shown. A variety of Vi-conjugate vaccines have been developed by a number of manufacturers, and efficacy studies of the most advanced of these vaccines have been encouraging.[99,100] Demonstrating novel vaccine efficacy against challenge could accelerate the development of these vaccines by serving as a bridge between immunogenicity trials and phase III efficacy, as occurred with Ty21a, saving time and money and ensuring that only the most promising

candidates are evaluated in large-scale pre-licensure efficacy field trials. As was seen in Maryland, new discoveries and trial methodology relating to one enteric pathogen can often be applied to related genera.[8,11,12,101,102]

## Conclusions

Typhoid fever remains a major cause of morbidity and mortality in many developing countries. Pioneering investigators in Maryland used a novel approach in developing a safe human challenge model of typhoid infection, thereby significantly advancing our understanding of *S.* Typhi infection and responses to vaccination.

These studies have provided a wealth of data on participant response and safety. Fifty years later, a further paradigm shift in our understanding of disease pathogenesis and host response is required in order to advance typhoid control. Combining clinical and experimental data from early typhoid challenge studies with the latest advances in laboratory science will allow the development of a modern typhoid challenge model that will assist global efforts to control typhoid fever.

## Search strategy and selection criteria

Relevant articles, published in English, Spanish, and German before April 2013, were identified by searching PubMed using the MeSH terms "S. Typhi", "*Salmonella* Typhi", or "typhoid", followed by "controlled human infection", "challenge", "human challenge", or "model". Relevant references cited in these articles, or highlighted by experts in enteric challenge studies were reviewed. Reports from the authors of the early challenge models were included. Studies in animal models were excluded.

## Conflicts of interest declaration

AJP conducts clinical trials on behalf of the University of Oxford, sponsored by vaccine manufacturers but does not receive personal payments from them; unrestricted grants for support of educational activities are paid to an educational/administrative fund held by the Department of Paediatrics, University of Oxford. MML is co-inventor of a single-dose live oral typhoid vaccine strain, CVD 909, and a live oral *S.* Paratyphi A vaccine strain, CVD 1902. Both of these vaccines have been licensed for developing country commercialization rights by Bharat Biotech International of Hyderabad, India.

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