Epidemiol. Infect. (2013), **141**, 223–232.
doi:10.1017/S0950268812001677

SYSTEMATIC REVIEW
The *Shigella* human challenge model

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Received 7 March 2012; Final revision 11 June 2012; Accepted 11 July 2012; first published online 21 August 2012

SUMMARY

Shigella is an important bacterial cause of infectious diarrhoea globally. The *Shigella* human challenge model has been used since 1946 for a variety of objectives including understanding disease pathogenesis, human immune responses and allowing for an early assessment of vaccine efficacy. A systematic review of the literature regarding experimental shigellosis in human subjects was conducted. Summative estimates were calculated by strain and dose. While a total of 19 studies evaluating nine strains at doses ranging from 10 to 1×10^{10} colony-forming units were identified, most studies utilized the *S. sonnei* strain 53G and the *S. flexneri* strain 2457T. Inoculum solution and pre-inoculation buffering has varied over time although diarrhoea attack rates do not appear to increase above 75–80 %, and dysentery rates remain fairly constant, highlighting the need for additional dose-ranging studies. Expansion of the model to include additional strains from different serotypes will elucidate serotype and strain-specific outcome variability.

Key words: Gastrointestinal infections, *Shigella*, travellers' infection.

INTRODUCTION

Infection with *Shigella* causes bacillary dysentery and has been recognized as a major cause of inflammatory diarrhoeal disease in endemic regions. The low infective dose and faecal–oral route of transmission facilitates spread through contaminated food and water and personal contact. *Shigella* infections remain problematical for young children in endemic regions, travellers and deployed military personnel [1, 2]. Since 1946, the *Shigella* human challenge model has been used to investigate pathogenesis and host immune responses induced by *Shigella* infection and to evaluate the efficacy of investigational products. In the inaugural study, subjects were challenged with *Shigella paradysenteriae* (Flexner W) strain in a series of dose-finding and vaccine efficacy studies [3]. While early preclinical data on these vaccines were promising, they failed to protect against shigellosis following challenge. Despite the lack of protective efficacy, this inaugural study paved the way for future development and utilization of the *Shigella* human challenge model.

Shigella is categorized into four species, each further classified into one or more serotypes: *S. dysenteriae* (17 serotypes), responsible for epidemic outbreaks;

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S. flexneri (14 serotypes), more common in the developing world; *S. sonnei* (one serotype), primarily found in developed countries; *S. boydii*, relatively rare and found mostly in the Indian subcontinent [2]. Presently, there is a lack of pathogen specific clinical data from field studies.

The purpose of this study was to conduct a systematic review of the published literature on experimental human shigellosis enabling an analysis of study-specific factors and their associations with clinical outcomes. This type of review can be an important resource for researchers considering experimental human challenge studies. Specific to human challenge models, continual review of the published literature is important to minimize risk to future study participants and to ensure the safe application of the experimental infection for evaluating novel study hypotheses.

METHODS

A systematic review of the published literature was conducted to examine specific outcomes in human subjects participating in experimental *Shigella* infection studies. A Pubmed database search was performed to identify all articles for potential inclusion. Searches began with the term '*Shigella*', and were followed by one of the following: 'challenge' or 'human challenge', 'efficacy' or 'vaccine efficacy', and 'model'. Only experimental infection studies in which human subjects received live, wild-type *Shigella* strains were included. Subjects receiving investigational products prior to, or during experimental infection were excluded. The search was limited to studies conducted in *Shigella* non-endemic regions and published in the English language prior to June 2010. Studies were also identified through a detailed review of references and through discussion with experts in the field.

Bibliographical information, inoculum and strain information, subject demographics, specific clinical outcomes, and immunogenicity data were indentified in the included studies and entered into a pre-tested Microsoft Access (Microsoft Inc., USA) database. Heterogeneity was assessed using a χ^2 heterogeneity statistic, and potential sources of heterogeneity were assessed graphically by Forest plots and using non-parametric methods (e.g. Kruskal–Wallis, Mann–Whitney *U* test) to compare differences in incidence between two or more groups of a given study characteristic. In the case of parameters where only a few studies were found, a median and range of estimates were reported. For summarization purposes, point estimates and standard 95% confidence intervals were combined using a random-effects model with methodology developed by DerSimonian & Laird [4]. As the principal purpose of this systematic review was to summarize studies reporting diarrhoea and dysentery incidence following experimental infection, publication bias was not assessed; as such, the concern for non-published findings due to negative studies or disappointing results was considered minimal. Individual study characteristics evaluated included strain and quantity of inoculum administered and inoculum administration procedures. These were assessed in relation to their effect on multiple outcomes such as diarrhoeal attack rates and disease severity. Statistical analyses were conducted using SAS version 8.2 (SAS Institute Inc., USA).

RESULTS

A total of 568 citations of published literature on experimental *Shigella* infection were identified through the Pubmed database search yielding a total of 18 individual studies and 47 dose/strain combinations for entry into a Microsoft Access database (Fig. 1). Because one reference included a review of a previous studies, but contained data of greater relevance than the original, only the review was included. A complete listing of all included studies is shown in Table 1.

The majority ($n=11$, 61%) of the studies were conducted between 1989 and 1999. Additionally, a majority ($n=10$, 55%) of the studies were conducted to evaluate vaccine efficacy, although one of the studies was also an infective dose-finding study [3]. Most of the remaining studies were performed to describe strain/dose pathogenesis ($n=6$, 33%), or evaluate the protection conferred by an oral antibiotic ($n=1$, 6%) or prophylactic ($n=1$, 6%). An additional study was conducted to assess the cross-reactivity of *S. flexneri* 2a antibodies generated subsequent to challenge [5].

Strains from three *Shigella* species were used in these studies: *S. flexneri*, *S. sonnei*, and *S. dysenteriae*. The following strains were used: 2457T (*S. flexneri* 2a), 53G (*S. sonnei*), M-131 and A-1 (*S. dysenteriae* 1), and five Flexner W (previous designation for *S. flexneri* 2a) strains, FW I, FW II, FW III, FW IV, and FW V. Summary information about each strain is provided in Table 2. The two most commonly used strains, 2457T and 53G, were included in 94% of the

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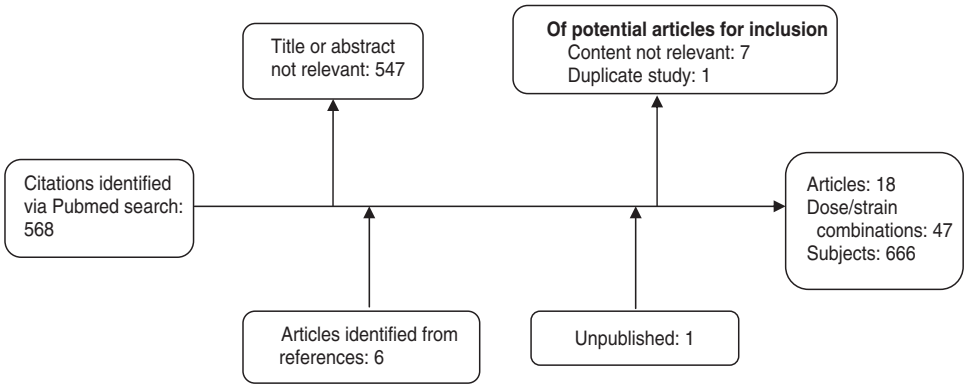

Fig. 1. Flow diagram for studies included in the systematic review.

published studies. The diarrhoea and dysentery rates following inoculation with various doses of those two strains are shown in Figure 2.

Several studies ($n=7$, 37%) reported the use of a sodium bicarbonate ($NaHCO_3$) pre-treatment prior to inoculation, although a considerable number ($n=11$, 58%) did not report any pre-treatment. The studies by Shaughnessy *et al*. in 1946, used water as the inoculum vehicle [3]. However, the majority ($n=12$, 63%) of studies, and nearly all before 1995, administered the challenge strain in milk. Subsequently, sodium bicarbonate has become the inoculum vehicle ($n=5$, 26%) following the 1995 study which found that this buffer yielded a higher and more consistent attack rate than milk [6].

A consistent definition for the primary clinical outcome, i.e. diarrhoea, was not used until the study published by Black *et al*. in 1987 [7]. Thereafter, excluding two studies, the definition for diarrhoea was most commonly ($n=9$, 50%) one loose or liquid stool (LLS) $\geqslant$300 ml or $\geqslant$2 LLS $\geqslant$200 ml over 48 h. The 1946 study by Shaughnessy *et al*. did not report a definition for diarrhoea, as dysentery was the primary clinical outcome [3]. Additional studies by Taylor *et al*. and Van de Verg *et al*. similarly failed to include diarrhoea definitions [5, 8]. For the remaining studies, most definitions were for LLS within 24 h: $\geqslant$3 LLS (11%), 2 or 3 LLS (11%), $\geqslant$4 LLS (6%), and $\geqslant$2 LLS or 1 LLS $\geqslant$300 ml (6%).

We found a significantly ($P<0{\cdot}05$) higher diarrhoea attack rate in studies of strain 2457T in which the inoculum was administered in $NaHCO_3$ [67%, 95% confidence interval (CI) 52–81] compared to studies utilizing milk (42%, 95% CI 32–53). Rates of dysentery were similar between inoculum vehicles. Fasting prior to inoculation may be associated with higher attack rates, although data are insufficient.

Excluding strain 2457T, a small range of doses has been evaluated for each strain. Despite no statistically significant dose response for diarrhoea or dysentery, a borderline positive association ($P=0{\cdot}1$) was noted between dysentery rates and dose at doses $\leqslant$1400 colony-forming units (c.f.u.) for strain 2457T. However, the rate of dysentery following administration of 2457T (41%, 95% CI 30–52) was lower than the other tested strains (56%, 95% CI 48–65).

Homologous protection has been assessed in three separate studies of two strains (2457T and 53G) [6, 9, 10]. The inaugural study by DuPont *et al*. showed a 64% reduction in disease in subjects receiving a second inoculum of 10^4 organisms of *S. flexneri* strain 2457T administered in skim milk [9]. Similar results were observed by Kotloff *et al*. following a second inoculation of 10^3 organisms of 2457T administered in $NaHCO_3$ [6]. A single study has assessed homologous protection using the *S. sonnei* strain 53G and shown 100% protection in subjects who received a prior dose (400 organisms) of the inoculum compared to 67% with disease in naive participants [10].

DISCUSSION

Since its inception, the *Shigella* human challenge model has been useful for researchers seeking to understand the disease pathogenesis, clinical manifestations, and immune responses associated with *Shigella* infection. Its utility has also been borne out in assessing the efficacy of vaccine candidates and antibiotics or other prophylactic products used to control bacterial infections. However, significant variability in study protocols including the methods utilized to administer the challenge inoculum, the outcome definitions and variable challenge strains confound result interpretation.

https://doi.org/10.1017/S0950268812001677 Published online by Cambridge University Press

Table 1. *List of experimental Shigella infection studies that met inclusion criteria for systematic review and meta-analysis*

Ref.	Pub. year	First-named author	Study type*	$NaHCO_3$ buffer	Inoculum solution	Diarrhoea definition†	Strain	Dose (c.f.u.)	*N*	*N* (%) diarrhoea	*N* (%) dysentery	*N* (%) fever	Mean no. LLS (volume)	Mean incub. (h)	Mean duration	% colonized	Immune response
[8]	2006	Taylor	C	Yes	NaHCO3	A	2457T	1.5×10^3	10	8 (80)	1 (10)	4 (40)	2 (0.3 l)	78.5		50	
[13]	1999	Coster	B	Yes	NaHCO3	A	2457T	1×10^3	15	6 (40)	4 (27)	6 (40)	11			40	ASC (LPS) –IgG: 40 % –IgA: 40 %
[5]	1996	Van de Verg	D	NR	NR	NR	2457T	1×10^3	14	NR	NR						Serum (LPS) –IgG: 50 % –IgA: 65 %
[14]	1995	Kotloff	B	Yes	$NaHCO_3$	A	2457T	1×10^3	14	9 (64)	9 (64)	12 (86)				93	Serum (LPS) –IgG: 50 % –IgA: 79 % ASC (LPS) –IgA:100 % –IgG:93 %
[6]	1995	Kotloff	A	Yes	$NaHCO_3$	A	2457T	1.4×10^3	12	10 (83)	10 (83)					100	
[24]	1995	Munoz	A	NR	Milk	A	53G	5×10^2	11	6 (55)	7 (64)			43.9		82	ASC (LPS) –IgA: 72 %
[25]	1992	Tacket	C	Yes	Milk	A	2457T	1.2×10^3	7	1 (14)	3 (43)	4 (57)	(3.4 l)		3 days		Serum (LPS) –IgG: 43 % –IgA: 29 % ASC (LPS) –IgG: 57 % –IgA: 71 %
								1.6×10^3	11	4 (36)	4 (36)	4 (36)	(0.8 l)		2.6 days	91	Serum (LPS) IgG: 45 % IgA: 82 % ASC (LPS) –IgG: 64 % –IgA: 73 %
[26]	1992	Kotloff	B	NR	Milk	A	2457T	1×10^3	21	8 (38)	7 (33)	8 (38)				48	
[27]	1992	Mackowiak	B	NR	Milk	A	53G	5×10^2	85	40 (47)	NR	33 (39)				54	
[28]	1990	Van de Verg	B	NR	Milk	NR	53G	5×10^2	12	5 (42)	NR						Serum (O Ag) –IgA: 50 % ASC (O Ag) –IgA: 50 %
[10]	1990	Herrington	B	NR	Milk	A	53G	4×10^2	12	7 (58)	8 (67)	6 (50)					
[29]	1989	DuPont‡	A	No	Milk	D	A-1	2×10^2	8	3 (38)	NR	3 (38)		144			
				No		D	A-1	1×10^4	6	2 (33)	NR	2 (33)		72			
				No		D	M-131	10	10	1 (10)	NR	1 (10)		36			
				No		D	M-131	2×10^2	4	2 (50)	NR	2 (50)		72			
				No		D	M-131	2×10^3	10	7 (70)	NR	7 (70)		146.4			
				No		D	M-131	1×10^4	6	5 (83)	NR	5 (83)		124.8			
				NR		C	53G	5×10^2	20	7 (35)	NR	7 (35)					

Table 1 (*cont.*)

Ref.	Pub. year	First-named author	Study type*	$NaHCO_3$ buffer	Inoculum solution	Diarrhoea definition†	Strain	Dose (c.f.u.)	*N*	*N* (%) diarrhoea	*N* (%) dysentery	*N* (%) fever	Mean no. LLS (volume)	Mean incub. (h)	Mean duration	% colonized	Immune response
[7]	1987	Black	B	NR	Milk	A	53G	5×10^2	38	20 (53)	19 (50)	20 (53)	9.2 (1.3 ml)				Serum (O Ag) –IgG: 45 % –IgA: 68 %
[30]	1977	Levine	B	NR	Milk	D	2457T	1×10^2	36	14 (39)	8 (22)	7 (19)					Serum (LPS) –HA: 22 %
								1×10^4	15	5 (33)	5 (33)	3 (20)				60	Serum (LPS) –HA: 60 %
[9]	1972	DuPont	B	NR	Milk	E	2457T	1×10^4	88	52 (59)	NR	52 (59)				75	Serum (LPS) –HA: 44 %
								1.8×10^2	36	9 (22)	NR	9 (22)				17	Serum (LPS) –HA: 27 %
								1×10^3	24	14 (58)	NR	14 (58)				63	Serum (LPS) –HA: 50 %
								5×10^3	49	28 (57)	NR	49 (28)				67	Serum (LPS) –HA: 49 %
[31]	1969	DuPont	A	NR	Milk	C	2457T	1×10^4 to 1×10^8	43	27 (63)	25 (58)	13 (30)		96	168 h	67	
[3]	1946	Shaughnessy	A	Yes	Milk	NR	FW I	1×10^8	4	0 (0)	NR					100	
					Milk		FW I	1×10^9	4	1 (25)	NR	0				100	
					Milk		FW I	1×10^{10}	4	2 (50)	NR	0				100	
					Water		FW I	1×10^{10}	4	2 (50)	NR	0				100	
					Water		FW II	6.25×10^8	3	2 (67)	2 (67)	2 (67)					
					Water		FW III	6.25×10^8	3	2 (67)	2 (67)	2 (67)					
					Water		FW IV	6.25×10^8	3	2 (67)	2 (67)	2 (67)					
					Water		FW V	6.25×10^8	3	2 (67)	2 (67)	2 (67)					
			B	Yes	Water		FW II	6.25×10^8	20	14 (70)	15 (75)	15 (75)					
							FW II	2.5×10^8	5	NR	2 (40)	2 (40)					
							FW II	1×10^9	5	NR	2 (40)	2 (40)					
							FW III	6.25×10^8	20	14 (70)	15 (75)	15 (75)					
							FW III	2.5×10^8	5	NR	2 (40)	2 (40)					
							FW III	1×10^9	5	NR	2 (40)	2 (40)					
							FW IV	6.25×10^8	20	14 (70)	15 (75)	15 (75)					
							FW IV	2.5×10^8	5	NR	2 (40)	2 (40)					
							FW IV	1×10^9	5	NR	2 (40)	2 (40)					
							FW V	6.25×10^8	20	14 (70)	15 (75)	15 (75)					
							FW V	2.5×10^8	5	NR	2 (40)	2 (40)					
							FW V	1×10^9	5	NR	2 (40)	2 (40)					

NA, Not applicable; NR, not reported, abx, antibiotic; HA, humoral antibody determined by haemagglutination; incub., incubation; LPS, lipopolysaccharide.

* Study types: A, pathogenesis; B, vaccine efficacy; C, treatment or prophylaxis; D, other.

† Diarrhoea definitions: A, 1 loose or liquid stool (LLS) $\geqslant$300 ml or $\geqslant$2 LLS $\geqslant$200 ml over 48 h; B, $\geqslant$2 LLS in 24 h or 1 LLS $\geqslant$300 ml; C, 2 or 3 LLS in 24 h; D, $\geqslant$3 LLS in 24 h; E $\geqslant$4 loose stools in 24 h.

‡ Data on strains A-1 and M-131 in the DuPont 1989 study was previously reported in Levine *et al.* [32].

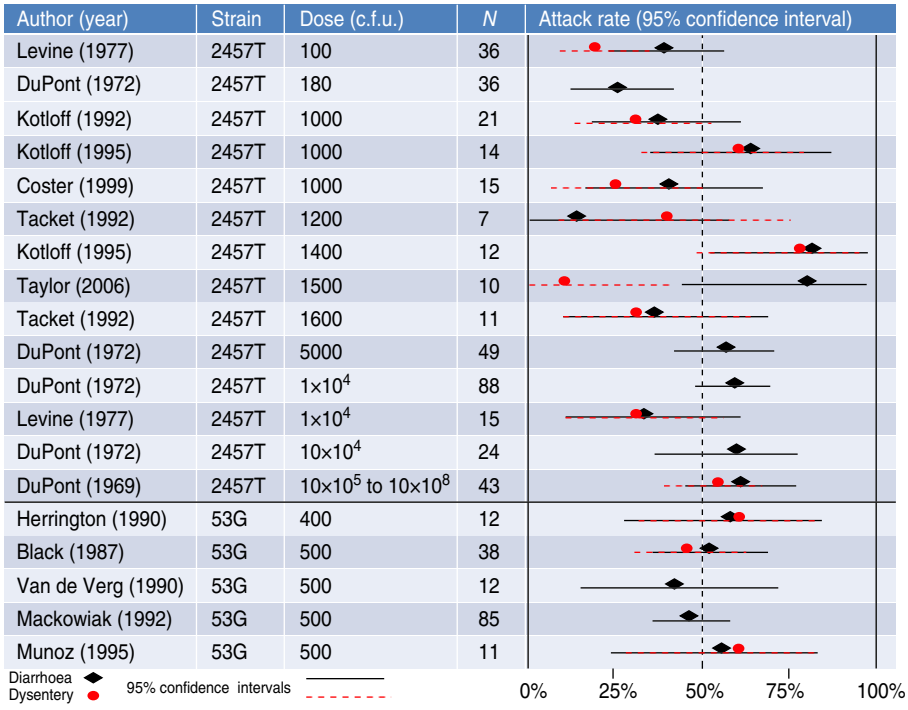

Fig. 2 [*colour online*]. Diarrhoea and dysentery rates (with 95% confidence intervals) for experimental infections with *Shigella flexneri* strain 2457T and *Shigella sonnei* strain 53G.

A major finding of this study is that diarrhoea attack rates appear somewhat dose-related but do not appear to increase above 75–80% with the two most commonly studied strains, 2457T and 53G. Higher doses generally did not result in higher diarrhoea or dysentery attack rates, nor are they representative of natural infection. Doses tested ranged from 10 to 1×10^{10} c.f.u., with doses as high as 1×10^4 to 1×10^8 tested for strain 2457T, even though as little as 10–100 organisms are needed to cause infection in the field [11, 12]. The dose range tested for the *S. paradysenteriae* (*flexneri*) strains from the inaugural 1946 study reached 1×10^9 to 1×10^{10} organisms, the highest doses tested for all studies; even this high dosage, however, failed to produce significant virulence in humans [3]. Unfortunately, detailed information on the origin or processing of these strains is unavailable. Recent studies have begun reporting rates of shigellosis which are further categorized by diarrhoea, vomiting and fever. No clear dose response for fever or dysentery is observable from the published results.

Only three studies to date have assessed homologous protection following an initial infection with an experimental challenge inoculum with clear evidence of a reduction in disease risk upon a second exposure [6, 9, 10]. There appears to be inherent variability in the induction of protective immune responses across the studied strains; however, more studies are needed to confirm this variability. Importantly, low attack rates in naive participants increase the sample size requirements to ensure such studies are adequately powered.

Despite a significant amount of effort to develop immune correlates of protection, data are lacking and limited to vaccination challenge studies where only a small number of immune parameters have been measured. A study in 1987 by Black *et al.* [7] identified a reduction in disease outcomes following challenge with *S. sonnei* strain 53G in subjects with prechallenge immune titres ($\geqslant 40$) against the O antigen of *S. sonnei*. Most recently, studies by Kotloff *et al.* and Coster *et al.* indicate a similar reduction in disease risk in subjects with anti-lipopolysaccharide (LPS) antibody-secreting cells circulating in peripheral blood [13, 14]. Despite these data, evidence of immune correlates against naturally occurring shigellosis is more limited. Serotype-specific immunity is supported by epidemiological studies as well [15]. However, only Cohen *et al.* identified a specific immune parameter (anti-LPS serum antibodies) associated with significant protection from naturally occurring disease [16].

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Table 2. *Strains of Shigella utilized in experimental human infection*

Species	Sero-type	Strain	Initial strain description	Country of origin	No. of subjects	Clinical information on index case	Dose (c.f.u.)
S. flexneri	2a	2457T	[31]	Japan	407	Isolated from a patient with clinical illness in Japan in early 1950s	8×10^2 to 1.6×10^3
S. sonnei	–	53G	[29]	Japan	178	Isolated from 5 year-old boy who had experienced diarrhoea for 2 days in a Tokyo, Japan hospital in August 1954	4×10^2 to 5×10^2
S. dysenteriae	1	M-131	[32]	Guatemala	30	Isolated from patient with severe dysentery acquired during pandemic of 1970 in Guatemala	2×10^2 to 1×10^4
S. dysenteriae	1	A-1	[32]	Guatemala	14	Isolated from patient with mild disease in Guatemala in late 1960s, early in pandemic	10 to 1×10^4
S. paradysenteriae (*S. flexneri*)	2a	FW I, FW II, FW III, FW IV, FW V	[3]	–	49	–	1×10^8 to 1×10^{10}

Increased dose-ranging for a variety of strains is needed in future studies. The greatest range of doses has been tested for strain 2457T, in which doses have ranged from 100 to 1×10^8. In comparison, the second most commonly studied strain (*S. sonnei*, 53G) has only reported inoculum doses ranging between 400 and 500 organisms, with relatively low diarrhoea and dysentery attack rates. Future studies should also focus on the development of models for other *Shigella* strains. Specifically, current vaccine development efforts have identified *S. flexneri* 2a, 3a, 6 and *S. sonnei* as likely targets that would provide broad-based coverage to travel and endemic populations [17]. Presently, there are no challenge models for either *S. flexneri* 3a or 6. Given the unpredictability of species-specific disease incidence in field settings, the human challenge is likely to play a significant role in vaccine development and highlights the need to expand efforts on *Shigella* challenge model development [17].

One underappreciated concern with the utilization of the human challenge model is the need for adequate sample sizes to ensure adequate confidence around estimates of disease outcomes. The confidence interval around a single estimate for disease decreases as sample sizes increase, but remains about 10% with as many as 60 subjects at a given inoculum dose. This is critical for adequately powering subsequent studies of vaccine efficacy. For example, assuming an 80% attack rate at a fixed inoculum dose in 60 people, the 95% CI is 70–90%. Presuming an efficacy study was adequately powered (80%) to detect 70% reduction in disease, total sample size requirements would range from 40 to 24 presuming a 70% or 90% placebo attack rate, respectively (assuming a 1:1 randomization).

Other challenges also complicate the development and utilization of these models. First, unlike challenge models with malaria, these studies are required to be conducted in an inpatient setting where appropriate oral and intravenous hydration fluids are readily available. Additionally, closed or treated water systems are often required to prevent release or spread of the experimental organism into the environment.

Other limitations include variability in challenge seed inoculum. Dose preparation is traditionally performed by preparing a bacterial suspension to a fixed optical density, a method with inherent imprecision, especially at low inoculum doses. Quantification of the number of organisms administered then relies on post-dose colony counts. One potential improvement

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in this area may be to include a more rapid technique for quantifying viable bacteria in the challenge inoculum. Implementation of a flow cytometry-based assay for viable bacteria may help ensure accurate target inocula are more frequently administered. Another approach could involve using lyophilized challenge inocula to more accurately estimate dose.

All clinical trials must weigh the ethical dilemmas involved with placing human subjects at risk against the potential benefit of a future drug, vaccine or other product designed to treat or prevent disease. This is no difference in experimental *Shigella* infections, which are used in drug and vaccine efficacy studies, but due to the unique aspects of experimental infection, challenge key aspects of this paradigm. The ethical framework developed by Miller & Grady offers a structure by which to evaluate experimental infection studies and can serve as a guide for future *Shigella* infection research [18]. These authors highlight seven ethical issues (rationale, risks, discomforts, vulnerable subjects, informed consent, financial compensation, and right to withdraw) that should be used to evaluate proposed studies and the application of several of these issues in relation to past and potentially future experimental *Shigella* challenge studies is warranted. One historical issue is that of the use of vulnerable subjects. Initial studies were conducted in inmates at local prisons, a vulnerable population that is unlikely to be utilized for future experimental infection studies. At present, however, factors associated with ensuring ongoing informed consent, financial compensation and the subjects' rights to withdraw seem no more intricate in human challenge studies than in other research in which subjects receive an experimental product.

An area that is an increasing topic of discussion related to the ethics of human challenge studies is that of risks and discomforts. While *Shigella*-associated diarrhoea in healthy adults is generally self-limiting and easily treatable in a controlled environment, there are known long-term health outcomes that can result from shigellosis. The most well-described of these sequelae is reactive arthritis (ReA), a sterile joint inflammation that occurs subsequent to a preceding infection [19]. The mechanisms by which a distal infection results in joint inflammation are largely unknown, although there appear to be genetic factors, specifically HLA-B27 predisposing some individuals to an increased risk. Efforts to exclude HLA-B27–positive subjects from experimental infection studies have been made and should persist in future studies.

In addition to ReA, recent literature has highlighted a potential association with other post-infectious sequelae. Three separate meta-analyses have shown a sevenfold increase in the risk of developing post-infectious irritable bowel syndrome (PI-IBS), following an episode of acute infectious gastroenteritis (IGE), of which *Shigella* is a cause [20–22]. Similar to ReA, the pathogen-specific attributable risk for these post-infectious sequelae is currently unknown; however, unlike ReA, PI-IBS occurs at a rate much higher than ReA and suggests a new paradigm in the understanding of the probability of long-term risks and discomforts possible following experimental *Shigella* infections. Given the rate of these outcomes, future studies should consider active surveillance for PI-IBS and other functional gastrointestinal disorders subsequent to experimental infections. It is important to note that the simple rate of these disorders in the general population will confound attribution to the experimental infection. Furthermore, the prompt treatment of infection in these models may obviate the sequelae risk. Moreover, there is growing evidence of immunological markers (IL-10, IL-12) which may predict risk of IBS following *Shigella* infection and could be used to screen for healthy volunteers (as is already done with HLA-B27).

Focusing on human challenge studies, Hope & McMillan [23] conclude that the simple act of experimentally infecting someone does not solely make a study morally wrong. They argue that the inherent concern arising from such studies is more associated with the general perception that experimental infection (and subsequent disease) leads to harm; however, in reality, that harm is no greater than what is widely acceptable in other human subjects' research. Future researchers should continue to strive for an adequate risk/benefit ratio and implement more stringent enrolment criteria to minimize the risk of long-term adverse health outcomes as well as increase more prolonged surveillance to identify incident outcomes.

The *Shigella* human challenge model has served as an invaluable tool in understanding the mechanisms of disease, clinical presentation, assessing vaccine efficacy and identifying immunological outcomes. While there has been variability in the model over time it is clear that several prototypic human challenge strains have emerged. Although more work is needed to refine methods for inoculum preparation and utilizing consistent, well-specified outcome definitions, it is clear that these models will serve an important role in furthering our understanding of this

intestinal pathogen and as an early screen for potentially viable vaccine candidates. Future studies designed to further refine the dose–response relationship and to expand the repertoire of available challenge strains should be considered a priority.

ACKNOWLEDGEMENTS

This study was conducted under support of Military Infectious Disease Research Program funding. The opinions and assertions herein should not be construed as official or representing the views of the Department of the Navy, Department of the Army, the Department of Defense, or the U.S. Government. This is a U.S. Government work. There are no restrictions on its use.

DECLARATION OF INTEREST

None.

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