Seroepidemiological investigations of *Salmonella enterica* serovar Typhi infection and the potential role of vaccination in the control of typhoid fever in Fiji

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ABSTRACT OF THESES

To be completed by the candidate – Please read the guidance notes on page 2

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TITLE OF THESIS

Seroepidemiological investigations of *Salmonella enterica* serovar Typhi infection and the potential role of vaccination in the control of typhoid fever in Fiji

DEGREE FOR WHICH THESIS IS PRESENTED ..... PhD .....

Typhoid fever is a potentially-life threatening systemic disease caused by *Salmonella* Typhi, a human-restricted bacterium, spread through the faecal-oral route. Following a sustained rise in observed incidence in Fiji from 2004, in 2013, I undertook a nationally-representative cross-sectional serological survey of 1,531 participants to determine infection by age, assess putative risk factors, and quantify social contact patterns. These data were utilised in the development of a transmission dynamic model.

The literature indicated that typhoid transmission models are relatively under-utilised, particularly in economic evaluation, with little to guide use of vaccination in place of or alongside water, sanitation and hygiene (WASH). The serosurvey found that iTaukei and non-iTaukei Fijians have similar risk of raised IgG antibodies to the Vi antigen expressed by *S.* Typhi. Seroprevalence increased with age, suggestive of endemic transmission or declining incidence. Unimproved sanitation may increase risk of seropositivity. Geospatial analysis suggested rainfall, proximity to major rivers and creeks, or flood-prone areas were risk factors for acquisition of anti-Vi IgG antibodies. Social mixing was assortative by ethnicity and age when assessed by mealtime contacts and highest in school-age children. Increasing number of age-adjusted contacts increases the odds ratio for being seropositive, though substantial uncertainties remain around the specificity and sensitivity of serological thresholds as indicators of past typhoid infection.

An age- and ethnicity-structured transmission dynamic model fitted the serology and case surveillance data well when including a substantially reduced force of infection for high-dose infection being passed to non-iTaukei Fijians, and high generation of asymptomatic non-infectious cases per new infectious case. Surveillance reporting of infectious cases was estimated as one in five infectious adult cases and one in twelve infectious child cases. The fit to the data suggested endemic rather than declining transmission, and there was better fit with age-ethnic assortative mixing than with ethnically-assortative or homogeneous mixing.

Vaccine scenarios suggested that of single dose routine programmes, school entry could be more effective than school leaver vaccination, reflecting age-contact transmission probabilities in the model. Modest reduction (10%) in per-case infectious transmission through effective WASH programmes offered substantial incidence reductions of around 25%, comparable to two-dose (school entry and exit) ViPS vaccination programmes. Potential benefits of conjugate vaccines were projected to be similar to more effective WASH programmes, with administration alongside other vaccines in the second year of life projected to offer approximately 50% incidence reduction, the most benefit of any single dose regimen; with the impact being greater if typhoid carrier daily infectious risk is lower than the daily infectiousness of acute typhoid fever cases.

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Abbreviations

AIC	Akaike's Information Criterion
AusAID	Australian Agency for International Development. Since 2014, part of the Australian Government's Department of Foreign Affairs and Trade
CCDM	Control of Communicable Disease Manual (ed. Heymann), 2015
CDC	Centers for Disease Control and Prevention
CFR	"Case-fatality rate": the proportion of cases that are fatal (with a specified time period)
ELISA	Enzyme-linked immunosorbent assay
FCCDC	Fiji Centre for Communicable Disease Control, Mataika House
FHSSP	Fiji Health Sector Support Programme
FNRERC	Fiji National Research Ethics Review Committee
GNI	Gross national income
H / Hd	Salmonella flagellar antigen (type d expressed by *S.* Typhi)
HWWS	Handwashing with soap
ICC	intra-cluster correlation coefficients
LMIC	Low/middle income country
LSHTM	The London School of Hygiene & Tropical Medicine
MDR	Multi-drug resistant
MLE	Maximum likelihood estimation
MOH	Fiji Ministry of Health. Renamed in 2014 MOHMS
MOHMS	Fiji Ministry of Health and Medical Services
O / O9 / O12	*Salmonella* somatic antigen (type 9 and 12 expressed by *S.* Typhi)
OUCRU	Oxford University Clinical Research Unit
S. Typhi	*Salmonella enterica* subspecies *enterica* serovar Typhi
SBA	Serum bactericidal activity
SIR	Susceptible Infectious Removed model structure
SIRCAV	Susceptible Infectious Removed Carrier Asymptomatic model structure with Vaccination
STRATAA	The Strategic Typhoid Alliance across Asia and Africa
TCV	Typhoid conjugate vaccine
UN	United Nations
Vi	"Virulence" capsular polysaccharide antigen, as expressed by *Salmonella* Typhi
ViPS	Vi polysaccharide (vaccine)

WASH	Water, Sanitation and Hygiene
WHO	World Health Organization
XDR	Extensively drug resistant

iTaukei pronunciation

b	“mb”	The town “Labasa” is pronounced “Lam-ba-sa”
c	“th”	“Moce” (goodbye) is pronounced “Mow-they”
d	“nd”	The airport “Nadi” is pronounced “Nan-dee”
i	“ee”	The language and people “iTaukei” is pronounced “ee-tau-kee”
q	“nga”	The rugby ground “Siqatoka” is “Singa-toe-kah”

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1. Introduction

1.1 Background

This doctoral thesis describes my seroepidemiological investigations into typhoid in the Republic of Fiji, outlined in a series of linked scientific papers. This research follows an invitation from the Fiji Ministry of Health (MOH) to academic and public health partners to examine an apparent upturn in typhoid fever cases and to determine appropriate control strategies. It was conducted as a secondment from UK National Health Service specialty training in public health as a Medical Research Council doctoral scholarship in vaccine science.

My investigations primarily comprised of a national serological survey with collection of demographic data, social contact data and putative epidemiological and environmental risk factors. These data have been utilised in the development of a transmission dynamic model. This seroepidemiological approach seeks to address some of the information gaps on typhoid infection that cannot be attained through routine clinical and laboratory surveillance.

The overall aims of this research are (i) to strengthen epidemiological understanding of the transmission of Salmonella enterica Typhi, and (ii) to suggest potential evidence-based approaches to effective, sustained typhoid fever public health interventions in the Fijian archipelago.

1.1.1 Typhoid fever

Typhoid fever is caused by the gram-negative bacterium *Salmonella enterica* subspecies *enterica* serovar Typhi (*S*. Typhi). It is a faecal-orally transmitted systemic disease which is considered exclusive to humans and may present with prolonged fever, influenza-like-illness, headache, malaise, anorexia and abdominal symptoms.[1] Complications are associated with delayed administration of effective antibiotics[1] and can include encephalopathy, meningitis, myocarditis and intestinal perforation, with an estimated "case-fatality rate" (CFR, more strictly: proportion of clinical cases which are fatal) of 1% amongst the risk-adjusted estimated 12 million cases (95% confidence interval 10 to 15 million) arising annually in low and middle income countries.[2,3] While treatable with antibiotics, increasing antimicrobial resistance is a worrying trend internationally.[4] Notably, in November 2016, an extensively

drug resistant (XDR) strain emerged in an outbreak in Sindh, Pakistan with resistance to chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole, quinolones and third-generation cephalosporins, including plasmid-mediated resistance.[5] The potential for typhoid fever to return to CFRs of the pre-antibiotic era of approximately 15% is a significant concern.[6] Vaccination against typhoid has often been given a secondary role to sanitary interventions, which many suggest is a missed opportunity for disease prevention. [4,7–9]

The use and nomenclature of both *Salmonella* Typhi and typhoid fever are subject to debate and evolution. The taxonomic history of the organism previously known as *Salmonella typhosa*[10] and *Salmonella typhi*[11] is not pertinent to this thesis; the use of "typhoid" and its derivatives is. Some authoritative typhoid papers use "typhoid fever" to refer to any systemic *infection* with *Salmonella* Typhi: [1,4] by definition this includes subclinical and asymptomatic infection as well as the disease. Others use a stricter definition of typhoid fever to refer to the clinical disease only.[12] This thesis uses "typhoid fever" to refer to the clinical disease and the public health problem caused by *Salmonella* Typhi and "typhoid" to refer to any infection by *Salmonella* Typhi.

1.1.1.2 Epidemiology and modes of transmission of Typhoid (fever)

Disease Burden

Typhoid incidence is considered high if >100 per 100,000 population, medium if 10 to 100 per 100,000 and low if <10.[13] In Western Europe and North America, typhoid fever has been largely eliminated or controlled, with water quality improvements and other public health reforms variously attributed to the disease's decline.[14–16] In these settings, most contemporary cases are travel-associated.[17–19]

Recent typhoid fever research has predominantly been sited in South Asia, where the disease burden is considered the highest globally,[2,4,13] with multiple epidemiological studies and vaccine trials in urban slums as the highest incidence settings.[20–23] The incidence of typhoid fever in Africa, where enteric fever surveillance is sparse, is gradually being unmasked.[24,25] In the Pacific islands, typhoid has been recognised as a long-standing health concern in Fiji, Samoa and Papua New Guinea, with a Pacific clade of *S.* Typhi identified through phylogenetic analysis of sequenced genomes.[26–32] Recent global meta-regression estimates assign Oceanian states the highest typhoid incidence rates though it is unclear if predictor indices such as flood risk may over-estimate typhoid incidence in small-island populations.[33]

Modes of transmission

A consideration in communicable disease control in any setting are the predominant modes of transmission. For interventions to be effective in interrupting transmission, they should typically be well matched to these. These can be serendipitous: addressing malodorous sewage as a source of miasmic airborne illness in London was erroneous in theoretical construct but the resultant sewage system effective in cutting faecal-oral diseases such as cholera.[34] For typhoid control, whilst vaccination works by reducing susceptibility to infection, with additional indirect protection through reduction in case numbers, individual components of WASH (water quality, water supply, sanitation and hygiene; and specific elements within each) must align appropriately to transmission modes between portals of exit and entry.

The F-diagram model of diarrhoeal disease transmission paths may be informative to typhoid transmission modes (figure 1.1, adapted).[35] Public health engineers note that water quality interventions may have a lesser role than sanitation and hygiene for many faecal-oral diseases: better to prevent water contamination through effective sanitation (with avoidance of other contaminations) than to clean polluted water (personal communication: Val Curtis and Sandy Cairncross). Water access and water supply as facilitators of hygiene may take precedence over water quality as a policy intent.[36]

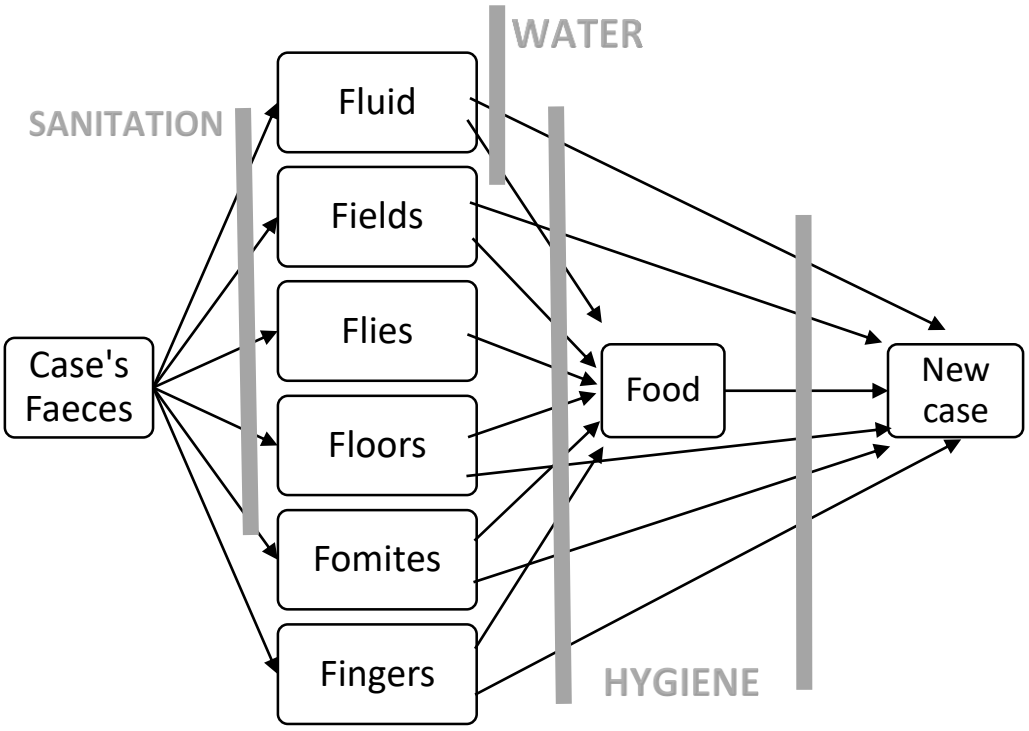

Figure 1.1. F-diagram

Black arrows indicate potential transmission mechanisms; vertical grey bars denote potential transmission-blocking factors.

Typhoid fever following consumption of food or water that has been contaminated by the faeces of a case or carrier may dominate contemporary discourse.[37] However, as outlined in this subsection, a critical read of the historical and other more recent literature indicates a diversity of mechanisms of transmission, more consistent with the breadth of modes in the F-diagram. This suggests epidemiological investigations should not be focused on these to the exclusion of all other modes. The predominant modes of transmission are also thought to vary with incidence, as will also be discussed in this subsection.

Furthermore, an important concern for public health practitioners is to identify those at high risk of becoming cases and/or transmitting disease to others, if resources are insufficient for universal coverage of interventions, or if interventions are better deployed in a targeted manner. Whilst peak incidence is often cited as being under five years or in school-aged children,[38,39] a transmission dynamic perspective notes that average age of infection falls as incidence and force of infection rise: such that typhoid is a disease of young childhood in high incidence settings, and with first infection most likely arise in older groups in settings with less intense transmission.[13]

Typhoid fever is further described as being transmitted through “short cycle” and “long cycle” routes.[40] The most recent background paper by the World Health Organization characterises these as “contamination of food and water in the immediate environment through inadequate hygiene and sanitation measures, either by shedding from temporary or chronic carriers” and “contamination of the broader environment, such as pollution of water supplies by sewage, inadequate treatment of piped water or use of raw human feces as a crop fertiliser.”[39] Mathematical modellers of typhoid have used these groupings to reflect direct person-to-person and indirect transmission.[40,41]

Under the wide-used Bradley classification of water-associated disease, typhoid fever is the archetypal classical waterborne disease.[42] Though influential over the last 40 years, this classification draws attention to a single mode of typhoid transmission only, omitting food and other typhoid transmission routes. Bradley himself reiterates the intent of this work as a functional classification to inform disease control in East Africa, and to shift debate, from industrial-nation focus on quality of piped water when considering interventions, toward other aspects of WASH.[43] Colleagues of Bradley found an absence of impact on typhoid fever for piped-water interventions in Lesotho, southern Africa, suggesting ingestion of remotely-contaminated water was not the principle route of transmission.[44]

The historical literature on typhoid indicates a diversity of modes of communication for typhoid and their relative contributions in settings with different incident rates. The seminal 1873 work on typhoid by William Budd, an English medical epidemiologist, sought to demonstrate typhoid fever as a communicable disease from intestinal discharges, when medical contemporaries did not consider it so.[45] Budd noted that his country practice enable elucidation of such aetiologies, which were obscured to city practitioners, detailing in turn a range of incidents which demonstrated typhoid’s contagious properties and modes of transmission. A chain of transmission of typhoid fever spread through the villages served by Budd, from one case to the next, only associated with household contacts of cases, while no cases arose in what might be deemed control households without cases but with otherwise identical circumstances, including exposure to noxious aromas from pit latrines and other putative causes of the disease. This indicated that typhoid fever was transmitted person-to-person, often affecting those who tended to the sick. Whether this is direct transmission or by fomites is not elucidated. Such transmission can be avoided by appropriate faecal and hand hygiene and infection control measures.[46,47]

Budd also observes a latent period between infection and disease onset, and that an attack appeared to confer lifelong immunity. Alongside “propagation by contagion” Budd also notes

typhoid's "quasi-miasmic" ability to "infect the ground… which has misled so many observers as to their true mode of spreading". Budd documented an outbreak in Bristol associated with a contaminated well serving "houses of a good class" whose neighbours remained free of fever. An outbreak at a ball in Cowbridge in Wales was traced to a well with adjacent cesspit which had received "the bulk of the diarrhoeal discharges" of a typhoid fever patient where "percolation from one to the other was almost inevitable". Amongst modes of communication, Budd notes contaminated hands and linen to transmit typhoid, describing a local case in a washerwoman contracting from bed clothes of two typhoid fever patients, and noting observations from the Fever Hospital in London that the washing profession was struggle to recruit due to the inevitability of succumbing to the disease. This is tempered by a reflection that the public increasingly appreciated the need to sterilise soiled products before sending for washing. Pawnbroker transmission through linen of the deceased is also reported, as is transmission from contact with the clothing of those nursing cases.

In the United States of America (USA) the most well-known case of typhoid infection is that of Mary Mallon, a New York chef. Her fiery disbelief that she could have asymptomatic chronic faecal carriage of *S.* Typhi thereby causing a series of cases and deaths in households saw her continue to work against official advice and resulted in her incarceration for public protection. The investigation report by George Soper details associations in persons, time and place, demonstrating epidemiologically Mallon to be the source and foodborne transmission the mode of communication when householders and others suspected sanitation defects and contaminated water supplies.[48]

Other studies from the USA demonstrate the important role for waterborne typhoid to contribute to the typhoid fever burden, where sewage systems have not protected the drinking water supply. Arguably, substantial contamination of piped drinking water is the most effective means for high incidence of typhoid fever to be attained, bringing large doses and so water borne transmission is typically associated with the highest incidences of typhoid fever. Sears found sewage leakage from a factory intro piped water to be responsible for a highly-localised outbreak.[49] Sedgwick and McNutt's paper on typhoid fever incidence in Massachusetts state and Hamberg, Germany, is informative.[50] Sedgwick had intervened to introduce sewage filtration to the Merrimac river in an effort to cut typhoid fever mortality rates in Lowell and Lawrence, with rates dropping from 112 per 100,000 in each to 24 and 25 per 100,000 respectively.[51] Interestingly the temporal association of water filtration and the decline in illness is not always strong: the figures published in the paper suggest decline preceded the introduction of filtration but does not preclude public awareness of the hazards of piped water reducing the illness rates. Stronger evidence of the impact of water filtration

on US waterborne typhoid is provided by Johnson's 1916 review, noting this to be greater in the north than the south, where other routes were considered to have a proportionally greater contribution.[52]

Sedgwick also, with Winslow,[51] coined the phrase "prosodemic transmission" to refer to faecal-oral typhoid transmission from person-to-person by direct or indirect means (regardless of the specific elements of the transmission chain), citing Budd's study of the same in England. It is taken from the Greek, "pros" meaning "from" and "to" and "demos", well known to epidemiologists as "the people". Sedgwick and Winslow reserve "epidemic" as "that special case in which circumstances permit the transfer of infection to a large number of persons through the same medium, and at approximately the same time" equivalent to "point-source" epidemics in current usage.

Importantly, Sedgwick and Winslow note that prosodemic mortality rates of typhoid in Massachusetts cities is of a range 13 to 25 per 100,000. Allowing a six to ten fold increase for clinical cases (Budd allows 9.5 recoveries per fatality[45], Levine 6.67[6]) gives an incidence rate in the range 78 to 250 per 100,000. This indicates that incidence rates classified today as very high can be attained without transmission through municipal piped water. Furthermore, this suggests that towns in Massachusetts, USA had incidence 125 years ago that would be considered very high by current standards.

The contribution of flies (e.g. housefly *Musca domestica* (Diptera: Muscidae)) to typhoid transmission is disputed. Typhoid infection requires relatively high inoculating doses of *Salmonella* Typhi (see table below) when compared to diseases such as shigellosis requiring just tens of *Shigella* spp. organisms.[53] Experimental work has shown *S*. Typhi to persist for up to ten days on the surface or gut of houseflies;[54] nevertheless, diseases with lower infecting doses that typhoid may be more amenable to transmission by the small feet and alimentary systems of flies travelling from exposed faeces to prepared food. Many have historically ascribed typhoid fever to fly-borne contamination of food, including Budd, and Walter Reed and colleagues assessing sanitary failings in US military bases[55], with Rosenau et al attributing 15% of cases in military bases to flies.[56]

Typhoid may be contracted through eating contaminated crops. Wastewater is an acceptable fertiliser for human-consumed vegetables, within guidelines, with contamination by pathogens such as *Escherichia Coli* more likely at markets than in fields.[57,58] Experimental assessment has found *S.* Typhi persistence in soil for months, and detection of viable organism in root and leaf vegetables one month after harvesting from growth in contaminated soil.[54] In a major outbreak in Santiago, Chile in the late 1970s and early 1980s,

70% of cases were attributed to locally-grown vegetables, with important corroborating evidence from S. Typhi detection by Moore swabs in sewers that flowed to crop fields.[59–61] Interventions to improve wastewater quality alongside educational programmes and other interventions to reduce consumption of raw vegetables have been given credit for driving down incidence.[59]

Data from Tokyo in the first half of the 20th century further demonstrated the importance of safe disposal of faecal matter, with an economically-driven interrupted time series natural experiment reported by Nagashima in 2004.[62] Collection of night-soil had previously been undertaken for free due to the commercial value of human faeces as a fertilizer. When chemical fertilizers became popular, night soil collection declined, and typhoid became established in affluent areas of Tokyo until government intervention to provide a night-soil collection service.[62]

More recent decades have seen a number of case-control studies and other epidemiological investigations into the transmission of typhoid. It should be noted that outbreak transmission and endemic or prosodemic transmission may not follow the same modes, with endemic transmission less likely to be investigated than if there is a perceived excess of cases, potentially biasing the literature towards transmission modes associated with epidemic disease. Similarly, transmission in high incidence settings may be more likely to be investigated than in lower incidence settings. These recent studies reveal a diversity of transmission mechanisms.

Mermin and colleagues reported in 1999 on a multi-drug resistant typhoid outbreak in Tajikistan associated with drinking unboiled water following equipment failure at a water plant, with back-siphonage causing faecal contamination.[63] In 2001, Olsen and colleagues reported on a restaurant outbreak on Nauru that was attributed to two food handlers with faecal carriage of *S.* Typhi. In a 2001 study in southern Vietnam, Luxemburger and colleagues found recent contact with a typhoid fever patient and low socioeconomic status were associated with disease in an adjusted logistic regression analysis with community controls.[64] Vollaard and colleagues examined typhoid fever in Jakarta in 2001-3 using case-control methods.[65] Against community controls, they found adjusted risk factors to be “mostly related to the household” including recent cases in the household, absence of soap for handwashing, sharing food from the same plate and absence of toilet in the household. With control selection matched by geography but not age, the study also found typhoid fever cases to be younger than controls. Studying Kamalapur slum in Dhaka, Bangladesh, Ram et al’s 2006 case control study identified on multivariable analysis drinking unboiled water at home

and consumption of foul-smelling water to be independently associated with illness, as was consumption of papaya, whilst latrine use was protective.[21] Srikantiah's 2007 study in Samarkand, Uzbekistan, found on adjusted logistic regression that risk factors were consumption of unboiled water outside the home, consumption of antimicrobials in the two weeks prior to disease onset and being a student. Routinely washing vegetables and drinking in tea houses were protective. Most houses in Samarkand draw household water from deep underground sources unlikely to harbour significant *S.* Typhi concentrations, in contrast to sources outside the home which are more likely to draw surface water and risk contamination. In Kathmandu, Nepal, low income and use of a household rather than community toilet were associated with typhoid fever in a 2013 adjusted case-control analysis by Karkey and colleagues.[66] Molecular methods applied in Kathmandu have identified household transmission and detected *S.* Typhi in municipal water spouts used as a supply of drinking water.[67] However, no bacterial culture and isolation was successfully done from any water sample: as polymerase chain reaction (PCR) can detect killed/dead bacteria as well as live organism, such findings do not conclusively support transmission through this mode.

A WHO expert-elicitation exercise on foodborne transmission global burdens estimated that in Oceania, foodborne transmission accounted for median 49% (95% "uncertainly" 10 to 84%) of typhoid fever incidence, person-to-person transmission (not defined) 13% (0 to 51%) and water 33% (1% to 66%).[68] Sustained transmission of typhoid in another Pacific island, Samoa, led to this being the setting for a ground-breaking transmission dynamic computational model developed by WHO in the 1970s, discussed further in the literature review chapter.[30,69,70]

Asymptomatic gallbladder carriage is established in a small percentage of cases, more commonly in women, older patient and those with gallstones, leading to prolonged faecal shedding (less commonly urinary shedding, typically if tract damage exists, such as through schistosomiasis) over months or years.[1] Gunn and colleagues divide carriage in three categories:

- Convalescent: three weeks to three months post-infection (presumably "post-ingestion" or "following the moment of infection" as carriage is a form of infection)
- Temporary: three to twelve months
- Chronic: more than one year.[71]

Experimental work has shown that pathogenesis is dose-dependent: the probability of developing clinical disease increases and the time to onset decreases with higher ingested doses.[72,73] This is consistent with the above epidemiological findings suggestive of

symptomatic infection from heavily sewage-contaminated water or crops, household transmission from acute cases, transmission from defective sanitation and contamination of fruit. The WHO notes that water consumption is more likely to deliver low inocula while foodborne inocula are usually high dose.[74]

Ingested dose of *S.* Typhi	Proportion of volunteers developing clinical disease	Days to onset of clinical disease, geometric mean with 95% CI
10^9	0.95	4.7 (4.1 to 5.4)
10^8	0.89	
10^7	0.5	7.4 (4.9 to 11.2)
10^5	0.28	9.3 (8.4 to 10.4)
10^3	0	N/A

Table 1.1. Ingested dose of S. Typhi, attack rates and incubation periods
Source: Hornick et al 1970, Glynn et al 1995 [72,73]

Diagnostics

Current typhoid diagnostic tools are considered inadequate.[75] The current gold standard test is blood culture, which has high specificity for typhoid but limited sensitivity, though this may be increased by larger blood draws with modern media and monitoring.[76] Bone marrow culture is more sensitive but invasive and not commonly practiced.[76] The outdated Widal test and modern rapid antibody test kits (often utilising Widal-based technology) are of limited clinical utility in differentiating typhoid from other febrile illnesses.[77] The weakness of these diagnostic tools impairs surveillance efforts as well as tilting clinical management towards empirical treatment.[75]

1.1.1.2 Immunobiology of Salmonella Typhi

The pathogenic process from *S.* Typhi ingestion to clinical disease is complex.[78–80] Bacteria passing through the low pH environment of the stomach into the small intestine arrive at the

lamina propria through M cells in Peyer's patches or through trans-enterocyte passage in endocytic vacuoles. In the lamina propria of the susceptible host, macrophages engulf *S.* Typhi largely without killing them; these either remain in localised lymph tissue or drain into the mesenteric lymphatic system, from where *S.* Typhi can multiplying and enter the circulation. This results in a primary bacteraemia, from where the bacteria can reach reticuloendothelial system organs and continue multiplication. A sustained second bacteraemia is associated with clinical disease, typically 8 – 14 days after ingestion.

Salmonellas are classified based on the O (somatic) and H (flagellar) antigen under the Kauffman White scale: *S.* Typhi express d-flagellin (Hd), O9 and O12. Widal tests for typhoid using O and H antibody are strongly cross-reactive with other species and consequently of insufficient specificity for acute diagnosis or serological surveillance.[81] Most *Salmonella* Typhi obtained from clinical samples also expresses the Vi capsular polysaccharide antigen, an abbreviation of "virulence". Vi-expressing strains are more pathogenic than those which do not express it, such that disease caused by an *S.* Typhi strain lacking Vi is rare.[72] Of bacterial species and serovars expressing Vi, only *S.* Typhi is recognised as a common pathogen in immunocompetent humans, in contrast to *Citrobacter freundii* and *Salmonella enterica* serovar Dublin.[82] Urinalysis of Vi antigen by ELISA has been examined as a potential rapid diagnostic test for typhoid fever in a 2004 Egyptian-American study,[83] however, equivalent sensitivity in examination of urine from other febrile patients suggests issues with the assay and limited validity.

The utility of Vi antibody in diagnosis has been found to be limited in endemic settings due to slow onset in acute disease and high background prevalence.[75,84] In a study in Vietnam, illness for two weeks or more was associated with a raised anti-Vi IgG response.[85] Vi antibody detection has greater utility in population level studies in determining these background prevalences, where subclinical infection is likely to go untreated - one study in Kathmandu, Nepal, found higher prevalences in adults than children, suggestive of endemic transmission, or high historical rates of childhood infection and later decline.[86] Another study using Kathmandu residual clinical samples from non-typhoid fever case patients found peak titres in young children and young adults and ELISA unit inter-quartile ranges in the region of 10 to 100 EU across all ages, suggesting hospital attendees to have sustained high prior risk of *S.* Typhi exposure.[66]

Very high anti-Vi titres have also been utilised in serological identification of chronic typhoid carriers such as in Chile, with a 75% sensitivity (27 of 36 carriers; 95% CI: 57 to 87%) at threshold 1:160 and 92% specificity amongst 388 stool-culture negative women.[87] Studies in

the USA, a low incidence setting, found Vi serology specific for carriage but insensitive (29 of 38 total carriers; 76%, 95% CI: 59 to 88%) relative to stool culture.

A 1986 expert meeting proposed wider use of serosurveillance to inform lifetime prevalence of symptomatic and asymptomatic infection using long-lived IgG Hd antibodies.[88] At the time most Vi antigen preparations were insufficiently pure to offer adequate sensitivity or specificity, though more sensitive and specific Vi antibody tests were in development, in parallel with efforts to develop a Vi-based vaccine.[88] Serosurveillance for Hd IgG antibodies in Santiago, Chile, in 1978, had found 25% of 76 15-19 year olds to be seropositive at 1:40 titre, higher than the same age groups in other regions not affected by epidemic typhoid fever.[89] Age-based analysis in Santiago found less than 10% of 163 children under 15 years to have Hd seropositivity, rising to over 50% of 23 sampled 25-29 year olds. Older ages has seroprevalence around 40% from 36 samples. Interpretation of this may include endemic transmission with waning antibody in older age-groups, or recent outbreak with differential risk by age.

Natural infection elicits both humoral and cell mediated immunity (CMI). Whilst cell-mediated immunity is considered likely to be the major defence mechanism against typhoid, the fact that Vi is an effective vaccine indicates that anti-Vi has a role in protection during natural infection.[90]

Correlates of immune protection are not clearly defined following infection or vaccination.[91–93] In highly endemic South African settings, a study by Klugman and colleagues [94] found that a Vi antibody titre correlating with protection was likely to lie within a range of 0.6 micrograms to 1.2 micrograms per millilitre. From this they considered 1 microgram an arbitrary and conservative estimate of the level necessary for protection. More recently 1.4 to 2 micrograms/ml has been suggested as a correlate.[95] Standardisation of assays internationally is unfortunately incomplete.[91]

Anti-Vi serology was reviewed by Robbins and Robbins in 1984, motivated by consideration of Vi as a vaccine candidate.[96] They challenge some prior research (focused on carrier identification) that suggested no relationship between Vi antibodies, susceptibility or recovery, which held that the most typhoid fever patients do not produce antibodies within weeks of illness. Robbins and Robbins note instead that higher titres and higher proportions positive are observed later in the course of illness[97] and suggesting impurity in Vi preparation used for assays rendered them invalid as unable to distinguish from background noise. Studies such as those by Landy and Lamb using purified Vi and passive haemagglutination in a highly endemic setting found seropositivity in current and recent cases but no response in

non-cases.[98] A further issue identified with historical studies is the identification of protective effects driven by anti-Vi incomplete antibody – that which binds antigen but does not agglutinate.[99] This could result in individuals who have been naturally infected being protected and yet have a serum antibody titre that cannot be identified on agglutination assays; such antibodies could be detectable by other means.

There remains limited data on Vi antibody kinetics following natural infection. House et al found elevated Vi antibody response by ELISA optical density in Vietnamese patients who had been ill for at least two weeks.[85] Lanata and colleagues found in Santiago raised titres in approximately half of acute cases,[87] whilst women aged ≥25years with confirmed typhoid fever one to four years previously had GMT and titre distributions more comparable to the general population from whom they were drawn (the high community Hd seroprevalence by late adolescence in the same setting should be noted).[89] Brodie's study of antibodies following a typhoid outbreak in Aberdeen, UK, found a bimodal distribution of Vi antibody by agglutination assay in acute cases with only 20% seronegative. At 3 months, 70% had become seronegative, and at 6 months 40% were seronegative.[100] Such findings may be consistent with an early IgM response and slower IgG response.

Vi vaccinees' antibody kinetics have been studied but are not readily interpretable in informing serological surveillance of natural infection. A small study by radioimmunoassay (RIA) in US volunteer vaccinees showed substantially raised titres three weeks after Vi-PS vaccination, with persistence at lower levels (but still above baseline) after three years.[101] Medium and long-term kinetic data is available from pilot participants in a vaccination trial in a typhoid-endemic area of South Africa Klugman and colleagues used ELISA optical density and RIA to examine serum pre- and post- vaccination, with these Vi assays considered more sensitive than the haemagluttination assays used previously for carrier detection.[9,102] Both those in groups with and without pre-vaccination antibodies saw rise in titre at one month and wane at six and twelve months. At twelve months, titres had returned to comparable levels in those with baseline titres. For those without baseline titres, sustained response was observed by RIA and, for those receiving higher vaccine doses, by ELISA, though these were less than baseline titre in those with pre-vaccination antibodies. At three year follow-up, geometric mean titres were higher than those of controls.[94] The 10 year follow-up of the South African vaccinees and controls appears to show that antibody had waned in 40 vaccinees to 1.23 (range 0.21 to 2.30) microgram/ml geometric mean titre by RIA whilst 43 control participants' serology had reached GMT of 1.30 0.15 to 3.91) microgram/ml.[102] Keddy

et al also report observing raised Vi in recent typhoid fever cases, including two control participant. Most notable for Vi seroepidemiology from the South African trial is that 40% of control participants at the age of nine had titres predicted to be protective, suggestive of natural infection.

Work is ongoing for novel biomarkers of typhoid infection.[103,104] Notably the recent characterisation of the typhoid toxin opens the future possibility of diagnostic methods, surveillance tools, therapeutics and vaccines based on the toxin and immune response to it.[105]

1.1.2 Setting

Fiji is a populous Pacific island state and classified by the World Bank as an upper-middle income country.[106] It is located approximately 2000km north of New Zealand in the Pacific Ocean.

At the last census (2007) the total population of Fiji was 837,271, of whom 57% are iTaukei (see below), 37% Indo-Fijian, and 6% other ethnicities. Most of the population reside in the two main islands, Viti Levu, where the port capital Suva is found, and Vanua Levu in the north. The administrative divisions of the islands are shown in figure 1.1.

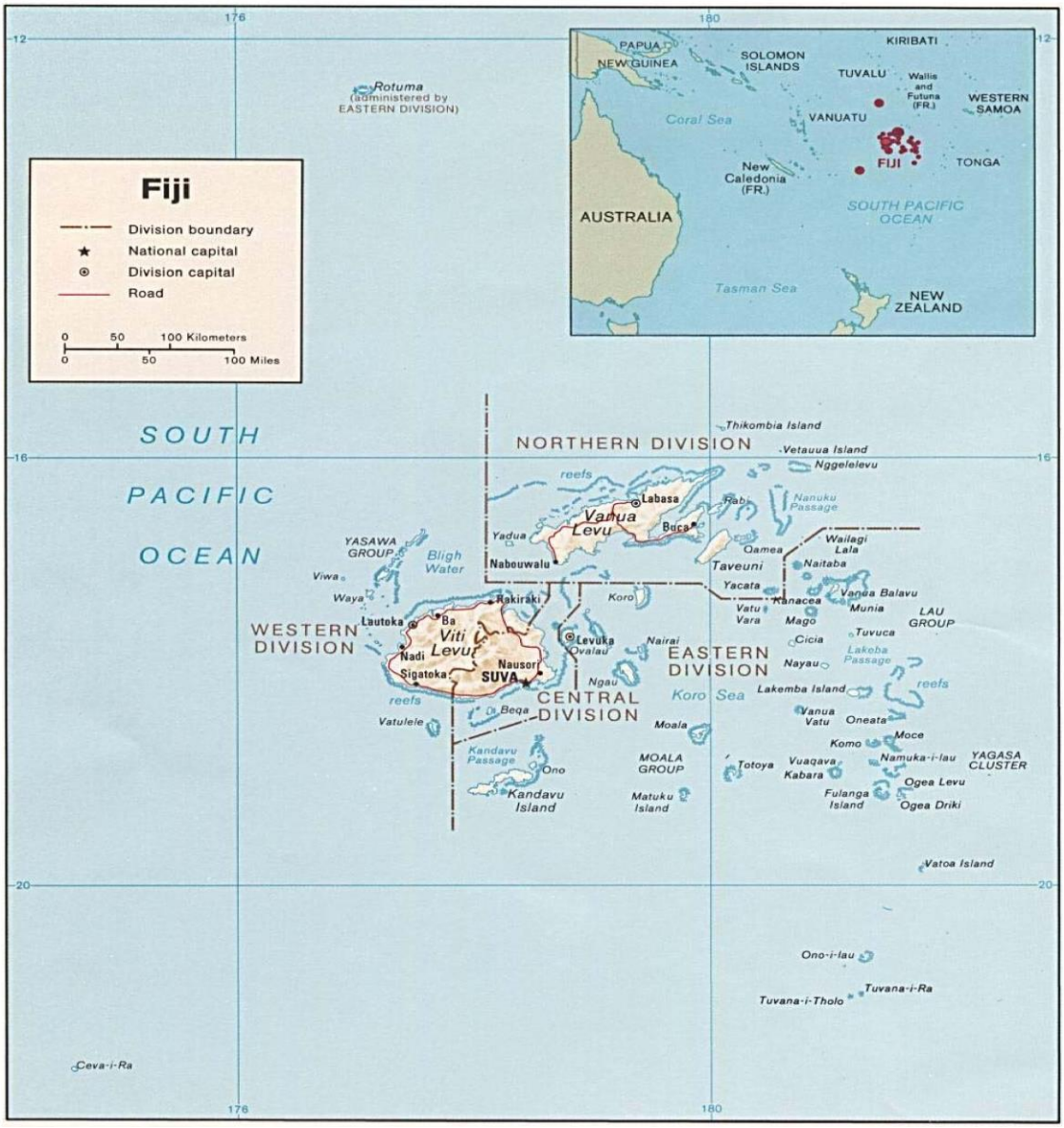

Figure 1.2. Fiji administrative map.

Source: Fiji Bureau of Statistics [107]

1.1.2.1 Ethnicity in Fiji

Ethnicity is an important consideration in the epidemiology of typhoid in Fiji, and the recent political and demographic history of Fiji are relevant in this regard. The language and terminology of ethnicity in Fiji is contested. In efforts to promote a singular national identity, on 30 June 2010 the government enacted a new law requiring all government agencies to replace "the word 'Fijian' or 'indigenous' or 'indigenous Fijian' with the word 'iTaukei' in all written laws, and all official documentation when referring to the original and native settlers of Fiji."[108] "Fijian" now denotes any citizen of the islands. This thesis uses the iTaukei word "iTaukei" to denote indigenous Melanesian Fijians (57% of the population), and "Indo-Fijian" to denote Fijians of Indian descent (38%). The term "Indo-Fijian" is widely used and understood by Fijians of all ethnicities including by influential Indo-Fijians such as the exiled academic Brij Lal.[109]

Fiji's multi-ethnic society is a product of its time as a British colony between 1874 and 1970. The period 1879 to 1916 saw the immigration of approximately 60,000 indentured labourers from India to work on sugar plantations.[110] Many remained after their five-year contracts expired, with the Indian population also increasing due to the arrival to urban Fiji of smaller numbers of Gujarati free immigrants from 1900 as retailers or skilled professionals.[111] The British Governor sought to protect indigenous traditions and rights, including barring the sale of Fijian land to non-iTaukei, leaving Indo-Fijians to lease land for property development and farming.[112] Thus indigenous iTaukei Fijians spent much of the 20th century maintaining traditions and in subsistence farming, whilst Indo-Fijians sought to gain security and position through wealth creation.[111]

By the 1980s Indo-Fijians had become the majority population and were making ever stronger calls for land and political rights. A coup in 1987 was led by an iTaukei military officer calling for iTaukei dominance of parliament and resulted in a period of racial unrest. Land leases were not renewed and Indo-Fijians were displaced from homes and farmland. Emigration of (skilled) Indo-Fijians has been a secular trend since this time.[113] A period of political instability came to a head in 2000 when iTaukei businessman George Speight took hostages in parliament including Fiji's first Indo-Fijian leader, Mahendra Chaudhry. A counter-coup led by Commodore Josaia Voreqe (Frank) Bainimarama saw the installation of an interim military government. A coup was led by Bainimarama in 2006 to strengthen Indo-

Fijian rights.[114] In elections in September 2014 Bainimarama's FijiFirst party became the largest party with Commodore Frank elected prime minister.[115]

1.1.2.2 Population structure

Regardless of political circumstance, iTaukei and Indo-Fijians live largely separate daily existences[111] and experience different demographic forces, with birth and death rates higher in iTaukei than in Indo-Fijians, and rural residency more common amongst iTaukei Fijians.[113] Urban and rural population split is close to 50:50, with iTaukei Fijians comprising 64% of the rural population.[113] Markers of material wealth are higher in Indo-Fijians.[113]

Figure 1.2 shows the age structure of the iTaukei and Indo-Fijian populations. It is notable from a health, demographic and modelling perspective that the iTaukei Fijian population appears to have an "exponential" population curve indicative of a constant mortality rate, while the Indo-Fijian age structure is more "rectangular" and consistent with epidemiological transition.[116]

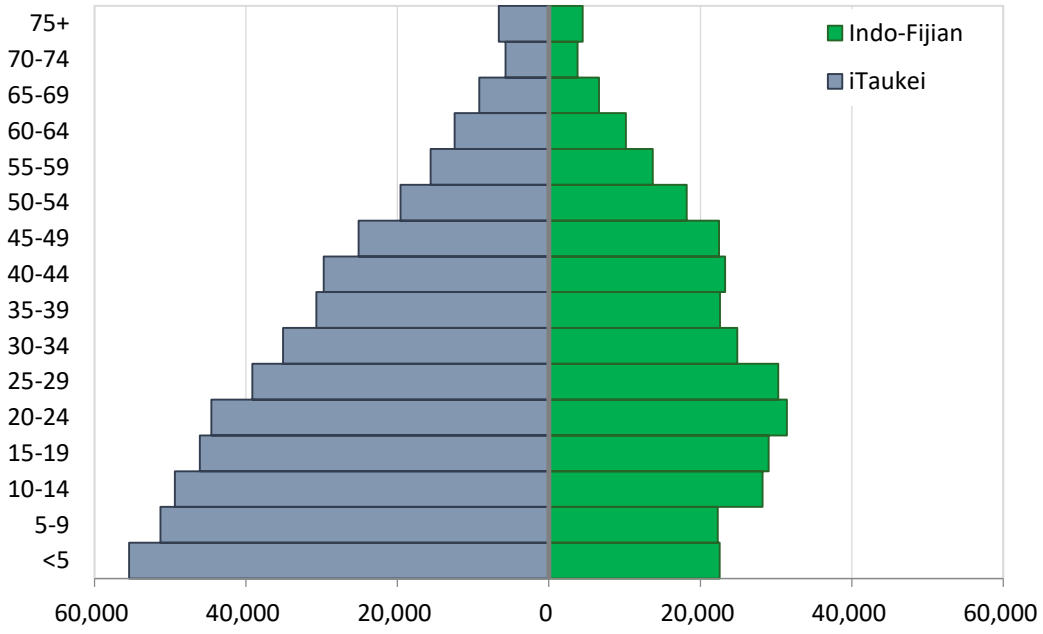

Figure 1.3. Population pyramid of iTaukei Fijian and Indo-Fijians by age group. *Data source: Census 2007.*

1.1.2.3 WASH in Fiji

WASH infrastructure and behaviours are important determinants of typhoid risk in Fiji. A 1914 epidemiological report on dysentery (shigellosis) considered the reticulated water system in Suva to be "beyond reproach".[117] The most recent census (2008) indicated that both ethnic groups have similar access to safe water supply (metered, communal and roof-tank) covering approximately 90% of households, up from 84% in 1996.[113]

The Fiji Water Authority is responsible for the reticulated water system, with the 2007 SOPAC report noting surface water to be the primary source for most towns in Fiji, with groundwater use on smaller islands only.

The United Nations note that due to an absence of primary legislation, "there is no clear ownership within any single government department when it comes to … regulating, managing, and delivering water resources and services."[118]

A recent systematic review of WASH in Pacific islands noted the importance of the cultural context in interventions, such as acceptability of household-level water harvesting schemes in iTaukei villages where community and communality are prized.[119]

Census reported ownership of "modern" toilets (private or shared flush toilets, regardless of sewage connection) is higher amongst Indo-Fijians (83% vs 63% of iTaukei in 2007, up from 54% and 35% respectively in 1996). In 2007, water seal toilets were used by 21% of iTaukei households and 3% of Indo-Fijian households, with pit latrines used by 14% and 14% of households respectively.

Sewerage systems were described in a 2002 report, which found that even in highly densely populated Suva-Nausori, only one third of the population were connected to the mains sewage system, with 270,000 utilising septic tanks. [120] Overflow from undersized sewers, blockages and poorly maintained plants were considered to contribute significantly to faecal contamination of water.

Socio-cultural aspects of hand hygiene have been examined in iTaukei Fijians by interview and focus group research.[121] Confusion was found in some participants between diseases such as typhoid and lymphatic filariasis, with media focusing on diseases rather than common risk factors. Handwashing was viewed with ambivalence regarding health benefits, and more likely to occur in the context of bathing and washing rather than associated with meal

preparation or post-toileting. Intermittent water access and general water-scarcity in a peri-urban settlement led to water being conserved for uses other than handwashing.

1.1.3 Typhoid in Fiji

Fiji has seen a sharp upturn in notified, blood-culture confirmed typhoid cases since 2004 (figure 1.3), rising from low tens of cases to approximately 360 per annum (approximate crude annual incidence 43 per 100,000 population) with some provinces considered high incidence by international standards at over 100 case per 100,000.[122–124] The Fijian enteric fever burden is almost exclusively due to typhoid, rather than paratyphoid fever, a clinical indistinguishable disease which has become more prevalent in Asia, where *S.* Paratyphi A is the most common causal organism.[125,126]

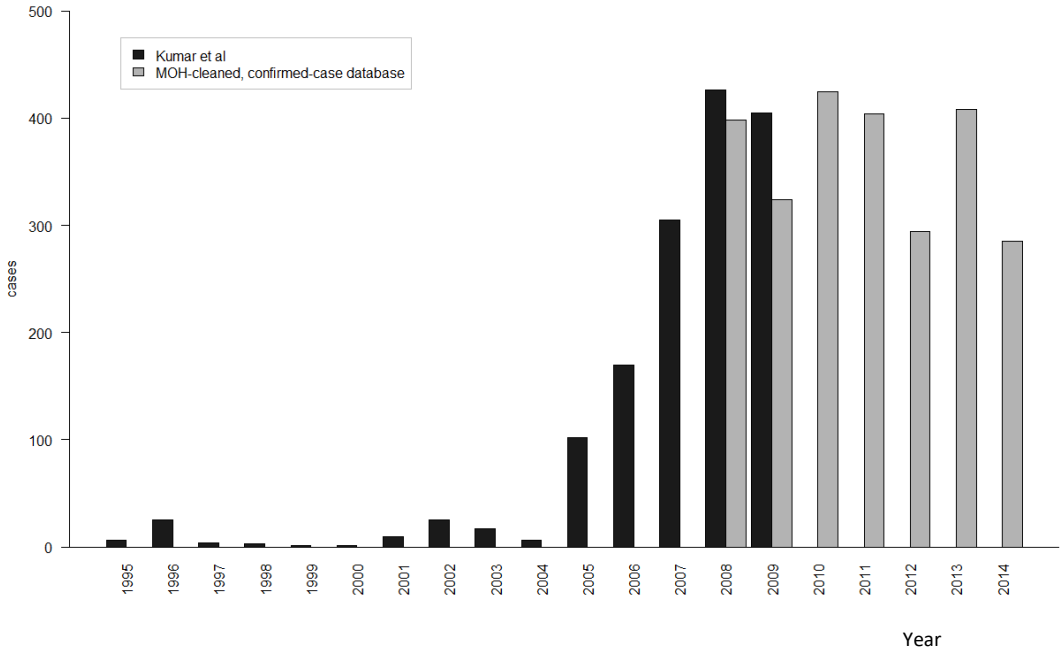

Figure 1.4 Typhoid fever notified case incidence 1995 to 2014

Data sources: Kumar et al (MOH data);[122] Cleaned confirmed-case national surveillance database, Fiji Centre for Communicable Disease Control, Ministry of Health and Medical Services

An Australian AID sponsored vaccination campaign was implemented in areas deemed at highest risk for typhoid following Cyclone Tomas in 2010, covering approximately 7% of the population, primarily Taveuni island and the southern coast of Vanua Levu, with smaller campaigns elsewhere. Achieving close to 100% coverage across all ages in some of these areas, the campaign was successful in reducing localised incidence, but appeared to have negligible effect on the overall typhoid epidemic.[127]

The overwhelming majority of reported cases are in iTaukei Fijians (94%), with a male excess (57%), a median age of 25 and an inter-quartile range of 15-36 years.[128] See figure 1.4. Antimicrobial governance is considered relatively strong in Fiji, though stock shortages and empirical treatment are common, and antimicrobially-resistant typhoid fever is rare at present.[123,129] A convenience sample survey of Fijian festival attenders found 41% reported antibiotic use in the previous month, including for colds, flu and other viral diseases which do not respond to antibiotics. Government health facilities were the main source of antibiotics, though almost 2% reported obtaining these from pharmacies without prescription.[130] The understanding of a number of survey terms and validity of prevalences require further assessment through in-depth qualitative research.

That the average age of infection appears to be in young adulthood suggests a lower annual force of infection compared with settings in the Indian subcontinent where disease is seen at high incidence rates earlier in childhood [38,86,131]. Statistical and causal inference is limited, not least by surveillance biases, such as the potential for differential reporting in different age groups. Under-ascertainment in children is a distinct possibility, where empirical treatment may be more common than invasive diagnostic testing and the sensitivity of blood culture further reduced by reduced blood-volume draw.[33,76]

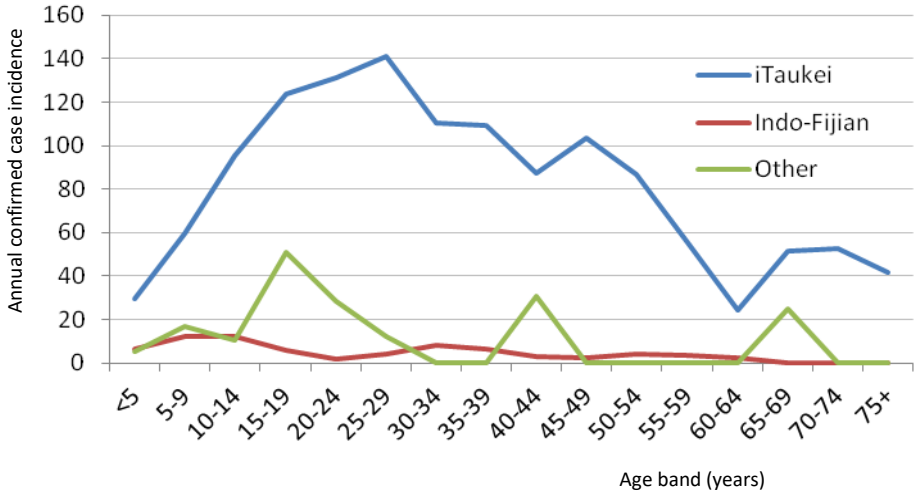

Figure 1.5 Age- and ethnicity-specific incidence rates of laboratory confirmed typhoid in Fiji 2008-11

(per 100,000 person years; 2007 census population; MOH data)

Kumar and colleagues[122] suggest possible causal mechanism for typhoid transmission in Fiji, noting association with the higher incidence of disease, including outbreaks, in the Northern Division and Suva peri-urban area. The proposed risk factors are poor sanitation, water supply and personal hygiene; slum settlements where sharing of toilets and water supply is

common; and the use of infected water in preparation of the herbal drink kava, which is consumed in groups with a communal bowl and cups. The sharing of farm tools is also asserted as increasing the rate of disease transmission, though no evidence is provided to implicate these particular fomites; public health officials have suggested poor sanitation facilities for seasonal sugar cane croppers was a driver of increased incidence in the Northern Division.[132] Handwashing campaigns have been implemented but not resulted in any appreciable decline in disease.[133]

1.1.4 International typhoid vaccination and control context

Whilst this thesis is oriented toward the typhoid public health problem in Fiji, it is not independent of the international research and policy context. Multiple iterations of World Health Organization (WHO) typhoid position papers support consideration of typhoid vaccination in high risk population groups and for outbreak control.[37,134,135] In practice, the cost of vaccines, limited duration of protection and inadequate immunogenicity in children under 2 have limited uptake by national authorities.[23,136] At the time this thesis was submitted, international recommendations for programmatic typhoid vaccination, particularly with typhoid conjugate vaccines, were being reviewed through the WHO Strategic Advisory Group of Experts on Immunization (SAGE),[39] and through the GAVI Alliance, an international finance coordination body for vaccination programmes in countries with GNI up to USD 1,580 per capita (Fiji is not GAVI-eligible).[137,138]

There are two internationally marketed typhoid vaccines: an injectable Vi-polysaccharide vaccine suitable from age 2 years and an oral three or four dose live Ty21a vaccine suitable from age five or six. Efficacy of each is modest, at around 50% over three years.[139] Even with low efficacy vaccines, school-based programmes have shown success in controlling typhoid.[140]

Much of the current typhoid control research effort is directed towards the development of Vi-conjugate vaccines which appear to offer greater efficacy, immunogenicity in the under-twos and longer duration of protection. These use Vi antigen conjugated to an immunogenic protein such as recombinant exoprotein A of *Pseudomonas aeruginosa* (rEPA), the mutant diphtheria protein CRM197, tetanus toxoid (TT) or diphtheria toxoid (DT).[141] Of these, Bharat Biotech's single-dose typhoid-tetanus toxoid conjugate vaccine has gained licensure in India[142] as has Bio-Med's two-dose PedaTyph,[143] and following submission of this thesis, Bharat's product attained the WHO-prequalification status that will enable more widespread utilisation in low and middle income countries such as Fiji, consistent with the 2018 update to

the WHO position on typhoid vaccines.[135] Oral typhoid vaccines that may offer greater efficacy or shorter regimens than Ty21a are also in development.[141]

Policy-oriented research efforts including development of surveillance programmes for disease burden estimates,[144,145] including serosurveillance;[146] and vaccine trials undertaken or in-planning for typhoid Vi-conjugate vaccines.[147] A notable recent development has been the revival in Oxford of the human challenge study model for vaccine efficacy testing. Originally undertaken in Maryland, USA, in volunteer prisoners, these controlled human challenge studies have also provided rich biological data as well as vaccine efficacy assessments.[72,73,148–152]

1.2 Research project and thesis

To address typhoid in Fiji, an international expert meeting was convened by the MOH and Australian Aid in August 2012, approximately a month before the start of this doctoral project. The meeting's aims were to make disease control recommendations and to identify the knowledge gaps that would merit addressing in order to assist control efforts.[123]

This doctoral project took forward a meeting recommendation of a serological survey to inform age-specific incidence rates and to guide vaccination policy. Vaccination accordingly receives a specific focus alongside consideration of public health case management of cases and interventions to improve adequate access to sufficiently clean water, effective sanitation and handwashing with soap. Findings from individual paper-format chapters have been shared with the Fijian Ministry of Health and Medical Services (MOHMS) during the development of each paper. The summarised thesis findings will have been presented to MOHMS in October 2017, alongside findings for other research groups undertaking case-control and environmental analyses in accordance with the 2012 meeting.[153,154]

1.2.1 Aims

The overall aims of this research are (i) to strengthen epidemiological understanding of the transmission of typhoid fever, and (ii) to suggest potential evidence-based approaches to effective, sustained public health interventions in the Fijian archipelago. These overall aims were broken down into four sets of sub-aims, each corresponding with the appropriate chapter.

1.2.1.1 Serological study

Aim:

To determine the age-specific incidence and cumulative incidence of typhoid in Fiji in order to inform transmission modelling, testing the hypothesis that surveillance data under-ascertains childhood infection.

Serological study hypotheses:

a) That individuals with recent exposure to *Salmonella* Typhi, or vaccination with Vi polysaccharide vaccine, are likely to have higher Vi-antibody titres than uninfected controls (obtained from a non-typhoidal setting).
b) That these titres will fall over time, and can be measured by re-testing individuals with previously high titres.

With knowledge of the Vi-antibody titre kinetics, a number of further hypotheses can be investigated:

c) That antibody responses suggest a higher proportion of children have had exposure to *Salmonella* Typhi than is suggested by surveillance data
d) That antibody responses vary between age groups, indicating different historical and recent exposure to *S.* Typhi.
e) That high antibody titres are associated with drinking water outside the home, the absence of improved sanitation, male sex, iTaukei ethnicity and rural residency, and that there will be interaction between two or more of these factors.

Serology Study Objectives:

1. Design appropriate sampling frames to identify a representative sample of Fijians resident in the two main islands, Viti Levu and Vanua Levu.
2. Prospectively collect 1600 blood samples from this population sample for serological analysis.
3. Gather risk factor data from the 1600 study participants.
4. Validate a Vi antibody ELISA serology test against a passive haemagluttination test to the long-acting d-epitope of *S.* Typhi flagellin, using negative control blood from Europe and positive control blood from Fijian typhoid cases.

5. Statistically analyse the results of the serological survey to assess past exposure to typhoid in different age groups.
6. Statistically assess different risk factors for typhoid serological titre differences, including between iTaukei Fijians and Indo-Fijians.
7. Determine wane of typhoid vaccine antibody protection in a Fijian island (Taveuni) where mass vaccination was done in 2010 and antibody testing done in 2011, by testing serum sample from 300 volunteers in a repeat cross-sectional analysis.

1.2.1.2 Social mixing study

Aim:

To determine social contact patterns in different age groups, and settings, as relevant to enteric infections and other person-to-person communicable diseases in Fiji, and as may be applicable in other Pacific island settings.

Hypothesis

a) That in urban and rural settings in Fiji, reported social contact will follow age-based mixing patterns consistent with social mixing studies in Europe.

Objectives:

1. Develop and pilot a social mixing survey tool for enteric infection research, based on those used for POLYMOD and SMILI studies.[155,156]
2. Complete social mixing surveys with serology study participants.
3. Analyse social mixing data to developing context-specific contact matrices (e.g. participants in urban and rural settings or by iTaukei Fijian and Indo-Fijian ethnicity) suitable for modelling disease transmission.

1.2.1.3 Transmission dynamic model

Aim:

To develop and validate an age-structured transmission dynamic model, using this to inform typhoid control by modelling likely impacts of vaccination programmes and interventions on sanitation, hygiene or water quality in Fiji.

Hypotheses:

a) That spread of infection in children contributes substantially to disease in people aged 15 to 29.
b) That vaccination of children of primary school age with annual vaccination of a school-year cohort would be predicted to reduce but not eliminate disease incidence in the whole population, and that this reduction would be greater if preceded with a multi-cohort campaign
c) That improving sanitation would be predicted to control typhoid.

Objectives:

1. Collect routine surveillance data and other data sources for model data fitting.
2. Using surveillance data, serological data, and social mixing data, develop and validate a transmission dynamic model in the R statistical environment.
3. Model the likely impact of interventions with polysaccharide vaccines, Ty21a vaccines or Vi-conjugate vaccines on typhoid transmission and incidence, including programmes and campaigns administered to child cohorts.
4. Model the likely impact of other interventions that may interrupt typhoid transmission, including sanitation improvements, hygiene campaigns and water quality improvements, alone and in combination with vaccination options.

1.2.2 Thesis structure

This thesis is in the "by publication" format, comprising paper-format chapters in their published or peer-reviewed formats and a chapter detailing the ongoing development of a typhoid transmission dynamic model for Fiji. Each paper-format chapter is preceded by a short bridging section giving the rationale for the chapter, the role of the contributors, its place in the narrative of steps towards typhoid control in Fiji and details of other considerations that lie outside the scope of the published piece. The nature of the thesis by publication necessitates some repetition in chapter introductory and methods sections, but these largely bring different angles to the problem, specific to the aims of each paper.

The thesis chapters are as follow:

1. **Introduction.** Describing the problem of typhoid in Fiji, and the context of the research.

2. **A review of typhoid fever transmission dynamic models and economic evaluations of vaccination.** This literature review was published in 2015 in a *Vaccine* journal special edition coinciding with the 9th International Conference on Typhoid and Invasive Non-Typhoidal Salmonelloses.

3. **A cross-sectional seroepidemiological survey of typhoid fever in Fiji.** This is the main paper of the thesis and is an analysis of my seroepidemiological fieldwork conducted in Fiji in 2013 and 2014, published in *PLOS Neglected Tropical Diseases* in 2017. This cross sectional study focuses on age-based patterns in unvaccinated areas of the Fijian mainland, but also includes findings from a Vi-polysaccharide vaccinated island and uses a cohort of convalescing cases to provide positive control data and insight into post-infection antibody waning.

   There is an extended bridging section prior to this paper which contains further details of the design, preparation and implementation of the serosurvey.

4. **Environmental factors drive the spatial distribution of Salmonella Typhi in Fiji: a Vi-antigen seroprevalence study.** This is a geospatial analysis of environmental factors associated with seropositivity for typhoid Vi antibody. Analysis and writing were led by MSc student Rukie de Alwis, and the paper is under second round review at *Emerging Infectious Diseases* journal.

5. **Social mixing in Fiji: who-eats-with-whom contact patterns and the implications of age and ethnic heterogeneity for disease dynamics in the Pacific Islands.** A paper on social mixing in Fiji, which was under second round of review at *PLOS ONE* at the time of writing this introduction (September 2017). The seroepidemiological survey included questions on whom the participant ate with the previous day, by age and ethnicity, in order to determine mixing patterns of potential relevance to the transmission of typhoid and parameterisation of transmission models.

6. **Transmission dynamics of typhoid fever in Fiji: a modelling framework.** A paper, in preparation for submission to Vaccine journal, of a deterministic transmission dynamic model of typhoid approximating the situation in Fiji, utilising national surveillance data, the serological findings and the social mixing data.

7. **Discussion.** The thesis concludes with discussion of the findings and the merits and limitations of the approaches used in this thesis in investigating typhoid in Fiji. Alongside synthesising the country-specific consideration, it addresses the applications of these to wider understanding and control of typhoid fever and potential further research directions.

1.2.3 Appendices and additional related research

The appendices of the thesis comprise of both supplemental information and additional research papers related to the Fiji typhoid investigations.

In this final section of the introduction I outline the main papers of the appendices and outline concurrent or ongoing research that is not included in the bound thesis, including those pieces of work directly linked to the doctoral work and other concurrent research that is noteworthy in the narrative of the doctorate but not directly contributing to it.

Appendix paper A1:

Informed consent form and survey questionnaire.

1.2.3.1 Additional research on typhoid

I have peer-reviewed seven papers concerning typhoid or typhoid vaccination during the conduct of the thesis. One resulted in publication of an editorial.

Appendix paper A2:

Evaluating Typhoid Vaccine Effectiveness in Travelers' Vaccination. J Travel Med. 2015;22:76-77. doi:10.1111/jtm.12185. An editorial arising from peer-reviewing a Broome-method analysis of typhoid vaccine efficacy in returning travellers.

I am included as a co-author in a series of poly-authored papers by Dr Vanessa Wong (Sanger Institute & University of Cambridge) and colleagues by dint of contributing to sharing of *Salmonella* Typhi DNA from Fiji and through the reference laboratories of the UK national communicable disease surveillance centre, for an international phylogenetic mapping collaboration.

Non-enclosed papers:

Wong VK, Baker S, Pickard DJ, Parkhill J, et al. **Phylogeographical analysis of the dominant multidrug-resistant H58 clade of Salmonella Typhi identifies inter- and intracontinental transmission events.** *Nat Genet*. May 2015. doi:10.1038/ng.3281.

Wong VK, Baker S, Connor TR, Pickard D, et al. **An extended genotyping framework for Salmonella enterica serovar Typhi, the cause of human typhoid.** *Nat Commun.* 2016;7:12827.

International Typhoid Consortium, Wong VK, Holt KE, Okoro C, et al. **Molecular Surveillance Identifies Multiple Transmissions of Typhoid in West Africa.** *PLoS Negl Trop Dis.* 2016;10(9):e0004781.

1.2.3.2 Additional Fiji-Pacific Research

I have been engaged in collaborative projects stemming from the serological survey and other research activities, in part reflecting my ongoing role as a public health registrar.

Leptospirosis

The doctoral project also addressed leptospirosis, a waterborne bacterial disease transmitted through mammalian urine. I extended the serosurvey to run as a joint investigation into leptospirosis, strengthening the survey's cost-utility. Typhoid, leptospirosis and dengue are described by local physicians as the "three plagues of Fiji": flooding in 2012 led to leptospirosis outbreaks with 40 deaths amongst 576 reported cases (an observed case-fatality risk of 7%).[157] An expert-panel approach to leptospirosis was initiated by MOH and the

WHO shortly after the typhoid expert meeting, with synergies identified in serological information needs. Epidemiological risk factor collection was extended to include putative risk factors for leptospirosis, and additional survey clusters selected from high-risk areas. This has been led by Dr Colleen Lau of the Australian National University, and includes a fieldwork paper and the development of analytical methods at the interface of epidemiology and ecology.

Appendix paper A2:

Lau CL, Watson CH, Lowry JH, David MC et al. **Human Leptospirosis Infection in Fiji: An Eco-epidemiological Approach to Identifying Risk Factors and Environmental Drivers for Transmission.** *PLoS Negl Trop Dis.* 2016;10(1):e0004405.

Non-enclosed papers:

Lau C, Mayfield H, Lowry JH, Watson CH et al. **Unravelling Infectious Disease Eco-epidemiology using Bayesian Networks and Scenario Analysis: A Case Study of Leptospirosis in Fiji.** *Environ Model Softw.* 2017; 97: 271-286

Lau C, Mayfield H, Lowry JH, Watson CH, Kama M and Nilles EJ. **Using geographically-weighted regression to understand spatial variation in the influence of environmental drivers of infectious disease transmission: A case study of human leptospirosis in Fiji.** Under review at *The Lancet Planetary Health*

Dengue

The second additional disease investigated by this research programme came as a result of establishing the survey as a serum bank for other public health research for concerns such as dengue fever and other arthropod-borne viral diseases. The field survey concluded in the same month as an epidemic of dengue serotype 3 began in Central Division Fiji, and so a repeat visit to survey participants in 2015 enabled paired serology to be done pre- and post-epidemic to support understanding of epidemic dynamics and participant antibody dynamics against a backdrop of intermittent dengue outbreaks. This work is led by Dr Adam Kucharski of the London School of Hygiene & Tropical Medicine.

Non-enclosed paper:

Kucharski AJ, Watson CH, Kama M, Hue S, et al **Dynamics of dengue transmission and control during a large outbreak in Fiji.** In preparation.

Other Arboviral diseases

Arboviral disease research has been twice-further extended using these serosurvey samples. Investigations into Ross River virus are being conducted by Dr Mike Kama, Fiji national advisor for communicable disease control, at the laboratories of Prof John Aaskov at the Queensland University of Technology, Australia and with Dr Van-Mai Cao-Lormeau of Institut Louis Malardé, French Polynesia.[158] A third and likely final round of serum collection from the original survey participants in Central Division was completed in June 2017 by Alasdair Henderson, a doctoral candidate at the London School of Hygiene & Tropical Medicine. These investigations extended the Fiji dengue research programme and began Fijian serological investigation into an emergent infection in the Pacific islands, Zika.[159,160] This was previously considered to have little public health importance beyond uncommon occurrence of Guillain-Barré Syndrome but has now been associated with a major epidemic of microcephaly in South America, which saw the temporary declaration of a public health emergency of international concern.[161–163]

Trachoma

Field research on trachoma was being conducted by Colin Macleod at the time of my field investigations. Through advising on field operations and social science research methods, I was invited to be a co-author on a paper investigating the Fijian custom of eyelash-plucking, a major confounder in the surveillance of trichiasis and ocular *Chlamydia trachomatis*.

Non-enclosed paper:

Macleod C, Yalen C, Butcher R, et al. **Eyelash Epilation in the Absence of Trichiasis: Results of a Population-Based Prevalence Survey in the Western Division of Fiji**. *PLoS Negl Trop Dis*. 2017;11(1):e0005277. doi:10.1371/journal.pntd.0005277.

1.2.3.3 Concurrent Unrelated Research

Ebola

A further set of investigations in infectious disease epidemiology and vaccine science unrelated to typhoid but conducted during the timeline of the thesis warrants particular mention.

The devastating and tragic outbreak of Ebola virus disease in West Africa from 2013 to 2016 first closed off typhoid investigation using flagellin Hd immunoassays (intended to be run on a

10% survey serum subset for assay comparison), as the University of Maryland Center for Vaccine Development, the only institution running the assay, diverted all available resources to Ebola vaccine development at declaration of an international public health emergency in August 2014. Soon after this, I was seconded from the London School of Hygiene & Tropical Medicine to the WHO Ebola vaccine response based in Geneva.

The principle output of my Geneva collaboration was the design and implementation of a novel cluster randomised trial (a ring vaccination trial entitled “Ebola: Ça Suffit!”). The trial had a fighting chance at demonstrating vaccine efficacy by utilising an appreciation of infectious disease dynamics and the epidemic operating environment to create a trial with could be feasibly implemented under demanding field conditions. Interim and final analysis demonstrated high efficacy for the VSV-ZEBOV vaccine (Merck) in Guinea.

The second major piece of Ebola response research was to design and implement for the WHO (October 2015 to summer 2016) a VSV-ZEBOV vaccination cohort study in Guinean communities in which survivors resided. These communities were thought to be at risk of late transmission due to Ebolavirus persistence in semen and other body fluids. This study, “les proches des survivants” (those close to survivors), examines vaccine safety and immunogenicity in community members with baseline Ebola antibodies, asymptomatically acquired, relative to those without. Alongside leading the design and protocol writing of the vaccine cohort investigations, a substantial component of my work for “les proches des survivants” was to design, pilot and implement with field partners a comprehensive electronic data collection system compliant with Good Clinical Practice (GCP) trials standards. This used Android tablet computers running google.org’s OpenDataKit software and a system I designed of QR barcodes for recording participants, field staff and samples – this offers substantial potential for improving field deployment times for vaccine trials in future epidemics.

An ongoing extension of the ring vaccination trial research has been to examine the dynamics of transmission in and around the trial, including reconstruction of epidemiological chains of transmission and comparison to putative molecular phylogenetic trees. This examines the epidemiological assumptions of the original trial and their applicability for future epidemic trial design. My Ebola research portfolio also includes contributions to modelled projections of epidemic scenarios for response planning and trial planning, appraisal of the risks of semen-mediated late transmission of Ebola virus, and contribution to vaccine deployment under GCP conditions to contain post-epidemic flare-ups. These papers and projects are listed below.

Non-enclosed papers:

1. Ebola ça suffit ring vaccination trial consortium. **The ring vaccination trial: a novel cluster randomised controlled trial design to evaluate vaccine efficacy and effectiveness during outbreaks, with special reference to Ebola**. *BMJ.* 2015;351:H3740. doi:10.1136/bmj.h3740.

2. Henao-Restrepo AM, Longini IM, Egger M, Dean NE, et al. **Efficacy and effectiveness of an rVSV-vectored vaccine expressing Ebola surface glycoprotein: interim results from the Guinea ring vaccination cluster-randomised trial.** *Lancet*. 2015;386(9996):857-866. doi:10.1016/S0140-6736(15)61117-5.

3. Kieny MP, Longini IM, Henao-Restrepo AM, Watson CH, Egger M, Edmunds WJ. **Changes in the primary outcome in Ebola vaccine trial: Authors' reply.** *Lancet.* 2016;387(10027):1509-1510. doi:10.1016/S0140-6736(16)00686-3.

4. Henao-Restrepo AM, Camacho A, Longini IM, Watson CH et al. **Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ça Suffit!).** *Lancet.* January 2017. doi:10.1016/S0140-6736(16)32621-6.

5. Eggo RM, Watson CH, Kucharski AJ, Camacho A, Funk S, Edmunds WJ. **Duration of Ebola virus RNA persistence in semen of survivors: population-level estimates and projections.** *Eurosurveillance.* 2015;20(48):pii=30083. doi:10.2807/1560-7917.ES.2015.20.48.30083.

6. Kucharski AJ, Eggo RM, Watson CH, Camacho A, Funk S, Edmunds WJ. **Effectiveness of Ring Vaccination as Control Strategy for Ebola Virus Disease.** *Emerg Infect Dis J.* 2016;22(1). doi:10.3201/eid2201.151410.

7. Camacho A, Eggo RM, Goeyvaerts N, et al. **Real-time dynamic modelling for the design of a cluster-randomized phase 3 Ebola vaccine trial in Sierra Leone.** *Vaccine.* 2016. doi:10.1016/j.vaccine.2016.12.019.

8. Camacho A, Eggo RM, Funk S, Watson CH, Kucharski AJ, Edmunds WJ. **Estimating the probability of demonstrating vaccine efficacy in the declining Ebola epidemic: a Bayesian modelling approach.** *BMJ Open.* 2015;5(12). doi:10.1136/bmjopen-2015-009346.

Research in progress

Gsell PS*, Camacho A*, Kucharski A*, Watson CH* et al **Ring vaccination of adults and children with rVSV-ZEBOV under Expanded Access in response to an outbreak of Ebola virus disease in Guinea, 2016: an operational and vaccine safety report.** In press, Lancet Infectious Disease. *contributed equally

Eggo R*, Roberts A*, Watson C, Hue S, et al. **Chains of Ebola transmission in Guinea and the ring vaccination trial: findings from epidemiology and phylodynamics.** *contributed equally. In preparation.

Watson CH, Camacho A, Gsell PS, Carroll M et al. **Vaccination des proches des survivants**. An immunogenicity and safety cohort study in people connected to survivors of Ebola Virus Disease comparing those with and without baseline non-zero Ebola antibody titres. In preparation.

Watson CH, Marks M, Abdourahamane D, Roberts C, Gsell PS et al. **Design of a GCP-compliant electronic data collection system for vaccine and clinical trials using Android tablets and OpenDataKit (ODK).** In preparation.

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Chapter 2. A review of typhoid fever transmission dynamic models and economic evaluations of vaccination. Watson CH, Edmunds WJ. Vaccine. 2015. doi:10.1016/j.vaccine.2015.04.013.

2.1 Bridging section

This non-systematic review, completed in 2015, considers the role of published models in informing typhoid fever epidemiology and decisions around typhoid vaccine utilisation, including the economic appraisal of vaccination programmes. From the literature, data gaps that could support model estimates were identified. The Fiji fieldwork programme reflects efforts to close these gaps.

As noted in the thesis introduction, my proposed overall aims included a sub-aim on economic evaluation aspect to the transmission dynamic modelling of Fiji, consistent with the approaches used in national immunisation technical advisory groups such as the UK's Joint Committee on Vaccination and Immunisation. Cost-effectiveness analysis of possible Fiji typhoid vaccination programmes was curtailed on the grounds of feasibility within the timeframe of a PhD and with an eye to utility for in-country partners. This chapter offered a mechanism through which to engage with the health economic literature on typhoid vaccination. In terms of informing the formation of a model appropriate to the situation in Fiji, this literature review was a starting point for identifying relevant parameters and prior approaches to typhoid modelling.

Whilst structured frameworks exist for reviews, systematic reviews and meta-analyses in a number of areas of health and medical research, including PRISMA and its extensions,[1] the literature is thinner on methods specific to reviews of modelling. This chapter was informed by a review by Esther van Kleef on healthcare associated infection models,[2] and by seminar notes by Richard White.[3]

The review identified comparatively few transmission dynamic models of typhoid relative to similar burden infectious diseases, none using Bayesian methods, and found no economic analyses based on dynamic models, such as would meet current recommendations in accounting for indirect protection.[4,5]

There have been developments in typhoid models and economic evaluation since the review was published. Of reviewed typhoid models, those by the Pitzer group at Yale are foremost in informing current typhoid vaccination and control policy discussions.[6,7] The Pitzer group have

more recently published work on drivers of dynamics in Malawi[8] and Nepal[9] and on the cost-effectiveness of typhoid conjugate vaccines.[10] The latter, undertaken by Marina Antillón and colleagues ahead of updates to the WHO and GAVI position papers, used Bayesian parameter estimation (Hamiltonian Monte Carlo methods[11]) in a transmission dynamic model to produce typhoid vaccination economic evaluations in line with current recommended approaches to vaccine cost effectiveness analysis. This found 28%-43% of benefits were from indirect protection, which would not be captured in a static economic model.[10] Vaccination versus improvements to WASH components remains an unanswered question in the economic evaluation of in typhoid control.

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PLEASE NOTE THAT A COVER SHEET MUST BE COMPLETED FOR EACH RESEARCH PAPER INCLUDED IN A THESIS.

SECTION A – Student Details

Student	Conall Watson
Principal Supervisor	John Edmunds
Thesis Title	Seroepidemiological investigations of typhoid fever in Fiji and the potential role of vaccination in control

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?	Watson CH, Edmunds WJ. A review of typhoid fever transmission dynamic models and economic evaluations of vaccination. **Vaccine.** 2015.		
When was the work published?	doi:10.1016/j.vaccine.2015.04.013.		
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SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	CHW conducted the review & interpretation, wrote the initial draft and revisions. WJE contributed to the interpretation, and reviewed & edited the draft manuscript.

Student Signature: [redacted] **Date:** 12 Sep 2017

Supervisor Signature: [redacted] **Date:** 12 Sep 2017

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Review

A review of typhoid fever transmission dynamic models and economic evaluations of vaccination

Conall H. Watson, W. John Edmunds

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2. A review of typhoid fever transmission dynamic models and economic evaluations of vaccination.

Authors:

Conall H Watson and W. John Edmunds

Ethics:

Literature review, ethics approval not required.

Conflict of interest:

CHW and WJE have had travel and expenses paid for by the Coalition against Typhoid to attend meetings on the modelling of typhoid vaccination programmes. WJE has undertaken consultancy for the Coalition against Typhoid, which was paid to a fund held the London School of Hygiene & Tropical Medicine.

Role of the authors

CHW conducted the review and interpretation, wrote the initial draft of the manuscript and revisions. WJE contributed to the interpretation, and reviewed and edited the draft manuscript.

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Keywords

Typhoid, typhoid fever, enteric fever, *Salmonella* Typhi, vaccination, immunization, transmission dynamics, mathematical model, economic evaluation, cost utility analysis.

Highlights

- There are relatively few dynamic models or economic analyses of typhoid vaccination.
- The relative contribution of carriage to transmission is a key uncertainty.
- Published economic analyses use static models that omit indirect protection of vaccines.
- Nevertheless, vaccines appear highly cost-effective against WHO criteria in high-incidence settings.
- No economic model was found to compare vaccine and sanitation.

Abstract

Despite a recommendation by the World Health Organization (WHO) that typhoid vaccines be considered for the control of endemic disease and outbreaks, programmatic use remains limited. Transmission models and economic evaluation may be informative in decision making about vaccine programme introductions and their role alongside other control measures. A literature search found few typhoid transmission models or economic evaluations relative to analyses of other infectious diseases of similar or lower health burden.

Modelling suggests vaccines alone are unlikely to eliminate endemic disease in the short to medium term without measures to reduce transmission from asymptomatic carriage. The single identified data-fitted transmission model of typhoid vaccination suggests vaccines can reduce disease burden substantially when introduced programmatically but that indirect protection depends on the relative contribution of carriage to transmission in a given setting. This is an important source of epidemiological uncertainty, alongside the extent and nature of natural immunity.

Economic evaluations suggest that typhoid vaccination can be cost-saving to health services if incidence is extremely high and cost-effective in other high-incidence situations, when compared to WHO norms. Targeting vaccination to the highest incidence age-groups is likely to improve cost-effectiveness substantially. Economic perspective and vaccine costs substantially affect estimates, with disease incidence, case-fatality rates, and vaccine efficacy over time also important determinants of cost-effectiveness and sources of uncertainty. Static economic models may under-estimate benefits of typhoid vaccination by omitting indirect protection.

Typhoid fever transmission models currently require per-setting epidemiological parameterisation to inform their use in economic evaluation, which may limit their generalizability. We found no economic evaluation based on transmission dynamic modelling, and no economic evaluation of typhoid vaccination against interventions such as improvements in sanitation or hygiene.

2.2 Introduction

Typhoid fever is an exclusively human enterically-transmitted systemic disease caused by infection with the bacterium *Salmonella enterica enterica* serovar Typhi. Although largely controlled in Europe and North America, typhoid remains endemic in many parts of the world, notably Asia, where it is an important cause of febrile illness in crowded, low-income settings.[1] A notable feature of typhoid is the carrier state – asymptomatically infected individuals who continue to shed *S.* Typhi in their stool or urine for many years, thereby sustaining transmission.[2]

Despite a recommendation by the World Health Organization in 2008 that typhoid vaccination be considered for the control of endemic disease and outbreaks, programmatic use remains limited.[3]

In the early twentieth-century, public health officials were debating the best methods of evaluating typhoid vaccine effectiveness, and whether vaccination was a distraction from improvements in sanitation and hygiene.[4] These remain contemporary policy issues for ministries of health and other health partners who may be considering programmatic anti-typhoid vaccination as a counterpart to other anti-typhoid measures such as improvements to income distributions, sanitation, water supplies and hand washing with soap (post-defecation and before the preparation of food in the home or sold in the street) as well as identification and management of carriage.[5–8] Transmission dynamic modelling and economic evaluation are two informative tools to support such decisions.[9,10]

Where health budgets are limited, allocation of resources to activities which generate the best value for money maximises the population's health (not withstanding other health programme criteria such as equity). To compare between and across health states, cost utility analysis (CUA) can be employed using a common metric of health, such as disability-adjusted life-year (DALY). The World Health Organization's Choosing Interventions that are Cost–Effective project (WHO-CHOICE) describes interventions as "cost-effective" if they add a DALY at a cost of less than three times Gross Domestic Product (GDP) per capita, and "highly cost-effective" if each DALY costs less than GDP per capita. These are arbitrary thresholds and meeting them does not necessarily lead to the intervention being adopted, as health decision-makers are often required to make choices between multiple interventions that fall below these thresholds. Furthermore, even highly cost-effective activities may be too expensive overall for a health service to provide within budget: a hypothetical drug adding a year of life and costing GDP per capita for each person treated would require the entire national economy to be spent giving the drug to every member of the population.[11]

By building on the germ theory of disease, and mass-action principles from the physical sciences,[12] mechanistic mathematical modelling enables extrapolation beyond observed data, and can be used to project the expected trends of disease in a population or the potential impact of control strategies such as vaccination. Through capturing indirect effects of immunization – the reduced incidence of disease in members of a population not themselves immunized, commonly described as “herd immunity” – these transmission dynamic models capture the impact of such interventions more completely than static economic models measuring only the direct effects in vaccinees.[13]

In this review, typhoid transmission dynamic models and typhoid vaccine economic evaluations are examined for their potential contributions to informing disease control, identification of gaps in knowledge and indication of directions for further research.

2.3 Methods

PubMed was searched on 23 October 2014 without date restriction using the following terms: ("Typhoid Fever"[Mesh]) AND ("Nonlinear Dynamics"[Mesh] OR "Models, Theoretical"[Mesh] OR "Models, Statistical"[Mesh] OR "Computer Simulation"[Mesh] OR "Models, Economic"[Mesh] OR "Least-Squares Analysis"[Mesh] OR "Likelihood Functions"[Mesh] OR "Resource Allocation"[Mesh] OR "Cost-Benefit Analysis"[Mesh]) AND (Humans[Mesh]) NOT "Mice"[Mesh].

Personal libraries were reviewed and reference lists in papers searched for modelling and economic studies that may not have been identified by the above search strategy. Results were restricted to those available in English. We obtained information about unpublished studies through the Coalition against Typhoid and International Vaccine Institute.

Studies were included if they modelled typhoid transmission and/or analysed the cost-effectiveness of vaccination in endemic settings. Endemic settings were identified using recent high-quality reviews.[14,15] We included cost of illness (COI) studies if they were linked to an analytical study, and willingness-to pay (WTP) studies if they included an economic evaluation or were linked to an analytical study. Studies were excluded if they used geographical or statistical modelling, including time-series analysis, without transmission dynamics, or if they addressed transmission or cost-effectiveness in non-endemic populations, such as international travellers.

Transmission models were assessed for their model structure, data sources, parameter estimates, use of fitting methods, sensitivity analysis and the contribution of their approach to epidemiological understanding of typhoid. Economic studies were evaluated by data sources, economic evaluation approach, perspective, comparator programmes, use of sensitivity analysis and capture of indirect effects of vaccination.

2.4 Results

Seventy-nine titles were retrieved. Ten modelling papers were selected for review based on title or abstract. One was discarded as a non-mechanistic time series study[16], one as it modelled outbreaks in a non-endemic setting[17], while two papers were of the same model and considered together.[18,19] These are summarised in tables 2.1 and 2.2.

Table 2.1. Summary of typhoid transmission model types

Characteristic	**Number of models (n=7)**	**Reference**
Type of model		
• Compartmental	6	[18–24]
○ Deterministic		
○ Stochastic	0	
• individual-based stochastic	1	[25]
Scope of model		
• Analytical/mathematical,		
○ Without data	1	[22]
○ Uses data without fitting	1	[23]
• Exploratory/epidemiological,		
○ uses data without fitting	1	[21]
○ fitted to data	1	[25]
• Policy-oriented/public health,		
○ uses data without fitting	2	[18–20]
○ fitted to data	1	[24]
Parameter-fitting method		
• Maximum likelihood estimation	2 of 2	[24,25]
• Bayesian	0	
Investigates vaccination	4	[18–21,24]
• Compares with improved sanitation, hygiene or water supply	4 of 4	[18–21,24]
• Include economic evaluation of vaccination	2 of 4	[18–20]

Table 2.2a. components and main findings of typhoid transmission models

	First author and year	Ref	Model type	Disease states	Data source(s)	Fitting process	Interventions modelled	Time horizon	Sensitivity analysis	Findings	Comments
1	Cvjetanović 1971, 1978	[18] [19]	Compartmental deterministic with births = deaths, without age-structure	N S E_s E_a I_s I_a C_t C_l R_t R_l	Parameters estimated using literature and expert opinion. Considers an eidemiological scenario approximating Western Samoa.	None	Vaccination with whole-cell inactivated vaccines, VE 60%, 75% or 90%, coverage 60, 80 or 100%. As one-off or 5 yearly campaigns. Sanitation	60 years	Epidemiological/ clinical parameters fixed. Effective contact rate (per capita per day) varied.	For both low and high VE, single vaccination campaigns achieve temporary reduction in incidence rates before return to a rate determined by the force of infection, where force of infection is above an elimination threshold. Sustained reduction in force of infection reduces incidence. Multiple vaccination campaigns reduce incidence will campaigns are sustained.	Multiple parameters are included without fitting. Outputs should be considered illustrative.
2	Briscoe 1980	[22]	Deterministic analytical SIS	S I	N/A	N/A. Reviews Cvjetanović models. Analysis of role of force of infection and recovery on equilibrium prevalence.	N/A	N/A	N/A	Force of infection determines prevalence, and vice versa. Stochasticity may prevent disease eradication.	Intended as an analytical model rather than epidemiological simulation.

	First author and year	Ref	Model type	Disease states	Data source(s)	Fitting process	Interventions modelled	Time horizon	Sensitivity analysis	Findings	Comments
3	Bailey 1982	[23]	Compartmental deterministic with births = deaths, without age-structure	S E I C R	[18]	Rule-based simplification of Cvjetanović 1971 model[18] with direct mathematical solution of steady-state equations.	N/A	N/A	N/A, suggests an approach to sensitivity analysis [26]	For a steady-state model, structural simplification results in compartment population estimates consistent with the unsimplified model for a given effective contact rate.	Reducing the number of compartments makes a model more suitable for validation with data.

	First author and year	Ref	Model type	Disease states	Data source(s)	Fitting process	Interventions modelled	Time horizon	Sensitivity analysis	Findings	Comments
4	Cvjetanović 1986	[20]	Age-structured compartmental deterministic SIRS. Birth and death rates from Chile	N S I C_t C_l R_t R_l	Demographic and typhoid surveillance data for Santiago and rest of Chile	Effective contact rate per capita per unit day (age-specific for acquisition) from linear interpolation of age-specific incidence. Visual goodness-of-fit. Strong assumption that 20% of all cases are clinical.	Vaccination with Ty21a, 95% VE at 75% or 95% coverage of under 25s with 5yrly revaccination. Food sanitation in schools reducing force of infection by 1/3 in ages 6 to 16y. Sanitation with annual 2% or 5% improvement in force of infection over 10 years.	Interventions analysed over 25y after run-in to equilibrium.	None	Vaccination campaigns would reduce age-specific incidence and increase the age of peak incidence Vaccination would not eliminate disease over 25y but would result in year on year reduction in incidence if sustained. 10y sanitation campaigns likely to reduce prevalence and continue to reduce prevalence after cessation.	Somewhat simplified model structure, though now age structured. The model is not validated sufficiently against data, nor are outputs sufficiently clear to make strong policy conclusions. Age-based changes in incidence with vaccination are consistent with epidemic theory.

	First author and year	Ref	Model type	Disease states	Data source(s)	Fitting process	Interventions modelled	Time horizon	Sensitivity analysis	Findings	Comments
5	González-Guzmán 1989	[21]	Compartmental deterministic SIS structure with births and deaths	S I V with environmental transmission	Parameter estimates for Chile	None, analytical model	Reductions in combinations of: • carrier prevalence • indirect contact rate • direct contact rate • environmental life of the bacterium • bacterial count in the environment. Vaccination with Ty21a, coverage scaled for equivalence to VE 74% or 95%.	10y	N/A	Decline in incidence is not rapid, even with highly effective combined control measures. Reduction in chronic carriage most effective control procedure. Vaccination as a permanent programme would require a high proportion of the population to become immune to control typhoid within a meaningful timeframe.	Author cautions against using the model to estimate the effect of a vaccination programme but that it indicates areas for further epidemiological parameter determination.

	First author and year	Ref	Model type	Disease states	Data source(s)	Fitting process	Interventions modelled	Time horizon	Sensitivity analysis	Findings	Comments
6	Saul 2013	[25]	Individual-based stochastic, random-mixing.	S E I_s I_a C_t C_l R_t R_l, R_c, ;R_s; V_c V_s	Surveillance data from Dhaka, Bangladesh, and Kolkata India. Migration, birth and death rates from Matlab, Bangladesh. Other parameter from literature and expert opinion.	Maximum likelihood and visual inspection	None	40y to equilibrium and 40y follow-up. 20y for effects of carriage.	Sensitivity analysis on refractory period from birth.	Distinguishes between sterile immunity and clinical Immunity (in which individuals can be infected but not develop disease). Multiple infections needed to develop sterile immunity. Natural immunity is likely to be long-lasting but needs further field investigation. Carriage stabilizes dynamics, and is particularly important in lower incidence settings.	Complex agent based model, limited availability of epidemiological data results in issues of parameter identifiability. Plausible combinations of parameters identified.

	First author and year	Ref	Model type	Disease states	Data source(s)	Fitting process	Interventions modelled	Time horizon	Sensitivity analysis	Findings	Comments
7	Pitzer 2014	[24]	Compartmental, age-structured deterministic	S_1 S_2 I_1 I_2 R C W	Surveillance case series, Vellore, Tamil Nadu, India	Two-stage fitting with Latin hypercube sampling of starting parameters. Maximum likelihood estimation, simplex method.	Vaccination with: Ty21a, (VE 48%, duration =natural immunity), Vi polysaccharide (VE 80%, 3y), Vi conjugate (VE 95.6%, 19.2y). Vaccination of school age children as a campaign, routine vaccination of 6 year olds, or both together. Improvements in water and sanitation over 30y	50y to quasi-steady state and 25 y follow-up	Multi-parameter sensitivity analysis in model fitting.	Basic reproduction number is around 3 in Vellore and 7 in Dhaka. Natural immunity is likely to be long-lasting. Vaccination campaigns would not eliminate disease in Vellore but instead see disease rebound in 5 to 10 y. A campaign plus routine immunization could result in a new lower incidence disease state. High baseline carriage rates reduce the indirect protection of vaccines – understanding carriage prevalence should be a disease control priority. In most circumstances modelled, improvements in hygiene & sanitation have more impact than vaccination.	Best fitting parameter sets were highly sensitive to initial parameter selection. Identifies carrier transmissibility and relative contributions of short- and long- cycle as import epidemiological sources of uncertainty.

VE = vaccine efficacy. Effective contact rate is the rate at which two individuals come into contact per unit time, with the nature of the contact being such that if one was infectious and the other susceptible, infection would be transmitted.

Table 2.2b. Disease states in typhoid models

Abbeviation	Disease state	Comment
N	Newborn	Susceptible in Cvjetanović's model, refractory in Saul's
S S_1 S_2	Susceptible; Fully and partially susceptible	
E; E_s; E_a	'Exposed'; Symptomatic or asymptomatic	Infected but not (yet) infectious
I; I_s I_a I_1 I_2	Infectious; Symptomatic or asymptomatic Primary or subclinical infection	Primary infection of a fully susceptible individual or asymptomatic/ subclinical infection of a previously partially susceptible individual
C; C_t; C_l	Carrier; temporary; long-term	
R; R_t; R_l; R_c, ;R_s	Removed/resistant/refractory/recovered; Temporary immunity; long-term immunity; Natural immunity to clinical disease; natural sterile immunity)	Not able to be infected, immune. Clinical immunity is against disease but allows infection and onward transmission. Sterile immunity is against any infection.
V V_c, ; V_s	Vaccinated Vaccine-induced immunity to clinical disease; Vaccine-induced sterile immunity	
W	'Water'	Long-cycle transmission from water or environmental contamination, contributed to by all infectious or carrier classes.

A further, as yet unpublished, transmission model has been developed by the International Vaccine Institute (personal communication, Jin Kyung Park) and is not reviewed here.

Seven titles were identified as economic evaluation and obtained for full-text review (tables 2.3 and 2.4) alongside two underpinning COI studies and one underpinning WTP study (table 2.5).

Table 2.3. Summary of typhoid vaccine economic evaluation types

Characteristic	**Number of studies (n=7)**	**Reference**
Based on field studies	5	[27–31]
Perspective:		
• Public sector only	2	[27,32]
• Private only	1	[29]
• Societal (public and private) ○ Include intangible costs of pain, suffering and disability	4 3	[28–31,33] [28,30]
Analytical approach: (a study can include more than one approach) • Cost-benefit analysis component • Cost-effectiveness analysis component • Cost-utility analysis component • Willingness-to-pay component • Price-optimisation model	 4 2 2 4 1	 [29–32] [27,32] [30,33] [27–29] [27]
Include indirect protection of vaccines • Include transmission dynamics	1 0	[27]
Evaluates improve sanitation, hygiene or water supply as an alternative to or adjunct to vaccination	0	

Table 2.4 Components and main findings of typhoid vaccine economic evaluations

	First author, year, reference	Analytical approach	Economic perspec-tive	Setting	Burden of disease	Costs	Vaccine intervent-ion modelled	Vaccine effective-ness	Time horizon	Discount-ing	Disease dynamics	Sensitivity analysis	Data source(s)	Findings
1	Musgrove 1992 [32]	CBA CEA	Public sector	PAHO SIREVA countries	150 cases per year per 100k population. CFR1% Does not cost pain, suffering or death.	Vaccine programs and clinical/ field trials or pilots.	Mass vaccination; reducing number of doses over time.	Estimated 90%	14 and 24 years	10%pa,	No	Program administration costs, vaccine costs, delay between accrual of costs and benefits	Expert opinion	Describes incidence, treatment costs and vaccination costs at which a program would be cost-neutral
2	Shepard 1995 [33]	CUA, cost per QALY.	Public sector costs; societal benefit captured as QALYs	Countries with middle, high or very high U5MR	1.5 cases per person per lifetime. CFR 1.8% Morbidity is excluded from QALY estimates	Marginal costs of additional vaccination within a childhood programme	By birth cohort, two doses	Anticipated 80% over 10y	10 years	3%, costs and benefits	No. Steady states pre- and post-vaccine program start. Assumes disease most common in late childhood or early adulthood.	1. Dose cost at USD50/ QALY. 2. Vaccine development costs.	Expert opinion; extrapolation of high incidence epidemiological studies [34]	Preliminary estimate of highly CE (<USD50 per QALY, 1992 price) if data assumptions are valid. Critical parameters are incidence, CFR, VE, vaccine costs
3	Poulos 2004 [31]	CBA	Multi-dimensional public sector and societal	Kalkaji slum, New Delhi, India	As per [35]. Does not cost pain, suffering or death.	As above. Public funded vaccine programme.	Campaign with 80% coverage of: age 2-5, age 6-19, or all-age.	70% for 3 years	3 years	10%	No	Incidence; Vaccine cost; Ratio of total economic benefit to measured COI	Bahl 2004 [35]	Immunization of 2-5year olds is cost saving to the public sector in a high incidence setting. Sensitivity analysis and inclusion of private costs suggest vaccination of other ages may also be highly CE.

	First author, year, reference	Analytical approach	Economic perspec-tive	Setting	Burden of disease	Costs	Vaccine intervent-ion modelled	Vaccine effective-ness	Time horizon	Discount-ing	Disease dynamics	Sensitivity analysis	Data source(s)	Findings
4	Canh 2006 [29]	WTP, contingent valuation, CBA.	Private	Hue, Vietnam	Raised incidence 1995-9; associated with outbreak in 1996 Benefits measured by WTP.	Proposed USD 0.67 1.70 3.30 6.70 13.30	N/A	Proposed: 70%, 3y; 70%, 20y; 99%, 3y; 99%, 20y	N/A	Inherent	Typhoid perceived to be in decline by 67% of participants	N/A	Cross sectional survey in 2002 of households with children	Survey participants are more sensitive to price than to expected vaccine efficacy or duration of protection. Modest user fees could support a vaccination programme.
5	Cook 2008 [30]	CUA	Public sector and societal	Kolkata, India; Karachi, Pakistan; North Jakarta, Indonesia; Hue, Vietnam	Highest in the sites within Karachi and Kolkata, lowest in Hue. Reported incidence double to account for false negative blood cultures. DALY weight 0.27, illness duration 7d CFR 1%.	Private direct and indirect cost of illness obtained in interviews with confirmed cases, public costs obtained from health facilities. Public and private vaccination costs from literature and estimation.	Campaigns: 1. School children 5 to 14y 2. Children aged 2 to 15y 3. All 2y+	65%, 3y	Over duration of vaccine	3%	No	Single parameters and Monte Carlo across all parameters, triangular distribution. VE 55% to 75%, duration 2to4y,vaccine cost USD 0.40 to 0.80 (2007 prices), delivery cost variable. CFR 0.5% to 3%, illness duration 4d to 3w, DALY weight 0.08 to 0.47.	DOMI	No programmes would be cost saving but (school-) child immunization would be very CE to health services or society in all but Hue, including under sensitivity analysis. Adult vaccination in Kolkata and N.Jakarta is less CE but still meet thresholds. Surveillance likely reduced illness costs through early diagnosis.

	First author, year, reference	Analytical approach	Economic perspec-tive	Setting	Burden of disease	Costs	Vaccine interven-tion modelled	Vaccine effective-ness	Time horizon	Discount-ing	Disease dynamics	Sensitivity analysis	Data source(s)	Findings
6	Cook 2009 [28]	CBA total economic benefits vs costs 1. Societal COI 2. above + Value of statistical life (VSL) saved 3. WTP (contingent valuation) + public costs	Societal	Tiljala and Narkeldanga slums, Kolkata, India	3.4 case per 1000 2 to 4 y 4.9 per 1000 5to15y 1.2 per 1000 16y+ DALY weight 0.27 CFR 1%,	Total marginal vaccine cost USD (2007) $1.11 WTP as per [36] VSL from literature.	Campaigns: 1. School children 5 to 14y 2. Children aged 2 to 15y 3. All 2y+	65%, 3y	1 year cost, 3 year benefits	3%	No	As per [30]. VSL varied by 50%.	Kolkata [37]	Economic perspective 1 is not cost neutral, but perspectives 2 and 3 indicate benefits greater than cost across all campaign strategies. Sensitivity analyses suggest WTP and VSL models show net benefit for all campaign strategies across most parameter sets.
7	Lauria 2009 [27]	Optimization model: different adult & child pricing, implicit CEA	Public sector	Hypothetical population	3.5 annual cases per 1000 children and 1per1000 adults	As per [38]	Price-dependent uptake	70%, 3y	3y	8%	Possible herd protection described in a sensitivity analysis, with variable adult and child transmissibility.	Monte Carlo simulation, allowing most parameters to vary.	Five Asian countries[37]	There is minimal advantage to different vaccination charges for children and adults under the static model. Herd protection greatly influences case numbers and value.

CBA = cost-benefit analysis; CE = cost-effective(ness); CEA = cost-effectiveness analysis; CFR = case-fatality rate (proportion of cases that result in death); CUA = cost-utility analysis; COI = cost of illness; DALY = Disability adjusted life-year; DALY weight = a scale from 0 (perfect health) to 1 (death). DOMI =Diseases of the most impoverished programme [39]; PAHO = Pan-American Health Organization; SIREVA = Sistema Regional de Vacunas (Regional Vaccine System); U5MR = under-five mortality rate; USD = United States Dollars; VE = vaccine effectiveness; WTP = willingness to pay

Table 2.5 Components and main findings of cost of illness studies and willingness to pay studies used in typhoid vaccine economic evaluations

	First author, year, reference	Analytical approach	Economic perspec-tive	Setting	Burden of disease	Costs	Vaccine interventio n modelled	Vaccine effective-ness	Time horizon	Discount-ing	Disease dynamics	Sensitivity analysis	Data source(s)	Findings
1	Bahl 2004 [35]	Cost of Illness	Multidimen sional public sector and societal costs	Kalkaji slum, New Delhi, India	Culture confirmed incidence per year: 17 per 1000 under 5s; 12 per 1000 5-18y; 1 per 1000 >19y	Public sector/ institutional and private costs, comprising direct medical, direct non-medical and indirect costs; for hospitalized and non hospitalized	N/A	N/A	One year surveillance	N/A	No. Decline in incidence rate with age is informative of an immunizing infection.	With both most conservative and least conservative cost estimates, and with incidence both on confirmed and clinically suspected disease.	Cohort study 1995-6, weekly interviews and passive surveillance.	Costs are high per episode regardless of age, both private and public/ institutional. Hospitalization and non-response to antimicrobials increase costs
2	Poulos 2011 [38]	COI	Public and private (direct and indirect)	Hechi, China; North Jakarta, Indonesia; Kolkata, India; Karachi, Pakistan; Hue, Vietnam.	Highest in the sites within Karachi and Kolkata, lowest in Hechi and Hue.	Measured by questionnaire, with estimates for nonmarket activities. Karachi costs from expert information.	N/A	N/A	N/A Interviews at 7, 14 and 90d from disease onset.	N/A	N/A	N/A	Interviews with cases or carers.	Total episode costs range from USD 15 to132. Private costs exceed public costs unless reimbursed. Hospitalization adds to costs substantially. 14 to 49% of households borrowed money to pay for treatment. Costs of drug resistant infection are higher, but not significantly so.

	First author, year, reference	Analytical approach	Economic perspec-tive	Setting	Burden of disease	Costs	Vaccine interventio n modelled	Vaccine effective-ness	Time horizon	Discount-ing	Disease dynamics	Sensitivity analysis	Data source(s)	Findings
3	Whittingto n 2009 [36]	WTP	Private	Tiljala slum and Beliaghata neighbour hood, Kolkata, Inida	2 case per 1000 population per year, peak incidence in older children and teenagers	Proposed USD (2007) 0.22 0.56 1.11 11.11 And sliding scale.	Price-dependent uptake	70%, 3y	N/A	Inherent	N/A	N/A	Cross sectional survey of households with children.	9% would decline a vaccine, with a further 7% only accepting free vaccine. WTP is at least USD2. Vaccines for children were valued higher than those for adults. Time to think reduces willingness to purchase vaccine.

A further COI study was identified but excluded as not linked to a published economic evaluation.[40]

There was minimal overlap found between transmission modelling and economic evaluation. Of the transmission dynamic models, only those by the Cvjetanović group also had cost-effectiveness components.[18–20] One economic study included quantitative consideration of indirect protection.[27]

2.4.1 Transmission dynamic models

The seven typhoid models identified range from two-state analytical tools to complex individual-based simulation or multi-state compartmental models (see table 1). Only two models were formally fitted to data.[24,25]

The structures of models (table 2a and 2b) are based on different assessments or representations of the natural history of typhoid fever, particularly in how immunity to *S.* Typhi is considered. González-Guzmán suggests that natural partial immunity is likely to arise but simplifies to a model with vaccine immunity only, noting that sufficiently high infectious doses can overcome immunity.[21] Pitzer uses population compartments to separately represent immunity against typhoid infection ('sterile immunity'), and immunity against typhoid disease ('clinical immunity'), allowing transition from the latter to either full susceptibility or to a subclinical infection that in turn restores full sterile immunity in the individual. This corresponds to immunity boosting repeated infection cycles without overt disease, particularly in adults after recovery from clinical disease in childhood, and allows bacterial shedding during subclinical infection to contribute to sustained transmission.[24] Saul similarly models both sterile and clinical immunity, with infection resulting in sterile immunity that wanes to clinical immunity (potentially after zero time) and then to susceptibility, and explores a range of hypothetical state-transition scenarios based on multiple infections, though he does not clearly resolve a most-likely scenario.[25] Despite asymptomatic boosting being a long-standing hypothesis, or perhaps because of it, there is a paucity of data from microbiological, immunological or epidemiological studies to parameterise models or to validate assumptions.[41]

While noting leaky immunity in those naturally infected (each individual has a reduced probability of further infection), González-Guzmán models Ty21a oral vaccine protection as

all-or-none, giving each vaccinee a probability of developing immunity or not. In this model, those who develop immunity following vaccination have 100% protection against typhoid, until vaccine wanes and they return to full susceptibility, an approach also applied by Cvjetanović.[18–21] Pitzer handles injected Vi vaccination the same, noting results were similar in a sensitivity analysis assuming leaky vaccine immunity. Pitzer represents oral Ty21a vaccination as akin to natural immunity, transitioning vaccinees to clinical immunity after full immunity wanes.[24]

While vaccination programmes are predicted to reduce typhoid incidence, uncertainty around carriage prevalence, duration and contribution to the force of infection substantially affects vaccines' projected impact.[21,24,25] In reviewing Cvjetanović's 1978 model,[19] Anderson and May observe that the implicit assumption that the effective contact rate for carriers is equal to that of acute cases, combined with other fixed parameter estimates, gives carriers a contribution to transmission ten times that of other cases.[42] While illustrative of the potential contribution of carriage in sustaining disease, for policymaking it has been recommended that such assumptions should be tested against data.[43,44] An approach might be to conduct systematic, detailed investigation of incidence cases to identify potential sources, using suitable screening methods to look for carriers as well as acute cases, and combine this with population-level carriage surveys and water quality studies. While labour-intensive, such investigations could be integrated into wider control efforts.[45]

Chronic *S.* Typhi carriage can be treated with antibiotics and/or cholecystectomy for gallstone-associated infection, but there is no demonstrated role for vaccination in clearance of carriage.[46,47] Premised on this, Cvjetanović's and Pitzer's models demonstrate that where carriage contributes substantially to transmission in an endemic settings, vaccination is unlikely to result in elimination in the short-to-medium term.[18,24] Similarly, where carriage rates are high the indirect protective effects of vaccination are reduced, as the risk to the unvaccinated of acquiring disease from carriers is not diminished.[24,42] The contribution of carriage, however, requires further epidemiological assessment, as does the role of short-cycle and long-cycle (environmental) transmission.[21,24,25]

If immunization of the susceptible population does not bring about typhoid elimination, then measures to reduce per case or per carrier infectivity, such as improved sanitation or hand washing with soap, might be considered instead of or in conjunction with vaccination.[48,49] The multi-compartment models suggest such a reduction in effective contact rates could lead to important reduction in prevalence.[21,23,24] This is consistent with Briscoe's analytical model.[22]

Another feature of transmission dynamic studies is that the average age of infection increases as the force of infection decreases, for example, with the introduction of vaccine.[20] This is consistent with burden of disease studies which find earlier average age of infection in settings with higher disease incidence.[14,35]

2.4.2 Economic evaluation

Our literature search found seven papers evaluating typhoid vaccine cost effectiveness. The earliest two of which were based on values derived from expert opinion and are less informative to current policy considerations than the most recent five which were based on field studies, as outlined in tables 3 and 4. Two supporting COI studies and one supporting WTP study are outlined in table 5. These field-informed analyses share multiple common authorships with collaboration through the Diseases of the Most Impoverished (DOMI) programme. Of the seven economic evaluations, four included a cost-benefit analysis (CBA), two a cost-effectiveness analysis (CEA) and two a cost-utility analysis (CUA). Four of these used a societal perspective [28,30,31,33]. Only one evaluation considers indirect protection quantitatively, but uses hypothetical values for herd immunity from different coverage levels rather than estimates from dynamical modelling.[27] No studies considered improvements in sanitation, hygiene or water supply as an alternative or adjunct to typhoid vaccination.

In a one-year study of a very-high typhoid incidence area – Kalkaji slum, Delhi – Bahl found average costs per episode of illness were high to both the health sector and families, excluding intangible costs such as pain, with hospitalization an important component of health service costs.[35] A CBA by Poulos on this data reported that a vaccination programme for children under five years of age would be cost-saving to the public sector. Analysis from a societal perspective, incorporating private costs, indicated that vaccination in high incidence settings with modestly-priced vaccines could also have net benefits in other age groups.[31]

Cook and colleagues conducted a CUA based on field data from multiple Asian sites,[30,38] and found that while typhoid vaccination using the Vi-polysaccharide across adults and children would be unlikely to be cost saving to the public sector, in high incidence settings it was likely such a programme would meet the standard for “very cost-effective” health interventions of less than per-capita gross domestic product (GDP) per DALY gained. In these settings, targeting vaccination to the highest incidence age-groups improved cost-effectiveness substantially. Through sensitivity analysis, the main determinants of cost-effectiveness identified were vaccine cost, case-fatality rates (CFR), vaccine duration of

protection, (baseline) incidence and vaccine efficacy. Cost-effectiveness was insensitive to vaccine coverage as no indirect protection was assumed.[30]

WTP has also been used in economic evaluation of typhoid vaccines as an alternative approach to COI in valuing private or societal benefits. Such an approach is considered to demonstrate the value individuals place on the total benefit of the vaccine, though is confounded by ability to pay, and ability to value public sector activities foregone if vaccines are supplied through the state.[50] One study from Hue, Vietnam suggested that typhoid vaccination would pass a social cost-benefit test (total costs less than total societal benefit), based on demand estimates from a contingent valuation survey addressing hypothetical vaccine purchases for householders and their children.[29] Analysis by Cook of a similar WTP study done in Kolkata, India, by Whittington and colleagues, suggested that vaccination of children or all-ages would not pass a social cost-benefit test using COI , but costing benefits using WTP plus public costs would likely find that such programmes pass such a test. [28,36]

WTP studies are also informative to vaccine uptake, with 9% of respondents in the Kolkata survey stating they would not accept a free vaccine, with data suggesting these individuals are more likely to be older, have lower income and never boil drinking water.[36] In the Hue survey, Canh suggests a number of issues affect validity of economic evaluation using WTP estimates, noting that householders were most sensitive to price, with proposed vaccine efficacy making no detectable difference to individual or household demand at a given price.[29]

Observing that typhoid vaccines are equivalent to one-sixth of per-capita public sector health spending in India, Cook notes the potential for user fees in financing a state-administered programme.[28] Whittington's Kolkata WTP survey suggested vaccine protection for children was given greater value than vaccinating adults.[36] Drawing on data from this study, a price optimisation model by Lauria of different vaccine prices for children and adults did not find a strong case for differential pricing, but in a non-dynamic sensitivity analysis of potential indirect protection scenarios found herd immunity to be a significant influence on incidence and cost-effectiveness.[27]

2.5 Discussion

This review found a relatively sparse literature on typhoid modelling and vaccine economic evaluations. Of seven transmission models found, only two were published in the last 25

years and use contemporary data-fitting methods. All five field-based economic evaluations we found shared multiple common authorships around the DOMI collaboration.

Although the Global Burden of Disease is a much-criticised ranking, it provides some comparator infectious diseases, with measles and syphilis ranked close to typhoid and paratyphoid fever, and cholera attributed around one-third of the annual number of DALYs.[51] Repeating our (non-comprehensive) PubMed typhoid search strategy for these returned over six times as many measles papers, three times as many syphilis papers, and twice as many cholera papers. Typhoid fever transmission and economic evaluation appears relatively under-studied. Typhoid's low profile-to-burden ratio has variously been attributed to ubiquity in developing countries, inadequate diagnostic tools, the absence of champions in health agencies, and affecting mostly the poor and the underclasses. [52] Effective antibiotics and previous non-availability of a long-acting infant vaccine have also been cited as reasons for the absence of internationally-funded typhoid vaccination programmes.[52] For budget-constrained national health agencies, typhoid vaccination programmes may appear unattractive unless public sector cost saving can be demonstrated, for example, if the incidence rate is very high.[31,35]

Considering only a health services perspective – of treating typhoid and providing vaccine – omits private costs associated with the disease and therefore underestimates the societal impact of typhoid. Various approaches have been taken to address these costs and more completely capture the benefit of vaccination, from cost of illness studies for private expenses, estimates of health utility forgone due to illness, or by determining the extent to which people value vaccination in willingness-to-pay studies. While user fees and WTP are controversial,[10,50] economic evaluations that consider these have been consistent with typhoid economic analysis using more widely-accepted cost-utility analysis and private cost of illness. These CUAs suggest typhoid vaccine programmes are likely to be highly cost-effective (against international norms) where disease is highly endemic, particularly when targeted to the age-groups at highest risk of disease. The economic analyses, which do not include mechanistic components for transmission, emphasise as key drivers of cost-effectiveness the vaccine cost, case fatality rates, baseline incidence, vaccine efficacy and duration of protection.

We did not find any economic evaluation comparing typhoid vaccination programmes against other potential means of typhoid control, such as measures to improve sanitation, hygiene or water supplies. It is still not possible to answer the century-old question of whether, in a given setting and within a limited budget, vaccination should be adopted over improved

sanitation and hygiene, or what combinations are optimal for control under what circumstances. Such analysis would need to be based on a transmission dynamic model, with extensive epidemiological surveillance for detailed burden of illness measurement, and comprehensive costing for CUA or CBA approaches that allow other diseases to be included in the evaluation.

The only published data-fitted transmission model of typhoid vaccination suggests that while vaccination is effective in reducing disease incidence, if other measures are not enacted to reduce the ongoing force of infection, particularly from asymptomatic carriage, short or medium-term vaccination campaigns are unlikely to result in elimination and would see disease rebound if vaccination stopped.[24] The authors of recent dynamic models emphasise our lack of understanding of certain aspects of the natural history of typhoid (particularly around acquisition of immunity, the role of carriers, and the contribution of short- and long-transmission cycles). [21,24,25] A model intended to examine what role the putative different forms of *S.* Typhi immunity have in determining typhoid incidence rates found an absence of suitable immuno-epidemiological data on which to fit parameters and make strong inferences, a challenge further compounded by the absence of age structure in the model.[25]

Transmission dynamic modelling and a non-mechanistic economic analysis have shown that the level of indirect protection may have an important impact on vaccine effectiveness and cost-effectiveness respectively.[24,27] None of the economic models mechanistically consider disease dynamics and so cannot scientifically appraise the indirect effects of vaccination in cost-effectiveness calculations. Indeed, while a number of economic analyses readily acknowledged indirect effects as an important phenomenon, they specifically excluded them, citing the absence of evidence for Vi polysaccharide vaccine herd immunity pending the publication of cluster randomised controlled trials.[28,30,53,54] Early work by Cvjetanović is the only meeting point we found of mechanistic typhoid transmission modelling and economic analysis, but the complexity of this model and absence of fitting make it difficult to apply findings to contemporary disease control problems. [18,19]

Using a static economic model premised only on direct protection in vaccinees may be a reasonable approximation in some situations, such as if vaccine-preventable new typhoid cases (symptomatic or otherwise) make a relatively small contribution to the force of infection compared with carriers and the unimmunised. Even in such circumstances, prior assessment with mechanistic modelling of field epidemiological data would be appropriate in estimating the relative contributions of each group to transmission.

While there seems limited inter-disciplinary dialogue between typhoid modellers and economists, a unifying concern is the importance of accurately determining age-based incidence rates, which can be highly variable within-country or between otherwise similar settings, and are central to estimates of vaccine impact and cost-effectiveness. Heterogeneity in disease rates, transmission mechanisms and health service provision may limit external validity of both typhoid modelling studies and economic evaluations.

Accurate assessment of disease burden could be done with large, population-based studies to inform incidence, complications and case-fatality rates, using blood culture confirmation of cases, or altogether improved diagnostics.[30,55–57] It should be noted that even well-conducted studies are unlikely to provide unbiased estimates, due to the positive health consequences of introducing disease surveillance. Bahl notes that active surveillance with early treatment gave rise to disease that is less severe, and less expensive, than disease detected through passive surveillance.[35] This is echoed in the DOMI disease burden study, which rather than the 1% case-fatality rate widely cited in literature, had a zero percent CFR amongst the 475 cases detected (which gives an upper 95% confidence interval of around 0.63%).[30,37,58]

Other field epidemiology and laboratory investigations could further inform typhoid transmission dynamics:[59]

- Large, population-based, *S.* Typhi carriage studies, similar to those done in Chile in the early 1980s, [5] to determine prevalence in a range of endemic settings, potentially with serological surveys, [60] alongside investigation of potential sources of infection amongst new cases.
- Serological assay development and population-based serological surveys to determine past infection to *S.* Typhi, natural immunity and waning of this immunity. Seroprevalence could be linked to surveillance records to estimate the proportion of infections that are clinically apparent and notified to national authorities.
- Epidemiological time series with consistent, transparent methodology and/or cross-referencing between methods.[61]
- Age-based social contact pattern surveys, which may inform short-cycle transmission.[62]

For models to assess vaccination against other enteric fever control measures, findings could be incorporated from interventional field studies on the role of improvements to sanitation, hygiene and water supply in changing disease incidence and transmission. Ongoing scrutiny of vaccine efficacy and duration of vaccine protection may also be informative. Estimates of

efficacy of Ty21a vaccines in recent systematic reviews are less than in the data sources for early modelling, with the reviews focusing on individual RCTs rather than cluster field studies.[20,63–66] Estimates for Vi-polysaccharide effectiveness have also been modified downward.[31,64] Analysis of differences between cluster and individual randomised trials may be informative on indirect protection.

This review has a number of limitations. In the absence of licensed human vaccines, the review does not cover paratyphoid fever or non-typhoidal salmonelloses. It covers only material in the English language, limited searches to a non-systematic enquiry of a single database and does not attempt to synthesis qualitatively or quantitatively any of the studies reviewed. Comparator studies have not been sought that consider investments in water, sanitation and hygiene as alternatives to typhoid vaccination.

One possible feature observed in the course of this review is a less pessimistic assessment of disease burden, perhaps reflecting true decline, as well as a more sceptical perspective on vaccine efficacy estimates, with fewer inputs based on expert opinion alone. Any such trend towards assessment of vaccine costs and benefit firmly grounded in data is beneficial to equitable, scientifically-informed health-policy setting.

It has been suggested that for a model to have sufficient complexity to enable robust cost-effectiveness analysis, substantial data collection may be required.[67] When data is in short supply, theoretically-informed modelling may still be a particularly appropriate tool to support decision-making.[68,69] Transmission modelling using existing data explains patterns seen in average age of infection, demonstrates the importance of carriage, suggests optimal strategies for vaccination, and appraises the potential role of other interventions to reduce transmissibility.

2.6 Conclusion

Transmission dynamics have not yet been integrated into a comprehensive cost-utility analysis of typhoid vaccination and, as such, there is no economic evaluation that would meet contemporary gold standards.[9,70] Given the costs and time involved in further field study, constructive efforts could be made to integrate existing transmission modelling and cost-effectiveness analyses, such as utilising the extensive collation of typhoid epidemiological and clinical parameters by Saul[25] with the transparent, reproducible modelling approach of Pitzer,[24] and DOMI project economic data.[30,38] While such endeavours would not address the fundamental limitations on health service budget in endemic areas, an analysis of typhoid vaccination that enables economic comparison across health arenas could help bring into the public gaze the full potential of measures to control enteric fever, and improve the

prospects of protection from typhoid for people living with daily risks from a disease eliminated from most of the affluent world.

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Chapter 3. A cross-sectional seroepidemiological survey of typhoid in Fiji.

Watson CH, Baker S, Lau CL, et al. PLoS Negl Trop Dis. 2017;11(7):e0005786. doi:10.1371/journal.pntd.0005786.

3.1 Serosurvey bridging section

3.1.1 Introduction

This section of the thesis describes the planning and operational background of the Fiji serology and social mixing survey which I conducted between September 2013 and January 2014. The version of the serosurvey paper enclosed in this thesis is the last-but-one draft of the version published in PLOS Neglected Tropical Diseases,[1] as cuts to methodological detail were made in condensing the final manuscript for the journal. Even in long-form, the paper omits details that are of relevance to describing the process leading to the implementation of the field research, and this bridging section is intended to complete those gaps.

The subsections in this bridging section are in roughly chronological order.

3.1.2 Preliminary planning

Following the Fiji typhoid expert panel meeting recommendations in August 2012, the study was conceived and designed by me in autumn 2012 and early 2013, with input from colleagues and collaborators. Discussion and partnership with the Ministry of Health (MOH) and World Health Organization (WHO) Division of Pacific Technical Support was established through Australian Aid's (AusAID) Fiji Health Sector Support Programme (FHSSP). I visited Fiji between November and December 2012 to develop collaboration with local partners in drafting a research proposal and establishing the local mechanisms through which to take forward the proposals, such as ethical approval.

The primary aim of the serosurvey was to determine antibody prevalence by age, with the study powered towards this. Secondary aims were to identify risk factors for raised antibody titres, based on a questionnaire interview. Also embedded in in the serological survey was a social contact survey from which to produce a mixing matrix that might weight risks of person-to-person transmission events.

3.1.3 Design

I sought to conduct a nationally-representative study, and to include a study population that would reduce the selection biases that could limit internal and external validity and policy-relevance. A national survey is preferable to convenience samples such as only sampling Suva, the capital, omitting large geographies; or using residual blood samples from hospitals which selects for the sick rather than the general population; or using blood donor residua which omit children entirely.

A simple random survey with a whole-population sampling frame is desirable for simplifying statistical inference but logistically unfeasible and so a cluster design was utilised. UN handbooks on field sampling methods[2,3] and the work of the late Steve Bennett[4,5] and others[6,7] were consulted on practical sampling methods for areas where details for structured sampling frames are incomplete, including Pacific islands.[8] These supported the use of multi-stage sampling based on available administrative data as described in the methods section of the paper. Analytical handling of clustering is also further described in the paper. As the survey was planned as a serum banking study, this further emphasised the importance of strong methods to support representativeness of the general population.

The cluster size was set as 25 participants. This was based on the daily survey capability of a field team in prior serological research conducted by the US Centers for Disease Control and Prevention (CDC) and WHO on Fiji's Taveuni island (Heather Scobie, Kashmira Date & Eric Nilles, unpublished work) a year after the cyclone Tomas vaccination campaign.[9] One random participant was selected per household to reduces correlation. Age-stratified sampling was initially considered to ensure age-representativeness; however, with the random-selection approach, this was considered to on average have had balanced samples. Experience on Vanua Levu island (11 clusters) was that imbalanced clusters were readily feasible and so age-stratified sampling was introduced for Viti Levu island where the majority of the survey was to take place.

Taveuni samples would be collected as vaccinated comparators to the unvaccinated population. Mooted examination of waning post-vaccination antibody titres by repeat cross-sectional analysis of Taveuni samples (2013 plus the 2011 CDC/WHO samples) was not taken forward.

As noted in the introduction, a proposal was made by the WHO, who were funding the fieldwork costs, to extend the survey scope to encompass leptospirosis, following an expert meeting in Fiji in May 2013 on control of the zoonotic pathogen, and was a welcome

opportunity to increase the utility of the sero-survey.[10] The leptospirosis serosurvey approach was to be risk-analytical rather than population-representative in primary intent. This would require additional cluster sites purposefully selected in the Western Division for their known leptospirosis outbreak history, as well as those randomly selected for the typhoid survey. Multiple residents would be sampled per household unit in these clusters to maximise serological comparisons. These additional clusters were done within available time and resource. They are not included in the typhoid analysis.

3.1.4 Serological testing plan

A plan was also developed for serological testing. After field collection, venous blood samples would be allowed to clot, centrifuged at the field site, in a local hospital or a local operating base, and pipetted into cryovials. These would be transported to the MOH reference laboratories at the Fiji Centre for Communicable Disease Control (FCCDC) at Mataika House, Suva for freezing at -80˚C. From there they could be shipped on dry ice to international partners. Proposed domestic testing for IgG anti-Vi as a carrier detection tool was not expected to become available (and did not) in the timeline of the project. Use of anti-Vi IgG testing has been proposed since the 1950s as an alternative to faecal testing for asymptomatic carriage detection, though successive studies show less than ideal sensitivity and specificity, which has limited operational uptake.[11] An alternative role for anti-Vi IgG serology may be in detection of past infection in seroepidemiological surveillance.

The use of anti-Vi ELISA was proposed at the AusAID/MOH meeting by Stephen Baker at the Oxford University Clinical Research Unit (OUCRU) Enteric Pathogen Laboratory in Ho Chi Minh, Vietnam. Previous serosurveillance in Nepal using anti Vi ELISA in 2006 and 2009-11 using residual serum samples indicated the potential for this to be an informative approach in the Fijian context, particularly on age-based serology.[12,13] OUCRU would be the principal laboratory partners for the investigations. These samples would be examined using pharmaceutical grade Vi donated by Novartis, Italy.

Testing was planned with Myron Levine of the University of Maryland using agglutination assays for antibody to d-flagellin (Hd), or an ELISA for anti-Hd that was in development in Maryland but not completed in the timeline of this thesis. This assay comparison was proposed to be on a 10% subset of samples, due to the cost of the assays. As mentioned in the thesis introduction, this aspect of the study was not taken forward due to Ebola response commitments on both sides of the Atlantic.

3.1.5 Geographical coverage

The national survey was conducted in Central, Western and Northern Divisions, with sampling frames based on unvaccinated populations. This excluded the southern side of Vanua Levu island, and pockets in

We excluded Eastern Division small island groups for operational feasibility. The Eastern population is small at 37,000 and there are few reported typhoid fever cases, though this could reflect reporting as readily as reflecting the limited infection-sustaining ability of a small island population. A feature of Fiji is the mobility of the population between urban and rural, including to the eastern islands, giving adequate opportunity for transmission events.

The other excluded areas were those that had been vaccinated in the 2010 campaign which followed Cyclone Tomas. Lists were obtained from AusAID FHSSP of vaccinated areas and their coverage and these areas excluded from sample frames.

3.1.6 Funding and financial management

It was initially unclear where funding might be obtained from in order to conduct a serological survey. There was no further funding available from AusAID to take forward this recommendation from the AusAID-MOH expert meeting, nor from within the AusAID funded Fiji Health Sector Support Programme. My MRC budget was insufficient. Funding was attained through Eric Nilles, team leader for WHO's Pacific emerging infection surveillance and response team, with whom I worked to develop a detailed budget plan for submission to the WHO Western Pacific Regional Office for approval.

The support of Stephen Baker at OUCRU Vietnam to undertake the anti-Vi serology at no cost greatly improved the feasibility of the project. Additional competitive funding from the Chadwick Trust for personal research costs was also sought and obtained.

With the addition of the leptospirosis extension, the budget was distinctly tight; tighter still with an additional requirement of a preparatory visit to rural field sites by Kitione Rawalai, the MOH-appointed local study coordinator. One cost pressure was addressed when the University of Queensland, as host institute of Colleen Lau, lead for the leptospirosis study, was able to purchase two portable mains centrifuges for the survey, on provision these were returned to the institution to facilitate further Pacific fieldwork, which was done following the study.

Financial management of the GBP 90,000 budget was one of the most challenging and time-consuming aspects of the study; including large-scale cashflow management for staff and

research costs in a country where LSHTM had no research base. In particular, cash withdrawal limits and prohibitive international transfer fees would have made it impossible to pay for the field team from a foreign based account on the required weekly basis. This necessitated arrangement to be made with the School's Chief Financial Officer for use of a personally-held bank account in Fiji as the operational account for staff costs from where cheque payments could be made. Attaining a bank account as a *kaivalagi*, "a person from the land of foreigners", was also an informative exposure to navigating Fijian administrative pathways. LSHTM funds were duly transferred to the account and accountability maintained through the emailing of photographs of payment receipts and cheques to LSHTM departmental administration colleagues along with detailed records and bank statements.

Field research costs such as vehicle hire, ferry tickets and accommodation continued to put through receipted, itemised claims forms, which totalled tens of thousands of pounds. With careful management, the study was completed within budget.

3.1.7 Field team recruitment

As per the costed operational plan and following the appointment of Kitione Rawalai in July 2013 as local coordinator, we established two independently operational field teams under direction of each of us, with rotation of staff between the teams to share best practice and good morale. Each team comprised two field workers and a phlebotomist.

Each team requiring a field worker able to represent the team effectively at iTaukei village *sevusevu* ceremonies, detailed below. Two such field workers were recruited, Ilai Koru, a former Navy environmental health officer, and Leone Vunileba, a census officer, who would also be able to drive one of the vehicles (to contain staff costs, I drove the other vehicle during research in Vanua Levu and Western Division). Ilai and Leone were joined by two very capable and experienced graduates of the Bachelors in Public Health programme at Fiji National University, Jeremaia Coriakula and Mere Taufa. The teams were completed by the recruitment of newly-qualified phlebotomists Sala Ratulevu and Ala Salesi, identified through the MOH blood service. Substitutes were found to cover periods of unavailability through local MOH facilities. Figure 3.1 is a photograph of the field team in an iTaukei village.

Figure 3.1 Field team outside a village hall

(L to R) Jeremaia Coriakula, Conall Watson, Sala Ratulevu, Mere Taufa, Kitione Rawalai, Leone Vunileba, Ala Salesi and Ilai Koru.

3.1.8 Fieldwork plan

After completing piloting in the Suva area in August 2013, our planned approach was to do the fieldwork as an anti-clockwise lap of Fiji. We would sail to Taveuni island in the north east in September 2013 and completing sampling on the larger neighbouring island Vanua Levu before returning to Suva for rest and and re-stocking supplies.

The second leg would be to completing an anticlockwise loop of Viti Levu, to cover the Western Division. The final section would be to complete fieldwork in Central Division by December, which could be managed as day trips in or from Suva. This frontloaded the demanding elements of fieldwork, with the understanding in the field team that the work would get progressively more straightforward, and avoid remote travel during the onset of the rainy season in December.

3.1.9 Ethical approval

Ethics approval was sought in Fiji in December 2012 from the National Health Research Committee (NHRC) and the Fiji National Research Ethics Review Committee (FNRERC), both hosted by the Ministry of Health. Reviews from NHRC were received in April 2013 requesting substantially more information than was apparent from the ethic committee application forms. I submitted a 40-page response document and fifteen supporting files in May 2013.

Verbal approval from the NHRC was received following their meeting in July 2013. The committee had approved the scientific and technical aspects of the study and was passing the study to the FNRERC for further consideration due to the inclusion of human participants. Approval was received on 13 September 2013 from the FNRERC (reference 2013 03). We sailed to Taveuni that night.

Ethics approval from the London School of Hygiene and Tropical Medicine was sought and received in 2013 prior to commencement of the study (LSHTM reference 6344).

3.1.10 Equipment procurement

An inventory of resources and equipment was drawn up based on the anticipated needs for the typhoid and leptospirosis components plus contingency. Questionnaires were printed by a local company. A manual centrifuge was brought from London for use in remote sites without power. Fiji is a regional centre of medicine and commerce in the Pacific islands and has a number of medical suppliers from whom phlebotomy equipment could be procured, as had been done for previous WHO studies. Other standard medical consumables such as gloves, swabs and alcohol gel were sourced through local pharmacies.

Due to the Pacific geography and market demand, a number of items had to be specially ordered from other parts of the wholesale company in Australia and New Zealand with a lead-time of several weeks, and only just arrived in time. This include “gold-top” serum separator blood tubes that would be used to reduce red-cell contamination in transit and safety shielded needles used to reduce the risk of needlestick injuries. Unused equipment was donated to the MOH at the end of the study. For subsequent dengue fieldwork, equipment was procured in London and flown to Fiji.

3.1.11 Training, questionnaire development and survey piloting

We spent time in Suva and Central Division training on study processes and developing the proposed questionnaire (figure 3.2), including individual-level piloting and community-level

piloting. This included strategies for sensitive question areas including toilets, handwashing habits, income and social mixing by ethnicity, as necessitated by the socially conservative norms in Fiji. This led to modifications of the questionnaire such as dropping physical contact as a form of social contact. See thesis appendix A1 for the questionnaire.

With further refinement, we would have shortened the survey length by removing the distinction in the leptospirosis question sets between small-scale farming/animal keeping for domestic use and equivalent questions on these being done as a source of income or employment. Whilst this was a distinction made in other Pacific island settings examined in prior leptospirosis research, many Fijian participants did not differentiate domestic and commercial farming.

Study processes included methods of community level random selection to reduce selection bias, using random number selection grids, pen-spin methods adapted from EPI for villages and settlements, and equivalent methods for urban and road-side communities.

Figure 3.2 Questionnaire development

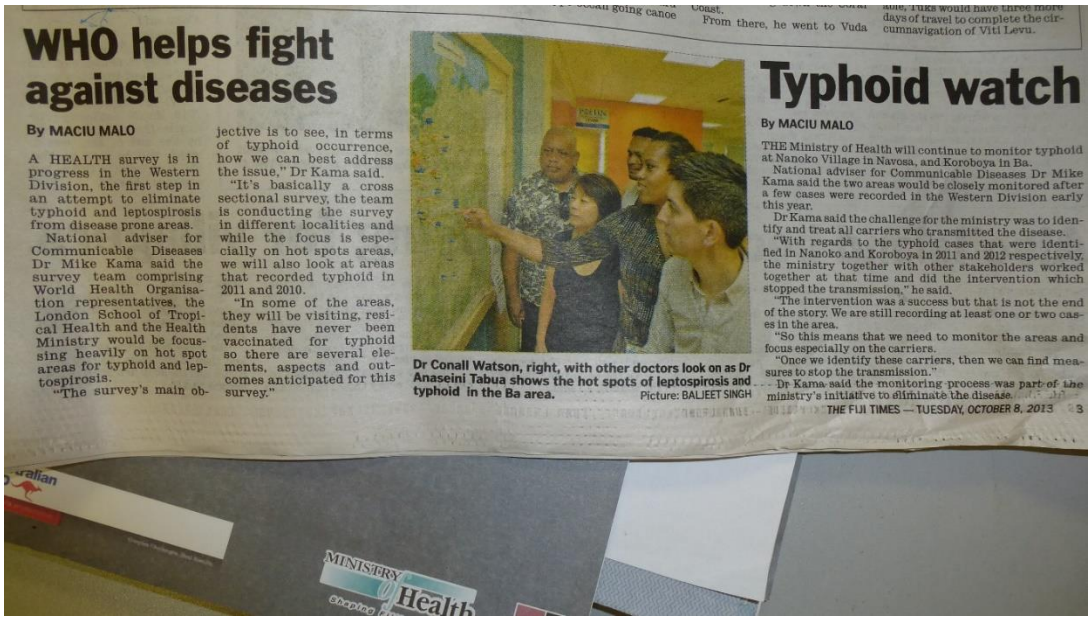
3.1.12 Risk management

Standard operating procedures were put in place and training given on management of risks to patients and study staff, including needlestick injury management, and reporting mechanism for study participants to the field team and the MOH of any complaints or

adverse events. One member of field staff required an overnight hospital stay for an unspecified illness during the course of the research but was discharged without sequelae. No needlestick injuries occurred, and no complaints or adverse event reports were received from participants.

3.1.13 Permissions and consent

At the administrative level, we notified divisional medical officers of plans to work in their division, and were often able to enlist the assistance of zonal community nurses through subdivisional medical officers and the public health nursing system. Media outreach was done through divisional MOH to inform people of the survey and support local disease control efforts (figure 3.3).



going canoe Coast. From there, he went to Vuda ... days of travel to complete the circumnavigation of Viti Levu.

WHO helps fight against diseases

By MACIU MALO

A HEALTH survey is in progress in the Western Division, the first step in an attempt to eliminate typhoid and leptospirosis from disease prone areas.

National adviser for Communicable Diseases Dr Mike Kama said the survey team comprising World Health Organisation representatives, the London School of Tropical Health and the Health Ministry would be focussing heavily on hot spot areas for typhoid and leptospirosis.

"The survey's main objective is to see, in terms of typhoid occurrence, how we can best address the issue," Dr Kama said.

"It's basically a cross sectional survey, the team is conducting the survey in different localities and while the focus is especially on hot spots areas, we will also look at areas that recorded typhoid in 2011 and 2010.

"In some of the areas, they will be visiting, residents have never been vaccinated for typhoid so there are several elements, aspects and outcomes anticipated for this survey."

Dr Conall Watson, right, with other doctors look on as Dr Anaseini Tabua shows the hot spots of leptospirosis and typhoid in the Ba area. Picture: BALJEET SINGH

Typhoid watch

By MACIU MALO

THE Ministry of Health will continue to monitor typhoid at Nanoko Village in Navosa, and Koroboya in Ba.

National adviser for Communicable Diseases Dr Mike Kama said the two areas would be closely monitored after a few cases were recorded in the Western Division early this year.

Dr Kama said the challenge for the ministry was to identify and treat all carriers who transmitted the disease.

"With regards to the typhoid cases that were identified in Nanoko and Koroboya in 2011 and 2012 respectively, the ministry together with other stakeholders worked together at that time and did the intervention which stopped the transmission," he said.

"The intervention was a success but that is not the end of the story. We are still recording at least one or two cases in the area.

"So this means that we need to monitor the areas and focus especially on the carriers.

"Once we identify these carriers, then we can find measures to stop the transmission."

Dr Kama said the monitoring process was part of the ministry's initiative to eliminate the disease.

THE FIJI TIMES — TUESDAY, OCTOBER 8, 2013 3

MINISTRY OF Health

Figure 3.3. Public engagement through the Fiji Times

Fijian residential settings can be broadly divided into three: residential housing, informal settlements and traditional iTaukei villages. Before household could be approached in an iTaukei village, permission was required from a village official, usually through a *sevusevu* ceremony. At these, we explained the purpose of our visit and sought permission from headmen (akin to chief executives or mayors) or hereditary chiefs to conduct the study, handing over the traditional gift of a large wrapped bundle of dried *yaqona* roots, which are pounded to make the traditional *kava* drink, a putative sedative anxiolytic and local anaesthetic consumed socially and ritually from a wooden *tanoa* (figure 3.4).[14]

Figure 3.4. Preparation of *kava* from ground *yaqona*

Once village permission had been established, fieldwork was conducted in the village as it would be in other settings, with the community supportive of research endorsed by the village leadership enabling rapid progress. Community health workers and zonal nurses (figure 3.5) were frequently available in villages to support the study through personal connections, local knowledge and detailed, up-to-date village health censuses.

Fig 3.5. Assistance from MOH zonal nursing staff

Individual informed consent was obtained as described in the main paper, including the opportunity to decline questions or withdraw from any or all parts of the study. Individual

consent was not replaced by village-level permissions. During informed consent, potential participants were often surprised to hear that the blood would be sent to Vietnam for the typhoid immunity testing. We would explain that there was a lot of typhoid in Vietnam, so colleagues there were the international experts on the disease and its tests, which reassured participants. Leptospirosis immunity testing in Australia was readily accepted.

3.1.14 Field implementation

The cross-sectional field survey was implemented as described. Some of the more remote mountainous villages selected by the random sampling required 4x4 vehicles (figure 3.6) and overnight stays for access.

Figure 3.6. Travel by 4-wheeled drive vehicle.

The most remote sites required travel on horseback or by raft (figure 3.7).

Figure 3.7. Travel by horse and raft to remote field sites

3.1.15 Patient cohort

The patient cohort was identified as described in the main paper. Serum collection was initiated following return from the survey work in the Western Division and completed in 2014 after the Central Division cross-sectional sampling was completed. Most of these visits were done by me with a phlebotomist after dropping off one team at a Central Division cluster survey site. We organised the teams so that the local study coordinator, a physician, was able to do the phlebotomy for one team, freeing up a phlebotomist for the patient visits. Recovering patients were contacted by phone or in person, and informed consent obtained as per the main study procedures. Follow-up appointments were arranged and patients visited at a location convenient for the patient, including at or near workplaces.

3.1.16 Serum management

The field team were initially responsible for serum processing during fieldwork on Taveuni and Vanua Levu. Samples were stored refrigerated at Tamavua Hospital, Taveuni, and sent to Mataika House with routine clinical samples. Vanua Levu samples were stored refrigerated at Labasa Hospital, Vanua Levu, and taken to FCCDC at Mataika House by the team when we returned to Viti Levu. With more favourable budget forecast outturn by the time the survey reached the Western Division, we were able to arrange the assistance of laboratory

colleagues at Lautoka Hospital to complete centrifugation and aliquotting/labelling of samples for Western Division. These were held at Lautoka Hospital and driven back to Suva on the final day of Western fieldwork. In Central Division, sample processing was done entirely at Mataika House.

Samples were also labelled to match the study participant identified and divided into four alphabetically coded aliquots:

A) Sent to Ho Chi Minh, Vietnam, for typhoid analysis;
B) Sent to Queensland, Australia, for leptospirosis analysis;
C) Held for d-flagellin analysis in Maryland, USA; and
D) Reference serum bank sample held at Mataika House.

Customs permissions were obtained for sample transport. Air shipping was arranged through a local company, with dry ice sufficient for the journey plus delays, and samples packaged in accordance with International Air Transport Association requirements for biological samples. Samples were received frozen, intact and on schedule in Vietnam and Australia.

3.1.17 Serum analysis

A first run of ELISAs was completed in June 2014. Examination of the titre results with the field data suggested the possibility of an operational issue with the ELISA. A second run was completed in December 2014, as per the methods described in the paper, and are the results presented here.

3.1.18 Data management

Data was checked during fieldwork and feedback given to the teams as necessary. This included the modification to use age-stratified sampling on Viti Levu. Completed paper questionnaires were stored in opaque folders during fieldwork, and transported to Mataika House for data entry. Data entry team members were recruited through collaborating partners at FCCDC and WHO and are named in the acknowledgements section of the paper. Training was given and entry was done in EpiData with secure handling of patient identifiable data. Original paper records were stored securely at Mataika House following completion of data entry. Encrypted patient identifiable records are held on a secure server at LSHTM. Data analysis is of an anonymised dataset. Data cleaning was undertaken in 2014 and shared with Colleen Lau for the leptospirosis analysis.

3.1.19 Data analysis

I analysed the serological data in the R statistical environment in 2015-16.[15] For examination of possible seropositivity we discussed possible analytical approaches with Niel Hens at the University of Hasselt, Belgium pre- and post-fieldwork.[16] On Niel's advice, I examined the patient cohort serology data using a mixed model of antibody log titre wane with fixed rate towards candidate thresholds, examining best fit to determine the most likely threshold.[17]

Additional to the primary age-based, ethnicity-stratified analysis, an epidemiological risk factor logistic regression model was developed.[18–20] This was a secondary research aim, intended to provide supportive evidence of possible risks of previous *Salmonella* Typhi exposure. Examination of the dataset showed minimal clustering once data were stratified by age group and a risk factor analysis was undertaken without variance adjustment. After examination of a number of methods for cluster analysis in R,[6,21–24] I repeated this analysis with allowance for clustering, using the RMS package.[24] This required writing of a specific R function to convert RMS model output into the epidemiologically-framed results reported in the paper.

3.1.20 Dissemination

Preliminary serological findings were reported to MOHMS in January 2015, and results of the formal non-clustered analysis in September 2015. Final results were presented to the Pacific Islands session of the Tropical Medicine and Malaria Conference in Brisbane, Australia, in September 2016 and published in July 2017.

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PLEASE NOTE THAT A COVER SHEET MUST BE COMPLETED FOR EACH RESEARCH PAPER INCLUDED IN A THESIS.

SECTION A – Student Details

Student	Conall Watson
Principal Supervisor	John Edmunds
Thesis Title	Seroepidemiological investigations of typhoid fever in Fiji and the potential role of vaccination in control

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?	Watson CH, Baker S, Lau CL, et al. A cross-sectional seroepidemiological survey of typhoid fever in Fiji. PLoS Negl Trop Dis. 2017;11(7):e0005786.		
When was the work published?	doi:10.1371/journal.pntd.0005786.		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	N/A		
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Stage of publication	

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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	See attached

Student Signature: [redacted] **Date:** 12 Sep 2017

Supervisor Signature: [redacted] **Date:** 12 Sep 2017

Indication of Open Access

Author contributions

Conall H. Watson: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing

Stephen Baker: Conceptualization, Funding acquisition, Investigation, Resources, Supervision, Writing – original draft, Writing – review & editing

Colleen L. Lau: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing – review & editing

Kitione Rawalai: Investigation, Methodology, Supervision, Writing – review & editing

Mere Taufa: Data curation, Investigation, Methodology, Writing – review & editing

Jerimaia Coriakula: Data curation, Investigation, Methodology, Writing – review & editing

Nga Tran Vu Thieu: Investigation, Writing – review & editing

Tan Trinh Van: Investigation, Writing – review & editing

Dung Tran Thi Ngoc: Investigation, Writing – review & editing

Niel Hens: Formal analysis, Methodology, Software, Validation, Writing – review & editing

John H. Lowry: Visualization, Writing – review & editing

Ruklanthi de Alwis: Formal analysis, Visualization, Writing – review & editing

Jorge Cano: Formal analysis, Visualization, Writing – review & editing

Kylie Jenkins: Conceptualization, Funding acquisition, Methodology, Writing – review & editing

E. Kim Mulholland: Conceptualization, Writing – review & editing

Eric J. Nilles: Conceptualization, Funding acquisition, Methodology, Resources, Writing – review & editing

Mike Kama: Funding acquisition, Supervision, Writing – review & editing

W. John Edmunds: Conceptualization, Formal analysis, Funding acquisition, Methodology, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing

A cross-sectional seroepidemiological survey of typhoid in Fiji

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Abstract

Fiji, an upper-middle income state in the Pacific Ocean, has experienced an increase in confirmed case notifications of typhoid fever, a bacterial disease caused by *Salmonella enterica* serovar Typhi (*S*. Typhi). To characterize the epidemiology of *S*. Typhi exposure in Fiji, we conducted a cross-sectional sero-epidemiological survey measuring IgG against the Vi antigen of *S*. Typhi to estimate the effect of age, ethnicity, and other variables on seroprevalence. Epidemiologically relevant cut-off titres were established using a mixed model analysis of data from recovering culture-confirmed typhoid fever cases. A total of 1,787 participants were enrolled and their plasma assayed for anti-Vi IgG; 1,531 of these were resident in mainland areas that had not been previously vaccinated against *S*. Typhi (seropositivity 32.3% (95%CI 28.2 to 36.3%)), 256 were resident on Taveuni island, which had been previously vaccinated (seropositivity 71.5% (95%CI 62.1 to 80.9%)). The seroprevalence on the Fijian mainland is one to two orders of magnitude higher than expected from confirmed case surveillance incidence, suggesting substantial subclinical or otherwise unreported typhoid. We found no significant differences in seropositivity prevalences by ethnicity, which is in contrast to disease surveillance data in which the indigenous iTaukei Fijian population are disproportionately affected. Using multivariable logistic regression, seropositivity was associated with increased age (odds ratio 1.3 (95% CI 1.2 to 1.4) per 10 years), the presence of a pit latrine (OR 1.6, 95%CI 1.1 to 2.3) as opposed to a septic tank or piped sewer, and residence in settlements rather than residential housing or villages (OR 1.6, 95% CI 1.0 to 2.7). Increasing seropositivity with age is suggestive of low-level endemic transmission in Fiji. Improved sanitation where pit latrines are used and addressing potential transmission routes in settlements may reduce exposure to *S.* Typhi. Widespread unreported infection suggests there may be a role for typhoid vaccination in Fiji, in addition to public health management of cases and outbreaks.

Author summary

Fiji has experienced a decade-long increase in typhoid fever cases, a potentially life-threatening systemic bacterial disease caused by *Salmonella* Typhi. We undertook a representative blood-serum community survey to measure antibodies (IgG) against the Vi antigen of *Salmonella* Typhi using a rigorous survey design. We found one in three residents of mainland, unvaccinated Fiji had detectable antibody against Vi in comparison to antibody levels in recovered typhoid fever cases. This was higher than would be expected from confirmed case notifications received by the national surveillance system. Additionally, similar antibody responses were detected in Fijians of all ethnicities, which contrast to surveillance cases in which indigenous iTaukei Fijians were disproportionately affected. Serology on a Fijian island in which a significant proportion of the population has been vaccinated found that three-quarters of residents were seropositive three years after vaccination with a Vi-antigen typhoid vaccine. Importantly, in mainland participants, seroprevalence increased with age, suggesting long-standing, low-level, endemic transmission. Pit latrines were associated with seropositivity when compared with septic tanks, and settlements compared with residential housing. Very high antibody titres in a small percentage of participants may suggest carriage of *Salmonella* Typhi. The seroprevalence findings suggest eliminating typhoid from Fiji by focussing on cases and outbreaks alone will be challenging. Our results support typhoid vaccination and further development of water, sanitation and hygiene infrastructure in Fiji.

Keywords: typhoid fever, *Salmonella* Typhi, Vi antibody, risk factors, vaccine, Fiji, serological survey, immunoepidemiology, serum bank

3.2 Introduction

Typhoid fever is a systemic disease resulting from infection by the bacterium *Salmonella enterica* subspecies *enterica* serovar Typhi (*S.* Typhi). *S*. Typhi is restricted to humans and transmitted in the faeces of infected individuals, which can contaminate water and food or spread by contact and fomites. [1,2] Infection with *S*. Typhi can present as a range of syndromes, from asymptomatic (including carriage) to a disease with life-threatening complications, including intestinal perforation, encephalopathy, and haemodynamic shock [3,4]. The majority of typhoid fever cases present as non-specific acute febrile illnesses that may be difficult to differentiate from other common tropical infectious diseases such as dengue and leptospirosis. There were an estimated 11.9 million (9.9 to 14.7) cases of typhoid fever in low and middle income countries in 2010, resulting in 129,000 (75,000 to 208,000) deaths [5].

The Republic of Fiji is an upper middle income country, which had an estimated population of 892,000 in 2015 [6]. Fiji is comprised of approximately 300 islands (100 inhabited) in the Pacific Ocean of which the largest and most populous are Viti Levu and Vanua Levu [7]. Viti Levu is administratively separated into the Central Division, containing the capital Suva and its suburbs, and the Western Division. The Northern Division comprises of Vanua Levu and Taveuni island, whilst the Eastern Division comprises of smaller islands including the Lomaiviti and Lau groups (figure 3.7).

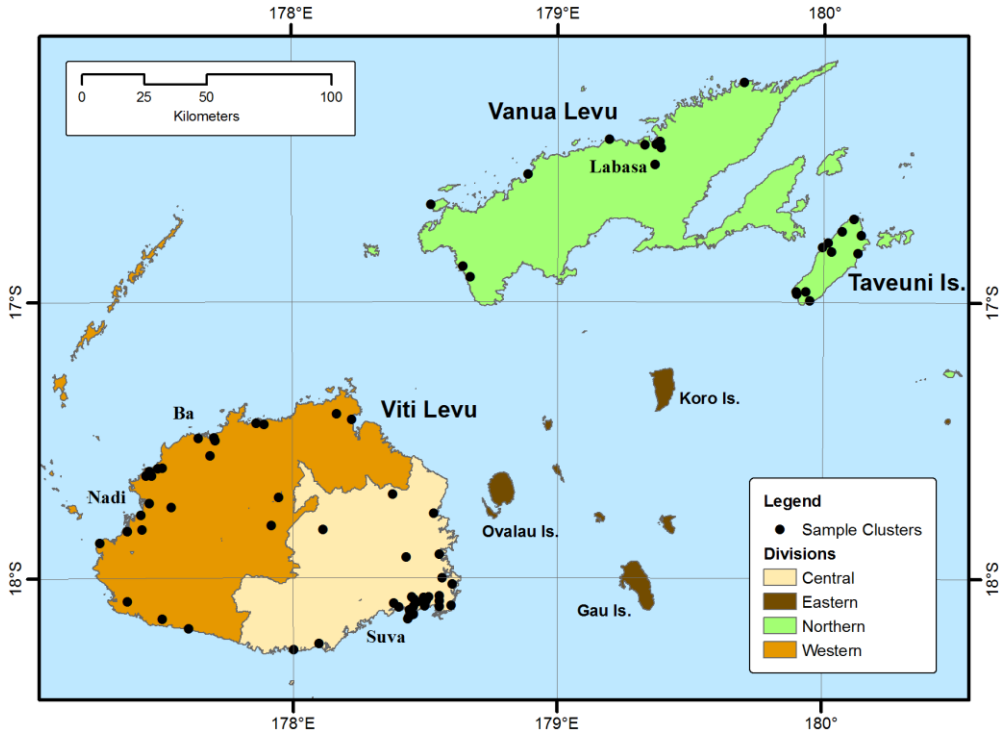

Figure 3.7. Administrative Divisions and Cluster sites on mainland Fiji (Viti Levu and Vanua Levu) and Taveuni islands

Phylogenetic evidence from genome sequencing suggests that *S.* Typhi has existed as a distinct clade in the Fiji Islands for some time, though notified blood-culture confirmed cases numbered fewer than 30 per annum for the decade up to 2004 [8,9]. From 2005, Fiji has experienced a substantial increase in blood-culture confirmed cases of typhoid fever notified through Divisional hospitals to the national surveillance centre [9,10], rising from 4.4 cases per 100,000 population in 2004 to 45 cases per 100,000 population per year during 2008-2011 [11]. Highest incidence was in the Northern Division; 121 cases per 100,000 in 2009 vs 28 per 100,000 in the Western Division and 19 per 100,000 in the Central Division [11]. A proportionate increase in clinically diagnosed typhoid fever was reported over the same period [12].

Notably, >90% of blood-culture confirmed cases are amongst indigenous Fijians (iTaukei), who make up approximately 57% of the population, with relatively few cases reported in Fijians of Indian descent (Indo-Fijians, 38% of the population) or Fijians of Asian or European descent[12]. The causes of this disparity are unknown [12]; health seeking behaviours would be expected to lead to higher relative ascertainment in Indo-Fijians than iTaukei [13].

The peak incidence of typhoid fever in Fiji occurs around the wet season (November to April) [14], with a lag of approximately two months (January to June) [15]. Outbreaks of waterborne and water-washed diseases also arise following cyclones in Fiji, although the causal association between cyclones and typhoid is unclear [12,16,17]. Inadequate access to effective sanitation or treated water supplies alongside low uptake of hand washing with soap may predispose individuals to *S*. Typhi infection [12,18]. It was estimated that sewage disposal in Fiji in 2005 occurred via reticulated systems for 23% of the population, 40% by septic tank, and 37% by pit latrine or other direct disposal [19]. Access to internationally defined “improved water sources” is near universal,[20] however, this definition includes untreated piped surface water in rural areas, which may be a conduit for several enteric pathogens[11,20,21]. While historically the majority of the population lived a rural subsistence lifestyle, based on fishing and small-scale agriculture, more than half the population now reside in urban areas, notably the capital Suva and its suburbs; these areas include informal settlements close to riverbanks and other flood-prone areas with limited access to water and sanitation infrastructure [22].

The Vi-polysaccharide (Vi-PS) vaccine is considered to offer partial direct protection against typhoid fever for two to three years [23]. Typhoid vaccine is not routinely used in Fiji; however, in 2010, following cyclone Tomas, a Vi-PS vaccination campaign was conducted in the highest incidence areas of Fiji. This campaign was conducted predominantly in the

Northern Division, with high coverage on Taveuni island in the Cakaudrove subdivision on Vanua Levu. Targeted geographical vaccination was conducted within subdivisions in other parts of Fiji, including Ra, Nadrogo and Ba in the Western Division, Suva in the Central Division, and Lomaiviti in the Eastern Division [11]. The campaign immunised 64,000 people, representing 8% of the total national population. A reduction in disease incidence rates was observed in the targeted areas whilst rates increased or were unchanged in other areas[11]. Given the ongoing transmission of typhoid in Fiji and the short duration of Vi-PS protection, an expert meeting was convened in 2012 by the Fijian Ministry of Health, with support from Australian Aid, to "develop, prioritise and implement a comprehensive control and prevention strategy" [12]. Analysis of knowledge gaps identified that a serological survey across multiple demographic groups could inform vaccination policy [12].

Seroepidemiological surveys can be used to determine population immunity, pathogen exposure and disease susceptibility, as well as determining disease and exposure related risk factors [24]. Conducted alongside clinical and/or laboratory surveillance, seroepidemiology can help quantify surveillance under- or over-ascertainment, including for enteric diseases [25–28]. Setting-specific immunity and carriage are important in determining typhoid transmission dynamics [29–31]; however, the seroepidemiological methods to attain this are underexploited [25]. This may be in part due to concerns about the sensitivity and specificity of serology for typhoid, which historically has not demonstrated sufficient discriminatory power for individual-level clinical diagnosis [32], (though recent methods may offer promise [33]) as well as concerns about the specificity of assays for carriage detection [34–36] and the existence of multiple immunological pathways to immunity against typhoid fever [37]. Seroepidemiological surveys utilising assays based on purified, pharmaceutical-grade Vi polysaccharide, the "virulence" factor expressed by *S.* Typhi [35], for detection of anti-Vi IgG antibody may offer a more reliable approach by avoiding cross-reactivity that arises when Vi antigen preparations contain other bacterial antigens [38,39]. Furthermore, high anti-Vi titres may indicate chronic carriage in response to prolonged immune stimulus [38,40–42].

To characterize and better define the immunoepidemiology of typhoid infection in Fiji, with the aim of informing effective and efficient control measures, we surveyed three groups of people in Fiji: group 1) a representative, multi-stage, clustered, cross-sectional seroepidemiological survey of the Fijian mainland for *S*. Typhi, including demographic data and data on potential risk factors for typhoid [18,43–56]; group 2) residents of Taveuni island, where 92% of the population was vaccinated in 2010, surveyed by the same methods [11]; and group 3) a cohort of culture-confirmed typhoid fever cases, to enable estimation of a threshold for seropositivity.

3.3 Methods

3.3.1 Ethics

The study was approved by the Fiji National Research Ethics Review Committee (2013-03) and the London School of Hygiene & Tropical Medicine observational study research ethics committee (6344).

3.3.2 Study design

Three groups of participants were recruited for the study: Group 1, Mainland: Sixty-four communities were randomly selected by multi-stage sampling on the two most populous Fijian islands, Viti Levu and Vanua Levu. These were visited for serological sampling and questionnaire interviews from September to December 2013. Vaccinated areas on these two islands were excluded to avoid confounding of interpretation of serological responses to natural exposure. Eastern Division, with a population of under 40,000 spread across multiple small islands [13], was not visited for logistical reasons. Group 2, Taveuni: A vaccination campaign had been conducted on Taveuni Island in 2010; 11 communities from this location were randomly selected by multi-stage sampling as vaccinated comparators and surveyed in September 2013. Group 3, Convalescent cases: Sequential recently blood-culture confirmed typhoid fever cases in the Central Division were identified in October 2013 from national surveillance and hospital records and approached to seek informed consent for blood sampling. Further blood-culture confirmed cases diagnosed previously were identified during visits by the field team to the convalescent cases' villages or residences and invited to participate after validation with national surveillance records. Between November 2013 to April 2014 up to three blood samples were collected from cases at a minimum of one-month intervals.

Headmen, health workers and other community leaders were visited in advance to seek agreement to participate in the study and make arrangements to visit with the full study team. No communities declined participation in the study. A team of experienced, multilingual Fijian field workers was trained and questionnaires piloted prior to the survey. Interviews were conducted in iTaukei, English or Hindi at the preference of the interviewee. Trained phlebotomists collected venous blood samples.

For group 1 (mainland) and group 2 (Taveuni), a multi-stage cluster-sample survey was conducted [57]. For group 1, cluster numbers per geographical Division (Northern, Central, Western) were proportional to the resident typhoid non-immunised population. Within subdivisions, nursing zones are contiguous health administrative areas, each serving a population of approximately 1,000 to 10,000 people. For the groups 1 and 2, nursing zones were selected with probability proportional to population size [57] by random number

generation in Microsoft Excel 2007, using Ministry of Health administrative records. Communities were randomly selected from within the nursing zones from unweighted lists, in the absence of detailed population data.

Households were randomly selected within each community and a single occupant aged one year or older per household was randomly invited to join the study (to reduce correlation compared with recruiting multiple residents), with selection using random number tables. Registries held by nurses or community health workers were used for household and participant sampling where available; when not available, geographical sampling was performed. In rural village-like clusters, Expanded Programme on Immunization-derived sampling of houses was conducted in randomly selected directions (by pen-spin) from the community's central point [58]. For clusters on streets, random sides of roads, starting points, and directions of progress were selected following a rapid appraisal of house numbers to enable selection of all households with equal probabilities.

Household occupancy was on *de facto* residency in the household the previous night as per Fiji census methods. Information regarding the study was provided to the households, the participants and/or the parent/guardian of all child participants (under the age of 18 years old). All adult participants provided written informed consent. Parents/guardians provided written informed consent on behalf of all child participants (under the age of 18 years old). Written assent was also provided by children aged 12 years and above. Exclusion criteria were clotting disorders, medical anticoagulation or severe medical disorders that would preclude safe participation in the study.

For group 1 residents in Viti Levu, age- stratified sampling (strata size proportional to national population) was used to ensure representation across all age groups after field data review identified potential age imbalances arising in some clusters on Vanua Levu and Taveuni. Once the required number of participants within an age-band was attained, further members of that age-band were ineligible for selection in subsequent households. If the selected participant was temporarily absent from a house e.g. for work or school, data collectors would revisit later in the day after their expected time of return. If a whole household was absent, an alternative house was randomly selected from the health registry, or the next nearest non-sampled dwelling in the same geographical sampling frame.

3.3.3 Sample size

A study sample size of 1,600 was proposed for the mainland group, as this would allow for 7% confidence intervals for seroprevalence for age band groups of 200, if seroprevalence was 40%, at alpha = 0.05. If non-independence within age bands within clusters gave rise to a

design effect of two, then the confidence intervals would be 10%, which was deemed sufficient precision. Expected seroprevalence levels were informed by prior work on Taveuni (Eric Nilles, data on file).

3.3.4 Laboratory methods

Enzyme-linked immunosorbent assays (ELISAs) to detect *S.* Typhi Vi-polysaccharide antigen-specific IgG in human serum samples were performed as described previously [59] (provided by Sclavo Behring Vaccines Institute for Global Health, Siena, Italy). Briefly, ELISA plates were coated overnight with 1µg/ml of Vi polysaccharide antigen. Coated plates were washed and blocked with 5% fat-free milk solution. Following blocking, plates were washed and incubated with serum diluted at 1:200 at room temperature (RT) for 2 hours. Plates were washed and incubated with secondary antibody, alkaline phosphatase-conjugated anti-human IgG at RT for one hour. Finally, p-Nitrophenyl phosphate (SigmaFAST N1891, Sigma-Aldrich, United Kingdom) substrate was added for 30 minutes at RT and absorbance was read at dual wavelengths (405 nm and 490 nm) using an automated microplate reader (Biorad). Optical densities (OD) from blank control wells were subtracted from all sample absorbance values prior to estimation of serum titers from a standard curve. We selected 96 random Fijian plasma samples and subjected them to the anti-Vi ELISA . Twenty samples of these samples (with an OD >2.5) were pooled (standard plasma) and used to generate a standard curve. One ELISA Unit (EU) was defined as the reciprocal of the standard dilution (made by 10 2-fold dilutions of the standard plasma) that gave an absorbance value equal to 1 in this assay. All samples were tested at the Oxford University Clinical Research Unit in Ho Chi Minh City, Vietnam.

3.3.5 Data analysis

A surveillance seropositivity threshold was determined using a mixed-effects model of serial titres in the convalescent cases group. A log-normal mixture model approach to the data as per del Fava et al [60] had been examined previously but was considered non-informative. Mixed-effect models were fitted by maximum likelihood estimation (ML), using a random-intercept fixed-slope above a putative threshold value and random intercept time-invariant model below, signifying antibody returning to a baseline level, with each convalescent case's data assigned to either the fixed slope component or the time invariant component. Data from two patients with titres at the upper limit of detection (25,000 EU) were excluded leaving 70 titres from 28 patients. Model fit was examined at a range of threshold values with Akaike's information criterion (AIC) used for threshold comparison, summing the AIC from the fixed-slope and time-invariant models.

Data were entered in EpiData 3.1 [61] and analysed in R 3.3 [62]. Seroprevalences were calculated using intra-cluster correlation coefficients (ICCs) and design effects determined on log titres with clustering at the primary sample unit. Putative risk factors for seropositivity were estimated with Huber-White robust standard errors, clustered on the same, using the "rms" package[63]. A multivariable model was developed from univariable risk factors with *p*-values of less than 0.25, after-regrouping sparse cells for numerical stability, using a backward stepwise approach fitted by AIC, with deletion of observations with missing data. Potential collinearity was assessed by linear-adjusted generalized variance inflation factors in the "CAR" package [64,65], and variables were removed if GVIF^(1/(2*Df)) was over 2 and not considered epidemiologically important to retain. Data were considered insufficient to examine possible risk factors associated with titres that may indicate typhoid carriage. Self-reported vaccination was assessed as non-informative and participants analysed on vaccine status of their residential geography (see supplementary information, section 3.7.1). For comparison to age-based seroprevalence, typhoid fever cumulative incidence expected across the life-course was estimated with binomial confidence intervals from confirmed cases notified in 2008-2014 to the national surveillance system at the Fiji Centre for Communicable Disease Control, Mataika House, Suva.

3.4 Results

Group 1: Sixty-four mainland clusters in unvaccinated areas of Viti Levu and Vanua Levu were visited for this sero-survey (Figure 3.7). Of 1,565 people approached, five declined and 1,560 were enrolled. There were no exclusions on medical grounds. A serum IgG titre against Vi polysaccharide (anti-Vi IgG) could not be attained for 29 participants (median age 23, IQR 6 to 34; 19/29 female; 25/29 iTaukei) but was determined in 1,531 individuals (98%). The age of the sampled population ranged from one to 85 years (median 29; IQR 16 to 48, Table 3.1). Of these, 820/1,530 (54%) were female and 76% (1,164/1,530) were iTaukei (non-responses excluded).

For Group 2, on Taveuni Island, the location for a vaccination campaign, 277 people were approached and 256 participants (127 (49.6%) female) in 11 clusters enrolled, with nil excluded, and all providing samples that were successfully assayed for anti-Vi IgG (Table 3.2 and Figure 3.7).

Group 3: Thirty-seven patients with blood-culture confirmed typhoid fever provided one or more samples that were assayed for anti-Vi IgG (19 (51.4%) female, median age 30, IQR 14 to 42) (Table 3.3); thirty provided two or more blood samples (15 (50%) female, median age 30, IQR 15.5 to 44.5); and 19 provided three samples (10 female, median age 32, IQR 17 to 46.5). The median duration from reported fever onset to first sample collection was 187 days (IQR 132 to 272 days).

Table 3.1. Group 1. Demographics of mainland Viti Levu and Vanua Levu (unvaccinated areas) survey participants

Variable	**Value**
Number of participants assayed	1531
Age (median, IQR)	29 (16 to 48)
1 to 14	343 (22·4%)
15-34	554 (36·2%)
35-54	384 (25·1%)
55+	250 (22·9%)
Female	820 (53·6%)
Clusters	64
Central Division	28
Northern Division	11
Western Division	25
Participants per cluster (SD)	23·9 (2·5)
iTaukei	1164 (76·1%)
Indo-Fijian	338 (22·1%)
Other	28 (1·8%)
House income <100 FJD/wk	548 (36.3%)
100-199	490 (32.5%)
200-299	296 (19.6%)
300-399	61 (4.0%)
400+	81 (5.4%)
Self-report previous vaccination against typhoid	103 (6.7%)
Self-report previous typhoid fever	20 (1.3%)

Table 3.2. Group 2. Demographics of Taveuni island (Vi-polysaccharide vaccinated area) survey participants

Variable	**Value**
Number of participants assayed	256
Age (median, IQR)	36 (24 to 52)
1 to 14	32 (12.5%)
15-34	90 (35.3%)
35-54	85 (33.3%)
55+	48 (18.8%)
Female	127 (50%)
Clusters	11
Participants per cluster (SD)	23.3 (3.3)
iTaukei	220 (86.3%)
Indo-Fijian	27 (10.6%)
Other	8 (3.1%)
House income <100 FJD/wk	91 (36.5%)
100-199	99 (39.8%)
200-299	22 (8.8%)
300-399	12 (4.8%)
400+	14 (5.6%)
Self-report previous vaccination against typhoid	54 (21.1%)
Self-report previous typhoid fever	5 (2.0%)

Table 3. Group 3. Demographics of convalescent confirmed typhoid fever cases

Variable	Value
Number of participants assayed	37
Age (median, IQR)	30, 14 to 42
5 to 14	10 (27.0%)
15-34	12 (32.4%)
35-54	12 (32.4%)
55-74	3 (8.1%)
Female	19 (51.4%)
iTaukei	36 (97.3%)
Indo-Fijian	0
Other (Pacific Islander)	1 (2.7%)

Threshold estimation using a mixed model of sero-reversion amongst the Group 3 recovering typhoid fever cases exhibited best fit at 64EU (Supplementary Figure S3.1 and Supplementary Table S3.1). The ICC and design effect per Group 1 mainland cluster were 0.09 and 3.03, respectively. Across the five-year age bands (Supplementary Table S3.2), the Group 1 mean ICC and design effect were 0.13 and 1.09, respectively.

Using the defined seropositivity cut-off (64 EU threshold), 32.3% of Group 1 mainland participants (95%CI 28.2 to 36.3%) were seropositive for anti-Vi IgG (Supplementary Table S3.3 Table), compared to 71.5% (95% CI 62.1% to 80.9%) of Group 2 (Taveuni island). As a sensitivity analysis, when a higher anti-Vi IgG threshold of 100 EU was used, 17·7% of the Group 1 mainland participants (95%CI 14·4 to 21.0%) were seropositive, compared to 58.6% (95% CI 48.4% to 68.8%) of Group 2 Taveuni islanders (Supplementary Table S3.3). To determine a rough estimate of carriage prevalence within Group 1 (mainland), we additionally examined those with the highest anti-Vi IgG titres; 2.8% (1·4 to 4·2%) of the sampled population had an antibody titre of 500 EU or above and 1.4% (0.4% to 2·4%) of the sampled population had an antibody titre of 1,000 EU or above (Supplementary Table S3.3).

The anti-Vi IgG titre distributions are shown in Figure 3.8 for group 1, mainland unvaccinated areas (A); group 2, Taveuni island (B); and group 3, convalescent cases (C). The distribution of antibody titres in group 2 (Taveuni island) was shifted rightward relative to the Group 1

(mainland) titres, as would be predicted with the mass administration of Vi-PS vaccine. Thirty-nine (15%, 11.2 to 20.4%) of the 256 Group 2 (Taveuni) participants had Vi IgG titres at the upper limits of detection. In the recovering typhoid fever cases the data were bimodal, with the higher peak above the modal titre for the mainland group.

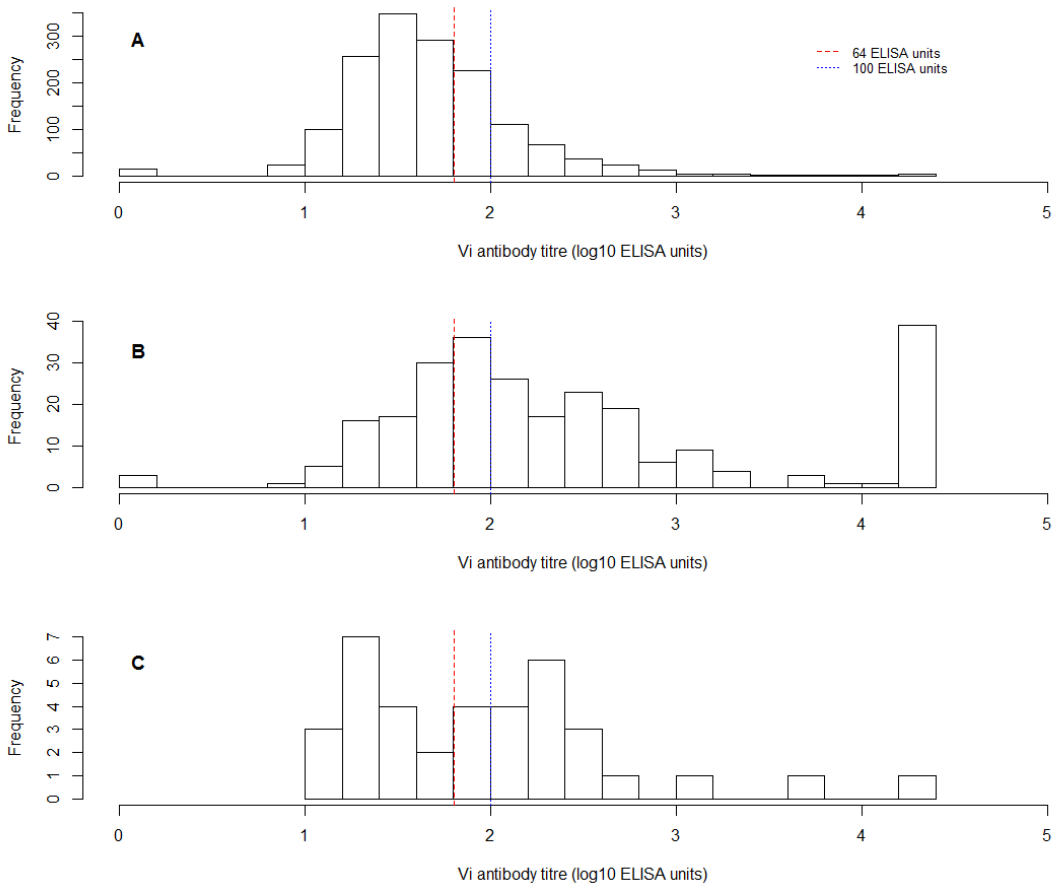

Figure 3.8. Distributions of log10 anti-Vi IgG antibody titres in three Fijian groups.

A) Group 1: residents of Fiji mainland Viti Levu and Vanua Levu islands; B) Group 2: residents of Taveuni island, where a vaccination campaign with Vi-polysaccharide injection was conducted three years previously; and C) Group 3: recovering cases of culture-confirmed typhoid fever. Red vertical line denotes 64 ELISA unit seropositivity threshold determined from case antibody kinetic analysis; blue line denotes 100 ELISA unit threshold used in sensitivity analysis. Case titres are mean log titre if multiple samples collected, range 68 to 645 days from fever onset.

In Group 1 (mainland), the age trends for unvaccinated iTaukei and non-iTaukei ethnic groups (Figure 3.9) both showed increasing seroprevalence with age based on the patient-fitted threshold. These increased from approximately 20% in the youngest age bands to 50% in the oldest, at threshold of 64 EU. Age and ethnicity trends were comparable when a sensitivity

analysis at a higher threshold (100 EU) was performed with seroprevalence rising from <10% in younger groups to approximately 30% in the oldest age brackets. At both thresholds there was some suggestion that iTaukei seroprevalence may be higher than non-iTaukei seroprevalence, though differences between ethnic groups were not statistically significant at the 0.05 level. Notably, for both ethnic groups, seroprevalence by age band was substantially higher than the equivalent cumulative incidence that would arise if considering only confirmed cases, more than ten-fold in iTaukei Fijians and several hundred-fold in non-iTaukei Fijians.

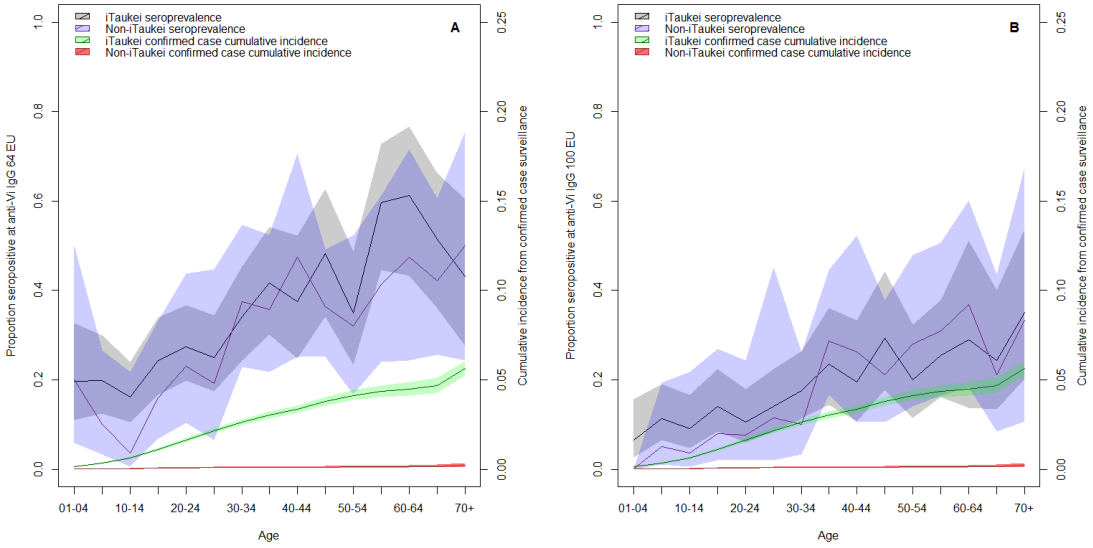

Figure 3.9. Seroprevalence of anti-Vi IgG by age and ethnicity

Seroprevalence in iTaukei and non-iTaukei groups at A) 64 ELISA units (case-fitted threshold) and B) 100 ELISA units (sensitivity analysis). Each panel also indicates confirmed case cumulative incidence by ethnicity. Shared areas denote 95% confidence intervals.

A multivariable analysis of group 1 (mainland) indicated that several factors were significantly associated with seropositivity at a 64 EU anti-Vi IgG threshold after adjusting for potential confounders (Table 3.4). Residents of Western Division had an odds ratio (OR) of 0.6 (95%CI 0.4 to 0.8) for seropositivity in comparison to the Central Division. Age was associated with seropositivity, with an adjusted OR of 1.3 (95% CI 1.2 to 1.4) for every ten-year increase. Additionally, we found that individuals with pit sewage systems had an adjusted OR of 1.6 (95% 1.1 to 2.3, p=0.01) for seropositivity in comparison to participants with septic tanks. Residence in a settlement rather than residential housing had an adjusted OR 1.6 (95% CI 1.0 to 2.7) for seropositivity.

Table 3.4. Risk factors by adjusted odds ratios for anti-Vi IgG seropositivity at 64 ELISA units for mainland Viti Levu and Vanua Levu by cluster-robust multivariable logistic regression.

Variable	Value	OR	95% CI	p-value	
Division or island	Central Division	Baseline			
	Western Division	0.58	0.41 to 0.83	0.0027	**
	Vanua Levu	0.74	0.46 to 1.17	0.19	
Age	Per decade	1.31	1.23 to 1.40	<0.0001	***
Ethnicity	Other vs iTaukei	0.79	0.54 to 1.14	0.21	
Community type	Residential				
	Village	1.07	0.61 to 1.89	0.82	
	Settlement	1.63	1.00 to 2.65	0.048	*
Rurality	Urban	baseline			
	Peri-urban	0.65	0.41 to 1.01	0.055	
	Rural	1.17	0.72 to 1.88	0.53	
Home sewage	Septic tank	Baseline			
	Piped sewer	1.07	0.77 to 1.48	0.69	
	Pit	1.62	1.12 to 2.32	0.01	*
	Elsewhere	0.82	0.39 to 1.72	0.60	
Typhoid vaccination, self-report	Yes	1.34	0.91 to 1.96	0.14	
Typhoid fever diagnosed, self-report	Yes	1.66	0.77 to 3.50	0.18	

* p<0.05, **p<0.01, ***p<0.001
n=1436 complete records

Lastly, after adjustment, no significant association with seropositivity was observed at p<0.05 for ethnicity, community type, rural residence, self-reported typhoid vaccination, or self-reported diagnosis of typhoid fever. “Home toilet type” was excluded from consideration for multivariable analysis: pour-flush (water seal) toilets were found to be associated with seropositivity on univariable analysis, however these are installed in response to disease outbreaks and so are confounded by indication (Fiji National Taskforce on Control of Outbreak-Prone Diseases, personal communication 2015). Other candidate risk factors identified on univariable screening (Supplementary table S3.4 Table) were not retained in the final model, including sex, drinking water sources, kava consumption, bathing or washing in rivers and typhoid fever cases within the household or social network.

3.5 Discussion

This seroepidemiological survey of the Fijian mainland, established in response to a rise in confirmed typhoid fever case notifications, found seroprevalence of IgG against the Vi antigen of *S*. Typhi of 32.3% (95%CI 28.2 to 36.3%) based on an threshold of 64 IU determined from waning antibody in recovering patients. This seroprevalence, if indicative of past infection, is one to two orders of magnitude higher than would be predicted from case notifications. Seroprevalence increased with age, suggesting established endemic transmission; an alternative hypothesis may be that typhoid in Fiji is predominantly a childhood infection and that incidence has dropped over the decades, leaving older cohorts with serological signs and younger cohorts unexposed. Such an effect has been seen in hepatitis A in Indo-Fijians.[66,67] The serology would be consistent with typhoid primarily infecting older adults in a recent upturn, though this seems unlikely. Both iTaukei and non-iTaukei ethnic groups exhibit similar typhoid seroprevalences across age groups, in contrast to typhoid fever, the disease, which is disproportionately reported from iTaukei Fijians. A small number of very high titres suggests that carriage occurs. Multivariable logistic regression found seropositivity was associated with pit latrines compared with other sewage systems, and living in a settlement compared with residential housing or a village. Central Division and Vanua Levu island had higher seroprevalences than Western Division.

Our investigation used a population-representative survey design rather than a convenience sample such as blood donors or hospital patients, or recent outbreak areas. This strengthens external validity of seroprevalence estimates, particularly for age-based inference, as children are rarely blood donors or inpatients. A limitation of the study is the use of a single antigen due to resource availability, though mitigated by the use of antibody-waning data from recovering patients to determine a surveillance threshold. Serum-banking enables future investigation of other *S.* Typhi immune biomarkers.

The proportion of the survey participants that was non-iTaukei (24%, specifically Indo-Fijian (22%)), was lower than expected from the last census [13]. This may be due to a greater proportion of Indo- Fijians residing in larger communities within nursing zones than documented in the sampling frame, due to migration from rural to urban area, or in areas that had been vaccinated; secular trends of a higher emigration rate and lower fertility rate for Indo-Fijians than iTaukei may also be have contributed [13]. Post-stratification weighting was not considered appropriate given uncertainties in demographic changes since the 2007 census and sparse population records within nursing zones which could result in inappropriate adjustment. Representativeness was addressed through the design of the survey, and clustering through use of design-effects (which were modest) and cluster-robust

regression. The slight excess of females in the survey may be due to different residency patterns, such as male overnight residency in agricultural shelters, as encountered by the survey team.

The serological results from the mainland survey, using highly purified Vi, were strengthened by benchmarking against serum from two other Fijian groups. Alongside threshold-estimation using a mixed model of antibody waning in convalescent cases, the higher anti-Vi seroprevalence from Vi-PS vaccinated residents of Taveuni island also informs the use of anti-Vi IgG as a surveillance marker in the unvaccinated mainland population. Typhoid serology remains relatively understudied and further work could be done to strengthen comparability between settings and to inform thresholds used for seroprevalence surveys. Hospital-based serology studies of fever patients in acute illness and convalescence would be valuable in assessing the specificity of Vi antibody response to typhoid against patients with other laboratory confirmed diagnoses (notably Enterobacteriaceae family bacteraemias) and without specific clinical signs of concurrent typhoid fever or positive *S.* Typhi culture.

Despite incomplete international standardisation of Vi assays [68], the distribution of IgG titres can be compared across studies. The Fiji results contrast to those from two Vi ELISA serosurveys done in Kathmandu, Nepal. The first compared Vi to serum bactericidal activity (SBA) found rising SBA with age, suggesting a similar acquisition of exposure with age, but found no age trend in anti-Vi IgG. [69] The second, using an assay similar to that applied in Fiji, reported high anti-Vi IgG in all age groups, suggesting hyperendemicity [70]. In a study in Cape Town, South Africa, where typhoid was considered endemic, 40% of unvaccinated 9 year olds were found to have anti-Vi IgG titres believed to be protective [71]. In contrast, we found in Fiji mean seroprevalence for children of similar age (5 to 9 and 10 to 14 years) was not more than 20% (Figure 3.9) suggesting lower force of infection than Cape Town, if these antibody thresholds are comparable. This lower serological force of infection would also be consistent with lower confirmed case incidence [12,71].

The large difference in AIC between the best-fit convalescent titre threshold and higher or lower thresholds provide good support the choice of threshold statistically,[72] but needs considered in the biological context. Determining and making inference from the IgG waning rate is challenging due to statistical uncertainty in the fixed effect slope estimate which allow a wide range of biologically plausible scenarios. Incomplete seroconversion (as seen in other settings[73]) and waning anti-Vi IgG titres observed amongst convalescent cases in our study gives rise to the possibility that the seroprevalence estimates in this survey are under-

estimates and thus conservative with respect to sero-incidence. Multiple infections, symptomatic or otherwise, may be required for the establishment of sustained immunity to typhoid fever;[74,75] such a mechanism may explain non-response in patients and be consistent with experimental study of booster vaccination with killed *Salmonella* Typhi [76] as well as recent models used to estimate vaccine impact in India [29] and elucidate transmission determinants in Malawi [31]. In Fiji, model-fitting using age-structured contact patterns could shed light on whether the age-dependent increase observed in seropositivity is more likely due to long-term endemic transmission or a more recent outbreak with higher propensity to affect older age groups. A similar upturn in typhoid fever notifications occurred in Papua New Guinea in the 1980-90s, also from a low-level, sporadic baseline [77]. A rise in population O antigen was observed [78], suggesting an overall increase in transmission; longitudinal serological investigations may likewise be informative to current transmission in Fiji.

There are a number of epidemiologically-plausible, model-testable hypotheses for the differences between our seroepidemiological findings and the epidemiology of notified cases in Fiji, in which peak incidence is observed in adolescents and young adults of iTaukei ethnicity. Ingestion of a large dose of *S.* Typhi can overwhelm naturally-acquired immunity (as well as that from vaccination) [74,76,79,80], and so age- and ethnically-differential exposure to high and low dose inocula is one mechanism by which these data may be explained, if for example, iTaukei adolescents and young adults ingest larger inocula through exposure to particular foods. Such patterns might also be compatible with genetic differences in typhoid susceptibility, potentially mediated by HLA-type [81], with reduced susceptibility in Fijians of Indian descent, whose South-Asian ancestors may have experienced many millennia longer exposure to *S.* Typhi than iTaukei Fijians have historically had [82–84].

Findings from our multivariable analysis suggest that living in a settlement and the use of pit latrines may be risk factors for S. Typhi infection. Whilst there are likely to be public health benefits from improving conditions in settlements and upgrading sewage systems, specific interventions for typhoid prevention should be planned with consideration for findings emerging from case-control and environmental health research [48,85]. Widespread subclinical infection, both transient and chronic, such as may be inferred from these serological findings, suggests that whilst systematic public health management of cases and outbreaks and early diagnosis and treatment of patients remain of vital importance to reduce morbidity and mortality from typhoid fever in Fiji, a focus on these alone may be insufficient to eliminate transmission. Alongside continued socio-economic development and expanded access to infrastructure for sanitation, water supplies and handwashing with soap,

programmatic vaccination may be amongst interventions necessary to bring about effective typhoid control in Fiji.

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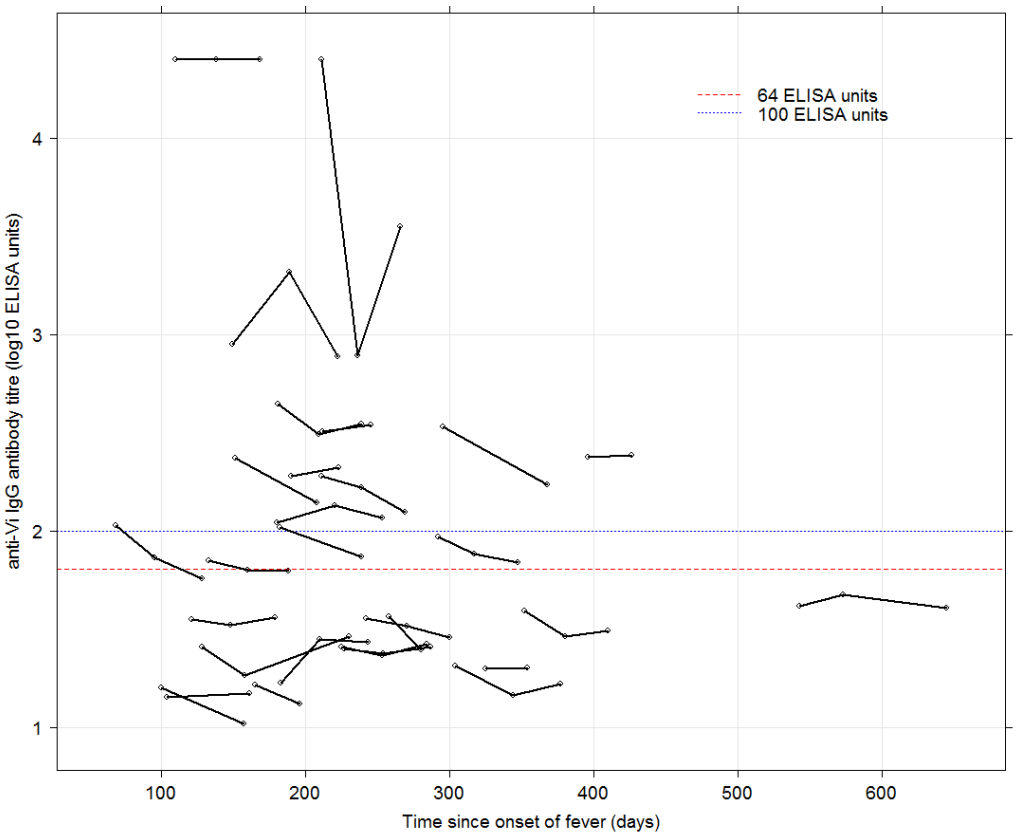
3.7 Supporting Information



Supplementary Figure S3.1. Serial anti-Vi IgG titres from convalescent confirmed typhoid cases

Supplementary Table S3.1. AIC by maximum likelihood for anti-Vi IgG antibody waning fixed effect model thresholds in culture-confirmed typhoid cases

Titre threshold (ELISA units)	AIC	Δ AIC from best fit
16	-24.48	*29.95*
32	-46.29	*8.13*
64	-54.43	*0.00*
100	-45.07	*9.36*
150	-44.59	*9.84*
200	-34.22	*20.20*
250	-21.07	*33.36*

Supplementary Table S3.2. Design effects and ICC by 5-year age band, mainland Viti Levu and Vanua Levu

Age band	Design effect	ICC
01-04	1.07	0.30
05-09	1.15	0.14
10-14	1.11	0.10
15-19	1.09	0.08
20-24	1.06	0.05
25-29	1.06	0.06
30-34	1.40	0.39
35-39	1.08	0.11
40-44	1.07	0.24
45-49	1.24	0.46
50-54	1.06	0.13
55-59	0.97	-0.10
60-64	1.00	-0.02
65-69	1.03	-0.30
70+	0.94	0.45

S3 Table. Seroprevalence for anti-Vi IgG at different ELISA unit thresholds Amongst Group 1 mainland residents (by self-reported vaccine status), Group 2 Taveuni island residents and Group 3 convalescent cases.

Group	**Number (proportion, 95% confidence interval) (by design effect for mainland and Taveuni)**
1) All mainland survey participants	1531
Titre ≥ 64	494 (32.3%, 28.2 to 36.3%)
Titre ≥ 100	271 (17.7%, 14.4 to 21.0%)
Titre ≥ 500	43 (2.8%, 1.4 to 4.2%)
Titre ≥ 1000	21 (1.4%, 0.4 to 2.4%)
1a) Mainland unvaccinated (self-report)	1304 (excludes don't know)
Titre ≥ 64	410 (31.4%, 27.0 to 35.8%)
Titre ≥ 100	223 (17.1%, 13.5 to 20.7%)
Titre ≥ 500	37 (2.8%, 1.4 to 4.4%)
Titre ≥ 1000	18 (1.4%, 0.3 to 2.5%)
1b) Mainland vaccinated (self-report)	103
Titre ≥ 64	42 (40.8%, 31.0 to 50.5%)
Titre ≥ 100	25 (24.3%, 15.7 to 32.8%)
Titre ≥ 500	5 (4.9%, 0.6 to 9.1%)
Titre ≥ 1000	3 (2.9%, 0 to 6.3%)
2) Taveuni island	256
Titre ≥ 64	183 (71.5%, 62.1 to 80.9%)
Titre ≥ 100	150 (58.6%, 48.4 to 68.8%)
Titre ≥ 500	72 (28.1%, 18.8 to 37.5%)
Titre ≥ 1000	57 (22.3%, 13.6 to 30.9%)
3) Convalescent typhoid cases	37
Mean titre ≥ 64	21 (56.8%, 39.6% to 72.5%)
Mean titre ≥ 100	17 (45.9%, 29.8% to 62.9%)
Mean titre ≥ 500	4 (10.8%, 3.5% to 26.3%)
Mean titre ≥ 1000	3 (8.1%, 2.1% to 23.0%)

Supplementary Table S3.4. Univariable risk factors for seropositivity
with anti-Vi IgG on mainland Viti Levu & Vanua Levu at 64 ELISA unit threshold

Variable	Value	Count	OR	95% CI	P-value	
Division or island	Central Division	667	Baseline			
	Western Division	607	0.68	0.48 to 0.97	0.035	*
	Vanua Levu	257	1.24	0.85 to 1.81	0.26	
Ethnicity	iTaukei	1164	Baseline			
	Other	366	0.91	0.64 to 1.29	0.58	
Age	Per decade	1530	1.28	1.21 to 1.36	<0.0001	***
Sex	Male	710	Baseline			
	Female	820	0.95	0.75 to 1.20	0.67	
Household size	Unit increase	1499	0.93	0.88 to 0.98	0.0078	**
Community type	Residential	430	Baseline			
	Village	656	1.24	0.86 to 1.79	0.26	
	Settlement	444	1.41	0.99 to 2.02	0.06	^
Rurality	Urban	500	Baseline			
	Periurban	262	0.62	0.44 to 0.88	0.0067	**
	Rural	763	1.30	0.90 to 1.88	0.16	^
Income (FJD household^{-1} week^{-1})	0-99	548	baseline			
	100-199	490	0.98	0.31 to 1.64	0.91	
	200-299	296	0.96	0.72 to 1.35	0.77	
	300-399	61	0.78	0.48 to 1.27	0.32	
	400+	81	0.91	0.49 to 1.67	0.75	
Drink tap water at home	4+d/wk	1427	baseline			
	Never	87	1.68	1.15 to 2.47	0.0075	**
	Monthly	3	1.09	0.10 to 12.17	0.95	
	1-3d/wk	8	1.3	0.32 to 5.33	0.71	
Drink river water	Never	1451	Baseline			
	< monthly	12	0.72	0.24 to 2.13	0.55	
	Monthly	11	1.23	0.45 to 3.31	0.69	
	1-3d/wk	9	2.68	0.73 to 9.93	0.14	^
	4+d/wk	36	1.92	1.04 to 3.55	0.037	*
Drink kava	Any vs never	608:913	1.43	1.12 to 1.84	0.0048	**
	At least monthly vs less than monthly	1018:503	1.28	1.02 to 1.61	0.037	*
Kava shared with how many people at last consumption?	Not applicable	895	Baseline			
	Zero to nine	387	1.28	(1.02 to 1.62)	0.0367	*
	Ten or more	235	1.79	(1.24 to 2.58)	0.0018	**
Bath or swim in rivers	Never	1065	Baseline			
	< monthly	187	0.89	0.59 to 1.34	0.57	
	Monthly	99	0.82	0.52 to 1.29	0.39	
	1-3d/wk	83	0.90	0.51 to 1.56	0.70	
	4+/wk	92	1.40	0.81 to 2.41	0.23	^
Home toilet	Flush	1174	Baseline			
	Water seal (pour flush)	244	1.52	1.03 to 2.25	0.035	*
	Pit or bucket	106	1.38	0.98 to 1.93	0.62	
Sewage	Piped sewer	285	Baseline			
	Septic tank	991	1.21	0.90 1.62	0.20	^
	Pit	138	2.07	1.31 3.26	0.0019	*
	Elsewhere	42	1.17	0.62 2.22	0.63	
Toilet location	Indoor	913	Baseline			
	Detached	610	1.22	0.94 to 1.58	0.13	^
Shared toilet	Private	1379	Baseline			

Variable	Value	Count	OR	95% CI	P-value	
	Shared	106	0.86	0.59 to 1.26	0.44	
Soap available after household toilet use	No	133	baseline			
	Yes, reported	1199	0.84	0.52 to 1.35	0.47	
	Yes, seen	189	0.78	0.45 to 1.35	0.38	
	Yes, seen/reported	1388	0.83	0.52 to 1.33	0.44	
Self-reported soap use	No	414	Baseline			
	Yes	1091	1.00	0.77 to 1.29	0.99	
Household tap	No	102	Baseline			
	Yes	1422	0.72	0.46 to 1.12	0.15	^
Typhoid vaccination self-report	No + Don't know	1304+12	Baseline			
	Yes	103	1.51	1.07 to 2.12	0.019	*
Typhoid diagnosis self-report	No	1453	Baseline			
	Yes	20	2.15	0.96 to 4.82	0.062	^
Typhoid in the household, self-report	No + Don't know	1447+6	Baseline			
	Yes	20	0.63	0.19 to 2.12	0.46	
Know at least one person who has had typhoid	No + Don't know	1341+36				
	Yes	93	1.51	1.01 to 2.25	0.042	*

3.7.1 Supporting information on self-reported vaccination.

Self-reported typhoid immunisation history was considered to be non-informative, as only 21% of Taveuni islanders reported vaccination against an expected coverage of over 90%.[11] Furthermore, 100 (6.7%) mainland residents reported receiving a typhoid vaccine, in locations where coverage was predicted to be zero with only five of these participants having possibly previously resided in areas covered by the 2010 vaccination campaign. A subgroup sensitivity analysis was conducted in the 1,428 mainland participants who reported no history of typhoid immunisation. Seropositivity for anti-Vi IgG in this group was observed at thresholds of 1:64, 1:100, 1:500 and 1:1,000 for 31.4% (27.0 to 35.8%), 17.7% (13.5% to 20.7%), 2.8% (1.4 to 4.4%) and 1.4% (0.3 to 2.5%) respectively, indicating no difference from the full mainland survey group.

Chapter 4. Environmental factors drive the spatial distribution of Salmonella Typhi in Fiji: a Vi-antigen seroprevalence study. de Alwis R, Watson CH, Nikolay B, et al. Emerging Infectious Diseases. Under Rev. 2017.

4.1 Bridging section

In parallel to the main typhoid serological survey analysis, the data was provided to Rukie de Alwis, a postdoctoral laboratory scientist retraining in public health through an MSc at LSHTM, to undertake an analysis of geospatial risk factors for anti-Vi seropositivity as her MSc project. This was done recognising that there would not be scope within the PhD for me to undertake a geospatial element to the typhoid sero-epidemiological analysis, particularly given unfolding Ebola response activities, but that there might be epidemiological and public health utility in Fiji to also considering findings from such an analysis. The data was made available for analysis in academic year 2014-2015 on the understanding of the primacy of the main serosurvey paper. The geospatial analysis continued to be developed through to 2017. Analysis was led by Rukie de Alwis under the supervision of Jorge Cano and Birgit Nikolay of LSHTM.

The serosurvey was designed from the outset to include collection of coordinates of participants' residential location by handheld GPS devices, enabling geospatial analysis to be included. This was a particular consideration for serum banking, and for the ecological-epidemiological approaches that were to be applied to leptospirosis when this component was brought into the serosurvey.

Two GPS devices were brought to Fiji from London, and two loaned by John Lowry, a GIS specialist at the School of Geography, University of the South Pacific, Suva. Teams were trained in their use, including need to be outside, and leaving sufficient time to allow satellite detection and position lock. Coordinates were entered as Degrees-Hours-Minutes-Seconds to reduce transcription errors compared with a degree decimal system when recording onto survey questionnaires. Coordinates were converted to degree decimal during data cleaning. Geographical data are displayed at a level that avoids individual participant identification.

John Lowry had painstakingly assembled rich geospatial data of Fiji including data on agriculture, rainfall, fluvial systems and soil types, and provided technical input, both of which were critical to the analysis. My main contributions were in identifying the scope for geospatial analysis, designing and delivering a field survey that could be analysed in such a manner. I also contributed to the interpretation of the analysed data, particularly towards

caution in interpreting and reporting observed findings, given uncertainties in serology, and multiple testing, particularly with respect to clustering.

This chapter is the only research paper in the thesis on which I am not lead author. It sits between a thesis chapter and an appendix. It is closer to an appendix paper in ownership, and may be read as such. But because it contributes to the narrative of understanding of typhoid in Fiji, it is placed in the main body of the thesis.

There was a further consideration in collecting the coordinates of study participants. In a country without a comprehensive address system, this was an important contributor in being able to follow-up study participants. This has been utilised in the arbovirus serological cohort study that has continued for two follow-up rounds in the Central Division.

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PLEASE NOTE THAT A COVER SHEET MUST BE COMPLETED FOR EACH RESEARCH PAPER INCLUDED IN A THESIS.

SECTION A – Student Details

Student	Conall Watson
Principal Supervisor	John Edmunds
Thesis Title	Seroepidemiological investigations of typhoid fever in Fiji and the potential role of vaccination in control

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	Environmental factors drive the spatial distribution of Salmonella Typhi in Fiji: a Vi-antigen seroprevalence study. *Emerging Infectious Disease*
Please list the paper's authors in the intended authorship order:	de Alwis R, Watson CH, Nikolay B, et al. See manuscript for full authorship.
Stage of publication	Under second review

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	Designed and implemented the field study, led the research collaboration. Critically reviewed all drafts of the manuscript.

Student Signature: [redacted] **Date:** 12 Sep 2017

Supervisor Signature: [redacted] **Date:** 12 Sep 2017

Environmental factors drive the spatial distribution of Salmonella Typhi in Fiji: a Vi-antigen seroprevalence study

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Keywords:

Salmonella Typhi; Fiji; Environmental factors; Risk Factors; Multilevel Analysis; flooding; Vi antibodies.

Abstract

Fiji recently experienced a sharp increase in reported typhoid fever cases. To investigate the geographical distribution and environmental risk factors associated with *Salmonella enterica* serovar Typhi (*S*.Typhi) infection, we conducted a cross-sectional cluster survey with associated serological testing for Vi-specific antibodies (a marker of *S*.Typhi exposure) in Fiji in 2013. High Vi-specific seroprevalence "hotspots" were identified in northeast of mainland Fiji. Risk of Vi-seropositivity increased with increasing annual rainfall (Odds Ratio, OR: 1.26 per quintile increase, 95% CI: 1.12-1.42), and decreased with increasing distance to major rivers and major creeks (OR: 0.89 per km increase, 95% CI: 0.80-0.99) and distance to modeled flood-risk areas (OR: 0.80 per quintile increase, 95% CI: 0.69-0.92), after being adjusted for age, typhoid vaccination and home toilet type. Risk of *S*.Typhi exposure and its spatial distribution in Fiji are strongly driven by environmental factors. This study's findings can directly impact typhoid-control efforts in Fiji.

4.2 Introduction

With an estimated disease burden of 20.6 million cases in low and middle-income countries (LMICs) in 2010, typhoid fever remains an enteric disease of public health importance (1, 2). Typhoid cases largely arise in LMICs, as marked improvements in water, sanitation and sewage are considered to have helped reduce typhoid incidence in most developed countries (3-6). *Salmonella enterica* subspecies Typhi (*S.* Typhi) is the causative agent of typhoid fever. *S.* Typhi is specific to the human host and is typically transmitted faecal-orally between humans through the ingestion of contaminated food and water (3, 7). Typhoid infections are usually acute, although in around 3-5% of cases *S.* Typhi establishes an asymptomatic and persistent (chronic) infection. These individuals are commonly referred to as typhoid carriers, and are capable of shedding bacteria and sustaining transmission within the community (3, 8).

Pathogenicity of *S.*Typhi is conferred by virulence factors such as Vi-polysaccharide. The Vi-polysaccharide is an outer capsular antigen that enables greater human infectivity than those *S.*Typhi strains not expressing the antigen (9). Due to the highly antigenic nature of Vi, infection with Vi-positive *S.*Typhi strains elicits Vi-specific antibodies in humans (10). Therefore, detection of Vi-specific IgG antibodies can be used to measure *S.* Typhi exposure, either past infection(s) or chronic infection (11). Furthermore, the current human-approved typhoid vaccines are primarily Vi antigen-based (such as Vi polysaccharide and Vi conjugate vaccines) (12). Despite antigenicity of the Vi-polysaccharide, antibodies and immunity conferred by the Vi-vaccine is short lived (13).

Fiji is an archipelago of over 300 islands situated in the Pacific Ocean, with a majority of its population living on the two islands of Viti Levu and Vanua Levu. Between 1991 and 2000, less than 5 typhoid cases/100,000 people were reported per year, and mostly in Vanua Levu (14, 15). However, since 2005, the number of typhoid fever cases have been rising (16) and reached a peak of over 50 cases/100,000 inhabitants per year following the widespread destruction and flooding caused by Cyclone Tomas in 2010. As a result, the Fiji Ministry of Health increased surveillance and implemented additional prevention strategies, such as vaccination against typhoid fever in the worst affected regions (17, 18).

The risk factors for typhoid transmission in Fiji are only partially understood. Inadequate hand-washing practices, poor sanitation, lack of access to safe water, dumping of untreated waste/sewage are thought to contribute to typhoid transmission in Fiji (17, 19). In addition, every year between November and April Fiji experiences powerful cyclones, which have led

to destruction of homes and contamination of water sources by extensive rainfall and flooding, followed by a rise in diarrheal diseases (20, 21). Although flooding has been shown to lead to outbreaks of other food and waterborne diseases (22-24), a direct link between flooding and increased typhoid fever incidence has not been confirmed in Fiji.

Public health efforts to control typhoid have been hampered by the lack of information regarding the epidemiology, spatial distribution and risk factors of typhoid exposure in Fiji. Therefore, we used the presence of Vi-specific antibodies as a biomarker for typhoid exposure, and combined both geospatial and statistical approaches to identify environment-associated risk factors in the general population of Fiji. Due to the yearly occurrence of cyclones in Fiji, we gave special attention to the potential contribution of flooding (and flood-promoting factors) to *S.* Typhi Vi-seropositivity.

4.3 Methods

4.3.1 Study design

This study was a cross-sectional cluster survey with an associated serological analysis, which was conducted across three Fijian divisions, i.e. Northern, Central and Western divisions. Administrative areas where the 2010 typhoid vaccination campaign (18) had been implemented were excluded. Nursing zones were selected using probability-proportional-to-size (PPS) random sampling based on census data. Cluster sites ("communities") within nursing zones were selected using random list sampling, followed by random sampling of households within community cluster sites using community health worker censuses or modified World Health Organization's Expanded Program on Immunization sampling (25), and then random sampling of an individual per household. Children under 1 year of age were excluded from the study. Community visits and data collection took place during September-December 2013, and entailed questionnaire administration, blood sample collection and geolocation of surveyed households. Geographical coordinates were collected using handheld geographical positioning system (GPS) devices at the participant's house or the nearest community center. Sample size was calculated at alpha=0.05 using expected seroprevalence informed from prior studies (26). Further details on study design and sampling are described in chapter 3 (26).

Informed consent was obtained in writing or thumb-print from all adult participants and parents or guardians of participating children, with written assent from children aged 12 and

over. This study was approved by the Fiji National Research Ethics Review Committee (#201303) and the London School of Hygiene and Tropical Medicine's ethics committees (#6344 and #9187).

4.3.2 Survey data

The cross-sectional survey collected information on forty-four variables, as previously described (26). Thirteen survey variables were chosen for the present typhoid risk factor analysis based on potential environmental risk factors of interest and potential confounding covariates (26). These variables included age, education, self-reported typhoid vaccination status, type of toilet at home, type of sewage, work location, urbanization and several flooding-related variables (Figure 4.1 and Technical Appendix Table TA4.2).

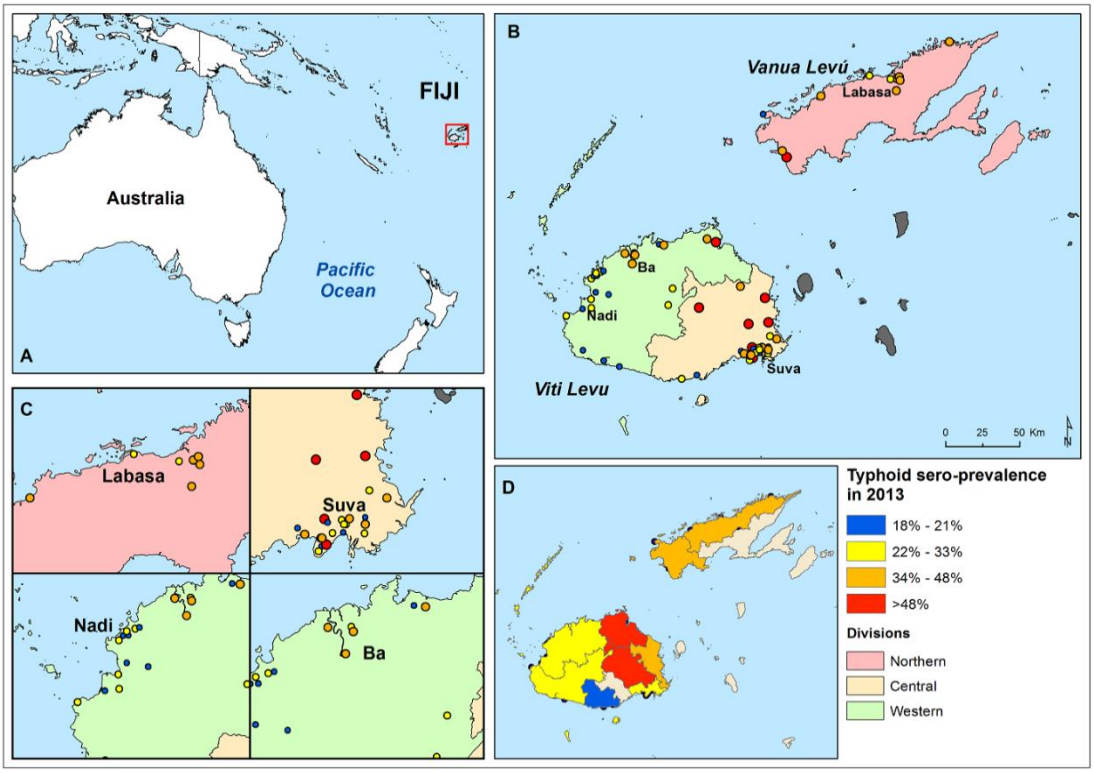

Figure 4.1. Geographical distribution of anti-Typhoid Vi seroprevalence in 2013 in Fiji.

Location of Fiji islands in the Pacific and Vi-seroprevalence in sampled communities in 2013 (A and B). Details of typhoid seroprevalence in large Fijian cities (i.e. Labasa, Suva, Nadi and Ba) (C). Typhoid seroprevalence estimated for Fijian subdivisions (D).

4.3.3 Vi-specific serology

Vi-specific antibody levels were determined using an enzyme-linked immunosorbent assay (ELISA) (methods adapted from Rondini et al 2011) (27). Briefly, ELISA plates were coated

with Vi-polysaccharide antigen, blocked with non-fat milk buffer, followed by incubation with participant sera (dilution 1:200), proceeded by alkaline phosphatase-conjugated anti-human IgG. Antibody binding was detected using p-Nitrophenyl phosphate substrate (Sigma-Aldrich) and absorbance measured at 405nm. As previously established in Watson, C et al (2017) (26, 28), we used a cut-off of ≥64 ELISA units (EU) to classify as Vi-seropositive.

4.3.4 Geospatial mapping and clustering

The geographical centroid of each community was estimated by averaging latitude and longitudinal coordinates of households sampled within each community. Typhoid seroprevalence for each geo-referenced community was computed using the Vi-seroimmune status of participating individuals who resided in each community. Confirmed-typhoid case incidence data was obtained from the Fijian Ministry of Health and mapped per subdivision. All geographical coordinates of communities were presented in the local projected coordinate system, Fiji Map Grid 1986.

Global and Anselin local Moran's *I* tests were used to identify statistically significant spatial clusters and conducted using GeoDa v1.6.7 (Technical Appendix text TA4.2) (29, 30). Vi-seroprevalence was log-transformed, separate row-standardized spatial weight matrices were calculated based on an inverse-distance relationship, and global and local spatial associations were analyzed within each division.

4.3.5 Environmental variables

Fijian administrative boundaries were downloaded from Global Administrative Divisions Map (GADM) (31). The largest administrative boundaries are known as Divisions (i.e. Central, Western, Northern and Eastern), and the island of Viti Levu is made up of Central and Western Divisions, while the island Vanua Levu is the Northern Division. Smaller island groups make up the administrative Eastern Division (where the present study did not collect samples). The Divisions are further broken down into 14 subdivisions.

Geospatial environmental data were provided by the University of South Pacific (Suva, Fiji): topography data (elevation and slope), climate data (annual rainfall, rainfall of the wettest month, total rainfall for cyclone season), hydrology data (rivers and creeks) and soil data (soil type according to composition and drainage quality) (32, 33). Euclidean distance maps of straight-line distance to major rivers and creeks, and poorly drained soils were generated from hydrology and soils maps, respectively. Further details of spatial data used in the study are provided in Technical Appendix Table TA4.3.

A deterministic flood-risk model was generated based on the principle that depressions and poorly drained soils are more likely to collect rainwater and be flooded (34). Further details on the development of this flood-risk map are provided in Technical Appendix text TA4.1 and Technical Appendix Figure TA4.1.

Except for the rainfall variables that were extracted at the community level, the remaining environmental data were extracted at the individual geo-spatially coded household level using bilinear interpolation. All geospatial processing and mapping was done using ArcGIS v10.2 (Redlands CA, USA).

4.3.6 Multilevel mixed-effect logistic regression

Risk factors for typhoid Vi-seropositive status were identified using multilevel mixed-effects logistic regression (also known as a generalized linear mixed-effect model, GLMM) by including environmental and individual-related covariates as fixed-effect and a random intercept. First, a null multilevel mixed-effects logistic model was run with the typhoid sero-immune status (binary variable) as the dependent variable. The variance partition coefficient (VPC) and a caterpillar plot (Technical Appendix Figure 2) were generated using community residuals.

Sixteen environmental covariates (Table 4.1) were tested in the univariable analysis. Regarding continuous independent variables, if analysis showed at least moderate evidence of an association with seropositivity ($p<0.05$), then the variable was used in the multivariable analysis as a continuous variable. However, if analysis showed weak or no evidence of an association with typhoid seropositivity ($p>0.05$), then the continuous variable was divided into quintiles (Technical Appendix Table 4.4) and re-tested in the univariable model separately as categorical or ordered-categorical variables. All continuous variables associated with Vi-seropositivity with a $p<0.10$ were tested for collinearity. Variables with high collinearity (correlation coefficient>0.8) were grouped, and the variable with the smallest *p*-value from each group was included in the multivariable analysis.

Table 4.1. Association between environmental factors and typhoid seropositivity using univariable multilevel mixed-effects logistic analysis

Environmental Variable	*n*	Variable Type	Odds Ratio (95% CI)	*P* value
Survey Data				
Is there a stream nearby? (0=No, 1=Yes)	1,508	Binary	1.09 (0.82-1.46)	0.528
No	*616*			
Yes	*892*			
No. of times house has flooded in the past 3 years	1,483	Categorical		
0	1,380		1.00 (reference)	-
1 to 2	97		0.87 (0.52-1.47)	0.604
3 to 5	6		0.89 (0.15-5.13)	0.897
No. of times land has flooded in the past 3 years	1,496	Categorical		
0	1,264		1.00 (reference)	-
1 to 2	174		1.13 (0.77-1.66)	0.534
3 to 5	58		1.21 (0.66-2.22)	0.542
Work Location*	1,359	Categorical		
Indoors	636		1.00 (reference)	-
Outdoors	267		1.59 (1.15-2.19)	0.005†
Both indoor and outdoor	456		1.22 (0.93-1.60)	0.160
Urbanization*	1,510	Categorical		
Urban	500		1.00 (reference)	-
Periurban	247		0.61 (0.37-1.01)	0.054
Rural	763		1.27 (0.89-1.81)	0.185
Geospatial Data				
Elevation (by quintiles)	1462	Ordered Categorical	1.02 (0.90-1.15)	0.793
Slope (by quintiles)	1462	Ordered Categorical	1.04 (0.93-1.15)	0.519
Temperature (by quintiles)	1462	Ordered Categorical	0.95 (0.84-1.07)	0.398
Annual Rainfall (by quintiles)*	1462	Ordered Categorical	1.13 (1.01-1.28)	0.039†
Rainfall in wettest month (by quintiles)	1462	Ordered Categorical	1.15 (1.02-1.30)	0.020†
Rainfall during cyclone season (by quintiles)	1462	Ordered Categorical	1.14 (1.01-1.29)	0.029†
Distance to major rivers (by quintiles)	1462	Ordered Categorical	1.07 (0.95-1.20)	0.255
Distance to major rivers and major creeks (Km)*	1462	Continuous	0.99 (0.99-1.00)	0.081
Distance to major rivers, major and minor creeks (by quintiles)	1462	Ordered Categorical	0.96 (0.86-1.07)	0.439
Distance to poorly drained soils - major & secondary flood plains (by quintiles)	1462	Ordered Categorical	0.92 (0.80-1.06)	0.275
Distance to poorly drained soils - major flood plains only (by quintiles)	1462	Ordered Categorical	1.00 (0.87-1.17)	0.949
Distance from modelled flood-risk area (by quintiles)*	1462	Ordered Categorical	0.90 (0.78-1.03)	0.134

*Variables included in the multivariable multilevel analysis. †Variables that quite strongly associated with typhoid sero-immune status in the univariable analysis (i.e. $p<0.05$).

In addition to five environmental variables (as indicated in Table 4.1), several non-environmental risk factors (i.e. age, education, self-reported typhoid vaccine status, type of home toilet, type of sewage, and know people who have had typhoid) of *S.* Typhi Vi-seropositivity were confirmed as significant risk factors by univariable analysis (Technical Appendix Table TA4.2) and included in the multivariable analysis. Parsimonious regression models were developed using a backward stepwise variable selection approach, eliminating one variable at a time based on the highest *p*-value in a likelihood ratio test and retaining only variables with $p \leq 0.05$. The final fitted multivariable statistical model was validated using the Hosmer-Lemeshow test and by generating predicted typhoid seroprevalence values for sampled communities (Technical Appendix Figure TA4.2). Data were analyzed using Stata v14 (Statacorp, College Station).

4.3.7 Boosted regression trees (BRT) modeling

A *base* model was developed using the location of communities (longitude and latitude) and those variables that were found to be associated with Vi-seropositivity in the univariable analysis. A simplification of the *base* model was conducted by removing redundant or non-informative variables. An ensemble of 50 BRT models with 11 of the most influential predictors and random sampling from a total of 1,305 samples (a minimum of 750 sampled at one time) was conducted to estimate relative contributions and marginal effect plots of the most influential variables. Further details on the BRT model are given in Technical Appendix Text TA4.3. BRT modeling was conducted in R (v3.2.2, www.R-project.org) using the '*gbm*' library (35).

4.4 Results

4.4.1 Detection of typhoid hotspot communities in Fiji

Approximately one-third of the serum samples (485/1,516) were sero-positive for Vi-specific antibodies (Technical Appendix Table TA4.1). Vi-seroprevalence among sampled communities in Fiji ranged from 8% to 65%, with 35% and 24% estimated for Central and Western divisions, respectively (Figure TA4.1). Furthermore, although northern division (Vanua Levu) has a smaller population, it had almost 40% Vi-seroprevalence.

Global Moran's *I* analysis showed strong evidence of geographical clustering of Vi-seroprevalence among sampled communities in the Western division (I=0.49, p=0.002), and weak evidence for Central and Northern divisions (I=0.08 and -0.42, p=0.08 and 0.10, respectively). Anselin Local Moran's *I* test showed that although Vanua Levu had high typhoid

seroprevalence, there was no apparent typhoid hot-spot clustering among communities in this island (Figure 4.2A). However, four high-high (hot-spot) seroprevalence cluster communities were detected in the north and northeast of Western and Central divisions, respectively (Figure 4.2B and 4.2C), while cold-spots were primarily detected in the Western division (Figure 4.2B).

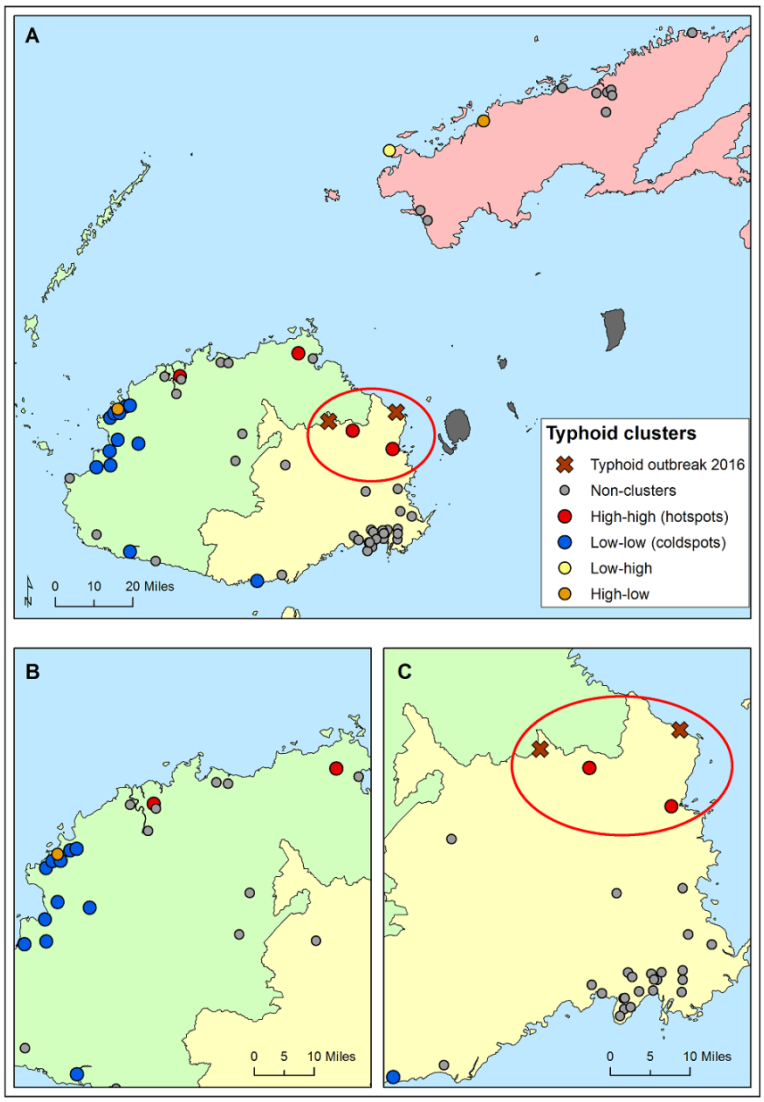

Figure 2. Local clustering of typhoid seroprevalence within divisions in Fiji. *Local Anselin Moran's I analysis conducted for each division separately using an inverse-distance weighting for the communities within the three divisions, north (A), western (B) and central (C). High-high clusters (also known as hotspots) are high Vi-seroprevalence communities that are close to other high Vi-seroprevalence communities. Similarly, low-low clusters (also known as coldspots) are low Vi-seroprevalence communities that are in close proximity to other low Vi-seroprevalence communities.*

4.4.2 Multilevel univariable and multivariable analysis

Univariable analysis identified four environmental variables (i.e. work location, annual rainfall, rainfall in the wettest month and rainfall in the cyclone season) and four non-environmental variables (i.e. age, education, sewage disposal, typhoid vaccination status) with significant association with Vi-seropositivity ($p<0.05$) (Table 4.1 and Technical Appendix Table TA4.2). Furthermore, there was suggestive evidence of an association with Vi-seropositivity (i.e. $0.1>p>0.05$) for several other environmental and non-environmental variables (i.e. urbanization, distance to major rivers and major creeks, toilet type, knowing people who has had typhoid) (Table 4.1 and Technical Appendix Table TA4.2).

One rainfall variable and all other environmental and non-environmental factors with at least a suggestive association (i.e. $p<0.01$) were included in the multivariable multilevel logistic regression analysis (indicated in Table 4.1 and Technical Appendix Table TA4.2). Proximity to modelled flood-risk areas was included as a fixed-term in the final fitted multivariate model regardless of its evidence of association on the univariable analysis, since other environmental factors (such as rainfall and proximity to rivers) maybe confounding the univariable analysis. The final multivariable statistical model contained six variables that significantly explained the variation in Vi-seropositivity for sampled individuals and communities. After adjusting for potential confounders (i.e. age, typhoid vaccination and flush toilets), annual rainfall showed positive association (OR=1.26 per quintile increase, $p<0.001$, respectively), while distance to major rivers and major creeks and to modeled flood-risk areas showed negative associations with Vi-seropositivity (OR: 0.89 per km increase $p=0.031$ and OR 0.80 per quintile increase $p=0.002$, respectively) (Table 4.2).

Table 4.2. Association between social and environmental factors with Typhoid sero-immune status using a multivariable multilevel model

Variable	Odds Ratio (95% CI)*	*P* value
Annual Rainfall (by quintiles)	1.26 (1.12-1.42)	<0.001
Distance to major rivers and major creeks (km)	0.89 (0.80-0.99)	0.031
Distance to modelled flood-risk areas (by quintiles)	0.80 (0.69-0.92)	0.002
Age of participant (yr)	1.03 (1.02-1.03)	<0.001
Vaccination status (0=Not vaccinated, 1=Vaccinated)	1.62 (1.02-2.57)	0.041
Type of toilet at home		
Flush	-	
Water seal/ pour-flush	1.66 (1.16-2.38)	0.006

Pit (with/without slab) & bucket	1.51 (0.91-2.52)	0.110

*The multivariable model was run using 1,338 observations in 61 communities.

The fitted model not only explained fixed-effect variation across individuals, but also some of the variation across sampled communities. Comparison of the null and final models showed a reduction in VPC from 7.6% (p<0.0001) to 2.1% (p<0.0001), which means that the final statistical model explained 72% of the variation in seropositivity between communities. The final multivariable model fitted was validated using the Hosmer-Lemeshow test, where the predicted proportions computed at the individual level were not significantly different from the observed proportions (p=0.558) (Technical Appendix Figure TA4.2).

4.4.3 Boosted Regression Tree modelling

Age, GPS location and the three environmental factors, i.e. distance to major rivers and major creeks, distance to flood-risk areas and annual rainfall were estimated to be the major predictors of Vi-seropositivity in Fiji (Table 4.3). These six covariates accounted for almost 90% of the estimated relative contribution to *S*.Typhi Vi-seropositivity.

Table 4.3. Relative contributions (%) of predictor variables from an ensemble of 50 boosted regression tree models for typhoid seropositivity developed with cross-validation on data from 1,305 samples and 11 variables

Variable	Data type	Relative Contribution (95% CI)
Age (yr)	Continuous	33.0 (31.1 – 34.8)
Longitude (Degrees)	Continuous	15.5 (14.7 – 16.0)
Distance from major rivers & creeks (m)	Continuous	14.5 (13.6 – 15.3)
Annual rainfall (mm)	Continuous	9.3 (8.5 – 10.0)
Distance from flood-risk areas (m)	Continuous	7.7 (6.8 – 8.4)
Latitude (Degrees)	Continuous	6.9 (5.6 – 7.9)
Education	Categorical	4.2 (3.8 – 4.6)
Urbanization	Categorical	3.3 (2.9 – 3.8)
Typhoid vaccination	Binary	2.3 (2.1 – 2.5)
Sewage disposal	Categorical	1.8 (1.5 - 2.2)
Toilet type at home	Categorical	0.8 (0.6 – 1.2)

The marginal effect plot for age showed that a majority of initial exposure to *S*.Typhi occurs before 40 years of age and plateaus above 60 yrs (Figure 4.3A). Distances of less than ~1,300 m to major rivers and major creeks were predicted to increase Vi-seropositivity, with distances less than ~200 m having the largest effect (Figure 4.3B). Annual rainfall had minimal effect on Vi-seropositivity until around 1,700 mm, above which the risk increased

dramatically (Figure 4.3C). Furthermore, shorter distances to modeled flood-risk areas showed some contribution to typhoid seropositivity (Figure 4.3D).

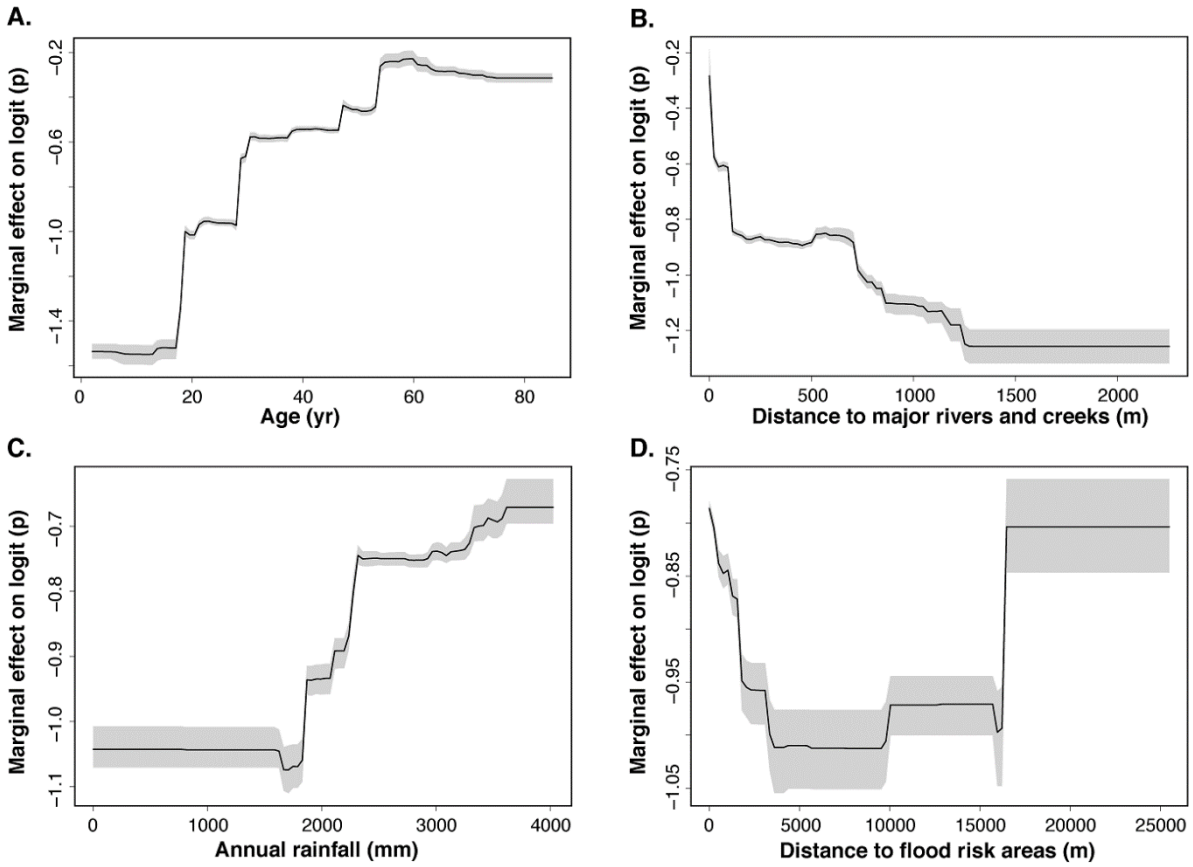

Figure 4.3. Partial dependence plots for the four most influential variables in the BRT model for typhoid seropositivity.

The partial dependence plots of age (A), Distance to major rivers and creeks (B), Annual rainfall (C) and Distance to flood-risk areas (D). The final ensemble BRT was built upon 50 BRT models, 11 environmental and social covariates, using data from 1,305 samples. The grey area depicts the 95% confidence intervals of the plots.

4.5 Discussion

In the past two decades Fiji has observed a steady rise in confirmed typhoid fever cases (16-18). However, little is known about the geospatial distribution and underlying risk factors of typhoid fever in Fiji. Our study demonstrated a spatially heterogeneous exposure to typhoid fever across Fiji, with Vanua Levu island having the highest seroprevalence. High-seroprevalence communities (hotspots) were only detected in Viti Levu whilst typhoid fever appeared to be more homogeneously distributed in Vanua Levu, suggesting a different transmission pattern in the two islands. Annual rainfall, and proximity to major rivers, creeks

and potentially floodable areas were found to be important environmental risk factors of serological evidence of exposure to *S.*Typhi in Fiji.

The Vi-seroprevalence distribution closely resembled the typhoid case incidence pattern reported by the Fijian Ministry of Health for the period 2008-2013 (Technical Appendix Figure 4.3), with Vanua Levu and the northeast of Viti Levu having the largest typhoid burden. Interestingly, in April 2016 following the wake of cyclone Winston hitting Fiji, there was a sudden outbreak of typhoid fever in the villages of Qelekuro and Nabulini (36), which were both located in northeastern Viti Levu. This latest typhoid outbreak in Fiji supports our findings of high-risk areas for *S.*Typhi exposure particularly in the northeastern region of Viti Levu island (Figure 4.2A) and reinforces the hypothesis of increased exposure to typhoid due to environmental anomalies in the aftermath of a cyclone.

Similar to our findings, other studies have found positive associations between faecal-orally transmitted diseases (such as cholera and typhoid) and waterborne diseases (such as leptospirosis) with heavy rainfall and proximity to major rivers (37-41). Heavy rains in Fiji, particularly during the cyclone season (November-April) (21), may lead to the overflowing of septic tanks and contamination of the local environment and drinking water sources. Furthermore, our study indicated proximity to major rivers and creeks as a risk factor for acquiring *S.*Typhi probably due to major rivers and creeks being used in Fiji (similar to many other middle-income countries) as places for washing clothes, taking baths and swimming (42). In addition, streams near populated areas can become contaminated as a result of cyclones or heavy rains causing overflow of sewage and waste systems. Therefore, future studies investigating environmental risk factors should sample surrounding water sources for water quality assessment.

Many food and waterborne diseases have been shown to increase soon after heavy flooding (22-24, 43). Fiji experiences typhoid fever and leptospirosis outbreaks following devastation and flooding by cyclones (16, 18, 41, 44). Our multivariate model demonstrated an increased risk for *S.*Typhi infection for those individuals living closer to the modeled flooding areas. Annual cyclone season and heavy rainfall combined with a majority of the Fijian population living in low-lying coastal areas make exposure to flooding a very common phenomenon in Fiji and a potential conduit of *S*.Typhi transmission.

A major strength of this study is the unbiased, individual-level assessment of environmental factors specific to each participant based on their residential GPS coordinates. Furthermore, the large sample number analyzed enabled inclusion of a large number of independent

variables (consisting both major non-environmental risk factors and environmental variables) in the statistical modeling. Despite many strengths, the present study also has several limitations. Although Vi-specific antibodies were measured as a proxy for *S*.Typhi infection, the role and dynamics of Vi-specific antibodies following *S*.Typhi infection is unclear. For example, anti-Vi antibodies have been found to numtyphoid vaccination. Furthermore, geospatial cluster analysis was partially hampered by an uneven distribution of surveyed communities. To mitigate this potential spatial bias, we conducted spatial clustering analysis separately for each division.

The present study is an in-depth study of the spatial epidemiology of typhoid in Fiji. It also investigates flooding as a risk factor for typhoid transmission. Findings of this study can be used to inform future typhoid control programs. Recent outbreak detection in high seropositivity areas (36) suggests that anti-Vi IgG sero-surveillance offers potential for identification of areas and communities at higher risk of typhoid fever. This spatial epidemiology analysis suggests flood-prone areas and other communities lying close to major rivers and creeks or in high-rainfall areas could be prioritized for stricter flood-control and typhoid-preventative measures, such as improved sanitation, provision of secure water sources, and typhoid vaccination campaigns.

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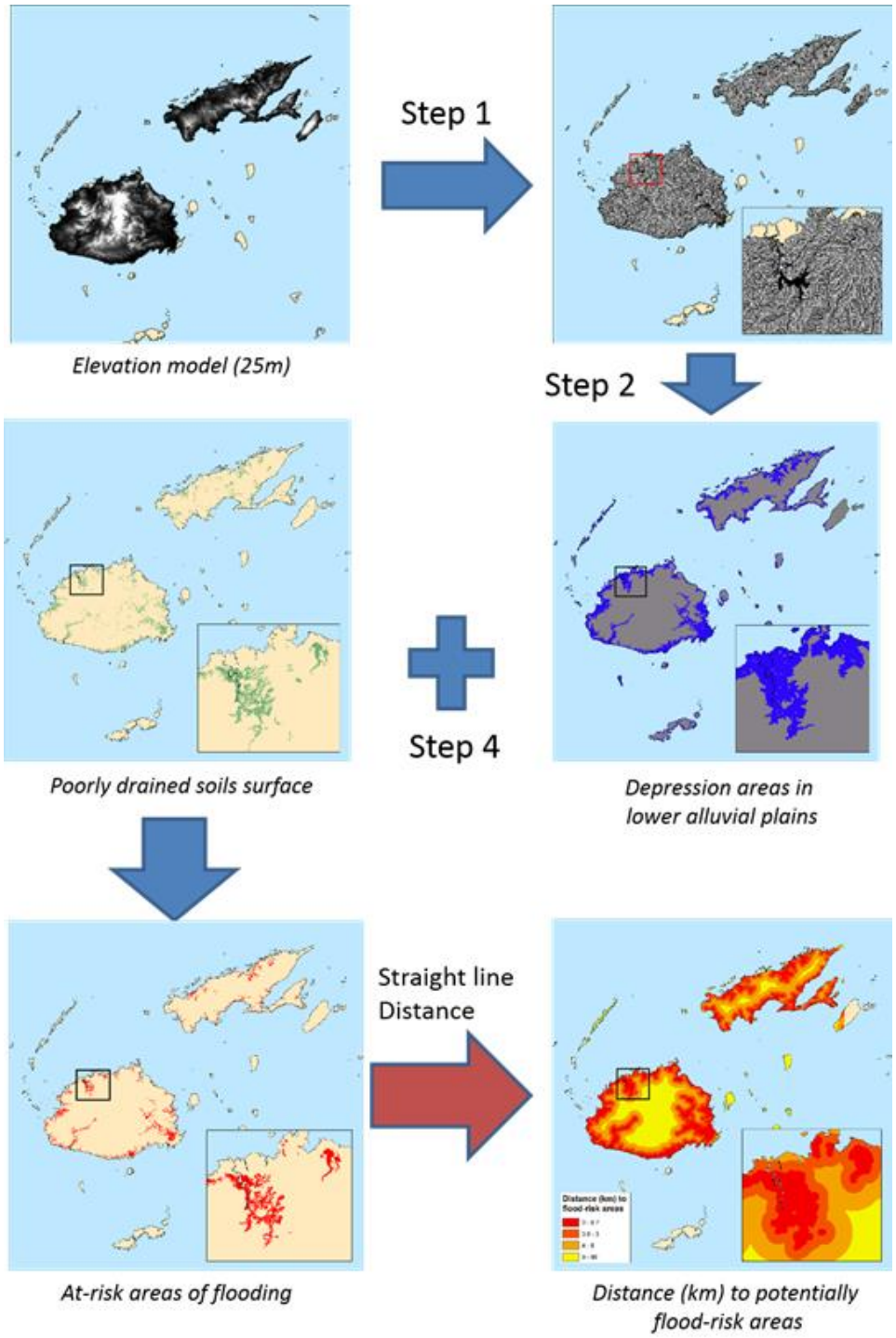
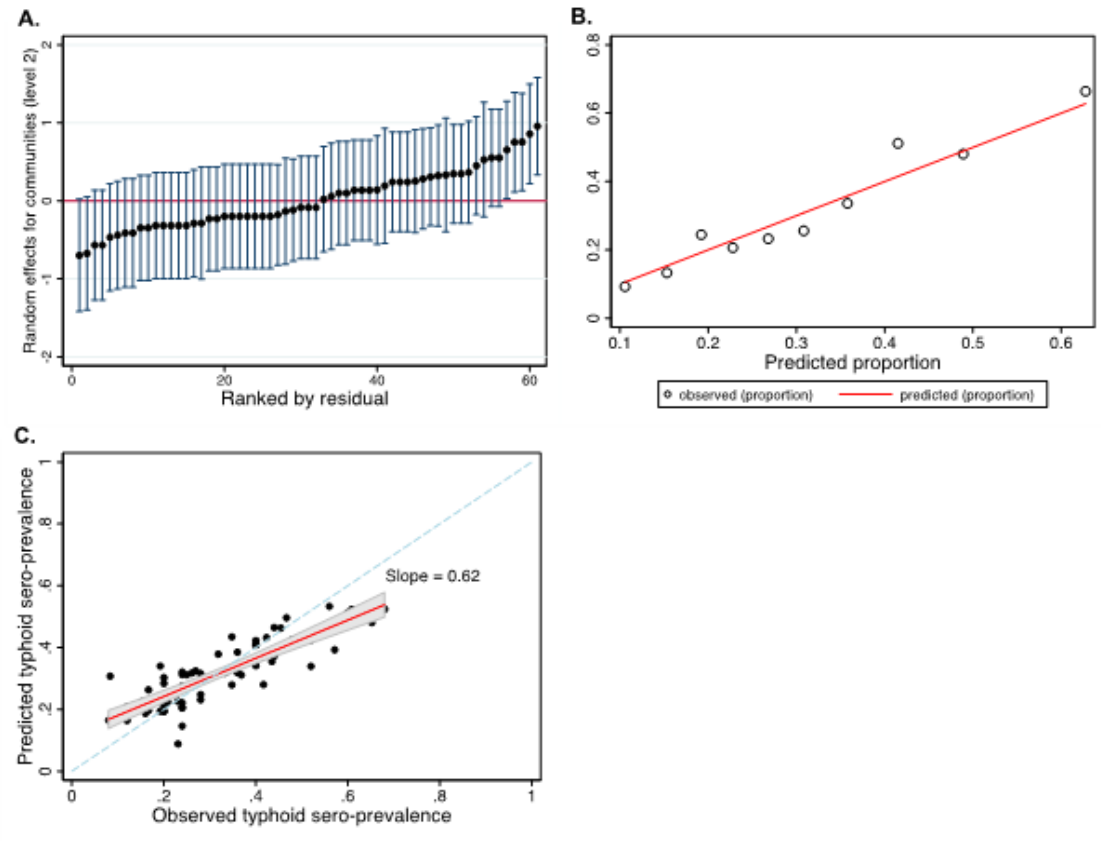
4.7 Technical Appendix



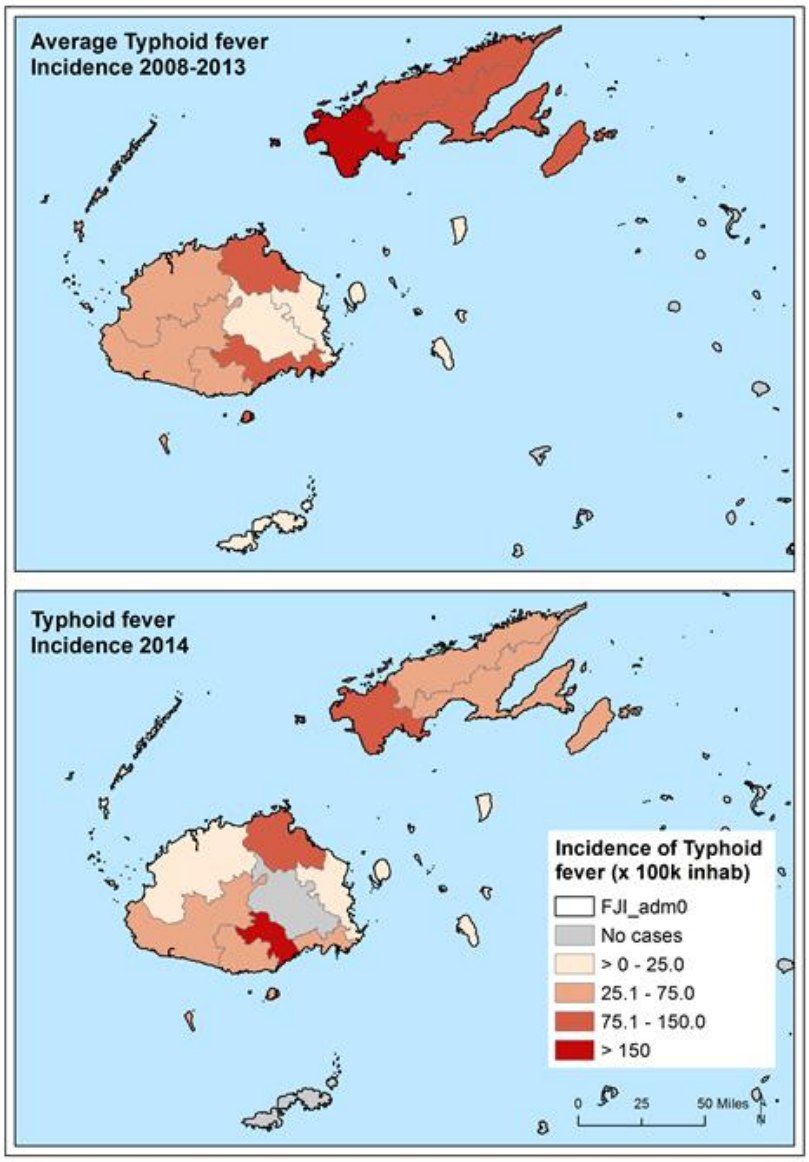
Technical Appendix Figure TA4.1. Development of a flood-risk model.

Detailed methods are described in Technical Appendix text TA4.1



Technical Appendix Figure TA4.2. Validation of the fitted multilevel mixed-effect logistic regression model.

Distribution of community random effect residuals with 95% CI to justify the use of a multilevel model (A). Validation of the final multilevel regression model to explain variation in sero-immune status to typhoid Vi antigen using the Hosmer-Lemeshow test (p-value= 0.558) (B). Assessing the final statistical model by comparing the predicted and observed typhoid sero-prevalence at the community level (C).



Technical Appendix Figure TA4.3. Confirmed typhoid fever case incidence per 100,000 inhabitants reported for each subdivision during 2008-2013 and 2014.

Technical Appendix Table TA4.1. Characteristics of samples collected by the survey and those included in the statistical analysis

Variable	*n*
Survey Samples	
Individuals	1,560
Communities	65
Anti-Typhoid IgG	1,531
Individuals per community, mean (range)	24 (15-28)
Samples included in analysis†	
Individuals	1,516
Communities	63
Anti-*S*. Typhi Vi IgG‡	1,516
Sero-negative (<64 EU)	1,031
Sero-positive (≥64 EU)	485
GPS coordinates	1,463
Community cluster area, km^2 (IQR)*	0.04 (0.02-0.13)

*Cluster area of each community was assessed using the sampled household locations of each community.

†Samples from pilot study were not included in present analysis.

‡Samples with missing Anti-*S*. Typhi Vi IgG titres were excluded from analysis.

Technical Appendix Table TA4.2. Univariable analysis of non-environmental risk factors of S. Typhi Vi-seropositivity used in the present study

Variable	Variable Type	Odds Ratio (95% CI)	*P*-value
Age (yr)	Continuous	1.03 (1.02-1.03)	<0.001*
Education	Categorical		
No Schooling		1.00 (reference)	
Primary		1.47 (0.94-2.30)	0.091
Secondary		1.71 (1.11-2.64)	0.015*
Vocation & University		1.17 (0.71-1.93)	0.546
Toilet at home	Categorical		
Flush		1.00 (reference)	-
Water seal/pour-flush		1.40 (1.00-1.95)	0.051*
Pit (with/without slab) & bucket		1.22 (0.75-1.99)	0.425
Sewage disposal at home	Categorical		
Piped sewer system		1.00 (reference)	-
Septic tank		0.59 (0.35-0.99)	0.048*
Pit latrine		0.65 (0.43-0.99)	0.043*
elsewhere		0.61 (0.28-1.33)	0.215
Typhoid vaccination status (*0=No, 1=Yes*)	Binary	1.67 (1.07-2.59)	0.023*
Do you know people who have had typhoid? (*0=No, 1=Yes*)	Binary	1.56 (0.96-2.54)	0.073*

*These non-environmental variables were included in the multivariable analysis.

Technical Appendix Table TA4.3. Characteristics of the topographical and environmental data variables used

Variable	Resolution (meters)	Mean (Std Err.)	Range
Geospatial data			
Elevation (m)	25	41.1(±89.3)	0-761 m
Slope (degree)	25	3.02(±3.81)	0-25.0
Mean Temperature (°C)	100	25.1(±27.5)	0-26.1
Annual Rainfall (mm)	100	2490(±660)	0-4040
Rainfall in wettest month (mm)	100	372(±76)	0-789
Rainfall during cyclone season (mm)	100	1032 (±195)	0-2055
Distance to major rivers (km)	25	1.21(±1.74)	0-9.8
Distance to major rivers and major creeks (km)	25	0.360(±0.343)	0-2.250
Distance to major rivers, major and minor creeks (km)	25	0.148(±0.177)	0-1.280
Distance to poorly drained soils - major & secondary floodplains (km)	25	0.722(±1.710)	0-11.250
Distance to poorly drained soils - major floodplains only (km)	25	2.370(±3.670)	0-17.410
Distance from modelled flood-risk area (km)	25	1.890(±4.260)	0-25.540

Technical Appendix Table TA4.4. The range of each category for the continuous variables that were broken into quintiles

Variables		Range
Elevation	Q1	0-7 m
	Q2	8-15 m
	Q3	16-19 m
	Q4	20-39 m
	Q5	≥40 m
Slope	Q1	0.00 deg
	Q2	0.40-1.21 deg
	Q3	1.28-2.29 deg
	Q4	2.36-4.45 deg
	Q5	≥4.46 deg
Temperature	Q1	0-25.19°C
	Q2	25.20-25.37°C
	Q3	25.38-25.64°C
	Q4	25.65-25.81°C
	Q5	≥25.82°C
Annual Rainfall	Q1	0-1909 mm
	Q2	1910-2265 mm
	Q3	2266-2582 mm
	Q4	2583-3104 mm
	Q5	≥3105 mm
Rainfall in wettest month	Q1	0-338 mm
	Q2	339-360 mm
	Q3	361-379 mm
	Q4	380-408 mm
	Q5	≥409 mm
Rainfall during cyclone season	Q1	0-943 mm
	Q2	944-1001 mm
	Q3	1002-1053 mm
	Q4	1054-1125 mm
	Q5	≥1126 mm
Distance to major rivers	Q1	0-0.150 km
	Q2	0.151- 0.459 km
	Q3	0.460-0.908 km
	Q4	0.909-1.726 km
	Q5	≥1.727 km
Distance to major rivers and major creeks	Q1	0-0.090 km
	Q2	0.091-0.195 km
	Q3	0.196-0.320 km
	Q4	0.321-0.506 km
	Q5	≥0.507 km
Distance to major rivers, major and minor creeks	Q1	0-0.025 km

Variables		Range
	Q2	0.026-0.075 km
	Q3	0.076-0.111 km
	Q4	0.112-0.200 km
	Q5	≥0.201 km
Distance to poorly drained soils (major & secondary floodplains)	Q1	-
	Q2	0-0.044 km
	Q3	0.045-0.152 km
	Q4	0.153-0.776 km
	Q5	≥0.777 km
Distance to poorly drained soils (major floodplains only)	Q1	-
	Q2	0-0.276 km
	Q3	0.277-1.521 km
	Q4	1.522-4.310 km
	Q5	≥4.311 km
Distance from modelled flood-risk area (km)	Q1	-
	Q2	0-0.127 km
	Q3	0.128-0.576 km
	Q4	0.577-1.681 km
	Q5	≥1.682 km

4.7.1 Technical appendix text

Technical Appendix Text TA4.1. Building of a flood-risk model.

The flood-risk model was created in four main steps as described below:

1. A map depicting depression sites (or sink areas) was created using the DEM raster. A convex or depression surface was obtained with the formula; original DEM – mean DEM, where values < 0 were identified as convex zones. First, a mean DEM raster was created by averaging the elevation of 10x10 neighbouring (i.e. a 250x250m area). Then, the depression map was obtained by subtracting the mean DEM raster from the original DEM map, and selecting only the regions with negative pixel values.
2. Areas selected as potential flooding areas where those that were convex and fall within an elevation range between 0-40 m, which is approximately the elevation range corresponding to the lower alluvial plains, which is generally affected during severe flooding (Townsend PA, Walsh SJ: Modeling floodplain inundation using an integrated GIS with radar and optical remote sensing. *Geomorphology* 1998, 21:295-312.).
3. Then a raster map with poorly drained soils was created using the polygon features ranging from imperfectly to very poorly drained soils.
4. A new raster flood-risk map was created using only the overlapping regions of the depressions map and the poorly drained soils map. These overlapping regions were marked as regions at high-risk of flooding. Finally, a surface map estimating Euclidean distances to these high-risk flooding regions was created.

Global Moran's *I* statistic (Moran PAP: Notes on continuous stochastic phenomena. *Biometrika* 1950, 37:17-23) was used to account for the global spatial autocorrelation of typhoid fever sero-prevalence. For the Moran's *I* statistic, the sum of covariations between the sites for the distance *d(i,j)* was divided by the overall number of sites $W(d_{i,j})$ within the distance class *d(i,j)*. Thus, the spatial autocorrelation coefficient for a distance class *d(i,j)* was the average value of spatial autocorrelation at that distance.

$$I = \frac{\mathrm{n}}{\mathrm{S}_p} \frac{\sum_{i=1}^{n}\sum_{j=1}^{n} W_{ij}(\gamma_i-\bar{\gamma})(\gamma_j-\bar{\gamma})}{\sum_{i=1}^{n}(\gamma_i-\bar{\gamma})^2}, \text{ where}$$

n = the sample size;

$W_{ij} = \begin{cases} 1 \text{ if sites i, j are neighbours} \\ 0 \text{ otherwise} \end{cases}$ = row-standardized spatial weights matrix of sites *i* and *j;*

$\mathrm{S}_p = \sum_{i=1}^{n}\sum_{j=1}^{n} W_{i.j} =$ sum of the number of sampling locations per distance class;

$\gamma_i =$ the value at community i; and $\bar{\gamma} =$ global mean value

The actual value for Moran's *I* was then compared with the expected value under the assumption of complete randomisation.

$$E(I) = -\frac{1}{n-1}$$

Moran's *I* values may range from -1 (disperse) to +1 (clustered). A Moran's *I* value of 0 suggests complete spatial randomness. To verify that the value of Moran's *I* was significantly different from the expected value, a Monte Carlo randomisation test was applied with 9,999 permutations to achieve highly significant values. This statistic is a global statistic in that it averages all cross outcomes over the entire domain.

A local version, called Local Indicator of Spatial Association (LISA) or Anselin Local Moran's *I* statistic (Anselin L: Local Indicators of Spatial Association—LISA. *Geographical Analysis* 1995, 27:93-115) allows us to test for statistically significant local spatial clusters, including the type and location of these clusters. It is calculated as follows:

$$I_i(d) = \frac{(\gamma_i-\bar{\gamma})}{\frac{1}{n}\sum_{i=1}^{n}(\gamma_i-\bar{\gamma})} \sum_{i=1}^{n} W_{ij}(d)(\gamma_i-\bar{\gamma}), \text{ where}$$

$W_{ij}(d)$ is the row-standardized weights matrix given a local neighbourhood search radius *d.* The conceptualization of spatial relationship (i.e. neighbourhood definition) was the same as the global statistics were applied. Unlike the global Moran's *I,* which has the same expected

value for the entire study area, the expected value of local Moran's *I* varies for each sampling location because it is calculated in relation to its particular set of neighbours.

$$E(I_i) = -\frac{1}{n-1}\sum_{j=1}^{n} W_{i,j}$$

The significance of the local Moran's *I* was calculated using a randomization test on the Z–score with 9,999 permutations to achieve highly significant values. Positive spatial autocorrelation occurs when, a community with a specific typhoid sero-prevalence is surrounded by neighbouring communities with similar outcome value (low-low, high-high), thus forming a spatial cluster.

Technical Appendix Text TA4.3. Implementation of BRT modelling approach on typhoid sero-positivity data.

First, a single BRT model was constructed with the individual typhoid sero-immune status binary data, cross-validation optimization and accounting for multi-way interactions. As per guidelines (Elith J, Leathwick JR and Hastie T. A working guide to boosted regression trees. *J Anim Ecol* 2008, 77:802-813) the learning rate (l*r)* and tree complexity (*tc*) were set according to the number of observations and testing different values on a subset of samples (75%), using deviance reduction as the measure of success. After several test, lr of 0.0025 and tc of 5 were identified as optimal parameters, thereby enabling the model to account for up to 5 potential interactions and slowing it down enough to get the model converged without over-fitting the data. The *base* model was constructed including location of communities (longitude and latitude) and the eleven variables that were found to be associated with typhoid sero-positivity in the univariable logistic regression analysis (Technical Appendix Table TA4.2).

A simplification of the *base* model was conducted by removing redundant or non-informative variables without compromising the predictive performance of the model. This simplification process (implemented using the function *gbm.simplify*) was run within a 10-fold cross-validation (CV) procedure, progressively simplifying the model fitted to each fold, and using the average CV error to decide how many variables could be removed from original model without affecting predictive performance. Then an ensemble BRT (i.e. 50 BRT models) was run with the simplified model using five parallel CPUs to attain 95% confidence intervals in both the relative contributions of the variables and the marginal effect plots. Relative contributions of variables to typhoid sero-positivity were estimated using the ensemble BRT model. Fitted functions of the ensemble BRT model was visualized by graphing the marginal effect curves or partial dependence plots, which demonstrate the effect of each independent variable on the typhoid sero-positive outcome while all other variables in the model are held constant at its average.

Chapter 5. Social mixing in Fiji: who-eats-with-whom contact patterns and the implications of age and ethnic heterogeneity for disease dynamics in the Pacific Islands. Watson CH, Coriakula J, Dung TTN, et al

5.1 Bridging section

Social contact data has a well-established role in improving mathematical model predictions on transmission of spread of "close-contact" infections, such as measles, tuberculosis and meningococcus, where such data was previously estimated [1–3]. The introductory chapter of this thesis reviewed the historical and contemporary literature on transmission of *Salmonella* Typhi infection as a necessary prerequisite for the development of typhoid fever. Some of the challenge of typhoid epidemiology and control comes from its potential for multi-modal transmission. Faecal contamination of drinking water has contributed to high incidence in many settings.[4–6] Typhoid fever is recognised as a disease of "contagion" [6], "contact"[7], and "prosodemic" (person-to-person) transmission[8], which might be further separated into direct transmission, without intermediaries, and indirect transmission, such as through food prepared by a typhoid carrier [9]. A World Health Organization expert elicitation exercise found a diverse range of estimates of the contemporary contribution of water, foodborne and "person-to-person" disease across WHO regions [10].

Social mixing data does not directly disentangle of the important modes of transmission in any given setting. It cannot say if any association between social contact rates and incidence is attributable to direct transmission, or reflects, for example, common exposure of a particular social group to contaminated food or surface water. Melegaro and colleagues have noted the utility of social contact data in describing the strength of social relations, finding more intimate connections to be better predictors than more causal forms of social contact in influenza. As such, this data on social structure is potentially an informative proxy for transmission risks. The role of social mixing data in typhoid may be in examining whether socially structured contact patterns better predict infection than less structured transmission matrices that may reflect ubiquitous exposures.

This chapter describes the social mixing data used in the chapter 6, which examines models of transmission in Fiji. The social mixing survey was embedded in the seroepidemiological survey, obtaining data from the same participants. The preparatory work and field operations are described in the bridging section for the serosurvey paper (3.1). I conceived the idea of

using mealtime mixing as an appropriate contact matrix for enteric infection following the preliminary visit to Fiji in November-December 2012. Whilst it was potentially critically important to determine the ethnicity of participants' contacts for epidemiological understanding, I recognised this to be a potentially highly sensitive question and sought input from local partners. With the field team, I developed a suitable approach to this line of enquiry, with this typically asked as "all [self-reported ethnicity of participant]?" after completing the participant's report for each meal's co-diners. This gave the participant a simple yes/no answer with a natural route to correct the interviewer with which contacts were of a different ethnicity if this was the case. This was piloted successfully and implemented uncontroversially.

For analyse of the data, I was fortunate to have advice and some relevant pieces of R code from Stefan Flasche and Olivier Le Polain, who had recently completed social mixing analysis for pneumococcal disease (manuscript in preparation). I adapted this code to provide mixing pattern estimates for the survey mixing groups and population of Fiji, and undertook logistic regression analysis with the serological data to examine association with contact rates to examine whether serology supported the use of social mixing patterns for typhoid modelling.

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Student	Conall Watson
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If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?			
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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	See attached

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Social mixing in Fiji: who-eats-with-whom contact patterns and the implications of age and ethnic heterogeneity for disease dynamics in the Pacific Islands

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Ethics

The study was approved by the Fiji National Research Ethics Review Committee (2013-03) and the London School of Hygiene & Tropical Medicine observational study research ethics committee (6344).

Abstract

Empirical data on contact patterns can inform dynamic models of infectious disease transmission. Such information has not been widely reported from Pacific islands, nor strongly multi-ethnic settings, and few attempts have been made to quantify contact patterns relevant for the spread of gastrointestinal infections. As part of enteric fever investigations, we conducted a cross-sectional survey of the general public in Fiji, finding that within the 9,650 mealtime contacts reported by 1,814 participants, there was strong like-with-like mixing by age and ethnicity, with higher contact rates amongst iTaukei than non-iTaukei Fijians. Extra-domiciliary lunchtime contacts follow these mixing patterns, indicating the overall data do not simply reflect household structures. Inter-ethnic mixing was most common amongst school-age children. Serological responses indicative of recent *Salmonella* Typhi infection were found to be associated, after adjusting for age, with increased contact rates between meal-sharing iTaukei, with no association observed for other contact groups. Animal ownership and travel within the geographical division were common. These are novel data that identify ethnicity as an important social mixing variable, and use retrospective mealtime contacts as a socially acceptable metric of relevance to enteric, contact and respiratory diseases that can be collected in a single visit to participants. Application of these data to other island settings will enable communicable disease models to incorporate locally relevant mixing patterns in parameterisation.

5.2 Introduction

Infectious disease models synthesise epidemiological data and germ theory to understand and predict disease transmission. Non-homogeneous contact patterns are widely used in estimating the spread of an infection within a population (Hethcote and Yorke, 1984; Bansal, Grenfell and Meyers, 2007). For public health policy making, the prior practise of inferring social contact patterns as part of the model fitting has increasingly been replaced with data collection on social contact patterns (Edmunds, O'Callaghan and Nokes, 1997; Hens *et al.*, 2012). This can strengthen model validity when assessing the potential impact of interventions such as school closures or vaccination (Baguelin *et al.*, 2013).

Whilst social mixing has been studied in Europe (Mossong *et al.*, 2008); Africa, including South Africa (Johnstone-Robertson *et al.*, 2011; Dodd *et al.*, 2016), Kenya (Kiti *et al.*, 2014), Zambia (Dodd *et al.*, 2016) and Zimbabwe (Melegaro *et al.*, 2017); Asia, including Vietnam (Horby *et al.*, 2011), Taiwan (Fu, Wang and Chuang, 2012), southern China (Read *et al.*, 2014) and Japan (Ibuka *et al.*, 2015); and Australia (Rolls *et al.*, 2015), there is a paucity of social contact data for Pacific island states. This lack of data is despite the enormous historical mortality impact of diseases such as measles and bacillary dysentery in Pacific populations (Schmitt and Nordyke, 2001; Shanks, 2016), and contemporary burdens such as streptococcal diseases (Steer *et al.*, 2008; Temple *et al.*, 2012) and scabies (Romani *et al.*, 2015). Such data could also inform ongoing programmes such as trachoma elimination (Marks *et al.*, 2015), emerging infection preparedness (Moss *et al.*, 2016) and surveillance-response system strengthening (Craig, Kool and Nilles, 2013), and insights from island outbreaks of pathogens such as Zika (Duffy *et al.*, 2009; Cao-Lormeau *et al.*, 2016; Kucharski *et al.*, 2016). A sustained upturn in notified enteric fever cases caused by *Salmonella* Typhi in Fiji (Thompson *et al.*, 2014), prompted this investigation of social-mixing patterns.

Social mixing epidemiological research has predominantly considered conversational contact relevant to respiratory diseases such as influenza, or sexual contacts for infections such as HIV. Faecal-orally transmitted diseases such as typhoid are not transmitted by droplet or aerosol routes, (Cvjetanovic, Grab and Uemura, 1978; Feachem, Mara and Bradley, 1983) making conversation less relevant to transmission than mechanisms that involve food, fomites, direct contact or waterborne transmission (Bakach *et al.*, 2015). Sexual transmission of typhoid is rare, and associated with penile-anal or penile-oral rather than vaginal sex (Reller *et al.*, 2003). Methods for social contact patterns estimation of relevance to enteric pathogens are required. Quantifying food-sharing contacts may be one approach (Bates *et al.*, 2007; Phimpraphai *et al.*, 2017).

Furthermore, whilst rarely a reported feature of social contact surveys, ethnicity – which encompasses perception of common ancestry or homeland, kinship, language, culture, physical characteristics, religion and history (Cornell and Hartmann, 2007)– may also be critical to understanding disease dynamics in specific epidemiological circumstances, though requires sensitive consideration in biomedical research (Osborne and Feit, 1992; McKenzie and Crowcroft, 1996).

The contribution of different modes of transmission to typhoid fever incidence has been reviewed in the historical literature. Rosenau uses "contact infection" as a "convenient term" spanning direct and indirect spread from close association in time and place between the infectious and the susceptible, whether through physical contact, contaminated linen, medical equipment, shared food, drink, cutlery and crockery or other household transmission.(Rosenau MJ *et al.*, 1913)

Budd demonstrated the potential for typhoid person-to-person transmission along social networks through his detailed description of an outbreak in rural England, reported alongside other outbreaks involving water contaminated by faeces from typhoid fever cases (Budd, 1873). Sedgwick and Winslow (1902) showed that once piped water had been cleaned in New England towns, the residual or prosodemic (person-to-person) transmission of typhoid had a mortality rate of around 25 per 100,000 person years; adjusting for historical death-to-clinical-disease ratios puts these settings into the very high incidence range by today's standards, suggesting waterborne disease is not the only mechanism by which sustained high incidence can be attained.

The contribution of typhoid transmission by direct or indirect person-to-person in the Pacific islands is highly uncertain, with a World Health Organization expert elicitation exercise deriving a median estimate of 13% and a 95% uncertainty interval of 0% to 51% with similar variability in estimates of transmission from food and water. (Hald *et al.*, 2016). Social mixing surveys may not directly address this but may inform another form of conceptualisation in typhoid epidemiology: short- and long cycle transmission. Though the exact definition is not agreed,(González-Guzmán, 1989; Pitzer *et al.*, 2013; The SAGE Working Group on Typhoid Vaccines & the WHO Secretariat, 2017) "short cycle" transmission is typically through contamination of the immediate environs, which is more likely to be socially-structured, and "long-cycle" through the broader environment, which is typically not related to social contact patterns.

Additional to day-to-day contacts, diseases with person-to-person communicability may be spread by population movement within a country, if infection does not entirely impede

mobility. A further public health threat is the spillover of disease from livestock or wildlife to humans, such as leptospirosis (Lau *et al.*, 2012). Zoonotic diseases may give the impression of sustained human-to-human transmission when in fact there are multiple spillover events from an epizootic (Bausch *et al.*, 2006). Knowledge of human-animal contact patterns may inform zoonotic transmission models.

This social-mixing survey, conducted as part of a seroepidemiological survey, sought to determine 1) the distribution of social contacts by age and ethnicity 2) travel and internal migration patterns and 3) animal ownership and contact as relevant to the spread of communicable diseases in Fiji and other Pacific island settings.

5.3 Methods

5.3.1 Ethics approval

The study was approved by the Fiji National Research Ethics Review Committee (2013-03) and the London School of Hygiene & Tropical Medicine observational study research ethics committee (6344).

5.3.2 Setting

Fiji is an upper-middle income state of 837,000 people in the South Pacific Ocean (Fiji Bureau of Statistics, 2012). Administratively, Viti Levu, the largest island, is divided into Central Division (population 342,000 including the capital, Suva) and the Western Division (population 320,000). The northern Division (population 136,000) comprises the next largest two islands, Vanua Levu and Taveuni. Eastern Division (population 39,000) comprises of many smaller island groups.

An international expert meeting was convened in 2012 by the Fijian Ministry of Health and Australian Aid to investigate an upturn in typhoid fever cases from the mid-2000s. Over 90% of typhoid cases are reported in indigenous iTaukei Fijians who comprise 57% of the population,(Thompson *et al.*, 2014) giving an odds ratio >6 relative to other ethnic groups, which include Fijians of Indian descent (Indo-Fijians, 38%) and Fijians of Chinese or European descent, thus suggesting ethnicity is important in understanding transmission. Communal eating, beyond the immediate family, was commonly observed in iTaukei villages and amongst paid workers and students of both major ethnicities in Fiji. Co-dining and food-sharing was thus identified as a means of recording epidemiologically-relevant mixing patterns for enteric infections.

5.3.3 Survey methods

A multistage, clustered, cross-sectional survey was done in the Central, Northern and Western Divisions of Fiji between September and December 2013 as a joint serological, risk-factor and social mixing investigation. The Eastern Division, was excluded for logistical reasons, and we did not attempt to assess seasonal variation in contact patterns.

The community clusters were randomly-selected from Ministry of Health and Medical Services administrative lists for nursing zones, a contiguous health geography, with the zones selected randomly with probability proportional to population size. Within each cluster, 25 households were randomly selected. If registers were held by community health workers or nurses, these were preferentially used. Otherwise, in street-based settings, rapid enumeration of households was done with random start points and set sampling intervals. In rural villages/settlements extended program on immunization (EPI)-derived methods were used, enumerating households in random (pen-spin) directions from community centroids. One participant was randomly selected from each household. Fieldwork was done from Monday to Saturday, thereby recording social mixing for Sunday to Friday. Days for visits were determined by operational feasibility, not by randomisation and results are not reported by day. If a randomly selected household member was temporarily absent from the household at the time of the visit due to e.g. work or school, the survey team revisited later in the day after their expected return. The full survey methods have been described elsewhere (Lau *et al.*, 2016; Watson *et al.*, 2017) Sample size was calculated based on expected typhoid seroprevalence in 10 year age bands, the linked serosurvey's primary endpoint. Whilst a sample size was not calculated for the social mixing survey aspect and would be inappropriate to do post-hoc, the study size is consistent with or larger than other social mixing surveys (Mossong *et al.*, 2008; Horby *et al.*, 2011; Fu, Wang and Chuang, 2012; Read *et al.*, 2014). Where others report individual year contact rates we used broader age bands in the survey's implementation and analysis to provide appropriate precision in ethnic and age strata.

The purpose of the survey was explained to community leaders if applicable, to the head of the household and the selected participant, and their permissions sought for inclusion in the survey. Written informed consent was sought and obtained from adult participants and parents of child (under 18 years) participants. Children aged 12-17 years provided written informed assent.

Interviews were done face-to-face by a trained, multilingual Fijian fieldworker in iTaukei, Hindi or English at the preference of the participant, at the participant's home or in a community centre. Venous blood was collected by a trained phlebotomist or physician. Participants provided demographic details, including their age, sex and self-reported ethnicity. They were first asked to recall where they had lunch and dinner the previous day, to enable recording of both close extra-household and household contact rates. We then asked how many people of each age group (defined below) they ate each meal with and asked how many of the lunch and dinner contacts were the same individuals (to enable calculation of unique daily meal contacts), and asked to give their assessment of the ethnicity of co-diners. Ages of contacts were categorised into 0 to 4 years (preschool children), 5 to 14 years (school-age children), 15 to 34 years (young adults), 35 to 54 (older working-age adults) and 55+ (retirement-age adults). If more than fifteen contacts were reported in an age group, then ranges 16 to 24, 25 to 49 and 50 to 99 were recorded and midpoints of these bands used in analysis. Parents answered on behalf of young children. Infants under one were omitted from participation our study, as ineligible for the serological aspect of the field survey, though were included in the under 5s as contacts in participant responses. Participant ages were categorised as above, with the youngest band 1 to 4 years accordingly. In domestic eating settings, including villages, participants were asked to report the details of people with whom they actually shared food i.e. cooking pots or buffet meals. For those eating in settings where cooking pot sharing would be impossible to estimate and not an appropriate measure of social contact (such as at a restaurant or canteen), they were asked to report with whom they shared a table or shared a table-like setting. See thesis Appendix A1 for the mealtime social contact questionnaire tool.

Participants were further asked about travel outside of their neighbourhood (including villages or settlements) in the past week, ever having lived in a different neighbourhood, and about animal ownership or physical (touch) contact with select wild animals.

We also sought information on physical contacts of participants. During survey piloting, candidate questions about skin-to-skin human physical contact were often met with embarrassment, and often received reports of zero contact with anyone other than between parents and infant offspring, despite observance of frequent social contact such as handshaking or arm-touching in villages and settlements. This line of enquiry was dropped to reduce participant survey fatigue and risk of social response biases in other part of the survey. Similarly, breakfast contacts were not sought due to expected overlap with dinner

contacts. Fomite contacts are hard to quantify (Read *et al.*, 2012) and were not sought. Water-related exposures are described elsewhere (Watson *et al.*, 2017).

5.3.4 Data analysis

Data were entered in EpiData (Lauritsen and Bruus, 2005) and analysed in R version 3.3.2 (R Core Team, 2017). Bootstrap 95% confidence intervals (CI) were estimated for mean contact rates. Total daily contacts between age and ethnicity subgroups were estimated based on census populations and participant-reported rates and used to construct a reciprocal-contact adjusted, symmetrical mixing matrix (Wallinga, Teunis and Kretzschmar, 2006). Binomial 95% confidence intervals were estimated for travel and animal ownership as prior analysis had found minimal influence of clustering on variances for age-structured data on similar exposures (Watson *et al.*, 2017).

To assess possible association between mealtime contacts and biological markers of enteric infection transmission, for participants resident in unvaccinated areas, we estimated by logistic regression the age-adjusted odds ratios for iTaukei and non-iTaukei contacts and *S.* Typhi seropositivity using anti-Vi IgG titres from a linked serosurvey at thresholds 64 ELISA units (EU) and 100 EU alongside examining potential confounders. Previous research in Fiji (Watson *et al.*, 2017) established 64 EU as the threshold towards which case titres decay; ≥100 EU is used to indicate a recent (months to a few years) infection such as may be influenced by the reported social mixing patterns.

5.4 Results

5.4.1 Study population

We received 1,814 analysable responses from 1,816 interviewees (two withdrew before contributing sufficient data, 1,842 were documented as having been approached, response rate = 98.6%). Of these, 1,409 (78%) were iTaukei ethnicity and 53.3% were female (Table 5.1). The median age was 30 years (IQR 17 to 48 years), with a median age of 29 years (IQR 26 to 47 years) in iTaukei and 35 years (IQR 21 to 52 years) in non-iTaukei. In comparison to the 2007 census, the non-iTaukei Fijians were under-represented amongst survey participants (Supplementary Figure S5.1). Over half of participants resided in rural areas, with rural living more common for the iTaukei population (60%), typically living in formal village settings (47%), than non-iTaukei (42%) who resided almost exclusively in settlements (59%) or residential housing (37%).

Table 5.1. Participant demographics

		All participants	**iTaukei**	**Non-iTaukei**
		1,814	1,409 (78)	405 (22)
Sex (%)	Female	966 (53.3)	744 (53.3)	222 (54.8)
Age (%)	1 to 4	87 (4.8)	74 (5.3)	13 (3.2)
	5-14	299 (16.5)	246 (17.5)	53 (13.1)
	15-34	654 (36.1)	523 (37.1)	131 (32.3)
	35-54	473 (26.1)	347 (24.6)	126 (31.1)
	55+	301 (16.6)	219 (15.5)	82 (20.2)
Setting (%)	Urban	505 (27.8)	386 (27.4)	119 (29.4)
	Peri-urban	289 (15.9)	175 (12.4)	114 (28.1)
	Rural	1,020 (56.2)	848 (60.2)	172 (42.5)

5.4.2 Contact patterns

The 1,814 participants reported a total of 9,650 mealtime contacts. The distribution of daily mealtime contacts reported by participants was right-skewed (Figure 5.1A). Whilst both the iTaukei and non-iTaukei modal value was two (Figure 5.1B and C), the iTaukei participant's contacts distribution had higher median (4 vs. 3) than the non-iTaukei participants and a higher interquartile range (2-7 vs. 2-5, respectively). After stratification by age and ethnicity (Figure 5.1D), heavy-tailed distributions were apparent for the iTaukei in comparison with equivalent-age non-iTaukei. The iTaukei aged 5 to 14 years and 15 to 34 years were the most likely to report between 5 and 10 mealtime contacts. Few respondents of any age or ethnicity reported more than ten such contacts.

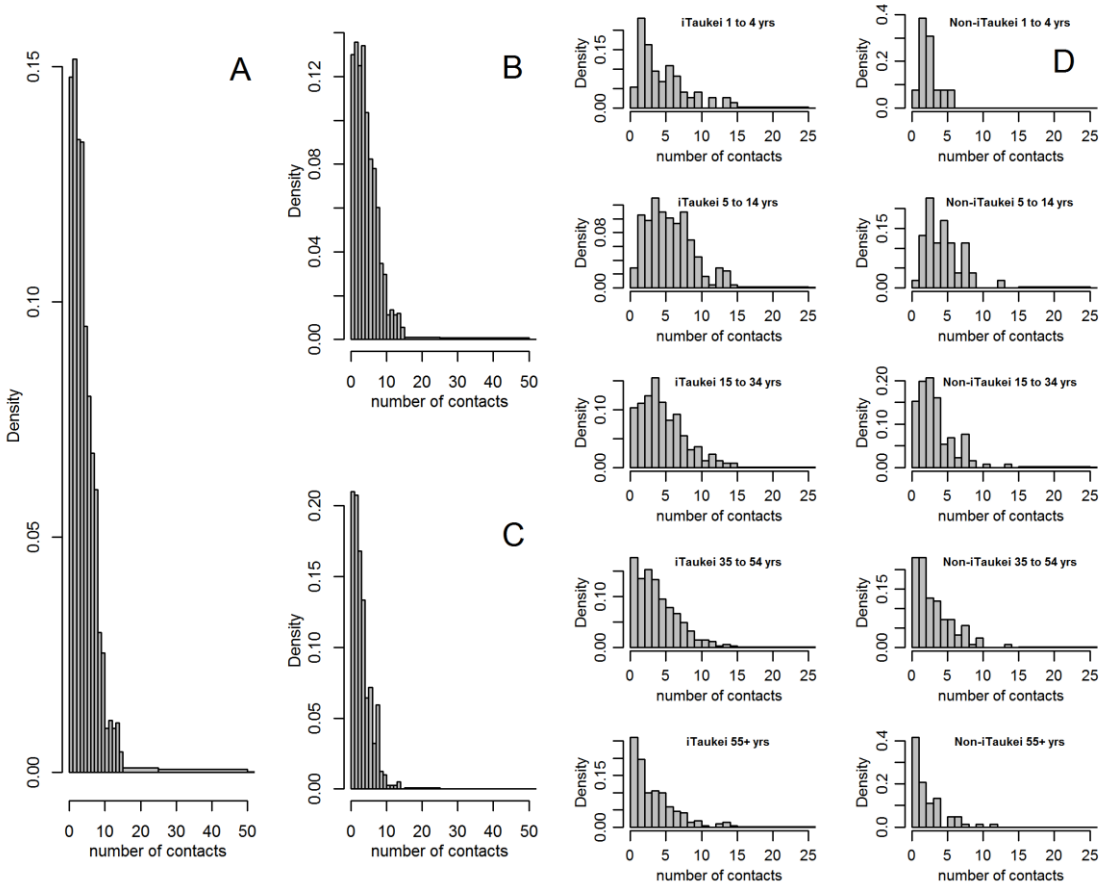

Figure 5.1. Distribution of daily contacts

Reported by A) all participants, B) iTaukei participants, C) non-iTaukei participants and D) participants stratified by age and ethnicity. Fig 1 A to C panels truncated at 50 contacts and D panels at 25 contacts for clarity as there were few reports of contact numbers in higher bands; densities are for the full range of reported value.

Residents of Fiji exhibited strong assortative mixing by age within the two ethnicity categories (Table 5.2). The highest mean reported contact rate was for iTaukei participants aged

between 5 to 14 years who shared a meal with people of the same age and ethnicity (3.2 contacts per day; 95% CI 2.67 to 3.98). Few contacts were reported with people of different ethnic groups to the respondents; all iTaukei participant age groups had confidence intervals that included zero for contacts of different ethnicity. The highest mean reported heteroethnic contact rates were reported by Non-iTaukei participants aged between 5 and 14 years with their iTaukei counterparts of the same age at 0.61 contacts per day (95% CI 0.25 to 1.14).

Table 5.2. Unweighted mean number of daily contacts by age and ethnicity and 95% bootstrap confidence interval

		Contacts									
		iTaukei					Non-iTaukei				
	Age	0 to 4	5 to 14	15 to 34	35 to 54	55+	0 to 4	5 to 14	15 to 34	35 to 54	55+
Participants: Non-iTaukei	1 to 4	0	0.07 (0 to 0.23)	0	0.29 (0 to 0.73)	0.2 (0 to 0.62)	0.13 (0 to 0.5)	0.29 (0 to 0.67)	0.77 (0.26 to 1.3)	0.54 (0.2 to 1)	0.45 (0.09 to 0.93)
	5 to 14	0.09 (0.02 to 0.2)	0.61 (0.25 to 1.14)	0.18 (0.06 to 0.34)	0.31 (0.06 to 0.8)	0.02 (0 to 0.07)	0.17 (0.07 to 0.27)	1.56 (1.09 to 2.15)	0.75 (0.42 to 1.31)	0.93 (0.68 to 1.19)	0.33 (0.18 to 0.53)
	15 to 34	0.16 (0.06 to 0.31)	0.13 (0.04 to 0.25)	0.28 (0.16 to 0.43)	0.1 (0.02 to 0.23)	0.14 (0.05 to 0.28)	0.36 (0.25 to 0.51)	0.48 (0.32 to 0.67)	1.18 (0.91 to 1.5)	1.02 (0.8 to 1.27)	0.41 (0.24 to 0.63)
	35 to 54	0.04 (0 to 0.09)	0.08 (0.02 to 0.16)	0.17 (0.07 to 0.3)	0.11 (0.05 to 0.18)	0.02 (0 to 0.06)	0.35 (0.18 to 0.57)	0.56 (0.4 to 0.77)	1.07 (0.81 to 1.36)	1.27 (0.9 to 1.76)	0.57 (0.3 to 1.03)
	55+	0.01 (0 to 0.04)	0.03 (0 to 0.13)	0.08 (0.01 to 0.17)	0.07 (0.01 to 0.16)	0.07 (0.01 to 0.15)	0.1 (0.03 to 0.18)	0.19 (0.07 to 0.35)	0.82 (0.55 to 1.14)	0.57 (0.39 to 0.76)	0.59 (0.43 to 0.79)
Participants: iTaukei	1 to 4	1.15 (0.84 to 1.49)	1.05 (0.63 to 1.62)	1.66 (1.32 to 2.09)	0.85 (0.61 to 1.1)	0.51 (0.33 to 0.72)	0.01 (0 to 0.05)	0	0	0.03 (0 to 0.1)	0
	5 to 14	0.63 (0.47 to 0.81)	3.2 (2.67 to 3.98)	1.25 (1.09 to 1.4)	1.28 (1.16 to 1.4)	0.33 (0.24 to 0.45)	0.01 (0 to 0.02)	0.03 (0 to 0.07)	0 (0 to 0.02)	0.01 (0 to 0.03)	0 (0 to 0.01)
	15 to 34	0.75 (0.64 to 0.87)	0.99 (0.86 to 1.13)	2.31 (2.05 to 2.58)	1.34 (1.14 to 1.56)	0.67 (0.54 to 0.83)	0 (0 to 0.01)	0.01 (0 to 0.01)	0.01 (0 to 0.02)	0.01 (0 to 0.03)	0
	35 to 54	0.47 (0.37 to 0.58)	1.19 (1.02 to 1.36)	1.42 (1.18 to 1.69)	1.54 (1.28 to 1.84)	0.59 (0.45 to 0.75)	0	0 (0 to 0.01)	0.02 (0 to 0.04)	0.01 (0 to 0.04)	0.01 (0 to 0.02)
	55+	0.48 (0.36 to 0.61)	0.84 (0.67 to 1.04)	1.08 (0.89 to 1.32)	0.92 (0.71 to 1.19)	0.91 (0.74 to 1.1)	0	0	0.01 (0 to 0.02)	0 (0 to 0.02)	0.01 (0 to 0.02)

iTaukei household sizes had a median of 5 residents and mean of 4.9 residents compared with a median of 4 residents and mean of 4.0 residents for non-iTaukei households. Data from lunchtime contacts indicated that contact rates did not only reflect household structure. Whilst eating dinner at home was almost universal (95%), 22% of iTaukei respondents had lunch contacts away from home, as did 21% of non-iTaukei. Those reporting lunch away from home also reported more contacts than those lunching at home (mean, median and IQR 8.0, 6, 3 to 9 and 4.5, 4, 2 to 6 contacts, respectively, $p<0.0001$). Participants eating lunch away from home (Table 5.3) had contact patterns indicating similar age and ethnically assortative mixing as seen in the overall contact pattern.

Table 5.3. Unweighted mean number of non-household lunch contact by age and ethnicity (bootstrap 95% confidence intervals)

			Contacts									
			iTaukei					Non-iTaukei				
		Age	0 to 4	5 to 14	15 to 34	35 to 54	55+	0 to 4	5 to 14	15 to 34	35 to 54	55+
Participants	Non-iTaukei	1 to 4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		5 to 14	0.08 (0 to 0.18)	0.7 (0.19 to 1.34)	0.19 (0.03 to 0.36)	0.43 (0.05 to 1.14)	0	0.14 (0.03 to 0.26)	1.78 (1.21 to 2.45)	0.78 (0.3 to 1.48)	0.84 (0.54 to 1.12)	0.38 (0.17 to 0.64)
		15 to 34	0.19 (0 to 0.67)	0.22 (0 to 0.69)	0.25 (0 to 0.67)	0.22 (0 to 0.78)	0.19 (0 to 0.67)	0.56 (0.24 to 1)	0.5 (0.09 to 1.06)	2.38 (1.68 to 3.21)	1.69 (1.08 to 2.46)	0.72 (0.22 to 1.5)
		35 to 54	0	0	0	0.05 (0 to 0.15)	0	1 (0.06 to 2.14)	1 (0.33 to 1.83)	1.95 (0.92 to 3.18)	3.3 (1.28 to 6)	2.1 (0.35 to 4.4)
		55+	0	0	0	0	0	0	0.5 (0 to 2)	0.75 (0 to 3)	1.25 (0 to 4)	0.25 (0 to 1)
	iTaukei	1 to 4	2 (0.33 to 4)	2.67 (0 to 8.59)	0.83 (0 to 1.67)	1.33 (0.33 to 2.33)	0.33 (0 to 0.8)	0	0	0	0	0
		5 to 14	0.65 (0.42 to 0.93)	4.86 (3.72 to 6.51)	1.3 (1.07 to 1.56)	1.27 (1.08 to 1.45)	0.38 (0.23 to 0.57)	0.01 (0 to 0.03)	0.04 (0 to 0.12)	0 (0 to 0)	0.01 (0 to 0.03)	0.01 (0 to 0.03)
		15 to 34	0.86 (0.54 to 1.24)	1.28 (0.96 to 1.68)	3.42 (2.79 to 4.11)	2.15 (1.56 to 2.95)	0.86 (0.45 to 1.48)	0	0	0.03 (0 to 0.07)	0.03 (0 to 0.06)	0
		35 to 54	0.54 (0.17 to 1)	1.37 (0.82 to 2.08)	2.44 (1.48 to 3.59)	2.76 (1.77 to 3.94)	0.93 (0.41 to 1.56)	0	0	0.05 (0 to 0.17)	0.05 (0 to 0.17)	0
		55+	1 (0.48 to 1.62)	1.39 (0.76 to 2.11)	1.93 (1.17 to 2.9)	1.79 (0.91 to 2.87)	1.32 (0.75 to 2)	0	0	0	0	0

After adjusting for reciprocity of contacts (census population and survey participant pyramids are shown in Supplementary Figure S5.1), total daily contact data indicated sparse mixing between iTaukei and non-iTaukei ethnicity categories in all but school-age children (Figure

5.2). Age-assortative mixing was apparent within the two ethnicity categories, along with off-diagonal mixing, indicative of parent-child contact. In both ethnic categories, school-age children had the highest mean contact rates, followed by working-age adults. In contrast to the iTaukei pre-school children, the non-iTaukei children aged 1 to 4 years exhibited disassortative mixing, with more contacts reported within the working age adults than children of the same age.

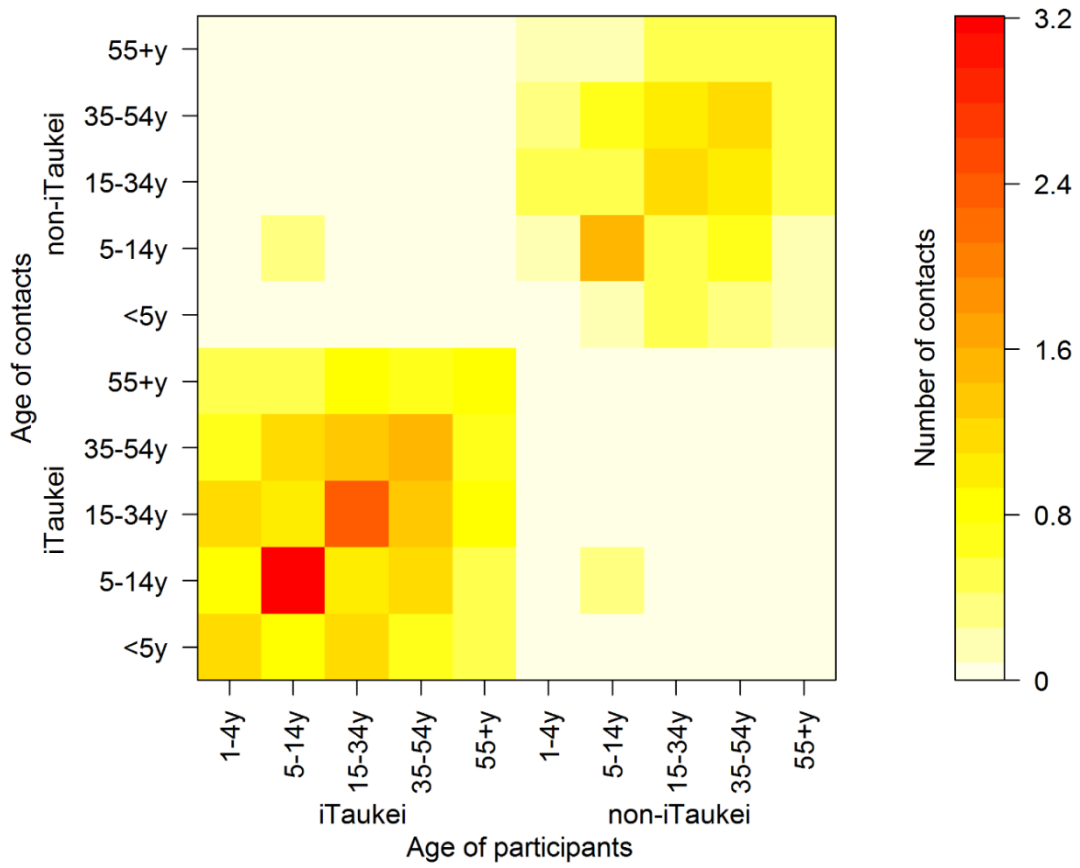

Figure 5.2. Age and ethnicity structured mixing matrices of reciprocity-weighted unique mealtime contacts per day.

We next examined the influence of unit increases in number of contacts on seropositivity for anti-Vi IgG *S.* Typhi amongst 1,559 participants (1,530 with complete data) from unvaccinated areas of Fiji. Age-adjusted seropositivity showed no correlation at threshold 64 EU used as a marker of any previous or current infection (odds ratio (OR) 1.01; 95% CI 0.99 to 1.02; p=0.3) but some evidence of association at 100 EU, posited as indicative of recent infection (OR 1.02; 95% CI 1.00 to 1.03; p=0.002) (supplementary text S5.1).

We performed further age-adjusted analyses to identify potential confounding variables and ascertain drivers for any such observed effect (supplementary text S5.1). Examination by

ethnicity of contacts across both participant ethnic categories combined identified elevated odds ratios for association between unit increase in number of iTaukei contacts and the 100 EU recent infection serological threshold. No association was found for number of non-iTaukei contacts and seropositivity. The iTaukei contact rate association was not influenced by adjustment for participant ethnicity, the number of non-iTaukei contacts, or eating lunch outside the home when examined by multivariable regression (supplementary text 5.1). Analysis stratified by participant ethnicity found an effect of increasing contact rates in increasing seroprevalence amongst iTaukei participants but not non-iTaukei participants.

A parsimonious epidemiological model was constructed for serological association of recent infection with age-adjusted iTaukei contact rates in the unvaccinated iTaukei group, with non-iTaukei excluded from the model due to the absence of observed association between any contact rates and seropositivity, finding evidence of association at the 100 EU threshold ("recent infection") for a per contact increase in odds of seropositivity, after adjusting for age A final model for recent was developed adjusting for epidemiological risk factors found previously to be associated with seropositivity at 64 EU: after adjusting for these, iTaukei contact remained associated with seropositivity, with adjusted odds of 1.027 (1.008 to 1.045) (table 5.4).

Table 5.4. Logistic regression model of association between contact rates and seropositivity For anti-Vi IgG seropositivity (100 EU), iTaukei daily contact number and participant age in 1,189 iTaukei participants from areas of Fiji never vaccinated against typhoid, adjusted for co-variables found significant in the seroepidemiology survey (chapter 3).

Variable	**Adjusted odds ratio (95%CI)**	**p-value**
iTaukei contact (per)	1.027 (1.008 to 1.045)	0.003
Age (per year)	1.025 (1.018 to 1.032)	<0.001
Sewage – septic tank	Reference	
Sewage – piped sewer	1.05 (0.69 to 1.60)	0.8
Sewage – pit latrine	1.10 (0.67 to 1.73)	0.7

Sewage – elsewhere	0.97 (0.42 to 2.03)	0.9
Community - Residential	Reference	
Community - Village	1.07 (0.72 to 1.63)	0.7
Community - Settlement	1.35 (0.91 to 2.01)	0.14

5.4.3 Travel and internal migration patterns

Local travel was common amongst the survey participants, with over half reporting travel outside of their residential community in the past week for all but the youngest and oldest age groups (figure 5.3). Similar proportions of iTaukei and non-iTaukei reported travel in the past week. Recent travel was similar for urban, rural, and peri-urban residents (data not shown). Most travel was within the geographical administrative division (i.e. Central, Eastern, Northern, or Western Division of Fiji); of 974 participants reporting travel, 43 (4.2%, 95%CI 3.2% to 5.6%) reported travel to another Division in the past week.

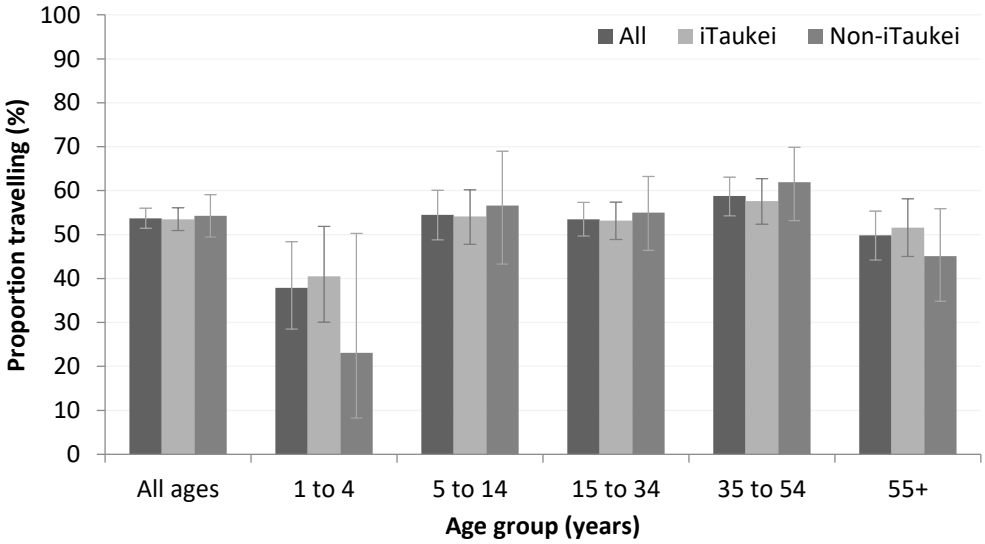

Figure 5.3. Travel outside of the community in the past week

Overall 37.2% (670/1803, 95% CI 35.0 to 39.4%) reported having moved residential community in their lifetimes. Participants aged 40 to 44 were most likely to have moved from a different place (Supplement Fig S5.2A) to their current community of residence. Those aged 20 to 24 years were most likely to report having moved in the past 5 years, regardless of ethnicity category (Supplement Figs S5.2B and S5.2C). Non-iTaukei children were more likely

to have moved than iTaukei children, while adult iTaukei were more likely to have moved recently than non-iTaukei.

5.4.4 Animal ownership and contact

Animal ownership was common in both ethnicity categories and across urban/peri-urban and rural households (Supplementary Tables S5.1A and S5.1B). Pigs were the most commonly owned animal (28% of households (95% CI22 to 26%) despite being infrequently kept by non-iTaukei Fijians (5.7%, 95% CI 3.8 to 8.4%). Chickens were also widely kept, particularly by non-iTaukei (36%, 95% CI 31.5 to 40.8%). In rural communities, 20.8% (95% CI 18.4 to 23.4%) owned horses, with comparable ownership in both ethnic groups. This association contrasted with goat ownership, which was predominantly amongst rural non-iTaukei (30.8, 95%CI 24.4 to 38.1%, compared with mean prevalences <10% amongst other groups). Few Fijians reported keeping sheep (1.2% 95% CI 0.8 to 1.8%). Physical contact with wild rats or mongooses was reported infrequently (Supplement Table S5.1C) despite sightings of these by 90% and 75% of participants, respectively.

5.5 Discussion

Empirical research on contact patterns for infectious disease modelling has to date primarily considered epidemiological contacts for transmission of sexual or respiratory diseases. Social mixing patterns of direct relevance to enteric infections, or patterns of animal contact relevant to zoonotic spillover are less studied.

Using unique daily mealtime contacts, our social contact survey of Fiji found that within iTaukei and non-iTaukei ethnic groups there is age-assortative mixing, even within broad age categories, similar to contact patterns studied in the transmission of respiratory diseases seen in Asian or European settings (Mossong *et al.*, 2008; Horby *et al.*, 2011; Fu, Wang and Chuang, 2012; Read *et al.*, 2014). We found minimal social mixing between people of the two ethnic categories, with inter-ethnic mixing most common amongst school-age children. iTaukei participants had higher mean daily contact rates than non-iTaukei participants. Examination of extra-domestic lunchtime contacts indicates that these patterns are replicated outside the home, showing that data do not simply reflect household structure. High levels of mobility in the population for all ages from 5 years upwards (overall >50% travelling in the previous week) suggest that communities on these larger Fijian islands are not isolated and transmission between urban and rural populations is readily feasible.

These data suggest it is plausible that effectively independent epidemics could occur in iTaukei and non-iTaukei residents of Fiji, for pathogens whose transmission can be approximated by mealtime contacts, given the low rates of substantive hetero-ethnic contact. The higher contact rates amongst iTaukei Fijians would more readily sustain short-cycle transmission than the rates in non-iTaukei Fijians. Analysis of age-adjusted contact rates and anti-Vi IgG to *Salmonella* Typhi found association with inter-iTaukei contacts and titres above a threshold that may be indicative of recent past infection but no association for contact rates involving non-iTaukei, further supporting use of these ethnically-structured social contact data in infectious disease modelling. This association remained after adjusting for covariables found to be significant in prior work, (Watson *et al.*, 2017) suggesting social structure to be important. Our recording of animal ownership by ethnicity enables estimation of the impact of differential seeding of zoonotic diseases such as avian influenza were they to first arrive in the Pacific as an epizootic.

The absolute contact rates obtained in this study cannot readily be compared with those from Mossong and others which are primarily conversational in nature and not restricted to mealtimes. Nor do we attempt to document changing social contact patterns during acute illness (Eames *et al.*, 2010; Van Kerckhove *et al.*, 2013). However, their origins in mealtime

contact do not limit these data to application in enteric disease only. As Goeyvaerts and colleagues (2010) note, the importance of empirically-obtained social mixing rates is that they represent relative mixing patterns between population subgroups as proxies for the distribution of mechanisms of disease transmission. This study does not attempt to elucidate the relative contributions these components of the potential chain of typhoid transmission from portal of entry to exit but suggests these distributions may be reflected in social contact patterns. Similarly, Melegaro and colleagues' 2011 study of airborne viral pathogens found "that intimate types of contacts explain the pattern of acquisition of serological markers by age better than other types of social contacts".

In the absence of setting-specific data, these data might be very cautiously applied to use in other Pacific Island countries and territories, though more applicable to larger states than to low-lying small islands given that data collection excluded Fiji's Eastern Division. Unadjusted iTaukei contact rates could be applied in many settings; unadjusted inter-ethnicity contact patterns could have potential application in settings such as French Polynesia where the estimated ethnically non-Polynesian population is relatively large at 22% (Anon, 2016), though do not account for the different social and cultural norms of such settings.

Our survey demonstrates that it is feasible and socially acceptable to gather data on social mixing not only by age but by ethnicity, in settings where heterogeneity may be of relevance to transmission networks and dynamics. Interestingly, we found non-iTaukei pre-school children had non-assortative mixing, in that they had greater contact with older age groups rather than with children of the same age, suggesting mealtime contact within a small family structure. Similar findings were reported in a UK study of under-ones (van Hoek *et al.*, 2013). This contrasts to assortative mixing in iTaukei pre-schoolers, consistent with sustained high birth rates/large extended families in iTaukei Fijians (Fiji Bureau of Statistics, 2012) and the divergent demographic trends in iTaukei and non-iTaukei Fijians (Supplementary Fig S5.1). Compared with social contact survey settings overseas, low relative contact rates in older adult Fijians may reflect both lower adult life expectancy (Taylor *et al.*, 2013) as well as different social mixing patterns.

We found that non-iTaukei participants, predominantly comprising Indo-Fijians, were under-represented in the survey relative to census estimates, despite use of a structured sampling method. This may to some extent reflect continued outmigration and potentially within-country rural-to-urban migration differentially increasing nursing zones populations in majority Indo-Fijian areas above the numbers used in the sampling frames. Boosted surveys of non-iTaukei residents could address this, though value of the expected potential gains in

precision would need consideration. The high reported enrolment rate likely reflects incomplete documentation of candidate participants approached who declined involvement.

Theoretical mixing structures that are not informed by data are largely being replaced in infectious disease modelling by contact patterns derived from data. Traditional, line-listing, prospective, paper-based, contact diaries can be demanding for participants and in data entry/analysis. Methods of measuring contact utilising portable electronic devices, such as mobile phone tracing (Wesolowski *et al.*, 2012; Bengtsson *et al.*, 2015) and RFID tagging (Cattuto *et al.*, 2010; Stehlé *et al.*, 2011) increasingly offer methods for collecting rich data on contact patterns but can involve substantive cost and/or complexity. There can be an advantageous degree of simplicity in asking people with whom they ate yesterday and where they travelled in the last week. Data for model parameterisation can be collected in a single-contact survey potentially alongside serology and behavioural or environmental risk data. Although retrospective survey responses may risk recall bias, we found that the previous day's lunch and dinner partners were readily recallable by participants. This also reduces respondent fatigue and avoids potential for behaviour modifications that a prospective diary might trigger. Social response bias is reduced by reassurance that individual responses are kept confidential, and the socially-acceptable nature of enquiry, including careful structuring of ethnicity questions.

Whilst eating patterns themselves are an important public health topic with regards to the enormous impact of the epidemic of non-communicable disease in the Pacific and worldwide (Taylor, 1983; Ng *et al.*, 2017) they also offer insights for infectious disease epidemiologists and modellers. "Who-eats-with-whom" reflects social intimacy as well as specific food-borne and fomitic transmission risks and can effectively document ethnic- as well as age-assortative mixing. The universality of food-sharing as a human experience lends this approach, developed for enteric infectious diseases in Pacific Islands, to a range of settings where people interact and infections may transmit.

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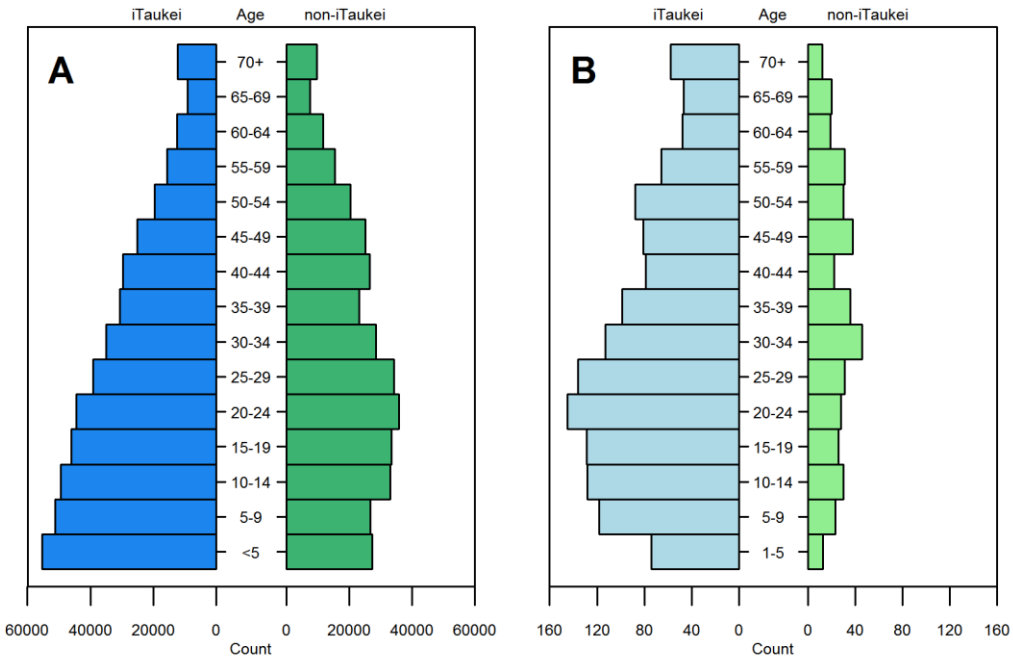
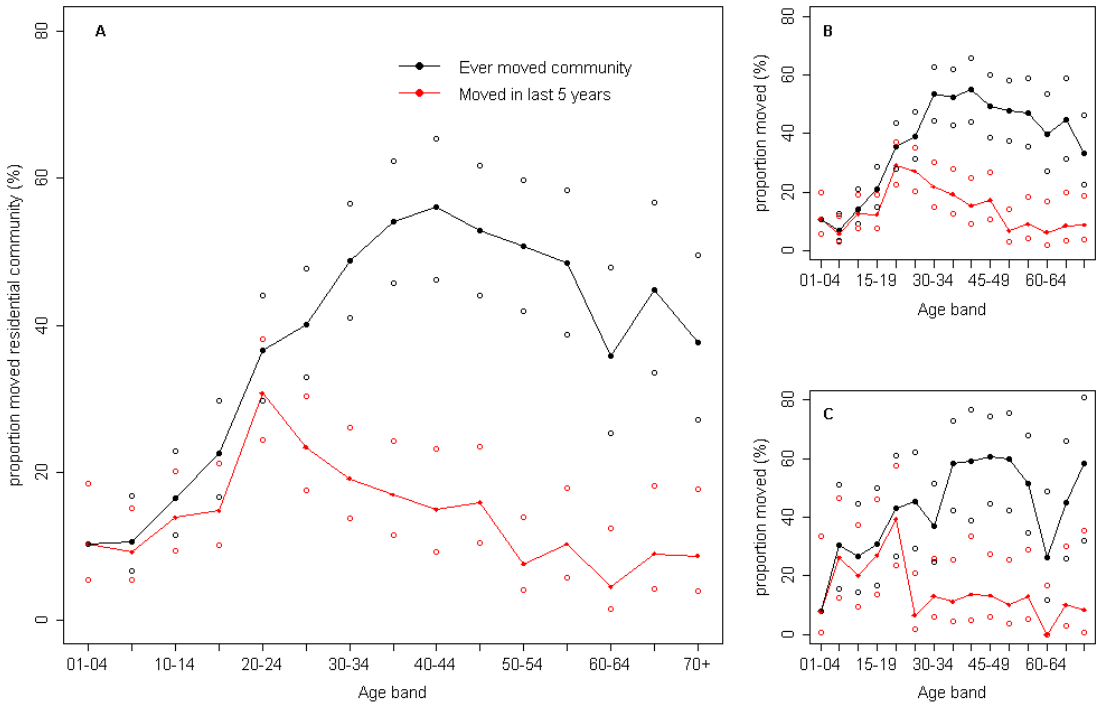
5.7 Supplementary material



Supplementary Fig S5.1. Age distribution (count) of iTaukei and non-iTaukei in Fiji in A) 2007 census and B) 2013 social contact survey.



Supplementary Fig S5.2. Lifetime prevalence of having moved community and moved in the last five years

for A) all participants, B) iTaukei participants, C) non-iTaukei participants, by five-year age bands. Hollow points denote 95% confidence intervals.

Supplement Table S1. Animal contact by ethnicity and geography
(A: owned livestock, B: other owned domesticated animals, C: physical contact with wild rodents)

A		Overall		iTaukei		Non-iTaukei	
	Geography	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
Chicken	All	441	24.3 (22.4 to 26.3)	295	20.9 (18.9 to 23.1)	146	36 (31.5 to 40.8)
	Urban	23	4.6 (3.1 to 6.7)	16	4.1 (2.6 to 6.6)	7	5.9 (2.9 to 11.6)
	Peri-urban	55	19 (14.9 to 24)	24	13.7 (9.4 to 19.6)	31	27.2 (19.9 to 36)
	Rural	363	35.6 (32.7 to 38.6)	255	30.1 (27.1 to 33.2)	108	62.8 (55.4 to 69.7)
Cows	All	388	21.4 (19.6 to 23.3)	316	22.4 (20.3 to 24.7)	72	17.8 (14.4 to 21.8)
	Urban	44	8.7 (6.6 to 11.5)	42	10.9 (8.2 to 14.4)	2	1.7 (0.5 to 5.9)
	Peri-urban	41	14.2 (10.6 to 18.7)	27	15.4 (10.8 to 21.5)	14	12.3 (7.5 to 19.6)
	Rural	303	29.7 (27 to 32.6)	247	29.1 (26.2 to 32.3)	56	32.6 (26 to 39.9)
Goats	All	155	8.5 (7.3 to 9.9)	91	6.5 (5.3 to 7.9)	64	15.8 (12.6 to 19.7)
	Urban	29	5.7 (4 to 8.1)	27	7 (4.9 to 10)	2	1.7 (0.5 to 5.9)
	Peri-urban	26	9 (6.2 to 12.9)	17	9.7 (6.2 to 15)	9	7.9 (4.2 to 14.3)
	Rural	100	9.8 (8.1 to 11.8)	47	5.5 (4.2 to 7.3)	53	30.8 (24.4 to 38.1)
Pigs	All	508	28 (26 to 30.1)	485	34.4 (32 to 36.9)	23	5.7 (3.8 to 8.4)
	Urban	64	12.7 (10.1 to 15.9)	62	16.1 (12.7 to 20.1)	2	1.7 (0.5 to 5.9)
	Peri-urban	57	19.7 (15.5 to 24.7)	47	26.9 (20.8 to 33.9)	10	8.8 (4.8 to 15.4)
	Rural	387	37.9 (35 to 41)	376	44.3 (41 to 47.7)	11	6.4 (3.6 to 11.1)
Sheep	All	21	1.2 (0.8 to 1.8)	14	1 (0.6 to 1.7)	7	1.7 (0.8 to 3.5)
	Urban	0	0 (0 to 0.8)	0	0 (0 to 1)	0	0 (0 to 3.1)
	Peri-urban	11	3.8 (2.1 to 6.7)	10	5.7 (3.1 to 10.2)	1	0.9 (0 to 4.8)
	Rural	10	1 (0.5 to 1.8)	4	0.5 (0.2 to 1.2)	6	3.5 (1.6 to 7.4)

B		Overall		iTaukei		Non-iTaukei	
	Geography	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
Cats	All	320	17.6 (16 to 19.5)	219	15.5 (13.7 to 17.5)	101	24.9 (21 to 29.4)
	Urban	84	16.6 (13.6 to 20.1)	59	15.3 (12 to 19.2)	25	21 (14.7 to 29.2)
	Peri-urban	63	21.8 (17.4 to 26.9)	38	21.7 (16.2 to 28.4)	25	21.9 (15.3 to 30.4)
	Rural	173	17 (14.8 to 19.4)	122	14.4 (12.2 to 16.9)	51	29.7 (23.3 to 36.9)
Dogs	All	577	31.8 (29.7 to 34)	397	28.2 (25.9 to 30.6)	180	44.4 (39.7 to 49.3)
	Urban	146	28.9 (25.1 to 33)	101	26.2 (22 to 30.8)	45	37.8 (29.6 to 46.8)
	Peri-urban	98	33.9 (28.7 to 39.5)	48	27.4 (21.4 to 34.5)	50	43.9 (35.1 to 53)
	Rural	333	32.6 (29.8 to 35.6)	248	29.2 (26.3 to 32.4)	85	49.4 (42 to 56.8)
Horses	All	265	14.6 (13.1 to 16.3)	233	16.5 (14.7 to 18.6)	32	7.9 (5.7 to 10.9)
	Urban	35	6.9 (5 to 9.5)	33	8.5 (6.2 to 11.8)	2	1.7 (0.5 to 5.9)
	Peri-urban	18	6.2 (4 to 9.6)	18	10.3 (6.6 to 15.7)	0	0 (0 to 3.3)
	Rural	212	20.8 (18.4 to 23.4)	182	21.5 (18.8 to 24.4)	30	17.4 (12.5 to 23.8)

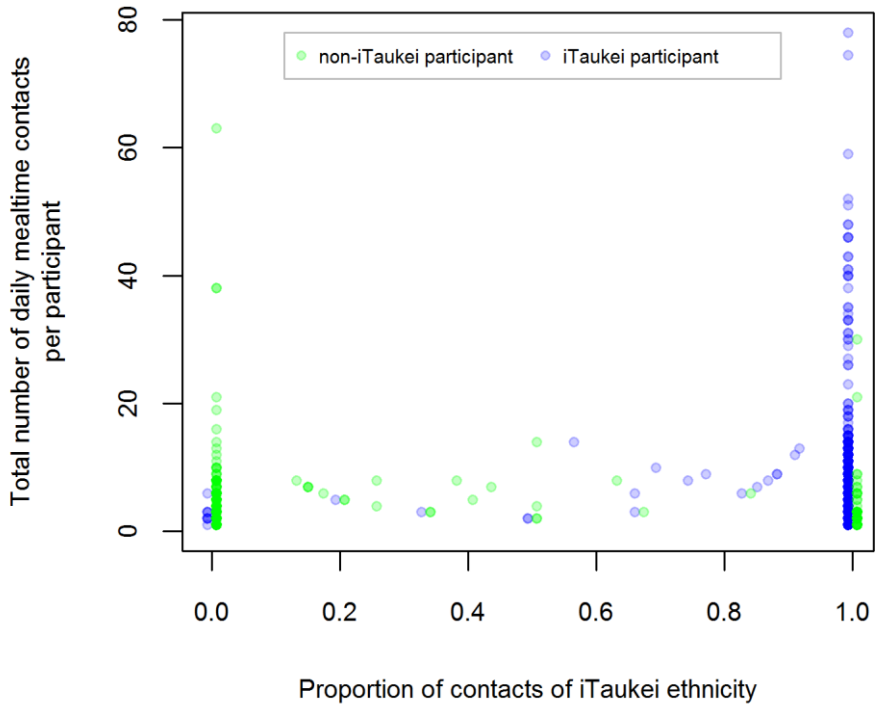
C		Overall		iTaukei		Non-iTaukei	
	Geography	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
Mongooses	All	110	6.1 (5.1 to 7.3)	103	7.3 (6.1 to 8.8)	7	1.7 (0.8 to 3.5)
	Urban	19	3.8 (2.4 to 5.8)	18	4.7 (3 to 7.3)	1	0.8 (0 to 4.6)
	Peri-urban	13	4.5 (2.6 to 7.5)	9	5.1 (2.7 to 9.5)	4	3.5 (1.4 to 8.7)
	Rural	78	7.6 (6.2 to 9.4)	76	9 (7.2 to 11.1)	2	1.2 (0.3 to 4.1)
Rats	All	247	13.6 (12.1 to 15.3)	220	15.6 (13.8 to 17.6)	27	6.7 (4.6 to 9.5)
	Urban	56	11.1 (8.6 to 14.1)	50	13 (10 to 16.7)	6	5 (2.3 to 10.6)
	Peri-urban	38	13.1 (9.7 to 17.5)	26	14.9 (10.3 to 20.9)	12	10.5 (6.1 to 17.5)
	Rural	153	15 (12.9 to 17.3)	144	17 (14.6 to 19.7)	9	5.2 (2.8 to 9.6)

5.7.1 Supplementary text.

Supplementary text S5.1. Seropositivity and social contact regression analysis

This supplementary text describes logistic regression analyses that were undertaken to develop understanding of association between mealtime social contact rates and seroprevalence in Fiji when taking into account potential confounding variables and ethnicity-based contact rates. These analyses follow an initial strong signal for association between total contact rates and seropositivity in the total survey group. These analyses are of residents of unvaccinated areas of mainland Fiji only, to reduce noise from vaccine-associated seropositivity on Taveuni island.

Figure ST5.1 Fig 1 shows participants' contact rates, by participant ethnicity and proportion of contacts that were of iTaukei ethnicity. These data show that most participants have contact with only one ethnicity category of contact, which is usually the same category as the participant. There are a number of participants with mealtime contacts from the other ethnic category only, and relatively few with mealtime contacts across both ethnic categories.



ST5.1 Fig 1. Number of contacts per iTaukei and non-iTaukei participant, by proportion of contacts that were iTaukei.

We first examined odds ratios for association between anti-Vi IgG seropositivity and total number of contacts, adjusting for participant age, without ethnicity adjustment for contacts

or participants. This association was examined at both a seropositivity threshold of 64 EU, determined in prior research to be suggestive of previous or current infection, and 100 EU, more suggestive of recent or current infection. The recent infection 100 EU marker was considered to be more likely to be influenced by the participants' reported contact rates, as contact rates may have changed over time, particularly given the variation in contact rates demonstrated across age groups in the social mixing survey. Data for 64 EU are reported here for completeness. Data were available for 1559 participants from unvaccinated areas of Fiji mainland.

Evidence of association between seropositivity and total contact rates (contacts of any ethnic category) was observed at 100 EU, with an effect size of approximately* 2% additional likelihood of seropositivity per additional contact. No association was demonstrated at 64 EU (ST5.1 Tables 1 and 2).

* *Cummings P. The relative merits of risk ratios and odds ratios. Arch Pediatr Adolesc Med. 2009;163(5):438-445.*

The data were then analysed by ethnicity of contacts (ST5.1 Table 1 and 2). Some evidence was observed for association with iTaukei contacts at 64 EU, with stronger evidence of association at 100 EU, where data showed a similar effect size per contact to the above total contact rates analyses and greater strength of association. Weak association between increasing non-iTaukei contact rates and sero*negativity* was observed at 64 EU but not supported by examination at 100 EU. This suggested there was no association between non-iTaukei contact rates and seropositivity but that these data might contribute to non-differential misclassification when included in analysis of total contact rates.

ST5.1 Table 1. 100 EU Vi IgG threshold, participants from unvaccinated areas, all ethnicities.

Model	Variable	Odds ratio	p-value	AIC
1.	Any contact Age	1.02 (1.00 to 1.04) 1.025 (1.019 to 1.032)	0.02 <0.0001	1376
2.	iTaukei contact Age	1.025 (1.007 to 1.042) 1.026 (1.019 to1.033)	0.005 <0.0001	1373.5
3.	Non-iTaukei contact Age	0.96 (0.89 to 1.02) 1.025 (1.018 to 1.031)	0.2 <0.0001	1378.9

ST5.1 Table 2. 64 EU Vi IgG threshold, participants from unvaccinated areas, all ethnicities.

Model	Variable	Odds ratio	p-value	AIC
4.	Any contact Age	1.01 (0.99 to 1.02) 1.026 (1.020 to 1.032)	0.33 <0.0001	1854.1

5.	iTaukei contact Age	1.01 (1.00 to 1.03) 1.026 (1.020 to 1.032)	0.08 <0.0001	1852.1
6.	Non-iTaukei contact Age	0.96 (0.91 to 1.00) 1.026 (1.020 to 1.031)	0.1 <0.0001	1851.7

Analyses were next undertaken to determine whether an effect remained when data were stratified by the ethnicity of the survey participant. Amongst the 1,189 iTaukei participants, strong association with 100 EU seropositivity was observed for increasing total or iTaukei contact rates (ST5.1 Table 3). This is consistent with the ST5.1 Table 1 models and ST5.1 Fig 1, as iTaukei participants comprise the large majority of respondents and primarily report iTaukei contacts. At 64 EU, models of iTaukei participants including total contacts or iTaukei contacts showed some association (ST5.1 Table 4). Non-iTaukei contact rates in iTaukei participants showed weak evidence of association for 64 EU seropositivity and this association further weakened at 100 EU.

Amongst the 370 non-iTaukei participants, no association between seropositivity and contacts of any or either ethnic category was observed at 100 EU or 64 EU thresholds (ST5.1 Tables 5 and 6).

ST5.1 Table 3. 100 EU Vi IgG threshold, participants of iTaukei ethnicity from unvaccinated areas.

Model	Variable	Odds ratio (95% CI)	P value	AIC
7. Any contact, age adjusted	Any contact Age (year)	1.026 (1.007 to 1.045) 1.023 (1.015 to 1.030)	0.005 <0.0001	1046
8. Ethnically-stratified contact, age adjusted	iTaukei contact non-iTaukei contact Age (year)	1.026 (1.007 to 1.044) 1.24 (0.84 to 1.77) 1.023 (1.015 to 1.031)	0.005 0.2 <0.0001	1047

ST5.1 Table 4. 64 EU Vi IgG threshold, participants of iTaukei ethnicity from unvaccinated areas.

Model	Variable	Odds ratio (95% CI)	P value	AIC
9. Any contact, age adjusted	Any contact Age (year)	1.01 (1.00 to 1.03) 1.026 (1.019 to 1.032)	0.1 <0.0001	1416.7
10. Ethnically-stratified contact, age adjusted	iTaukei contact non-iTaukei contact	1.01 (1.00 to 1.03) 1.40 (1.00 to 2.04)	0.1 0.06	1415.1

	Age (year)	1.026 (1.019 to 1.033)	<0.0001	

ST5.1 Table 5. 100 EU Vi IgG threshold, participants of non-iTaukei ethnicity from unvaccinated areas.

Model	Variable	Odds ratio (95% CI)	P value	AIC
11. Any contact, age adjusted	Any contact Age (year)	0.95 (0.86 to 1.02) 1.035 (1.020 to 1.052)	0.3 <0.0001	328.42
12. Ethnically-stratified contact, age adjusted	iTaukei contact non-iTaukei contact Age (year)	0.98 (0.80 to 1.10) 0.95 (0.85 to 1.02) 1.035 (1.020 to 1.052)	0.8 0.2 <0.0001	330.28

ST5.1 Table 6. 64 EU Vi IgG threshold, participants of non-iTaukei ethnicity from unvaccinated areas.

Model	Variable	Odds ratio (95% CI)	P value	AIC
13. Any contact, age adjusted	Any contact Age (year)	0.95 (0.88 to 1.01) 1.027 (1.015 to 1.041)	0.1 <0.0001	436.05
14. Ethnically-stratified contact, age adjusted	iTaukei contact non-iTaukei contact Age (year)	0.92 (0.75 to 1.04) 0.95 (0.88 to 1.01) 1.027 (1.015 to 1.040)	0.3 0.2 <0.0001	437.83

Having observed an age-adjusted association between increasing daily number of iTaukei contacts and recent *Salmonella* Typhi infection, driven by contacts made by iTaukei participants, we examined the full participant dataset for the influence of covariates such as lunching away from home on this association (S3 Tables 7 and 8). As these covariates did not substantially influence the effect size or evidence of association, the model presented in the main paper is the parsimonious model accounting for iTaukei contacts and participant age only. As non-iTaukei participants showed no association between contact rates and seropositivity, the model shown in the main paper is that applying to iTaukei only.

ST5.1 Table 7. 100 EU Vi IgG threshold multivariable regression models, unvaccinated areas, participants of all ethnicities

Model	Variable	Odds ratio (95% CI)	P value	AIC
15.	iTaukei contact non-iTaukei contact Age (year)	1.023 (1.005 to 1.041) 0.98 (0.91 to 1.03) 1.026 (1.019 to 1.033)	0.01 0.4 <0.0001	1374.8

16.	iTaukei contact non-iTaukei contact Age (year) Non-iTaukei ethnicity	1.025 (1.006 to 1.044) 0.96 (0.87 to 1.02) 1.025 (1.018 to 1.032) 1.2 (0.8 to 1.8)	0.007 0.3 <0.0001 0.4	1376
17.	iTaukei contact non-iTaukei contact Age (year) Lunch away from home	1.01 (1.00 to 1.04) 0.97 (0.91 to 1.02) 1.026 (1.019 to 1.034) 1.2 (0.8 to 1.6)	0.022 0.4 <0.0001 0.3	1375.4
18.	iTaukei contact non-iTaukei contact Age (year) Non-iTaukei ethnicity Lunch away from home	1.023 (1.004 to 1.042) 0.95 (0.87 to 1.02) 1.026 (1.019 to 1.033) 1.2 (0.8 to 1.8) 1.2 (0.8 to 1.7)	0.015 0.3 <0.0001 0.4 0.3	1376.5

ST5.1 Table 8. 64 EU Vi IgG threshold multivariable regression models, unvaccinated areas, participants of all ethnicities

Model	Variable	Odds ratio (95% CI)	P value	AIC
19.	iTaukei contact	1.01 (0.99 to 1.03)	0.17	1851.8
	non-iTaukei contact	0.97 (0.91 to 1.01)	0.17	
	Age (year)	1.026 (1.020 to 1.032)	<0.0001	
20.	iTaukei contact	1.01 (0.99 to 1.03)	0.2	1853.6
	non-iTaukei contact	0.97 (0.91 to 1.02)	0.3	
	Age (year)	1.026 (1.020 to 1.032)	<0.0001	
	Non-iTaukei ethnicity	0.92 (0.69 to 1.29)	0.6	
21.	iTaukei contact	1.01 (1.00 to 1.03)	0.2	1852.5
	non-iTaukei contact	0.97 (0.91 to 1.02)	0.3	
	Age (year)	1.026 (1.021 to 1.033)	<0.0001	
	Non-iTaukei ethnicity	0.93 (0.67 to 1.3)	0.7	
	Lunch away from home	1.04 (0.78 to 1.37)	0.8	
22.	iTaukei contact	1.02 (0.99 to 1.03)	0.2	1854.4
	non-iTaukei contact	0.97 (0.91 to 1.02)	0.3	
	Age (year)	1.026 (1.021 to 1.033)	<0.0001	
	Non-iTaukei ethnicity	0.93 (0.67 to 1.3)	0.7	
	Lunch away from home	1.04 (0.78 to 1.37)	0.8	
	Interaction for contacts	1.03 1.00 (0.94 to 1.05)	1	

Chapter 6. Transmission dynamics of typhoid fever in Fiji: a model of vaccination and WASH. CH Watson, AJ Kucharski, WJ Edmunds

London School of Hygiene & Tropical Medicine.

6.1 Bridging section

This final research paper chapter describes the fitting of, and findings from, a transmission dynamic model. The model was conceptualised by me in late 2016 in terms of a compartmental structure that could synthesis case and serological data by means of dose-dependency of pathogenicity, with input from the co-authors. It was developed by me in summer 2017 after a break to return to professional duties at Public Health England. I have been able to estimate model parameters by maximum likelihood estimation, and produce a number of informative intervention impact scenarios and sensitivity analyses. The interpretation of findings is intended to be appropriately cautious, particularly with regard to precision, but the results are indicative of relative importance of parameters and may be indicative of impacts of putative interventions. The development of the model framework opens the prospect for further analysis of the situation in Fiji and to further explore the transmission drivers, uncertainties in parameterising the model and plausible programme impacts.

Conceptualisation and coding of the model was done by me in the R statistical environment. I had used the analysis of the serological survey and the social mixing data to improve my R literacy, rather than opting for quicker and easier Stata or Excel based analyses and this proved extremely useful in coding up the model. Under the tutelage of Adam Kucharski and supervision of John Edmunds, the model was substantially extended by me from an SI example model, to one with six compartments (SIRCAV), 80 ages and two ethnicities. Adam coded the aging process to reflect the demographic structure, which was our joint conceptualisation but beyond my numeracy, and checked the compartment-transition differential equations. I parameterised the aging process model to visual fit with census data.

I developed the likelihood function with input from Adam Kucharski and John Edmunds, adapting a log likelihood function originally developed for the Fiji dengue fever model of adult and child dengue case data and arbovirus immunity data from the serosurvey and 2015 follow-up. The modelled typhoid incidence case count was initially to be fitted to case data through a negative binomial function. However, the negative binomial overdispersion parameter was found to be intractable and the function unsuitable and so a Poisson

distribution was utilised. An important step was identifying means of reducing model run times from over an hour to seconds, by taking advantage of time step handling properties in the ODE45 implementation of the Runge Kutta differential equation solver. This opened up scope to run maximum likelihood estimation using the bbmle package rather than through a low-resolution grid search. My analysis, sensitivity analysis, interpretation and drafting of the manuscript was done with review by John Edmunds.

Preliminary findings have been discussed with the Ministry of Health and research partners in October 2017 in support of typhoid control.

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Student	Conall Watson
Principal Supervisor	John Edmunds
Thesis Title	Seroepidemiological investigations of typhoid fever in Fiji and the potential role of vaccination in control

If the Research Paper has previously been published please complete Section B, if not please move to Section C

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Please list the paper's authors in the intended authorship order:	Conall H Watson, Adam J Kucharski, W John Edmunds
Stage of publication	In preparation

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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	See attached

Student Signature: [redacted] **Date:** 12 Sep 2017

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Transmission dynamics of typhoid fever in Fiji: a model of vaccination and WASH.

CH Watson, AJ Kucharski, WJ Edmunds

London School of Hygiene & Tropical Medicine.

6.2 Introduction

Typhoid fever is a systemic human bacterial disease, caused by *Salmonella enterica* serovar Typhi (*S.* Typhi), spread through the faecal-oral route. Initial symptoms of fever and malaise can be followed by debilitating abdominal pain, headache, cough, anorexia, nausea, myalgia and confusion, with life-threatening complications in prolonged illness including intestinal perforation, gastrointestinal haemorrhage and encephalopathy.[1] As in many parts of Asia-Pacific,[2,3] typhoid fever is a pressing public health concern in Fiji. An emergency typhoid Vi-polysaccharide (ViPS) mass vaccination campaign was implemented in the highest-risk areas of Fiji (7% by population) following Cyclone Tomas in 2010, demonstrating effectiveness consistent with vaccine efficacy and coverage.[4] Ministry of Health and public health partners have sought to understand and control the disease, convening an expert meeting to assess long-term strategies for control, and knowledge gaps that would support this. Serologically-informed modelling was proposed to inform vaccination programme considerations.[5]

Typhoid has an incidence of approximately 380 blood-culture confirmed cases per annum in Fiji, or 45 per 100,000 person-years.[5] Over 90% of reported culture-confirmed cases are of the indigenous iTaukei community (57% of the census population), predominantly affecting young adults, with few cases reported in Fijians of Indian descent (Indo-Fijians) or other non-iTaukei ethnicities.[5,6] This contrasts to findings of our serological survey, which identified similar seroprevalences in both iTaukei and non-iTaukei ethnic groupings of anti-Vi IgG, a putative marker of current or past *S.* Typhi exposure, based on a case cohort-fitted threshold.[7] The survey indicated seroprevalence of approximately 1 in 3, though there remain considerable uncertainties as to the sensitivity and specificity of such thresholds, and found increasing seroprevalence by age and a non-significantly higher seroprevalence in iTaukei than non-iTaukei.

Different conceptual frameworks exist for typhoid transmission models,[8,9] with different analytical intent, described previously.[10] One conceptualisation of typhoid immunogenicity and pathogenicity is that the occurrence and presentation of typhoid fever is dependent on the inoculating dose of *S.* Typhi. A simplified schematic for the role of dose-dependency in

the “clinical iceberg” of typhoid fever is shown in figure 6.1. With known surveillance case numbers and sero-survey data supporting estimates for the total size of ever-infected population in Fiji, a transmission dynamic approach offers potential insight into the relative size of the infectious case population that would drive disease dynamics, and the impact of underlying natural immunity on interventions such as vaccination.

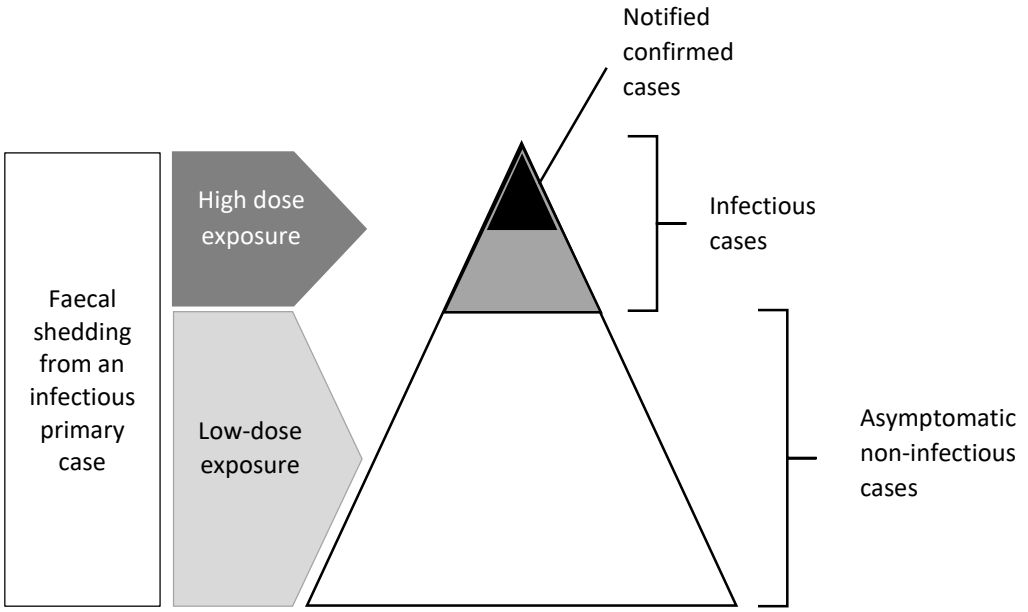

Figure 6.1. Clinical iceberg conceptual schematic for acute typhoid fever.
The dose response gradient for typhoid infection is dichotomised into high and low doses exposures from S. Typhi shedding in a primary case. These result in infectious (grey triangle) or non-infectious (white triangle section) secondary cases depending on the ingested dose. Of infectious cases, a proportion are unwell enough to seek medical care, and of these some have blood taken, test positive and are notified to public health surveillance (black triangle).

As outlined in the introduction to this thesis, high inoculating doses were established in the Maryland typhoid human challenge studies as more likely to result in symptomatic disease, as assessed by various diagnostic approaches, with earlier onset compared to low-dose challenge.[11,12] In community settings, high inoculating doses may be derived from prepared-food faecal contamination; direct person-to-person and fomite transmission in the absence of adequate infection control and hygiene practices; or consumption of drinking water with high volumes of faeces containing S. Typhi, most typically stored or surface water in the immediate environment around a case. Symptomatic cases from such events are very likely to shed *S.* Typhi in faeces.[11] Immunity occurs following recovery, with infectious asymptomatic carriage established in a small proportion of cases.[1]

Low-dose inoculation, such as may be derived from contamination of the environment, water contaminated with lower volumes of faeces (such as that further away from cases) and

unprepared foods, or other similar distal, non-direct transmission, may give rise to asymptomatic infection and natural immunity without observed or surveillance-notified development of systematic disease. The Maryland challenge studies found an inoculating threshold below which clinical disease did not develop. Low dose exposure may act as an immunising force, creating immunity without faecal shedding. Mild or asymptomatic typhoid infection in childhood can preclude severe disease later in life,[13] consistent with classical models of tropical infection.[14]

Both typhoid immunisation and other interventions to reduce transmission offer potential to reduce the burden of typhoid in Fiji; though relative impact may depend on both intervention efficacy and the underlying immuno-epidemiological landscape.

6.2.1 Aims

We developed a deterministic, compartmental, dynamical model of typhoid fever in Fiji utilising field data on social mixing patterns and seropositivity, alongside national surveillance data. This study aimed to describe the observed serological and case data for Fiji and examine a range of model structures reflecting different epidemiological conceptual constructs of typhoid transmission in Fiji, and the goodness-of-fit of these to the data. We sought to estimate the potential impact on typhoid fever incidence of immunisation programmes and other programmes to reduce transmission, such as adequate water, sanitation and (hand & food) hygiene (collectively: WASH).[15,16] We considered different vaccination strategies such as school-entry vaccination programmes and one-time school-age campaigns, which were examined with and without WASH interventions.

6.3 Methods

6.3.1 Ethics approval

This study was approved by the Fiji National Research Ethics Review Committee (2013-03) and the London School of Hygiene and Tropical Medicine's ethics committees (6344).

6.3.2 Data sources

Anonymised, blood-cultured confirmed, national surveillance case data were provided by the Fiji National Centre for Communicable Disease Control for the years 2008 to 2014, the

complete range of cleaned, disaggregated data available for analysis. There were mean 363 (range 285 to 425) cases annually, which were relatively stable with an average decline of 11 cases per year (r^2 = 0.15). Ninety-seven percent of cases were iTaukei.

Serological data was obtained through a field survey, described previously, of 1,531 residents aged 1year and over in unvaccinated areas of the two main Fijian islands (Viti Levu and Vanua Levu) conducted between September and December 2013. An anti-Vi IgG ELISA titre threshold of 64 was considered indicative of natural immunity, based on a mixed model of antibody decay in recovering confirmed cases.[7]

6.3.3 Model structure

The full model structure comprises of six compartments representing five disease states plus vaccination (figure 6.2). Each compartment is age- and ethnicity- structured (0 to 79 years; iTaukei and non-iTaukei). Daily movements between compartments are specified by a system of ordinary differential equations (equations 1 to 6). Simpler model structures can be created by setting relevant parameters to zero.

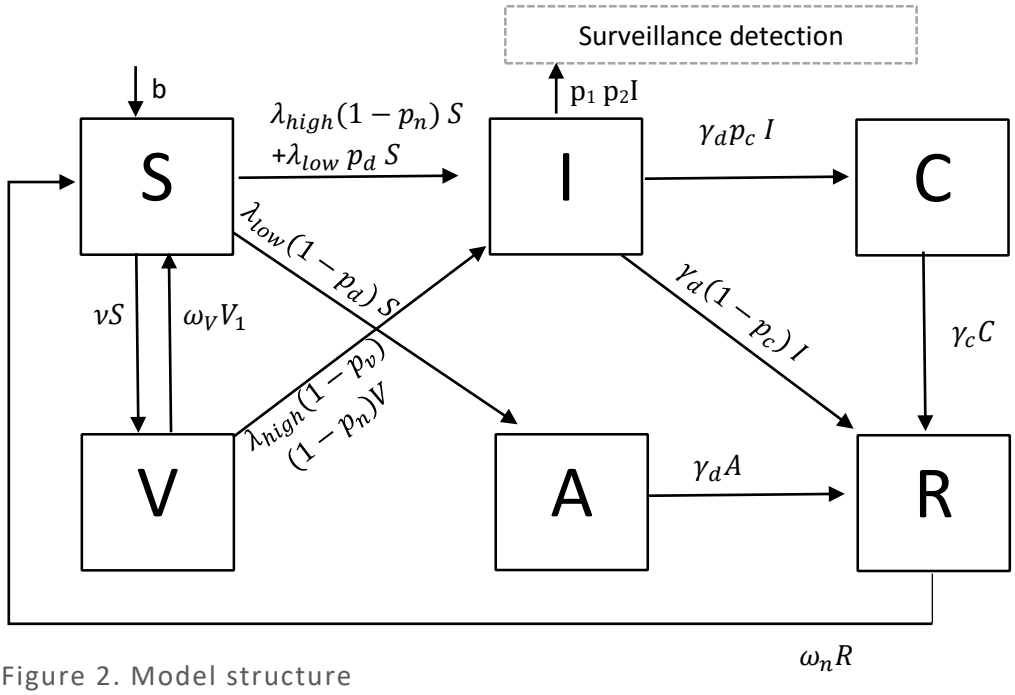

Figure 2. Model structure

Susceptible (*S*) non-immune individuals can move into the Infectious (*I*) compartment at a rate λ_{hi} following inoculation from a high dose of *S.* Typhi from a case (*I*) or long-term infectious carrier (*C*), due to proximal or short-cycle transmission including direct contact and

localised transmission from locally-contaminated water, food, fomites and environment with force of infection determined by social contact patterns. They can be joined in *I* by *S* compartment individuals arriving at a rate $\lambda_{low}p_d$, indicating a proportion of individuals receiving a low inoculating dose (generalised force of infection independent of contact patterns) that results in becoming infectious (with or without symptoms).

$$\frac{dS_{i,j}}{dt} = b_{i,j} + \phi_{i-1,j}\zeta_{i,j}\, S_{i-1,j} + \omega_n R_{i,j} + \omega_v V_{i,j} - \lambda_{high,i,j}(1-p_{n,j})S_{i,j} - \lambda_{low}S_{i,j} - \nu_{i,j}S_{i,j} - \phi_{i,j}S_{i,j} \quad (1)$$

$$\frac{dI_{i,j}}{dt} = \phi_{i-1,j}\zeta_{i,j}\, I_{i-1,j} + \lambda_{high,i,j}(1-p_{n,j})S_{i,j} + \lambda_{low}\, p_d\, S_{i,j} + \lambda_{high,i,j}(1-p_v)(1-p_n)V_{i,j} - \gamma_d I_{i,j} - \phi_{i,j}I_{i,j} \quad (2)$$

$$\frac{dR_{i,j}}{dt} = \phi_{i-1,j}\zeta_{i,j}\, R_{i-1,j} + \gamma_d(1-p_c)\, I_{i,j} + \gamma_c C_{i,j} + \gamma_d A_{i,j} - \omega_n R_{i,j} - \phi_{i,j}R_{i,j} \quad (3)$$

$$\frac{dC_{i,j}}{dt} = \phi_{i-1,j}\zeta_{i,j}\, C_{i-1,j} + \gamma_d p_c\, I_{i,j} - \gamma_c C_{i,j} - \phi_{i,j}C_{i,j} \quad (4)$$

$$\frac{dA_{i,j}}{dt} = \phi_{i-1,j}\zeta_{i,j}\, A_{i-1,j} + \lambda_{low}(1-p_d)\, S_{i,j} - \gamma_d A_{i,j} - \phi_{i,j}A_{i,j} \quad (5)$$

$$\frac{dV_{i,j}}{dt} = \phi_{i-1,j}\zeta_{i,j}\, V_{i-1,j} + \nu_{i,j}S_{i,j} - \lambda_{high,i,j}(1-p_v)(1-p_n)V_{i,j} - \omega_v V_{i,j} - \phi_{i,j}V_{i,j} \quad (6)$$

$$\frac{dF_{i,j}}{dt} = \lambda_{high,i,j}(1-p_n)S_{i,j} + \lambda_{low}\, p_d\, S_{i,j} + \lambda_{high,i,j}(1-p_v)(1-p_n)V_{i,j} \quad (7)$$

Where:
$S_{i,j}$, $I_{i,j}$, $R_{i,j}$, $C_{i,j}$, $A_{i,j}$ and $V_{i,j}$ are the number of susceptible, acute infectious, recovered (naturally immune), chronic infectious carrier, asymptomatically infected non-infectious and vaccinated individuals respectively in the population, by year of age and ethnicity.
$F_{i,j}$ records incident acute infectious typhoid cases, by year of age and ethnicity.

i denotes year of age (0 to 79)
j denotes ethnicity (iTaukei or non-iTaukei)
$b_{i,j}$ is ethnicity-specific daily births into for *i*=0 (0 for i>0)
γ_c is the daily recovery rate from carriage *C* class infection
γ_d is the daily recovery rate from infectious *I* and asymptomatic *A* class infection
$\phi_{i,j}$ is daily aging rate, by year of age and ethnicity ($\phi_{i-1,j}$ = 0 if *i*=0)
$\zeta_{i,j}$ is survival proportion following aging, by year of age and ethnicity
$\nu_{i,j}$ is the daily rate of vaccination to achieve a specified vaccine coverage over the course of a year in a specified year of age and ethnic group
p_c is the proportion of cases that become carriers
p_d is the proportion of low dose infections that lead to the infectious *I* class
$p_{n,j}$ is the proportionate reduction in high-dose force of infection for non-iTaukei relative to iTaukei (p_n=0 if *j* = iTaukei)
p_v is the vaccine efficacy
ω_n is daily waning rate of natural immunity
ω_v is daily waning rate of vaccine immunity
$\lambda_{high,\,i,j}$ is the daily high-dose force of infection and λ_{low} is the low-dose force of infection

An age and ethnic group structured *F* compartment records incident *I*-compartment cases (equation 7). A subset of *F*-compartment cases is reported in culture-confirmed national surveillance at a reporting rate p_1 which is further modified by p_2, the reduction in reporting rate in children. See "surveillance reporting" section for details.

S compartment individuals transition to an asymptomatic, non-infectious (*A*) compartment at rate $\lambda_{low}(1-p_d)$ indicating receipt of a low inoculating dose without becoming infectious. Individuals exit *A* and enter a recovered (*R*), naturally immune compartment at a rate γ_d. The same exit rate applies to the *I* class, with a proportion p_c entering the *C* compartment and the remainder entering *R*. Carriers leave *C* and enter *R* at a rate γ_c. The transition rate for *R* to *S* (loss of natural immunity) is ω_n. The model does not include a latent period. Force of infection is described by equations 8 and 9.

Force of infection (high-dose) is

$$\boldsymbol{\lambda_{high,i,j}} = \boldsymbol{\beta} \sum_{m=1}^{5} \sum_{n=1}^{2} \boldsymbol{M_{ij\,kl}} \left(\frac{\boldsymbol{I_{k,l}} + \boldsymbol{C_{k,l}}}{\boldsymbol{N_{k,l}}} \right) \tag{8}$$

And force of infection (low-dose) is

$$\boldsymbol{\lambda_{low}} = \frac{\boldsymbol{\alpha\beta} \sum_{k=1}^{5} \sum_{l=1}^{2} (\boldsymbol{I_{k,l}} + \boldsymbol{C_{k,l}})}{\sum_{k=1}^{5} \sum_{l=1}^{2} \boldsymbol{N_{k,l}}} \tag{9}$$

Where:

i is year of age and *j* is ethnic group of susceptible and vaccinated individuals
k is age band (0-4, 5-14,15-34, 35-54 and 55-79 years) and *l* is ethnic group of infectious and carrier individuals
$M_{ij\,kl}$ is the contact matrix by year of age (i) and age band (k) and ethnic group (j and l), weighted for reciprocal contact, for transmission from kl to ij
β is the effective contact rate for high-dose transmission
α is the low-dose β ratio
$N_{k,l}$ is the population size by age band and ethnic group.

Vaccination transition *S* to *V* occurs at a rate v with *V* to *S* loss of vaccine immunity at rate ω_v. *V* compartment individuals enter *I* with λ_{high} modified by 1-p_v where p_v is vaccine efficacy, but do not transition to *A* as vaccine is assumed to fully protect against low dose typhoid infection.

6.3.3.1 Structural sensitivity analysis

Alternative epidemiological models were examined as follows:

- With high-dose social mixing homogeneous within iTaukei and non-iTaukei groups
- With high-dose social mixing homogenous across age band and ethnic groups
- Without low-dose force of infection and thus no asymptomatic non-infectious compartment; and high dose force of infection social mixing:
    - heterogeneous by age and ethnic group
    - homogeneous within iTaukei and non-iTaukei ethnic groups.
    - homogeneous across age band and ethnic groups

6.3.3.2 Waning force of infection sensitivity analysis

In chapter 3, the possibility was raised that the observed seroepidemiological age patterns could be explained by endemic transmission, or decreasing force of infection across several decades, potentially with transmission concentrated in childhood. Using an approach adapted from Gay for hepatitis A,[17] a five-fold higher baseline equilibrium was fitted and models of decreasing force of infection were run as a sensitivity analysis using a monotonic decline function (equation 10).

$$\psi = 3 - 2\tanh\left(\frac{2(t - med\,(t))}{T}\right)$$

(10)

Where:

Ψ is the Beta scaling factor

t is the time period of analysis in years

T is the time period over which force of infection predominantly declines in years.

In the sensitivity analyses t is 100 years and T is 50 years.

6.3.4 Software

Analysis was conducted in R version 3.4.1.[18] The model was established as a series of ordinary differential equations which were solved using the ODE45 adaptive time step Runge-Kutta method in package deSolve.[19]

6.3.5 Demographics

A demographic process operates across each model compartment to approximate the 2007 census population structure for each ethnicity category.[20] Individuals are born into the iTaukei or non-iTaukei age zero strata of the susceptible compartment, with daily births equal to the sum of the ethnicity-specific all-age deaths. Individuals exit each age stratum with a daily aging rate and a number enter the next age stratum proportional to an age- and ethnicity- specific survival probability. This survival proportion is less than one for all ages of iTaukei and one for non-iTaukei to the age of 39, after which it declines. Comparison between the census population and the equilibrium synthetic population are shown in figure 6.3 below.

The demographic process is identical across each compartment; there is no adjustment to death rates for disease states. The model population structures are attributable only to constant rates of births and deaths with no adjustment for migration or historical trends in birth and death rates.

6.3.6 Social contact

A reciprocity-weighted contact matrix of mealtime social contacts, determined by a field survey and described previously (chapter 5),[21] was used for daily contact rates for high-dose transmission events. Age groups were 0-4, 5-14,15-34, 35-54 and 55+ years and ethnic categories were iTaukei and non-iTaukei. In the analysis of association between social mixing and seropositivity for anti-Vi IgG, we found that an increase in number of age-adjusted contacts was associated with increased odds of seropositivity, driven by inter-iTaukei contact. This supports the utilisation of the field-sourced mealtime contact rate matrix in this modelling analysis.

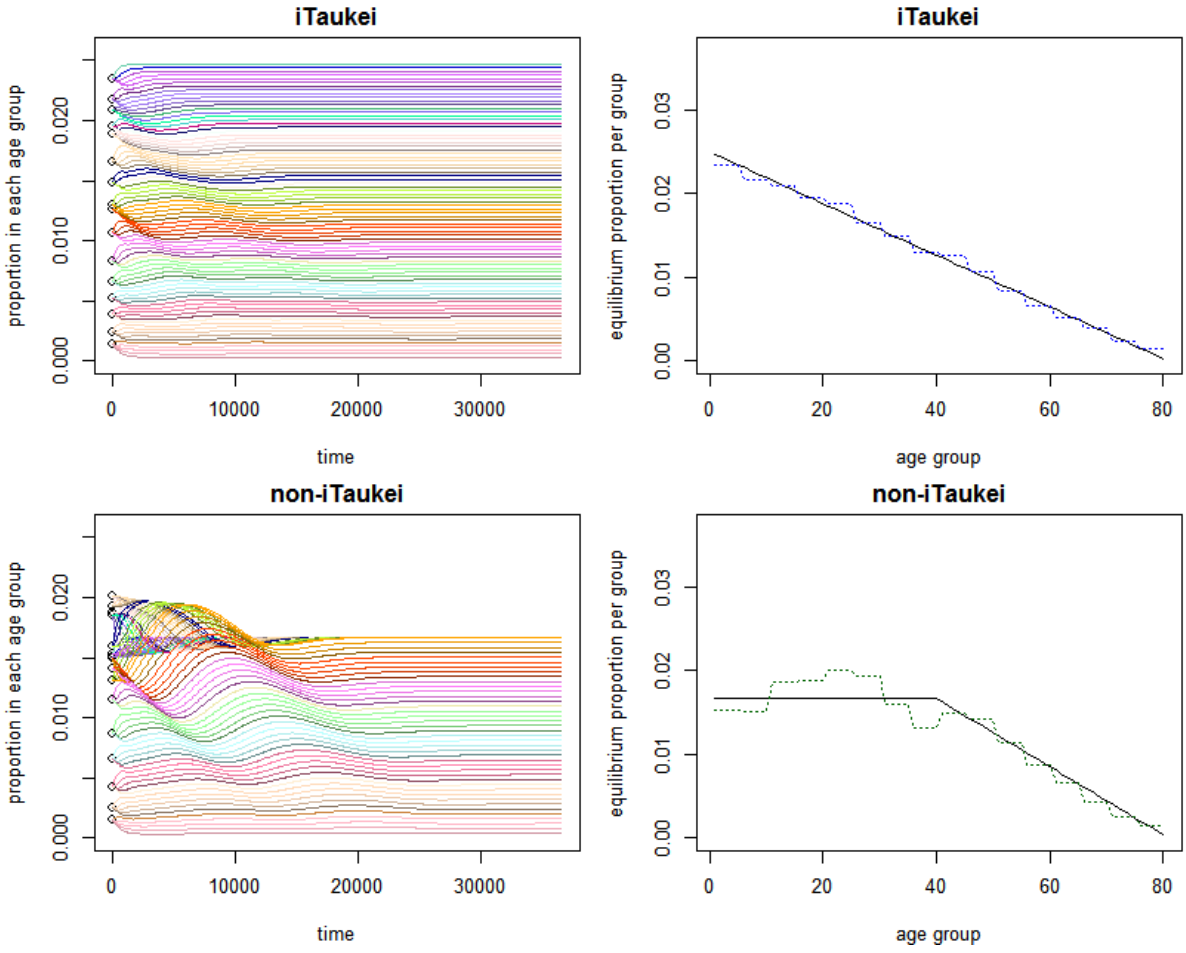

Figure 6.3. Model demographic structure and census population (2007) by age and ethnicity and after 100-year equilibration.
Panels denote iTaukei (top row) and non-iTaukei (bottom row) demographics. Starting parameters (left panel dots and right panel dashed lines) indicate census population relative to the model equilibrium demographic state (right panels' solid lines).

6.3.7 Model fitting

Three transmission parameters and two reporting parameters were fitted by maximum likelihood estimation (MLE) using the mle2 function in the package bbmle.[22] Parameter space exploration was first undertaken with visual fitting to surveillance data and serology. MLE was performed using Nelder-Mead optimisation.

The model was fitted to case and serology data. Simulation surveillance-reported cases were compared to data for annual (2008-2014) national surveillance blood culture confirmed case reporting, disaggregated by age group (as per the social mixing matrix) and ethnic category. Simulated *R* compartment proportion by year of age and ethnic category was compared to IgG Vi seropositivity from the 2013 serological survey. Fixed parameters are shown in table 6.1.

Model Parameter	**Description**	**Value**	**Source**
γ_d	Recovery rate from acute infection (14 days)	1/14 per day	CCDM[23]
γ_c	Recovery rate from carriage (10 years)	1/(365*10) per day	Assumed from [24,25]
p_c	Proportion of infectious cases becoming carriers	1%	Adapted from [26]
p_n	Proportion of low-dose inoculation recipients developing infectious illness	1%	Assumed
ω_n	Loss of natural immunity (40 years)	1/(365*40)	Inferred from [27] and examined by sensitivity analysis

Table 6.1. Fixed parameter values

The log likelihood function is given in equation 10 below.

$$L(\theta|X,Z) = \sum_{b=1}^{5}\sum_{e=1}^{2}\left(\left(\sum_{y=1}^{7} x_y log p_1\, p_{2,b}\Lambda_{b,e}\right) - 7\Lambda_{b,e}\right)$$

$$+ \sum_{a=1}^{80}\sum_{e=1}^{2}\left(z_{a,e}\log p_{a,e} + (n_{a,e} - z_{a,e})\log(1-p_{a,e})\right) \quad (11)$$

Where:

x are national surveillance case data across five age groups (b), (0-4, 5-14,15-34, 35-54 and 55+ years) two ethnic groups (*e*) (iTaukei and non-iTaukei) and seven surveillance years from 2008 to 2014 (*y*);
Λ is the simulation equilibrium annual case count by age and ethnic group;
p_1 is the surveillance reporting fraction and p_2 is the reporting fraction modifier for children ($b \leq 2$; 0 to 14 years, 1 for $b > 2$)
z is the observed seropositive count from n serosurvey participants in each of 80 one-year ages (*a*) and two ethnic groups (*e*); and $p_{a,e}$ is the simulation equilibrium *R* compartment prevalence by year of age and ethnic group.

6.3.8 Surveillance reporting

Propensity to seek care, propensity to obtain blood for culture, poor blood culture sensitivity and incomplete notification to national authorities leads to under-ascertainment of cases in national culture-confirmed surveillance.[3,28] There is no known differential care-seeking behaviour (including antimicrobial utilisation, which may avert seroconversion arising in prolonged infection)[29] in different ethnic groups in Fiji and so we do not modify reporting by ethnicity.[20,30] Data we collected in 2015 for a dengue fever serosurvey found no difference in health-seeking behaviour between iTaukei and non-iTaukei Fijians who had had recent fever (34 of 43 and 17 of 20 respectively; odds ratio for non-iTaukei 1.5; 95% confidence interval 0.4 to 9.7; p-value = 0.6). Under-ascertainment in children relative to adults is well established internationally[3,31] and so we include both a surveillance reporting fraction p_1 for adults (age 15+) and a further modifier to this for children (p_2).

6.3.9 Carriage

Typhoid is noted for its asymptomatic carriage, which contributes to sustained transmission in low endemicity settings.[32] Anderson and May[33] posit that typhoid carriers should not have the same β as acute cases, as this gives them a contribution in excess of ten times that of acute cases after taking into account duration of infection. Feachem and colleagues[14] note that typhoid carriers shed very high concentrations of bacteria in faeces, of the same orders of magnitude as cases. Shedding events may be intermittent, making detection challenging,[34] and making it challenging to estimate the infectiousness of a carrier relative to an acute case (notwithstanding the depletion of susceptibles socially-connected to a carrier, which may become saturated during their infectious period).

Recent data on the proportion of cases who become carriers is sparse, particularly for low and middle income countries.[25,35] Surveillance data from Baltimore, USA, found 0 carriers from 55 child typhoid cases while in Scotland 32 carriers were identified relative to 267 acute

cases though potential differential ascertainment precludes assuming this to be a ratio.[36] US data from the pre-antibiotic era found 2.9% of cases became carriers with increasing risk with age[26] – approximating for the Fijian population distribution, we modelled 1% of acute infectious cases becoming carriers, but do not vary this proportion by age.[37]

Carriage duration varies from months to years with the longest known carriage 41 years.[24,25] We model recovery from carriage as an exponentially waning rate with mean duration 10 years rather than assuming lifelong carriage.[37]

6.3.9.1 Carriage sensitivity analysis

Sensitivity analysis of the full model were undertaken with carrier β set as one-tenth and one-hundredth of case β.

6.3.10 Natural immunity

Typhoid immunity is generally considered partial in that it can be overwhelmed by a large inoculum.[38] However, data on second attacks suggests this is infrequent, and most likely to occur 10 years or more after the first attack.[27] We reflect this by modelling natural immunity as initially complete but waning over a long time period. Sensitivity analysis of duration of natural immunity was done. We do not include acute relapse in the model.[39]

6.3.11 Control scenarios

Two Vi-containing vaccines were modelled based on parameters from a systematic review.[40] The characteristics of these vaccines in the baseline analyses are:

1) Licensed Vi-polysaccharide (ViPS) of 70% (leaky) initial efficacy, 3 year mean duration
2) A typhoid Vi-conjugate (TCV) of 95% (leaky) initial efficacy, 15 year mean duration.

The following scenarios were modelled for each vaccine.

1) Baseline scenario (no vaccination)
2) Routine vaccination at school entry (6 years old)
3) Routine vaccination at school exit (15 years old)
4) Routine vaccination at school entry and exit

5) Routine vaccination at school entry and exit with a one-time school catch up campaign (ages 7 to 14 year).
6) A one-time school catch-up campaign (age 6 to 15 years).

A sensitivity analysis was done on the most impactful single-dose routine vaccination scenario to examine the impact of different vaccine efficacies and durations of immunity. These allow for impact assessment for vaccines such as different regimen and formulations of the Ty21a live oral typhoid vaccine, which might otherwise be considered comparable to ViPS,[40–42] and for vaccines in clinical trials.[43,44]

A further scenario was modelled for TCV only, reflecting efficacy data,[40] and the current WHO position paper which recommends TCV vaccination in endemic areas at age six months, or nine months or in the second year of life (to align with measles-containing vaccination and other vaccinations that may be given at the same time.[45]

7) Vaccination at 12-23 months of age.

Vaccination coverage was set at 95% per school year (modelled as a rate applied to the *S* compartment over 365 days) and per campaign, consistent with the UNICEF estimate (94%) and 2016 official figures for measles-rubella vaccine first dose and school entry dose.[46]

The above were run with WASH scenarios:

1) Baseline transmission
2) 10% reduction in transmission parameter β
3) 25% reduction in transmission parameter β
4) 50% reduction in transmission parameter β.

These are consistent with previous typhoid modelling studies.[8,37] WASH programmes were not disaggregated by component elements (nor readily can be) but treated as a single intervention in the model. While not directly applicable to *S.* Typhi and typhoid fever, systematic reviews of the available, limited, data on WASH for diarrhoeal disease control suggest risk reduction for handwashing with soap (HWWS), improved water quality and excreta disposal as 48%, 17% and 36% respectively,[15] suggesting these are a plausible range of modelled scenarios for effectively implemented programmes.

A further set of sensitivity analysis was done examining the impact of a hypothetical WASH intervention reducing the low-dose transmission scaling factor α by the above proportions

with no change to β. Equivalent changes to the surveillance reporting fraction *p1* were also examined graphically.

6.4 Results

6.4.1 Model fit

The full model was able to reproduce the seroprevalence patterns and case-surveillance data for typhoid in Fiji. Figure 6.4 shows the model output for the best fit parameters attained through MLE. Convergence occurred from a range of starting parameters, suggesting no local minima were found. The step-changes observed in figure 6.4 in simulation case data indicate transition across age-boundaries of the social contact matrix.

There are two particularly notable findings in the fitted parameters (Table 6.2). The first is that α, the low-dose force of infection scaling parameter was clearly non-zero, indicating that the movement of susceptible individuals into the asymptomatic non-infectious compartment was an important contributor to the model fit. The second is that due to the low incidence of non-iTaukei cases in surveillance data, model best fit arose when p_n, the reduction in force of infection from high dose exposures acting on susceptible non-iTaukei relative to iTaukei counterparts, was 99%. This suggests high-dose exposures almost never occur to non-iTaukei, or that if they do, these exposures do not lead to surveillance-detectable disease. The surveillance reporting fractions for acute infectious cases were 20% in adults and 8% (overall) in children. Sensitivity analysis of duration of natural immunity found no statistically significant difference between 40 year and 50 year immunity, which were better fits than other durations of natural protection (table 6.3) and returned the same fitted parameter values. We used 40 year natural immunity for scenario projections.

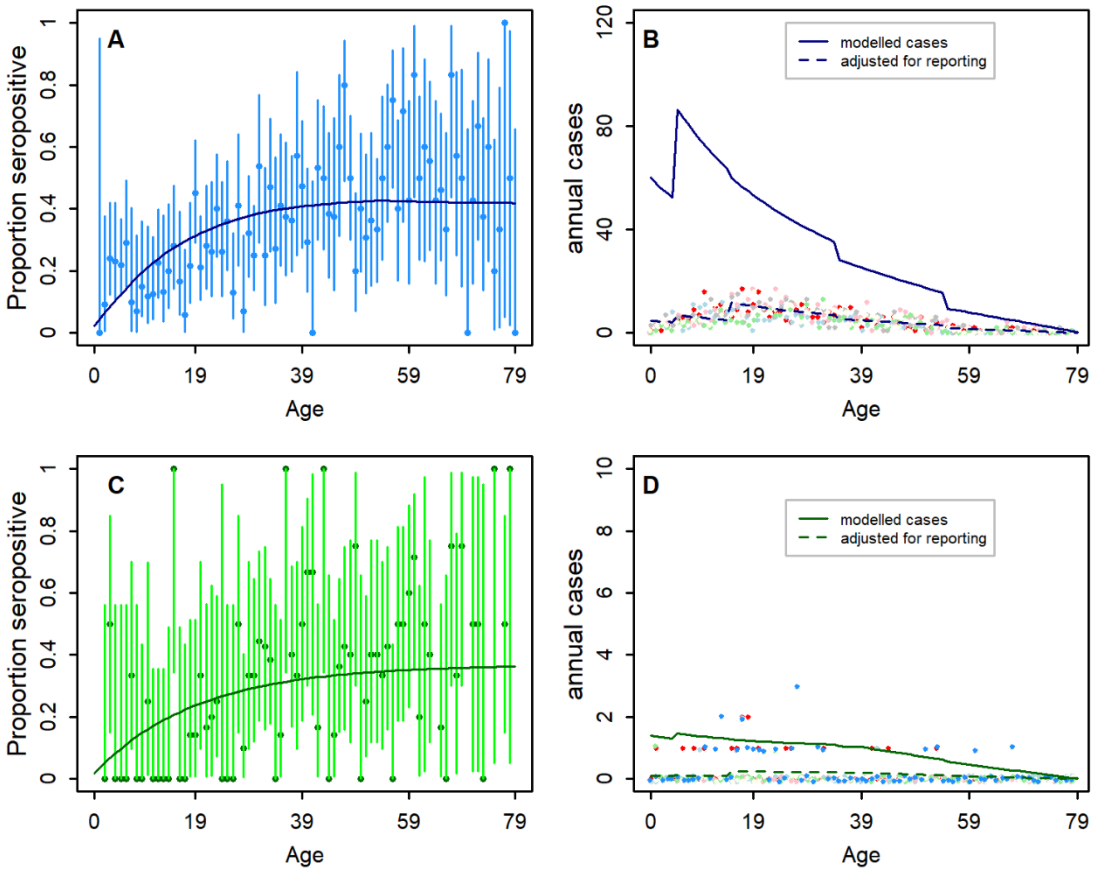

Figure 6.4. MLE-fitted age- and ethnicity-specific model equilibrium outputs with anti-Vi serological and confirmed case data.

A: iTaukei serology (anti-Vi IgG>=64 ELISA units) and immunity at model equilibrium.
B iTaukei modelled cases (solid line) and modelled cases scaled for reporting (dashed line). Dots represent cases data by age with each colour representing a different year.
C: Non-iTaukei serology (anti-Vi IgG>=64 ELISA units) and model output line for immunity.
D: Non-iTaukei modelled cases (solid line) and modelled cases scaled for reporting (dashed line). Dots represent cases data by age with each colour representing a different year.

Model Parameter	Description	Fitted Value
β (Beta)	Per contact daily effective transmission risk for "high-dose" exposure from I and C individuals	0.00558
α (Alpha)	Ratio parameter for the low dose force of infection relative to the high dose force of infection. The fitted value indicates that for each symptomatic secondary case generated, approximately five asymptomatic infections would occur in a susceptible population, all else being equal.	5.42
p_n	Proportion reduction in high-dose Beta in non-iTaukei relative to non-iTaukei	99.1%
p_1	Reporting fraction 1. Proportion of adult (15+) I-compartment symptomatic infectious cases that appear in national surveillance confirmed-case surveillance.	19.6%
p_2	Reporting fraction 2. Further reduction in the proportion of child (0-14) I-compartment symptomatic infectious cases that appear in national confirmed-case surveillance. This gives I-compartment children a 19.6% x 40.1% = 7.9% probability of appearing in national surveillance.	40.1%

Table 6.2. Fitted parameter values

Mean duration of natural immunity	Negative log likelihood
20 years	512
30 years	500
40 years	498
50 years	497
Lifelong	617

Table 6.3. Sensitivity analysis of duration of natural immunity

6.4.1.1 Sensitivity analysis of model structure

Model fit was examined under different mixing matrices and in the absence of low-dose infection giving rise to a non-infectious asymptomatic compartment (table 6.4). As would be anticipated from the best-fit value of α, models without low-dose force of infection had significantly worse fit than those with this. For models with or without a low-dose force of infection and non-infectious asymptomatic compartment, examining mixing patterns for high dose force of infection found that a matrix with age-ethnic heterogeneity fitted the data significantly better than homogenous or ethnically heterogeneous matrices. Interestingly, models with ethnically heterogeneous mixing did not have improved fit over those with homogeneous mixing.

Model	**Asymptomatic non-infectious component**	**Mixing pattern in high-dose force of infection**	**Negative log likelihood**
1	No	Homogeneous	784.4
2	No	Ethnic only	784.6
3	No	Age-ethnic	713.6
4	Yes	Homogeneous	576.0
5	Yes	Ethnic only	594.5
6 (full)	Yes	Age-ethnic	498.1

Table 6.4. Model fit under different epidemiological assumptions

6.4.1.2 Sensitivity analysis of declining force of infection

For models without low dose force of infection, fit was improved by including historically higher incidence through a time-dependent scaling parameter (table 6.5, models 7 and 9 vs table 6.4, models 1 and 3). However, these were still significantly poorer fit than models with an asymptomatic component. In a variant (model 8) of the homogenous mixing model (model 7), allowing transmission to be predominantly in childhood did not improve fit. Against the best-fit model of stable transmission (model 6), inclusion of historically high transmission did not fit as strongly (model 10).

Model **With declining force of infection**	**Asymptomatic non-infectious component**	**Mixing pattern in high-dose force of infection**	**Negative log likelihood**
7	No	Homogeneous	740.3
8	No	Homogeneous with adult beta one-tenth of child beta	795.2
9	No	Age-ethnic	688.3
10	Yes	Age-ethnic	508.7

Table 6.5 Model fit under declining force of infection

6.4.1.2 Sensitivity analysis of carriage

In sensitivity analysis of carrier infectiousness, the fit of models with carriers' infectiousness reduced by 90% or 99% relative to acute cases was very close to that of the best fit model (table 6.6, models 11 and 12 vs table 6.4, model 6). Scenario projections were therefore taken forward of interventions under reduced carrier contribution to transmission.

Model	**Carrier beta relative to acute infectious case beta**	**Negative log likelihood**
11	10%	499.0
12	1%	499.1

Table 6.6 Model fit under reduced carriage beta

6.4.2 Interventions

With peak surveillance incidence in young adults, we first examined the projected impact of a school-leavers ViPS vaccination programme, which cut cases by an estimated 8% (notified cases by 9%) (figure 6.5 and table 6.5). A school-entry vaccination programme was more effective, reducing cases by 16% (notified: 13%) indicating the role of school-aged mixing in contributing to the high-dose force of infection in the model suggesting this is a preferable timing if the model assumptions are valid. Combined school entry and leaver vaccination resulted in 24% (notified: 22%) reduction in projected cases. The projected gain for a one-time catch-up campaign of other school years was modest, an additional 2% over 50 years, with benefits largely accruing in the first decade and incidence returning to baseline after two decades. This was the same case reduction seen for a one-off school-age ViPS campaign, with incidence returning to baseline after two decades.

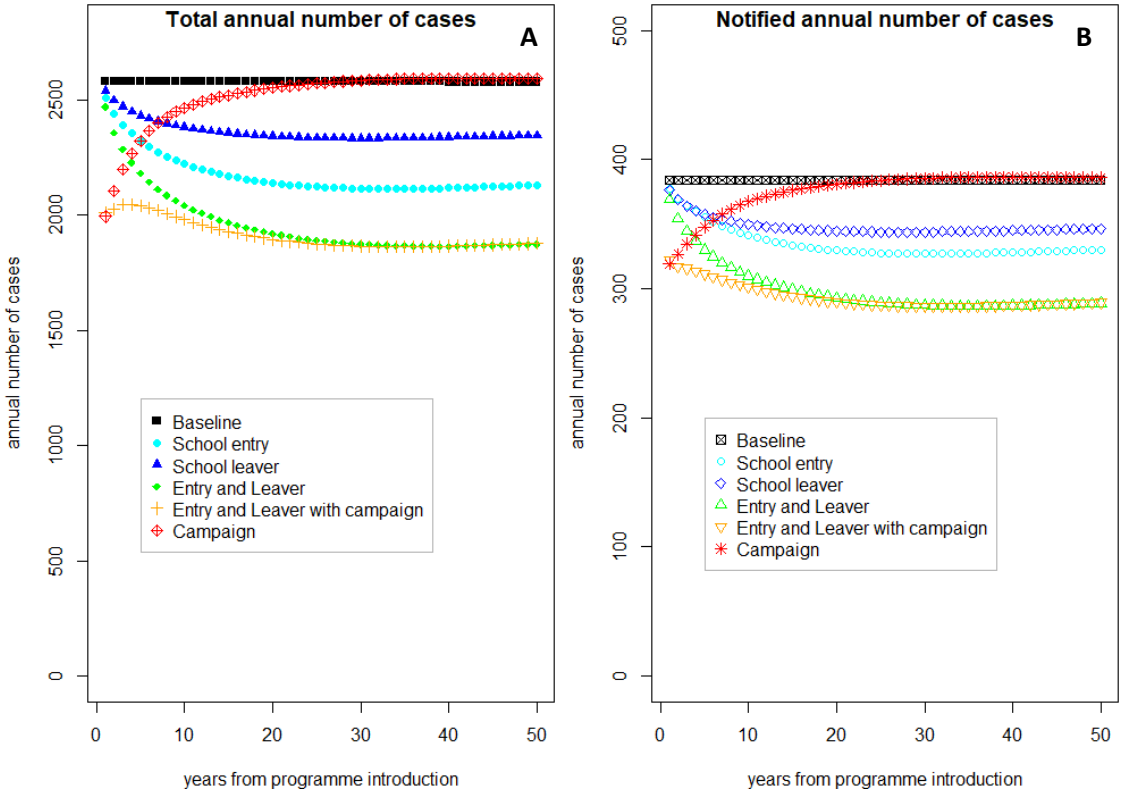

Figure 6.5. Projected total (A) and notified (B) typhoid fever cases over 50 years by ViPS vaccination scenario.

A WASH intervention that reduced transmission per case by 10% would be estimated to result in a 25% reduction in annual cases (notified: 24%) (table 6.4 and figure 6.6); equivalent to the most effective ViPS programmes.

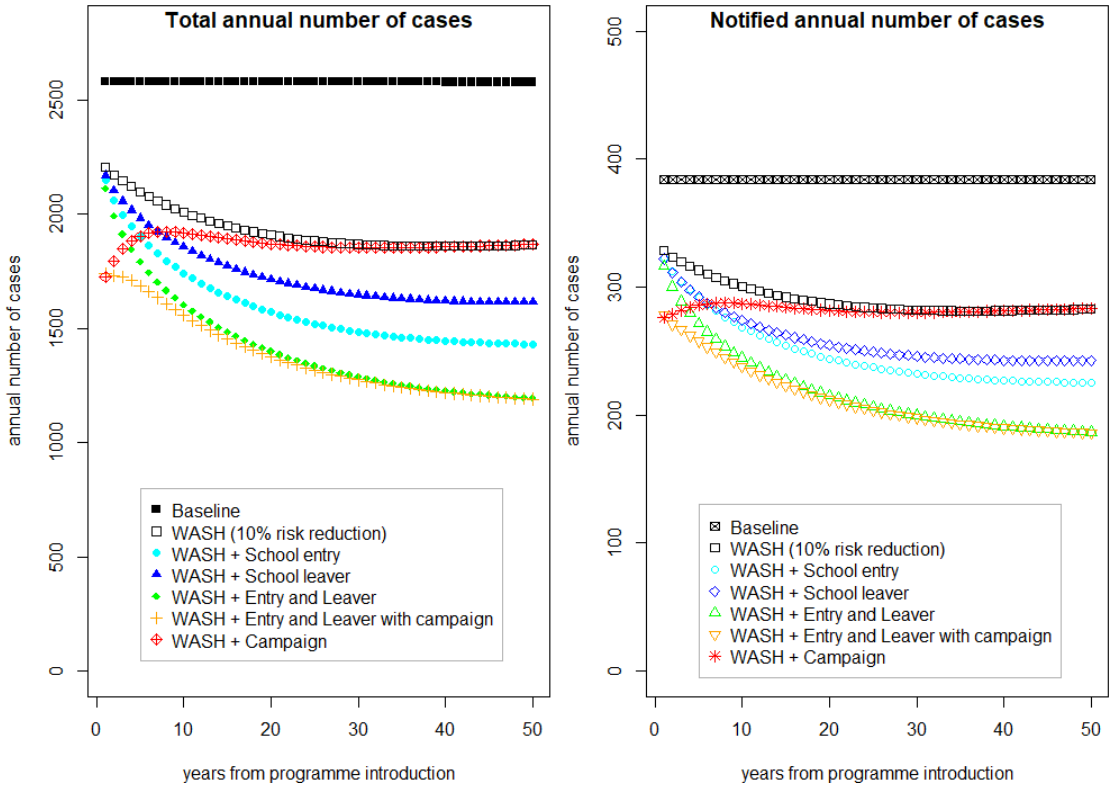

Figure 6.6. Projected total (A) and notified (B) typhoid fever cases over 50 years by ViPS vaccination scenario with 10% transmission reduction through WASH.

Vaccine	Intervention scenario	Total annual cases	Averted total annual cases	%	Notified annual cases	Averted notified annual cases	%
None	Baseline	2579	0	0	384	0	0
None	WASH 10% risk reduction	1929	650	25	290	94	24
None	WASH 25% risk reduction	1072	1506	58	163	221	58
None	WASH 50% risk reduction	350	2229	86	54	330	86

Table 6.7. Projected impacts of transmission reduction through WASH

Adding the above ViPS programmes to a 10% WASH programme brings projected incremental benefits approximately equivalent to the arithmetic sum of the WASH and vaccine programme (table 6.5, figure 6.6).

Vaccine	Intervention scenario	Total annual cases	Averted total annual cases	%	Notified annual cases	Averted notified annual cases	%
None	Baseline	2579	0	0	384	0	0
ViPS	School entry	2172	407	16	335	49	13
ViPS	School leaver	2360	219	8	348	36	9
ViPS	Entry and Leaver	1957	622	24	299	85	22
ViPS	Entry & Leaver + campaign	1911	668	26	293	91	24
ViPS	Campaign	2515	64	2	376	8	2
ViPS	WASH 10% + School entry	1591	988	38	247	137	36
ViPS	WASH 10% + School leaver	1733	846	33	258	126	33
ViPS	WASH 10% + Entry &Leaver	1414	1165	45	218	166	43
ViPS	WASH 10% + Entry & Leaver + campaign	1374	1205	47	213	171	45
ViPS	WASH 10% + Campaign	1868	711	28	282	102	27
ViPS	WASH 25% + School entry	890	1689	65	140	244	64
ViPS	WASH 25% + School leaver	955	1624	63	144	240	62
ViPS	WASH 25% + Entry & Leaver	793	1786	69	123	261	68
ViPS	WASH 25% + Entry & Leaver + campaign	765	1814	70	119	265	69
ViPS	WASH 25% + Campaign	1025	1554	60	157	227	59
ViPS	WASH 50% + School entry	313	2266	88	49	335	87
ViPS	WASH 50% + School leaver	323	2256	87	49	335	87
ViPS	WASH 50% + Entry &Leaver	290	2289	89	45	339	88
ViPS	WASH 50% + Entry & Leaver + campaign	279	2300	89	44	340	89
ViPS	WASH 50% + Campaign	332	2247	87	51	333	87

Table 6.8. Projected impacts of ViPS vaccination programmes with transmission reduction through WASH

If WASH resulted in 25% or 50% reduction in the per-case transmission parameter (figure 6.7 and figure 6.8, table 6.4), the model suggests this would reduce cases by 58% and 86%

respectively, down to tens of notified cases for a 50% WASH intervention. Projections suggest adding vaccination programmes to a 25% WASH programme has further benefits, for a total case reduction of 65% with school-entry vaccination and marginal gain for more intensive programmes. The projected incremental gains of ViPS vaccination programmes on 50% WASH were very modest compared with use in other modelled WASH scenarios (table 6.5).

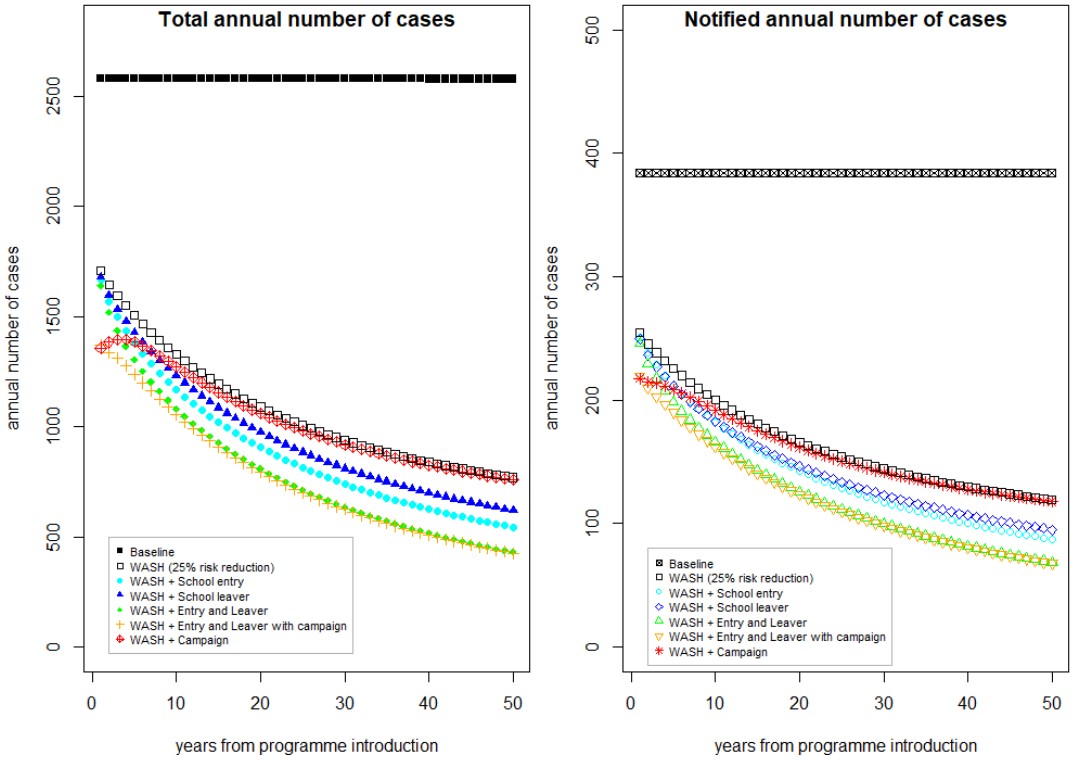

Figure 6.7. Projected total (A) and notified (B) typhoid fever cases over 50 years by ViPS vaccination scenario with 25% transmission reduction through WASH.

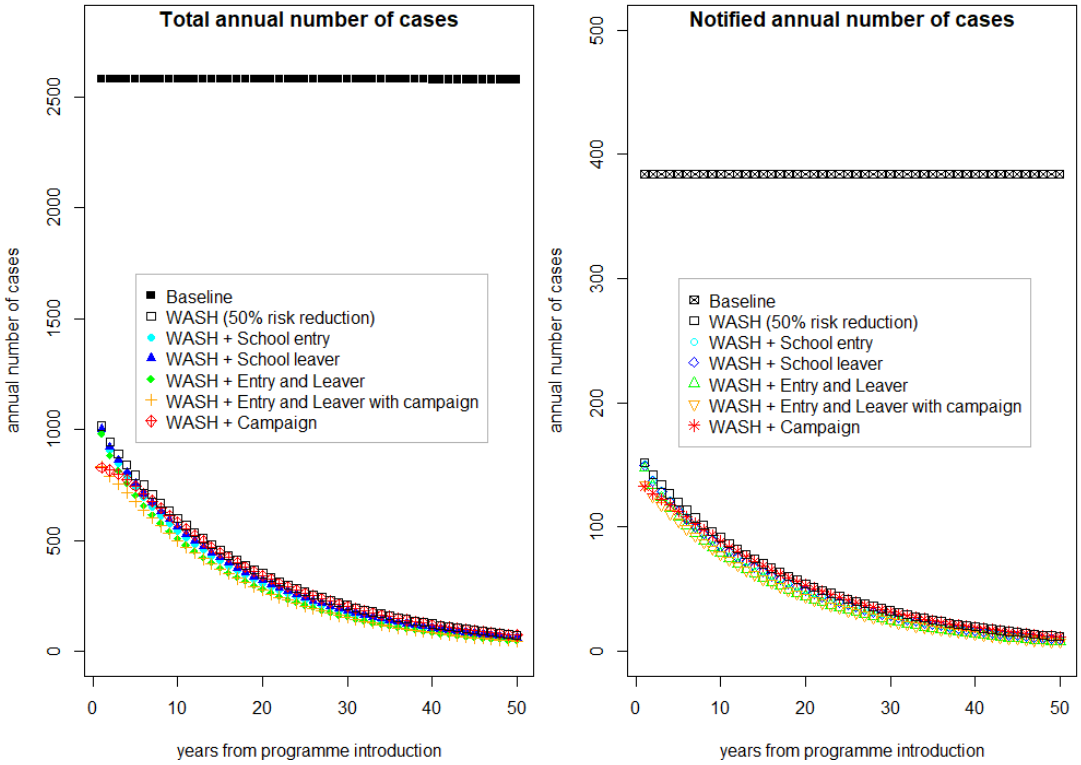

Figure 6.8. Projected total (A) and notified (B) typhoid fever cases over 50 years by ViPS vaccination scenario with 50% transmission reduction through WASH.

If a TCV vaccine was available with the efficacy and durable immunity utilised in our model, it could have substantial impact (table 6.6, figures 6.9 to 6.12).

Vaccine	Intervention scenario	Total annual cases	Averted total annual cases	%	Notified annual cases	Averted notified annual cases	%
None	Baseline	2579	0	0	384	0	0
TCV	Infant	1311	1268	49	214	170	44
TCV	School entry	1348	1231	48	216	168	44
TCV	School leaver	1771	808	31	252	132	34
TCV	Entry and Leaver	961	1618	63	149	235	61
TCV	Entry & Leaver +campaign	837	1742	68	132	252	66
TCV	Campaign	2252	327	13	337	47	12
TCV	WASH 10% + Infant	977	1602	62	159	225	59
TCV	WASH 10% + School entry	992	1587	62	159	225	59
TCV	WASH 10% + School leaver	1268	1311	51	182	202	53
TCV	WASH 10% + Entry &Leaver	720	1859	72	112	272	71

TCV	WASH 10% + Entry & Leaver + campaign	626	1953	76	99	285	74
TCV	WASH 10% + Campaign	1627	952	37	246	138	36
TCV	WASH 25% + Infant	603	1976	77	98	286	74
TCV	WASH 25% + School entry	603	1976	77	97	287	75
TCV	WASH 25% + School leaver	718	1861	72	104	280	73
TCV	WASH 25% + Entry & Leaver	461	2118	82	72	312	81
TCV	WASH 25% + Entry &Leaver + campaign	402	2177	84	64	320	83
TCV	WASH 25% + Campaign	867	1712	66	133	251	65
TCV	WASH 50% + Infant	256	2323	90	42	342	89
TCV	WASH 50% + School entry	253	2326	90	41	343	89
TCV	WASH 50% + School leaver	273	2306	89	40	344	90
TCV	WASH 50% + Entry & Leaver	210	2369	92	33	351	91
TCV	WASH 50% + Entry & Leaver + campaign	186	2393	93	30	354	92
TCV	WASH 50% + Campaign	285	2294	89	44	340	89

Table 6.9. Projected impacts of TCV vaccination programmes with transmission reduction through WASH

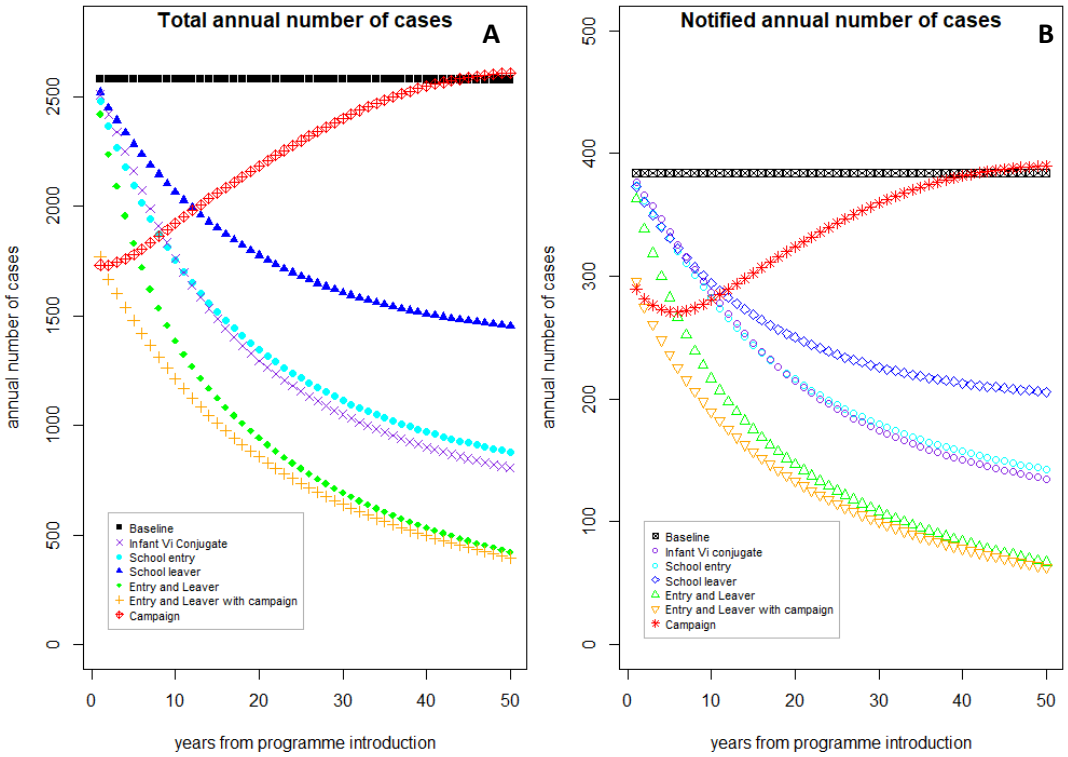

Figure 6.9. Projected total (A) and notified (B) typhoid fever cases over 50 years by TCV vaccination scenario.

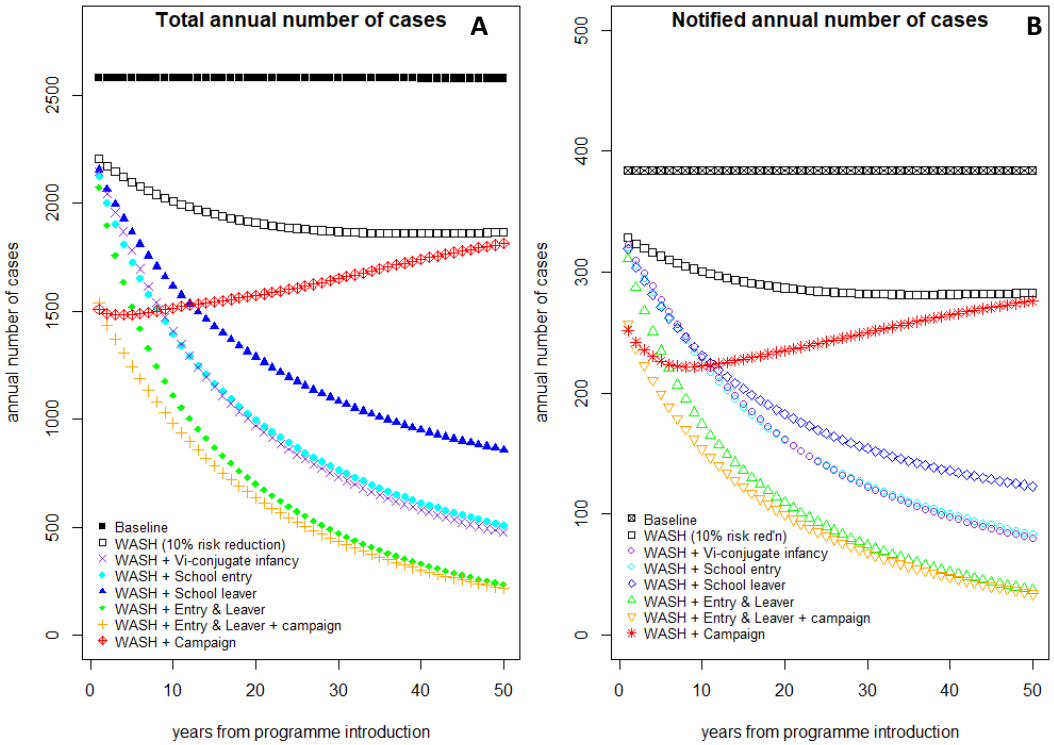

Figure 6.10. Projected total (A) and notified (B) typhoid fever cases over 50 years by TCV vaccination scenario with 10% transmission reduction through WASH.

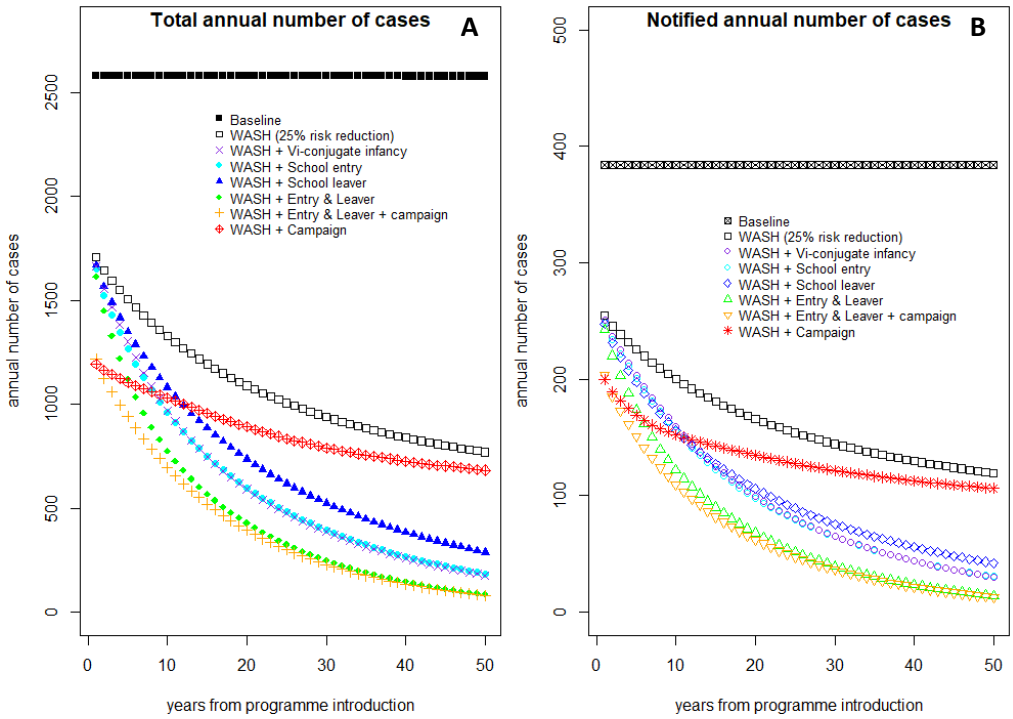

Figure 6.11. Projected total (A) and notified (B) typhoid fever cases over 50 years by TCV vaccination scenario with 25% transmission reduction through WASH.

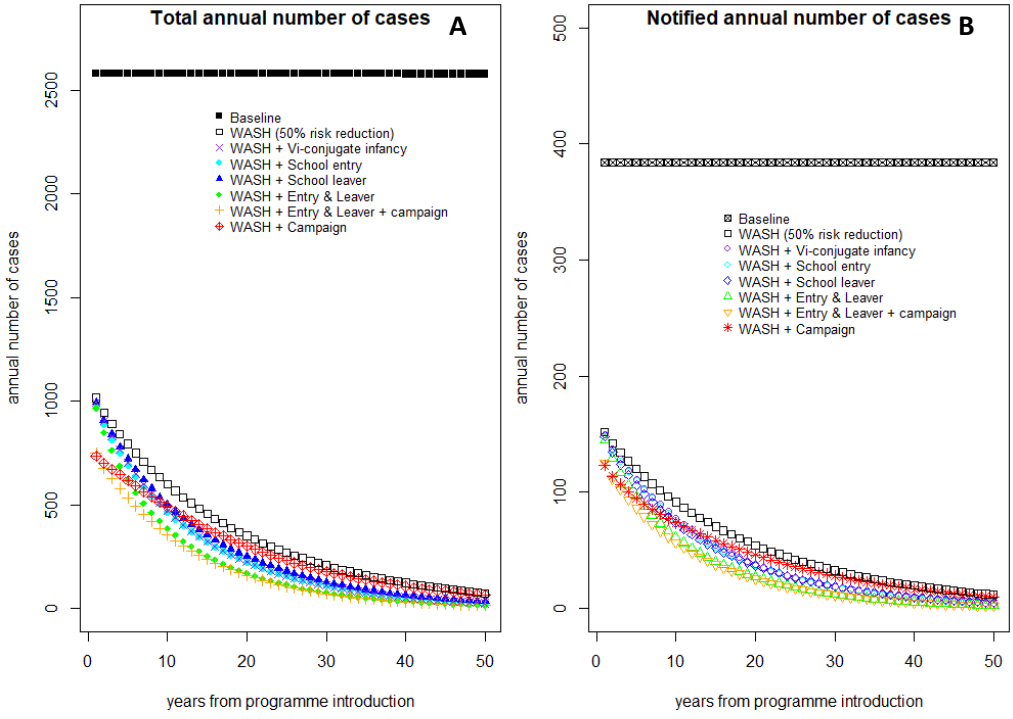

Figure 6.12. Projected total (A) and notified (B) typhoid fever cases over 50 years by TCV vaccination scenario with 50% transmission reduction through WASH.

The optimal single-dose programme of those modelled would be vaccination in the second year of life rather than school age, reducing cases by an estimated 31% (notified 34%) in the absence of WASH intervention. A two dose school programme could cut incidence by an estimated 63% with the possibility of greater gain from an infant plus school leaver programme given the projected effect of the single dose infant programme and durability of vaccine immunity. Combining TCV annual programmes with WASH interventions suggests incidence reduction of over 60% over a 50-year horizon, with TCV programmes projected to be more effective than ViPS in reducing incidence over the gains accrued from major WASH interventions.

A sensitivity analysis of vaccine efficacies and durability of vaccine immunity modelled incidence reduction in a school-entry routine immunisation programme (figure 6.13) and allows comparison across combinations of effects. For example, a 95% initial efficacy 3-year vaccine offers similar incidence reduction to a 70% initial efficacy 5-year vaccine.

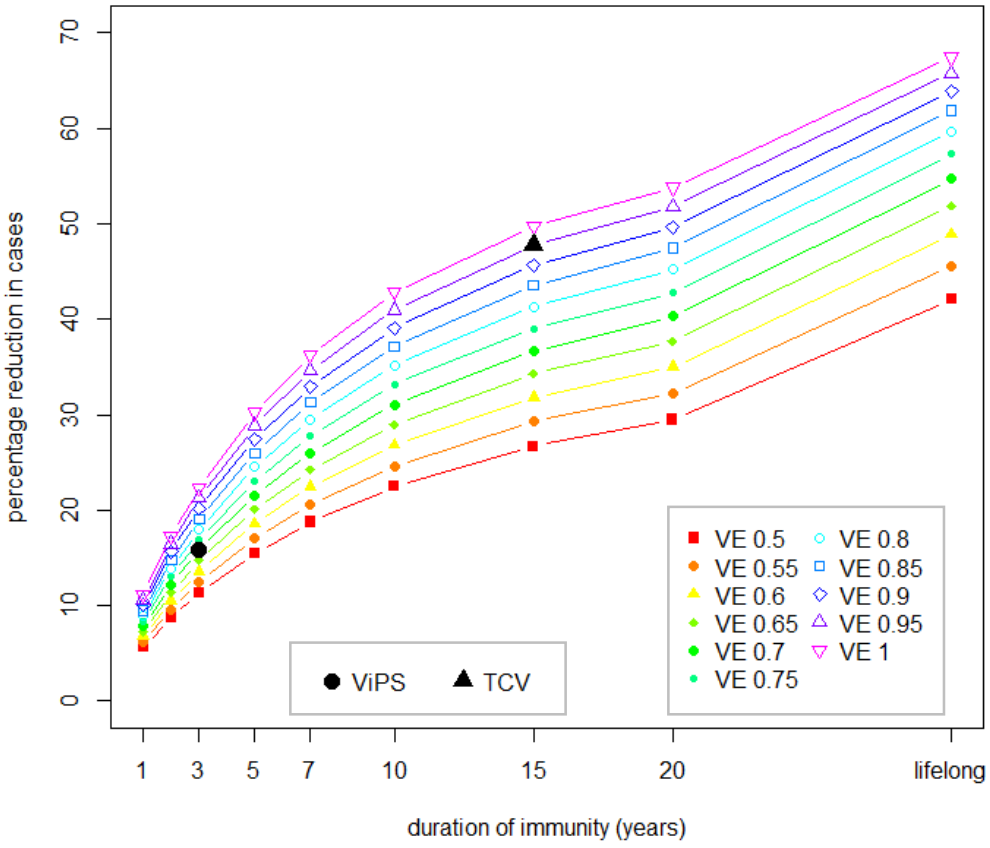

Figure 6.13. Impact of vaccine efficacy and duration of vaccine immunity. *Percentage reduction in cases over 50 years for a school entry vaccination programme.*

Projections were done for scenarios in which carriers' infectiousness per contact per day (beta) was 90% less than acute cases. To achieve the same reported case incidence requires a higher annual incidence of total acute infections than the baseline model: 3768 vs 2579. Examining TCV vaccination and 10% WASH improvements, without the "damping" effect of so many carriers, interventions resulted in more rapid drop in incidence, but also "rebound" effects following one off-campaigns (figure 6.14).

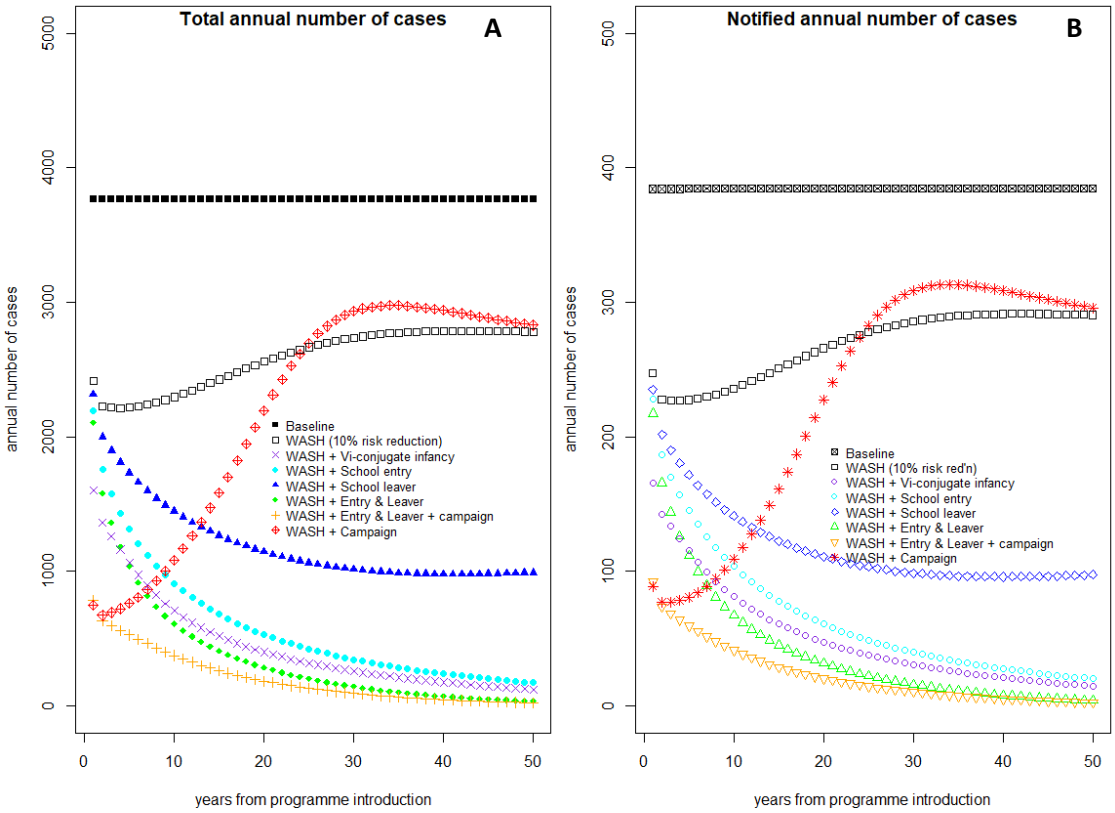

Figure 6.14. Projected total (A) and notified (B) typhoid fever cases over 50 years in scenarios with carrier beta at 10% of acute case beta.

Over the 50 year projected time horizon, a greater reduction in incidence would be observed than under equivalent scenarios with carrier beta equal to acute case beta. For example, infant TCV vaccination with 10% WASH improvement results in 87% reduction in notified cases (table 6.10) versus 59% reduction (table 6.9), with the gain from vaccination proportionately greater than the gain from WASH (30% vs 24% effectiveness for 10% WASH improvement), as a result of indirect protection. Carriage contribution therefore represents a considerable degree of uncertainty in projected impacts, particularly for vaccination, and could affect the balance of prioritisation within WASH versus vaccination. There is no change to order of effectiveness in terms of vaccination strategies.

Intervention scenario	Notified annual cases	Averted notified annual cases	%
Baseline	385	0	0
WASH 10%	269	116	30
WASH + Vi-conjugate infancy	51	334	87
WASH + School entry	66	319	83
WASH + School leaver	119	266	69
WASH + Entry and Leaver	40	344	89
WASH + Entry and Leaver with campaign	23	362	94
WASH + Campaign	231	154	40

Table 6.10 projected reduction in annual notified cases of typhoid fever over 50 years in scenarios with carrier beta at 10% of acute case beta.

Whilst this paper is directed towards interventions from Fiji's current situation, the mechanism by which the current epidemiological situation arose bears consideration in assessing assumptions implicit in the model. There has been a notable upturn in confirmed case incidence from pre-2004 when incidence was <5 per 100,000 person-years[47] to the approximately 45 per 100,000 levels seen in 2008 onwards.[5] These analyses offer some possible insight into the observed epidemic dynamics. These projections start from the current modelled equilibrium rather than projecting forward to the present day from unknown historical immunological situation. We examined a scenario set in which the low-dose per-case α transmission parameter was reduced while keeping constant the β parameter that determines high-dose transmission (figure 6.15 and table 6.11). These scenarios suggest that the resultant increase in number of susceptibles would be projected to increase case numbers by 5% in a 10% α-reduction scenario over 50 years, and 31% if α was reduced by 50%. Loss of immunity through a reduced α parameter and declining asymptomatic infection leads to a build-up of susceptible individuals and subsequent increase in cases. Such scenarios are plausible in settings in which infectious disease is in decline. The modelled timeline over which this takes place and scale – approximately three to four decades for a 40% increase in cases at a halved α is not consistent with the observed

epidemic increase of typhoid in Fiji. In an illustrative set of models, increasing the reporting fraction p_1 scaled notified cases linearly (figure 6.16) suggesting this is a feasible mechanism to explain the observed increase in notifications.

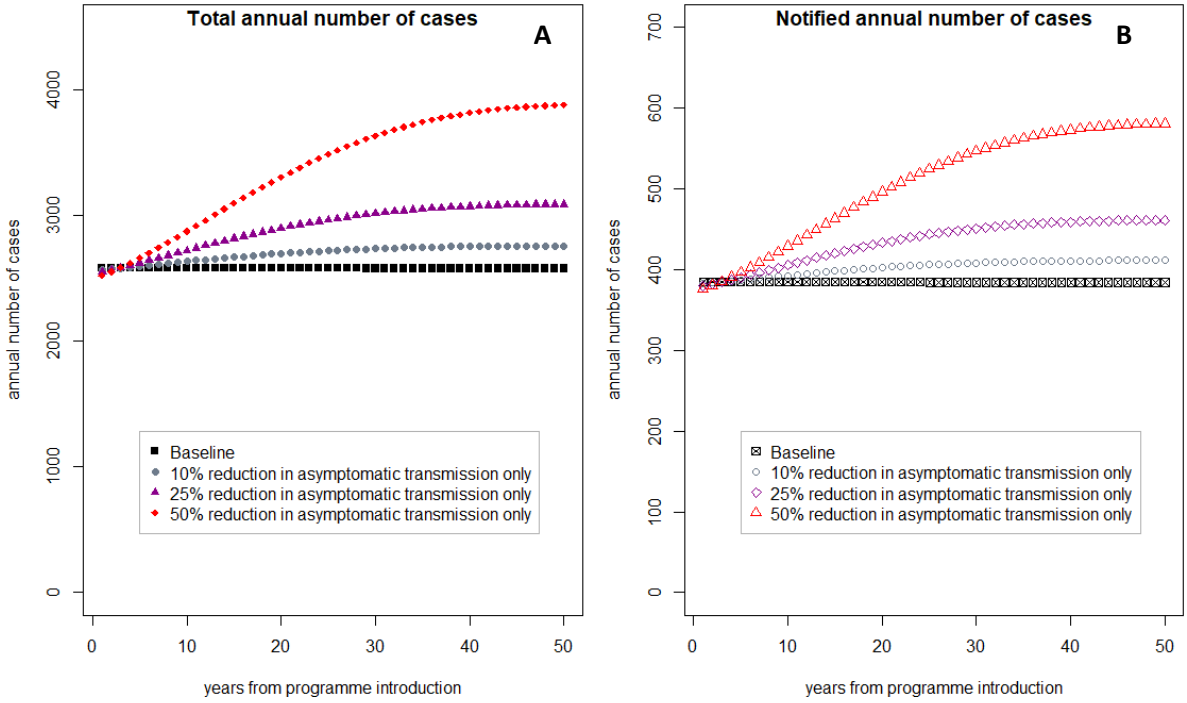

Figure 6.15. Projected total (A) and notified (B) typhoid fever cases over 50 years in scenarios reducing low-dose force of infection only.

Reduction in low dose force of infection only	Total annual cases	Additional annual cases	%	Reported annual cases	Additional annual reported cases	%
Baseline	2579	0	0	384	0	0
10% α reduction	2699	120	5	402	18	5
25% α reduction	2910	331	13	435	51	13
50% α reduction	3381	802	31	506	122	32

Table 6.11. Projected impact of reduction in low dose force of infection

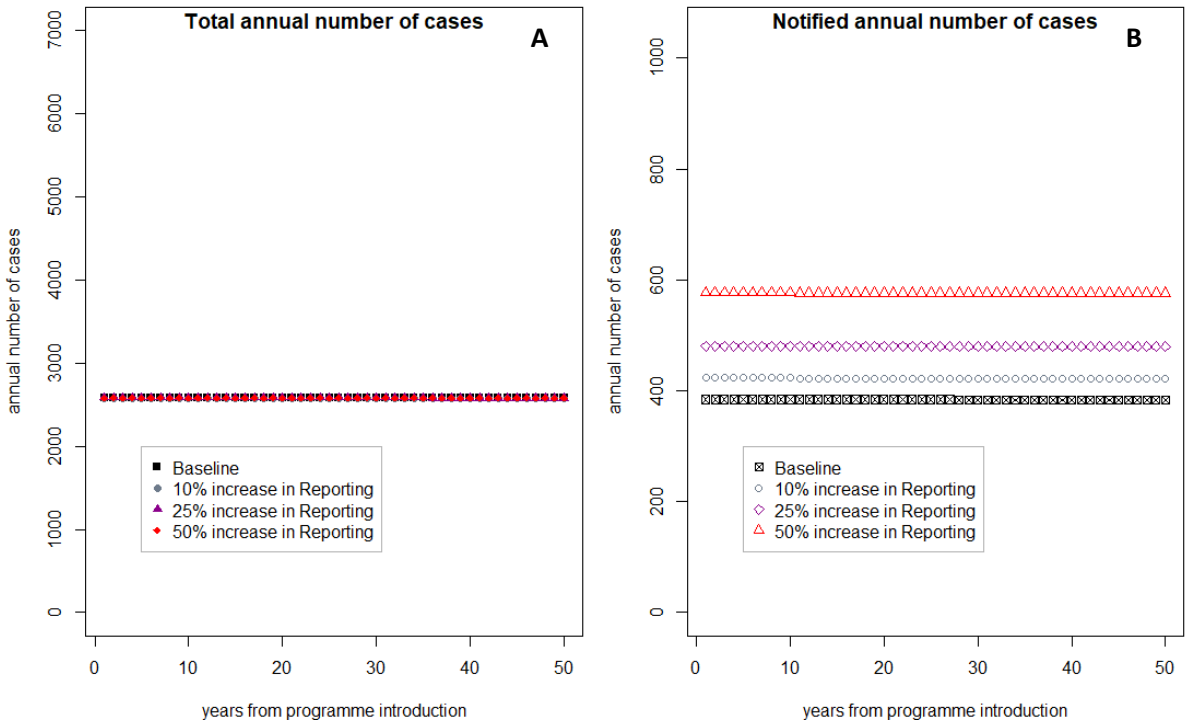

Figure 6.16. Projected total (A) and notified (B) typhoid fever cases under different reporting scenarios.

A final scenario set projection of an increase in the β per-case transmission parameter was undertaken for consideration alongside the sensitivity analyses of the α parameter and reporting fraction (figure 6.17). An increase in the effective β per-case transmission parameter may be suggested as associated with increased inter-person contact rates with travel, housing and work pattern changes, changes to transmission mechanisms such as sanitation breakdown or increases in the pathogen's innate biological transmissibility. An increase in β appears more consistent with the observed trend in notified cases than changes to α, with a slight decline in cases following the sharp epidemic upturn that may or may not reflect such a change in transmissibility.

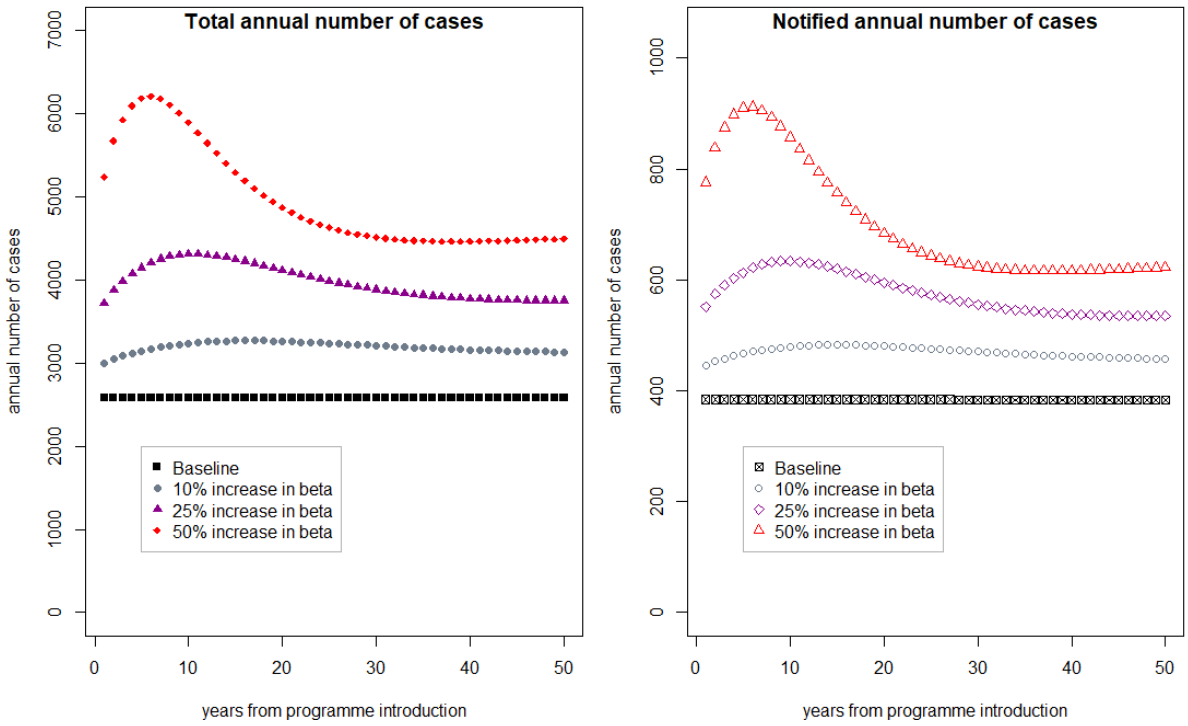

Figure 6.17. Projected total (A) and notified (B) typhoid fever cases under different increases to β transmission parameter.

6.5 Discussion

This study examines models of the epidemiology of typhoid fever in Fiji and explores the potential impact of control measures in age- and ethnicity-structured transmission dynamic model projections. The findings are strengthened by model-fitting under a range of epidemiological constructs, which found that generation of asymptomatic, non-infectious cases, and assortative mixing for case generation were important in reproducing the field serology and surveillance data. The study combines a conceptual framework incorporating a literature-grounded assessment of typhoid dose-dependence of infection with Fijian national surveillance data and a nationally-representative field survey for serology and social contact patterns.

Projecting over a fifty-year time horizon, we found that ViPS vaccination programmes offered modest reduction in incidence when implemented as school-age programmes. The largest incidence reduction, of around 25%, was seen with a programme utilising school entry and school leaver vaccination, with equivalent reduction in incidence from a 10% reduction in per-case transmission parameters, such as through effective enhancement of WASH infrastructure and norms. School vaccination campaigns and catch-ups offered some short-term additional benefits to programmatic use without long-term impact. A high efficacy TCV programme could substantially reduce incidence, including through implementation alongside measles-rubella vaccination at 12 months of age and in parallel to WASH strengthening. Notably, vaccination at 12 months, as recommended by WHO, was projected to have less impact than a schedule with two doses (one in early childhood and a second dose at school-exit age); though this is based on speculative duration of vaccine effect, which remains an important area for research. There is important uncertainty in the model projections due to the role of carriers in transmission. If carriers are substantially less infectious per day than cases, then the impacts projected in this paper may underestimate the impact of vaccination and improvements to WASH, and the balance of these, with lower carriage contribution enhancing the impact of vaccination.

The finding of best fit at a 99% reduction in high dose inoculation in non-iTaukei Fijians was unexpected in its magnitude. Descriptive data collected during the linked serosurvey on putative risk factors such as HWWS, socio-economic status, piped water and sanitation systems would not seem to account for such a scale of difference in risk between iTaukei and non-iTaukei Fijians, though social response bias is a consideration. Differences in health-seeking behaviour of this scale did not seem borne out in public sector hospital attendances observed during fieldwork, nor in responses to a dengue fever survey. This model finding adds weight to the hypothesis that the notified disease difference may be in part due to

reduced susceptibility in Indo-Fijians. There is a known protective HLA type,[48] and the ancestral exposure of Fijians of Indian descent to *S.* Typhi through millennia of double-digit typhoid case fatality rates would be a potent driver of selection for immunity. This contrasts to the known vulnerability of iTaukei and other Melanesians to dysenteric infections.[49] Budd reports the population of Tahiti, a Polynesian island, being "swept off" by typhoid imported from Europe 150 years ago.[50]

Our approach is similar to but conceptually distinct from short-cycle (direct) and long-cycle (environmental, indirect) transmission modelling.[37,51] Our model explicitly includes the different pathogenic outcomes following low or high dose inoculation, building on the Maryland challenge studies,[11,12] focussing on the inoculating infectious event and its consequences rather than the source of the inoculum. Long-cycle may be more likely to typically convey low-dose inoculum with low probability of pathogenesis and short cycle to convey high dose, though this is not an explicit component of this model. The high dose contact patterns and transmission events in the model do not preclude an element of long-cycle transmission and vice versa, given known localised environmental bacterial detection around cases,[52] and serological association with wetter areas described previously[53] as well as reports of river-borne transmission between iTaukei villages.

We examine WASH as a reduction in the β per contact transmission parameter, without prescribing components for intervention, which are be better determined by case-control studies, such as a recent paper by Prasad and colleagues.[54] Prasad's study found risk factors to be (by decreasing population attributable risk (PAR)) unimproved sanitation (72%), non-washing for produce before consumption, intermittent water supplies, and consumption of surface water as a secondary water source. Protective factors were HWWS and high handwashing frequency after defecation. All of these are compatible with the findings of this modelling study – the two studies together suggest substantial impact would be possible by addressing safe sanitation systems as a priority.

The balance of infectious and immunising forces in the model was examined by a sensitivity analysis of the α scaling parameter for low-dose transmission. Reducing this increases the susceptible population and caused a rise in cases, though not on a timescale that explains the upturn in cases seen in the last decade. It is unlikely that interventions for WASH might independently act to reduce α without impacting primarily on β: follow up serosurveys and national typhoid fever surveillance would be important means of observing changes to population immunity and any association with case incidence.

A number of mechanism may explain the rise in cases in Fiji in the 2000s. In Malawi, the arrival of the globally-expanding virulent multidrug resistant H58 subclade of S. Typhi has been suggested as increasing transmission.[55] The absence of emerging antimicrobial resistance[5] or of persistence of H58 isolated in Fiji in the early 1990s[56,57] against the endemic Oceanian *S.* Typhi clade suggests transmission has not risen as a result of H58 expansion. Loss of population immunity is not consistent with the timescale and extent of increase in notified cases. Of scenarios we tentatively examine with this model, socio-epidemiological changes increasing transmission could fit with the timescale as could changes in disease reporting. Dunn and colleagues suggest that the increase in notification was a result in the change in laboratory detection following a scale-up in hospitals' use of blood culture diagnostics in the 2000s.[47] This modelling analysis, in assuming a stable long-term disease equilibrium, implicitly accepts Dunn's assessment, supported by serology consistent with an endemic transmission pattern.[7] When fitted to declining transmission parameters from a historically higher baseline, examined models were less able to reproduce the data than under equilibrium assumptions. Pre-2004 serum samples could be strongly informative to further analysis. The modelling framework we have developed may offer a mechanism for further investigation of these events.

There are a number of limitations to our study. Limitations on the interpretation of serology are a key consideration, and are reviewed in more detail elsewhere in this thesis, centring on sensitivity and specificity of anti-Vi IgG and the fitted threshold for seropositivity used as an indicator of past infection and subsequent immunity. Parameter estimates are best fit values but could have uncertainty quantified by likelihood profiling. Bayesian methods could alternatively be applied to parameter estimation. Our model considers typhoid transmission to occur as single time point events. This simplification does not exclude the possibility or probability that exposure to Typhi can occur over a prolonged period, with such occurrences plausibly associated with raising anti-Vi titres to observed levels. The carriage risk following an acute infectious episode was fixed across age groups; this could be varied, though would be expected to have only modest impact on dynamics. The reporting fraction adjustment for children could be varied further by age in line with a recent meta-regression analysis.[3,31]

A further model extension would be to include seasonality through a varying β parameter. Typhoid incidence tracks rainfall in Fiji by approximately a two month lag,[58] though the relative contribution to this of environmental factors or seasonally varying social mixing patterns and other seasonal factors is unclear, and is likely to have modest impact in long-run projections.

Much remains to be determined in the pathogenicity and immunogenicity of natural typhoid infection in informing its control; the interplay of human challenge studies, field epidemiology and modelling offer potential insights alongside new developments in surveillance, laboratory research and vaccine trials.[44,59] Stool shedding of Typhi can occur in those who ingest Typhi but do not develop overt disease, though may not arise in the majority of such cases. Hornick and colleagues note this was seen at the ID50 of 10^7 bacilli, whilst low dose exposure in community settings might be considered in the range 10^3 to 10^5. Recent blood culture-PCR in the Oxford typhoid human challenge study tested positive for 6 of 17 culture negative participants who were not diagnosed with typhoid, further indicating asymptomatic bloodstream infection occurs, as might lead to raised plasma anti-Vi IgG. Five of the six Oxford asymptomatic DNAaemic participants did not have *S.* Typhi detected in stool: this broadly supports our handling of an asymptomatic case compartment as non-infectious whilst also have infectious cases potentially subclinical in their presentation.

In efforts to understand the transmission of typhoid in Fiji, we have developed a parsimonious age structured model that incorporates ethnicity and inoculation dose-dependency in its structure, reflecting some of the complexity of Fijian society and the pathology of *Salmonella* Typhi. Our modelling study, grounded in a sero-epidemiological and social contact survey, suggests the potential role of established and recently-recommended vaccines, whilst further supporting the use of effective WASH interventions in control of the persistent scourge of typhoid fever.

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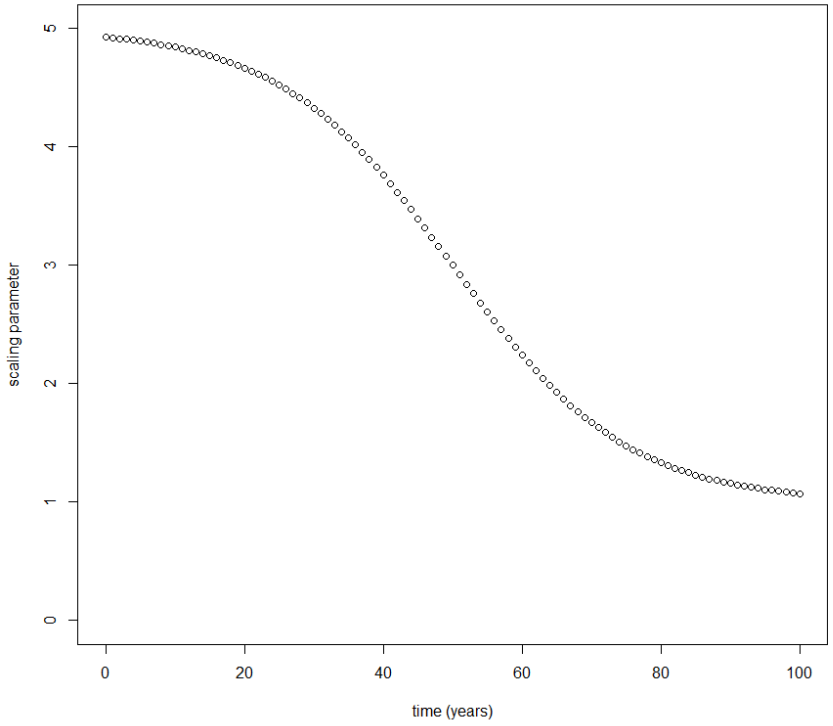
6.7 Supplementary material

Supplementary Figure S6.1. Time-dependent scaling parameter for declining-incidence sensitivity analysis



Chapter 7. Discussion

The aim of this thesis has been to examine the seroepidemiology of human *Salmonella* Typhi infection in Fiji and potential impacts of vaccination programmes or other public health interventions to reduce typhoid transmission and typhoid fever cases.

This discussion chapter seeks to:

- briefly restate the principal findings of this research project;
- review strengths and limitations;
- compare findings to the state of knowledge prior to the work;
- to suggest implications of the research; and
- to indicate possible research future directions.

At the commencement of these studies, surveillance data indicated the overwhelming majority of cases were in iTaukei Fijians, most commonly those aged 15-34 years.[1] There was limited data by which the extent of under-reporting could readily be assessed.[2] Alongside universal demographic factors such as age and sex, a particular consideration for Fiji is the role of ethnicity in the epidemiology of health and disease,[3] including for typhoid fever and other communicable diseases.

The thesis papers are approximately sequential in development with prior papers informing the more recent. The following summarises the key findings of the papers, with those for the serosurvey, contact study and model described in relation to the aims presented in the introduction to the thesis.

In chapter 2, the literature review,[4] I identified that transmission models are relatively under-utilised in typhoid control, particularly in economic evaluation, with little guide to relative cost-effectiveness of vaccination in place of or alongside with WASH. The particular relevance of the review to Fiji was in identifying model conceptualisations and the need for setting specific parameterisation.

Chapter 3 covered the serological survey.[5] This has the principal field research finding, which is that iTaukei and non-iTaukei Fijians have similar risk of developing raised IgG antibodies to the Vi antigen expressed by *S.* Typhi, and that seroprevalence increases with age. This is suggestive of endemic transmission, though also compatible with historically higher incidence in childhood cohorts.

Antibody decline towards baseline was determined in a patient cohort examined alongside the cross-sectional survey. Risk factor analysis suggests there may be possible roles for unimproved sanitation and settlement residency in antibody acquisition, whilst the study did not indicate different risks by sex, ethnicity, rurality or water sources. The implications of the serosurvey for the epidemiological model of Fiji is that there appears to be substantial un-reported typhoid transmission in Fiji, affecting both major ethnic groups, which requires a mechanistic explanation.

The geospatial paper[6] in chapter 4 identifies rainfall, proximity to major rivers and creeks, and proximity to flood-prone areas as environmental risk factors for acquisition of anti-Vi IgG antibodies, after adjusting for age, self-reported vaccination status and home toilet type.

In the social contact survey (chapter 5),[7] I found that social mixing is assortative by ethnicity and age when assessed by mealtime contacts. These are not unexpected findings but provide quantitative estimates of daily contact rates. Similar to conversation-based contact studies in other countries, these were found to be highest in school-age children. Increasing number of age-adjusted contacts increases the odds ratio for being seropositive, driven by number of iTaukei contacts of iTaukei participants, with no evidence from other ethnicity-based contacts, after adjusting for age, sanitation and settlement residency.

The transmission dynamic model (chapter 6) was designed to synthesise the findings from the prior studies. The model fitted the serology and case data well when fitted parameters included a substantially reduced force of infection for high-dose infection being passed to non-iTaukei Fijians, and there was high generation of homogeneously-distributed asymptomatic non-infectious cases per new infectious case. Surveillance reporting of infectious cases was estimated as one in five infectious adult cases and one in twelve infectious child cases. Vaccine scenarios suggested that of single dose routine programmes, school entry could be more effective than school leaver vaccination, reflecting age-contact transmission probabilities in the model. Modest reduction (10%) in per-case infectious transmission through effective WASH programmes offered substantial incidence reductions of around 25%, comparable to two-dose (school entry and exit) ViPS vaccination programmes. Potential benefits of conjugate vaccines were projected to be similar to more effective WASH programmes, with administration alongside other vaccines in the second year of life projected to offer approximately 50% incidence reduction, the most benefit of any single dose regimen.

A strength of the research programme is the collection of field data specifically for the purpose of informing epidemiological understanding, to support modelled assessments of

vaccine impact. Epidemiological fieldwork without a theoretical framework and data-free theoretical models are increasingly recognised as less productive than approaches utilising both.[8,9] The absence of serology, imperfect is it might be, would have left parameter space much freer and greatly increased the difficulties in attempting to assess the extent of under-reporting in Fiji, particularly given the important differences observed in case ethnicity. Social mixing data further strengthens the model in this regard.

The research findings may be informative to local communicable disease control policy, though require caution in interpretation, not least for serology in this study, including the fitted threshold. This study represents an early endeavour in the use of anti-Vi IgG as a typhoid serosurveillance tool. The surveillance threshold fitted from convalescent case data is used as indicative of exposure and immunity but requires further development in assessing sensitivity and specificity, and thus the degree to which the derived threshold may under- or over-ascertain past infection. The decision to pursue an anti-Vi serosurvey was supported by a relatively modest body of research including immunological analysis,[10] field research,[11] and vaccine trials.[12,13] Since the start of the study, there has been increased immunological support for Vi as a marker of natural protection[14] including supportive finding on the role of modest Vi titres in protection from human challenge studies.[15,16] The use in the serosurvey of purified pharmaceutical grade Vi for ELISA may contribute to reduced cross-reactivity and strengthen test validity relative to less refined Vi preparations.[17] Recent findings from the Oxford human challenge studies suggest serum bactericidal activity (SBA) protects against severe disease but not infection[18] – this is not field data but nonetheless supports the use of Vi ELISA as a more suitable current tool for typhoid seroepidemiology.

Further comparison with d-flagellin assays would have been welcome in triangulating findings. Re-examination with recently developed reference serum, even in the absence of agreed correlates of protection,[19–21] and for typhoid toxin[22] or other novel biomarkers are potential extensions to such seroepidemiological work. We reached a limit in the number of confirmed typhoid patients from Colonial War Memorial, the national teaching hospital, that could feasibly be traced and recruited but a larger Fijian patient cohort prospectively identified and with longer follow-up could potentially inform population serology.

An important consideration in seroepidemiology is that serology is not necessarily a marker of past or current disease. As suggested in the serology study and modelling study, a large proportion of typhoid cases are mild or asymptomatic. Those enrolled as patients may be a frailer subset (in the statistical hazard sense) of the general population and not representative of population immunity. There are potential differences in kinetics between

patients and those whose typhoid (mild or otherwise) goes untreated in the community. Clinical serology from Vietnam[23] (with supporting evidence from the Oxford challenge study)[24] suggest infection longer than two weeks may be necessary for sustained Vi response; in the Fijian patients, no antibody differences was seen when grouped by self-reported clinical duration. The antibody kinetics in seroconverted patients suggests a more rapid decline towards baseline than durations fitted in the model. Modelling typhoid in Fiji with short natural immunity resulted in very poor serological fit relative to multi-decade or lifelong immunity. It is possible that decline becomes asymptotic towards the threshold but a further explanation is asymptomatic boosting or prolonged exposure: unlike the Yale Asia model,[25] the Fiji model simplification does not capture boosting processes. As noted in the introduction, two further rounds of serum collection have been undertaken with Central Division participants for arboviral disease investigation. These additional survey rounds offer the possibility of examining true population typhoid antibody kinetics such as wane and boosting, by paired or cohort analysis of anti-Vi titres.

International comparison with representative population serology from high and low incidence settings may offer the most informative means to examine typhoid natural immunity distributions. This work uses a dichotomised immunity state, in a conventional medico-epidemiological approach to simplification of biological processes for analytical purposes. What is suggested from synthesis of the literature on dose dependency in infection, inconclusive thresholds for correlates of immunity, and the serological study's population distributions of antibody titre, is that typhoid immunity could or should be treated as a distribution accounting for protection to different inoculating doses. As noted in the model chapter, multiple dose-pathology responses could be developed; sensitivity analysis of thresholds and allocation to compartments could also be done. Some approaches to modelling vaccine immunity distributions alongside inoculation distributions have been suggested in recent analysis of simulated susceptible-infectious data by Gomes and colleagues.[26] To really begin to examine immunological thresholds and distributions of natural anti-Vi protection, adaptations of the human challenge study could be done based on known baseline antibody titres of participants. Challenge studies in endemic areas would be a major undertaking and likely require sustained engagement with local research ethics committees.

In terms of within-population risk factors, the mechanisms for the transmission of seropositivity are not necessarily the mechanisms by which disease is transmitted. This may particularly be the case if high dose and low dose are transmitted separately – for example, if municipal waterborne typhoid transmission is more likely to be of a low inoculum, as noted in

Glynn and Bradley's study of historical outbreaks.[27] Thus it is important to be very cautious in interpretation of findings from the serosurvey (including geospatial component) as to how these might apply to typhoid fever, the disease. A further consideration in this domain is that the mechanism by which the geospatially-determined water-based exposures might give rise to seropositivity is not necessarily ingestion of water. No variables for river exposure (bathing/swimming, walking through, cleaning clothes, cleaning pots) or drinking water sources were identified as associated with seropositivity in the main serosurvey paper. This may point towards the saturated physical environment as a conduit, consistent with other research in Fiji.[28]

In the social mixing study, the association of seropositivity with age-adjusted inter-iTaukei contact rates is supportive of a hypothesis of socially-structured transmission being important to the spread of typhoid in Fiji, which is not synonymous with direct person-to-person transmission but potentially reflects common exposure to indirect transmission within a social context, which may include contamination of food or surface water supplies, and contaminated fomites, in line with contemporaneous studies and historical reports of typhoid transmission. The age-assortative social contact data has highest rates in the school-aged, which steers our model towards accepting childhood transmission as important in typhoid, consistent with established theories of transmission in developing countries of typhoid and non-typhoidal enteric bacterial infections.[29,30] Other mixing matrices' fit to data were less good when examined in the model.

The last full year of available national surveillance data was 2014. Analysis with more recent data could also be informative. There was suggestion in simple linear regression of case data of a trend of an 11-cases per year decline from 2008 to 2014. This would be consistent both with an increased transmission coefficient (β) as a driver of the epidemic, and consistent with improved WASH interventions reducing β as part of outbreak response. These were premised on endemicity – models of declining transmission rates across decades from historical highs did not fit the data as well. Access to historical serum sets would support assessment of the mechanisms underpinning the rise in cases.

The findings from this project have implications for public health practice, even with these limitations. Whilst economic evaluation was left beyond the scope of the doctoral project, the model findings are informative to the relative potential contribution of vaccines and sanitation, and their sensitivity to factors such as the relative infectiousness of carriers. Whilst some policymakers may use these to signify the necessity of vaccination, the model is

intended to give an appreciation of levels of effective intervention necessary for disease control of different degrees though both vaccination and WASH.

Of possible risk factors generated in the analysis of the serosurvey, unimproved sanitation systems are consistent with findings of disease association from recently completed case-control and case-environmental analyses of Fiji's Central Division, primarily implicating unimproved or damaged sewage systems, with attributable risk also from unwashed produce, intermittent water, surface water as a secondary drinking water source and the absence of handwashing with soap.[31,32] Interestingly, these typhoid fever findings are consistent with systematic reviews[33] on diarrhoeal disease (which, as a classification, excludes typhoid fever), rather than suggesting different predominant modes of transmission for *Salmonella* Typhi compared with enteric bacterial pathogens considered to have lower inoculating doses such as *Shigella* spp. Collectively, the transmission model and these studies create a consistent picture of the importance of addressing sanitation and hygiene to reduce *Salmonella* Typhi transmission from infectious cases and carriers to protect those around them, water courses and food produce, in line with the F-diagram approach to sanitary engineering outlined in the introduction to this thesis, the historical literature,[34–36] and Asia-Pacific case control studies.[37–40]

A policy consideration should be the cost and opportunity cost of establishing a long-term typhoid vaccination programme versus other interventions, particularly if baseline typhoid fever incidence may be falling. Unlike a vertical vaccination programme, WASH strengthening also contributes to control of other communicable diseases. Were vaccine campaigns to be considered for short term control, potentially as a stop-gap to improvements in WASH, our modelling suggests effective school based campaigns can result in substantial incidence reductions without the potential health service disruption of all-age vaccination drives. Consistent with the national narrative on citizenship and identity, the modelled analyses do not examine the impact of immunisation or targeted WASH interventions based on ethnic group.

The age- and ethnic- structured modelling framework could be adapted for other public health threats in Fiji. A recent outbreak of the faecal-orally transmitted hepatitis A virus primarily affected Indo-Fijians in Western Division.[41] Serological studies have shown markedly different viral hepatitis seroprevalences by ethnicity in Fiji[42,43] and could be integrated into a dynamic, socially-structured model, potentially with serological testing of the 2013 samples.

Direct application of the modelled vaccine effectiveness results to other settings, or specific comparison to results from other models,[25,44] is limited by setting-specific epidemiology and model parameterisation other than in general terms described above, such as the likely relative effect of different vaccine characteristics and the interplay of WASH and vaccination. The project demonstrates that it is feasible to undertake serological data collection and use this to inform model projections that account for otherwise unobserved transmission and

immunity. Serologically informed typhoid modelling could readily be applied in other settings for vaccine impact estimation, such as those included in international typhoid surveillance programmes.[45,46] The major Wellcome- and Gates- funded STRATAA study uses anti-Vi IgG ELISA for detection of seroconversion in household contacts of cases to determine secondary attack rates.[47]

In terms of research unknowns, long-term carriage remains one of the most interesting characteristics of typhoid transmission. Carriage supports persistence of S. Typhi in low transmission settings and may make elimination more challenging.[4] The literature review found that carriage prevalence or risk of carriage following acute illness was an important determinant of indirect vaccine protection in models combining lifelong carriage with or without a β modifier. A similar finding was observed in the Fiji model, with the β of carriers relative to cases having important influence on vaccine effectiveness, and no difference in fit observable across two orders of magnitude variation in carrier β. Despite the concept that Vi carriage can be determined by Vi serosurveillance, there was insufficient data in the field survey (and no known Fijian carriers from which to have a positive control set) to begin to determine a serological threshold suggestive of carriage, beyond the speculative observations in the field survey paper. As noted in the introduction, historical efforts to use Vi serology for carrier detection have not been universally successful as a convenient alternative to repeat stool culture.

A number of programmes could support the identification of carriers and the development of carriage serological thresholds, some of which were proposed for Fiji but not yet taken forward from the 2012 expert meeting. An anti-Vi screening programme for carriage was proposed to replace stool screening, on the grounds that case and peri-case stool screening is wasteful, though this would be inconsistent with recent UK guidelines which still give primacy to stool culture over serological methods.[48] Undertaking these developments as research rather than a programme would seem appropriate. Serological testing of cholecystectomy patients alongside suitable gallbladder or gallstone culture could identify carriers and possible thresholds for screening and inform carrier seroprevalence from the national serosurvey.

The social mixing survey develops this field of research in two dimensions – the use of meal-based contact for enteric infectious disease modelling (rather than conversational contacts for respiratory diseases), and in enquiry about ethnicity. By quantifying mealtime social contacts, this study is one of the first social mixing studies specifically designed to inform transmission of faecal-orally transmitted pathogens, though it is important to note the intent of such data is to inform intensity of social contact as a proxy for risk rather than being

prescriptive about the mechanism.[49] On this basis, the mixing matrices could readily have wider application in Fiji for addressing data gaps in epidemiological modelling of diseases such as scabies (personal communication, David Regan), again utilising the social intimacy of eating as a proxy for transmission risk.[7,49] The contact survey has potential utility beyond Fiji, both as Pacific island mixing data, and in methodological development. The study warrants further investigation and validation against a range of markers of infection for a range of enteric diseases and in other settings.

Consistent with a broader health utility perspective, the serosurvey was designed for application across multiple diseases. Since undertaking this study, there has been a call for increased use of such serum banking for infectious disease investigation, endorsed by leading Anglo-American public health institutes.[50] Establishing the Fiji serum bank has opened up a multiplicity of uses and contribution to communicable disease research beyond typhoid fever, most notably to date through the leptospirosis investigation led by Colleen Lau,[51,52] but also arboviral disease, including dengue outbreaks and the emerging threat of Zika virus.

A final point I had been pondering during my doctoral research was where the intersection might lie of these typhoid control investigations and my parallel research in Ebola vaccine field trials and novel trial designs for control of diseases of epidemic potential.[53,54] A reanalysis by Mohammad Ali and colleagues of a cholera vaccine cluster randomised trial as a ring vaccination trial showed one possible approach.[55] Another answer came from Kim Mullholland (personal communication): ring vaccination trials against typhoid, with the possibility of demonstration projects in Fiji or other Oceanian island states. Such studies, whether individually- or cluster-randomised, would be compatible with current WHO recommendations on typhoid vaccination in outbreak control,[56] internationally declining typhoid incidence trends,[57] and case response by environmental health teams in some low to moderate incidence settings. The serial interval between cases makes this a plausible, if enormously challenging, means of examining vaccine efficacy and effectiveness and an intriguing parallel approach to ongoing large-scale cluster randomised trials of typhoid conjugate vaccines in Asia and Africa.[58] The prospects for scientifically-informed typhoid fever control look increasingly promising.

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Appendix A1. Informed consent and questionnaire

Typhoid and leptospirosis in Fiji

Research Consent form for adult and child participants

Participant ID Number: **FJT** ☐☐☐☐☐☐
letter site person

I/we have read and understood the information sheet. I/we understand that participation is voluntary and I/we can withdraw assent/consent at any point without giving a reason.

I consent to the following: *(please tick yes or no to each question)*		*Yes*	*No*
1.	*A sample of my/my child's blood may be taken and used for typhoid and leptospirosis research.*		
2.	*The answers I/we give to questions can be used for public health research, including typhoid and leptospirosis.*		
3.	*The Ministry of Health, or researchers working with the Ministry, can contact me/us again about typhoid and leptospirosis research.*		
4.	*My/my child's blood sample can be used for other health research.*		
5.	*The Ministry of Health, or researchers working with the Ministry, can contact me/us about other health research.*		

Name of participant: *(please print)*	*Age in years:*
Signed by participant if age 12+:	*Date (dd/mm/yyyy)*
Name of PARENT or CARER for child participants (age 17 or less): *(please print)*	
Signed by parent/carer:	*Date (dd/mm/yyyy)*

Consent taken by research staff member: *(please print name)*	
Signed by research staff member:	*Date (dd/mm/yyyy)*

If the participant/parent/carer does not speak English:

Witnessed by: *(please print name)*	
Signed by witness:	*Date (dd/mm/yyyy)*

Participant ID ***FJT*** (letter site person) ☐☐☐☐☐☐ Data entered☐ Re-entered☐ checked☐

Date today: *__ __ / __ __ / 20__ __ (dd/mm/yyyy)*

Day of the week *(circle number)**:*** *1=Mon 2=Tue 3=Wed 4=Thu 5=Fri 6=Sat 7=Sun*

Interviewer *(circle)* ***:*** *JC IK MT LV SR AS KR CW*
Other:_____

How many homes did you have to visit before this one? *____ empty*
_____did not want to take part.

Location

Q1a. ***Location name*** *(Village, Settlement, or Street):*
_____

Q1b. ***Community type:*** *1= Village (registered), 2= Village (unregistered Fijian settlement),*
3= Settlement (Indo-Fijian), 4= Settlement (Mixed) 5= Squatter, 6=Residential (private housing/flats),
7=Residential (government/social housing) 8 = other: _____

Q1c. ***Geography:*** *1=urban, 2=peri-urban, 3=rural*

Q1d. ***Nursing Zone:*** *_____ Q1e.* ***Medical Area****:*
_____

Q1f. ***Division (circle):*** *1= Northern, 2= Central, 3= Western, 4=Eastern*

Q1g. ***GPS coordinates of*** *(circle)**:*** *1= front door of house, 2= community centroid, if not possible to visit house*

Q1h. South ***0☐☐°☐☐.☐☐☐'*** *(circle)* ***West / East***
☐☐☐°☐☐.☐☐☐'

Q1i. ***GPS elevation:*** *_____metres* *Q1j.* ***Garmin 1 or 2*** *(circle) &* ***Waymark no*** ***☐☐☐***

Household occupants

Q2. ***Please tell me the age and gender of each person who stayed in the household last night.*** *(circle sex)*

No	Age	Sex	
1		M	F

No	Age	Sex	
6		M	F

No	Age	Sex	
11		M	F

No	Age	Sex	
16		M	F

2		M	F
3		M	F
4		M	F
5		M	F

7		M	F
8		M	F
9		M	F
10		M	F

12		M	F
13		M	F
14		M	F
15		M	F

17		M	F
18		M	F
19		M	F
20		M	F

Q2a. ***Random number from tables to select the participant (reselect if <1year old):*** ☐☐

Q2b. Record any participant number(s) selected but who could not be contacted:

_____

The selected participant

Q3a. ***First name:***_____ *Q3b.****Family Name :*** _____

Ask for initials if this is declined, or record "-2" if refused.

Q4a. ***Age (****from above)* ☐☐*years* *Q4b.* ***Date of birth :*** __ __ / __ __ / __ __ __ __ ***(dd/mm/yyyy)***

Q5. ***Sex: (****from above) 1= Male, 2= Female, -2= refused.*

Q6. ***Were you born in Fiji?*** *1=yes (skip to Q7a), 0=no, -1=don't know, -2=refused*

If no, don't know, refused: Q6a. ***Roughly how long have you lived in Fiji?***

_____*years*

Q6b. ***Where did you live before that:*** _____

Q7a. ***How long have you lived in this village/settlement/town****?* _____*years*

-3= all my life (skip to Q8) -1 =don't know, -2=refused

Q7b. ***What was the last place you lived before here?***

Name of place: _____*-1 d/k -2=refused*

Q7c. Province/area/town _____ *-1 d/k -2refused*

Q7d. Division (circle): 1= Northern, 2= Central, 3= Western , 4=Eastern, 5=outside Fiji: _____

Q7e. ***Which of the following best describes that place:*** *1=urban, 2=peri-urban, 3=rural, 4=remote island*

Q7f. ***Community type:*** *1= Village (registered), 2= Fijian settlement (e.g. unregistered village),*

3= Settlement (Indo-Fijian), 4= Settlement (Mixed) 5= Squatter, 6=Residential (private housing/flats),

*6=Residential (government social housing) 7 = other*_____ *-1=don't know, -2=refused*

Q8. ***What is your ethnicity?***

1 = Fijian / iTaukei / indigenous Fijian 2 = Indo-Fijian / Fijian of Indian Descent / Indian

3 = European / Fijian of European Descent 4 = Chinese / Fijian of Chinese Descent

5 = Melanesian/Polynesian/Micronesian/other Pacific island

6 = Mixed 7=other -1 = don't know -2 = refused

Q9. ***What is your religion?***

1 = Christian - Methodist

2 = Christian - Catholic 5 = Christian - Anglican 6 = Christian - Other 8 = Muslim 9 = Sikh 0 = none -1 = don't know

3 = Christian - Assembly of God
4 = Christian - 7th Day Adventist
7 = Hindu
10 = other
-2 = refused

Q10. ***What is the highest level of school you have completed?*** *Either Class _____ or Form _____ or:*

0= No formal schooling 1= Primary 2= Secondary 3= Technical / Vocational School 4=University/tertiary -1= don't know -2= refused

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Work and Farming

Q11a. ***What is your job title or job description, if any?***

_____

Q11b. ***Do you mostly work:*** *1=indoors, 2=outdoors, or 3=a mix of both? -1 = don't know, -2 = refused -3 not applicable*

Q11c. ***Which of the following best describes your main job?*** (Suggest one based on above description)

(The occupation that the participant spends the most time on. Circle one number only):

1 = pre-school child;	*6 = manual labourer;*	*11= retired;*
2 = pupil/student;	*7 = skilled manual worker;*	*12 = unemployed;*
3 = professional/office worker;	*8 = farming;*	*13 = other;*
4 = shop worker;	*9 = fishing;*	*-1 = don't know;*
5 = Market/outdoor trader;	*10 = housewife/husbandman;*	*-2 = refused.*

Q12. ***What is the total household income each week***? (combined total of everyone who lives there)

0= less than FJD100, 1=100 to 199, 2=200 to 299, 3=300 to 399, 4 = 400 to 499, 5=500 to 999, 6=1000 or more. -1 = don't know, -2 = refused

Q13. If the main job is not farming: ***Have you done any part-time/casual farming in the last 12 months?*** *(not just growing vegetables in the yard or keeping a few animals) 1=yes, 0=no, (go to Q16) -1=don't know -2=refused*

*Q14. If a farmer (main job or part-time/casual)****, which of the following do you farm?*** *(tick all that apply)*

a	*Chickens*	☐	*d*	*Goats*	☐	*g*	*Vegetables/root crop*	☐
b	*Pigs*	☐	*e*	*Cows*	☐	*h*	*Fruit*	☐
c	*Sheep*	☐	*f*	*Sugar Cane*	☐	*i*	*Other:_____*	☐

Q15. If farming: ***To go from home to where you mostly farm, is it:*** *1=**uphill**, 2=**downhill**, 3=**up+down** or 0=**on the same level?***

Travel and meeting people

Q16. ***How often do you visit places outside your village/settlement/town?*** *(not just for farming/fishing)*

Every day	*4 to 6 days per week*	*1 to 3 days per week*	*One a month or more, (but not once a week)*	*Less than once a month, but not never*	*Never (go to Q18)*	*Don't know*	*Refused*
5=	*4=*	*3=*	*2=*	*1=*	*0=*	*-1=*	*-2=*

Q17a. ***Could you please name all the villages, settlements or towns you have visited in the last 7 days?***

(either for work or visiting friends and relative) ***0=*** *Visited no village, settlement or town.* ***-1=*** *don't know*

	Name	***Division*** *(circle)*				***Community type***				***Tick furthest away***
						iTaukei Village	*Indo-Fijian Settlement*	*Town*	*Other:*	
1		*N*	*C*	*W*	*E*	☐	☐	☐		☐
2		*N*	*C*	*W*	*E*	☐	☐	☐		☐
3		*N*	*C*	*W*	*E*	☐	☐	☐		☐
4		*N*	*C*	*W*	*E*	☐	☐	☐		☐
5		*N*	*C*	*W*	*E*	☐	☐	☐		☐
6		*N*	*C*	*W*	*E*	☐	☐	☐		☐
7		*N*	*C*	*W*	*E*	☐	☐	☐		☐
8		*N*	*C*	*W*	*E*	☐	☐	☐		☐
9		*N*	*C*	*W*	*E*	☐	☐	☐		☐
10		*N*	*C*	*W*	*E*	☐	☐	☐		☐

If more than ten, please give ten examples, including the furthest away, and typical places visited.

Q17b. If more than ten, roughly how many? _____

I'd like to ask you about who you had meals with yesterday. If asked, this is because typhoid may be spread on food.

	Q18a. *First, where did you eat lunch yesterday?* ***Q18b.*** *And where did you eat dinner yesterday?*	***Lunch*** *(X one answer only)*	***Dinner*** *(X one answer only)*	***Q19***
1	*Home*	☐	☐	*Ask: "how many people of each age also ate the same food?"*
2	*Somebody else's home*	☐	☐	
3	*Buffet / lovo at church, village social gathering or similar*	☐	☐	
4	*Workplace/school – brought food from home/from somebody else's home*	☐	☐	
5	*Workplace/school – cafeteria food*	☐	☐	*Ask: "how many friends, family or colleagues of different ages did you share a table with?"*
6	*Restaurant/cafe/food court/takeaway (takeaway or ate there, includes hotel restaurants)*	☐	☐	
7	*Outdoor food stall (takeaway or ate there).*	☐	☐	
8	*Bought from a walking food seller*	☐	☐	
9	*Didn't eat lunch/dinner*	☐	☐	
10	*Other:_____*	☐	☐	
-1	*Don't know*	☐	☐	
-2	*Refuse to say*	☐	☐	

Q19. ***I'd like you to think about how many people of different ages you shared food with, first at lunch, then at dinner. (****The participant should not include him or herself.)*

(1) For people who ate at home, somebody else's home, at a buffet, or brought food to work, ask "how many people of each age also ate the same food?"
(food made in the same kitchen, shared at the meal, or from the same buffet)

(2) For people who ate at a work cafeteria, from a roadside stall/street seller or a restaurant/food court, ask "how many friends, family or colleagues of different ages did you share a table with?"
(or equivalent to a table if ate outdoors)

Q19a. ***First lunch, starting with children under 5:***
- of these people, how many were iTaukei Fijian, Indo-Fijian, or other?

Q19b. ***Then dinner, starting with children under 5:***
- of these people, how many were iTaukei Fijian, Indo-Fijian, or other?

Q19c. ***And finally, how many of these people were the same at both lunch and dinner?***
- of these people, how many were iTaukei Fijian, Indo-Fijian, or other?

Go through each age group. Record exact numbers for numbers 0 through to 15. If the participant is concerned about getting it exactly right, tell them it is ok if they are a little bit out or can't remember exactly.
If more than 15, use these bands: 16-24, 25-49, 50-99, 100+. Use also:-1=don't know, -2 = refused, -3 not applicable.

Age:	***0 to 4***		***5 to 14***		***15 to 34***		***35 to 54***		***55+***	
19a. Lunch		*Fjn* *Indo* *Othr*		*Fjn* *Indo* *Othr*		*Fjn* *Indo* *Othr*		*Fjn* *Indo* *Othr*		*Fjn* *Indo* *Othr*
19b. Dinner		*Fjn* *Indo* *Othr*		*Fjn* *Indo* *Othr*		*Fjn* *Indo* *Othr*		*Fjn* *Indo* *Othr*		*Fjn* *Indo* *Othr*
19c. Same people		*Fjn* *Indo* *Othr*		*Fjn* *Indo* *Othr*		*Fjn* *Indo* *Othr*		*Fjn* *Indo* *Othr*		*Fjn* *Indo* *Othr*

Contact with people

Q20. ***No longer being asked***

Water and Sanitation

Q21. ***How often do you drink the following?*** *(circle number)*		*Every day*	*4 to 6 days per week*	*1 to 3 days per week*	*One a month or more, but not once a week*	*Less than once a month, but not never*	*Never*	*Don't know*	*Refused*
Water from a...	*a. Tap at home*	*5*	*4*	*3*	*2*	*1*	*0*	*-1*	*-2*
	b. Tap somewhere else (e.g. village standpipe, cafe)	*5*	*4*	*3*	*2*	*1*	*0*	*-1*	*-2*
	c. Well, pump or borehole	*5*	*4*	*3*	*2*	*1*	*0*	*-1*	*-2*
	d. Rainwater container	*5*	*4*	*3*	*2*	*1*	*0*	*-1*	*-2*
	e. River, stream, pond or lake	*5*	*4*	*3*	*2*	*1*	*0*	*-1*	*-2*
f. Juice made by a cafe or street stall (not carton juice)		*5*	*4*	*3*	*2*	*1*	*0*	*-1*	*-2*
g. Grog/Kava		*5*	*4*	*3*	*2*	*1*	*0*	*-1*	*-2*

Q22. ***Do you treat your water in any way to make it safer to drink?*** (i.e. does anyone in the household treat the water, we are not asking if the water supplied to the household/participant is already treated)

3=Yes - always (at least 95% of the time)	*2=Yes - usually (50% or more of the time but not 95%),*	*1=Yes, sometimes (less than 50% of the time but not 5%)*	*0= no, never (5% or less of the time) (go to Q24)*	*-1= don't know*	*-2 = refused*

Q23. If yes, ***what do you usually do to the water to make it safer to drink?***

Prompt "Anything else?" (Record all that are mentioned, do not prompt specific methods)

☐*Boiling* ☐*Use a water filter* ☐*Other* _____

☐*Add bleach/chlorine* ☐*Solar disinfection* ☐*Don't know*

☐*Strain it through a cloth* ☐*Let it stand* ☐*Refused*

Q24, If the participant drinks grog/kava: ***The last time you had grog/kava, how many people did you drink it with?***

(circle the number, do not include the participant in the total)

Not app. -3=	*no one else 0=*	*1 to 4, 1=*	*5 to 9, 2=*	*10 to 14, 3=*	*15 to 19, 4=*	*20 to 24, 5=*	*25 to 49, 6 =*	*50+ 7=*	*Don't know -1 =*	*refused -2 =*

Q25. ***Thinking about lunch and dinner yesterday, was soap and water available, if you had wanted to wash your hands before eating?***
Did you wash your hands with soap and water before eating?

a. ***Lunch: soap & water available:*** *1=yes 0=no -1= don't know -2 = refused -3=not applicable*

b. ***Lunch: washed hands with soap & water:*** *1=yes 0=no -1= don't know -2 = refused -3=not applicable*

c. ***Dinner: soap & water available:*** *1=yes 0=no -1= don't know -2 = refused -3=not applicable*

d. ***Dinner: washed hands with soap & water:*** *1=yes 0=no -1= don't know -2 = refused -3=not applicable*

Q26. ***What sort of toilet do you have at home?*** *(circle)*

	not applicable	*Flush toilet*	*Water seal/pour flush*	*Pit with slab*	*Pit without slab*	*Compost toilet*	*Bucket latrine*	*Hanging latrine*	*Field/ bush*	*River / stream*	*Sea*	*don't know*	*refused*
Home	*-3*	*1**	*2**	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*-1*	*-2*

Q26a. If flush/water seal/pour flush,* ***where does it go to?* *1=piped sewer system, 2=septic tank, 3=pit latrine, 4=elsewhere (street, yard. plot, open sewer, ditch, drainage way). -1= don't know, -2=refused, -3=not applicable*

Q27. ***Where is your home toilet located:*** *1=indoor, 2=detached, -1= don't know, -2=refused, -3= not applicable.*

Q27a. ***Is it shared with any other households?*** *1=yes, 2=no, -1= don't know,-2 refused,*

Q27b. ***Is there soap and water available to wash hands after going to the home toilet?***
2=Yes, visually confirmed; 1=yes, but unable to check; 0=No, -1= don't know, -2=refused.

Q27c. ***The last time you used your home toilet, did you wash your hands with soap and water afterwards?***
1=yes, 2=no -1= don't know,-2 refused

Q28. ***When you are at work/school, what sort of toilet do you have access to?***

	not applicable	*Flush toilet*	*Water seal/ pour flush*	*Pit with slab*	*Pit without slab*	*Bucket latrine*	*Hanging latrine*	*Field/ bush*	*River / stream*	*Sea*	*don't know*	*refused*
a. ***Work Defecate (poo)***	*-3*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*-1*	*-2*
b. ***Work Urinate (pee)***	*-3*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*-1*	*-2*

Q28c. ***When at work/school, are there facilities for washing hands with soap and water after going to the toilet?***

1=yes, 2=no -1= don't know, -2 refused -3= not applicable.

Q28d. ***Last time you went to the toilet at work/school, did you wash your hands with soap and water afterwards?***

1=yes, 2=no -1= don't know, -2 refused -3= not applicable.

The house

Q29. ***What is the house made of?*** *1=concrete/brick, 2=wood, 3=corrugated iron, 4=bure, 5=bamboo, -1=don't know,*

-2=refused. If it is made of more than one, record the main material (e.g. concrete with metal roof =1).

Q30. ***What is the floor mostly made of?*** *1= earth/sand, 2=palm/bamboo, 3= wooden planks, 4= polished wooden floor, 5=vinyl, 6=ceramic tile, 7=cement, 8=carpet*

Q31. ***Is the floor raised at least one foot (30cm) from the ground?***

1=yes, 0=no, -1= don't know, -2= refused

If the home is on stilts on a hillside, decide based on the edge nearest the hillside. Answer yes only if all edges are at least one foot from the ground. An A4 piece of paper is one foot long.

Q32, ***In your home, how many living rooms and bed rooms are there?*** _____

Do not count bathrooms, landings, porches, kitchens, storage rooms ; record -1 for don't know, and -2 for refused.

Q33. ***Does your home have tap water?*** *1=yes, 0=no, -1= don't know, -2= refused*

Q33a. If yes, ***is the tap water treated by the government?*** *1=yes, 0=no, -1= don't know, -2= refused*

Q34. ***Does your home have a working indoor shower, or a tap to wash yourself under?***

1=yes, 0=no, -1= don't know, -2= refused

Waste disposal

Q35. ***How is garbage / rubbish mainly disposed of from the house?***

Dumped: 1= roadside / field / bush 2 = river/stream/lake 3= sea
Burnt: 4= incinerator, 5=bonfire
Buried: 6 = Household pit with raised mounting and tight cover,
7=household pit without these, 8 = communal pit, 9= Communal skip
Collection service: 10= bin with tight lid 11= bin without tight lid
12= bags on roadside 13= bags on raised rack
Other: 14=Composting 15=Recycling 16=Reuse
17= other _____
-1= don't know, -2= refused

Exposure to water

Q36. ***Is there a stream or river near your home?*** *1=yes, 0=no, -1= don't know, -2= refused*

Near is within the village/settlement for residents of these, or 100m (a rugby/football pitch) for other households

Q37a. ***Has your house been flooded in the last three years?*** *By inland water such as river or stream, not by seawater.*

1=yes, 0=no (skip to Q38), -1= don't know, -2= refused

Q37b. If yes, ***how many times?*** *1= One or two, 2= three to five, 3=more than five, -1= don't know, -2= refused*

Q38a. ***Has the land around your house been flooded in the last 3 years?***

1=yes, 0=no, -1= don't know, -2= refused

Q38b. If yes, ***how many times?*** *1= One or two, 2= three to five, 3=more than five, -1= don't know, -2= refused*

Q39a. ***Have you ever been swimming, playing, or bathing in flood water?***

1=yes, 0=no, -1= don't know, -2= refused

Q39b. ***Or walking in flood water?*** *1=yes, 0=no, -1= don't know, -2= refused*

Q40. ***How often do you do the following?***

	Every day	*4-6 days a week*	*1-3 days a week*	*One a month or more, but not once a week*	*Less than once a month, but not never*	*Never*	*Don't know*	*Refused*
a. Swim, play, bath in a river, stream, waterfall or lake? (not flood water, not the sea).	*5*	*4*	*3*	*2*	*1*	*0*	*-1*	*-2*
b. Walk through a river, stream, waterfall or lake?	*5*	*4*	*3*	*2*	*1*	*0*	*-1*	*-2*
c. Use river, stream, waterfall or lake water for washing ***clothes***	*5*	*4*	*3*	*2*	*1*	*0*	*-1*	*-2*
d. Use river, stream, waterfall or lake water for washing ***dishes***	*5*	*4*	*3*	*2*	*1*	*0*	*-1*	*-2*
e. Bath using an indoor shower/under an indoor tap?	*5*	*4*	*3*	*2*	*1*	*0*	*-1*	*-2*

Typhoid and leptospirosis

Q41. ***Had you heard of typhoid before this study?*** *1=yes, 0=no (ask* ***Q42*** *then skip to* ***Q46****), -1=d/k, -2=refused*

Q42. ***Have you ever been vaccinated against typhoid? It is NOT usually given in childhood.*** *(Some people were vaccinated in 2010 after the cyclone.)*

1=yes, 0=no, -1=don't know, -2=refused. 42a. ***If yes, which year?*** _____

Q43. ***Have you ever been diagnosed with typhoid by a doctor?***

1=yes, 0=no (skip to Q44) -1=don't know (skip to Q44) -2=refused (skip to Q44)

Q43a. If yes, ***roughly when did you get ill? Month*** *(-1 don't know)* _____***Year*** *(-1 don't know)* _____

Q43b. ***How or why do you think you got ill? (****-1=don't know);*

_____

Q44. ***Has anyone in your household been diagnosed with typhoid?***

1=yes, 0=no, (skip to Q45) -1=don't know, (skip to Q45) -2=refused (skip to Q45)

Q44a. If yes, ***roughly when did they become ill?***

(If more than one person, give the dates of illness of the first person to get ill, and the most recent)

a. ***First/ only ill person*** ***Month*** *(-1 don't know)* _____***Year*** *(-1 don't know)* _____

b. ***Most recent ill person*** *-3= not applicable* ***Month*** *(-1 don't know)* _____ ***Year*** *(-1 don't know)* _____

Q45. ***Thinking about friends, neighbours, colleagues and extended family in Fiji, how many people do you know who have had typhoid?*** *0= none, 1=one or two, 2 = three to five, 3=six to ten, 4= eleven+, -1=don't know, -2=refused*

Q46. ***Had you heard of leptospirosis (lepto) before this study?*** *1=yes, 0=no (skip to Q49), -1=don't know, -2=refused*

Q46a. If yes, ***Have you ever been diagnosed with leptospirosis by a doctor?*** *1=yes, 0=no (skip to Q47), -1=don't know, -2=refused*

Q46b. If yes, ***roughly when did you get ill? Month*** *(-1 don't know)* _____***Year*** *(-1 don't know)* _____

Q46c. ***how/why do you think you became ill?*** *(-1=don't know):*

_____

Q47. ***Has anyone in your household been diagnosed with leptospirosis?***

1=yes, 0=no (skip to Q48) -1=don't know(skip to Q48), -2=refused(skip to Q48)

Q47a. If yes, ***roughly when did they become ill?***

(If more than one person, give the dates of illness of the first person to get ill, and the most recent)

a. ***First/ only ill person*** ***Month*** *(-1 don't know)* _____***Year*** *(-1 don't know)*_____

b. ***Most recent ill person*** *-3= not applicable* ***Month*** *(-1 don't know)* _____ ***Year*** *(-1 don't know)* _____

Q48. ***Thinking about friends, neighbours, colleagues and extended family in Fiji, how many people do you know who have had leptospirosis?*** *0= none, 1=one or two, 2 = three to five, 3=six to ten, 4=eleven+, -1=don't know, -2=refused*

Contact with animals

Q49. ***Over the past three years, have you seen rats or mice…***

a. ***At or around your home?*** *1=yes, 0=no, -1=don't know,-2=refused*

b. ***At or around your work/school?*** *-3=not applicable 1=yes,0=no, -1=don't know, -2=refused*

c. ***Have you been in physical contact with rats or mice in that time?*** *1=yes, 0=no, -1=d/k -2=refused*

Q50. ***Over the past three years, have you seen mongooses…***

a. ***At or around your home?*** *1=yes, 0=no, -1=don't know,-2=refused*

b. ***At or around your work/school?*** *-3=not applicable 1=yes, 0=no, -1=don't know, -2=refused*

c. ***Have you been in physical contact with mongooses in that time?*** *1=yes, 0=no, -1=d/k -2=refused*

Q51. ***Over the past three years, have you been bitten by ticks or fleas?***

1=yes, 0=no, -1=don't know, -2=refused

(not bed bugs, which usually bite in a line of small dots at night, or mosquitoes. Ticks or fleas are often carried by furry mammals)

Local animals and vegetables

Q52. ***Please tell me which of the following animals or vegetables, are found locally, if any?*** *Select all that apply (mark with a cross). We are not asking about other types.*

		In garden / at home? (If applicable)	*In village/ settlement/ slum? (if applicable)*	*Animals kept in a pen / tied up?*	*Direction from house to the nearest penned/tied-up animals or to the fruit/vegetables*		
					Uphill	***Level***	***Downhill***
a	***Chickens***	☐	☐	☐	☐	☐	☐
b	***Pigs***	☐	☐	☐	☐	☐	☐
c	***Sheep***	☐	☐	☐	☐	☐	☐
d	***Goats***	☐	☐	☐	☐	☐	☐
e	***Cows***	☐	☐	☐	☐	☐	☐
f	***Horses***	☐	☐	☐	☐	☐	☐
g	***Dogs***	☐	☐	☐	☐	☐	☐
h	***Cats***	☐	☐	☐	☐	☐	☐
i	***Sugar Cane***	☐	☐		☐	☐	☐
j	***Vegetables/ root crop***	☐	☐		☐	☐	☐
k	***Fruit***	☐	☐		☐	☐	☐

Vinaka vaka levu! Thanks for taking part in this study.

Q53. ***Could we please take a phone number in case we have further questions?***

_____

Q54. ***Do you have any other comments?***

Remember to get GPS coordinates. Can soap be checked easily? Record any other important notes overleaf.

Appendix A2. Evaluating Typhoid Vaccine Effectiveness in Travelers' Vaccination. CH Watson. J Travel Med. 2015;22:76-77

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SECTION A – Student Details

Student	Conall Watson
Principal Supervisor	John Edmunds
Thesis Title	Seroepidemiological investigations of typhoid fever in Fiji and the potential role of vaccination in control

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?	Evaluating Typhoid Vaccine Effectiveness in Travelers' Vaccination. J Travel Med. 2015;22:76-77. doi:10.1111/jtm.12185.		
When was the work published?			
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Stage of publication	

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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	N/A

Student Signature: [redacted] **Date:** 12 Sep 2017

Supervisor Signature: [redacted] **Date:** 12 Sep 2017

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A2.1 Editorial

Evaluating typhoid vaccine effectiveness in travelers

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Typhoid fever exists somewhere in the borderlands of the neglected tropical diseases. Its history in Europe and North America, and market for vaccination of travelers means typhoid is not entirely in the pharmaceutical public health wilderness. Travel immunisation recommendations however are based on results of efficacy trials performed in typhoid-prone areas, rather than on evidence of direct effectiveness in those journeying from low-risk settings. Two recent epidemiological studies address the efficacy in travelers,[1,2] one of which is in this edition of JTM. Here, Karen Wagner and colleagues used the detailed enteric fever surveillance records of the English public health services to compare typhoid Vi-polysaccharide (ViPS) vaccine history amongst typhoid and paratyphoid cases. They estimated that vaccine effectiveness against typhoid was 65% over three years in travelers, after multivariable adjustment, consistent with efficacy trials.

Their approach, using the indirect cohort design or 'Broome method', first developed to examine pneumococcal vaccine efficacy across different serotypes,[3] is well-suited to the question. Paratyphoid cases are suitable controls for typhoid because the geographies in which they arise and routes of acquisition are similar.

Crucially, ViPS vaccine does not protect against paratyphoid: for unbiased estimates of typhoid vaccine effectiveness it is necessary to have equal probability of paratyphoid fever notification in ViPS-vaccinated and unvaccinated groups.[5] There is as yet no licensed vaccine against *S.* Paratyphi, though a number are in development,[6] while Ty21a oral typhoid vaccines, which have possible cross-protection with *Salmonella* Paratyphi A and B,[7] are uncommonly prescribed in the UK and do not feature in the analysis. Similar indirect cohort studies of forthcoming typhoid Vi-conjugate vaccines may be possible if there is an interval between widespread adoption of these and paratyphoid vaccines, or with detailed vaccine history-taking.

The suitability of paratyphoid controls bears further consideration: if the likelihood of pre-travel vaccination was different between the cohorts who ultimately end up as paratyphoid and typhoid cases then this could also bias estimates.[8] For example, travelers visiting friends and relatives may be less likely to seek advice or be vaccinated against typhoid than holidaymakers,[9] and they may also have different *Salmonella enterica* serovar exposure.

Case-control studies in urban Asian settings have suggested different risk factors for the two pathogens. In Jakarta, Indonesia, typhoid risk factors were characterised as "within the household": recent typhoid cases, shared plates, and the absence of soap or toilets.[10] Paratyphoid was associated with extra-household factors: eating street food or recent flooding. In Kathmandu, Nepal, a similar categorisation might be inferred: street-food consumption and migration were associated with paratyphoid fever, in contrast with typhoid for which risk factors were poor water and low income.[11]

So there might be some differences in enteric fever case cohorts in terms of backpackers and sunseekers eating out, and travelers staying with family and friends. This seems borne out to a modest extent in this study, with tourists making a slightly higher proportion of the paratyphoid group, which also has a higher mean age, though there was minimal difference in effect size after adjustment for age group, sex, country of birth and ethnicity.

Particular strengths of Wagner's paper are the comprehensive breakdown by traveler subgroup and by time, and the validation of vaccine histories by cross-checking a subset of enteric fever notifications with the patients' primary care records. One subgroup finding of interest is the possibility that vaccine efficacy may be reduced for travelers of white ethnicity, though low numbers make estimates imprecise and no significant difference was described. While genetic differences in typhoid susceptibility have been demonstrated elsewhere,[12] it would be premature to suggest this arises in UK travelers. Given the comprehensiveness of enteric fever surveillance in England, confirmation or refutation may be possible as more data accumulate. Further years' data could also determine if reduced-antigen Typhim ViPS batches, withdrawn by Sanofi Pasteur MSD from the UK, show impaired efficacy with time. It is reassuring that the first year of protection shows no difference from full-potency batches.

A similar indirect cohort study using paratyphoid controls was also completed recently by the US Centers for Disease Control and Prevention. Due to missing data, this assessed efficacy across both ViPS and Ty21a vaccines, and had less complete vaccine history ascertainment than in England. CDC found 80% vaccine effectiveness, higher than that in trials, which they ascribe to less intense exposure amongst American travelers compared with residents endemic-area studies, both in ingested dose and time at risk.[1] High infectious doses of *S.* Typhi can overcome vaccine-derived immunity[13] (*S.* Paratyphi is thought to require higher infectious doses than *S.* Typhi, with food a multiplier, contributing to the differential epidemiology[10,11]), while in one Australian study, all enteric fever cases had been abroad for at least three weeks.[14]

As well as measuring vaccine effectiveness within their own travelers, the two effectiveness studies provide ecological indicators of the effect of vaccine coverage on typhoid cases averted, adding to previous reports.[15] In the US, where just 8% of the notional cohort was vaccinated, this ratio was around four typhoid cases to each paratyphoid case, while in England, where 29% of studied enteric fever cases had been vaccinated, the case ratio was closer to 1:1.

Some of the difference in uptake may be due to typhoid vaccination being offered free-of-charge to travelers on the English National Health Service, as a public health measure intended to provide indirect protection to the families and communities of travelers, as well as direct protection to the vaccinees. The limited vaccine uptake amongst English travelers at risk of typhoid suggests that, on its own, removing financial barriers to access is not sufficient for all such voyagers to receive pre-departure vaccination, with opportunistic pre-travel advice from family physicians being one option suggested for improving uptake amongst travelers of south Asian ethnicity.[9] It also serves to remind us that while we await the arrival of paratyphoid vaccines and Vi-conjugate vaccines, the success of these interventions depends not just on their efficacy, but on the ability of public health systems to deliver vaccines to people who need them, whether residents of endemic areas or their visitors.

Acknowledgment

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Appendix A3. Human Leptospirosis Infection in Fiji: An Eco-epidemiological Approach to Identifying Risk Factors and Environmental Drivers for Transmission. Lau CL, Watson CH, Lowry JH, et al. PLoS Negl Trop Dis. 2016;10(1):e0004405.

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Author Contributions

Conceived and designed the experiments: CLL CHW SBC MK EJN. Performed the experiments: CLL CHW SBC SJW. Analyzed the data: CLL CHW JHL MCD. Contributed reagents/materials/analysis tools: SBC SJW. Wrote the paper: CLL CHW JHL MCD EJN.

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Student Signature: [redacted] **Date:** 12 Sep 2017

Supervisor Signature: [redacted] **Date:** 12 Sep 2017

An Eco-epidemiological Approach to Identifying the Main Determinants of Human Leptospirosis Infection in Fiji

Authors:

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Abstract

Leptospirosis is an important zoonotic disease in the Pacific Islands. In Fiji, two successive cyclones and severe flooding in 2012 resulted in outbreaks with 525 reported cases and 10% case-fatality. We conducted a cross-sectional seroprevalence study and used an eco-epidemiological approach to characterize the risk factors and drivers for human leptospirosis infection in Fiji, and aimed to provide an evidence base for improving the effectiveness of public health mitigation and intervention strategies. Antibodies indicative of previous or recent infection were found in 19.4% of 2152 participants (82 communities on the 3 main islands). Questionnaires and geographic information systems data were used to assess variables related to demographics, individual behaviour, contact with animals, socioeconomics, living conditions, land use, and the natural environment. On multivariable logistic regression analysis, variables associated with the presence of *Leptospira* antibodies included male gender (OR 1.55), iTaukei ethnicity (OR 3.51), living in villages (OR 1.64), lack of treated water at home (OR 1.52), working outdoors (1.64), rural areas (OR 1.43), high poverty rate (OR 1.74), living <100m from a major river (OR 1.41), pigs in the community (OR 1.54), cattle density in the district (OR 1.04 per head/sqkm), and maximum rainfall in the wettest month (OR 1.003 per mm). Risk factors and drivers for human leptospirosis infection in Fiji are complex and multifactorial, with environmental factors playing crucial roles. With global climate change, severe weather and flooding are expected to intensify in the South Pacific. Population growth invariably leads to more intensive livestock farming; and urbanization in developing countries is often associated with urban and peri-urban slums where diseases of poverty proliferate. Climate change, flooding, population growth, urbanization, poverty and agricultural intensification are important drivers of zoonotic disease transmission; these factors may independently, or potentially synergistically, lead to enhanced leptospirosis transmission in Fiji and other similar settings.

Author summary

Leptospirosis is a bacterial infection transmitted from animals to humans, and many outbreaks are associated with flooding. Globally, leptospirosis is responsible for at least 500,000 cases of severe illness each year, and many deaths. The bacteria are excreted in the urine of infected animals; humans can become infected through direct contact with animals or through contaminated water and soil. In Fiji, two successive cyclones and severe flooding in 2012 resulted in 525 cases and 52 deaths. We conducted this study to improve our understanding of the factors that increase the risk of leptospirosis transmission, so that public health control measures can be improved. Our study found that infection risk is related to many factors including individual demographics and behaviour, contact with animals, living conditions, poverty, and flooding risk. With global climate change, flooding is expected to become a bigger problem in the South Pacific. Population growth invariably leads to more intensive livestock farming; and urbanization in developing countries is often associated with slums with high risk of infectious diseases. Climate change, flooding, population growth, urbanization, poverty and livestock farming are important factors for leptospirosis transmission; these factors may combine to increase the risk of leptospirosis in Fiji in the future.

A3.1 Introduction

Leptospirosis is an emerging infectious disease worldwide, with particularly high incidence reported in the Pacific Islands [1,2]. Humans are infected through direct contact with infected animals, or through contact with water or soil that has been contaminated by urine of infected animals. Disease transmission is strongly driven by environmental factors including high rainfall, flooding, natural disasters, population growth, urbanisation, and poor sanitation and hygiene [2-4]. In addition, infection risk depends on individual behaviour (e.g. swimming in fresh water, working outdoors), and contact with animals including livestock, rodents, pets, & wildlife [2,4]. Risk factors for infections and drivers of outbreaks depend on interactions between humans, animals, and the environment, and vary significantly between locations based on environmental, cultural, and socio-demographic factors [4]. Transmission dynamics are therefore highly complex variable, and likely to evolve with global environmental change of both natural and anthropogenic environments [2,3].

In Pacific island nations, important risk factors for human leptospirosis include outdoor activities, tropical climate, flooding secondary to extreme weather events, and exposure to livestock [5-8]. Subsistence livestock are commonly kept in backyards, and veterinary expertise is generally limited. In some Pacific Islands, rapid population growth and urbanization exacerbate problems with sanitation, access to clean water, and waste management. Most islands have limited human or financial resources for the management and mitigation of the health impacts of natural disasters and climate change [9,10]. In Fiji, leptospirosis was identified as one of the four priority climate-sensitive diseases of major public health concern [11]. A recent systematic review of the global morbidity and mortality of leptospirosis identified tropical islands as particularly high-risk settings [2]. Apart from the tropical climate and high frequency of extreme weather events [3], factors that could contribute to the high risk of leptospirosis on tropical islands include the low biodiversity and delicate ecosystems that make islands vulnerable to invasive species such as rodents [12]; the outdoor lifestyle and associated intense exposure to the environment; and close contact with subsistence livestock animals [2,4].

Climate change is projected to increase the severity of extreme weather events including increased precipitation and flooding in the Pacific Islands [10], and such events have been associated with increased leptospirosis transmission and outbreaks around the world [3,7,13-16]. In 2012, two successive tropical depressions caused severe flooding and resulted in two outbreaks of leptospirosis in Fiji, with 525 reported cases and 52 deaths (10% case-fatality) (Fiji Ministry of Health and Medical Services [MHMS]). Cases were defined as positive

reactions to *Leptospira* ELISA IgM (Panbio, Brisbane, Australia); this laboratory test was only available at the national reference laboratory, and it was likely that reported cases were an underestimate of the true scale of the outbreaks. In comparison, previous studies reported a total of 576 cases during an 8-year period from 2000-2007 [17], and 487 cases during a 13-year period from 1969-1981 [18]. These studies identified a higher risk of infection in males, indigenous Fijians (iTaukei), young adults (aged 15 to 45 years), rural dwellers and abattoir workers; increase in reported cases in the rainy months and after a cyclone in 2001; and geographic variation in incidence.

Following the outbreaks in 2012, the Fiji MHMS and the World Health Organization convened a leptospirosis expert consultation to review the epidemiology of leptospirosis in Fiji and recommend priorities for control of endemic and epidemic disease. A key conclusion of the expert consultation was that significant knowledge gaps in the current epidemiology of leptospirosis in Fiji limited effective prevention and control. The study described in this paper was identified as one of several important steps to address the knowledge gaps. This study uses an eco-epidemiological approach and framework [19] to characterize the epidemiology and risk factors for human leptospirosis infection in Fiji, and aimed to provide an evidence base for improving the effectiveness and efficiency of public health mitigation and intervention strategies. Our findings would also be relevant to other countries with similar environments, particularly in the South Pacific.

A3.2 Methods

A3.2.1 Study location and population

The Republic of the Fiji Islands is an archipelago of 322 islands with a population of 837,217 in 2007; indigenous Fijians (iTaukei) and Indo-Fijians (Fijians of Indian descent) account for 57% and 35% [20] of the population respectively. Fiji is considered a 'small island developing state' by the United Nations [21] with a per capita GDP of US$4,712 [22]. The main island of Viti Levu has a landmass of 10,349 square kilometers and is home to >70% of the population. Vanua Levu is the second largest island in both population and land area, followed by Taveuni. The largest urban centre is the Greater Suva Area (population ~ 180,000) on the southeast coast of Viti Levu. The largest administrative units in geographical size are the Divisions (Central, Western, Northern, and Eastern) followed by Provinces (14 in total), Tikinas (86 in total), and Enumeration Areas (smallest unit for population census that typically include 80 to 120 households). Nursing zones are the smallest administrative unit of the

MHMS; they are under the care of a single nursing station and form a contiguous network across the Fijian Islands. Communities are residential clusters defined by MHMS and used for administrative purposes. The four main community types in Fiji are urban residential areas, villages, Indo-Fijian settlements, and mixed Indo-Fijian/iTaukei settlements.

A3.2.2. Seroprevalence study and sampling design

Field data were collected from September to December 2013 (January to March being the wettest months), and included the Central Division (on the eastern side of Viti Levu), the Western Division (on the western side of Viti Levu), and the Northern Division (the islands of Vanua Levu and Taveuni). The Eastern Division, with a population of ~40,000 spread across multiple small islands groups, was not included in the study because of logistical reasons. Field data were collected for a sero-epidemiological study of typhoid as well as the leptospirosis study described here.

We conducted a cross-sectional seroprevalence study, with a four-stage sampling design. An overview of the sampling plan is shown in Figure A3.1. In the first stage, both population-proportionate sampling and purposeful sampling approaches were used. The former was used to select 28 nursing zones from the Central Division, 21 from the Western Division, 12 from the Northern Division and 4 from the Ba Province which lies within the Western Division. Due to high incidence of reported leptospirosis and post-flood outbreaks in 2012, the latter sampling approach was used to select 6 more nursing zones from the Ba Province. Similar to Ba Province, Taveuni Island (part of the Northern Division) was oversampled because of a high incidence of typhoid in 2008-2009. Consequently, 11 additional nursing zones were selected from this region, resulting in 82 zones in total being selected from the five regions in the first stage of sampling. At the second stage of sampling, one community was randomly selected from each of the 82 nursing zones. Headmen, health workers and other community leaders were consulted to seek agreement to participate in the study; no community leaders declined participation. At the third stage of sampling, 25 households were randomly selected from each community using health census records, if available, or using the World Health Organization's Expanded Programme on Immunization (EPI) sampling method. For the fourth and final stage of sampling, household members (defined as a person who stayed at the house the previous night) were enumerated and one selected at random for inclusion, except in Ba subdivision where up to three randomly selected household members were included. If a selected household member was absent but returning later that day, the survey team would await their return or made a repeat visit. Wholly absent household members were substituted from within the household. Empty households were substituted by selecting the nearest house to the right of the front door. The above sampling

strategy aimed to include 25 households in each of 82 communities, with up to three participants per household in Ba, and one participant per households in other areas. We therefore aimed to recruit a total of 2050 to 2250 participants.

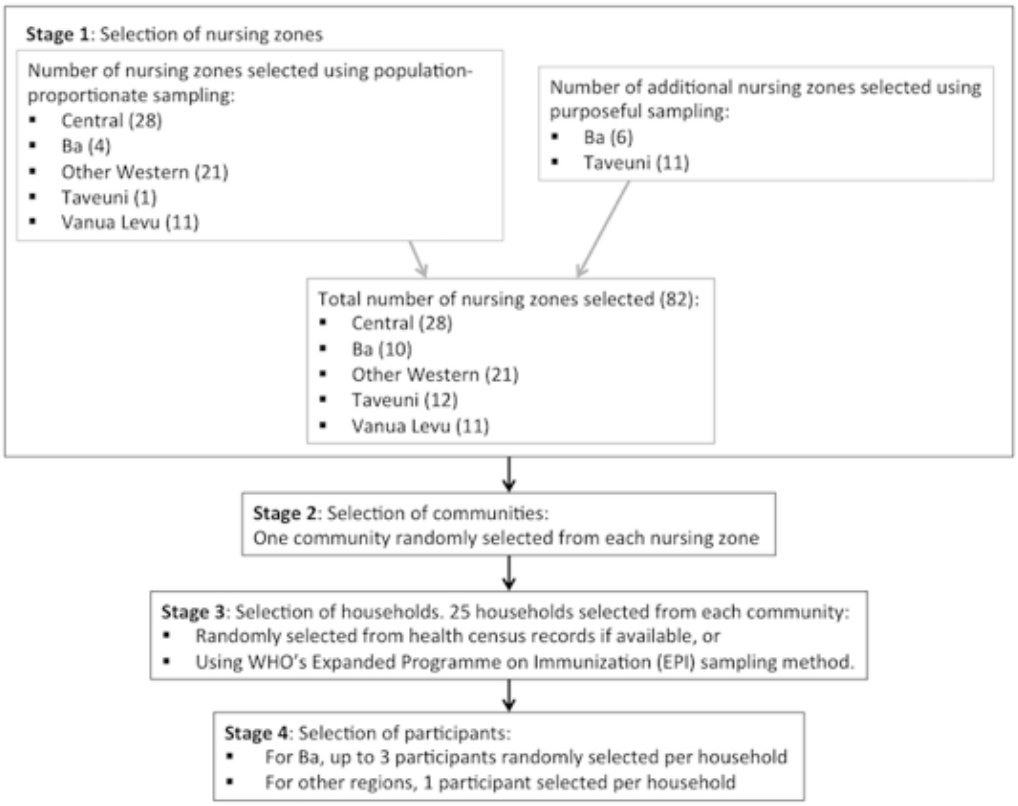
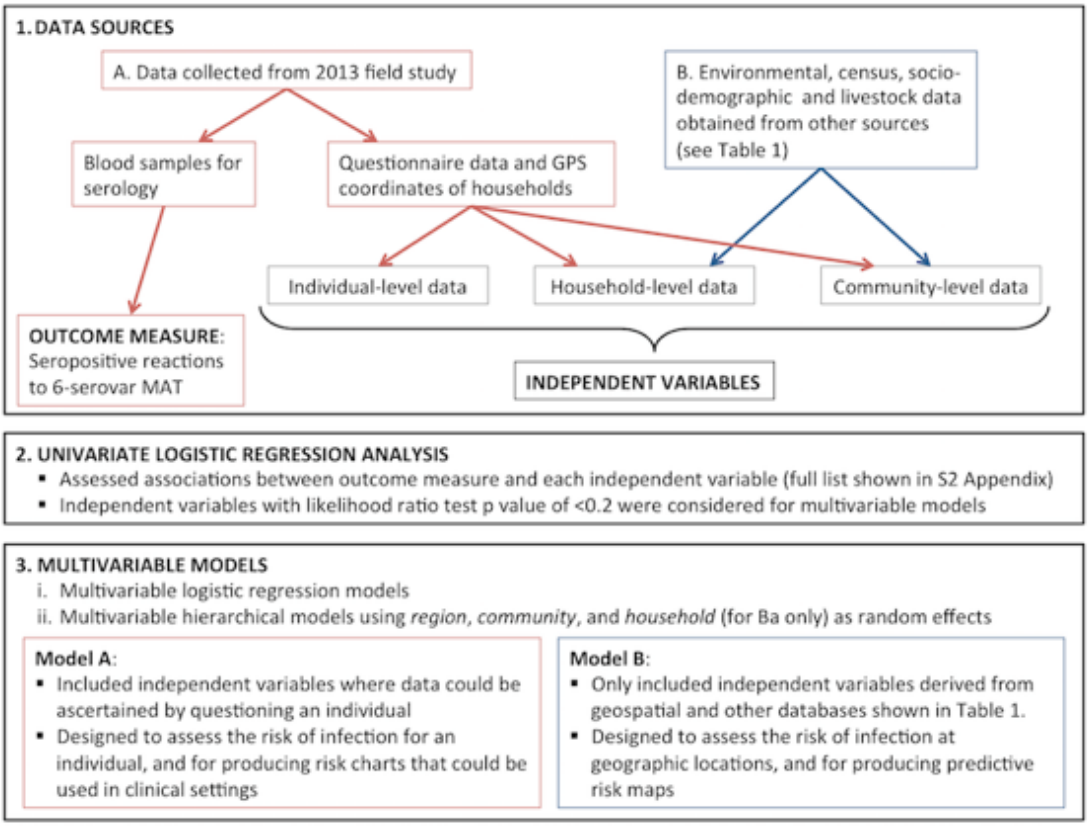


Figure A3.1. Overview of sampling strategy used in 2015 field study

Participants were eligible for inclusion if they were aged 12 months or older. Exclusion criteria included clotting disorders or medical anticoagulation, severe underlying medical conditions, significant acute illness, and fear of needles.

The communities included in the study represented the general population and different environments in Fiji (urban, peri-urban, rural), with higher sampling density in Ba and Taveuni. The study included a total of 82 clusters, with 28 in Central Division, 10 in Ba, 21 in other parts of the Western Division, 12 in Taveuni, and 11 in Vanua Levu. These areas will be referred to as the five 'regions' in this paper.

An overview of data sources and statistical methods is shown in figure A3.2.



. Figure A3.2. Overview of data sources and statistical methods

A3.2.3 Informed consent and ethics approvals

Ethics approvals were granted by the Fiji National Research Ethics Review Committee (2013 03), the Human Research Ethics Committee of The University of Queensland (2014000008) and the London School of Hygiene & Tropical Medicine (6344). Support was sought and obtained from divisional and sub-divisional Ministry of Health officers for community visits. To ensure that research activities were culturally acceptable and local customs respected, community visits were conducted with field teams that included multilingual local Fijians. The study was explained to the heads of each of the randomly selected households, or another competent adult, and permission sought to include the household in the study. Written or thumb-printed informed consent was obtained from adult participants. The ethics committees specifically approved the use of thumbprint informed consent in illiterate participants. Parental/guardian consent and informed assent was obtained for child participants.

A3.2.4 Data collection during field study

The following were collected from each participant:

1. Venous blood samples, collected by trained phlebotomists under sterile conditions (5-8mL depending on the age of the participant).

2. Questionnaire data, using standard questionnaires administered by field research assistants, and conducted in English or other local languages depending on each participant's preference. Questions related to demographics, income, occupation, recreational activities, household environment, contact with animals, and other potential risk factors for leptospirosis.

3. Geographic Positioning System (GPS) coordinates of the place of residence, using handheld GPS devices.

A3.2.5 Environmental, census, socio-demographic and livestock data

Environmental data on hydrology and roads were obtained from the Fiji Ministry of Lands and Mineral Resources [23]; and soils and land use/cover data from Fiji Ministry of Agriculture [24]. Climate (temperature and rainfall) and elevation data were obtained from the Landcare Research Institute [25]. Data on educational attainment, household construction, employment, ethnicity, and other socio-demographic variables were obtained from the 2007 Fiji National Census [20], and data on poverty rates from the 2011 World Bank Report [26]. Livestock data were provided by the Fiji Ministry of Agriculture's 2009 National Agricultural Census (unpublished data). All geospatial data were georeferenced to the Fiji Map Grid 1986 coordinate system. Table A3.1 provides a summary of the environmental, census, socio-demographic and livestock data used in the study. The five geographic regions used in this study and examples of the geo-referenced data are shown in Figure A3.3.

Household GPS coordinates from the study were projected on to the Fiji Map Grid 1986 coordinate system. Attributes from the geospatial predictor layers were linked to each household location by intersecting points through polygons for vector GIS data, and sampling the raster GIS data in a similar fashion. As a result, GIS attributes for each predictor layer were obtained for each household location. Attributes for a community were obtained by first calculating the location of the median centre of sampled households, followed by an approach similar to that which was carried out for individual households. All GIS data preparation and analysis was performed using ArcGIS v10.1 (Environmental Systems Research Institute, Redlands, CA).

Maps were drawn to present seroprevalence at each community; household-level results were not mapped in order to protect the identity of participants. Locations of communities were mapped to their median centre, calculated as the location nearest to all sampled households in the community while minimizing the effects of outliers.

All GIS data preparation and analysis was performed using ArcGIS v10.1 (Environmental Systems Research Institute, Redlands, CA).

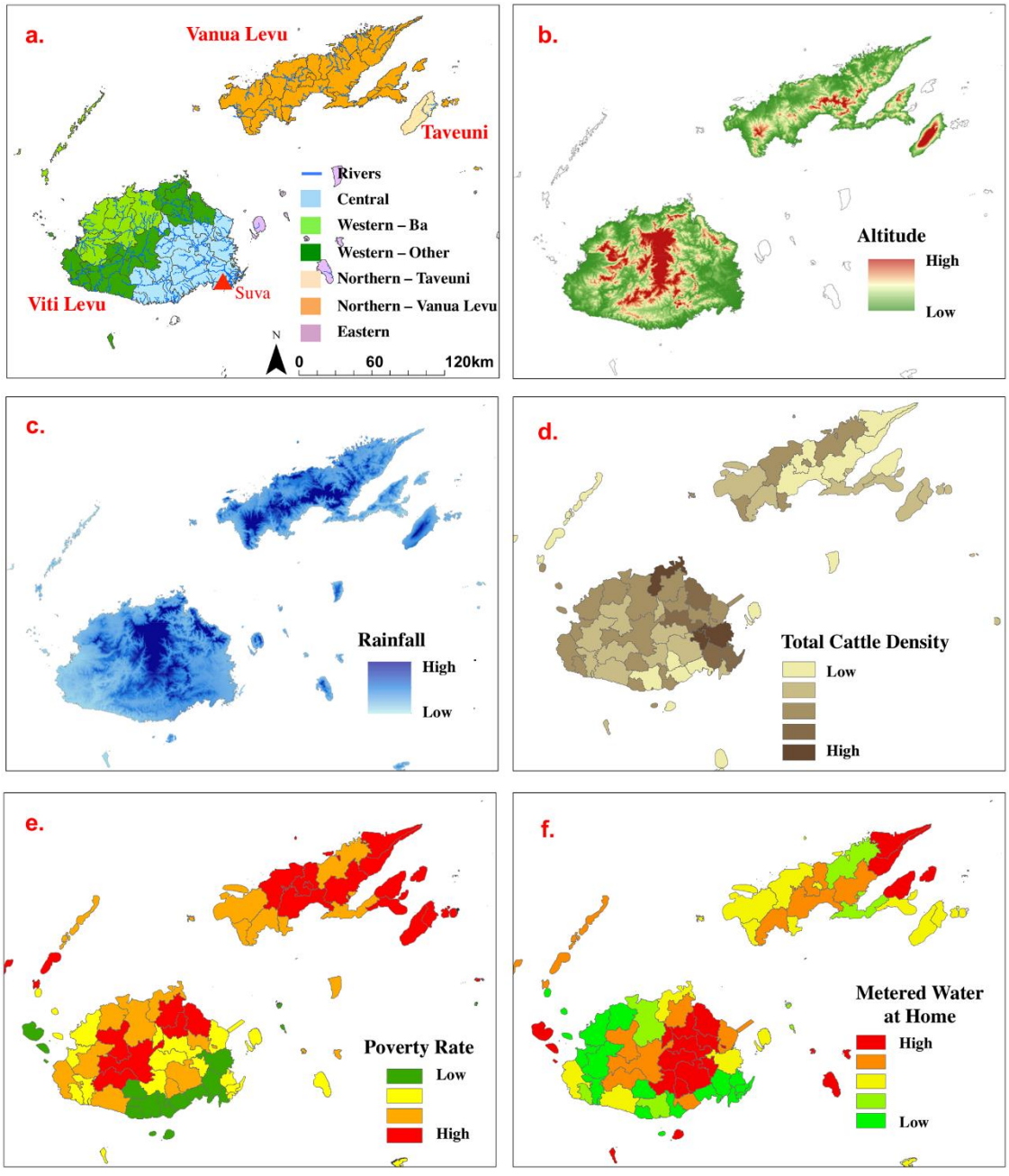


Figure A3.3. Fiji geography, and examples of environmental and census data
a) Divisions and 'regions' included in the study, major rivers; b) altitude; c) rainfall; d) total cattle density; e) poverty rate; f) proportion of households with metered (treated) water at home. See Table A3.1 for data sources.

Table A3.1. Summary of environmental, census, socio-demograhpic and livestock data used

Data	Source	Variables examined	Description & Resolution
Hydrology	Fiji Ministry of Lands and Mineral Resources. Digital data from FLIS (Fiji Land Information System). Original 1:50K topographic maps [23].	Distance to rivers, major, minor creeks.	Euclidean distance to rivers, major, minor creeks. 25 m raster data.
Roads	Fiji Ministry of Lands and Resources. Digital data from FLIS (Fiji Land Information System). Original 1:50K topographic maps.	Road density.	Number of roads per sq km within 1 km radius. 25 m raster data.
Soils	Fiji Ministry of Agriculture. 1980/85 National Soil Survey [24].	Soils of major and secondary floodplains, and depressions.	Soil units and distance from soil units. 25 m raster data.
Land use/cover	Ministry of Agriculture. Digital data from Secretariat of the Pacific (SPC). Circa 2010.	Multiple land use/cover types.	Visual interpretation of satellite imagery. 25 m raster data.
Climate	Landcare Research Institute [25].	Annual, maximum, minimum, and average temperature and rainfall	Spatially interpolated climate data meteorological station data from 1971-2000. 100 m raster data.
Elevation	Landcare Research Institute [3].	Altitude and slope	Elevation derived from 20 m contours. 25 m raster data.
Census	Fiji National Census 2007 [20].	Multiple measures of education attainment, house construction, employment, ethnicity, and other sociodemographic factors.	Census variables available at the Enumeration Area level (~80-120 households). Vector count data converted to proportions.
Economic status	World Bank (2011) report [26].	Poverty rate (% of population below poverty line) and poverty gap (how far on average. people are from the poverty line)	Poverty rates estimated using small area estimation method. Vector data at the Tikina level.
Livestock	Fiji Ministry of Agriculture. Unpublished data from the 2009 Fiji Agricultural Census.	Number of farms and farm animals by species: cattle, commercial beef, dairy, subsistence beef, etc.	Density per sq km calculated by Tikina (not available for all Tikinas).

A3.2.6 Stratification of independent variables

Independent variables were stratified according to the ecological level at which they could potentially influence the risk of leptospirosis transmission and infection. Individual-level data relate to risk factors that are specific to individual demographics or behaviour. Household-level and community-level data include risk factors that are common to all inhabitants of a household and community respectively.

- **Individual-level data**. Potential risk factors for leptospirosis were assessed using questionnaire-based interviews, including demographics, occupation, recreational activities, contact with animals, education, and knowledge about leptospirosis.
- **Household-level data**. Information on household income, house construction, access to utilities (toilets, water, sewage, electricity), and the presence of animals and crops around the home were obtained through questionnaires. In addition, data on environmental attributes (including rainfall, temperature, elevation, land cover, soil type, and distance to rivers) at household locations were extracted or calculated using geographic information systems (GIS) as described above.
- **Community-level data**. Community type, urban/rural settings, and the presence of animal species in each community were ascertained through questionnaires. Census and agricultural data were extracted or calculated using the geospatial databases described in Table A3.1. Data were available at the enumeration area resolution (~80-120 households) for a variety of socioeconomic and demographic measures, including the proportions of households with metered water, toilets, electricity, and sewage services; population ethnicity, level of educational attainment, and reliance on subsistence farming as the main source of income. At the Tikina level, data were available on World Bank estimates of poverty measures, and census of animal populations conducted by the Fiji Ministry of Agriculture.

A3.2.7 Maps

Maps were produced to show the locations of communities that participated in the field study, and the observed community-level seroprevalence in 2013. Although all household GPS locations were recorded, only community-level seroprevalence were depicted on maps to protect the identity of participants.

A3.2.8 Serological analysis

Blood samples were processed in Fiji, and frozen sera transported to Australia, for serological analysis. Microscopic agglutination tests (MAT) were used to detect anti-*Leptospira* antibodies, and determine the putative serogroups associated with infections. The MAT is the reference serological test recommended by the WHO and the International Committee on Systematic Bacteriology (Subcommittee on the Taxonomy of *Leptospira*) [27,28]. Serological analyses were conducted at the WHO/FAO/OIE Collaborating Centre for Reference & Research on Leptospirosis in Brisbane, Australia.

Based on the laboratory's knowledge of the epidemiology of leptospiral serovars in the South Pacific, 21 pathogenic serovars (see Appendix) were selected for the MAT panel for this study, and samples were tested at dilutions from 1:50 to 1:3200. The 21-serovar panel was used to test a random selection of ~10% of total samples to determine the most common serogroups responsible for infections in humans. In addition, the 21-serovar panel was to test 199 *Leptospira* ELISA-positive samples collected from patients with suspected clinical leptospirosis in Fiji in 2012 and 2013 to ensure that the most common serogroups to identify the most important serogroups associated with clinical infections. Based on the MAT results from the two sets of sera, six serovars were chosen for the final panel used to test the remaining samples from this study (A3.S1 Appendix): *Leptospira interrogans* serovars Pohnpei (serogroup Australis), Australis (serogroup Australis), Canicola (serogroup Canicola), Copenhageni (serogroup Icterohaemorrhagiae), Hardjo (serogroup Sejroe), and and *Leptospira borgpetersenii* serovar Ballum (serogroup Ballum).

The MATs assay is expensive and time-consuming, and the described strategy to limit the number of serovars included in the panel resulted in reduced project costs. Considering that one dominant serovar was identified in the preliminary tests, it was determined that the smaller MAT panel was unlikely to have significant impact on the overall epidemiological findings. MAT titres of ≥1:50 were considered reactive or seropositive, and indicative of recent or past infection. For samples that reacted to multiple serovars within a serogroup, the serovar associated with the highest titre was considered to be the reacting serovar. Samples that reacted to serovars in more than one serogroup were recorded as reacting to multiple serovars. Although serogroups are no longer used in the taxonomic classification of serovars, they remain useful for laboratory purposes and epidemiological comparisons.

A3.2.9 Statistical analysis

An overview of the statistical analyses is shown in Figure A3.2. The outcome measure used was seropositive reactions to any of the six serovars included in the final MAT panel. Firstly, crude associations between the independent variables and the outcome measure were obtained by univariable logistic regression. Independent variables associated with the outcome by a likelihood ratio test (LRT) p-value of <0.2 were then subjected to a stepwise backward elimination process ($p<0.05$) to select the final set of independent variables for the multivariable logistic regression models. In addition, the possible presence of effect modification in the multivariable modelling was investigated using the LRT. This was assessed using interaction terms, which consisted of all independent variables found to be significant in the univariable analysis. Interaction terms were added separately to the analyses to determine their joint effect on the outcome measure. Multilevel hierarchical modelling was used to take into account the clustering of participants, and allowed for correlation of observations by *region* (n=5), *community* (n=82), and *household* (up to 3 participants per household in Ba) as random effects. Intra-cluster correlation coefficients (ICCs) with corresponding 95% confidence intervals were obtained from final multivariable models. Biological plausibility and collinearity between variables were taken into account when selecting the variables to be retained in the final models. For example, if we observed strong collinearity between poverty rate and absence of electricity at home, poverty rate would be chosen for the final model because of more direct relationship to exposure risks.

Two multivariable models were built:

Model A: Used independent variables where data could be ascertained by questioning an individual, and included primarily individual-level variables, but also some household-level and community-level variables. Model A was designed to assess the risk of infection for an individual, e.g. for producing predictive risk charts to graphically depict the combined effects of variables in determining overall seroprevalence. The charts are designed for use in clinical settings, and are similar to cardiovascular risk charts used to predict the risk of cardiac events based on combinations of risk factors such as blood pressure, diabetes, smoking, and cholesterol levels.

Model B: Used independent variables derived from geospatial and other national databases described in Table A3.1, without using the field data collected in this study. Model B included household-level and community-level variables only, and was designed to assess the risk of infection at geographic locations, e.g. estimating community-level seroprevalence, identifying hotspots and producing predictive risk maps.

Independent variables found to be statistically significant on multivariable regression analyses were reported. Adjusted odds ratios (OR) with 95% confidence intervals obtained from regression coefficients were used to quantify associations between the independent and outcome variables. In addition, univariate results of variables associated with animal exposure and contact were reported. Statistical significance was considered at $p < 0.05$ and two-sided. Data analysis was performed using STATA 13 (StataCorp, 2013). Model fit was assessed using the Hosmer-Lemeshow test [29], while relative predictive performance was undertaken using the area under the receiver operating curve (AUC) was calculated for each model and compared for statistical differences. An AUC of 0.7 was deemed to indicate an adequate predictive ability of the model. Akaike information criterion (AIC) and Bayesian information criterion (BIC) were reported for the final models.

A3.3 Results

A3.3.1 Study population

A total of 2152 participants from 1922 households in 82 communities on the three main islands of Fiji were included in the study. The age of participants ranged from 1 to 90 years (mean 33.6, SD 19.8), and 985 (45.8%) were males. The age and sex distribution of participants are shown in Figure A3.4.

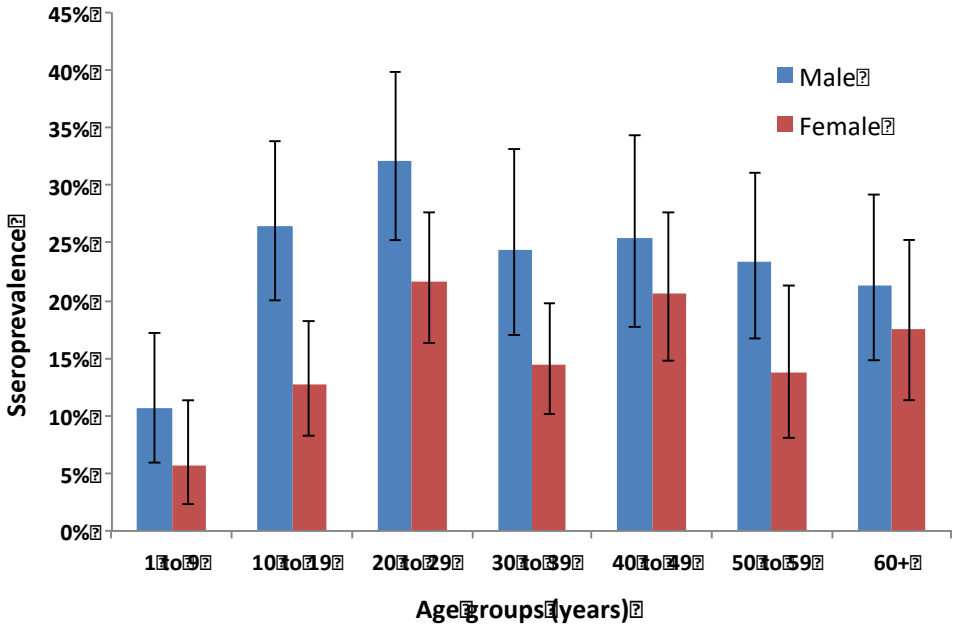


Figure A3.4. Seroprevalence by age group and gender.
Seroprevalence was defined as the percentage of participants with reactive MAT (≥ 1:50) to at least one of the 6 serovars used in the final panel. Blue=male. Red=female.

The study included 662 participants from the Central Division (28 communities), 453 from Ba (10 communities), 520 from other parts of the Western Division (21 communities), 261 from Taveuni (12 communities), and 256 from Vanua Levu (12 communities) (Table A3.2).

Table A3.2. Leptospira seroprevalence by age, gender, ethnicity, community types, and region

Variables		**No of participants**	**Reactive MATs***	**Sero-prevalence (%)**	**95% CI**
Total sampled		2152	417	19.4%	17.7 – 21.1%
Gender	Male	985	234	23.8%	21.1 – 26.5%
	Female	1160	182	15.7%	13.6 – 17.9%
Age groups (years)	0 – 9	256	21	8.2%	5.1 – 12.3%
	10 – 19	362	69	19.1%	15.1 – 23.5%
	20 – 29	387	101	26.1%	21.8 – 30.8%
	30 – 39	340	61	17.9%	14.0 – 22.4%
	40 – 49	279	63	22.6%	17.8 – 27.9%
	50 – 59	263	50	19.0%	14.5 – 24.3%
	≥ 60	263	52	19.8%	15.1 – 25.1%
Ethnicity	Indo-Fijian	459	34	7.4%	5.2 – 10.2%
	iTaukei	1651	374	22.7%	20.7 – 24.7%
	Other	39	8	20.5%	9.3 – 36.5%
Community type	Private residential	502	44	8.8%	6.4 – 11.6%
	Settlement (Indo-Fijian)	103	18	17.5%	10.7 – 26.2%
	Settlement (mixed ethnicity)	511	91	17.8%	14.6 – 21.4%
	Village	1036	264	25.5%	22.9 – 28.3%
Urban/Rural	Urban	579	64	11.1%	8.6 – 13.9%
	Peri-urban	287	44	15.3%	11.4 – 20.0%
	Rural	1286	309	24.0%	21.7 – 26.5%
Region	Central Division	662	107	16.2%	13.4 – 19.2%
	Western Division – Ba	453	82	18.1%	14.7 – 22.0%
	Western Division – Other	520	94	18.1%	14.9 – 21.7%
	Northern Division – Taveuni	261	59	22.6%	17.7 – 28.2%
	Northern Division – Vanua Levu	256	75	29.3%	23.8 – 35.3%

*Reactive MAT defined at titre of ≥1:50 for one or more serovars used in the 6-serovar panel

3.3.2 Seroprevalence and serovars

Details of the 21 serovars included in the screening panel and the seroprevalence of the initial 198 randomly selected samples are shown in A3.S1 Appendix, together with the six serovars included in the final MAT panel which accounted for 86.7% of reactive tests: *Leptospira interrogans* serovars Pohnpei, Australis, Canicola, Copenhageni, Hardjo, and and *Leptospira borgpetersenii* serovar Ballum. Using the 6-serovar panel, the overall seroprevalence was 19.4% (95% CI 17.7% - 21.1%), with 417 participants having reactive MATs to at least one serovar. One predominant serovar, Pohnpei, accounted for 351 (84.2%; 95% CI 80.3% - 87.5%) of reactive MATs. A total of 63 participants had MAT titres of ≥1:400 (47 for serovar Pohnpei, and 16 for other serovars), the cutoff used by our laboratory to indicate an acute infection. The distribution of MAT titres for Pohnpei and other serovars is shown in Figure A3.5.

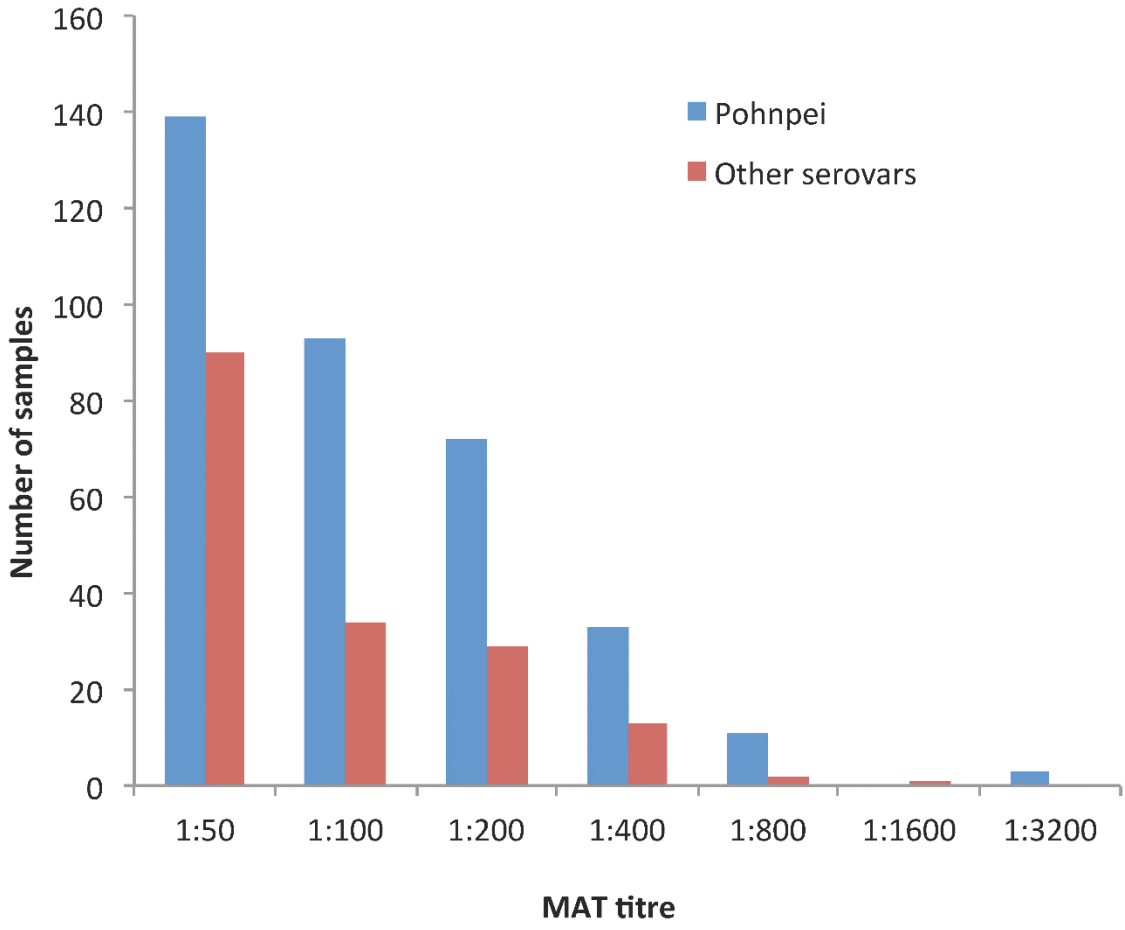


Figure A3.5. Distribution of MAT titres for serovar Pohnpei (blue) and other serovars (red); using the final panel of 6 serovars.

Table A3.2 shows that there were significant differences in seroprevalence by age, gender, ethnicity, community types, and region. Community-level seroprevalence ranged from 0% to 60%, and are shown on the maps in Figures A3.6a-d. Variations in seropositive reactions to each serovar by age groups and region of residence are shown in Figures A3.7a&b respectively.

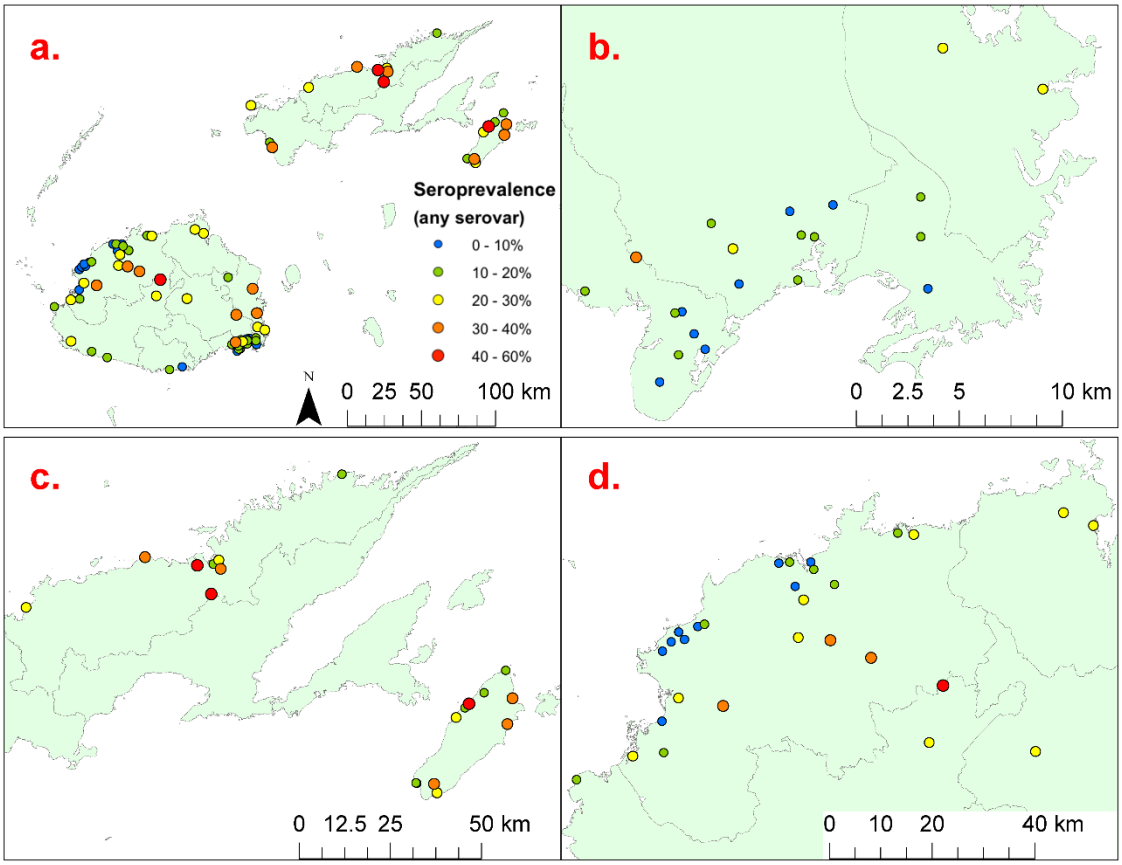


Figure A3.6. Community-level seroprevalence at the 82 communities included in the study

a) prevalence varied from 0% to 60%; b) enlargement of the Suva area in eastern Viti Levu; c) enlargement of Taveuni and eastern Vanua Levu; and d) enlargement of northwestern Viti Levu including Ba.

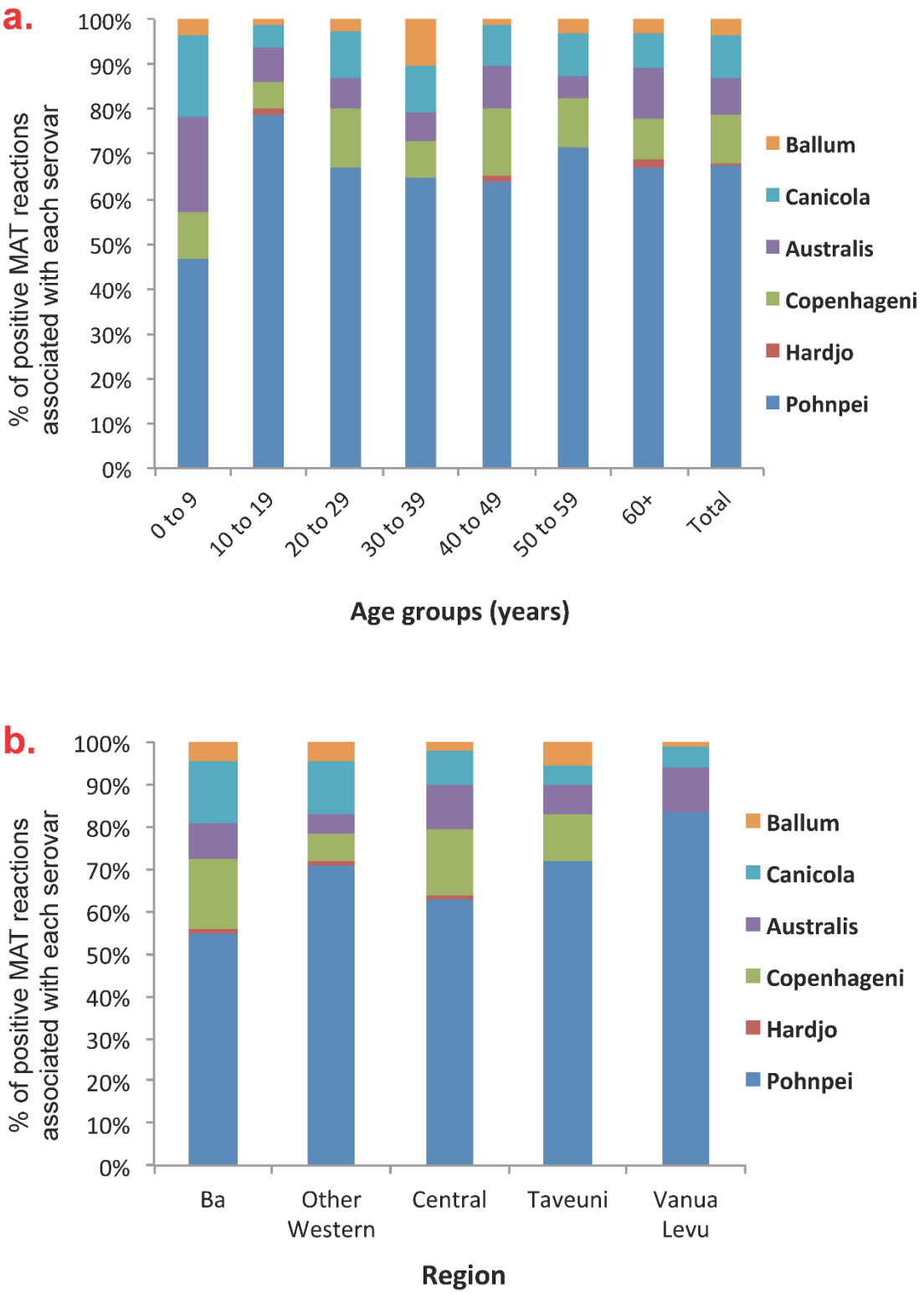


Figure A3.7. Percentage of positive MAT reactions associated with each of the 6 serovars included in the final panel by: a) age groups, and b) regions.
Positve MAT reactions defined as titre of ≥ 1:50.

A3.3.3 Risk factor analysis and multivariable models

A total of 118 independent variables were assessed on univariate analysis: 75 variables obtained from questionnaires, and 43 derived using GIS from the sources described in Table A3.1. Independent variables included 31 individual-level, 38 household-level, and 49 community-level risk factors described above. A3.S2 Appendix provides a list of the independent variables assessed at the univariate level. Variables that were statistically significant on univariate analyses were considered for the multivariable models, and included 19 individual-level, 21 household-level, and 25 community-level risk factors. Due to statistical significance not be reached, no interaction effect was included in the multivariable modelling.

Multivariable Model A (using variables where data could be ascertained by questioning an individual) included five variables that were independently associated with the presence of *Leptospira* antibodies, with an AUC of 0.7 (Table A3.3) including: male gender (OR 1.55 compared to females), iTaukei ethnicity (OR 3.51 compared to Indo-Fijians), living in settlements and villages (OR 2.13 and 1.64 respectively compared to urban residential areas), not having metered water at home (OR 1.52), and working outdoors (OR 1.64 compared to working indoors). Of the 434 participants who worked outdoors, 378 (87%) were full- or part-time farmers, indicating that outdoor work in Fiji is predominantly related to farming.

Multivariable Model B (using only variables derived from geospatial and other national databases) included six variables that were independently associated with the presence of *Leptospira* antibodies, with an AUC of 0.7 (Table A3.4) including: living in rural areas (OR 1.43 compared to living in urban or peri-urban areas), poverty rate ≥ 40% (OR 1.74), living <100m from a river or major creek (OR 1.41), presence of pigs in the community (OR 1.54), total cattle density in the Tikina (OR 1.04 per head of cattle per square km), and high maximum rainfall in the wettest month (OR 1.003 per mm of rain). Total cattle density (includes both commercial and subsistence livestock) ranged from 0.11 to 31.48 head of cattle per square km (mean 8.96, SD 5.31), and maximum rainfall in the wettest month ranged from 275 to 789mm (mean 375.02, SD 56.94). A similar multivariable model using total dairy farm density instead of total cattle density performed better than the final model (results not shown), but data on dairy farm density were only available for 57 of the 82 (69.5%) communities included in our study, and this variable was therefore not used in the final Model B.

Table A3.3. Variables significantly associated with positive MAT for Leptospira on univariable and multivariable analysis – Model A^ (individual-level variables)

Variables		**No of subjects**	**Reactive MAT***	**Sero-prevalence (%)**	**Univariable Odds Ratio (95% CI)**	**Adjusted Odds Ratio (95% CI)**	***p* value#**
Total sampled		2152	417	19.4%			
Gender	Female	1160	182	15.7%	1	1	
	Male	985	234	23.8%	1.67 (1.35–2.08)	1.55 (1.16–2.08)	0.003
Ethnic group	Indo-Fijian	459	34	7.4%	1	1	
	iTaukei	1651	374	22.7%	3.66 (2.53–5.29)	3.51 (2.23–5.54)	<0.001
	Other	39	8	20.5%	3.23 (1.38–7.56)	2.32 (0.82–6.58)	0.114
Community type	Urban residential	502	44	8.8%	1	1	
	Settlement	614	109	17.8%	2.25 (1.55–3.26)	2.13 (1.41–3.21)	<0.001
	Village	1036	264	25.5%	3.56 (2.54–5.00)	1.64 (1.08–2.51)	0.021
Metered water available at home	Yes	1412	221	15.7%	1	1	
	No	720	189	26.3%	1.92 (1.54–2.39)	1.52 (1.14–2.03)	0.004
Work location	Indoors	832	106	12.7%	1	1	
	Mixed indoors/ outdoors	639	123	19.2%	1.63 (1.23–2.17)	1.65 (1.23–2.20)	0.001
	Outdoors	434	119	27.4%	2.59 (1.93–3.47)	1.64 (1.15–2.34)	0.006

^ Model goodness of fit: AIC 1675.9, BIC 1731.3, df 10.

*Reactive MAT defined at titre of ≥1:50 for one or more serovars used in the 6-serovar panel

 # p value for adjusted odds ratios, multivariable model

Table A3.4. Variables significantly associated with positive MAT for Leptospira on univariable and multivariable analyses – Model B^ (environmental and census variables)

Variables		**No of subjects**	**Reactive MAT***	**Sero-prevalence (%)**	**Univariable Odds Ratio (95% CI)**	**Adjusted Odds Ratio (95% CI)**	***p* value#**
Urban/Rural	Urban/ Peri-urban	866	108	12.5%	1	1	
	Rural	1286	309	24.0%	2.22 (1.75–2.82)	1.43 (1.07–1.91)	0.016
Poverty rate	< 40%	1277	187	14.6%	1	1	
	≥ 40%	875	230	26.3%	2.08 (1.67–2.58)	1.74 (1.31–2.31)	<0.001
Distance between home and river or major creek	> 100m	1590	279	17.6%	1	1	
	≤ 100m	456	115	25.2%	1.58 (1.24 –2.03)	1.41 (1.09–1.83)	0.009
Presence of pigs in community	No	1587	266	16.8%	1	1	
	Yes	561	150	26.7%	1.81 (1.44–2.28)	1.54 (1.21–1.98)	0.001
		Mean (Standard deviation)					
Total cattle density in Tikina§ (per head of cattle per sq km)	Seronegative subjects	8.86 (5.18)					
	Seropositive subjects	9.38 (5.83)			1.02 (1.00–1.04)	1.04 (1.02–1.06)	<0.001
Maximum rainfall in wettest month (per mm)	Seronegative subjects	372.83 (50.05)					
	Seropositive subjects	384.18 (79.01)			1.003 (1.001–1.005)	1.003 (1.001–1.005)	0.002

^Model goodness of fit: AIC 1924.3, BIC 1963.6, df 7.

*Reactive MAT defined at titre of ≥1:50 for one or more serovars used in the 6-serovar panel

 # p value for adjusted odds ratios, multivariable model

§ Includes both commercial and subsistence cattle

Collection of biological samples from animals was outside of the scope of this study, but study questionnaires included detailed information about contact with animals (rodents, mongoose, pets, and livestock) at home and the presence of animals in the community. A number of animal-related exposures were significantly associated with the presence of *Leptospira* antibodies on univariable analysis (Table A3.5). The presence of rats, mice, and mongoose around the home was not significantly associated with seroprevalence, but higher infection rates were found in participants who reported physical contact with rats or mice (OR 1.58) and mongoose (OR 1.81). Table A3.5 shows that many Fijians have livestock animals at home and in the community. The presence of each livestock species was associated with a higher infection rates on univariable analysis, but only the presence of pigs in the community was significant on multivariable analysis, and included in Model B.

Table A3.5. Associations between positive MAT for Leptospira and animal exposure at home and in the community

Questions related to animal exposure and contact	**Number of subjects who answered 'yes'**	**% of subjects who answered 'yes'**	**Univariable Odds Ratio (95% CI)**	***p* value**
Seen rats or mice at or around your home	1844	85.9%	1.16 (0.84 –1.59)	0.371
Been in physical contact with rats or mice	323	15.3%	1.58 (1.20–2.09)	0.001
Seen mongooses at or around your home	1655	77.1%	1.08 (0.83–1.39)	0.574
Been in physical contact with mongooses	135	6.5%	1.81 (1.23–2.68)	0.003
Pigs at your home or in your garden	230	10.7%	1.55 (1.23–2.12)	0.007
Pigs in your community	561	26.1%	1.81 (1.44–2.28)	0.000
Cows at your home or in your garden	284	13.2%	1.53 (1.15–2.05)	0.004
Cows in your community	481	22.4%	1.52 (1.19–1.93)	0.001
Horses at your home or in your garden	200	9.3%	1.53 (1.09–2.14)	0.013
Horses in your community	377	17.6%	1.55 (1.19–2.01)	0.001
Are there goats at your home or in your garden?	107	5.0%	1.08 (0.67–1.75)	0.749
Goats in your community	242	11.3%	1.47 (1.08–2.01)	0.015
Chickens at your home or in your garden	431	20.1%	1.21 (0.93–1.57)	0.152
Chickens in your community	819	38.1%	1.39 (1.12–1.72)	0.003
Dogs at your home or in your garden	645	30.0%	1.00 (0.79–1.26)	0.992
Dogs in your community	998	46.5%	1.25 (1.01–1.55)	0.041
Cats at your home or in your garden	355	16.5%	0.78 (0.58–1.06)	0.115
Cats in your community	830	38.6%	1.38 (1.11–1.72)	0.003

Two multilevel hierarchical models were built to take into account spatial correlation of data: i) defining *region* and *community* as random effects, using the entire dataset, and ii) defining *community* and *household* as random effects, using Ba data only. The results of multilevel models were not statistically different to the results of multivariable Model A (chi2 = 0.01, p = 0.99) or multivariable Model B (chi2 = 0.02, p = 0.99), and therefore not reported here because coefficient estimates were very similar to the reported models.

A3.3.4 Seroprevalence estimation chart using Model A

Figure A3.8 shows a seroprevalence estimation chart that incorporates individual-level variables to show the combined effects of multiple independent risk factors on the prevalence of infection. Estimated seroprevalence were based on the five variables used in Model A. For example, the chart shows a range of seroprevalence from 2.0% for female Indo-Fijians who live in urban residential areas, have metered water at home, and work indoors; to 34.2% for male iTaukei who live in villages, do not have metered water and home, and work outdoors. It is uncommon for Indo-Fijians to live in villages or for iTaukei to live in Indo-Fijian settlements, and results were therefore not shown for these scenarios.

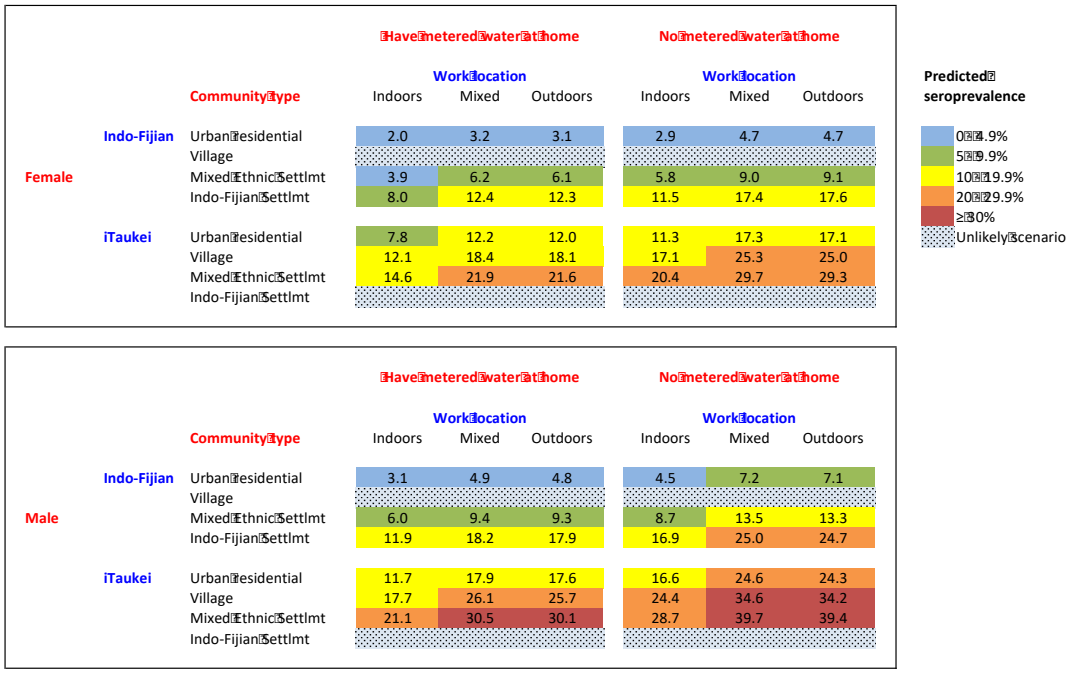


Figure A3.8. Seroprevalence estimation chart based on Model A, a multivariable logistic regression model of individual-level variables for a) females and b) males.

The chart shows the combined effects of independent risk on the estimated prevalence of leptospirosis infection. Seroprevalence was defined as as the percentage of participants with reactive MAT (≥ 1:50) to at least one of the 6 serovars in the final panel.

A3.4 Discussion

Our study identified a high risk of human leptospirosis infection in Fiji, with an overall seroprevalence of 19.4% using a 6-serovar MAT panel. One dominant serovar, Pohnpei, was associated with 84.2% of reactive MATs. The serovar was originally isolated from rodents and pigs during an animal leptospirosis study in the island of Pohnpei in the Federated States of Micronesia [30], and has been found to be an important cause of human infections [31]. Seroprevalence varied significantly between the five regions in our study, and ranged from 16.2% in the Central Division to 29.3% in Vanua Levu in the Northern Division. Community-level seroprevalence also varied significantly from 0% to 60% in the 82 communities included in our study. These findings indicate marked geographic variation in infection risk in Fiji and the presence of hotspots where disease transmission is more intense.

Globally, reported leptospirosis seroprevalence vary significantly between and within countries, based on environmental settings, behavioural risk factors, and socio-demographics; our results corroborate these findings. To put the Fiji results into a global context, examples of seroprevalence reported from known high risk settings [2,4] such as urban slums, tropical islands, and flood risk areas include 15.4% in an urban slum in Brazil [32], 37% of healthy adult males in the Seychelles [33], 18.8% in the Mekong delta in Vietnam [34], and 23.9% and 38.2% in flood-prone areas in Laos and Bangladesh respectively [35,36]. As found in Fiji, small-scale variations in seroprevalence within countries and differences between occupational groups have been reported. In American Samoa (a group of remote islands in the south Pacific), a community-based study reported an overall seroprevalence of 15.5% [8] and significant variation between islands with different environments, and between areas with different population density [37]. In Peru, seroprevalence varied from 28.0% in the Amazonian city of Iquitos and 16.5% in the surrounding villages (wet tropics), to 0.7% in a desert shantytown near Lima [38]. In the Andaman Islands, a study of high-risk populations found seroprevalence of 62.5% in agricultural workers, 39.4% in sewage workers, 37.5% in animal handlers, and 30.0% in butchers [39]. In contrast, a study of healthy blood donors in an area of high leptospirosis incidence in northern Queensland in Australia found a seroprevalence of only 1.4% [40,41].

Our study found that individual-level factors were important predictors of leptospirosis infection risk in Fiji. Model A shows that gender, ethnicity, community type, availability of water at home, and work location were independently associated with the presence of *Leptospira* antibodies. Higher infection rates in males corroborates findings in the majority of leptospirosis studies around the world, and is likely to be associated with higher frequency of

outdoor activities as well as higher risk occupational and recreational exposures. Reasons for the marked difference in seroprevalence between the two main ethnic groups in Fiji are unclear, but could be related to differences in genetic susceptibility or behaviours that were not elucidated by our questionnaire, e.g. differences in animal husbandry or slaughtering practices related to religion or culture. Further studies are required to explain the disparate risk between ethnic groups. Seroprevalence in villages was significantly higher than in urban residential areas or settlements, and is likely the result of more intimate contact with the natural environment and domestic animals. In our study, working outdoors was associated with a higher risk of infection, and the majority of outdoor work in Fiji involves farming. Agriculture is an important part of Fiji's economy, and apart from the livestock industry, there is commercial farming of a range of crops include sugarcane, coconut, copra, and a wide variety of fruits and vegetables. Occupational exposure in the agricultural industry is therefore likely to be an important source of leptospirosis infection in Fiji.

Of note, three of the predictors included in our final multivariable models were related to water: the *availability of metered (treated) water at home* (Model A), *distance between the home and the closest river or major creek* (Model B), and *maximum rainfall in the wettest month* (Model B). Considering that *Leptospira* can survive for weeks to months in fresh water, and are efficiently carried and disseminated by water (e.g. flooding, flowing downstream in rivers), the findings were not unexpected. Lack of metered water at home and proximity to rivers are likely to be associated with higher levels of contact with untreated freshwater, e.g. using rivers for bathing, cleaning, swimming, and recreational activities. Furthermore, poor access to water at home is generally associated with poverty (discussed below), and also influences personal hygiene, e.g. the ability to clean and wash after working outdoors, or after contact with mud, contaminated water, or animals. Two of the water-related predictors (*distance to river or major creek* and *maximum rainfall in the wettest month*) are also proxy measures of flooding risk. As seen with the post-flood leptospirosis outbreaks in 2012, flooding is an important driver of transmission in Fiji, as it is in many parts of the world.

Two of the predictors in Model B relate to livestock exposure: *total cattle density in the Tikina* and *presence of pigs in the community*. Data on cattle density in Tikinas includes both commercial and subsistence farming, and varied from < 1 to over 30 heads of cattle per square km. Infection risk could be related to direct occupational contact with cattle, or through more general contamination of the environment (especially rivers) with cattle urine. As shown in Table A3.5, many households in Fiji keep subsistence livestock. Backyard piggeries are commonly found in communities in Fiji and other Pacific Islands, and are usually

small pens with less than 10 pigs. The pens are often built on the edge of rivers and streams to allow convenient drainage of waste, but unfortunately also lead to contamination of freshwater at that community as well as further downstream. In American Samoa, similar backyard piggeries have been associated with the risk of human leptospirosis infection [8,42]. Dairy farmers are known to be at high risk for leptospirosis in many parts of the world because of close contact with cattle, and exposure to urine during milking. In our study, high density of dairy farms was strongly associated with infection risk, but was not included as a variable in the final model because data were only available for ~70% of the Tikinas in our study. As more data on dairy farms become available, associations with leptospirosis risk could be further explored and model performance potentially improved. Commercial dairy and beef farming could potentially intensify in the future with population growth, and increase the risk of leptospirosis.

Model B also shows that leptospirosis is a disease of poverty in Fiji and disproportionately affects the poorest. Leptospirosis has been associated with poverty in diverse settings around the world, including Brazilian and Indian urban slums [32,43,44], Peruvian Amazon [38], and areas of poor socioeconomic status in the USA and Europe [45,46]. Furthermore, the combination of poverty, livestock keeping, and global climate change are important drivers of zoonotic diseases transmission [47]. In our study, participants living in communities with high poverty rates (defined as ≥40% of households in the community) had almost twice the infection rate compared to other communities, independent of the other predictors in Model B. As discussed above, poor access to metered (treated) water at home was associated with a higher risk of infection for many reasons, and is also a proxy measure of socioeconomic status.

Although serovar Pohnpei was associated for 84.2% of reactive MATs, there were differences in serovar distribution by age and by region of residence, suggesting that the relative importance of animal species in disease transmission varies between subgroups. Variation in risk factors between age groups likely relates to age-specific behaviours, e.g. young children spend more time playing around the home, and have closer contact with pets and soil; teenagers have more frequent recreational freshwater contact from swimming in rivers and waterfalls; and adults have more intense contact with livestock through occupational exposure and managing animals at home. Variation in risk factors between regions likely relates to differences in environmental settings, with proportionately greater urbanization in the Central division, and more farming in the other regions. For example, rodents could be more important in transmission cycles in urban and peri-urban areas, and livestock more important in rural areas.

Many of the risk factors and environmental drivers identified in our study provide significant cause for concern about future risk of leptospirosis in Fiji, as well as other Pacific Islands with similar environments. Population growth is typically associated with agricultural intensification, leading to increase in livestock numbers (both commercial and subsistence) and occupational exposure. With global climate change, extreme weather events and flooding are predicted to become more frequent and intense in the Pacific Islands. Rapid population growth in developing countries is often associated with urban and peri-urban slums where diseases of poverty proliferate. Although our study found that leptospirosis seroprevalence was lower in urban areas, poverty rate was a significant risk factor independent of urban or rural settings. Climate change, flooding, population growth, urbanization, and agricultural intensification may independently, or potentially synergistically, lead to enhanced leptospirosis transmission in Fiji [3].

The findings should be considered in light of the study's limitations. Limitations of the MAT have been well documented; the test is considered to be serogroup rather than serovar specific, cross-reactions occur between serovars within a serogroup, and complex paradoxical reactions could occur in persons who have had previous infections [28]. Despite these limitations, the MAT is considered the gold standard test for identifying putative serogroups and serovars when isolates are not available [27]. Isolates of leptospires would be required to definitively confirm the serovars circulating in Fiji. Due to budgetary reasons, our study used a 6- rather than 21-serovar MAT panel to test the majority of samples. If the larger panel was used, additional less-common serovars may have been detected. However, the 6 serovars selected included the most reactive serovars when 198 randomly selected samples from this study were tested against the full 21- serovar panel; the 6 serovars selected accounted for 86.7% of the reactive samples, and one dominant serovar (Pohnpei) accounted for 65.9% of reactive samples. The reduced MAT panel size could have underestimated the overall seroprevalence by a factor of 0.13 compared to a 21-serovar panel, but unlikely to have significantly influenced the overall epidemiological patterns reported here because one serovar dominated the reactive MATs, and our data analyses in this paper were not stratified by serovars.

Our study measured antibodies to *Leptospira* to identify evidence of prior infection. However, many leptospirosis infections do not result in any apparent illness and are of no clinical significance. The severity of clinical disease depends on many factors including pathogen virulence and the individual's immune status, comorbidities, and age [48]. Serovar Pohnpei, the serovar associated with 84.2% of MAT-reactive cases, has been reported as an important cause of overt clinical disease in the Federated States of Micronesia [31], suggesting the

findings in this study are applicable to not only infection but also clinical illness. However, there are currently no available data on the proportion of serovar Pohnpei infections that result in clinical disease or severe complications.

Future studies could further improve our understanding of leptospirosis transmission in Fiji by examining serovar-specific risk factors; identifying the most important exposures in different subgroups such as age groups, gender, ethnic groups, and community types; determining the relative importance of livestock, rodents, pets and wildlife in transmitting leptospirosis to humans; and developing models to determine transmission (causal) pathways rather than just epidemiological links. For environmental and census variables, we used data at the place of residence, but infections could also have occurred at work or elsewhere. Future studies that focus specifically on work-related activities would provide more insight into the importance of occupational exposures in Fiji. The performances of models were partly determined by the accuracy of available environmental, census, and livestock data, and models could be updated and improved as more data become available. Models based on environmental factors, such as Model B, could be used to produce predictive risk maps for the whole of Fiji.

In summary, our study found that risk factors and drivers for human leptospirosis infection in Fiji are complex and multifactorial, and include climate, the natural environment, livestock (both subsistence and commercial), living conditions, socioeconomic status, demographics and individual behaviour. Some of these factors corroborate findings previously reported in other settings (e.g. male gender, working outdoors), but other factors appear to be specific to the cultural and environmental settings in Fiji, including ethnicity and presence of pigs in communities. By using an integrated eco-epidemiological approach and including a wide range of data sources in our analyses, we were able to quantify the relative importance of risk factors at different ecological scales. At the individual level, gender, ethnicity, and work location were strongly associated with infection risk. At the community level, important predictors of risk included rural setting, community type, poor access to clean water, close proximity to rivers, high rainfall in the wettest month, high poverty rate, presence of pigs, and high cattle density. From a wider perspective, significant spatial variations in risk and the ability to predict risk based only on environmental and census variables indicate that environmental factors play a crucial role in driving leptospirosis transmission in Fiji.

The above findings provide an important evidence base to guide the focus of public health and environmental health interventions at individual, community, and national levels. Health promotion activities and educational materials should be designed to reach the highest risk

groups including males, farmers, and iTaukei. Public health and environmental health interventions should target the highest risk communities (villages, rural areas, those in hotspots and high-risk regions), and include advice on proper management of livestock, avoiding contact with floodwaters, and minimizing flooding risk (e.g. adequate garbage disposal to reduce the risk of flooding from blocked streams and drains). At high risk times, e.g. post-flooding, communities should also be reminded about the risk of leptospirosis, protective measures, and the importance of seeking early medical care if unwell. In smaller communities in Fiji, where laboratory diagnostic tests are often not available, the predictive risk chart shown in Figure A3.8 could assist clinicians with determining the likelihood (pre-test probability) of leptospirosis infection based on a combination of individual-level variables. Broader environmental factors (both natural and anthropogenic) play a major role in leptospirosis transmission in Fiji, most of which are beyond the immediate control of individuals or small communities. Effective environmental health management at the public health and national level will therefore be crucial for the sustainable control of leptospirosis in Fiji and other countries with similar environmental and socio-demographic settings.

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A3.6 Supporting Information

A3.S1 Appendix. Initial 21 pathogenic serovars included in the microscopic agglutination test (MAT) panels, and the six serovars chosen for the final MAT panel.

Species	Serogroup	Serovar	198 randomly selected samples from this study#: % of seropositive reactions associated with each serovar	199 *Leptospira* ELISA-positive samples*: % of seropositive* reactions associated with each serovar	Used in final 6-serovar panel
Interrogans	Australis	Australis	7.3%	1.5%	
Interrogans	Australis	Pohnpei	65.9%	51.5%	
Interrogans	Autumnalis	Autumnalis			
Borgpetersenii	Ballum	Ballum	3.7%	6.1%	
Interrogans	Bataviae	Bataviae			
Interrogans	Canicola	Canicola	4.9%	10.6%	
Weilii	Celledoni	Celledoni		4.5%	
Kirshneri	Cynopteri	Cynopteri	1.2%		
Interrogans	Djasiman	Djasiman			
Interrogans	Grippotyphosa	Grippotyphosa			
Interrogans	Hebdomadis	Hebdomadis			
Interrogans	Icterohaemorrhagiae	Copenhageni	4.9%	21.2%	
Borgpetersenii	Javanica	Javanica			
Borgpetersenii	Mini	Mini	3.7%		
Noguchii	Panama	Panama	7.3%		
Interrogans	Pomona	Pomona			
Interrogans	Pyrogenes	Pyrogenes		1.5%	
Santarosai	Shermani	Shermani			
Weilii	Sarmin	Sarmin	1.2%		
Interrogans	Sejroe	Hardjo		3.0%	
Borgpetersenii	Tarassovi	Tarassovi			
TOTAL			100%	100%	

Overall, 32.3% of samples reacted to at least one of the 21 serovars used in the MAT panel; 17.7% with MAT titre of 1:50, 9.1% 1:100; 9.1% 1:200; 5.1% 1:400; 0.5% 1:800.

**Leptospira* ELISA-positive samples from patients with suspected clinical leptospirosis from April 2012 to November 2013 (spanning both epidemic and endemic periods). Samples were systematically selected from a total of 570 samples collected during this period; selection process was designed to maximise the probability of identifying the most common serogroups from all Divisions (geographic spread) and over the entire time period (temporal spread). Overall, 33.2% of samples reacted to at least one of the 21 serovars used in the MAT panel; 4.5% with MAT titre of 1:50; 10.5% 1:100; 5.5% 1:200; 7.0% 1:400; 4.0% 1:800; 1.0% 1:1600; 0.5% 1:6400.

A3.S2 Appendix. Independent variables stratified by data source and scale of ecological influence.

	Data source	
Scale of ecological influence	**Questionnaires**	**Derived using geographic information systems (GIS)**
Individual-level	Age Sex Country of birth Ethnicity Religion Highest school level completed Occupation Farming – none, part-time, full-time If farmer, type(s) of animal(s) Relative altitudes of home and farm Availability of soap & water at lunch Wash hands with soap & water after lunch Availability of soap & water at dinner Wash hands with soap & water after dinner Type of toilet at school or work Availability of soap & water at school or work Wash hands with soap & water after toilet at school or work Swimming, playing, or bathing in flood water Walking in flood water Contact with freshwater – recreation, walking, washing clothes, washing dishes Sighting rats or mice at home Physical contact with rats or mice Sighting mongoose at home Physical contact with mongoose Bitten by ticks or fleas Heard of leptospirosis before this study Diagnosed with leptospirosis Contacts (family, friends, colleagues) diagnosed with leptospirosis	
Household-level	Number of household members Household income Source(s) of drinking water Method(s) used to treat drinking water Availability of tap water in house Supply of government treated water to house Indoor shower or tap for washing Type of toilet at home (if any) Location of home toilet Sharing home toilet with other households Availability of soap & water at home toilet Wash hands with soap & water after using home toilet House construction material Floor construction material Floor raised at least 30cm above ground Number of rooms in house Garbage disposal method Stream or river near home (in community, or within 100m) Flooding at home Flooding of land around home Presence of animal species at home Grow crops, fruits, vegetables at home	Distance to rivers or major creeks Elevation above sea level Slope Road density Rainfall (multiple measures including maximum, minimum, average) Temperature (multiple measures including maximum, minimum, average) Land use Soil type

Community-level	Community type Urban rural classification Presence of animal species in community Grow crops, fruit, vegetables in community	Educational attainment House construction Sources of income (subsistence, salaried) Ethnicity Water supply Electricity Toilets Population density Population growth Poverty rate Poverty gap Commercial beef – numbers of animals and farms Commercial dairy – numbers of animals and farms Subsistence beef – numbers of animals and farms Subsistence dairy – numbers of animals and farms Total cattle – numbers of animals and farms Pigs – numbers of animals and farms Goats – numbers of animals and farms Horses – numbers of animals and farms Sheep – numbers of animals and farms Poultry – numbers of animals and farms Duck – numbers of animals and farms