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Revealing the disease dynamics of antigenically variable viruses

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Declaration

I declare that all of the work presented in this thesis is my own with some minor clarifications listed below. The work was completed under the supervision of Professor Steven Riley, Dr Michael White and Dr Nimalan Arinaminpathy. Chapters 3 and 4 have been published, and Chapter 5 has been submitted as a manuscript. Co-authors contributed to the published/submitted versions of these manuscripts, but all of the text, figures, methods and analyses in this thesis are my own. All published work is under the Creative Commons Attribution (CC BY) licence.

1. The Markov chain Monte Carlo (MCMC) code I use in Chapters 3 and 4 is based on an R package I developed available at <https://github.com/jameshay218/lazymcmc>. In Chapter 4, I use an extension of the code written by Ada Yan which incorporates parallel tempering MCMC, available at https://github.com/jameshay218/lazymcmc/tree/parallel_tempering.
2. The work in Chapter 5 has been developed into an R package available at <https://github.com/seroanalytics/serosolver>. This R package was written with collaborators at Imperial College London (Kylie Ainslie and Steven Riley) and the London School of Hygiene and Tropical Medicine (Adam Kucharski and Amanda Minter). I wrote the vast majority of the R and C++ code. This code base makes substantial additions to previously published methods that are appropriately credited in Chapter 5. I developed all of the methodology shown in Chapter 5.

Manuscripts

1. Hay JA, Nouvellet P, Donnelly CA, Riley S (2018) Potential inconsistencies in Zika surveillance data and our understanding of risk during pregnancy. *PLoS Negl Trop Dis* 12(12): e0006991. <https://doi.org/10.1371/journal.pntd.0006991>.
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Abstract

Many viruses exhibit substantial antigenic variation, resulting in phenotypic differences between related variants of the same species. This variability poses a substantial public health burden, as hosts can be reinfected many times over their lifetime. In this thesis, I use mathematical models to capture unobserved biological processes in two antigenically variable systems: Zika and influenza. In Chapter 3, I develop a model linking Zika virus infection and the gestational risk of microcephaly. Through fitting this model to incidence data from South America, I find inconsistencies in the gestational risk profiles inferred for different locations. Motivated by the question of how prior exposure influences subsequent disease, I turn to the problem of understanding influenza exposure histories. In Chapter 4, I fit antibody kinetics models to serological data from ferrets exposed to multiple infections and vaccinations. I generate estimates for immunological parameters that may be important in explaining human antibody dynamics arising from repeated unobserved exposures. Validating these models motivates their application in human populations. Chapter 5 develops a robust statistical framework to identify unobserved influenza infections using routinely collected serological data. Model assumptions for infection histories are an important consideration, and I thoroughly explore the underlying behaviour of the inference method. Finally, in Chapter 6, I use this model to infer influenza infection histories for 1,130 individuals in southern China. This enables the reconstruction of historical influenza A/H3N2 incidence, revealing epidemiological patterns that vary across individual ages and at a small spatial scale. Together, these results demonstrate how integrating modern statistical methods, immunology and seroepidemiology may help to answer public health questions. Improved analytical methods for serological data, such as those developed in this thesis, may provide a rich new stream of augmented data and generate new insights into the life course epidemiology of antigenically variable pathogens.

Abbreviations

Table 1: List of abbreviations

Acronym	Definition
Ab	Antibody
ADE	Antibody dependent enhancement
Ag	Antigen
AIC	Akaike information criterion
BCR	B cell receptor
BIC	Bayesian information criterion
BMA	Bayesian model averaging
CHIKV	Chikungunya virus
CI	Credible interval (unless otherwise stated)
CRS	Congenital rubella syndrome
CZS	Congenital Zika syndrome
DENV	Dengue virus
ELPD	Expected log predictive density
ESS	Effective sample size
GBS	Guillain-Barré syndrome
HA	Haemagglutinin
HI	Haemagglutination inhibition
Ig	Immunoglobulin
ILI	Influenza like illness
LOO-CV	Leave one out cross validation
MBC	Memory B cell
MCMC	Markov chain Monte Carlo
MDS	Multidimensional scaling
MN	Microneutralisation
MoH	Ministry of Health
NA	Neuraminidase (or not available)
OAS	Original antigenic sin
PAHO	Pan American Health Organisation
PHEIC	Public health emergency of international concern
PSIS	Pareto smoothed importance sampling
PSRF	Potential scale reduction factor
PT	Parallel tempering
RESP	Registro de Eventos em Saúde Pública
RT-PCR	Reverse transcriptase polymerase chain reaction
SE	Standard error
SEIR	Susceptible-exposed-infected-recovered
SIR	Susceptible-infected-recovered
TIV	Trivalent inactivated influenza vaccine
WAIC	Widely applicable information criterion
WHO	World Health Organisation
ZIKV	Zika virus

Contents

Abstract	v
1 Introduction	1
1.1 Life course epidemiology of infectious diseases	1
1.2 Life course of Zika and flavivirus infection	3
1.2.1 Teratogenic pathogens and Zika virus	3
1.2.2 CZS and prior exposures	4
1.3 Life course of influenza	6
1.3.1 Epidemiology and life history	6
1.3.2 Original antigenic sin, antigenic seniority and immune imprinting	8
1.4 Thesis overview	11
1.4.1 Terminology	12
2 Background	13
2.1 The complexity of antigenic variability	14
2.1.1 Overview	14
2.1.2 Population dynamics	14
2.1.3 The concept of antigenic space	15
2.2 Adaptive immune dynamics	19
2.2.1 The adaptive humoral immune response	19
2.2.2 Antibodies	21
2.2.3 The adaptive cellular immune response	23
2.2.4 Original antigenic sin, immune imprinting and related phenomena	24
2.2.5 Mechanism of OAS and related phenomena	27
2.2.6 Kinetics of the adaptive immune response	28
2.3 Seroepidemiology	31
2.3.1 Public health relevance of antibodies	31
2.3.2 Seroepidemiology for surveillance	34
2.3.3 Dynamical models and inference using serological data	36
2.3.4 Model fitting in multi-strain systems	38
2.4 Disease dynamic modelling and inference	41
2.4.1 Overview	41
2.4.2 The data generating process	41
2.4.3 Bayesian inference	42
2.4.4 The prior level	46
2.4.5 The transmission level	46
2.4.6 The observation level	51
2.4.7 Modern MCMC methods	52
2.4.8 Summary	56
3 Epidemiological dynamics of ZIKV-associated microcephaly risk	58
3.1 Introduction	59

3.2	Materials and Methods	62
3.2.1	Model description	62
3.2.2	Transmission model	62
3.2.3	Microcephaly risk model	66
3.2.4	Combined model	67
3.2.5	Data	67
3.2.6	Model fitting	71
3.2.7	Forecasting the second wave of microcephaly incidence	73
3.3	Results and Discussion	77
3.3.1	Patterns of incidence in the data	77
3.3.2	Different data sets suggest different risk profiles	78
3.3.3	Gestational age at peak risk	78
3.3.4	Duration of heightened gestational risk	81
3.3.5	Absolute risk of CZS	82
3.3.6	Understanding the missing second wave of microcephaly incidence	83
3.4	Conclusions and Implications	88
4	Antibody kinetics in a ferret model	91
4.1	Introduction	92
4.2	Models of antibody kinetics	94
4.2.1	Base model	94
4.2.2	Interaction of multiple exposures	96
4.2.3	Additional model mechanisms	96
4.2.4	Full model	100
4.3	Materials & Methods	103
4.3.1	Study Data	103
4.3.2	Model fitting	104
4.3.3	Model comparison	106
4.3.4	Simulation recovery experiments	107
4.4	Results	108
4.4.1	Antibody kinetics following a single exposure support biphasic waning	108
4.4.2	Variation in antibody kinetics driven by different exposure histories	108
4.4.3	Model comparison results	109
4.4.4	Comparison of homologous boosting by exposure type	115
4.4.5	Comparison of cross reactivity by exposure type	115
4.4.6	Magnitude and duration of waning phases	117
4.4.7	Impact of priming	118
4.4.8	Limited evidence for antigenic seniority and titre-dependent boosting	118
4.4.9	Sensitivity analyses	119
4.5	Discussion	121
5	A statistical framework to represent influenza infection histories	126
5.1	Introduction	127
5.2	Materials & Methods	129
5.2.1	Serological data	129
5.2.2	Antibody kinetics model	130
5.2.3	Additional immunological mechanisms	132
5.2.4	Infection history model	133
5.2.5	Observation model	133
5.2.6	Inference using MCMC	135
5.2.7	Model settings key	137
5.3	Motivation for understanding prior assumptions	139

5.4	The inference problem	141
5.4.1	Description of the full model	141
5.5	Refinement of methods for inferring latent infection states	143
5.5.1	Intuitive prior	143
5.5.2	The infection history prior is not defined in the original framework	144
5.5.3	Prior 1: Beta prior on per-individual infection probabilities	145
5.5.4	Prior 2: Prior on per-time infection probability	148
5.5.5	Prior 3: Beta prior on per-time infection probability	151
5.5.6	Prior 4: Beta prior on the probability of any infection event	154
5.6	Results	156
5.6.1	Comparison of infection history priors using simulation results	156
5.6.2	Impact of prior choice on model estimates	156
5.6.3	Refinement of real historical attack rate estimates	159
5.6.4	Systematic bias in measurements not captured by the model	163
5.6.5	Inference of virus-specific measurement offsets	165
5.6.6	Antibody kinetics estimates	170
5.6.7	Titre-dependent and age-dependent boosting	170
5.7	Discussion	175
5.8	Conclusion	179
5.9	Serosolver R-package	180
6	Patterns of spatial and individual variation in influenza incidence and immunity in a southern Chinese cohort	181
6.1	Introduction	182
6.2	Materials & Methods	184
6.2.1	Participant data	184
6.2.2	Serological data	184
6.2.3	Antibody kinetics and observation model	185
6.2.4	Infection history model and assumptions	185
6.2.5	Model fitting	186
6.2.6	Validation using simulated data	187
6.2.7	Statistical and post-hoc analyses	187
6.3	Results	189
6.3.1	Description of Fluscape data	189
6.3.2	Antibody titres vary by age and in space	189
6.3.3	Fitting of the antibody kinetics and infection history model	191
6.3.4	Inferred historical and contemporary attack rates	195
6.3.5	Attack rates varied in space but were not predicted by proximity	199
6.3.6	Age-specific infection patterns	202
6.3.7	Relationship between titre and probability of infection	202
6.4	Discussion	207
6.4.1	Study limitations	211
6.5	Conclusion	216
7	Discussion	217
7.1	Summary of findings and implications	218
7.2	Future directions	222
7.2.1	Expanding the infection history data structure and use of multiplex assays	222
7.2.2	Developing further antibody kinetics models and B-cell dynamics	223
7.3	Conclusion	225
	Bibliography	225

Appendices	256
A Epidemiological dynamics of ZIKV-associated microcephaly risk	257
A.1 Additional data methods	261
A.1.1 Summary of data	261
A.1.2 Detail of additional incidence data extraction	261
A.1.3 Birth data	263
A.2 Methods for forecasting the second wave of microcephaly incidence	265
A.3 Sensitivity analyses	267
A.3.1 Fitting to seroprevalence data from Salvador, Brazil	267
B Characterising antibody kinetics of influenza exposure using a ferret model	268
B.1 Model variants referred to in main text	268
B.2 Simulation-recovery results	268
B.3 Further model comparison results	273
C Adding titre-mediated immunity to <i>serosolver</i>	281
C.1 Adding immunity to the infection process	281
C.1.1 Titre-mediated immunity	283
C.1.2 Implementation of the immunity model	287
C.2 Approximations of $P(X, V, \theta)$	290
C.2.1 Numerical integration over Φ	290
C.2.2 Approximate integration by iterating over Φ	290
C.2.3 Importance sampling approximation	290
C.2.4 Comparison of approximation implementations	292
C.2.5 Computation time prevents these solutions from being useful	292
C.2.6 No closed form solution for the logit immunity model	294
C.3 Representing X and Φ explicitly	296
C.4 Representing X and Z explicitly	297
C.5 Results	298
C.5.1 Impact of titre-mediated immunity on model behaviour	298
C.5.2 Comparison of real parameter estimates and attack rates with titre-mediated immunity	300
D Additional figures for “A statistical framework to represent influenza infection histories”	302
E Algorithms for “A statistical framework to represent influenza infection histories”	308
E.1 Prior 1: Further considerations and Gibbs sampling	308
E.2 Proposal algorithm under prior 1: Beta prior on per-individual infection probability	310
E.3 Proposal algorithm under prior 2: Hyper-prior on probability of infection	311
E.4 Proposal algorithm under prior 3: Beta prior on per-time probability of infection	313
E.5 Proposal algorithm under prior 4: Beta prior on overall probability of infection	315
F Patterns of spatial and individual variation in influenza incidence and immunity in a southern Chinese cohort	316
G Discussion figures	332

List of Tables

1	List of abbreviations	vi
2.1	Summary of assays used for quantification of influenza antibody titres	33
3.1	Zika model parameters, sources and assumed parameter ranges	70
4.1	Description of model parameters	101
4.2	Description of model mechanisms and their potential formats	102
4.3	Description of experimental protocol	104
4.4	Description of models with $\delta\text{ELPD} < 20$	112
4.5	Parameter estimates for model ID 62, key YTY6BN	113
4.6	Parameter estimates for model ID 21, key NAY6BY	114
5.1	Comparison of the two HI titre datasets	129
5.2	Representation of infection histories for a population of three individuals from 1968 to 2014	133
5.3	Example simulation settings table	138
5.4	Simulation settings to compare different infection history prior assumptions	158
5.5	Settings for simulations matching the Ha Nam cohort	162
5.6	Settings for simulations matching the Fluscape cohort	163
5.7	Comparison of antibody kinetics parameter estimates under different model assumptions for ρ	169
5.8	Parameter estimates from fitting model to data from China and Vietnam using the original and refined framework	170
5.9	Comparison of parameter estimates using the base model, model with titre-dependent boosting and model with age-dependent boosting	174
6.1	Virus-specific offset terms used in the main model fits	186
6.2	Demographic characteristics of the 1,130 Fluscape participants	190
6.3	Estimated attack rates and infection patterns 2010–2014	198
6.4	Model predicted titres against circulating strains since birth	201
A.1	Transmission parameter estimates for the primary analyses based on estimated posterior distributions	258
A.2	Microcephaly risk parameter estimates for the primary analyses based on estimated posterior distributions	259
A.3	Parameter estimates for the analysis of the second ZIKV infection incidence wave	260
A.4	ZIKV and microcephaly incidence data included in the Zika analysis	262
A.5	Vital statistics and sources	263
C.1	Settings for simulations matching the Fluscape cohort with titre-mediated immunity	296
C.2	Comparison of parameter estimates and convergence diagnostics of the model with titre-mediated immunity and without	301
F.1	Demographics of the 651 households used.	317

F.2	Characteristics of the 40 Fluscape study locations.	318
F.3	Comparison of generalised additive model fits using raw HI from second serum sample or change in HI titre as outcome.	331
F.4	Antibody kinetics parameter estimates from the full model fit to the Fluscape data without strain-specific measurement bias.	331

List of Figures

1.1	Timeline of influenza pandemics and subsequent circulation in humans	8
2.1	Example antigenic map of influenza A/H3N2 strains	17
2.2	Schematic of B cell fates and antibody secreting functions	20
2.3	Schematics of various OAS effects and related phenomena	25
2.4	Timeline of the adaptive humoral response following primary and secondary exposure	29
2.5	Schematic demonstrating observations of an individual's antibody kinetics process . .	35
2.6	Schematic of potentially confounded serology due to cross-reactive antibody kinetics .	37
2.7	Relationship between individual-level antibody trajectories and population-level dynamics	38
2.8	The forward process	44
2.9	The life course forward process	45
3.1	Schematic depiction of the interaction of epidemic dynamics and gestational-age-varying risk of microcephaly given infection	63
3.2	Graphical representation of the SEIR model	64
3.3	Google search trends and model fits to the first wave of ZIKV and microcephaly in Bahia, Brazil	75
3.4	Schematic of forecasting analysis mechanism switch times	76
3.5	Notified and confirmed microcephaly and ZIKV infection incidence	79
3.6	Estimates of the microcephaly risk profile fit to different data sets	80
3.7	Model residuals for ZIKV infection and microcephaly incidence	84
3.8	Key regions of parameter space that are consistent with the observed ZIKV and microcephaly data	86
4.1	Base model	95
4.2	Summary of model mechanisms	97
4.3	Summary of experimental protocol	103
4.4	Observation error matrix	105
4.5	Comparison of four base model fits to data from three ferrets following exposure to a single immunogen	109
4.6	Ferret model trajectory fits	110
4.7	Estimated ferret model parameters	116
4.8	Estimated cross reactivity profiles by exposure type	117
5.1	HI titres for 3 randomly chosen individuals from the Fluscape cohort	130
5.2	Distribution of ages and strains in serological data	131
5.3	Conceptual overview demonstrating how an antibody landscape develops and may be observed over an individual's lifetime	136
5.4	Inferred annual A/H3N2 attack rates using simulated Fluscape data with original framework described in Kucharski <i>et al.</i> [1]	139
5.5	Example of individual model fits to simulated Fluscape-like titre data using original framework	140
5.6	Directed acyclic graph representation of the original model	142

5.7	Empirical priors on the total number of infections over a 10 year period in the inference framework compared to random samples from the beta-binomial density	149
5.8	Directed acyclic graph representation of the updated model	152
5.9	Simulated versus analytical infection history priors when $\alpha = \beta = 1$	157
5.10	Priors on cumulative number of lifetime infections when $\alpha = \beta = 1$	158
5.11	Posterior distribution of long-term boosting parameters μ_l under different prior and data scenarios	160
5.12	Accuracy of estimated attack rates under the various priors, prior strengths and data scenarios	161
5.13	Inferred annual A/H3N2 attack rates using real and simulated Ha Nam data comparing original and refined framework with prior version 2 (beta prior on per-time infection probability, Φ_j)	162
5.14	Inferred annual A/H3N2 attack rates using real and simulated Fluscape data with refined framework, prior version 2 (beta prior on per-time infection probability, ϕ_j) . .	164
5.15	Example of individual model fits to real Fluscape titre data using refined framework with prior version 2 (beta prior on per-time infection probability, ϕ_j)	165
5.16	Distribution of antibody titre prediction errors (observed - model predicted)	166
5.17	Model residuals against estimated attack rates grouped by year of circulation using the Fluscape data	166
5.18	Estimates for measurement bias parameters, ρ , by strain and model version	171
5.19	Individual model fits to Fluscape-like titre data with virus-specific measurement bias .	172
6.1	Distribution of log HI titres against 20 A/H3N2 strains circulating from 1968 to 2014	192
6.2	Proportion of individuals seropositive and seroconverted to 20 A/H3N2 strains circulating from 1968 to 2014 stratified by age	193
6.3	Distribution of log HI titres by study location at first serum sample	194
6.4	Quarterly incidence and individual infection histories from the Fluscape dataset	196
6.5	Quarterly attack rates across the 40 Fluscape study locations	200
6.6	Spatial variation in attack rate estimates over time and correlation of nearby locations	201
6.7	Age-specific patterns of infection	203
6.8	Model predicted titres against circulating strains since birth	204
6.9	Estimated relationship between HI titre and probability of infection	206
B.1	Antibody trajectories for group E from model variant 64	269
B.2	Posterior estimates for titre-dependent boosting relationship from the best supported model	269
B.3	Antibody trajectories for groups C&D from model variant 54	270
B.4	Simulation-recovery of the four base model fits to data from three ferrets following exposure to a single immunogen matching the real protocol	271
B.5	Re-estimated model parameters from simulated data	272
B.6	Summary of posterior distribution estimates for homologous boosting parameter, μ from models with $\delta\text{ELPD} < 20$	274
B.7	Summary of posterior distribution estimates for initial waning phase proportion, d from models with $\delta\text{ELPD} < 20$	275
B.8	Summary of posterior distribution estimates for duration of initial waning phase, t_s from models with $\delta\text{ELPD} < 20$	276
B.9	Summary of posterior distribution estimates for long-term waning rate, m from models with $\delta\text{ELPD} < 20$	277
B.10	Summary of posterior distribution estimates for cross reactivity gradient, σ from models with $\delta\text{ELPD} < 20$	278
B.11	Summary of posterior distribution estimates for priming cross reactivity gradient, β from models with $\delta\text{ELPD} < 20$	279

B.12	Summary of posterior distribution estimates for titre dependence gradient, γ and titre-dependent switch point, y_{switch} from models with $\delta\text{ELPD} < 20$	279
B.13	Summary of posterior distribution estimates for antigenic seniority parameter, τ from models with $\delta\text{ELPD} < 20$	280
C.1	Distribution of times between infections sampled from the infection history model . . .	282
C.2	Relationship between HI titre and immunity	284
C.3	Directed acyclic graph representation of the modified model with immunity	286
C.4	Directed acyclic graph representation of the modified model with separate exposure and infection states	288
C.5	Comparison of joint distribution approximations to known full joint distributions . . .	293
C.6	Comparison of simulated attack rates with and without titre-mediated immunity . . .	299
C.7	Comparison of inferred attack rates from the Fluscape data using the base model and the model with titre-mediated immunity	301
D.1	Priors on cumulative number of lifetime infections when $\alpha = 2$ and $\beta = 10$	303
D.2	Simulation-recovery of one individual's infection history using prior version 1 (beta prior on per-individual infection probability, P_i) with various strength priors, sparse data . .	304
D.3	Simulation-recovery of one individual's infection history using prior version 3 (beta prior on per-time infection probability, ϕ_j) with various strength priors, sparse data	305
D.4	Simulation-recovery of one individual's infection history using prior version 4 (beta prior on overall infection probability) with various strength priors, sparse data	306
F.1	Distribution of serum sampling times from the Fluscape cohort.	316
F.2	Relationship between age and titre.	320
F.3	Convergence diagnostics of antibody kinetics parameters from fitting the full model with virus-specific measurement bias.	321
F.4	Convergence diagnostics of quarterly attack rate estimates from fitting the full model with virus-specific measurement bias.	322
F.5	Example inferred latent antibody titres and infection histories.	323
F.6	Assessment of model fitting accuracy based on simulated data.	324
F.7	Quarterly incidence and individual infection histories from the Fluscape dataset using the model without virus-specific measurement bias.	325
F.8	Distribution of quarterly attack rates by location over time.	326
F.9	Age-specific patterns of infection under alternative infection history prior assumptions. .	327
F.10	Estimated relationship between HI titre and probability of infection based on unmodified model-predicted infection histories.	328
F.11	Logistic regression showing relative risk of infection as a function of titre stratified by age group	329
F.12	Comparison of virus-specific offsets used and estimates in Chapter 5	330
G.1	Schematic comparing the current antibody kinetics model and a prototype with antigen trapping	333
G.2	Comparison of prototype antigen trapping model with current model	334

Chapter 1

Introduction

1.1 Life course epidemiology of infectious diseases

Properties of individuals within a host population are determined by historical events related to the disease of interest, and many public health questions require a holistic view of an individual's life history to fully understand disease dynamics. For example, how susceptible is an individual given a history of prior exposures to related pathogens? This long-term approach to epidemiology can be described as “life course epidemiology”, the field of study describing how events earlier in life can impact disease risk later on [2]. In this thesis, I focus mainly on influenza A viruses, but also investigate the link between incidence of Zika virus (ZIKV) and congenital outcomes.

A life course approach is well established in chronic disease epidemiology by Barker *et al.*, who developed the idea that environmental exposures during fetal development impact disease risk in later life [3]. Although the importance of historical life events in infectious disease epidemiology is well known, its consideration in a life course epidemiology framework has received little quantitative attention [4]. For many public health questions this is not an issue: for example, understanding spreading patterns or potential intervention strategies during the 2014 Ebola epidemic in West Africa requires focus only on recent events [5]. However, understanding other disease systems requires the consideration of many past unobserved events, particularly where multiple lifetime exposures are common or the event of interest is observed long after the causative exposure. Kuh *et al.* described a glossary of terms relevant to life course epidemiology which are readily comparable to infectious diseases. For example, the concept of “birth cohort effects”, wherein the year and location of an individual's birth impacts their long-term risk profile, is analogous to immunological imprinting in influenza infection [2, 6]. Another

example might be how the concept of a “critical period” relates to the windows during gestation where teratogenic (agents that affect the normal development of the fetus) pathogens may cause congenital malformations [7, 8].

Improving our biological understanding of these unobserved, long-term, biological processes is usually driven by clinical or experimental work that isolate particular mechanisms. For example, the effectiveness of a vaccine administered to an individual relative to a matched individual who did not receive the vaccine. However, in the context of inference to understand population level disease dynamics, mathematical models need to incorporate multiple mechanisms to link known biology to observed data. This is particularly relevant when the observed data might be of public health significance, but only linked to an epidemiological event through a complex, hidden biological mechanism.

1.2 Life course of Zika and flavivirus infection

1.2.1 Teratogenic pathogens and Zika virus

Many pathogens are known to cause congenital abnormalities, leading to the recognition of the TORCH paradigm; a group of diseases that initially referred to (but is not limited to) *toxoplasma*, rubella virus, cytomegalovirus and herpes simplex viruses [9]. Rubella holds particular historical significance as one of the first viruses shown to act as a teratogen [10]. This led to the description of congenital rubella syndrome (CRS) early in the 20th century. A key feature of CRS is that there is a critical window of risk during gestation, as summarised by Santis *et al.* (Table 1 of [8]): when infection occurs early in gestation, the absolute risk of CRS is vastly increased relative to infection later in pregnancy.

The recent ZIKV epidemic in South America and its link to microcephaly represents a recent case study of how an infectious agent affecting the fetus at a crucial stage during gestation can have a significant impact on congenital malformation incidence [11, 12]. Although historically considered to cause mild disease, ZIKV was linked to a number of neurological conditions including Guillain-Barré syndrome (GBS) and microcephaly in 2016 [13–16]. Similar to CRS, the time since conception at which pregnant women are infected has a significant impact on their risk of developing congenital Zika syndrome (CZS), with exposure in the first trimester bearing the highest risk [17]. The fact that the public health emergency of international concern (PHEIC) called by WHO was in response to the rise in microcephaly cases rather than the rise in ZIKV cases highlights that many disease burdens are not necessarily apparent at the time of infection, but rather a result of some unobserved biological process [18]. Indeed, the importance of ZIKV was raised long after the initial wave of the epidemic was over in many countries, and surveillance data from the first wave was therefore poor and lacked virological confirmation [17]. Mathematical modelling was therefore potentially useful to increase understanding of the risk to women of child bearing age in ZIKV affected areas using available surveillance data [7, 19, 20]. It was not until later that cohort studies of pregnant women would be able to investigate this risk directly [21, 22].

However, early clinical reports put these modelling results in a puzzling light. A cohort study of pregnant women in Rio de Janeiro, Brazil reported increased adverse events in ZIKV-positive women throughout pregnancy, including 4 cases of microcephaly (3.4% of infected women) following infection at weeks 8, 12, 30 and 38 of gestation [11]. These results suggest that the window of microcephaly risk might extend beyond the first trimester, though proportionate microcephaly was only recorded

following infection in the first trimester. Another study based on surveillance data from Colombia found no evidence for ZIKV-associated congenital abnormalities following infection in the third trimester, though the majority of investigated women were still pregnant at the time of publication [23,24]. How recent clinical findings link to the complex picture portrayed by ecological data is still unclear, as second waves of microcephaly incidence in Brazil were not as severe as expected based on incidence from the first wave [25]. Nonetheless, final findings from cohort and surveillance studies in Brazil, Colombia, the USA and French Territories of the Americas have supported the association between ZIKV infection during pregnancy and adverse congenital outcomes beyond microcephaly, particularly following infection in the first trimester [21,22,26–28].

1.2.2 CZS and prior exposures

The link between ZIKV and CZS is now widely accepted, but one of the outstanding questions from the PHEIC is why the incidence of microcephaly was so much higher in some regions than others. Differences in microcephaly risk were apparent even after accounting for differences in ZIKV infection incidence [20]. Variation in prior flavivirus immunity, namely from prior dengue virus (DENV) exposure, was proposed as a possible explanation, because antibody-dependent enhancement (ADE) from prior infection with DENV is known to increase the risk of developing severe disease upon secondary exposure with a different DENV serotype [29–32]. Antibodies isolated from human sera following dengue exposure have been shown to cross-react with ZIKV, likely through shared structures in the envelope (E) protein which is a key target of the IgG to DENV and ZIKV, supporting the possibility of ZIKV-DENV ADE [33–35].

Despite these findings, there is still a lack of consensus across *in vitro* and *in vivo* studies regarding the role of pre-existing DENV antibodies in ZIKV infection (or vice versa), with some *in vitro* and small sample size mouse studies suggesting ADE effects [29,30,36,37] and others suggesting cross-protection [38,39]. The different impacts of low, intermediate, and high antibody concentrations or time since DENV exposure have been proposed as potential explanations for these differences in line with observations in DENV-DENV ADE [29,38,40,41]. Clinical evidence is currently lacking on the role of prior DENV exposure in ZIKV-associated congenital outcomes. Halai *et al.* tested for association between fetal outcomes from ZIKV-PCR positive pregnant women in Rio de Janeiro, Brazil, and the presence of prior dengue IgG and found no association. However, their cohort was small (131 women) with only 14 women who were seronegative for dengue IgG [42]. Another study of 64 infants born with probable microcephaly in Sergipe, Brazil also found no association between presence of congenital

microcephaly and prior dengue fever, though again their sample was small and prior dengue fever was based on patient recall [43].

It is clear that an individual's prior flavivirus exposure history has an impact on subsequent disease risk, an observation that is reminiscent of another notoriously antigenically variable virus: influenza. The ZIKV project in this thesis motivates development of methods to infer prior exposures, which are unobserved but result in a somewhat predictable serological profile by including known immunological processes. Individual-level serological data for ZIKV and DENV antibodies in Brazil were not available at the time, but comprehensive serological datasets for influenza were (alongside over a century of influenza immunology research). The latter part of this thesis is therefore focused on developing mathematical models linking observed influenza serology to known biological mechanisms, with the aim of developing a framework to infer latent exposure events.

1.3 Life course of influenza

1.3.1 Epidemiology and life history

Influenza viruses are a group of negative-sense, single-stranded, segmented RNA viruses from the *Orthomyxoviridae* family. There are four types of influenza virus that can cause disease in humans: Types A, B, C and D that differ in their host ranges, biochemistry, and evolutionary history [44, 45]. Types A and B routinely cause seasonal epidemics and influenza A causes occasional pandemics in humans when novel variants emerge, usually from a zoonotic source [46]. Type A is also endemic in many mammalian and avian populations and causes occasional spillover infections from animal populations. Type C causes occasional mild outbreaks in humans (particularly children) and some mammals, whereas Type D is not known to naturally infect humans [47].

Seasonal influenza is estimated to be responsible for between 3 and 5 million cases of severe illness and 291,000–646,000 deaths worldwide per year [48]. The disease associated with influenza infection is usually an acute respiratory illness (ARI) in humans due to infection of airway and alveolar epithelial cells in the upper respiratory tract. The severity of the disease is varied: mild symptoms and asymptomatic infections are common, with classic influenza like illness (ILI) symptoms including rapid onset fever, sore throat, coughing, myalgia, malaise, fatigue and headache [49]. Without further complications, influenza illness is considered an acute infection that lasts up to seven days [49]. However, pneumonia caused by secondary bacterial infection, cytokine storms and acute respiratory distress syndrome are relatively common complications that can lead to severe and even fatal disease [50]. In this thesis, I will focus primarily on seasonal influenza A viruses.

The influenza virus particle is comprised of 9 structural and 2 non-structural proteins encoded by 8 gene segments [46]. The immunodominant haemagglutinin (HA) and neuraminidase (NA) structural glycoproteins have key roles in the life cycle of the virus, and are thus under strong selection pressures for functionality as well as immune escape [51]. Influenza viruses recognise and bind to sialic acid receptors on the host cell surface, with different HA proteins having binding preferences for different sialic acid configurations. Sialic acid moieties with α -2-6 configurations are found on human tracheal cells in the upper respiratory tract, whereas α -2-3 configurations are predominately found in the intestinal tract of poultry and water fowl [52, 53]. Crucially, α -2-3 configurations are also found (though at a lower frequency) in the lower respiratory tract of humans, which likely enables rare but often severe infection of humans from non-human-adapted subtypes [54]. The remaining proteins, RNA polymerase

subunits 1 and 2 (PB1/2), viral nucleoprotein (NP), matrix protein (M1), membrane protein (M2), nonstructural proteins (NS1/2) and nuclear export protein (NEP) are of interest as (i) targets for antiviral drugs [55], (ii) determinants of host species ranges [54] and (iii) antibody targets [56].

Influenza A is notable for its antigenic variability, and is further sub-classified beyond the genotype level. At the first level are many different influenza A subtypes, characterised by their combination of two surface glycoproteins: HA and NA [57]. There are 18 HA and 11 NA subtypes (or serotypes), which are divided based on their antigenic cross-reactivity in serological assays. These are numbered based on the order of discovery, hence A/H1N1 contains the first recorded HA and first recorded NA, followed by A/H2N2 and A/H3N2. These subtypes are also placed into two phylogenetic groups: group 1, encompassing H1, H2, H5, H6, H8, H9, H11, H12, H13, H16, H17 and H18, and group 2, encompassing H3, H4, H7, H10, H14 and H15. Subtypes are further split into clades, which exhibit the same HA and NA but with some structural and genetic changes.

There are two main mechanisms that generate genetic and therefore antigenic novelty in influenza: reassortment with or direct spillover of viruses in animal reservoirs, and the evolution of existing human-endemic strains. The former mechanism, known as “antigenic shift”, has led to regular pandemics throughout the 20th and 21st centuries with varying degrees of morbidity, mortality and different age-distributions of cases. Antigenic shift events drastically change the antigenicity of circulating strains by gaining novel genetic material from animal strains, rendering any pre-existing population immunity largely ineffective. For example, the 2009 A/H1N1 pandemic occurred via reassortment of avian-derived Eurasian swine and avian-swine-human triple reassortment influenza A viruses [58]. Historically, pandemic strains have replaced existing strains, though A/H1N1, A/H3N2 and two influenza B lineages now co-circulate, shown in Fig 1.1.

Although the threat of another pandemic is constant and inherently difficult (if not impossible) to predict, the continual evolution of circulating human strains, known as “antigenic drift”, is another ongoing challenge [72]. The influenza RNA polymerase does not have a proof reading mechanism, and replication errors are therefore frequent [73–76]. This leads to the accumulation of point mutations in the HA and NA gene segments, which can lead to structural changes of these surface glycoproteins and thereby change the antigenicity of the virus [77]. Amino acid substitutions at only 7 key sites in the HA1 domain of HA account for the vast majority of observed antigenicity changes, suggesting that mutations to confer escape from host immunity are readily fixed [78]. Positive selection under immune pressure leads to the classic ladder-like phylogenetic tree of influenza A/H3N2, with a slender trunk

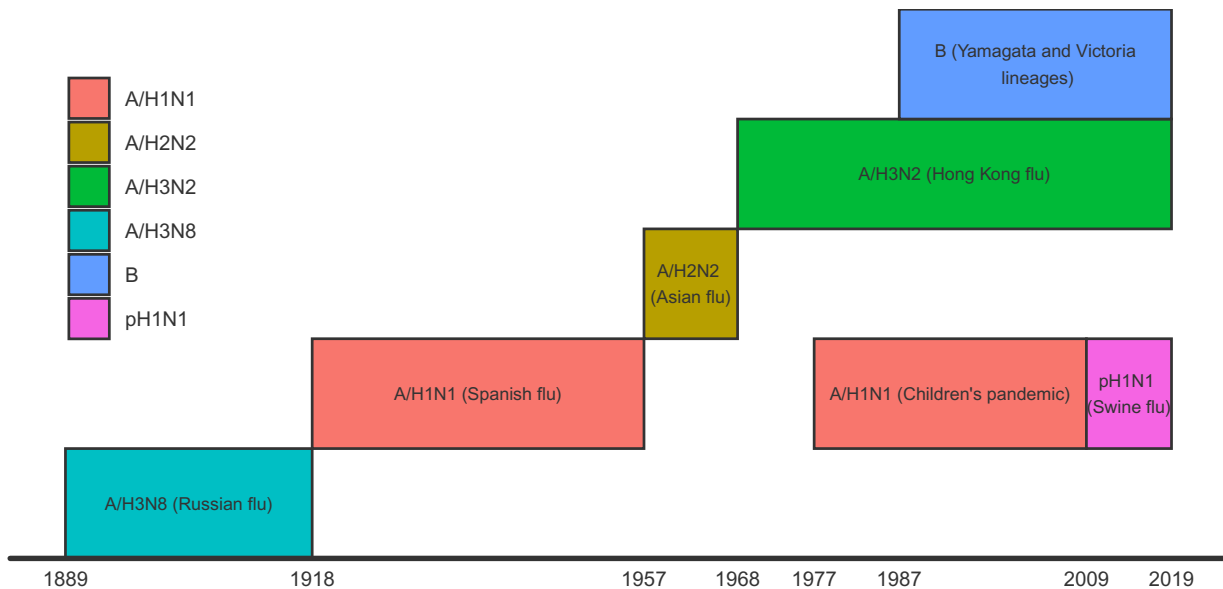


Figure 1.1: **Timeline of influenza pandemics and subsequent circulation in humans.** Adapted from [59,60]. The 1889 Russian flu pandemic was thought to be an A/H3N8 subtype [61]. The 1918 Spanish flu pandemic, which killed an estimated 50–100 million people, was attributed to a human A/H1 virus that acquired avian N1 neuraminidase and internal proteins, based on sequencing of a frozen lung tissue sample and molecular clock analysis [62–64]. The A/H1N1 lineage circulated until 1957, when it reassorted with and gained the HA, NA and PB1 from an avian A/H2N2 virus [65,66]. A/H2N2 replaced A/H1N1 and circulated until 1968, when it reassorted with another avian H3 virus and received a novel HA and PB1 [66,67]. A/H1N1 was reintroduced from a suspected laboratory escape in 1977 [68,69]. Since influenza B began circulating, A/H3N2, A/H1N1 and both influenza B lineages have co-circulated [70,71]. The previous A/H1N1 was replaced by pH1N1 following the pandemic [64]

and short branches [73,77]. This pattern is due to the high replacement rates of existing variants by immune escape mutants combined with presence of partial cross-immunity [79]. However, it should be noted that multiple clades of the same subtype do co-circulate and reassort [80,81]. In terms of long term patterns, genetic and antigenic characterisation suggests that while genetic drift is continuous, antigenic drift is punctuated by periodic larger jumps into new “clusters” due to disproportionately advantageous amino acid substitutions [82–84]. Overall, these evolutionary and ecological patterns lead to substantial genetic and antigenic diversity of seasonal influenza viruses from one epoch to the next, but with dominant clusters at any given time.

1.3.2 Original antigenic sin, antigenic seniority and immune imprinting

Because of this significant antigenic variability, influenza adaptive immunity becomes less effective over time as antigenically novel strains replace antigenically familiar ones [82,85]. Humans therefore experience multiple influenza exposures over the course of their lives [86,87]. Consequently, the

immunology of an influenza infection or vaccination is complex; repeated exposure to antigenically related yet distinct strains of the same type can result in cross-reactive boosting of antibodies, incomplete cross-protection from infection with a heterologous strain or subtype, and “original antigenic sin” (OAS) effects [88–91].

OAS and related immunological imprinting effects result in particularly complex epidemiological dynamics, wherein an individual’s prior experience with influenza has a significant impact on their future susceptibility and immune response, discussed in detail in Chapter 2.2.4. For example, Lessler *et al.* showed that age-specific differences in observed antibody titres to multiple A/H3N2 clusters could be better explained by taking into account the strain that circulated during an individual’s early childhood [92]. They described the resulting phenomenon as “antigenic seniority”, a refinement of the OAS hypothesis wherein antibody titres are higher against more ‘senior’ strains encountered early in life. Similarly, Gostic *et al.* used a statistical model to show that age-specific patterns of infection by A/H5N1 and A/H7N9 viruses could be explained by the phylogenetic group of the seasonal influenza virus circulating in particular years [6]. Individuals who had initial exposures to group 1 (A/H3N2) viruses early in life had some protection against A/H7N9 (a group 2 virus), whereas individuals who had initial exposures to a group 1 (A/H1N1, A/H2N2) virus had some protection against A/H5N1 (a group 2 virus).

Our understanding of the biological mechanisms behind these observations is improving thanks to extensive experimental work [93]. However, linking the transmission dynamics of influenza (recurrent, seasonal epidemics over many years) to the observed serological data has only recently received quantitative attention. A seminal study by Fonville *et al.* used locally weighted multiple linear regression to fit smooth surfaces to antibody titres against influenza strains arranged in a 2-dimensional map of antigenic space. This method generates a visualisation of an individual’s antibody landscape: a 3-dimensional representation of antibody mediated immunity across all influenza strains of a particular subtype [87]. Kucharski *et al.* were able to reproduce similar visualisations using a mechanistic model to link latent infection states to predicted latent titres in the same antigenic space [1, 86]. By capturing the key antibody boosting and waning processes that result from infection events in a mechanistic model, Kucharski *et al.* were able to jointly infer individual infection states and antibody kinetics parameters that were most congruent with observed antibody titres. Modelling these antibody landscapes mechanistically allows the inference of the unobserved latent infection states, which may contribute to cross-strain immunity independently of antibody titres, and also allows forward predictions

of an antibody landscape given a particular exposure or vaccination.

1.4 Thesis overview

The overall aim of this thesis is to develop Bayesian inference methods to uncover hidden, longitudinal processes between epidemiological events of interest and the point of observation. I approach this problem by first addressing the life course problem in a relatively small window of time (the duration of pregnancy) where individual meta-data is not available, and then proceed to consider a life-time perspective where an individual's identity and specific exposure history are important. In doing so, I answer specific questions in ZIKV and influenza epidemiology. The objectives of each chapter are:

- **Chapter 2:** Review the literature required for the subsequent chapters, covering topics on antigenic variation, adaptive immunity, immune imprinting, and seroepidemiology. The majority of this chapter focuses on the influenza literature in preparation for Chapters 4–6. I end with a review of infectious disease modelling and Bayesian inference, which leads into Chapter 3.
- **Chapter 3:** Develop a mathematical model and inference framework to quantify the gestational-time-varying risk of microcephaly given ZIKV infection. I use this framework to compare risk profiles inferred using surveillance data from different locations in South America and to propose explanations for the apparent inconsistency in microcephaly incidence following the two ZIKV incidence waves in Brazil.
- **Chapter 4:** Fit antibody kinetics models to antibody titre data from a ferret model. These ferrets have undergone a variety of infection and vaccination events with different influenza strains, and are considered as a model for varied human exposure histories. I generate estimates for a number of antibody kinetics parameters, including boosting and waning rates, extent of cross-reactivity, and variation between infection and vaccination.
- **Chapter 5:** Address a number of limitations in the framework proposed by Kucharski *et al.* to infer latent influenza infection states using serological data. In particular, I address the lack of an explicit transmission model and demonstrate that the current framework generates biased attack rate estimates. I then propose a number of potential solutions that allow the infection generating process to be parameterised explicitly. As part of this work, I led the development of an R-package to infer infection states and antibody kinetics parameters given a generic serological dataset.

- **Chapter 6:** Using the R-package developed in Chapter 5, I infer infection states, historical attack rates and antibody kinetics parameters using data from a large serological survey from Guangzhou, China. I use these estimates to explore heterogeneities in infections by time, geography and age.
- **Chapter 7:** Summarises the findings of the thesis and discuss directions for future work.

1.4.1 Terminology

I define the general term for any kind of influenza antigen exposure (eg. inoculation, vaccination) as an *exposure*. I also define *effective antibodies against strain A* or *measured strain A* as the log HI assay titre measurement that was effective at inhibiting haemagglutination by antigens of influenza strain *A*. Similarly, the term *exposure strain* is used to describe the strain of influenza antigens contained in a given exposure (eg. vaccine strain).

Chapter 2

Background

In this chapter, I review the literature on antigenic variability, influenza immunology, models for infectious disease dynamics and Bayesian statistics. First, I describe the concept of antigenic variability and how it can be modelled through the concept of antigenic space. I then provide an overview of adaptive immune dynamics in the context of repeated exposures to influenza. Although many of the concepts I describe also apply to Zika virus, I focus these two sections on the influenza literature in anticipation of Chapters 4–6. With an understanding of humoral immunity, I discuss the importance of measuring antibodies for public health surveillance and model fitting. Finally, I provide an overview of the methodological background that underpins the mathematical models and inference methods throughout the thesis.

2.1 The complexity of antigenic variability

2.1.1 Overview

Infectious pathogens are said to show antigenic variation when the antigens encountered by a host's immune system can undergo structural change, allowing them to alter their life history and avoid preexisting host immunity. Many of humanity's greatest public health success stories have come from tackling strongly immunising pathogens that show little structural plasticity, with classic examples including the eradication of smallpox and the drastic reduction of measles cases following routine vaccination in the 1960s [94,95]. Immunity from one exposure can provide long-term immunity [85]. Viruses such as measles, smallpox, mumps and rubella exhibit little relevant antigenic variability [96]. Conversely, combating pathogens that exhibit significant antigenic variation over time is an ongoing challenge for public health planning and epidemiological research, particularly for vaccine design. Malaria, HIV, influenza and the flaviviruses (including dengue virus (DENV) and Zika virus) all exhibit substantial antigenic variation within the host, across geographical space and over time, leading to persistent infections and recurrent epidemics [97–100]. Even if immune memory generated through vaccination or infection is long lived against a particular set of antigens, antigenically novel variants may emerge that are no longer recognised by the immune system. Predicting how these pathogens might evolve and developing prophylactic treatments that are robust to antigenic variation are therefore key outstanding challenges in contemporary infectious disease research [79,101].

Antigenic variability is not unique to viruses, and is a key consideration for many bacterial (eg. *Streptococcus pneumoniae*, *Neisseria meningitidis*) and parasitic (eg. *Plasmodium falciparum*, trypanosomiasis) diseases. However, this thesis focuses primarily on influenza A viruses, with one chapter on Zika, and these pathogens therefore form the focus of this chapter.

2.1.2 Population dynamics

Differences in the tempo of change and coexistence capacity of antigenic variants can lead to different pathogen ecology. For example, influenza A/H3N2 exhibits limited global diversity at any given time [102], but substantial diversity over time as dominant antigenic types are periodically replaced by antigenically novel viruses [82]. These dynamics lead to the characteristic ladder-like phylogenetic tree of influenza A/H3N2: a predominant single trunk lineage with side branches that persist for 1-5 years until existing strains are out-competed and replaced by novel ones [73]. Conversely, DENV demonstrates

co-existence of up to four closely related serotypes (DENV 1-4) despite some cross-immunity (and cross-enhancement of disease) and temporal fluctuations in relative incidence [103–105].

Pioneering work by Gupta and others considered the role of cross-immunity in generating a wide range of dynamical behaviour [106, 107]. Gupta *et al.* showed that *P. falciparum* dynamics, which had historically relied on the assumption of an extremely high basic reproductive rate of a single strain, could be explained by more realistic transmissibility when multiple, antigenically distinct strains were considered [108, 109]. This body of work has since been captured under the term “Strain Theory”, describing the observation that many pathogens exist as non-overlapping strains by modelling a fixed number of discrete immunological targets (epitopes) at multiple loci on the pathogen [110, 111]. Around the same time, Gog and others began modelling strains within a continuous strain space, where strains show antigenic relatedness to those nearby in strain space and dissimilarity to those further away [112–116]. A common feature of these frameworks is that multi-strain pathogen systems are under selection pressure by population immunity, which leads to the pressure to diversify or out-compete other strains.

Whereas early modelling work typically considered competition for hosts between only two strains [117, 118], advances in computational capacity have enabled contemporary models to consider a large number of interacting strains [119]. There are now a few clearly defined strategies for modelling multi-strain systems: history based models (where an individual's status of exposure for each strain is tracked) [119, 120]; status based models (track ability of host to respond to strains but not history) [114, 121, 122]; polarized immunity (infection confers complete immunity to some hosts); models with other complexity-reducing assumptions such as age effects or strain symmetry [123, 124]; and individual based models (where all factors related to susceptibility and infectiousness can be stored for each individual) [76, 125]. However, all of these approaches rely on making assumptions about what an individual's infection history “should” look like; if an individual was tracked from birth and their encounters with each variant of a particular pathogen were recorded, what would their immunity look like? As we will see in later sections, answering this question with empirical data is challenging, and requires an understanding of the interaction of the immune system with different strains.

2.1.3 The concept of antigenic space

One of the most useful concepts in antigenically variable pathogen dynamics that has joined empirical observations and theoretical models is that of antigenic or strain space [126]. Many antigenically

variable pathogens exhibit genetic variation across the population that leads (sometimes) to differences in the structural properties of their surface proteins, resulting in differing interactions with host immunity and potentially different functional properties [82, 83, 127]. Variants exhibiting different isoforms of these surface proteins can be usefully defined as different strains [108]. Although there may still be significant genetic variation within strains, grouping pathogens at this level of variability is useful for the purpose of modelling and predicting phenotypic relatedness between distinct pathogen populations. Two strains that are close together in strain/antigenic space are more similar than two strains that are far apart. The magnitude of this distance, which describes the structural and biochemical similarities of two strains (but not genetic similarity) is termed antigenic distance.

Influenza is a key case study for antigenic space that has led to a now prolific method for visualising and quantifying antigenic relatedness: antigenic cartography [128]. Based on the idea of shape space, Lapedes *et al.* showed that antigenic distance is linearly related to the log of antibody effectiveness as measured by the haemagglutination inhibition (HI) assay, and described both metric and ordinal multidimensional scaling methods (MDS) [129–131]. Briefly, MDS is an algorithm to reduce the number of dimensions used to map the relationship between pairs of points in an N -dimensional space to an M -dimensional space (where $M \ll N$) without losing significant information.

Smith *et al.* developed a version of metric MDS to visualise the antigenic space of influenza viruses on a two-dimensional map [82]. Antisera are produced by infecting ferrets with a particular influenza strain, and the reactivity of that antisera against a panel of influenza strains is measured using the HI assay as a measure of antibody reactivity. An algorithm then finds the M -dimensional coordinates of all strains and antisera that minimises $\sum_{ij} e(D_{ij}, d_{ij})$: an error function to describe the distance between each antiserum j and each strain i . D_{ij} is referred to as the target distance between antigen i and antiserum j , defined as $D_{ij} = b_j - \log_2(H_{ij})$, where b_j is set as the log maximum log titre measurement achieved by antiserum j and H_{ij} is the log HI measurement for antigen i from antiserum j . d_{ij} is the Euclidean distance between the antigen i and antiserum j when given coordinates on the map. The error function to be minimised is then the sum of squared differences between the Euclidean distance of pairings in the M -dimensional map and the observed reduction in HI reactivity relative to the maximum HI response for that antisera ($e(D_{ij}, d_{ij}) = (D_{ij} - d_{ij})^2$, though a different cost function is used for measurements under the limit of detection for the assay). By placing all measured antisera and viruses in the same space, the antigenic distance between all strain pairs can be indirectly calculated.

Antigenic maps and the concept of antigenic distance are now integral to the analysis of many multi-

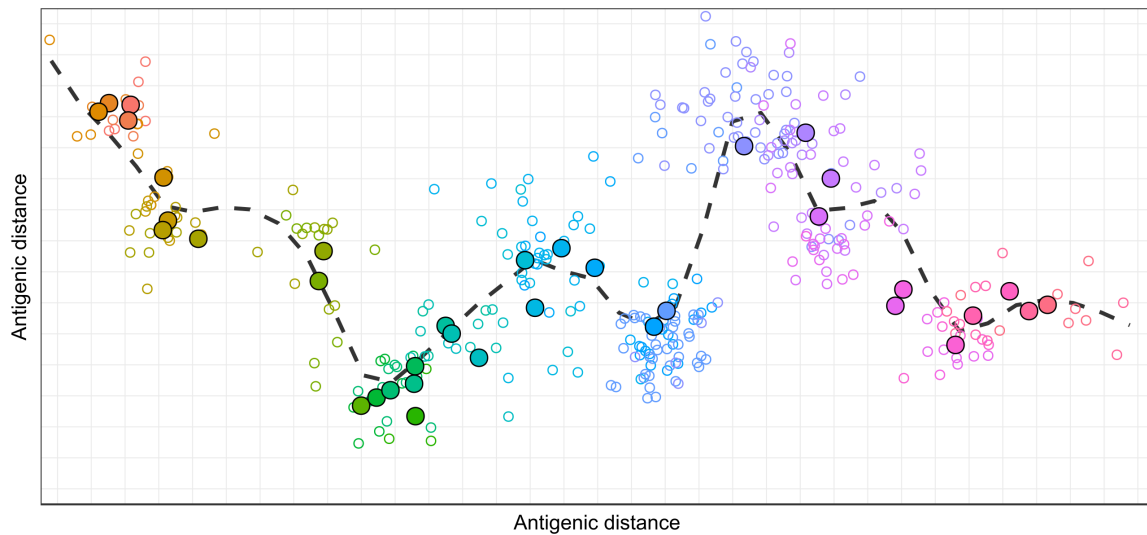


Figure 2.1: Example antigenic map of influenza A/H3N2 strains produced using coordinates from Smith *et al.* Coloured by year of isolation (not antigenic cluster), demonstrating the evolutionary path of influenza antigenicity through antigenic space. Dashed line shows a smoothing spline fit through the points. Points close together are highly reactive, whereas points far apart are less reactive. 1 grid cell represents 1 unit of antigenic distance, a single 2-fold titre difference.

strain pathogens, particularly influenza and dengue. Antigenic cartography is now a critical component of WHO's seasonal vaccine seed strain selection algorithm, where a shift of two antigenic units motivates update of the vaccine strain [132]. Non-human influenza strains also demonstrate vaccine escape due to antigenic change, and are therefore also suited to antigenic cartography visualisation [133, 134].

Subsequent work using antigenic maps has been invaluable in elucidating the temporal and spatial evolutionary dynamics of seasonal human and non-human influenza subtypes [82, 84, 135–137]. As part of their original paper, Smith *et al.* compared antigenic and genetic patterns of influenza to show that antigenic evolution is more punctuated and clustered than genetic evolution, where small genetic changes can lead to large antigenic changes [82]. More recently, antigenic cartography has also been used to understand the antigenic variation of dengue viruses [105, 138, 139]. Katzelnick *et al.* used antigenic maps produced using both monkey and human antisera to show that although strains of dengue virus do cluster into 4 serotypes, many strains are antigenically as similar to heterotypic strains as they are to strains of the same serotype [105].

Antigenic maps have also been extended to measure the magnitude of an individual's antibody profile across this space. These elevated surfaces across antigenic space are called antibody landscapes [87]. Antibody landscapes arise through the culmination of multiple infections and vaccinations that expand and strengthen the antibody response across antigenic space: elevated regions in the space represent the

abundance of reactive antibodies in that individual's sera and likely prior exposure to those antigens, and depressed regions represent the lack of reactive antibodies. Constructing these landscapes from sera taken at successive time points gives an idea of how an individual's antibody repertoire evolves over time.

Alternative models

Despite its limitations, the conceptualisation of antigenic distances into a continuous M-dimensional space is perhaps the most commonly used model for antigenic space. However, other theoretical models of antigenic space exist for influenza. One alternative (but not necessarily incompatible) model is to consider antigenicity as the properties of a fixed number of discrete epitopes with variable loci. The Hamming distance between strains across these epitopes is then used rather than Euclidean distance in discrete space [111, 140–142]. Interestingly, it was theoretical work modelling cross-strain immunity under this framework that motivated the search for such discrete, variable loci [143]. Wikramaratna *et al.* demonstrated how a limited number of epitopes and loci, some with high propensity for variability and some with low variability, were able to generate realistic influenza-like dynamics with strong single strain dominance [111]. They suggested that patterns observed under the continuous antigenic space model are readily reproduced under the discretised model. Empirical support for this hypothesis is currently the exception rather than the norm, but antigenic analyses of influenza A/H2N2 and more recent bioinformatic analysis of A/H1N1 suggest that some of these low-variability epitopes may be recycled over the past century [141, 144].

2.2 Adaptive immune dynamics

I will now discuss aspects of the immune response that are important to understanding long-term immunological dynamics, as it is adaptive immunity that responds to repeated exposures with antigenically related strains. I will primarily focus on the humoral adaptive immune response to influenza, as the mathematical models described later in this thesis are mostly fitted to anti-influenza antibody titre data. I will not consider the innate immune response, as it is (for the most part) invariant to antigenic variability [145].

2.2.1 The adaptive humoral immune response

Adaptive humoral immunity is governed by populations of B cells that produce antibodies, either immediately from the time of stimulation or through later reactivation. The full dynamics of the B cell response are complicated, given that they generate cytokines and interact with T cells in addition to secreting antibodies [146, 147]. In this thesis, I am interested in the production of antibody titres that can be measured by routinely used serological assays (eg. HI), and therefore restrict discussion to this main function. Fig 2.2 demonstrates the pathways through which B cells are activated and differentiate.

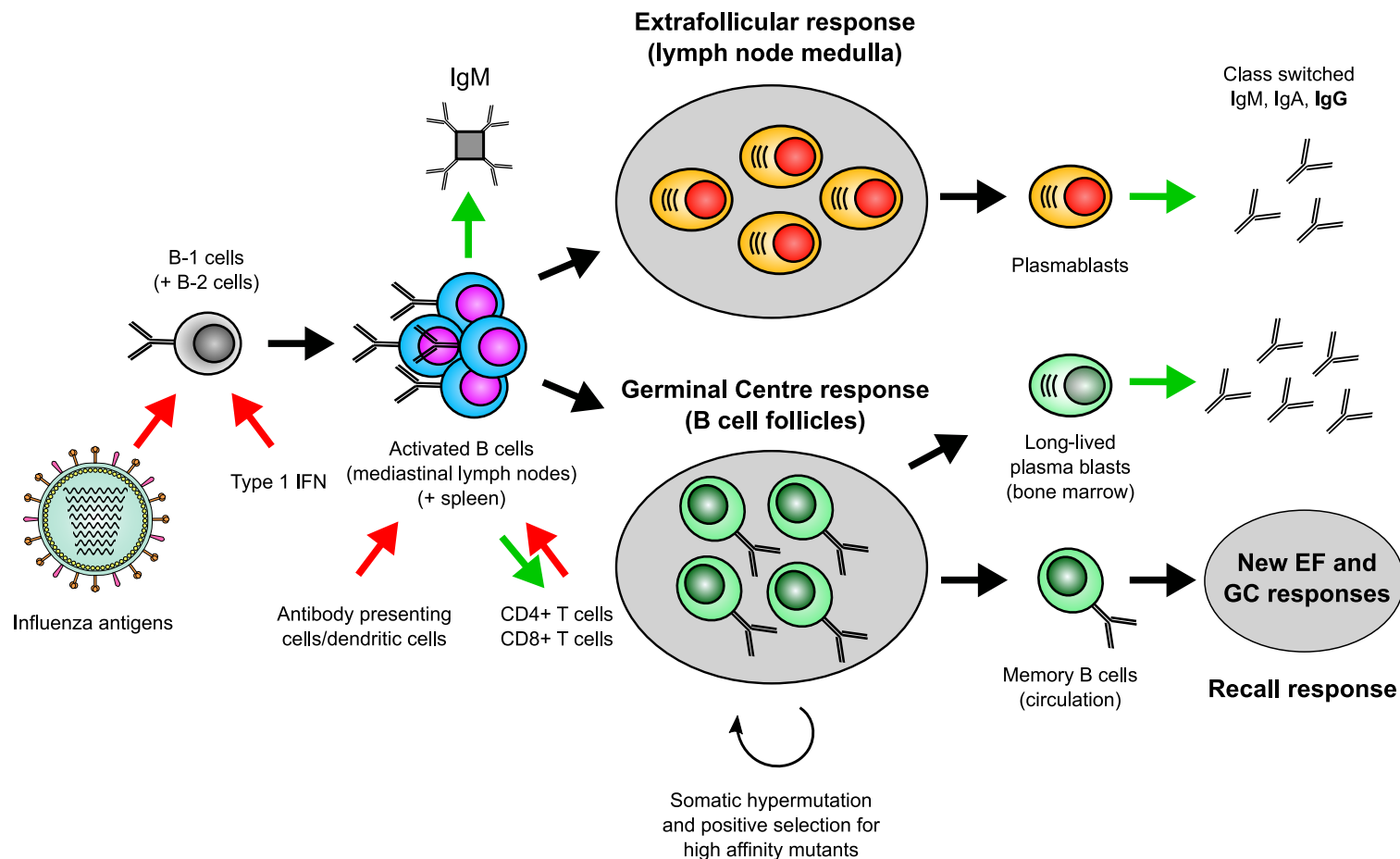


Figure 2.2: Schematic of B cell fates and antibody secreting functions. Pathway shown represents a typical primary infection with influenza. Red arrows show stimulatory/signalling interactions. Green arrows show production. Upon recognition of influenza (or any pathogen) antigens, naive B cells are activated and differentiate into either short-lived extrafollicular (EF) or long-lived germinal centre (GC) fates. Both lineages produce antibody secreting plasmablasts. Memory B cells from the GC lineage may be quickly reactivated upon re-exposure. General layout is adapted from [147].

Upon primary infection, pathogen antigens are made available to circulating naive lymphocytes in lymph and lymphoid tissues either as soluble antigens or by antigen presenting cells, namely dendritic cells. These antigens are recognised by one of the millions of diverse naive B cells that happen to have the complementary B cell antigen receptors (BCR) for that antigen. These successful naive B cells undergo clonal selection and expansion, leading to further proliferation and differentiation into mature lymphocytes. B cells may be activated in direct response to antigen binding (T cell independent), though more commonly require the help of T helper cells and cytokines at B cell follicles of secondary lymphoid tissue (T cell dependent) [147]. Some of these activated B cells quickly differentiate into extrafollicular (EF) short-lived plasmablasts, also known as antibody secreting cells (ASCs) [148, 149], which secrete antibodies that match the BCR. Others undergo affinity maturation at germinal centres (GC), whereby serial rounds of somatic hypermutation lead to B cells with increased antigen affinity again through clonal selection. Some of these high-affinity plasma cells enter the bone marrow to secrete antibodies for a few weeks as long-lived plasma cells, differentiate into circulating memory B cells (MBCs) [150, 151]. MBCs do not produce antibodies, but are long-lived and may be quickly reactivated and subsequently differentiate into ASCs upon exposure to a recognised antigen.

Immunity against secondary exposure takes advantage of these long-lived plasma cells. The antibodies produced by long-lived plasmablasts contribute to the serum antibody levels measured by routine assays, and can prevent or reduce the severity of infection if there is sufficient antigen recognition. MBCs are also rapidly activated upon antigen recognition; quickly differentiating into plasmablasts with a week delay and undergoing additional affinity maturation [150]. Depending on the number and properties of shared antigens between previous and subsequent exposures, the adaptive immune response to secondary exposure will be a mixture of the memory and naive response. If the re-exposure antigens are strongly recognised by the memory compartment, then the secondary response will be dominated by memory-derived plasmablasts, whereas novel antigens require a response from naive B cells. The memory response has a lower affinity but wider repertoire than the early response B cells, which hedges the memory response against potential mutations in the infecting antigen [152]. Indeed, repeated vaccination may lead to the continual recruitment of MBCs that undergo continued affinity maturation, leading to a memory response that is more robust to viral escape mutants [153].

2.2.2 Antibodies

Antibodies are small molecules with a two-armed variable region, which binds to the antigen, and a constant effector (Fc) region, which is involved in elimination of the bound antigen. By binding

to foreign antigens, antibodies can either directly neutralise the antigen or enable other effector mechanisms. There are five classes of antibodies determined by the constant region of the antibody, all of which are immunoglobulin (Ig) based in vertebrates. These are IgG, IgA, IgD, IgE and IgM [56]. These different antibody isotypes are typically found at different sites and times during the course of infection. For example, IgM is predominantly associated with primary exposure, whereas other classes, in particular IgG (which is further split into 4 subtypes), are rapidly produced upon secondary exposure [154]. The 2 IgA types are primarily found at mucosal surfaces, whereas IgG antibodies are found in serum. With respect to influenza, IgG antibodies are also typically found in the lower respiratory tract, whereas IgA are found in the upper respiratory tract [155]. Given that the assays and metrics that this thesis discusses concern measures taken from sera, reference to antibodies will implicitly apply to IgG antibodies [156].

Antigens are comprised of multiple epitopes that may be targeted by antibodies, and thus an individual's influenza antibody repertoire is diverse and complex [157]. In influenza, the HA, NA, M2 and NP proteins are all potential targets on the surface of infected cells [56]. However, not all epitopes have equal importance. The phenomenon whereby different epitopes are preferentially targeted in a hierarchical manner is termed immunodominance. For influenza, the HA and NA proteins are the most easily accessible for antibodies and BCRs, meaning that antibodies are primarily but not exclusively produced and maintained against these sites. Given the key roles of these proteins in target cell attachment and release, these are also effective antigens to target. Immunodominance applies at multiple structural levels; whereas HA is immunodominant to the M2 protein, immunodominance is also seen at a lower level within the HA glycoprotein, where the HA1 globular head is immunodominant to the HA2 stalk domain. Furthermore, there are distinct antigenic sites on HA1 that vary in their immunodominance: Sa, Sb, Ca1, Ca2 and Cb for H1 strains and A, B, C, D and E for H3 [158–162]. In contrast to the haemagglutinin of measles, which is relatively conserved, influenza has a number of possible variants at each of these antigenic sites [163]. Individuals will therefore have different antibody repertoires depending on which particular variants they have seen.

For influenza, as in many disease systems, past infection or vaccination with one strain can provide partial protection of varying strength to a range of other strains of the same subtype (and even across subtype) [6, 152, 157, 164, 165]. Empirical studies of this phenomenon in influenza have a long history, with many measuring the outcome of infectious challenge as a function of pre-existing immunity from heterologous and heterotypic exposure [166–168]. How much partial protection is provided from

pre-existing memory responses depends on how much of the exposure strain is immunologically familiar. As the HA head has many variable epitopes (as does the NA and HA stalk but to a far lesser degree), individuals may encounter strains to which they have substantial immune memory (ie. recognised epitopes), or strains that they have no memory (ie. novel epitopes) [89,169–171]. B cells are repeatedly stimulated upon repeated exposure, and the breadth of the B cell repertoire therefore changes over an individual’s life. However, because some influenza epitopes are more conserved than others, antibody levels against invariant regions become relatively more enriched over time than antibodies targeting variable regions, demonstrated by the increased abundance of anti-stalk antibodies in adults compared to children [172]. Antibodies against the conserved HA2 stalk do not exhibit neutralising activity, but rather are involved in the enhancement of other effector functions such as antibody-directed cell cytotoxicity (ADCC) [173]. The exposure kinetics in Fig 2.4 are shown as a recall response to an antigenically familiar virus, though *de novo* ASCs rather than memory derived ASCs responses would contribute more upon exposure to an unfamiliar virus.

2.2.3 The adaptive cellular immune response

Although not explicitly considered in this thesis, it is worth noting some general features of the adaptive cellular response. The cellular adaptive immune response is primarily mediated by CD4+ and CD8+ T cells. Broadly, CD4+ T cells have “helper” functions, whereas CD8+ T cells have cytotoxic functions. Naive T cells expressing a particular T cell receptor (TCR) react directly to foreign antigens presented by antigen-presenting cells (APCs) in secondary lymphoid organs and become activated [174]. CD8+ T cells help clear infection through a number of mechanisms: by inducing apoptosis or lysis of infected cells; signalling to macrophages to destroy phagocytosed microbes; activating B cells; and producing cytokines to initiate cell-signalling cascades [175]. During influenza infection, T cells tend to target epitopes of internal proteins, as these are presented on major histocompatibility complex (MHC) class 1 molecules by infected cells. These targets are important for heterosubtypic immunity, as internal proteins tend to be highly conserved across influenza strains and subtypes. Fig 2.2 shows the place of CD4+ T helper cells in aiding with development of the GC response, namely through a subset of T follicular helper (Tfh) cells [176]. Briefly, Tfh cells undergo a two-way interaction with presented antigens on MHC class II complexes of activated B cells, both reinforcing the Tfh lineage and promoting B cell differentiation into either EF or GC fates. Tfh cells continue to interact with B cells as they undergo multiple rounds of somatic hypermutation, providing the mechanism by which selection for high affinity BCR mutants occurs.

2.2.4 Original antigenic sin, immune imprinting and related phenomena

A consequence of repeated infections and vaccinations with viruses from a shared antigenic space is that each new exposure interacts with immunological memory induced from all previous exposures. These interactive effects were initially described following observations made in the 1950s and 60s, where human antibody titres to influenza haemagglutinin were typically highest against strains that circulated during childhood [177–180]. Not only were these titres higher, but they were also observed to be boosted further upon infection and vaccination with later circulating strains in humans and animal models [91, 181, 182]. This anamnestic response led to the loaded term “original antigenic sin” (OAS), where the term “sin” is often, but not always accurately, taken to imply negative consequences (Fig 2.3A). OAS has also been observed in children who experienced multiple sequential infections with dengue viruses, and to influenza neuraminidase as well as haemagglutinin [32, 183].

Research into OAS has since seen renewed interest because of unexplained changes in vaccine efficacy and improved assays to measure specific B cell lineages [93, 171]. With this renewed interest came a range of new terminology and refinements to account for the range of immunological and epidemiological consequences of this immune memory bias. The terms “antigenic imprinting” and “immune imprinting” are now widely used to capture the impact of an individual’s first exposure on all subsequent immune (both cellular and antibody) responses [6].

Referring specifically to the antibody response, “antigenic seniority” is a possible refinement of OAS (Fig 2.3B), wherein each successive strain in an individual’s exposure history is given a more junior position in the hierarchy of antigenic boosting preference. This mechanism leads to the accumulation of high titres against strains that circulated in childhood in line with original observations, but allows for strains met later in life to induce a significant antibody response [86, 92]. “Back-boosting” (Fig 2.3C) is related to OAS, and refers to the observation that antibodies against early encountered strains are increased following more recent exposure, likely due to the recognition of conserved epitopes [87, 187, 188].

OAS-related processes have been proposed as a potential explanation for age-cohort effects in syndromic incidence. Incidence of pH1N1 in 2013-2014 was far higher in middle-aged individuals than expected, possibly due to the acquisition of a mutation in an accessible region of the HA that was previously targeted by these individuals. Previous seasonal H1N1 strains were conserved in this same region, and these individuals were therefore never afforded the opportunity of generating antibodies against other sites, whereas younger individuals were (Fig 2.3D and shown in [93]) [189].

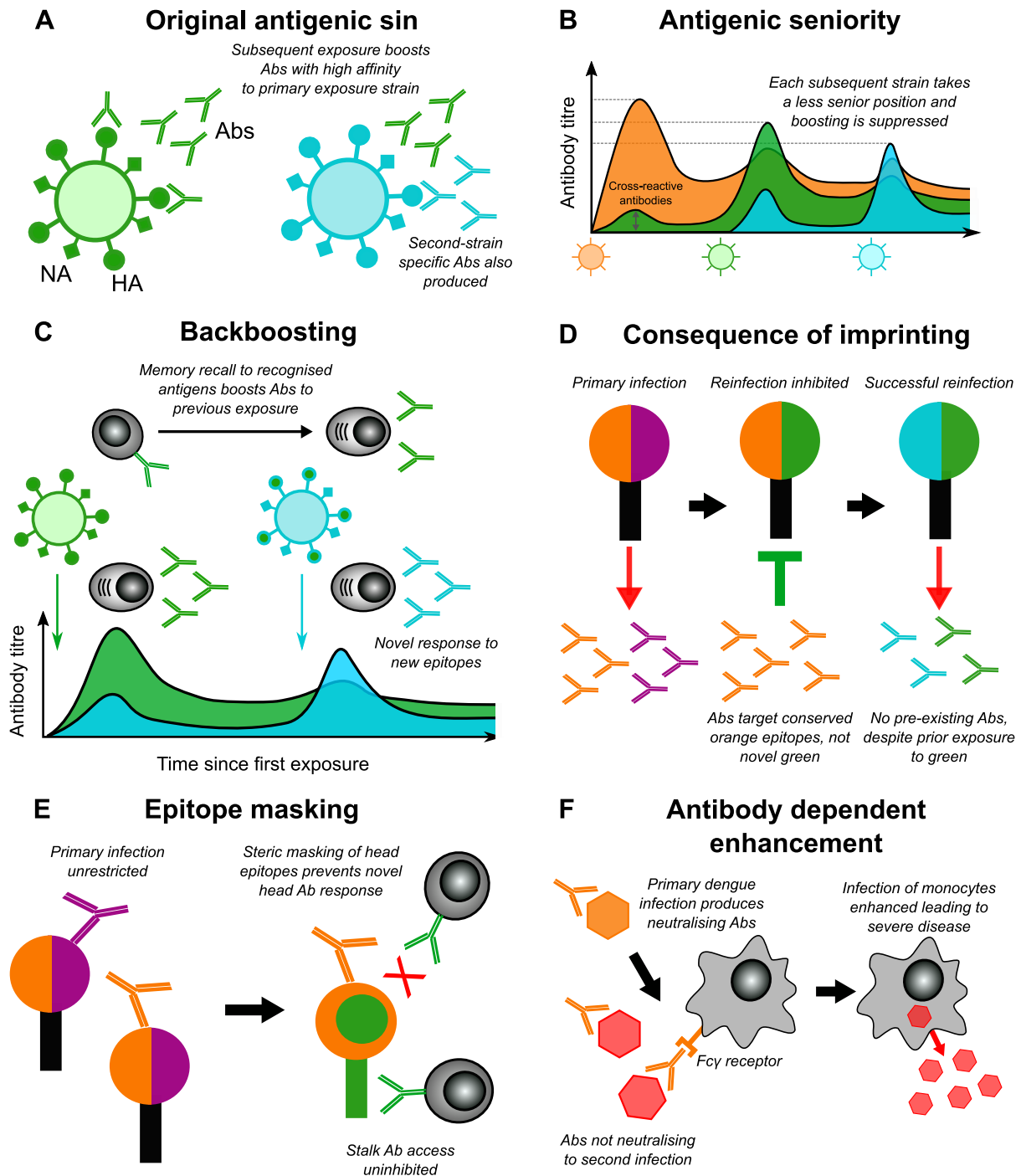


Figure 2.3: Schematics of various OAS effects and related phenomena. (A) Original antigenic sin occurs when antibodies (Abs) targeting previously encountered epitopes are boosted upon re-exposure to a different virus [91]. (B) Antigenic seniority describes the observation of Ab titres being highest against strains that circulated early in an individual's life, and successively lower against each more recent strain. The original model also postulates that cross-reactive Ab boosting proportional to antigenic distance occurs [86,92]. (C) Back-boosting of responses to previously encountered strains upon new exposure [87,184]. (D) Imprinting to particular epitopes can inhibit the production of a novel response in certain seasons. (E) Epitope masking. Abs against previously encountered strains prevent physical access to certain epitopes on the HA head but not stalk, inhibiting the production of novel Abs. (F) ADE occurring in secondary DENV infection through cross-reactive but not neutralising pre-existing Abs. Schematics interpreted and adapted from [56,93,185,186].

Variable vaccine efficacy is one of the most widely discussed topics related to immune imprinting [190]. Early work investigating the consequences of repeat vaccination initially appeared to be incompatible: the Hoskins study (study of a boy's boarding school over three influenza outbreaks 1970-1976) suggested that repeat vaccination did not provide long term protection from infection, whereas the Keitel study (5 year study of healthy adults in Houston, Texas) suggested that it did [191–193]. Smith *et al.* developed the “antigenic distance hypothesis” and terms “negative and positive interference” (proposed refinement to “antigenic interaction” by Monto *et al.* [193]) to explain how both of these observations may be compatible [187]. Smith *et al.* developed a mathematical model of an antigenic “ball of stimulation” taking into account the antigenic distance between vaccine strains and circulating strains: if the first and second vaccine strains are antigenically similar, then there may be negative interference when the circulating strain is antigenically distant to the first vaccine strain as response from the second vaccine targets the first vaccine strain, or positive interference if the vaccine and circulating strains are well matched and prior vaccine-induced immunity targets the correct antigen. Recent studies have also found a negative impact of serial annual vaccination on vaccine effectiveness relative to infrequent vaccination [194–197]. Systematic reviews on the topic suggest that repeated vaccination is still protective, but a better understanding of how and why reductions in efficacy arise remains a research priority [198].

Age-specific incidence and vaccine efficacy patterns are also thought to be related to age-cohort effects arising from immune imprinting. The 2009 H1N1 pandemic provided evidence for the role of immune imprinting on age-cohort effects: individuals who were young during circulation of the closely related 1918 H1N1 lineage (ie. older individuals) experienced lower mortality rates than those who were children during the dominant circulation of A/H2N2 and A/H3N2 viruses (younger individuals) [199, 200]. Immune imprinting to antigenically distinct subtypes has also been proposed to explain age-specific incidence and mortality patterns during the 1957 A/H2N2 pandemic, the 1918 pandemic, and during recent influenza seasons [189, 201–203]. In terms of general epidemiological trends, Kucharski *et al.* developed a mathematical model with cross-protective variable epitopes to show that immune imprinting can lead to strong age-cohort effects (age-specific patterns in so called “immune blind spots”). Different age groups are exposed to different combinations of epitopes, and because individual epitopes disappear from the virus population at different times, age groups will lose their protection at different times [123].

2.2.5 Mechanism of OAS and related phenomena

The exact mechanism and therefore predictability of OAS and its related phenomena is not yet fully understood, though work with animal models and measures of B cells have shed some light [169–171, 204, 205]. The competition between memory B cells and naive lymphocytes for access to antigens or specific epitopes is thought to be involved. Memory B cells have a lower threshold for activation and quicker activation, and therefore may sequester and neutralise the infecting antigen before a novel response can be mounted [206]. By binding to conserved epitopes on the HA head before a novel response can bind to unfamiliar drifted epitopes, pre-existing antibodies can cause steric interference, preventing other antibodies or receptors from accessing certain antigenic sites (Fig 2.3E) [185, 207]. Note that steric interference occurs across the HA head domain, but is thought to not interfere with binding to the stalk. Another hypothesis based on mathematical modelling is that T cells regulate the loading of novel antigen on dendritic cells due to preference for recognised antigens, thereby biasing the recruitment of naive lymphocytes towards previously encountered epitopes [208].

The mechanism of epitope masking is supported by the recent evolutionary behaviour of influenza itself. Despite appearing to be well matched, the chosen vaccine strain for the 2016–2017 elicited poor responses against circulating H3N2 strains, which had acquired a glycosylation site within epitope site B [209]. Adaptation during egg passaging has been suggested to result in antigenicity changes through the gain and loss of glycosylation sites, rendering vaccine responses against circulating strains ineffective [210]. The additional glycan on the 2016–2017 circulating strains may have masked epitopes to which vaccine-induced antibodies were generated [211]. In contrast, there is currently little biological evidence to support the regulatory T cell explanation, though OAS effects have been shown to be reduced through the inclusion of dendritic cell activating adjuvants or increased antigenic dose following vaccination, which is consistent with this theory [212].

Although not explored further in this thesis, it is worth noting that immune imprinting effects can have drastically negative consequences in dengue infection. Antibody dependent enhancement (ADE) occurs following re-exposure to a dengue virus (DENV) serotype different to the one from the primary exposure [32, 213]. Although the antibody response against the E protein following primary exposure is strongly neutralising to the primary serotype, it does not neutralise viruses of the other 3 serotypes at intermediate titres. Rather, these cross-reactive antibodies enable the DENV virus to bind to Fc γ receptor-bearing cells, enhancing access to these cells and thereby increase overall viral titres and thus clinical severity (Fig 2.3F).

2.2.6 Kinetics of the adaptive immune response

When referring to adaptive immunity in the context of longitudinal processes to be modelled, I am interested in the time-dependent immunology taking place within an individual. A common source of data in this thesis will be assays of antibody titres over time, and I therefore divide antibody kinetics into phases of boosting and waning. Although observed antibody boosting and waning is simply a proxy for complex underlying lymphocyte and antigen dynamics, it is still useful to produce simple models that can describe routinely generated serological data without the need to model or measure specific cell populations. It is also important to clarify what the antibody titre being modelled represents. Although antibodies are produced against specific epitopes by targeted B cell lineages, most routinely used antibody assays measure only an aggregated polyclonal response. All of the antibody titre data in this thesis will come from the haemagglutination inhibition (HI) assay, which measures the ability of serum antibodies to prevent viruses from agglutinating red blood cells and therefore captures the majority of the overall antibody response against HA1 [57, 214]. The HI assay measures the polyclonal response against a single virus, and even small changes to the assay virus can produce large changes in the assay reading [215]. Fig 2.4 provides a conceptual schematic of how overall and epitope-specific serum antibody titres develop over time following two infections in relation to the B cell dynamics described in Fig 2.2.

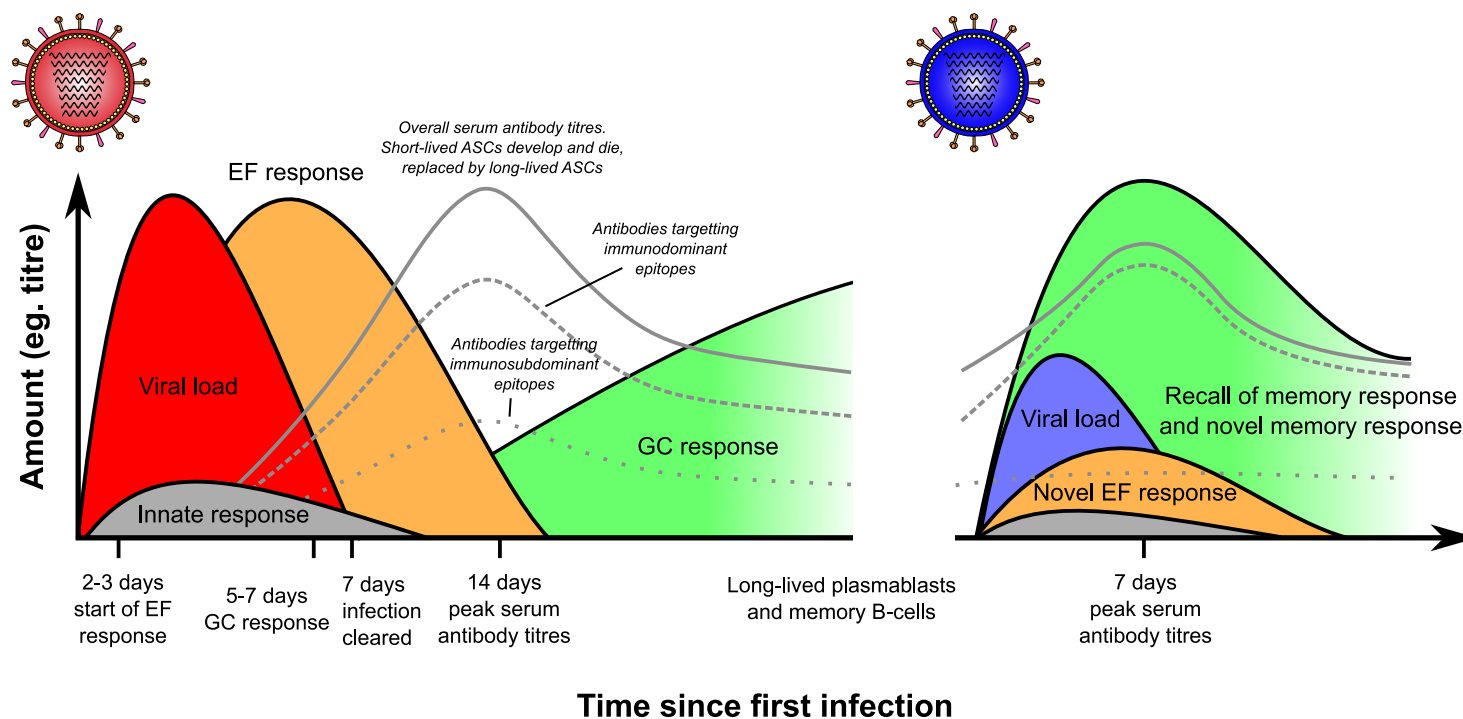


Figure 2.4: Timeline of the adaptive humoral response following primary and secondary exposure. Y-axis shows un-scaled intensity of response. This schematic represents the case of an individual becoming infected without complication for the first time with the red influenza strain, and later with an antigenically related blue strain. The grey line represents overall serum antibody titre as would be measured by a polyclonal antibody assay such as HI. Dashed grey lines show relative contributions of antibodies to specific antigens, though relative contributions will depend on OAS and related effects as described in the main text. General layout is adapted from [147,216]

Dynamical models of the adaptive immune response in controlled systems do exist, namely compartmental models based on the target cell, infected cell, virus (TIV) model and its many extensions [217–219]. These models tend to be better applied to the dynamics of infection and clearance, aiming to quantify the relative contributions of different arms of the immune response. The accompanying parameters of interest are on a short time scale. Furthermore, mathematical models to describe repeat-exposure systems, as are needed for multi-strain pathogens, are a very recent addition to this literature [220, 221]. Dynamical models focusing exclusively on the humoral immune response are uncommon [222].

However, recent years have seen a growing interest in making better use of serological surveillance data (discussed in the following section) by developing mathematical models of the humoral immune response – an arm of immunity that can be routinely measured using antibody assays [223, 224]. These studies have covered a number of disease systems, particularly influenza and DENV, but also malaria, meningococcus, cholera and various wildlife diseases. Although tailored to answer different questions, all of these studies attempt to capture immunological realism when analysing longitudinal antibody kinetics following (possibly multiple) exposure. Common features of these models include: inclusion of an unknown antibody boosting parameter, describing the magnitude of antibody titre increase following exposure [1, 40, 223, 225–228]; antibody or ASC waning, describing an exponential decrease in antibody titres or ASC half-life after peak serum titre [228–230]; cross-reactive boosting of antibodies to antigenically related strains [1, 40]; distinguishing between strong, transient short-lived antibody boosting and persistent boosting [1, 227, 228, 231, 232]; and individual-level variability in response [227, 228, 230].

In the following section, I discuss the use of serosurveillance and its combination with dynamical modelling and statistical inference to understand infectious disease dynamics.

2.3 Seroepidemiology

In this thesis, I am most interested in the story that antibodies tell us about an individual's history with a disease. I first discuss the use of antibody titres as a correlate of protection in influenza, and then turn to the interpretation of titre rises as markers of exposure.

2.3.1 Public health relevance of antibodies

The adaptive immune response is of particular interest for seasonal epidemic and pandemic preparedness, as it can (i) be induced in advance of an epidemic through vaccination and (ii) be measured and compared against correlates of protection to improve public health forecasting [233–236]. Despite limitations discussed below, measures of “seroprotection” (an HI titre above 1:40) or “seroconversion” (a 4-fold increase in HI titre) were traditionally required by the Committee for Medicinal Products for Human Use (CHMP) and Food and Drug Administration (FDA) as an endpoint in assessing seasonal influenza vaccine efficacy (though less rigid and broader analysis of serological metrics is now required) [237–239]. Table 2.1 describes some of the routinely used antibody assays in influenza research, though these assays are also used for flaviviruses. It should be noted that although these assays are used in serosurveillance, only the HI titre (and serial radial hemolysis assay to a lesser extent) is currently widely accepted as a robust correlate of protection against influenza infection [240]. Although there is good overall correlation between MN and HI titre readings (an HI titre of 1:40 roughly corresponds to an MN titre of 1:20), generating a direct equivalence is difficult due to virus-specific effects and the potential for changing antibody targets as individuals age [241].

Setting the $\geq 1 : 40$ HI titre endpoint dates back to a seminal study by Hobson *et al.* in the 70s, though use of anti-HA antibodies as an indicator of protection predates this [233,242]. In Hobson *et al.*, pre-exposure serum titres against the pandemic A/Hong Kong/1/68 virus were measured in volunteers who were then challenged with infection. The proportion of these individuals that were infected was then compared as a function of increasing antibody titre against the challenge strain, showing a clear decrease in probability of infection at higher titres. Here, and in most subsequent studies, reported thresholds for protection are the HI titre required to afford 50% reduction in infection likelihood (the 50% protective dose). Although more recent studies have attempted to tease out additional variables that may alter the $\geq 1 : 40$ threshold for certain populations (eg. children vs. the elderly), the general framework of a log-linear relationship between relative susceptibility and an HI titre of around 1:40 (or higher) still stands [235,243,244].

However, there are outstanding questions regarding the robustness of the HI threshold. First, different vaccines (namely the live attenuated influenza vaccine) stimulate different arms of the antibody and cellular response, providing protection despite inducing sub-threshold HI titres [214]. Second, not all individuals seroconvert in the HI assay following vaccination, and protection via other antigen targets (eg. NA) may be missed [245]. Third, a correlate of protection does not necessarily imply a causal relationship. Cowling *et al.* recently showed that 57% of the vaccine effect in a cohort of children receiving inactivated influenza vaccine could be attributed to post-vaccination HI titres, suggesting that other HI-independent effects played a protective role [244]. Similarly, Ranjeva *et al.* recently showed that although HI titres were a good marker of protection in children, time since last exposure was more strongly correlated with protection in adults [225].

Finally, it is worth noting that clinical outcomes of interest may not always be associated with a binary below-or-above threshold. In the case of dengue, although the probability of infection or hospitalisation decreases monotonically with log HI titre at the time of exposure, the probability of developing dengue haemorrhagic fever is higher at intermediate titres than low titres, and only decreases again at very high levels [40]. Understanding this non-linear relationship is crucial in the context of the available dengue vaccine, dengvaxia [246]. As a result, dengvaxia is currently licensed for use in individuals with serological evidence of prior dengue experience, given the potential for increased risk of severe disease through boosting seronegative individuals into the risk window [247].

Name	Description	Strengths	Limitations	Reference
Haemagglutination inhibition assay (HI)	Serial dilutions of sera measure the highest dilution that are able to inhibit agglutination of red blood cells (historically turkey or chicken) by influenza A virions	Cheap, fast assay; shown to be correlated with protection (titre above 1:40 50% protective in adults) [233]	Non-specific antibodies; between lab variability [248–250]; dependent on erythrocytes used; discrete data grouped by 2-fold dilution; measuring ability to bind to just HA head; ineffective for for contemporary (clade 3C.2a) A/H3N2 viruses [251, 252]	Pedersen, JC Methods Mol Biol 2014;1161:11-25 [253]
Microneutralisation assay (MN)	Mixture of influenza virus and sera at increasing dilutions are used to infect cell cultures. The highest dilution at which infection is inhibited is the resulting titre	Measures all inhibiting antibodies, not just those to HA head; Shown to be useful in detecting H5-induced antibodies [254]	Time intensive; limited evidence for correlates of protection; requires live wild-type virus; between and within lab variability [250]	Grund <i>et al.</i> J Virol Methods 2011;171(2):369-73 [255]
Enzyme-linked immunosorbent assay (ELISA)	Different types: indirect, direct, competitive and sandwich. Broad concept is to fix target antigen on multi-well plate. Serum samples are added and plates are washed, such that any antigen-specific antibodies bind. A second antibody, binding either the antigen or target antibodies, is linked to an enzyme and added. Colouration from enzyme-linked reaction through addition of substrate gives reading	Highly sensitive and specific by choice of antigen	Expensive; time consuming	Yuan, Q <i>et al.</i> Clinical Microbiology and Infection 2011; 17(10):1574-1580 [256]
Single radial haemolysis assay (SRH)	Erythrocytes are suspended in agarose gel containing influenza virus. Serum sample is placed in the well and antibodies diffuse radially The diameter of the cleared zone is the measure.	Requires only a small number of virions; sensitive for influenza B; [248] cleared diameter 25mm ² considered protective; simple assay; continuous measure	Dependent on erythrocytes used [257];	Schild, GC Bull World Health Organ 1975; 52(1):43-50 [258]

Table 2.1: Summary of different assays routinely used for quantification of influenza antibody titres

2.3.2 Seroepidemiology for surveillance

Epidemiological surveillance is important in informing public health planning both routinely and during an outbreak. Understanding when and where resources are needed, the nature of the pathogen, and the shape of the case severity pyramid all require information on who is infected and when [259–262]. If reporting occurs soon after infection, then the aetiological pathogen can be confirmed using virological assays, namely reverse transcription polymerase chain reaction (RT-PCR). However, being recorded as a suspected case and becoming laboratory-confirmed relies on detection of infected individuals. When the detection criteria is based on the presence of particular symptoms, asymptomatic or mild cases may not seek healthcare and be missed by surveillance sites. Asymptomatic infections may be important in determining attack rates in particular populations and key quantities of interest (eg. case fatality ratio, rate of clinical illness per infection), even if they do not themselves require treatment. Symptomatic surveillance is also non-specific, which can lead to under- and over-reporting. For example, Chikungunya and Zika may have been confounded between 2014 and 2016 due to similarities in symptoms [263], and influenza-like illness (ILI) can be caused by various other respiratory diseases [264]. Overall, reconstructing epidemiological dynamics based on non-specific, low coverage symptomatic case data with an unconfirmed causative agent can be extremely difficult [265].

Serological data have a number of advantages over virology and symptomatic surveillance, and there has been a recent drive towards a “World Serology Bank” to capture the immune landscape against common infectious diseases [266,267]. The long term waning rate is slow for most common pathogens, and a positive antibody titre can therefore usually be recorded long after the infection has been cleared [85]. Furthermore, asymptomatic cases may still mount a measurable antibody response [268]. Serological surveys are therefore often cited as the gold standard of surveillance for pathogens such as influenza [262]. Blood samples are either taken through cross-sectional (ie. from individuals at a single point in time) [269] or longitudinal (ie. from the same cohort at multiple time points) [270,271] surveys. Both are useful for detecting prior exposure: if an individual has a positive antibody titre to an antigen, then it is frequently assumed that the individual must have previously been exposed to said antigen through infection or vaccination.

There are two common ways of interpreting antibody titre data as a proxy for exposure: seropositivity and seroconversion. Following infection, serum antibody levels increase as the host’s immune system mounts a humoral response. This response is time-dependent; antibody levels take time to increase and peak and tend to wane over time, in distinct short and long term phases [231]. Fig 2.5A demonstrates

how an antibody assay measurement may indicate exposure under the criteria of seropositivity in the ideal case. If the measured antibody response at the time of sampling is above a pre-defined threshold, it is probable that a previously naive individual has been recently infected. Fig 2.5B demonstrates the use of seroconversion for detecting infection in the ideal case. Samples are taken from the same individual at two time points, and an increase above a certain amount suggests infection between the two sampling times.

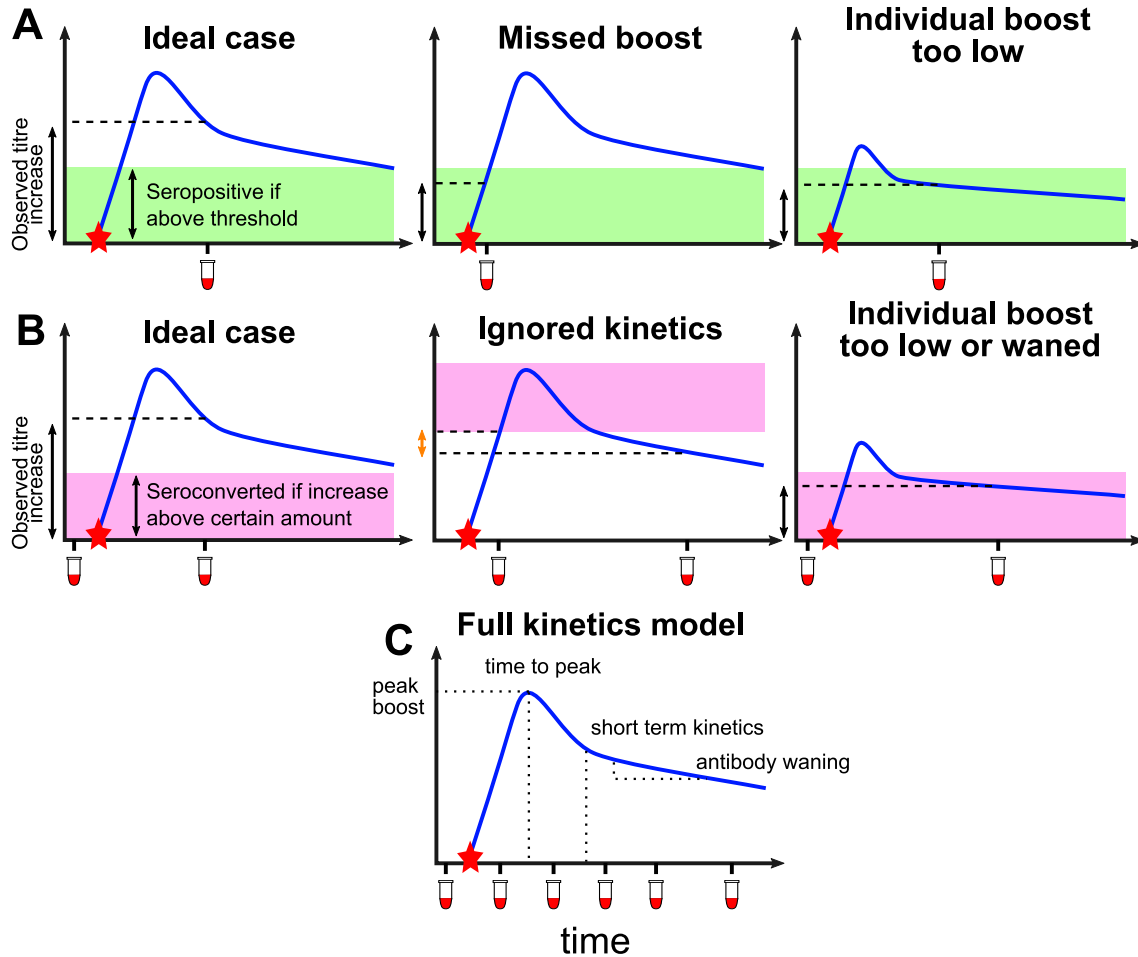


Figure 2.5: Schematic demonstrating observations of an individual's antibody kinetics process (blue line) following exposure (red star) given only a limited number of blood samples (blood vials). Dynamics are shown on a log scale, with linear long term waning on a log scale (exponential waning on a linear scale) A: Potential confounding of seropositivity criteria given variation in time between infection and sampling. B: Potential confounding of seroconversion criteria given paired serology. C: The full antibody kinetics process would be observable given a suitable mathematical model and frequent sampling.

There are clearly a number of instances where the interaction between an individual's antibody kinetics and the sampling time can confound both of these criteria. In the case of seropositivity (Fig 2.5A), taking the blood sample at a point in the kinetics process where the antibody response has either not yet reached or has subsequently waned below the threshold may lead to a false negative result. Given

that the antibody response to influenza infection takes 2-3 weeks to develop, such confounding is not uncommon [199, 272, 273].

Similar confounding may also affect paired serology. In Fig 2.5B second panel, antibody titres would not be observed to have increased, and thus a false negative result may be recorded by the seroconversion criteria. The final column of Figs 2.5A&B demonstrates how individual-level variation in the magnitude of an antibody response, or a response directed primarily against an antigen that is not measured, may also lead to a false negative result by either criteria [227, 244]. This fallibility may also result from the assay measurement itself, where sub-threshold rises may be missed through measurement error (ie. an observed titre lower than the true one) or setting the seropositivity/seroconversion threshold too high [274–276]. Censoring effects due to the discrete nature of titre measurements, antibody activity below the threshold for detection, and antibody boosting near the upper dilution limit may also mask true antibody kinetics, leading to false negative results.

Interpreting serological data for antigenically variable pathogens is further complicated by cross-reactivity from prior exposure to one or more serologically related strain. Fig 2.6 shows how infection with the blue virus may elicit a cross-reactive antibody response to the orange virus due to the presence of shared epitopes. If one were interested in the presence of exposure to only the orange virus, then the criteria for a positive exposure may be met and a false positive recorded. Failing to take into account effects arising from immunological memory may also confound interpretation of homologous antibody kinetics. Fonville *et al.* illustrate this nicely using longitudinal antibody landscapes, presenting an individual who likely experienced infection in 2009, but demonstrated the largest antibody boost to strains circulating a number of antigenic clusters previously (row 4, Fig 2 of [87]). Crucially, this individual did not have a 4-fold rise to the most recent strain. This may have been a result of strong back-boosting of memory responses, though boosting of antibodies to recent strains was still observed. Analyses that account for cross reactive responses against a panel of strains or epitope-specific assays are needed to control for these phenomena.

2.3.3 Dynamical models and inference using serological data

To address these limitations, mathematical models describing the link between observed antibody titres and latent infection states have seen a recent rise in interest [223]. The common aim of models with a dynamic antibody process is to capture individual antibody titre trajectories against an infecting pathogen, as summarised in Section 2.2.6. Observations of these latent antibody trajectories are

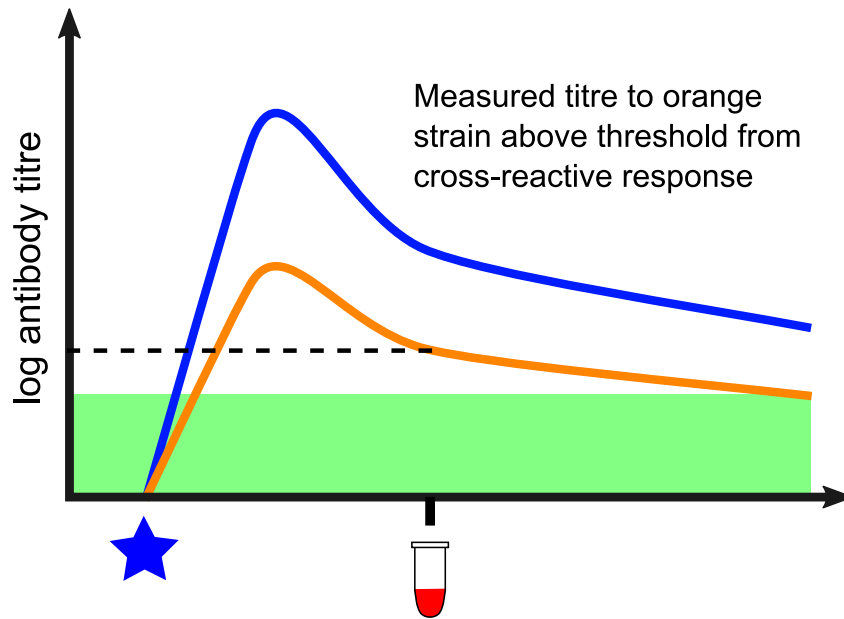


Figure 2.6: Schematic of potentially confounded serology due to cross-reactive antibody kinetics. An individual is infected with the blue strain, but only titres against the orange strain are observed and interpreted. As the threshold for seropositivity has been met, a false positive infection with the orange strain is inferred.

made using serological assays at various points in time and are used to calculate a likelihood for the presence/absence of infection and antibody kinetics parameter estimates. These inferred infection times may then be used to inform population-level trends in the force of infection (Fig 2.7 and summarised in Fig 1 of [223]). Serocatalytic models, which infer historical force of infection trends based on the proportion of seropositive individuals in different age groups, have also been used to interpret aggregated cross-sectional serosurvey data, but these do not account for individual-level antibody trajectories [265].

Two key features that consistently emerges from dynamic antibody models are the importance of capturing antibody waning and the variability of response between individuals. A recent modelling study by Zhao *et al.* fit antibody trajectories to longitudinal HI titre data, demonstrating that HI titres peaked a few weeks after pandemic H1N1 infection at high levels but returned to near baseline levels within a year [230]. Both the delay from infection to peak and substantial waning demonstrate the importance of choosing sampling times to avoid missing periods of elevated titres. Interpretation of antibody waning rates is further complicated by the longevity of different ASC populations [231]. Models that distinguish between short- and long-lived ASCs are well suited to some datasets (eg. malaria, influenza [1, 227]), though models with a single exponential or power-law decay rate are sufficient for others (eg. pertussis [232]). Choosing between alternative waning models depends on

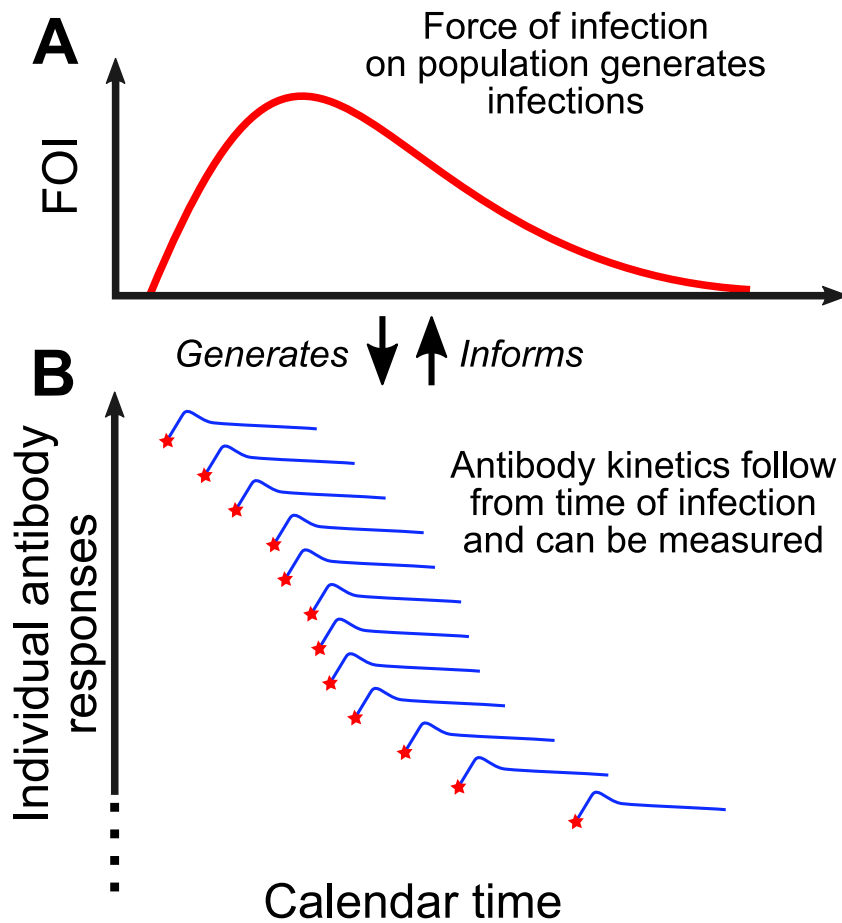


Figure 2.7: Relationship between individual-level antibody trajectories and population-level dynamics. (A) In the event of an epidemic, the force of infection (FOI) on the population leads to individual infections, with the probability of infection being highest when the FOI is high. (B) Individual antibody responses then undergo longitudinal kinetics which may be measured. By modelling the antibody kinetics process, antibody trajectories may be simulated back to the time of initial infection. The timing of these individual-level infections may be combined to generate a picture of the population-level force of infection.

the timescale under consideration and the availability of longitudinal samples to inform more complex models. Individual-level variation in antibody boosting and waning has also been captured using random effect distributions on model parameters [40,227,230]. However, this hierarchical structure can drastically increase the model space (the number of parameters to be estimated) and is therefore suited to datasets with multiple repeat observations per individual.

2.3.4 Model fitting in multi-strain systems

Although interest in dynamical modelling of antibody kinetics to inform epidemiological estimates has developed substantially, work on antigenically variable pathogen systems has been fairly limited to date due to the complexity of ecological and immunological interference between related variants of the same

species. This limits the ease of applying classical data augmentation and inference methods [277, 278]. For example, in the case of influenza, the probability of infection with a given strain depends on the level of immunity from all prior exposures, which are unobserved and would need to be included in the data augmentation step for a full life course model. Work to date has therefore focused on relatively simple models of co-circulation (eg. two pathogens [279]), scenarios where many infections are virologically confirmed rather than observed via a proxy measure (eg. RT-PCR and subsequent longitudinal observations of confirmed infections [40]), or restricted to short observation windows (eg. a few years rather than over the entire lifespan [225]).

Auranen *et al.* provide one of the first examples of applying data augmentation to the inference of infection states with multiple co-circulating pathogens [280]. Auranen *et al.* inferred the acquisition and clearance rates of *Streptococcus pneumoniae* (Pnc) bacteria using Pnc serotype carriage data by representing carriage at observation times using latent binary variables; each individual was assigned a 1 when they carried a particular serotype and a 0 otherwise. Although their methods were general, the setting was limited to 97 families in a 2 year window and to 3 (of 7 potential) Pnc serotypes. Furthermore, the observation model was not complex, given that data were direct observations of serotype carriage over time. A similar framework was later developed by Yang *et al.* that included a more complex observation model. Here, infection states for multiple co-circulating pathogens were inferred using non-specific symptomatic data (ILI in this case) with the complication of missing-at-random laboratory confirmation for many of the cases [281].

More recent work on multi-strain infection inference has focused on dengue due to the severity of reinfection and interaction with the dengvaxia vaccine. In a recent study, Salje *et al.* were able to reconstruct antibody profiles over time against the 4 DENV serotypes for 3,451 individuals, identifying 1,149 subclinical infections which were not detected by RT-PCR surveillance [40]. A boon of their dataset was that serum samples were taken at quarterly intervals for each individual, giving more power to estimate antibody kinetics at a detailed timescale. Many individuals also had RT-PCR confirmed infections following clinical disease, allowing antibody kinetics trajectories to be started from a known infection time for many individuals. Both of these factors allowed for a model with individual-level heterogeneity in antibody boosting and waning responses and inferred time of infection at a daily resolution. A similar analysis was recently undertaken by Tsang *et al.*, who used a statistical model to infer infection histories for the 4 DENV serotypes based on the presence of RT-PCR or ELISA confirmed infections [282]. A feature of this system that is not present for influenza is that the

maximum number of infections can be set for each individual *a priori* (once per serotype), whereas the upper bound on number of antigenically novel exposures is undefined for a single influenza subtype.

Overall, model fitting of dynamical antibody models to estimate epidemiological trends is a growing area, and more recent methods have revisited the statistical challenge of addressing multi-strain infection histories. These methods have largely focused on dengue, which is an excellent model system (and of high public health importance) due to the co-circulation of 4 serotypes and reporting of severe disease. These methods are yet to extend to influenza, and are hampered by the continuous nature of its antigenic space, possibility for many reinfections, and lack of RT-PCR confirmed infections alongside rich serological datasets. Recently, a life course model of A/H3N2 influenza exposure has been developed and continues to be adapted to address different immunological and epidemiological questions. This model was used to provide evidence and a proposed mechanism for antigenic seniority, and also to distinguish between short-term, antigenically broad, transient antibody boosting and long-term, antigenically narrow, persistent antibody boosting following infection [1, 86]. In this thesis, I develop this framework further in Chapter 5 and apply it to a new dataset in Chapter 6.

2.4 Disease dynamic modelling and inference

2.4.1 Overview

In this section, I discuss disease dynamic modelling and model fitting methods more generally. The combination of dynamical models of biological processes with observed data is crucial to generate realistic context and to parameterise model behaviour [283]. By finding the set of model parameters that are most consistent with the observed data through model fitting, epidemiological quantities of interest can be inferred. For example, the basic reproduction number (R_0), which describes the number of infections that a typical infectious individual is expected to generate in a population of entirely susceptible individuals during the course of infection, is possibly the most routinely reported and important parameter of interest. Data augmentation to estimate the times of unobserved infection events and transmission links (who infected whom) is also becoming routine as computational power and data availability have improved [274, 284–288]. In both of these examples, the overall aim is to link some mathematically described biological process that captures the dynamics of a system to empirical observations of that process.

2.4.2 The data generating process

Observed data from epidemiological processes are almost always proxy measures for the underlying, hidden process of interest. For example, surveillance data may record incidence as the rate at which symptomatic cases are reported by surveillance sites; the actual infection events are unobserved, and many failed infectious contacts and infections will be missed. Furthermore, there are multiple sources of uncertainty that must be captured when modelling the link between the epidemiological process and observed data. Models may be deterministic, meaning that model outputs are fully determined by the chosen parameter values and initial conditions, or stochastic, meaning that the same parameter set and initial conditions may lead to different outputs through process randomness [289]. There may also be error introduced in the observation and measurement process [274]. For example, observed infections may be under or over-reported relative to the true number of infections depending on the uniqueness of symptoms, or sensitivity and coverage of the surveillance system [290]. This observation process may be extended further to represent complicated properties of the underlying biological system, such as the progression from HIV through to acquired immunodeficiency syndrome (AIDS) via an unobserved incubation process [291].

In this thesis, I will term the collection of processes linking epidemiological events and observations as “forward processes”. By modelling the biological and epidemiological systems that stem from a particular event (usually infection) alongside Bayesian inference, I aim to reveal the dynamics of some of these forward processes.

2.4.3 Bayesian inference

Given sources of stochasticity but clear motivation to link observed data to models, it is necessary to account for this uncertainty when estimating underlying parameters of interest by integrating over possible states and parameter values [292]. This usually involves writing down the probabilistic relationship between the observed data and the parameters of interest. For example, the likelihood of the model-predicted number of new infections given an observation of the actual number of new infections at a given time. In the classical or frequentist framework, a typical analysis is to find the parameter values that maximise the likelihood through optimisation. In a Bayesian framework, a typical approach is to estimate the posterior distribution of the parameters of interest given the observed data.

Bayesian inference aims to calculate the posterior distribution of a set of model parameters, θ , conditional on some observed data D . Parameters are treated as random variables, and may include augmented data eg. latent states. Using Bayes theorem, the posterior distribution $\pi(\theta|D)$ is defined as:

$$\pi(\theta|D) = \frac{\mathcal{L}(D|\theta)P(\theta)}{\int_{\theta} \mathcal{L}(D|\theta)P(\theta)d\theta} \propto \mathcal{L}(D|\theta)P(\theta) \quad (2.1)$$

Where $\mathcal{L}(D|\theta)$ is the likelihood, $P(\theta)$ represents prior knowledge of the distribution of θ , and $\int_{\theta} \mathcal{L}(D|\theta)P(\theta)d\theta$ is the evidence. The evidence is constant with respect to θ and can therefore usually be ignored when approximating the posterior distribution.

It is often intractable to calculate or not possible to derive an analytic expression for the full posterior, and one solution is to estimate the posterior distribution through numerical approximation [293, 294]. A common solution is to use Markov chain Monte Carlo (MCMC) algorithms, which exploit features of Markov chains to estimate the posterior distribution (or more generally the target density) as the stationary distribution of a Markov chain [284, 295–301]. In the context of epidemic models and data augmentation, the likelihood function represents the probabilistic relationship between model parameters and observable data when filtered through some forward process model. This may capture the randomness of the transmission process (eg. the stochasticity of infection events), any latent

biological processes (eg. infection does not necessarily lead to disease) and in taking measurements in the observation process (eg. a laboratory confirmation may lead to false negative or positives). The full posterior is therefore often defined as a Bayesian hierarchical model split into three layers: the prior level, the transmission level and the observation level. Figure 2.8 depicts these levels in the context of a forward process. Here, the likelihood in Equation 2.1 corresponds to the transmission and observation levels, whereas the prior corresponds to the prior level. Figure 2.9 extends the conceptual framework described in Figure 2.8 to capture an arbitrary number of life events that may all interact and contribute to latent biological states and therefore to a particular set of observations.

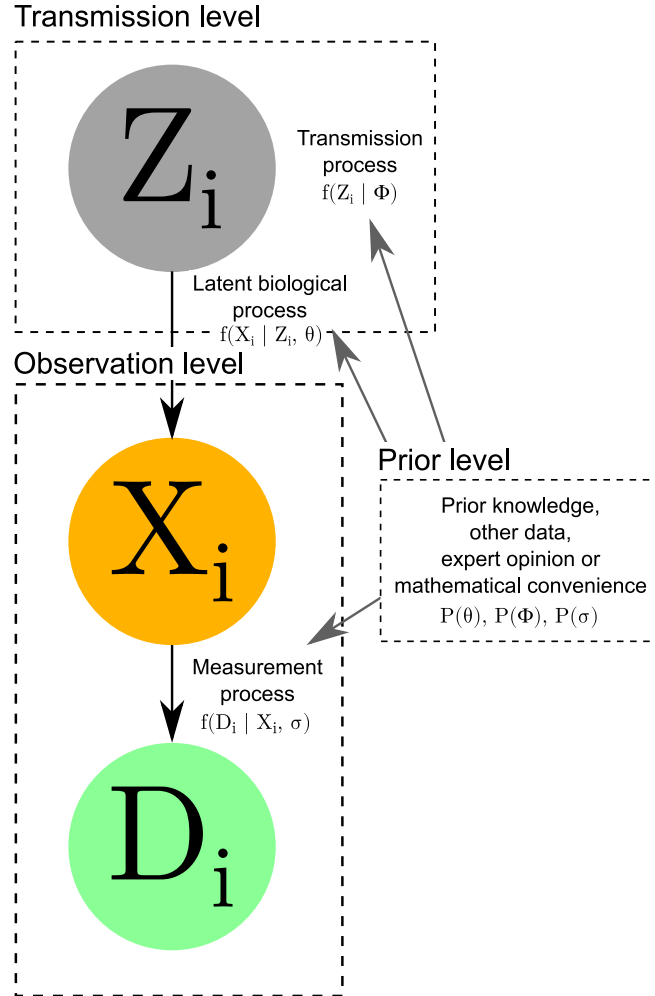


Figure 2.8: The forward process. At the *transmission level*, individual i experiences an epidemiological event to enter a particular state Z_i with respect to the disease eg. infection. This is generated by some biological process ($f(Z_i|\phi)$, where ϕ is the vector of transmission model and biological parameters), such as contact with infectious individuals in the community. This state change generates effects of some latent within-host process ($f(X_i|Z_i,\theta)$, where θ is the vector of within-host process parameters), such as the production of antibodies or progression to disease. The resulting biological state X_i may then be observed and measured through some proxy, such as an antibody assay or record of symptoms ($f(D_i|X_i,\sigma)$, where σ may include noise or variance parameters for the measurement process). The observed data D_i is therefore separated from the initial event Z_i through a number of unobserved processes. X_i and D_i are generated and observed in the *observation level*. Additional information and prior expectation may also be included in any inference through the *prior level*.

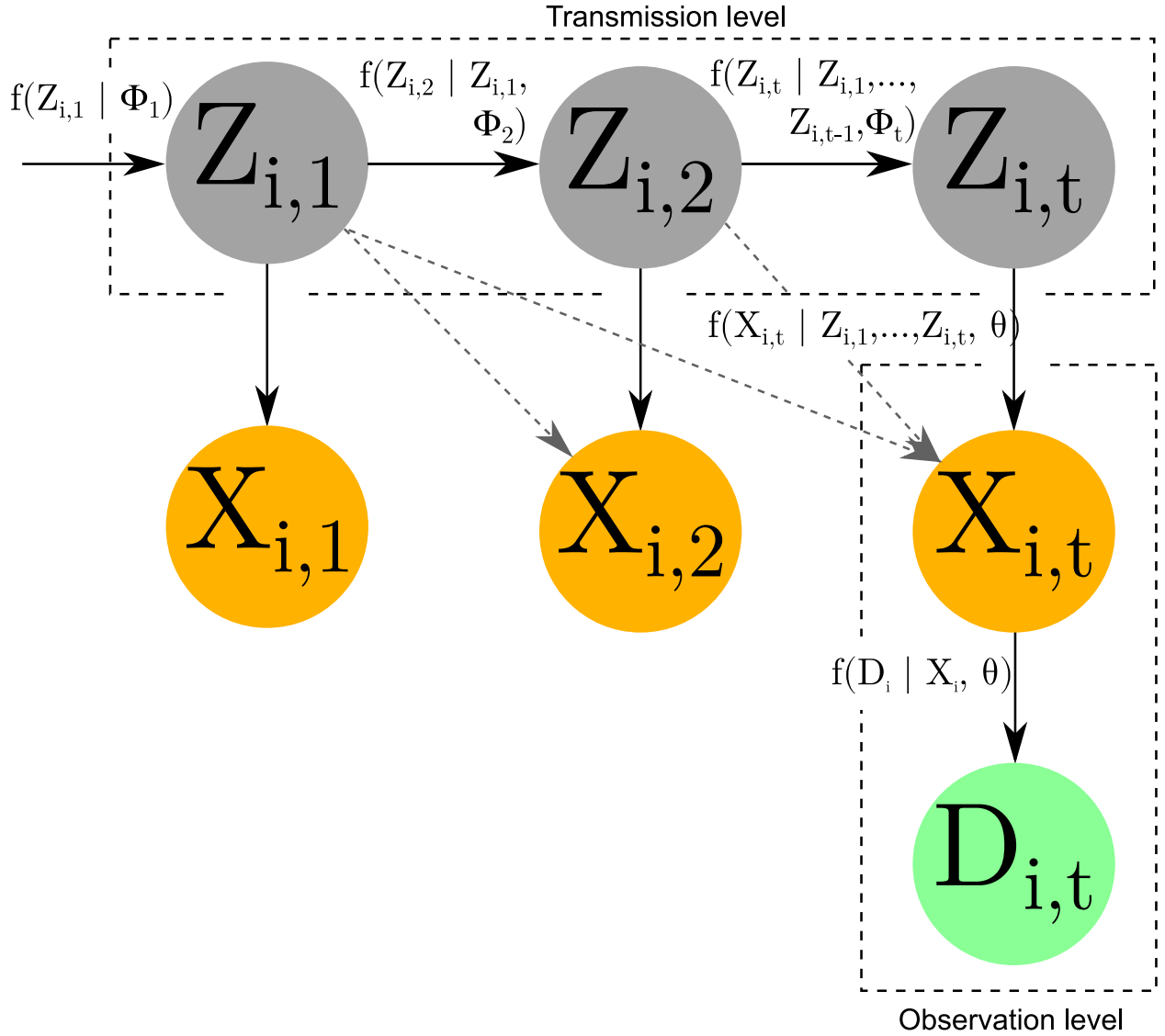


Figure 2.9: The life course forward process. The forward process model can be extended to incorporate multiple life events in generating some observable data D_i . Each epidemiological event $Z_{i,t}$ at different times t leads to some latent biological process that changes the individual's biological state $X_{i,t}$. For example, their antibody levels may change over time following each exposure. There are two crucial additions to this framework. First, each successive event may interact with the outcomes of all previous events, indicated by the grey dashed arrows and the formulation $f(Z_{i,t} | Z_{i,1}, \dots, Z_{i,t-1}, \phi_t)$. Second, not all intermediate biological states X_i may be observed, as the data D_i may only be obtained long after the contributing events eg. observations may be made in adulthood, but unobserved events in childhood contribute to the current state. Under this framework, parallels can be drawn with Hidden Markov models (HMM): an individual's relationship with a disease is described by their hidden states and transition probabilities. Although I denote these transition probabilities as conditional on all past states rather than just the current state (as in a HMM), the intuition is the same as assuming that the impact of prior states is summarised within the current state.

2.4.4 The prior level

The form of the prior level is extremely flexible. In theory, any prior probability distribution may be used to describe the prior knowledge (ie. before seeing the data) of the model parameters and latent variables. Informative priors may be used when previous empirical data or expert opinion should be captured within the model. For example, a clinician may have some experience regarding the distribution of disease duration prior to any quantitative assessment. Priors are very often chosen to be weakly informative using a uniform or normal distribution with high variance, meaning that the prior does not contribute information to the posterior relative to the likelihood [302]. Priors may also be chosen for mathematical convenience by choosing a conjugate prior: a distribution which when multiplied by the likelihood distribution results in a posterior distribution of the same distribution family as the prior. For example, a beta distribution is a conjugate of the a beta and a binomial distribution, as the combined posterior distribution is also beta distributed. Later in this thesis I will give extensive consideration to the way in which apparently weakly informative priors can interact with the model structure in a non-obvious way to substantially bias inference [303].

2.4.5 The transmission level

The aim of the transmission level is to capture the probabilistic nature of the non-linear process that generates events of interest, namely infections or symptom onsets. Historically, stochastic transmission models have been classified as either chain-binomial models (with the Reed-Frost model as a special case), or derivatives of the Markov Susceptible-Infected-Removed models [304–307] (see Box 4.1). In both cases, individuals are grouped based on their state with respect to the disease within compartments. Individuals transition between states through a Markov process. Both of these models are non-linear in the sense that the probability of a susceptible individual contacting an infected individual and therefore becoming infected initially scales exponentially with each subsequent generation of infections. I will use deterministic SEIR models (an extension of the SIR model with an exposed class) in Chapter 3 to model the incidence of Zika virus infection. I do not use the stochastic SIR model, though the framework in Chapter 5 has some conceptual similarities in described the random occurrence of infections.

Box 4.1: SIR models

The stochastic SIR model [308]: Stochastic Susceptible-Infected-Removed (SIR) models are continuous-time Markov chain models, meaning that they are defined by different states $((S(t), I(t)) : t \geq 0)$ and the transition probabilities between these states, which correspond to events of susceptibles becoming infected or susceptibles recovering (or dying) from infection:

$$\begin{aligned} P[(S(t + \delta t), I(t + \delta t)) = (s - 1, i + 1) | (S(t), I(t)) = (s, i)] &= \beta \delta t s i + o(\delta t) \\ P[(S(t + \delta t), I(t + \delta t)) = (s, i - 1) | (S(t), I(t)) = (s, i)] &= \gamma \delta t i + o(\delta t) \end{aligned} \quad (2.2)$$

These equations denote the probabilities of moving between states of the Markov chain. s is the number of susceptible individuals at time t ; i is the number of infected individuals at time t ; β is the infection or transmission rate; γ is the removal rate; δt indicates a very small time step; and $o(\delta t)$ defines a negligible remainder for the transition probabilities as $\delta t \rightarrow 0$, expressing the fact that the probability of two transitions happening in the same time interval is negligible. The number of new infections or removals at each time step can therefore be simulated using a binomial distribution where p is defined by the transition probabilities, or by a Poisson process with respective rates at time t equal to $\beta S(t)I(t)$ and $\gamma I(t)$.

The deterministic SIR model [307]: Unlike the stochastic SIR model, transitions between compartments are determined by rates rather than probabilities. This system can be described with a set of ordinary differential equations (ODEs) representing the rate of change of state variables over time:

$$\begin{aligned} \frac{dS}{dt} &= -\beta S(t)I(t) \\ \frac{dI}{dt} &= \beta S(t)I(t) - \gamma I(t) \\ \frac{dR}{dt} &= \gamma I(t) \end{aligned} \quad (2.3)$$

where β is the rate of transmission from infected to susceptible individuals and γ is the rate at which infected individuals recover. In this formulation, $S(t)$ denotes the number of individuals in the susceptible compartment at time t . β may be left as an independent parameter in the density-dependent formulation, wherein each individual makes a fixed number of contacts per unit time. Alternatively, the transmission rate may be defined as $\frac{\beta}{N}$ in the frequency-dependent formulation, wherein the number of contacts depends on the size of the population. The frequency-dependent formulation may also be defined by setting $S(0) + I(0) + R(0) = 1$ rather than $S(0) + I(0) + R(0) = N$.

In both the deterministic and stochastic cases, the models can be extended to include features such as: additional compartments (eg. an exposed but not yet infected class $E(t)$), births and deaths (overcoming the fixed population size assumption), temporary immunity (eg. returning to the $S(t)$ class after infection), intermediate vectors, complex transmission rate/probability terms (eg. dependent on prior immunity), host heterogeneity, non-random mixing, and spatial dynamics.

The basic reproduction number (R_0): R_0 is defined as the average number of secondary infections arising from one infected individual in a fully susceptible population over the course of the infectious period. In the simple SIR model above, this is given by:

$$R_0 = \frac{\beta}{\gamma} \quad (2.4)$$

where the denominator can be thought of as the expected time spent infected. It is useful to estimate R_0 early in an outbreak, as it can be used to calculate quantities such as the total proportion of people that are expected to be infected and the proportion of the population that would need to be vaccinated to confer herd immunity (by reducing R_0 to less than 1). Given that the number of susceptible individuals in the population changes over time, it is often useful to instead consider the effective reproduction number $R_e(t) = S(t)R_0$.

In the stochastic case, the transmission model is defined by the number of individuals in each state and the probabilities of transitioning between the states over time (eg. the rate at which individuals move from S to I , β , and the rate at which individuals move from I to R , γ) as a continuous-time Markov chain [308]. Usually only one of the state compartments is observable (eg. infected individuals, I), and the aim of Bayesian inference is therefore to integrate over the unobserved transition probabilities, transition events and individual states [306]. Including the inference of these latent individual states is termed data augmentation, where additional model parameters representing missing data are introduced such that equation 2.1 is modified to:

$$\pi(\theta, Z|D) \propto P(D|Z)P(Z|\theta)P(\theta) \quad (2.5)$$

where Z are the latent states, and $P(D|Z)$ relates to the observation model described below eg. see [309]. I will return to this formulation in Chapter 5 when linking attack rates to latent infection states.

Assuming that infections can happen through any pairwise contact within a large, homogeneously mixing population results in a huge number of potential latent infection states and transition events. Integrating over all of these possible states becomes intractable, and much of the original work applying MCMC methods to epidemiological data was therefore limited to household and community settings during single outbreaks [280, 285, 306, 310]. Under these models, the total hazard of infection for a susceptible individual is captured by summing the infectious contributions from all other household members, plus a parameter to capture all of the community-based transmission potential [310, 311].

Additional heterogeneity in the properties of each individual may also be included to test specific hypotheses, for example variable infectivity and susceptibility by age or vaccination status, the relationship between antibody titre and infection, and the relationship between virus shedding and infectivity [280, 285, 296, 312–314]. Data used in these analyses typically capture the number of infections, or at least detected infections, either at the end of the outbreak (final sizes [310, 311]) or as they occur over time (temporal [310]). Both are useful for inferring key quantities of interest (eg. R_0), but only temporal data allow the inference of time-varying processes such as the infectious period or latent period.

Another solution to the large population problem is to eliminate stochasticity altogether (eg. by using the deterministic SIR model in Box 4.1) Including stochasticity is necessary where randomness is likely to have a large impact on model predictions, for example due to a small population size or

the possibility of stochastic epidemic extinction [315]. However, stochastic behaviour converges to that of its deterministic counterpart as the population sizes increases. In a deterministic model, the probability of generating a set of model predictions (or latent states) given a set of model parameters is 1, and the likelihood can be thought of as the probabilistic link between latent states and observations. Fitting models under this assumption is now standard practice in many epidemiological analyses (eg. fitting a deterministic SIR model to epidemic curve data). Elder *et al.* provide one such example, demonstrating how inference of model parameters may be performed through combining a deterministic susceptible-exposed-infected-removed (SEIR) model with a normally distributed observation process and informative priors from the literature [316]. Such analyses are useful when the scale of the full stochastic transmission model is large, and each person-to-person contact cannot be explicitly modelled. When the transmission level of Figure 2.8 is deterministic, it does not contribute to the likelihood and model fitting becomes dependent on the observation level.

2.4.6 The observation level

The observation level is typically used to filter out augmented latent states and parameter values that are incongruent with the observed data. This may be as simple as applying an indicator function to set the probability of observing the data D given the incongruent model parameters (θ) and latent states (Z) to 0. Examples include filtering out augmented data that suggest carriage of a pathogen serotype that do not match serological data, or latent infection times that suggest too large a delay between the observed onset of symptoms and the time of infection [280, 285, 317]. A common form of the observation level is then:

$$p(D|Z, \theta) = \prod_{i=1}^n 1(Z_i, \theta \simeq D_i) \quad (2.6)$$

where $\simeq$ indicates that the augmented states for Z_i and parameter values θ are consistent with the observed data D_i for individual i , such that $p(D|Z, \theta)$ returns 1 if there is agreement for all individuals and 0 otherwise. Parallels can be drawn with Approximate Bayesian computation (ABC) when this term is used in place of a full evaluation of the likelihood, described in detail in Section 2.4.7 [318, 319].

A more flexible interpretation of the observation level is to model the process that links augmented model predictions to observable data. Under the nomenclature of Figure 2.8, this includes the latent biological processes $f(x_i|z_i, \theta)$ leading from latent infection states z_i to latent biological states x_i , and the measurement process $f(d_i|x_i, \sigma)$ leading from latent biological states x_i to observed data d_i . In the case of a deterministic transmission model with observed incidence data, this level may the Gaussian

of binomial variation in surveillance sampling (depending on the distribution of observations):

$$\mathcal{L}(D|\theta) = \prod_i f(Z_i|\phi) \cdot f(X_i|Z_i, \theta) \cdot f(D_i|X_i, \sigma) = \prod_i 1 \cdot 1 \cdot \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(D_i-X_i)^2}{2\sigma^2}} \quad (2.7)$$

It may also represent the case where only fallible proxy measures for infection may be available, such as the presence of non-specific symptoms, imperfect virological assays, or elevated antibody titres [40, 281, 314, 320, 321].

2.4.7 Modern MCMC methods

A common class of inference algorithms that are commonly used to fit epidemiological models to data are MCMC samplers. The common objective of MCMC samplers is to stochastically sample from and thereby estimate a distribution of interest. An estimated joint posterior distribution may then be integrated over to find the marginal distribution of each parameter to generate summary statistics, or to find the posterior predictive distribution of some unobserved data given a set of previous observations. For example, finding the marginal distribution of transmission model parameters θ that integrates out uncertainty in the latent states Z would be calculated using the integral:

$$P(\theta|D) = \int P(\theta, Z|D) dZ \quad (2.8)$$

Given that this is often intractable, MCMC estimates the marginal distribution by numerically sampling from the joint posterior distribution of θ and Z as shown in 2.5. Hence “Monte Carlo” refers to the integration aspect and “Markov chain” refers to the random sampling of the algorithm. A typical MCMC sampler performs a random walk through sample space (ie. the range of values that the parameters of interest can take), evaluating the posterior probability at each location (each state of the Markov chain). MCMC algorithms accept more samples from high posterior density regions and fewer from low posterior density regions, such that the sampling frequency is proportional to the posterior density. The algorithm is run until the chain is said to have converged on the stationary distribution and a high effective sample size (ESS) has been achieved. Calculating the effective sample size accounts for the fact that sequential draws from the posterior distribution using MCMC algorithms (the raw sample size) are often not independent. The sample size is therefore adjusted for the autocorrelation within the chain as:

$$\text{ESS} = \frac{n}{1 + 2 \sum_{k=1}^{\infty} \rho(k)} \quad (2.9)$$

where n is the number of samples and $\rho(k)$ is the correlation between samples at a lag of k .

More formally, a Markov chain is constructed that satisfies detailed balance [293, 322]:

$$\pi_i P(x_{t+1} = j | x_t = i) = \pi_j P(x_{t+1} = i | x_t = j) \quad (2.10)$$

where π_i and π_j are the probabilities of being in state i and j under the stationary distribution, and $P(x_{t+1} = j | x_t = i)$ and $P(x_{t+1} = i | x_t = j)$ are the transition probabilities of moving from state i to state j and vice versa. The MCMC sampler must construct a Markov chain that is irreducible (non-zero probability of getting from any state to any other) and aperiodic (no periodicity to transitioning between states, achieved by having non-zero probability of transitioning to the current state). Satisfying detailed balance ensures that the Markov chain also has positive recurrence, meaning that the time to return to any state is finite. With the criteria of irreducibility, aperiodicity and positive recurrence satisfied, the constructed Markov chain is said to be ergodic and is guaranteed to converge to the unique stationary distribution.

Sampling from the full conditional distribution of each individual parameter in turn conditional on each other parameter is known as Gibbs sampling [323]. However, it is often difficult to calculate these conditional distributions, and the more general multivariate MCMC samplers, which sample directly from the joint distribution of all model parameters, are more widely used. The Metropolis-Hastings algorithm is perhaps the most commonly used algorithm due to its ease of implementation, robustness, and automatic adaptation steps (see Box 4.2) [293, 322]. Although the basic random walk Metropolis-Hastings and Metropolis-within-Gibbs algorithms are more than adequate for most model fitting problems, there are now a huge number of MCMC methods implemented in various plug-and-play packages such as *POMP*, *BUGS*, *STAN* and *JAGS* that can be used to fit standard epidemic models [324–327].

Reversible-jump MCMC

Reversible-jump MCMC (RJ-MCMC) is used to sample from a posterior distribution when the dimensions of the sample space are unknown, and the dimensions of the model are inferred as augmented variables [328]. RJ-MCMC is therefore suited for variable and model selection problems, for example fitting a mixture distribution with an unknown number of components. Clear analogies can be drawn with inference of stochastic transmission models, where events (namely infections and removals) are unobserved binary events that may or may not occur [280]. Gibson *et al.* described an

early implementation of a RJ-MCMC sampler to infer individual state transitions in a stochastic SEIR model, where hidden events could either be inserted, deleted or given a different occurrence time at each iteration [306]. In this algorithm, a prior must be placed on the total number of hidden events, and proposals alternate between changing parameter values (eg. the recovery rate parameter) within the existing sample space and changing the dimensionality of the sample space (eg. the number of recovery events). RJ-MCMC algorithms are often implemented as Metropolis-Hastings algorithms with care given to the proposal probability and corresponding acceptance ratio when moving between states of differing dimensions to maintain detailed balance. In the context of transmission models, changes in model dimensions are straightforward: the proposal probability of adding an infection time is simply the proposal distribution for generating an infection time (eg. uniform between a and b such that $P(\text{add infection}) = \frac{1}{b-a}$), and the reverse proposal probability is simply the probability of choosing that infection time from $m + 1$ infections in the current model ($P(\text{remove infection}) = \frac{1}{m+1}$) [304].

Approximate Bayesian Computation

ABC methods are another popular set of algorithms for fitting dynamical models to observed data [318,319]. The rationale behind these methods is that many models are easy to simulate from, but not easy or impossible to calculate a likelihood for. Rather than sampling from the posterior distribution directly, proposals for θ are used to generate realisations y of the model that can be compared against the observed data D . A distance function d is then used to evaluate the agreement between D and y to some level of tolerance ϵ . If $d(y, D) \leq \epsilon$, the proposed value for θ is accepted, and rejected otherwise. Under this sampling regime, samples are taken from the distribution $\pi(\theta|d(y, D) \leq \epsilon)$ rather than $\pi(\theta|D)$. When ϵ is suitably small, $\pi(\theta|d(y, D) \leq \epsilon)$ is a good approximation for $\pi(\theta|D)$ [329]. Note that d does not need to compare y and D directly, but may compare the two sets based on some summary statistic. Rejection sampling by proposing from the prior $P(\theta)$ and ABC within an MCMC framework are two commonly used simple ABC algorithms [330].

Sequential Monte Carlo methods

Mechanistic models of infectious disease dynamics are often referred to as “state space models” (SSM), as they are represented by unobserved, dynamical states (eg. a Markov process). The posterior distributions of well characterised SSMs have historically been derived exactly using the Kalman filter or hidden Markov model filter, but these methods are not appropriate for the intractable SSMs of epidemic processes. Sequential Monte Carlo (SMC) methods, also called particle filters, are an

alternative to data augmentation methods for fitting complex SSMs with intractable likelihoods through iterative simulation of the model trajectory. The general approach is to sequentially take samples (called particles) at each simulated state (eg. the number of infected individuals at a particular time), weighted by their likelihoods, which are combined to approximate the posterior distribution or find the maximum a posteriori estimation of the parameter vector θ [331, 332]. By iteratively improving the sampling efficiency of these particles, the algorithm converges on the posterior distribution or maximum likelihood estimator. These methods are not described in detail here, as they are not used within this thesis. However, it is worth noting that SMC methods have expanded rapidly in the past 20 years, with methods now including particle MCMC [332], combining SMC with ABC [329], and iterated filtering [331, 333, 334]. A variety of plug-and-play software packages are also available, including *POMP* and the GPU-exploiting *LibBi* package [324, 335].

Hamiltonian MCMC

No discussion of MCMC algorithms in epidemiology would be complete without reference to the now infamous *Stan* software, a C++ implementation of a gradient-based Hamiltonian MCMC algorithm (specifically the no-U-turn (NUTS) sampler) [327, 336, 337]. Hamiltonian MCMC algorithms are conceptually similar to conventional MCMC algorithms, but exploit the geometry of the sample space using Hamiltonian dynamics (the sampler is given a position and momentum to control its movement, like a friction-less puck sliding over a surface of varying height) to generate more efficient proposals than a random-walk based algorithm [338]. Users write statistical models in *Stan*'s syntax and program layout, and the MCMC algorithm is then run with minimal input from the user. The advantage of this approach is that posterior distributions may be estimated extremely quickly with high effective sample sizes per iteration. However, it requires models to be written in a specific way and currently has limited support for discrete model parameters without marginalisation. This is a significant limitation if the posterior distributions of discrete parameters are of direct interest (eg. infection events). *Stan*'s support for discrete parameters and applications to mechanistic epidemic models has improved since the start of the projects in this thesis that require it (for example, using the forward algorithm to marginalise out discrete states when fitting hidden Markov models), and implementation of these models in *Stan* to exploit the power of Hamiltonian MCMC is therefore a possible direction for future work [339, 340].

2.4.8 Summary

Choosing between the above methods and packages is usually a case of finding the appropriate methodology for the model at hand, though comparisons of computational and statistical efficiency may also be taken into account [340,341]. Another consideration is the transparency of the algorithm; although these sophisticated algorithms exist, they may require substantial tuning and model adaptation where a simpler approach can yield comparable results. Throughout this thesis, I will develop my own MCMC samplers tailored to each problem. All of these frameworks are based on a Metropolis-Hastings/Metropolis-within-Gibbs (see Box 4.2) R-package that I developed to fit models implemented in the R programming language, available at <https://github.com/jameshay218/lazymcmc>. This package has been forked and extended by Ada Yan to implement parallel tempering, which is an extension of the Metropolis-Hastings algorithm that is better suited to exploring multi-modal posterior distributions [221].

Box 4.2: The Metropolis-Hastings algorithm

The Metropolis-Hastings algorithm estimates the desired posterior distribution by performing a random walk through sample space. A Markov chain is constructed with some initial state x_0 , where states x_t represent points in sample space, and transitions between states represent draws from the posterior distribution. The algorithm proceeds as follows:

Algorithm 1: The basic Metropolis-Hastings algorithm

```

1 Initialise the chain at some point in sample space,  $x_0$ ;
2 Set the current ( $t$ ) state to this first sample,  $x_t = x_0$ ;
3 for some specified number of iterations  $t = 1 : n$  do
4     Propose a new state from the pre-specified proposal distribution  $q(x'|x_t)$ ;
5     Calculate the acceptance probability as  $A(x'|x_t) = \min(1, \frac{P(x'|D)P(x')q(x_t|x')}{P(x|D)P(x)q(x'|x_t)})$ ;
6     Generate a uniform random number  $u$  on  $[0,1]$ ;
7     if  $u \leq A(x'|x_t)$  then
8         | set the current state to the proposed state  $x_{t+1} = x'$ ;
9     else
10    | reject the proposed state and set  $x_{t+1} = x_t$ ;

```

where $P(x|D)$ is the likelihood, $P(x)$ is the prior, and $q(x'|x)$ is the proposal distribution (ie. the conditional probability of moving from x' given x). By virtue of visiting different locations in the sample space proportional to their posterior probability, the distribution being sampled from converges to the desired posterior distribution. When $q(x|x') = q(x'|x)$, the proposal distribution is said to be symmetric and we retrieve a special case called the Metropolis algorithm (where these terms cancel out in the acceptance ratio) [293,322]. A multivariate Gaussian distribution is a common choice for the proposal distribution.

Chapter 3

Epidemiological dynamics of ZIKV-associated microcephaly risk

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This chapter aims to characterise a time-varying within-host process using population level data. Namely, the process of Zika virus (ZIKV) infection during pregnancy leading to congenital microcephaly. Here, I make use of a two component model, combining a within-host process model and transmission model to synthesise available data. In doing so, I estimate risk curves for the probability of ZIKV-associated microcephaly following infection over the course of pregnancy using data from different locations.

3.1 Introduction

ZIKV is a mosquito-borne positive-stranded RNA virus in the *flaviviridae* family, closely related to dengue virus (DENV), and more distantly to the yellow fever, Japanese encephalitis, West Nile and Saint-Louis encephalitis viruses [342]. The virus is named after the Zika forest in Uganda, where it was first isolated from a sentinel rhesus monkey in 1947 [343]. Despite seroprevalence of some anti-ZIKV antibodies, there was little early evidence of an associated disease in humans, though occasional human infections were described and detected throughout the 20th century [342, 344–346]. However, ZIKV came into public health focus in 2007 after being attributed to an outbreak of relatively mild disease on Yap Island, Federated States of Micronesia [347]. The virus is thought to have continued east through the Pacific, causing another major epidemic in French Polynesia in 2013–2014 [15]. This outbreak provided the first evidence that ZIKV could cause disease more severe than its usual influenza-like presentation, as the outbreak was associated with a ≈ 20 -fold increase in the incidence of Guillain-Barré syndrome (GBS) [348].

In 2016, the World Health Organisation (WHO) declared a public health emergency of international concern (PHEIC) following an unusual increase in the number of microcephaly cases in northeast Brazil [18, 349]. There is now a substantial body of experimental and clinical evidence implicating ZIKV infection in the sharp rise in the incidence of microcephaly cases in Brazil at the end of 2015 [21, 22, 43, 350, 351]. Previous population-level studies investigating the relationship between ZIKV and microcephaly incidence found consistent patterns of high first-trimester risk and lower risk later in pregnancy, which is consistent with early clinical findings for ZIKV-associated microcephaly [19]. The outbreak in French Polynesia provided some of the first quantitative estimates of microcephaly risk. Through retrospectively identifying microcephaly cases from medical records, Cauchemez *et al.* identified 8 cases that were linked to the ZIKV incidence curve through a statistical model, finding that only a model incorporating microcephaly risk in the first trimester gave a satisfactory fit. These early estimates suggested that the first trimester was a key period of risk, with a predicted risk given infection of 0.95% (95% CI 0.34 to 1.91) [7]. More recent clinical findings from cohort studies estimate that the absolute risk of any ZIKV-associated adverse congenital outcome may be far higher [28].

Early clinical studies investigating the link between ZIKV infection and congenital Zika syndrome (CZS) suggested that adverse outcomes may be associated with ZIKV infection throughout pregnancy, though more recent evidence corroborates the hypothesis that exposure early in pregnancy bears the

highest risk [11,16,21,22,352]. How these clinical findings link to the complex picture portrayed by the epidemiological data in Brazil is unclear and leaves a substantial knowledge gap for those counseling pregnant women in ZIKV-affected populations. For example, why did the majority of Latin America demonstrate a relatively small rise in microcephaly incidence rates compared to those seen in Northeast Brazil, and why was the second wave of microcephaly in Brazil much smaller than the first despite two similar waves of GBS [25]?

Observed population-level CZS incidence should be a reflection of underlying ZIKV transmission dynamics and the gestational-time-varying risk of CZS given infection with ZIKV. If a pregnant woman is infected at some point during gestation, her baby may present as a case of CZS with a probability conditional on the gestational age of her baby when infection occurred. Therefore, if recent clinical results from pregnancy cohort studies accurately characterise the risk of CZS through gestation, then population-level data should reflect this uniform risk profile. Characterising this link between underlying gestational risk of CZS and its presentation in epidemiological data has two potential benefits. First, an estimate of the underlying gestational risk profile from surveillance data may provide evidence to inform women of childbearing age to help plan pregnancy and mitigate exposure risk [353]. Second, if the risk profiles differ substantially between populations, those differences could support the study of alternative hypotheses of risk factors for CZS beyond ZIKV infection. For example, prior infection with another arbovirus has been suggested as a potential cofactor for risk of GBS; however, prior arbovirus infection has not yet been shown to play a role in increased neurological adverse event risk [15].

Research on other teratogenic pathogens shows the potential importance of gestational age to CZS risk [9]. For example, prospective cohort studies of pregnant women have shown that infection early in gestation greatly increases the risk of congenital rubella syndrome and cytomegalovirus-associated adverse fetal outcomes relative to infection later in pregnancy [354,355]. Although a similar pattern seems likely to be the case for CZS, the timing and magnitude of risk throughout pregnancy remains uncertain. However, quantifying this underlying gestational-age-varying risk profile should be possible given reliable data on infection and CZS incidence combined and a robust statistical approach. In this chapter, I fit a transmission model to reported incidence data. This model was used to infer the relationship between gestational age at the time of ZIKV infection and the risk of microcephaly. I found that different sources of data across different locations in South American suggested different microcephaly risk profiles. I then adapt this model to explore potential explanations, including changes

in reporting rates and abortions, for the lack of a second observed wave of microcephaly incidence in Brazil [25].

3.2 Materials and Methods

3.2.1 Model description

I developed a two-component model to describe the relationship between the incidence of ZIKV infection with the incidence of microcephaly-affected births, as depicted in Fig 3.1. The aim was to estimate the shape and size of the “window of risk” for developing ZIKV-associated microcephaly, and to test for differences in microcephaly incidence and risk between states.

3.2.2 Transmission model

The first component of the model captured the transmission dynamics of ZIKV in Brazil via the *Aedes aegypti* mosquito vector. The model was based on the Ross-MacDonald model for vector-borne disease, capturing deterministic Susceptible-Exposed-Infected-Recovered (SEIR) dynamics in humans with transmission via the mosquito vector experiencing SEI dynamics [356]. Through calculation of the force of infection over time, a per capita risk of infection was estimated per unit time. Each location (typically a Brazilian state) was assumed to be a closed, homogeneously mixing population with constant population size. All biological parameters pertaining to transmission characteristics and course of infection were assumed to be the same for all locations, whereas parameters relating to life expectancy, population size, vector density (the free component of R_0) and seeding time (t_0) were assumed to be location specific. Mosquito lifespan was fixed at 5 days, and other model parameters were fixed to give a ZIKV infection generation time of 20 days [246]. A sensitivity analysis was run where mosquito lifespan was fixed at 7 days, but this did not have a significant impact on the main model results, although R_0 estimates are conditional on the assumed generation time.

Humans were assumed to go through susceptible, exposed, infected and recovered compartments over the course of infection, and mosquitoes were assumed to go through susceptible, exposed and infected

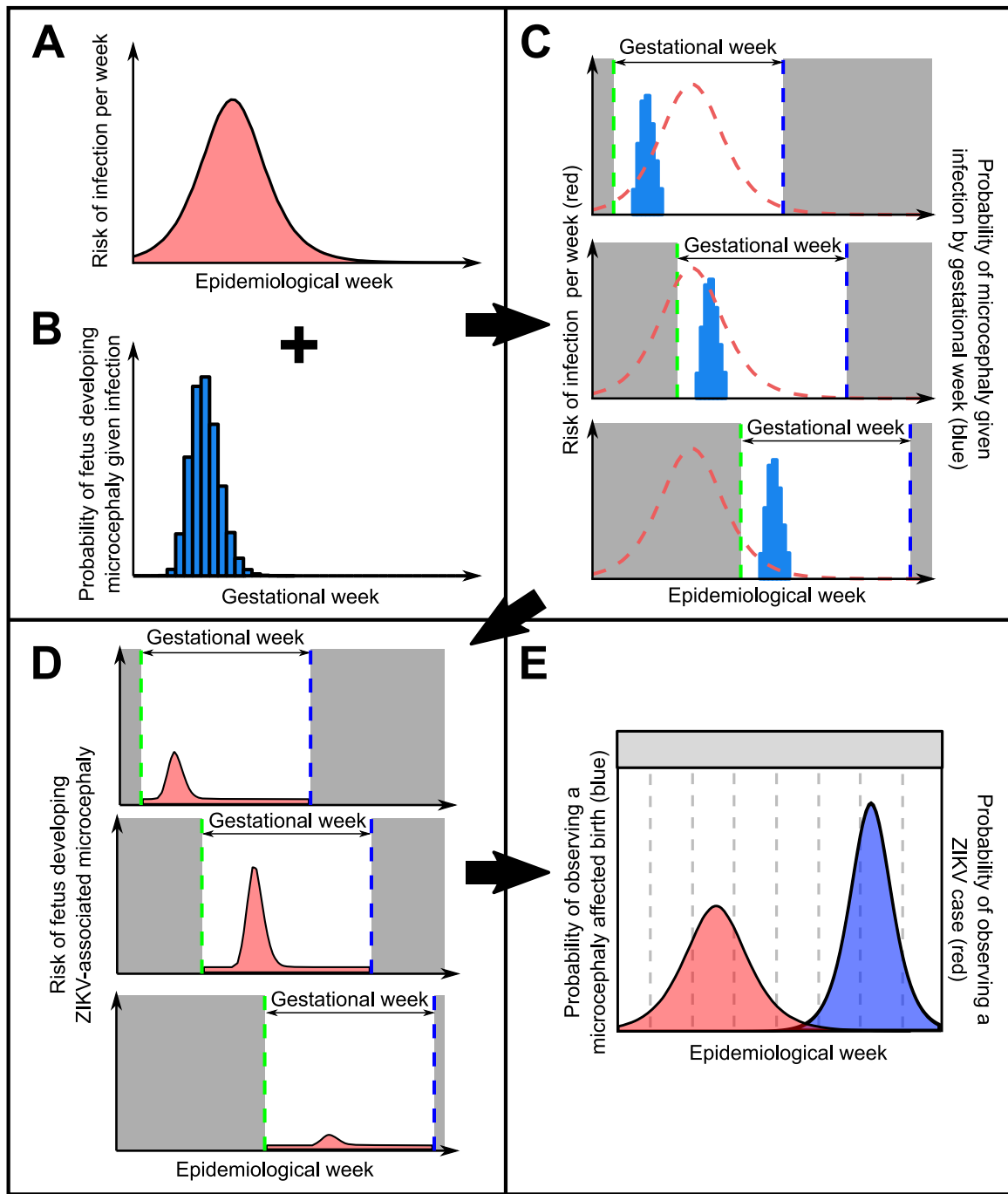


Figure 3.1: The interaction of the epidemic dynamics and gestational-age-varying risk of microcephaly given infection. (A) Time-varying (TV) risk of ZIKV infection generated from an SEIR model. (B) probability of a fetus developing microcephaly given infection by week of gestation at time of infection. (C) Link between risk of infection and microcephaly risk over gestational time. Dashed red line shows TV risk of ZIKV infection, blue bars show microcephaly risk given infection, green dashed line shows conception time, blue dashed line shows expected birth date. (D) TV risk of microcephaly given ZIKV infection by epidemiological week for the same three example pregnancies, found by multiplying the risk of infection in a given epidemiological week (curve A) by the risk of microcephaly given infection in that week of gestation (curve B). Dashed blue line shows expected birth (observation) date. (E) Combined probability of observing a microcephaly-affected birth in a given epidemiological week (blue), giving expected proportion of microcephaly-affected births. Red area shows probability of ZIKV infection by epidemiological week.

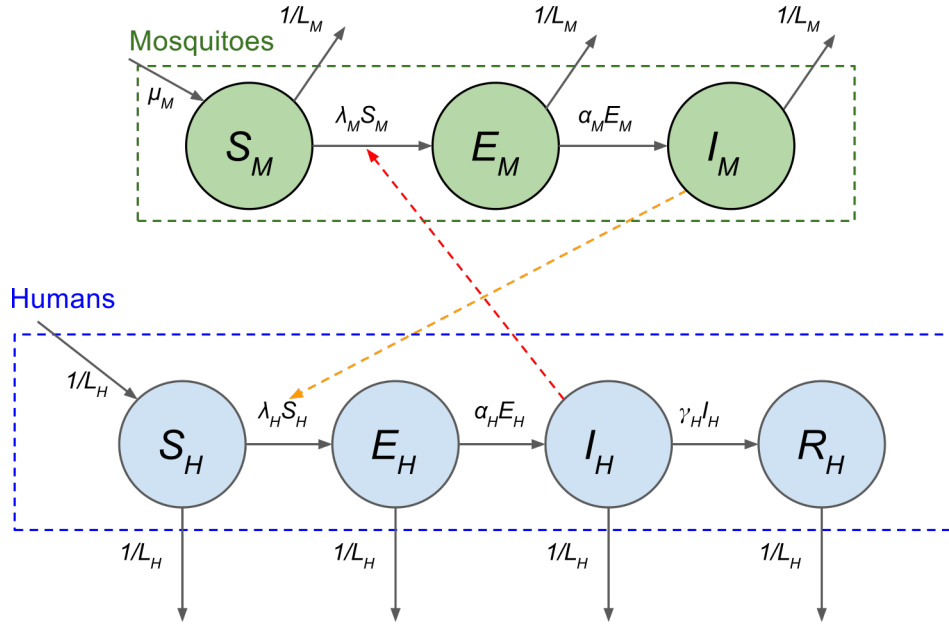


Figure 3.2: Graphical representation of the SEIR model. Mosquito vector population is shown in green, with new mosquitoes entering the susceptible class (S_M) and progressing through to the infected state. The human population is shown in blue, with new humans entering as susceptible (S_H). Humans become infected at a rate of λ_H , and become infectious at a rate of α_H . Humans then recover at a rate of γ_H . Note that the force of infection on humans comes from mosquitoes only, as represented by the orange arrows. All compartments experience a death rate of $1/L_H$ or $1/L_M$.

compartments as described in Equation 3.1 [345, 357, 358].

$$\begin{aligned}
 \frac{dS_M}{dt} &= \mu_M N_M - \mu_M S_M - \lambda_M S_M \\
 \frac{dE_M}{dt} &= \lambda_M S_M - \sigma_M E_M - \mu_M E_M \\
 \frac{dI_M}{dt} &= \sigma_M E_M - \mu_M I_M \\
 \\
 \frac{dS_H}{dt} &= \mu_H N_H - \lambda_H S_H - \mu_H S_H \\
 \frac{dE_H}{dt} &= \lambda_H S_H - \sigma_H E_H - \mu_H E_H \\
 \frac{dI_H}{dt} &= \sigma_H E_H - \gamma_H I_H - \mu_H I_H \\
 \frac{dR_H}{dt} &= \gamma_H I_H - \mu_H R_H
 \end{aligned} \tag{3.1}$$

where S , E , I and R indicate the number of individuals in the susceptible, exposed, infected or recovered compartment, and the subscript represents either human (H) or mosquito (M) populations; N is the total population size; μ is the birth/death rate; σ is the latent period; γ is the infectious period; and λ is the force of infection. Each location (typically a Brazilian state) was a closed, homogeneously mixing population with constant population size. The force of infection for mosquitoes and humans

respectively is given by:

$$\begin{aligned}\lambda_M &= bp_{HM}I_H \\ \lambda_H &= bp_{MH}I_M\end{aligned}\tag{3.2}$$

where b is the bite rate per vector; p_{MH} is the probability of a bite generating an infection in a human from an infected vector; p_{HM} is the probability of a bite generating an infection in a vector from an infected human; and I_H and I_M are the number of infected humans/mosquitoes.

Using this force of infection term, the probability of an individual not becoming infected at a given time, t , is [359]:

$$f(t) = e^{(-\lambda_H(t)\delta t)}\tag{3.3}$$

where $f(t)$ is the probability of remaining susceptible between t and $t + \delta t$. δt was set to 1 day (approximately 1/20th of the assumed generation time) to approximate the probability of remaining susceptible within a small, discrete period of time. This time step was chosen by testing values for δt between 0.1 and 2, which did not affect the model results. Smaller values of δt were not used for computational reasons. The probability of remaining susceptible from t_0 up to a given time, t , is:

$$F(t) = e^{\sum_{i=t_0}^t -\lambda_H(t)}\tag{3.4}$$

Finally, the probability of becoming infected at a given period of time, t , was defined as:

$$P_I(t) = F(t)(1 - f(t))\tag{3.5}$$

where $P_I(t)$ is the probability of becoming infected between t and $t + \delta t$, given by the probability of remaining susceptible up to that point multiplied by the probability of not remaining susceptible during the small time period defined by δt . Note that $F(t)$ refers to the period of time up to t , whereas $f(t)$ refers to the period of time between t and $t + \delta t$. Note also that t is treated as a discrete unit here to approximate the theoretical relationship between time-varying force of infection and infection risk [359].

The basic reproductive number, R_0 , was defined as the number of new human infections generated by the introduction of a single infected human into a naive human and mosquito population given

by [360]:

$$R_0 = \frac{b^2 p_{HM} p_{MH} N_M}{\mu_M (\sigma_M + \mu_M) (\gamma_H + \mu_H) N_H} \quad (3.6)$$

where N_M is the total number of mosquitoes; N_H is the total number of humans; μ_M is the birth/death rate of mosquitoes; σ_M is the rate at which mosquitoes leave the exposed class; σ_H is the rate at which humans leave the exposed class; γ_H is the rate at which humans leave the infected class; and μ_H is the birth/death rate of the human population.

3.2.3 Microcephaly risk model

The second component of the model described the risk of a fetus developing microcephaly given that the mother was infected in a particular week of pregnancy. Fitting this risk profile as a curve rather than a set of per-trimester risk estimates captures more information regarding the width and shape of the gestational-time-varying risk profile at the resolution of weeks or days rather than per trimester. The gamma distribution was chosen due to the flexible shape of the curve defined by a small number of parameters. A scaled gamma distribution was used to characterise the shape and scale of this curve with only 3 free parameters – the shape, scale, and an additional scaling constant to increase the magnitude of the curve. This additional scaling constant was required as the integral over the risk profile did not need to integrate to 1 as in the unmodified gamma function. The probability of developing microcephaly given infection was described as:

$$P'_m(x) = \frac{c}{\Gamma(x)\theta^k} x^{k-1} e^{-\frac{x}{\theta}} \quad (3.7)$$

where $P'_m(x)$ is the probability of developing microcephaly given infection in gestational week x (0 to 39, where 0 is the first week of pregnancy); c is an additional scaling constant; θ is the gamma scale parameter; and k is the gamma shape parameter. Note that θ and k can be manipulated to give the mean ($k\theta$), variance ($k\theta^2$) and mode ($(k-1)\theta$) of the gamma curve. The gamma distribution, Γ was defined as:

$$\Gamma(x) = \int_0^\infty t^{x-1} e^{-t} dt \quad (3.8)$$

3.2.4 Combined model

Based on the transmission model and microcephaly risk model, the expected proportion of microcephaly affected births (Fig 3.1E) was calculated by multiplying these two components together. The probability of a ZIKV-associated microcephaly affected birth at time, t , was therefore given by:

$$P_m(t) = \sum_{i=t-40}^t P_I(i)P'_m(i-t+40) \quad (3.9)$$

where $P_m(t)$ is the per live birth probability of a ZIKV-associated microcephaly birth at time t , $P_I(i)$ is the probability of an individual becoming infected at time i (and not before), and $P'_m(i-t+40)$ is the probability of fetus developing microcephaly given ZIKV infection at gestational week $i-t+40$. Essentially, the probability of a live birth being affected by ZIKV-associated microcephaly is the sum of all of the opportunities that the mother could have been infected and the fetus subsequently developed microcephaly in each of the 40 weeks of pregnancy preceding the birth.

Including a baseline microcephaly rate (ie. not associated with ZIKV) gives the probability of observing any microcephaly case at time t as:

$$P_{micro}(t) = \phi_{m,i}(P_m(t) + P_b - P_m(t)P_b) \quad (3.10)$$

where P_b is the baseline per birth microcephaly incidence rate and $\phi_{m,i}$ is the proportion of true cases that were reported in location i (less than one indicates under-reporting, greater than one indicates over-reporting). Multiplying this proportion by the total number of live births at time t , $B(t)$, gives the expected number of observed microcephaly-affected births at time t .

3.2.5 Data

ZIKV and microcephaly incidence data

I searched the literature and Brazilian state health authority websites for reports of suspected ZIKV incidence and microcephaly cases in 2015 and early 2016, building on a comprehensive literature search performed in 2016 [246]. These sources were: www.paho.org, www.who.int, Brazilian state-level ministry of health websites (eg. www.suvisa.ba.gov.br), and PubMed for the terms “zika” and “microcephaly”. Where confirmation status of cases was not recorded (eg. where incidence was only

shown as “reported cases”), data were classified as “notified” cases. Where suspected and confirmed cases were distinguished, the sum of suspected and confirmed cases was classified as “notified” cases. Some data sources were only available in graphical form, and these numbers were therefore extracted using a web digitiser (<https://automeris.io/WebPlotDigitizer/>). Data used for the main analyses were:

1. Aggregated weekly confirmed microcephaly and notified ZIKV infection incidence data in pregnant women from Northeast Brazil [25,361];
2. Weekly confirmed/notified ZIKV infection and notified microcephaly incidence data from Colombia [362,363];
3. State-reported weekly notified microcephaly and ZIKV incidence from Bahia, Brazil [364,365];
4. State-reported confirmed weekly microcephaly incidence from Pernambuco, Brazil [366];
5. State-reported monthly confirmed/notified microcephaly and notified ZIKV incidence from Rio Grande do Norte [367,368];
6. Reported acute exanthematous illness (AEI) and notified microcephaly incidence from the city of Salvador, Bahia, Brazil (see below) [369].

A description of all obtained data is provided in Table A.4 in Appendix A.1.1.

Vital statistics

In all analyses, life expectancy, birth numbers and population size were assumed to be fixed and known based on published vital statistics, shown in Table A.5 in Appendix A.

A key input to the model is the absolute number of births over time. Numbers of live births were obtained for Brazil from the SINASC/CGIAE/SVS/MS system [19,370]. For Colombia, live births were obtained from a publication of microcephaly and ZIKV incidence in Colombia and checked against country wide statistics [363,371]. Where reporting of live births was incomplete, the number of live births was estimated by averaging the number of births in the previous two years for the same dates. Where birth data was only available at a different time resolution than reported incidence data (eg. weekly birth data but monthly incidence data), the total number of births for that time period were assumed to be uniformly distributed across each day. Estimation of live birth data is described in further detail in Appendix A.1.3.

ZIKV transmission parameters

Model parameters related to ZIKV transmission were obtained from the literature as described in Table 3.1. Parameters were predominately chosen based on a previously published transmission model, with point values chosen to give a generation time of approximately 20 days [246]. Values used were as described in Table S6 of [246], with the intrinsic latency period taken as the intrinsic incubation period described in [246] less 1.5 days to reflect the assumption that infectiousness starts 1.5 days before symptom onset. Given a fixed generation time, the shape of the SEIR model predicted incidence curve was allowed to vary depending on the value of R_0 . As R_0 is comprised of multiple correlated parameters, all components of R_0 other than the vector density per human were fixed.

Table 3.1: Model parameters, sources and assumed parameter ranges. The component column refers to which part of the model or which part of the analysis that parameter relates. Values shown are the fixed point values used in the analysis or estimated. Where specified, lower and upper bounds refer to prior ranges imposed during the MCMC fitting.

Parameter	Description	Component	Value	Lower Bound	Upper Bound	Source	Notes
$\frac{1}{\mu_M}$	Lifespan of mosquitoes	SEIR	5 days	N/A	N/A	[246, 372]	
σ_H	Intrinsic latency period	SEIR	4 days	N/A	N/A	[246, 373]	
σ_M	Extrinsic latency period	SEIR	8.4 days	N/A	N/A	[246, 374]	
γ_H	Human infectious period	SEIR	6 days	N/A	N/A	[246, 374]	
p_{HM}	Probability of transmission from human to mosquito given bite	SEIR	0.5	N/A	N/A		Arbitrarily fixed – component of R_0
p_{MH}	Probability of transmission from mosquito to human given bite	SEIR	0.5	N/A	N/A		Arbitrarily fixed – component of R_0
b	Per vector per day bite rate	SEIR	0.5	N/A	N/A		Arbitrarily fixed – component of R_0
α	Microcephaly curve rate parameter (mean/variance)	Gamma risk curve	Estimated	0	10000		
β	Microcephaly curve shape parameter (mean*alpha)	Gamma risk curve	Estimated	0	100000		
c	Microcephaly curve scaling parameter	Gamma risk curve	Estimated	0	100		
$\frac{1}{\mu_H}$	Human lifespan	SEIR	Location-specific	N/A	N/A	[375, 376]	
N_H	Human population size	SEIR	Location-specific	N/A	N/A	[377, 378]	
d	Mosquito density per human	SEIR	Estimated	Varied	Varied		Free component of R_0 . Upper and lower prior range were such that R_0 was bounded between 1 and 7.
t_0	Epidemic seed time in days	SEIR	Estimated	0	10000		Effective seed time of the SEIR epidemic. 01/01/2013 taken as day 0
ϕ_m	Proportion of observed microcephaly cases	Likelihood	Estimated	0	2		Reporting rate
ϕ_i	Proportion of observed ZIKV cases	Likelihood	Estimated	0	1		Reporting rate
b_p	Baseline proportion of microcephaly affected births	Likelihood	Estimated	0	1		
b_{inc}	Baseline incidence rate of ZIKV case reporting	Likelihood	Estimated	0	1		
a_r	Proportion of microcephaly affected births that are aborted	Second wave	Estimated	0	1		
b_r	Proportion of potentially affected births avoided	Second wave	Estimated	0	1		
t_{switch}	Time of behavioural change	Second wave	01/02/16	N/A	N/A		
$t_{abortion}$	Latest pregnancy termination time in gestational weeks	Second wave	24 weeks	N/A	N/A	[25]	
ϕ_{i1}	ZIKV reporting rate before t_1	Second wave	Estimated				
ϕ_{i2}	ZIKV reporting rate after t_1	Second wave	Estimated				
t_1	Time of ZIKV reporting rate change	Second wave	11/11/2015			[379]	
t_2	Time of microcephaly reporting rate change	Second wave	13/03/2016	N/A	N/A	[380]	
ϕ_{m1}	Microcephaly reporting rate before t_2	Second wave					
ϕ_{m2}	Microcephaly reporting rate before t_2	Second wave	1	N/A	N/A		Assumed accurate reporting in 2016
Attack rate	Attack rate of SEIR model	SEIR	Estimated	N/A	N/A		
R_0	Basic reproductive value	SEIR	Estimated	N/A	N/A		

3.2.6 Model fitting

Microcephaly incidence likelihood

The log likelihood of observing a time series of microcephaly cases for a given location i is given by:

$$\mathcal{L}(D_i|\Psi, \theta_i) = \sum_t \log P(d_t|\Psi, \theta_i) \quad (3.11)$$

where D_i is the observed number of microcephaly cases over time; $D_i = \{d_t\}_{t=1}^T$; d_t is the number of microcephaly cases observed at time t ; θ_i is the set of location-specific parameters (eg. mosquito density, N_H) and Ψ is the set of universal model parameters that apply to all locations (eg. p_{MH} , α_H). Observed microcephaly incidence was assumed to be binomially distributed such that:

$$P(d_t) \sim B(n = n(t), p = P_{micro}(t)) \quad (3.12)$$

where $n(t)$ is the total number of births observed at time t which was known; and $P_{micro}(t)$ is the proportion of microcephaly affected births at time t as defined by the model parameters Ψ and θ_i ; and B is the binomial probability mass function. Note that the reporting rate scaling parameter, $\phi_{m,i}$ was assumed to be location-specific as described above.

ZIKV incidence and combined likelihood

The log likelihood is easily extended to incorporate ZIKV incidence data as well as microcephaly incidence data $I_i = \{i_t\}_{t=1}^T$ as:

$$\mathcal{L}(D_i, I_i|\Psi, \theta_i) = \omega \sum_{t_m} \log P(d_t|\Psi, \theta_i) + (1 - \omega) \sum_{t_i} \log P(i_t|\Psi, \theta_i) \quad (3.13)$$

where I_i is the set of ZIKV incidence data for location i ; θ_i is the set of location-specific model parameters; Ψ is the vector of universal model parameters; and ω is an optional weighting parameter that scales the contribution of the ZIKV incidence data to the likelihood. ω was set to 0.5 in all analyses presented here, representing the equal contribution of the microcephaly and ZIKV incidence data to the overall log likelihood. I specified the equation in this way to provide flexibility to weight the inference towards different data types, which may vary in their reliability and importance. Should there be strong evidence to prefer one data source over another, ω may be set to reflect this preference.

t_m and t_i indicate that microcephaly and ZIKV incidence data do not necessarily cover the same observation period (as ZIKV incidence would predate microcephaly incidence).

The binomial likelihood of observing a ZIKV case at a given time, t is given by:

$$P(i_t) \sim B(n = N, p = \phi_{I,i}P_I(t)) \quad (3.14)$$

where i_t is the observed ZIKV incidence at time t ; N is the total population size; $\phi_{I,i}$ is the location-specific proportion of true ZIKV cases that observed incidence represents (through under- or over-reporting, or misdiagnosis) and $P_I(t)$ is the model predicted probability of becoming infected at time t as described above.

The complete log likelihood function combining information from multiple locations is given by:

$$\mathcal{L}(D|\Psi, \theta) = \sum_{n=i} \mathcal{L}(D_i, I_i|\Psi, \theta_i) \quad (3.15)$$

where $D = \{D_i\}_{i=1}^n$; D_i is the microcephaly incidence data from location i ; $I = \{I_i\}_{i=1}^n$; I_i is the ZIKV incidence data from location i ; θ_i is the set of parameters specific to location i ; $\theta = (\theta_1, \theta_2, \dots, \theta_n)$ is the vector of all location-specific parameters; Ψ is the set of universal model parameters; and n is the number of locations included in the analysis.

Using the above log likelihood, the log posterior probability function is defined as:

$$\log \pi(\Psi, \theta|D, I) = \log p(\Psi, \theta) + \sum_{n=i} \mathcal{L}_i(D_i, I_i|\Psi, \theta_i) \quad (3.16)$$

where $p(\Psi, \theta)$ is the prior probability of the universal and location-specific model parameters; and $\pi(\Psi, \theta|D, I)$ is the posterior probability. Uniform priors were assumed for all free model parameters with upper and lower bounds described in Table 3.1.

Model fitting without ZIKV incidence data

It is possible to fit the model to microcephaly incidence data alone by setting the weighting of the ZIKV incidence component, ω , to 0. This is appropriate when fitting the model for locations with no ZIKV infection incidence. However, some knowledge of the timing of the ZIKV epidemic peak was available for locations without full reported incidence. For these locations, information on the timing of

ZIKV epidemic peak was incorporated to help constrain the timing of peak ZIKV infection risk. This peak time can be considered a function of the SEIR model parameters (ie. the peak of ZIKV incidence generated by the SEIR model). This was used to inform a uniform prior distribution as follows:

$$p(t_{peak}) \sim \text{unif}(a - \frac{b}{2}, a + \frac{b}{2}) \quad (3.17)$$

where t_{peak} is the model generated ZIKV peak incidence time in that location; a is the peak time of the ZIKV epidemic in that location based on the day of maximum reported ZIVK incidence; and b is the width of the uniform window around this peak time, representing uncertainty in the timing of the peak. b was set to be 120 days to represent a ~ 4 month window of uncertainty around the timing of the ZIKV epidemic peak. Parameter values that give $t_{peak} < a - \frac{b}{2}$ or $t_{peak} > a + \frac{b}{2}$ are therefore assigned a probability of 0. a was 06/05/2015 for Bahia; 03/02/2016 for Colombia; 16/03/2015 for Pernambuco; and 13/05/2015 for Rio Grande do Norte based on the sources shown in Table A.4.

MCMC algorithm

The model was fit to available incidence data for a single wave of ZIKV and microcephaly incidence to quantify the gestational-time microcephaly risk curve. I used an MCMC framework (`lazymcmc`, a package I developed) written in R and C++ with the `rlsoda` package [381,382]. Model fits were made to each location separately, though a sensitivity analysis was performed combining data across locations using Equation 3.15 and described in Appendix A.3. Chains were run for 2000000 iterations with a 750000 iteration burn in and adaptive period. The chains were run to ensure that a sufficient effective sample size was achieved for all model parameters or at least 200, with convergence assessed using the Gelman-Rubin diagnostic tool with the `coda` package, R [383]. Using the estimated parameters of the Gamma risk curves, it was possible to estimate the following quantities of interest: first week of gestation where ZIKV-infection confers a risk of microcephaly greater than 1 in 1000; last week of gestation where ZIKV-infection confers a risk of microcephaly greater than 1 in 1000; number of gestational weeks spent at risk; mean per-trimester risk; gestational week of greatest risk (gamma mode). All posterior estimates are shown in Table A.1 and Table A.2.

3.2.7 Forecasting the second wave of microcephaly incidence

In a separate analysis, the model was extended to quantify potential changes in behaviour and reporting rates that could explain the two seasons of observed data in Bahia, Brazil, where only one wave of

microcephaly incidence was observed despite two waves of ZIKV infection incidence. Time-dependent changes in behaviour and reporting were likely during the epidemic due to media hype and public awareness (demonstrated by changes in Google search behaviour shown in Fig 3.3A). Four mechanisms were included to account for these changes, described in detail in Appendix A.2 and depicted in Fig 3.4. First, I assumed that microcephaly reporting became 100% accurate from March 2016 (the most recent change in case definition in Brazil) and estimated the relative reporting rate prior to this as a model parameter [384, 385]. Second, I assumed that immediately following the WHO declaration of a PHEIC in February 2016, the rate of aborted pregnancies under 24 weeks gestation could have changed [386–388]. Third, I assumed that the number of ZIKV-affected births after this date may have changed, either due to avoided pregnancies or additional precautions taken by pregnant women to avoid infection relative to the rest of the population [387, 388]. Finally, I assumed that ZIKV infection reporting accuracy may have changed after 11/11/2015, when the Brazilian Ministry of Health declared a National Public Health Emergency, and just before WHO/PAHO issued an alert with laboratory detection guidelines for ZIKV [389, 390].

Parameter estimation was performed using the same likelihood function and MCMC framework described above. I fit the model assuming that either all four mechanisms were present simultaneously, that only one mechanism was present at a time, or that all mechanisms were present other than a change in ZIKV infection reporting rates. Where the contribution of each mechanism alone was estimated, I fixed the contribution of all but the one relevant mechanism parameter to be 0 and refit the model to the incidence data for Bahia, Brazil.

The model did not explicitly include seasonality, and the SEIR model was therefore only suitable for the single-season analyses. I therefore did not use the SEIR model component in the multi-season analysis, but rather assumed that the per capita risk of becoming infected with ZIKV was proportional to reported ZIKV infection incidence and that reported incidence of ZIKV infection represented a fraction of true cases scaled by one parameter up to 11/11/2015 and another from 11/11/2015 onward.

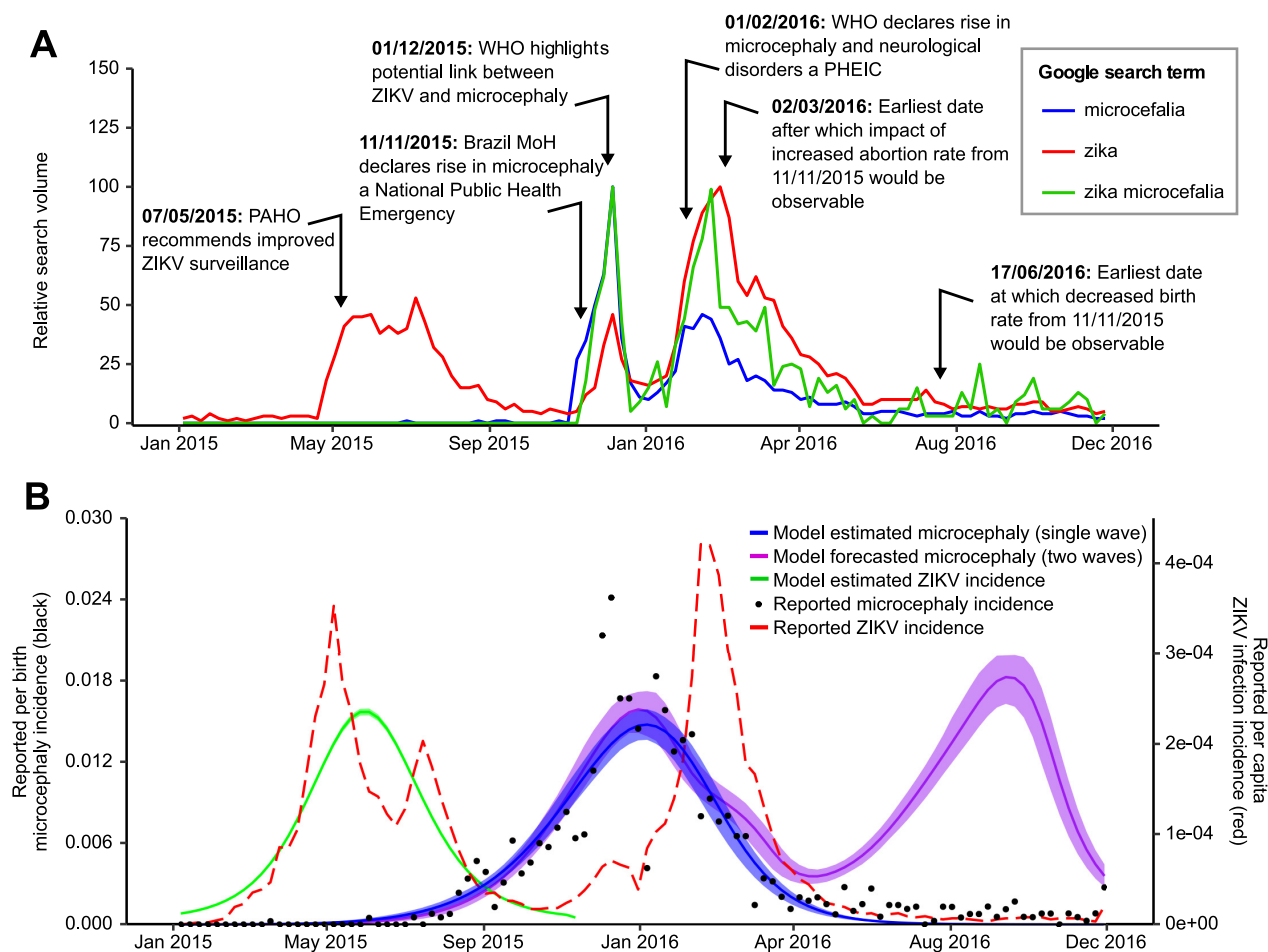


Figure 3.3: (A) Google search trends from 2015 to 2017 from Bahia, Brazil [391]. Y-axis shows the relative number of searches normalised by all searches at that time and location, with 100 indicating the highest search volume for that term across the entire time period. Red dashed lines highlight key epidemiological alerts that may have influenced public awareness and behaviour. MoH = Ministry of Health; PHEIC = Public Health Emergency of International Concern. (B) Model fits to the first wave of ZIKV and microcephaly incidence plotted with reported ZIKV and microcephaly incidence in Bahia, Brazil. Red dashed line shows weekly reported ZIKV infection incidence per capita; green line shows per capita ZIKV infection incidence predicted by the SEIR model based on the maximum a posteriori probability (MAP) parameter estimates from fitting to the first wave of ZIKV infection incidence; black dots show reported weekly microcephaly incidence per live birth; blue line and shaded region show the model estimated MAP and 95% credible intervals (CI) on microcephaly incidence per live birth based on ZIKV infection incidence predicted by the SEIR model fitted to the first wave of microcephaly incidence; purple line and shaded region shows MAP and 95% CI forecasted per live birth microcephaly incidence assuming that the infection-risk relationship as estimated in Fig 3.6 persisted through the second wave, that reported ZIKV infection incidence represents the true incidence for both waves and that case reporting remained the same from 2015 to 2016.

Model Name	Date of mechanism duration/ parameter value when mechanism excluded			Free parameter
Microcephaly reporting change only	$\phi_{m1}=1$		$\phi_{m2}=1$	ϕ_{m1}
Abortions only	None		$a_r=0$	a_r
ZIKV reporting change only	$\phi_{i1}=\text{estimated}$	$\phi_{i2}=\phi_{i1}$		ϕ_{i2}
Avoided births only	None	$b_r=0$		b_r
	11/11/2015	01/02/2016	13/03/2016	

Figure 3.4: Schematic of forecasting analysis mechanism switch times. The middle column demonstrates the time at which reporting or behaviour may have switched (green before the switch, red after). The values shown in the middle column show the assumed values or contributions of these parameters when the mechanism is excluded. The right hand column shows the parameter that is estimated as a free parameter for that analysis. For example, if microcephaly reporting is assumed to stay the same, then $\phi_{m1} = \phi_{m2} = 1$ for all dates. If microcephaly reporting change is the mechanism under consideration, then ϕ_{m1} is estimated as a free model parameter. In the joint impact analysis, all parameters in the “free parameter” column are estimated.

3.3 Results and Discussion

3.3.1 Patterns of incidence in the data

Both microcephaly and ZIKV incidence demonstrated clear epidemic-like signal in many Brazilian states and Colombia (Fig 3.5). Although the study data were from similar reporting systems, Fig 3.5 illustrates substantial differences in the key features of both ZIKV infection and microcephaly incidence patterns. Peak timing, width of the incidence curves, maximum per capita incidence and the lag between ZIKV infection and microcephaly incidence peaks differed by location and dataset. Variation in total and maximum per-birth microcephaly incidence indicates location-specific differences in the proportion of pregnant women that were infected with ZIKV or in the probability of developing microcephaly following infection. For example, weekly notified microcephaly incidence peaked at 32.6 cases per 10,000 births in Colombia, which was far lower than peak notified microcephaly incidence in Pernambuco, Brazil at 760 cases per 10,000 births. This suggests that microcephaly risk given infection and/or ZIKV attack rates were higher in Pernambuco. The lag between incidence peaks also varied, ranging from 23 weeks (bootstrapped confidence intervals: 19-32 weeks) for Colombia compared to 31 weeks (bootstrapped confidence intervals: 30-36) from state-level reports for Bahia, Brazil.

Given that only a small fraction of the Colombian population is at risk of arbovirus infection compared to the Brazilian population due to differences in vector ecology, it is unsurprising that the absolute per capita incidence of ZIKV infection and microcephaly are lower in Colombia than in Brazil [23]. However, as the population at risk of ZIKV infection is the same population at risk of microcephaly, we would expect the lag and relative magnitudes of ZIKV infection and microcephaly incidence to be the same between Brazil and Colombia. These differences in time lags and relative magnitudes therefore suggest that the time of peak risk during pregnancy may have varied between locations, potentially through differences in additional risk factors in these locations such as prior arbovirus exposure.

Differences in observed incidence patterns may also arise as a result of reporting bias, which may be reduced using confirmed rather than notified case data. Although some confirmed microcephaly case data were available for Brazil, the only available data of confirmed microcephaly cases in Colombia reported number of cumulative confirmed cases [392]. It should also be noted that only a subset of suspected cases were laboratory confirmed in Colombia to test for the presence of ZIKV in municipalities which had yet to confirm ZIKV infection, and case reporting was otherwise based on clinical symptoms [393]. Due to variation in confirmation delays, it is not possible to extract the

time of birth of these cases and I was therefore unable to use these data in model fitting. Between 03/01/2016 (epidemiological week (EW) 1 of 2016) and 05/06/2017 (EW 18 of 2017), approximately 70% of notified microcephaly cases were discarded in Colombia (328 cases confirmed, 874 discarded, 37 under investigation), highlighting that total notified case data likely overestimated true incidence [394]. The proportion of total notified cases that were discarded was similar for Rio Grande do Norte (138 confirmed vs. 475 notified) and higher for Pernambuco (365 confirmed vs. 2117 notified). Confirmed ZIKV infection incidence were available for Colombia but not Brazil due to the lack of reporting infrastructure during the first wave [395]. The lag between peak ZIKV infection and microcephaly incidence did not change when using notified or confirmed ZIKV infection or microcephaly data.

3.3.2 Different data sets suggest different risk profiles

Using the full model fitting framework, I inferred gestational risk profiles for 5 of the 6 datasets shown in Fig 3.5 (Fig 3.6, and posterior estimates for key parameters of interest are shown in Table A.2). Though clear estimates of the gestational-age-varying risk were obtained for each location, substantial differences are apparent in the inferred gestational age of peak risk, duration of the risk period, and maximum absolute risk. The model did not produce a biologically interpretable risk profile using data from Pernambuco, Brazil that was comparable to those inferred using the other 5 datasets. Sensitivity analyses excluding the ZIKV infection incidence data from the model fitting for other locations were able to produce plausible risk profiles, suggesting biases in reported microcephaly incidence data for Pernambuco that could not be explained by the model rather than the lack of ZIKV infection data. It is important to note that microcephaly is only one manifestation of CZS, and the risk profile of other adverse outcomes may differ. These risk estimates therefore apply only to the specific outcomes of proportionate and disproportionate microcephaly, which were not distinguished in these data [16].

3.3.3 Gestational age at peak risk

The time from the peak of ZIKV infection incidence to the peak of microcephaly incidence indicates the typical gestational age at which microcephaly cases were infected. When using both state and city level notified ZIKV infection and microcephaly incidence from Bahia, Rio Grande do Norte, and Salvador, Brazil, the peak week of gestational-age-varying risk was estimated to be in the middle of the first trimester (Fig 3.6). When notified ZIKV infection incidence in pregnant women and confirmed microcephaly incidence from Northeast Brazil were used, the estimated peak gestational-age-varying risk was towards the end of the first trimester. Notified case data from Colombia were also suggestive

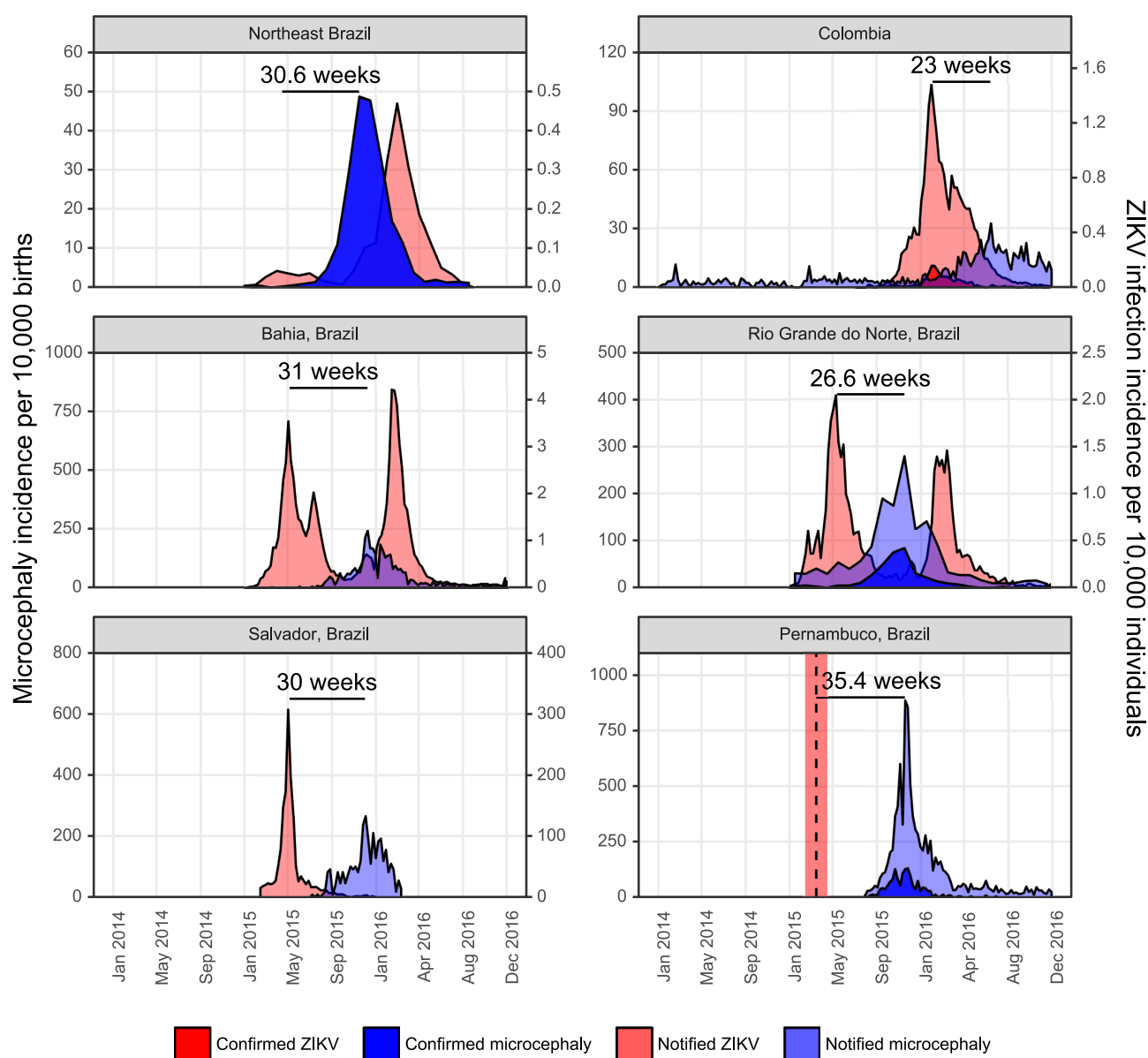


Figure 3.5: Notified and confirmed microcephaly and ZIKV infection incidence. X-axis shows date of report. Y-axis shows microcephaly incidence per 10,000 births (left) and ZIKV infection incidence per 10,000 individuals (right). Note different y-axis scales. ZIKV infection incidence for Northeast Brazil uses only reported cases in pregnant women with the entire population as the denominator. For Pernambuco, where comprehensive ZIKV infection incidence was not available, peak epidemiological week from which incidence was reported (black dashed line) is shown with a 4-month window (shaded red). Horizontal lines and labels show time in weeks between the initial peak of ZIKV infection incidence and peak microcephaly incidence. Confirmed and notified cases are distinguished by shading, where available. All incidence was reported by epidemiological week, apart from Northeast Brazil incidence and Rio Grande do Norte microcephaly incidence, which were reported by month. Note that no second y-axis is shown for Pernambuco, as no ZIKV infection incidence is shown.

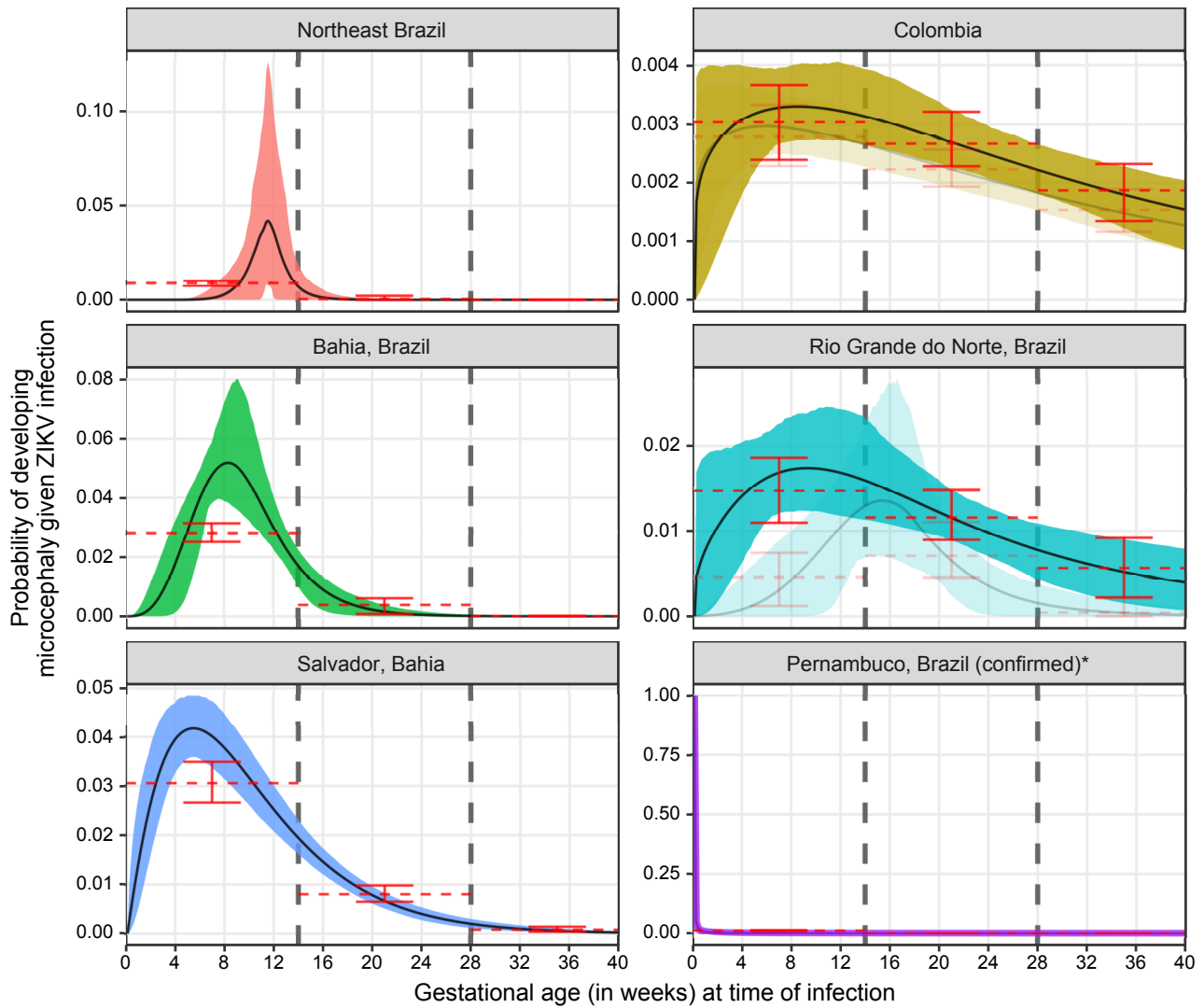


Figure 3.6: Estimates of the microcephaly risk profile fit to different data sets. Shaded regions show 95% credible intervals, black lines show posterior means. Vertical dashed lines show boundaries of the 1st, 2nd and 3rd trimester. Horizontal red dashed lines show the mean estimate for the mean model-derived risk (ie. average of the model suggested risk for that trimester) during each trimester, red bars show 95% credible intervals. Data sets were as follows (left-right). Subplots in order: Notified ZIKV infections in pregnant women and confirmed microcephaly incidence data from the entirety of Northeast Brazil combined [25, 361]; Notified ZIKV infection and microcephaly incidence data from Colombia (confirmed ZIKV faded) [362]; Notified ZIKV infection and microcephaly incidence from Bahia, Brazil state-level reports [364]; Notified ZIKV infection and microcephaly incidence from Rio Grande do Norte, Brazil state-level reports (confirmed microcephaly faded) [367, 368]; Notified AEI and microcephaly incidence from Salvador, Bahia, Brazil [369]; Confirmed microcephaly incidence from Pernambuco, Brazil state-level reports [366].

of peak risk in the first trimester. Inference did not change substantially using confirmed as opposed to notified ZIKV infection incidence data for Colombia; however, using confirmed microcephaly incidence data for Rio Grande do Norte resulted in a shift of the risk profile towards the start of the second trimester. When a ZIKV infection incidence peak in a 4-month window around March 2015 was assumed for Pernambuco, the inferred microcephaly risk profile was highly skewed towards the first week of pregnancy, suggesting that these data are incompatible with the other 5 data sets.

3.3.4 Duration of heightened gestational risk

There was substantial variation in the inferred window of heightened gestational risk between different populations. The window of heightened gestational risk is estimated from the relative durations of the ZIKV infection and microcephaly incidence curves (using an illustrative threshold of 1 in 1,000 infections leading to microcephaly to define the heightened gestational risk window). A narrow period of ZIKV infection incidence preceding a wide period of microcephaly incidence suggests a wide window of heightened gestational risk. If the period of heightened gestational risk is long, then infections at a particular point in time would present as cases of CZS across a wider interval of birth dates. Inferred risk profiles using notified case data from Rio Grande do Norte, the city of Salvador and Colombia all suggested heightened risk throughout pregnancy (Fig 3.6).

Conversely, two similarly narrow (or wide) periods of ZIKV infection and microcephaly incidence would suggest a relatively small window of heightened gestational risk, as all ZIKV-affected pregnancies would present as births after a similar delay. A true microcephaly incidence period that is narrower than the ZIKV infection incidence period should not be possible, as the narrowest microcephaly incidence curve would arise when all infected pregnant women give birth after the same delay. Aggregated confirmed case data from Northeast Brazil, state-level notified case data from Bahia and state-level confirmed case data from Rio Grande do Norte all suggested a more limited window of risk during pregnancy, with lower risk suggested towards the end of pregnancy (Fig 3.6, Northeast Brazil, Bahia and Rio Grande do Norte (confirmed cases)). Public awareness, media hype, changing criteria for case reporting and variation in laboratory testing capacity likely resulted in changing reporting rates throughout the epidemic [384,395–397]. Location-specific time-varying changes in reporting sensitivity and specificity are therefore one potential explanation for differences in the risk profiles inferred using data from Northeast Brazil and Colombia. Given that Colombia was expecting an increase in microcephaly cases during 2016, an increase in notified cases may have been reported before a true increase in confirmed cases, which would falsely suggest some gestational risk late in pregnancy. Time-varying reporting bias

may also explain the extremely narrow and early window of risk inferred using data from Pernambuco (Fig 3.6, Pernambuco, Brazil). The impact of reporting bias is clearly demonstrated by the contrasting results using confirmed or notified microcephaly case data for Rio Grande do Norte, wherein confirmed data suggested a narrower and later risk window than the notified data.

3.3.5 Absolute risk of CZS

The absolute risk of CZS is difficult to estimate as it depends on the true incidence of ZIKV infection in pregnant women and CZS cases as a proportion of live births. A high ZIKV infection attack rate with known microcephaly incidence would suggest a lower microcephaly risk per infection to the fetus than a low infection attack rate with the same observed microcephaly incidence [19]. Reported infection incidence data may be subject to under-reporting and over-reporting, potentially through missing asymptomatic or mild cases that might not present to surveillance systems (under-reporting), or misclassifying infections caused by other arboviruses as ZIKV infection, namely dengue and chikungunya virus (CHIKV) (over-reporting) [347,395]. These confounders present identifiability problems in inferring levels of true incidence and therefore microcephaly risk; surveillance data in a scenario of high risk with under-reporting would be similar to a scenario of low risk with over-reporting. For example, during the 2015 wave in Brazil many cases of illness likely caused by ZIKV were misclassified as dengue infection, resulting in under-reporting of ZIKV infection incidence [395]. Over-reporting of microcephaly incidence during the initial wave of cases was also possible, due to changing case definitions, reclassification of suspected cases and increased awareness in surveillance systems [384,385]. Estimating the proportion of true ZIKV infections that led to observed microcephaly cases is therefore dependent on knowing the true risk of ZIKV infection during the epidemic period. ZIKV IgG seroprevalence was estimated to have reached 63.3% (95% confidence interval, 59.4 to 66.8%) in Salvador, Brazil between 2015 and 2016 despite only 16,986 reported cases of AEI from a population of nearly 3 million (approximately 0.6%), suggesting that under-reporting of ZIKV infection incidence was a key problem in this location [369,398]

The absolute risk of ZIKV-associated microcephaly was inferred for each dataset by assuming that 100% of true microcephaly cases were reported but that reported ZIKV cases represented only a fraction of the true incidence (ie. estimating a location-specific ZIKV infection reporting rate parameter). The average first trimester risk of microcephaly given ZIKV infection was estimated to be 2.81% (mean; 95% credible interval (CI): 2.51-3.16%) based on data from Bahia, Brazil, but much lower in the second trimester at 0.365% (mean; 95% CI: 0.0715-0.588%). Conversely, the level of absolute risk estimated using notified case data from Colombia suggested that the risk was lower but consistent

throughout gestation at 0.303% (mean; 95% CI: 0.239-0.367%), 0.268% (mean; 95% CI: 0.228-0.322%) and 0.186% (mean; 95% CI: 0.135-0.232%) in the first, second and third trimesters respectively. The former estimate is slightly higher than risk estimates inferred based on seroprevalence data from French Polynesia which suggested a risk of 0.95% (95% confidence interval; 0.34-1.91%) in the first trimester, whereas the latter estimate suggests a lower risk [7].

I performed a sensitivity analysis with different constraint on the true ZIKV attack rate by taking microcephaly and AEI data from Salvador, Brazil for 2015 scaled by recent ZIKV IgG seroprevalence data, as described in Appendix A.3.1 [369]. Based on the ZIKV infection and microcephaly incidence data from Salvador, Brazil, I estimated the mean first trimester risk of microcephaly given ZIKV infection to be 3.06% (mean, 95% CI: 2.66-3.49%); the mean second trimester risk to be 0.805% (mean, 95% CI: 0.649-0.980%); and the mean third trimester risk to be 0.0833% (mean, 95% CI: 0.0407-0.142%). I did not scale incidence data for any other location due to the lack of seroprevalence data. However, given that the model is powered by the pattern of microcephaly incidence relative to the pattern of ZIKV infection incidence after accounting for differences in infection risk and reporting, these risk estimates may apply to other locations if no additional co-factors affect the risk of microcephaly given infection.

3.3.6 Understanding the missing second wave of microcephaly incidence

Despite a clear second wave of GBS incidence at the beginning of 2016, no second wave of microcephaly incidence in Northeast Brazil was observed in the latter half of 2016 [25]. Similar to [25], Fig 3.3B illustrates the incidence of microcephaly that would have been expected in Bahia, Brazil using the model framework and based on reported ZIKV infection incidence under the assumption that the underlying gestational-age-varying risk profile and reporting behaviour did not change from 2015 to 2016. Fig 3.7 shows the residuals between the model predicted ZIKV infection for the first wave using the SEIR model and the corresponding microcephaly infection incidence from this first wave. These residuals demonstrate that although the model generally captured the overall incidence trends, there were some periods around the peak where more highly resolved dynamics (eg. a bi-modal peak for ZIKV infection incidence) were present.

I used the population-level data fitting framework described above to test the hypothesis that plausible changes in behaviour or reporting are sufficient to provide a consistent narrative between the two waves of ZIKV and microcephaly case data. Fig 3.3A describes the timings of particular events that may have

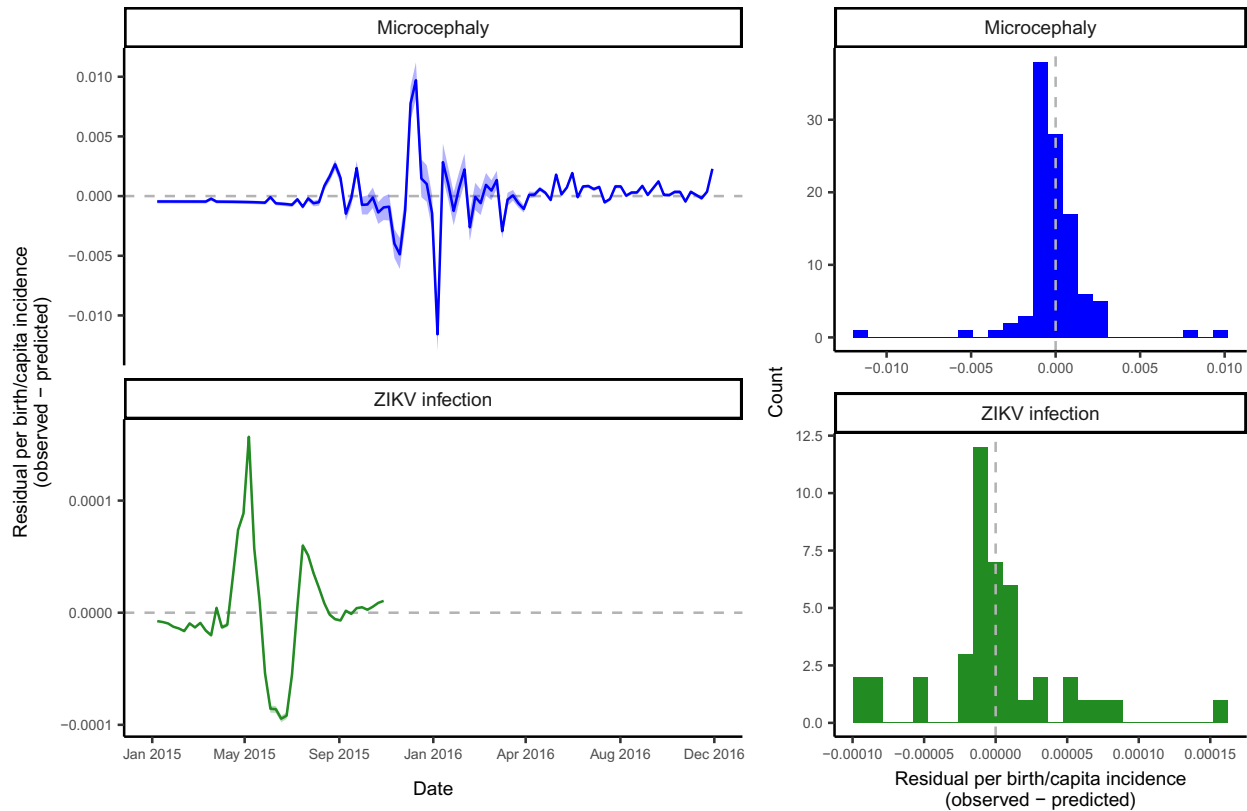


Figure 3.7: Model residuals for ZIKV infection and microcephaly incidence. Top row shows posterior median and 95% CI on observed minus model-predicted per birth microcephaly incidence over time and overall, derived from a single wave of ZIKV infection incidence from the SEIR model. Bottom row shows posterior median and 95% CI on observed minus model-predicted per capita ZIKV infection incidence derived from the SEIR model over time and overall.

led to these changes. I considered four hypotheses describing changes in behaviour and reporting rates:

1. Microcephaly reporting accuracy changed at week 11 of 2016, the most recent change in case definition in for microcephaly reported through the Registro de Eventos em Saúde Pública (RESP) database in Brazil) [380,385].
2. Immediately following the National Public Health Emergency announcement by the Brazilian MoH on 11/11/2015, the frequency of early abortions (up to 24 weeks gestation) due to early detection of CZS may have increased [389]. The earliest date at which targeted abortions would be observed as a drop in birth rate would be 16 weeks after this shift in behaviour (02/03/2016) [387,388,399]. A reduction in birth rate from delayed pregnancy would also be possible; however, this would only appear approximately 40 weeks after the behavioural shift.
3. The number of pregnant women affected by ZIKV after this date may have changed through additional precautions taken to avoid infection relative to the rest of the population [400].
4. ZIKV reporting itself may have changed on 11/11/2015 before the start of the second wave of ZIKV infection incidence through increased surveillance, increased awareness and/or increased misclassification of other arbovirus infections as ZIKV infection.

Based on state-level reports from Bahia, Brazil and assuming that ZIKV infection reporting did not change, these analyses suggest that the lack of a second microcephaly peak could be explained by the combined effect of: a 151% reporting rate of microcephaly cases prior to 13/03/2016 relative to fixed 100% accurate reporting after 13/03/2016; targeted abortions ending 88.4% of microcephaly-affected pregnancies prior to 24 weeks gestation; and a relative decrease in infection probability in pregnant women of 0.60% (values shown are the maximum a posteriori probability (MAP) estimates). It is important to note that many of these parameters are highly correlated, suggesting that these data could be explained by a combination of multiple mechanisms, or by a greater contribution of some mechanisms and a reduced effect from the others (Fig 3.8). If ZIKV infection reporting accuracy increased substantially between the two waves in addition to the behavioural changes described above, then a smaller increase in the proportion of terminated pregnancies would have been necessary. Similarly, targeted abortions and precautions to avoid infection by pregnant women would present a similar reduction in microcephaly incidence, and these estimates are therefore highly correlated (Fig 3.8C). All posterior estimates from these analyses are shown in Table A.3.

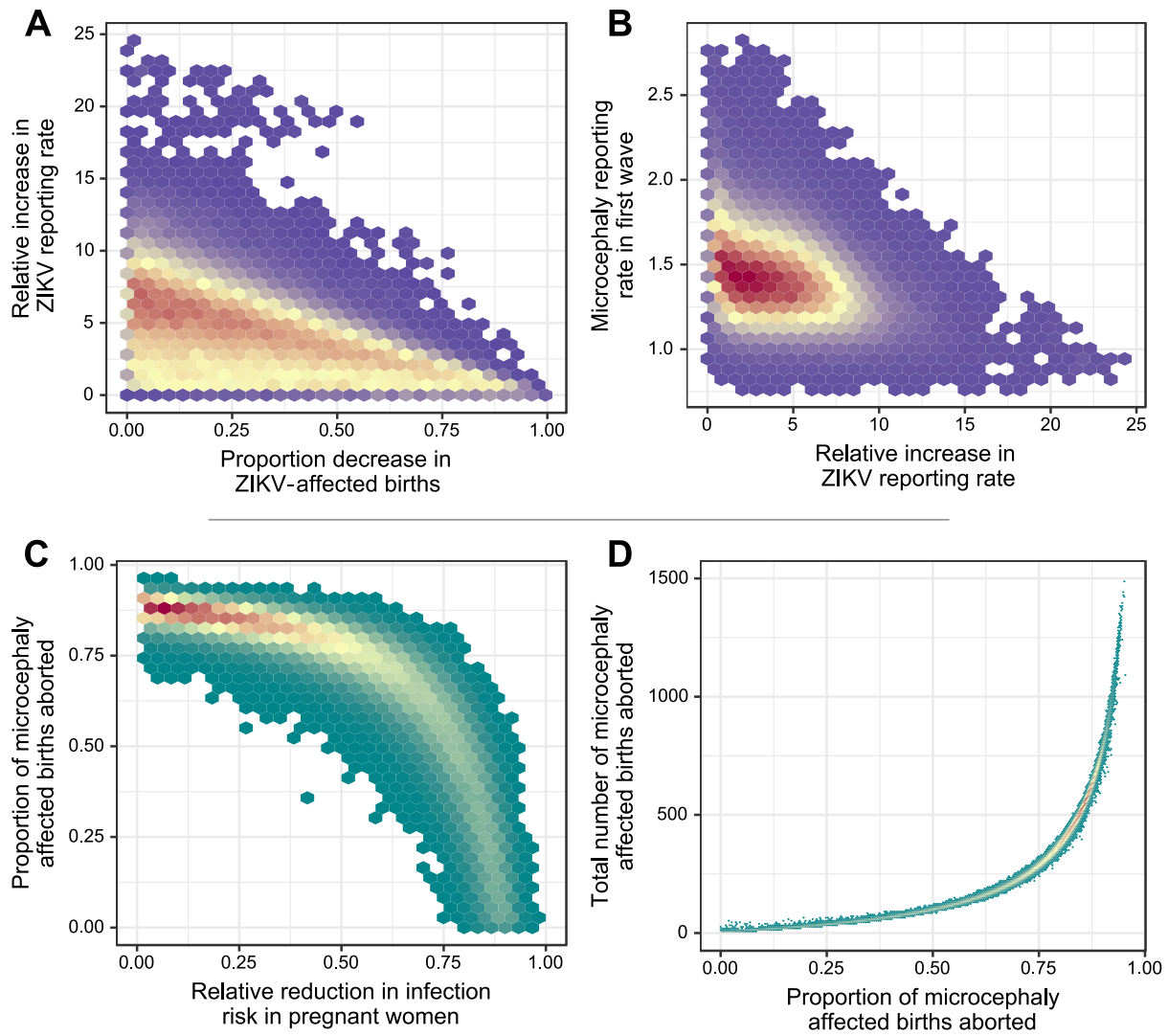


Figure 3.8: Key regions of parameter space that are consistent with the observed data. Unshaded regions show areas of parameter space that are less consistent with the observed data. Two-dimensional posterior density estimates for the behavioural and reporting changes necessary to explain the lack of a second microcephaly incidence wave in Bahia, Brazil. Better supported regions of parameter space are indicated in red/orange, whereas less well supported regions are purple/turquoise. Plots A and B were estimated assuming that ZIKV reporting behaviour could have changed between the two waves, whereas plots C and D were estimated assuming that ZIKV reporting behaviour stayed the same throughout the epidemic. All estimates presented assumed that microcephaly reporting, targeted abortions and infection avoidance behaviour may have been present in the second wave, as described in the main text. (A) Negative correlation between the increase in ZIKV reporting and increase in abortion rate, suggesting that the observed data could be explained by either mechanism in the absence of the other. (B) Lack of correlation between ZIKV reporting and microcephaly reporting change estimates suggest that both mechanisms may independently explain observations. (C) High correlation between the proportion reduction in ZIKV-affected pregnant women and the proportion of targeted abortions assuming no change in ZIKV reporting between the two waves, suggesting that high levels of either, or moderate levels of both mechanism are required to explain the data. (D) Relationship between total aborted births between 02/03/2016 and 31/12/2016 and the proportion of ZIKV-associated microcephaly-affected births aborted, highlighting the actual number of aborted pregnancies that would have occurred given a particular abortion rate.

Assuming that there were no targeted abortions, no additional precautions to avoid infection taken by pregnant women, and no change in microcephaly reporting accuracy, these data could be explained solely by a 18.9-fold (mean, 95% CI: 10.0-59.1-fold) increase in ZIKV infection reporting after 11/11/2015. Conversely, assuming that targeted abortions after 11/11/2015 were the only change, 92.5% (mean, 95% CI: 89.8-94.9%) of microcephaly-affected births would need to have been aborted to explain the lack of a second peak, corresponding to 1090 (803-1480) aborted pregnancies between 02/03/2016 and 31/12/2016. Fig 3.8D shows how the total number of aborted microcephaly-affected births, which may be observable, would change with different abortion rates of microcephaly-affected births. If microcephaly reporting accuracy were the only factor to change, then a 601% (mean, 95% CI: 492-726%) reporting rate of microcephaly cases prior to 13/03/2016 relative to fixed 100% accurate reporting after 13/03/2016 would have been necessary. Accurate data on the true number of abortions in this time period and information on the changes in ZIKV and microcephaly reporting would help to clarify the relative contributions of these mechanisms.

3.4 Conclusions and Implications

Overall, these results highlight the limitations of publicly available population-level data in explaining epidemiological trends during an outbreak. Different datasets suggested different risk profiles, some of which contrast with previous population-scale analyses. Whilst data from Bahia, Brazil were suggestive of a risk profile similar to that estimated using data from French Polynesia, data from Colombia and Rio Grande do Norte, Brazil suggested a much longer gestational risk period [7] .

Although reporting bias may explain the differences in inferred microcephaly risk in different locations, heterogeneity in the distribution of additional host risk factors of microcephaly may be important. Interpretation of epidemiological data for DENV infection requires an understanding of pre-existing immunity due to the presence of antibody-dependent enhancement, which may also be relevant to the interpretation of CZS incidence given the potential role of DENV antibodies in ZIKV disease enhancement [30,35,401]. Observations of increased prior DENV exposure in areas of disproportionately increased microcephaly incidence would support this hypothesis and be of importance for DENV- but not yet ZIKV-affected areas, highlighting the need for comprehensive serological studies [398,402]. An understanding of other potential host risk factors that may differ between affected areas, such as socioeconomic status or maternal smoking, will further aid the interpretation of contrasting incidence data [385]. Furthermore, a female bias in infection rates has been reported previously, potentially leading to a relatively greater reduction in susceptible women of childbearing age following the initial wave [403].

A limitation of the model is the aggregation of data into high-level administrative units, which may mask small-scale heterogeneity in infection risk and case reporting. This may be particularly problematic in the analysis for Colombia, as using the entire Colombian population and birth numbers as the susceptible population may underestimate the true risk should only a fraction of the population actually be exposed to ZIKV infection [404,405]. Similarly, differences in transmission peak times at a small spatial scale coupled with location-specific reporting accuracy may reduce the reliability of the population-wide inferred risk profile. Although I could not fit the model at a smaller administrative unit due to the lack of necessary meta-data for Colombia, doing so may reveal a similar risk profile to that estimated using data from Northeast Brazil.

These results should be interpreted with caution, as this risk profile applies only to completed pregnancies and does not describe a prospective study of the risk of all adverse outcomes. For example,

for tourists travelling to ZIKV affected areas, a low risk of microcephaly given infection in the first trimester does not necessarily mean that they are safe from adverse events, as it seems likely that ZIKV could cause miscarriages and other neurological complications throughout pregnancy [11,406]. However, for women with a robust ZIKV diagnosis and a conception date, these results may help pregnant women to understand the relative risk periods of ZIKV-associated microcephaly during gestation. In many setting it will be possible to confirm the likelihood of microcephaly with radiological examination.

Based on these estimates, ZIKV infection reporting rates would need to have increased 18.9-fold (mean, 95% CI: 10.0-59.1-fold) to explain the lack of a second microcephaly wave in Bahia, Brazil on its own, which may have been possible if awareness and diagnostic accuracy improved through the epidemic. Syndromic ZIKV reports may have included misclassified CHIKV infections which may not have represented an increased risk of ZIKV-associated microcephaly during the second wave in 2016 [407]. An 18.9-fold increase in ZIKV reporting as estimated here could therefore mean that ZIKV reporting was a more accurate representation of the true ZIKV attack rate in 2016, or that 18 chikungunya cases were misclassified as ZIKV for every 1 true reported ZIKV case with no change in the proportion of true ZIKV cases that were reported [395]. However, during the period in which second waves of ZIKV infection occurred, there was sufficient virological testing to justify confidence in the relative specificity of reported ZIKV cases [263]. Furthermore, in Salvador, Brazil, where serological data are available, the increase in CHIKV seropositivity from 2015 to 2016 was far lower than for ZIKV seropositivity [398]. Nonetheless, diagnostic tools with improved sensitivity and specificity in distinguishing these infections would help to clarify the proportion of true ZIKV infection incidence that observed incidence data represent.

The model estimated that 1090 (mean, 95% CI: 803-1480) microcephaly-affected births would need to have been aborted between 02/03/2016 and 31/12/2016 to explain the observed data through increased abortions alone. Given that approximately 1000 abortions are reported in Northeast Brazil weekly, it may be possible to identify the true increase in abortion rate during this time period if and when complete data become available (Supplementary Material of [25]) [388].

Estimating the true shape and magnitude of the underlying gestational risk profile therefore requires additional data that could either be gathered retrospectively or through surveillance in locations that are yet to experience a wave of ZIKV. A key limitation of the epidemiological data gathered in Brazil during 2015 and early 2016 is that surveillance systems were not in place to accurately describe the true per capita incidence of ZIKV. Retrospective serological surveys have been suggested previously as a

means of inferring attack rates, which would constrain estimates for the reporting rate of microcephaly and ZIKV and in turn constrain estimates both of actual risk and true changes in behaviour/reporting in the second wave [266,407]. For microcephaly and CZS, consistent and accurate case definitions would be advised such that sensitivity and specificity are high throughout the epidemic period – something which was not achieved in Brazil during 2015 [384].

A key question that still remains is whether the epidemiological data from Brazil represent the true relationship between ZIKV and microcephaly, and indeed CZS. Cohort studies for women of childbearing age to assess whether changes in behaviour regarding conception and infection avoidance occurred in 2016 would clarify whether the second season of ZIKV/microcephaly in Brazil is fully consistent with these estimates of gestational-varying risk from the first season. If reporting rates and behaviour changes are not sufficient to explain the apparent discrepancy between first-wave incidence in Brazil compared to later and elsewhere, additional cofactors would need to be identified. Coinfection with other arboviruses has been suggested as a potential cause, and as with antibody dependent enhancement in DENV infection, studies to identify such interactions are necessary [15].

Chapter 4

Characterising antibody kinetics of influenza exposure using a ferret model

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In this chapter, I develop a set of mathematical models describing antibody kinetics following influenza vaccination and infection. I extend this model to consider repeated exposures and incorporate a number of immunological mechanisms that are known to be important in explaining antibody responses. These models are fit to antibody titre data from ferrets with observed, varied exposure histories. Through a model comparison analysis, I demonstrate that complex models that take into account immunological mechanisms and exposure-type specific parameters are better supported than simpler models that don't. These results highlight the use of repeat exposure animal models in quantifying antibody kinetics parameters that are yet to be included in models of human antibody landscapes.

4.1 Introduction

A large body of experimental and observational work exists describing the contribution of immune histories to observed influenza susceptibility profiles, antibody landscapes and vaccination responses [6, 92, 185, 205, 408–411]. Next generation assays to characterise antibody diversity and B cell identity have provided a detailed understanding of immune dynamics, including short term immune kinetics, duration of the humoral response, and immunodominance of different antigenic sites [160, 412–416]. However, few studies have integrated these mechanisms into quantitative frameworks which can be used to explain and predict serological data from human populations, which often rely on simpler and less finely resolved assays [1, 86, 225, 230]. Furthermore, existing models of post-exposure antibody kinetics usually focus on the response to a single immunogen following one exposure or do not consider partial cross reactivity, which is insufficient to understand antibody kinetics in multi-strain pathogen systems [156, 185, 222, 231]. Few mathematical models exist that take into account immune cross reactivity across multiple epitopes [417, 418].

Animal models, in particular ferrets, have been used to generate much of our understanding of influenza immunology due to opportunity for intensive observation and control [413, 419–422]. For example, it has been shown in ferret and mouse models that priming infection elicits the production of broader cross-reactive antibodies and T cell recruitment compared to vaccination alone [423–425]. However, analytical methods for the data generated by these studies as it relates to humans are relatively underdeveloped and few ferret studies have been conducted in which ferrets are exposed to multiple influenza strains. Existing modelling studies of within host kinetics following influenza exposure have historically been limited to (i) understanding kinetics in a short time scale [220, 221] and (ii) experiments that use data that are not readily observable in human populations (eg. viral load, interferon and target cells) [218, 426–428]

To bridge the gap between animal models and human population analytical methods, I developed a set of models of antibody boosting and biphasic waning to describe antibody kinetics in a group of ferrets with varied but known exposure histories [420]. Quantifying immunological mechanisms that may be important in characterising antibody landscapes, yet are observable using only routine antibody assays, could be used to improve the predictability and interpretation of human antibody landscapes following exposure [429]. The mechanisms considered here included exposure type-specific homologous and cross-reactive antibody kinetics, the role of priming on subsequent vaccination, titre-dependent

antibody boosting (or titre ceiling effects) and reduced antibody boosting with each subsequent exposure (antigenic seniority). By fitting models with various combinations of these mechanisms to haemagglutination inhibition (HI) titre data from ferrets, the aim was to identify immunological mechanisms that are important in describing observed antibody profiles arising from multiple exposures.

4.2 Models of antibody kinetics

I defined a deterministic mathematical model describing the kinetics of homologous and heterologous antibody titres following exposure. Fig 4.1 depicts the example of an individual becoming infected and later vaccinated, though the model may characterise any sequence of exposures. Conceptually similar mathematical models of boosting followed by biphasic waning have been used previously to describe antibody secreting cell (ASC) and antibody kinetics [222, 228, 231, 430]. I then built on this base model to incorporate additional immunological mechanisms that are important in describing antibody boosting and waning. These included: biphasic or monophasic antibody waning; exposure-type specific or type non-specific cross-reactivity [431]; antigenic seniority resulting from suppression [92]; the impact of priming infection on subsequent vaccine response [432, 433]; and titre-dependent boosting [434]. I considered models with different numbers of exposure types to match the experimental design: either 3 (infection, trivalent inactivated influenza vaccine (TIV), TIV + adjuvant) or 6 (priming infection, secondary infection, initial TIV, secondary TIV, initial TIV + adjuvant, secondary TIV + adjuvant) [435–437]. The base boosting and waning model remains the same across model variants, but these mechanisms add complexity to the boosting parameter, μ , and link different exposures with common parameters.

4.2.1 Base model

Equation 4.1 describes a simplified version of the model, where antibody levels to a single strain are described over time following a single exposure, i , to influenza antigens.

$$f(\theta_i, t) = \left\{ \begin{array}{ll} y_i^0 & t \leq \xi_i \\ \frac{\mu_i}{t_{pi}} t - \frac{\mu_i}{t_{pi}} \xi_i + y_i^0 & \xi_i < t \leq \xi_i + t_{pi} \\ -\frac{d_i \mu_i}{t_{si}} t + \frac{d_i \mu_i}{t_{si}} (\xi_i + t_{pi}) + \mu_i + y_i^0 & \xi_i + t_{pi} < t \leq \xi_i + t_{pi} + t_{si} \\ -m_i t + m_i (\xi_i + t_{pi} + t_{si}) + (1 - d_i) \mu_i + y_i^0 & \xi_i + t_{pi} + t_{si} < t \leq \xi_{(i+1)} \end{array} \right\} \quad (4.1)$$

where:

$$\theta_i = \{\mu_i, d_i, t_{pi}, t_{si}, m_i, \xi_i\} \quad (4.2)$$

Prior to any form of exposure, the initial antibody titre, y_i^0 , is undetectable (log titre of 0). After an

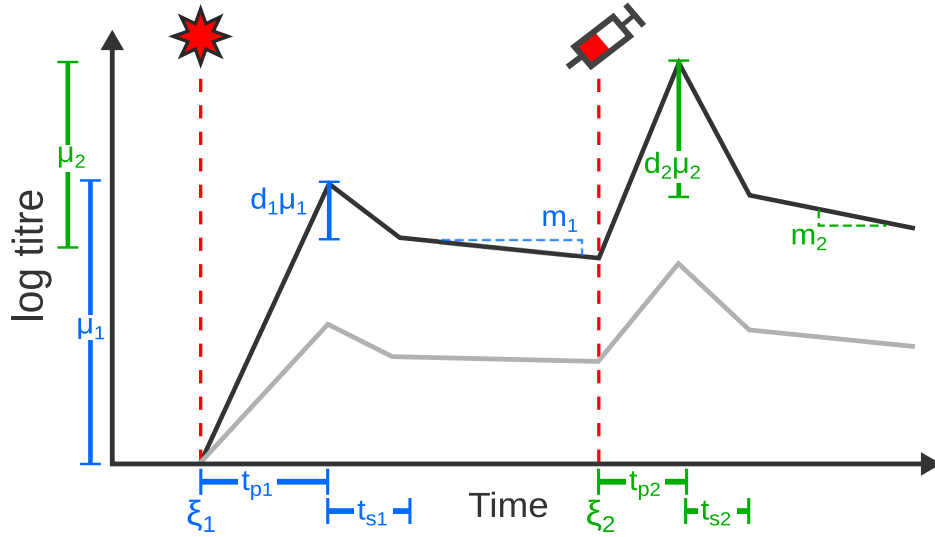


Figure 4.1: Base model. Schematic showing the relationship between model parameters and antibody kinetics over time. Black line shows antibody titres effective against one immunogen. Grey line highlights how antibody titres to a different influenza immunogen (that is less antigenically similar to the exposure immunogens than the black line) develop in parallel driven by cross-reactive antibodies. After each exposure, antibody levels undergo linear boosting on a log scale over a couple weeks, followed by an initial, short (about a week) waning phase and then a slower, long-term waning phase. This example demonstrates two exposures, initially with infection (star symbol) and subsequently with vaccine (syringe symbol), where antibody dynamics are governed by a set of parameters depending on the exposure type. Note that the y-axis is on a log scale and all observations are discrete and taken as the floor value.

infection event at time ξ_1 , homologous antibody titres undergo boosting rising linearly (on the log scale) to a peak of μ_1 after time t_p , ignoring any delay between exposure and the start of antibody production. Titres then quickly drop by a fixed proportion, d_1 , over time t_{s1} (in the timescale of a few days to weeks), representing the initial short-term waning phase as free antibodies and early short-lived ASCs begin to decay following clearance of the initial antigen dose [409]. Antibody waning then switches to a constant rate m_1 (log titre units lost per day) for the remainder of time (representing the population of persistent ASCs, lasting months to years) until subsequent vaccination (syringe at time ξ_2), when antibody dynamics become dominated by a new set of boosting and waning parameters. No third, steady state phase was included due to the short time frame of these experiments [438]. Antibodies effective against heterologous strains experience boosting and biphasic waning proportional to the antigenic distance between the measured and exposure strains.

The lower bound of detection of the HI assay (a log titre of 0) was assumed to be synonymous with a true absence of antibodies, such that model predicted titres could not wane below 0. Although antibodies may be detectable with a more sensitive assay, using a different lower bound for the true latent zero antibody titre would either require fixing the lower bound at an arbitrary value or estimation

as an unknown model parameter.

4.2.2 Interaction of multiple exposures

Each successive influenza exposure provides a dose of antigen that stimulates the adaptive immune response. An observed trajectory of antibodies over time is therefore the culmination of antibody responses generated from each exposure. Although there may be some interaction between overlapping exposures due to competition for T helper cell recruitment or interference in binding to antigen [185, 207], it was assumed here that all of the exposures were sufficiently far apart to be effectively independent [439, 440]. In the model, antibody kinetics follow one contiguous process, whereby the dynamics at a particular time are governed only by the parameters of the most recent exposure. In this sense, observed antibody titres are a direct reflection of independent antibody secreting cell (ASC) populations that undergo phases of boosting and waning depending on the time of the most recent exposure [438]. Each subsequent exposure supersedes the previous exposure such that:

$$y_A(t) = \left\{ \begin{array}{ll} f(\theta_1, t) & 0 \leq t < \xi_2 \\ f(\theta_2, t) & \xi_2 \leq t < \xi_3 \\ \vdots & \\ f(\theta_n, t) & \xi_n \leq t \end{array} \right\} \quad (4.3)$$

y_i^0 is defined as:

$$y_i^0 = \left\{ \begin{array}{ll} 0 & i = 1 \\ f(\theta_{i-1}, \xi_i) & i > 1 \end{array} \right\} \quad (4.4)$$

4.2.3 Additional model mechanisms

Additional structure was added to the model to describe biological mechanisms that may play a role in shaping an individual's antibody profile over multiple exposures. These model mechanisms are depicted in Fig 4.2 and are described below. In this section, I incrementally introduce each mechanism into the model, where the final expression shows the full model.

Biphasic and monophasic waning

Measurement of serum antibody titres after exposure are typically described as a biphasic waning process following peak titre, as short-lived ASCs (that account for the initial boost in titre) begin to

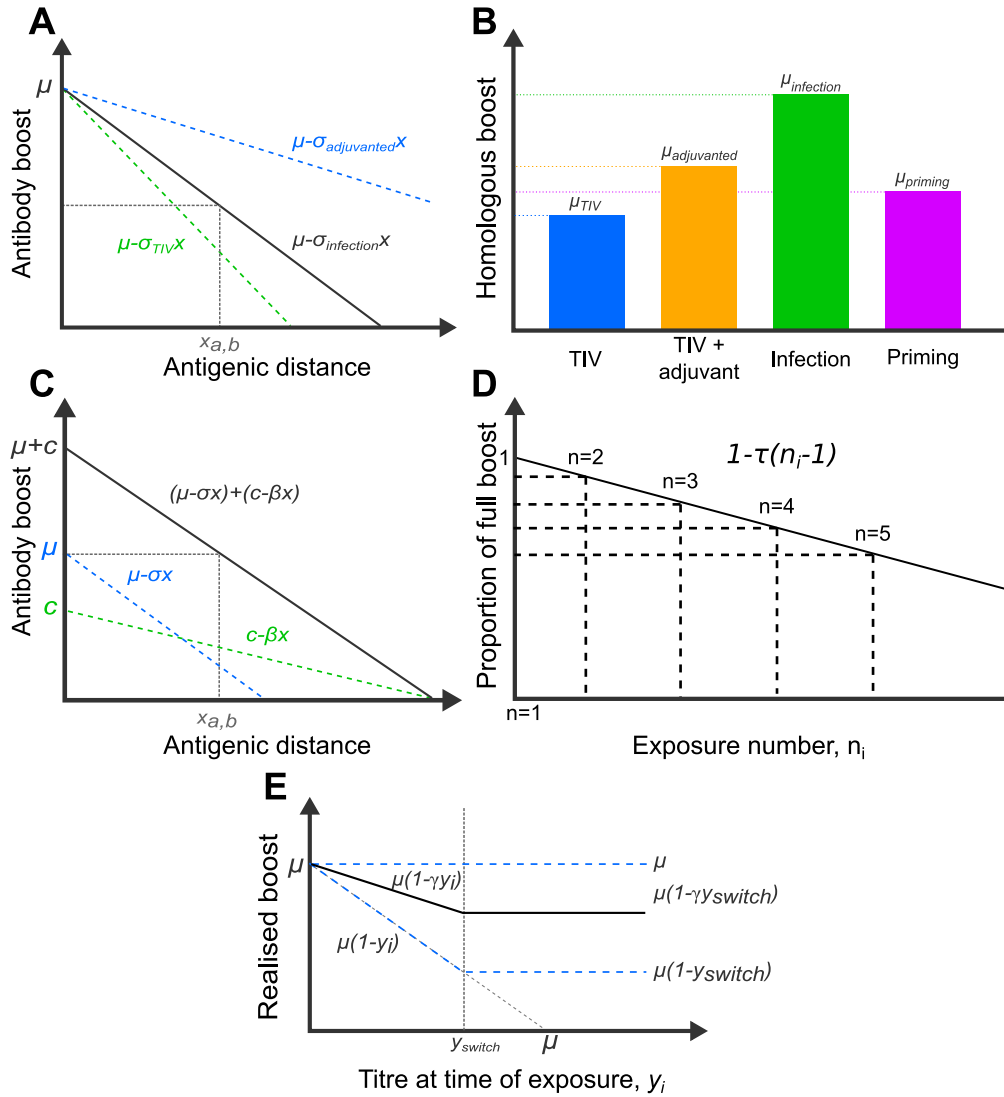


Figure 4.2: Summary of model mechanisms. (A) Cross reactive antibody boosting. The degree of boosting decreases as the antigenic distance between the exposure and measured strain increases. Different exposure types may have different gradients. (B) Illustrative example of exposure type specific parameter values. Level of homologous boosting may depend on the exposure type. Note that this may also apply to other parameters eg. waning rate. (C) Joint effect of exposure boosting and priming infection. Full boosting following a primed exposure is the sum of contributions of the exposure itself and the effect of priming. (D) Antigenic seniority mechanism. Amount of antibody boosting decreases linearly with the number of prior exposures. (E) Titre-dependent boosting. Amount of homologous boosting decreases as a function of titre at time of exposure. Solid black line shows example where $0 \leq \gamma \leq 1$. Blue dashed lines show boundary conditions. Note that the realized boost does not change when y_i is above y_{switch} .

die and are succeeded by more persistent antibody-secreting plasma cells [147]. This form of the model was chosen by comparison with simpler models with only a single waning phase (fixing $t_s = d = 0$), described below.

Cross reactive boosting

Assuming that infection of a naive ferret with strain A elicits a maximum antibody titre to strain A of μ log HI units, strain B has an antigenic distance from strain A of 1 if the antibody titre to strain B is $\mu - 1$ using sera from the same ferret. The parameter vector θ therefore captured the antigenic distance between the exposure strain and the measured strain (Fig 4.2A). The degree of boosting of antibodies effective against strain A following exposure with strain B, $\mu_{A,B}$, can be generalised as:

$$\mu_{A,B} = \mu' - \sigma x_{A,B} \quad (4.5)$$

where μ' is the level of homologous boosting following any exposure; σ is the cross reactivity gradient by which antibody effectiveness drops with increasing antigenic distance; and $x_{A,B}$ is the antigenic distance between strain A and strain B.

The cross reactivity gradient, σ , for initial infection was fixed at 1 in line with previous definitions of antigenic distance [82]. Estimating the cross reactivity gradient for other exposure types was therefore taken relative to this, such that a gradient of less than 1 suggests broader cross reactivity than priming infection, and greater than 1 suggests narrower cross reactivity. Antigenic distances were assumed to be fixed and known based on euclidean antigenic distance taken from previous data, and no cross reactivity in the HI assay was allowed between subtypes [164]. The antigenic distance between each pair based on previous data was: 6.23 between A/Panama/2007/1999 and A/Brisbane/10/2007; 6.12 between A/Panama/2007/1999 and A/Wisconsin/67/2005; 0.70 between A/Brisbane/10/2007 and A/Wisconsin/67/2005; and 1.55 between A/Fukushima/141/2006 and A/Solomon Islands/3/2006 [84].

Exposure types and type-specific parameters

Different influenza exposure types have been shown to generate different antibody boosting and persistence [437, 441–444]. Exposure-specific boosting and waning parameters were inferred for each of the exposure types in the protocol. In each model variant, either 3 (infection, vaccination and adjuvanted vaccination) or 6 distinct exposure types (one for each unique exposure formulation: primary infection; initial TIV; secondary TIV; initial adjuvanted TIV; secondary adjuvanted TIV; secondary

infection) were considered. Exposure type, l , was defined as:

$$l \in \{\text{infection, TIV, TIV + adjuvant}\} \quad (4.6)$$

or

$$l \in \{\text{infection}_1, \text{infection}_2, \text{TIV}_1, \text{TIV}_2, \\ \text{TIV}_1 + \text{adjuvant}, \text{TIV}_2 + \text{adjuvant}\} \quad (4.7)$$

Note that as the number of exposure events in the experiments was far greater than the number of different exposure types, this grouping into exposures types substantially reduces the number of parameters in the model (Fig 4.2B). Two scenarios were tested for the cross-reactivity gradient: cross reactivity was either conditional on exposure type, l , (ie. σ_l), or universal across all exposure types (ie. one value of σ for all exposures).

The effect of priming

Priming by infection has been shown to elicit both an increased magnitude and breadth of antibody boosting following subsequent vaccination [432,433]. The boosting parameter μ was modified to consider additional antibody boosting following vaccination if the ferret had previously received a priming infection. Vaccination following priming infection elicited an additional degree of homologous boosting as well as cross-reactive boosting with a different cross-reactivity gradient compared to un-primed vaccination. The term for antibody boosting including priming was defined as:

$$\mu_{A,B} = (\mu' - \sigma x_{A,B}) + \alpha(c - \beta x_{A,B}) \quad (4.8)$$

where c is the magnitude of additional homologous boosting due to priming; β is the gradient of cross reactivity related to priming infection (similar to σ); and α is either 1 or 0 depending on if priming had or had not occurred (Fig 4.2C).

Antigenic seniority

The number of previous exposures has been shown to impact antibody responses following exposure [92, 445]. Evidence for antigenic seniority suggests that the antibody response to each new exposure is smaller than the response from the previous exposure. This mechanism was included in the model by

modifying the boosting parameter, μ , to be conditional on the number of previous exposures (Fig 4.2D):

$$\mu_{A,B,n} = (1 - \tau(n - 1))((\mu' - \sigma x_{A,B}) + \alpha(c - \beta x_{A,B})) \quad (4.9)$$

where τ gives the proportion of the full boost lost relative to the first exposure ($0 \leq \tau \leq 1$) as a function of the number of previous exposures, $n - 1$. In these experiments, ferrets may have experienced up to 4 exposures.

Titre-dependent boosting

Ceiling effects have been observed in influenza antibody responses previously, where individuals already at a high antibody titre exhibit lower levels of boosting compared to naive individuals [434,446]. To capture this effect, the boosting parameter was modified as:

$$\mu_{A,B,n,y_i(\xi)} = \begin{cases} \mu_{A,B,n}(1 - \gamma y_i(\xi)) & y_i(\xi) < y_{switch} \\ \mu_{A,B,n}(1 - \gamma y_{switch}) & y_i(\xi) \geq y_{switch} \end{cases} \quad (4.10)$$

where $y_i(\xi)$ indicates the antibody titre against strain i at the time of exposure, ξ ; γ is the degree by which antibody boosting drops with preexisting antibody titre; and y_{switch} is a threshold on $y_i(\xi)$ above which antibody boosting remains unchanged (Fig 4.2E). γ was bounded between 0 and 1, where 0 implies no titre dependence and 1 implies strong titre-dependent suppression of boosting.

4.2.4 Full model

Incorporating all of the mechanisms above gives the full set of model parameters for a given exposure:

$$\theta_{i,A,B,l} = \{\mu'_l, d_l, t_{pl}, t_{sl}, m_l, \xi_i, x_{A,B}, \sigma_l, \alpha_i, c, \beta, \gamma, y_{switch}, \tau\} \quad (4.11)$$

The realized level of antibody boosting to a particular strain is given by:

$$\mu_{i,A,B,n,l,y_i(\xi)} = (1 - \gamma y_i(\xi))(1 - \tau(n - 1))((\mu' - \sigma x_{A,B}) + \alpha(c - \beta x_{A,B})) \quad (4.12)$$

where i is the exposure index (specifying only exposure time and whether the exposure was primed); A is the measured strain; B is the exposure strain; $n - 1$ is the number of previous exposures; and l is the type of exposure. All model parameters are described in Table 4.1. Note that the subscript for each parameter is crucial in highlighting where parameters are shared between different exposure events.

Table 4.1: Description of model parameters. Summary of parameter definitions and bounds. All bounds relate to lower and upper bounds of the uniform prior distribution used during model fitting.

Parameter Name	Definition	Lower bound	Upper bound	Notes
μ	Homologous boost on log scale	0	15	
t_p	Time from exposure to peak titre (days)	12	12	Fixed at 12 days
d	Proportion of boost waned in initial waning	0	1	Fixed at 0 for monophasic waning
t_s	Duration of initial waning phase	0	30	Fixed at 0 for monophasic waning
m	Long term titre waning rate (log units per day)	0	12	
σ	Cross reactivity gradient	0	100	A value above 5 would give a cross reactive boost of <0.1 log units at an antigenic distance of 0.5
τ	Antigenic seniority modifier	0	1	Antigenic seniority modifier given by $(1 - \tau(n-1))$ where $n-1$ is the number of previous exposures
γ	Titre dependence gradient	0	1	0 gives no titre dependence. 1 gives strong titre-dependent suppression of antibody boosting from higher titres
y_{switch}	Maximum titre at which titre-dependent boosting is in effect	0	12	Initial titres above this have the same titre dependence as a titre of y_{switch}
β	Priming cross reactivity gradient	0	100	A value above 5 would give a cross reactive boost of <0.1 log units at an antigenic distance of 0.5
c	Additional boost due to priming	0	15	Fixed at 0 to remove priming
ϵ	Standard deviation of truncated normal distribution	0	10	

It is possible to include or exclude all of these mechanisms by fixing or removing certain parameters: setting $t_s = d = 0$ incorporates monophasic waning, biphasic waning otherwise; cross reactivity may be either universal or type specific; typing may include either 3 or 6 distinct exposure types; fixing τ to 0 removes antigenic seniority; titre-dependent boosting can be removed by setting $\gamma = 0$; and fixing c to 0 removes priming. The result is 6 mechanisms each with 2 settings, giving 64 different model combinations to be fitted (Table 4.2). For convenience, a model key is derived from the first letter of each option. For example, the model with priming, type-specific cross reactivity, no antigenic seniority, 3 exposure types, biphasic waning and titre-dependent boosting has the key “YTN3BY”.

Table 4.2: Description of model mechanisms and their potential formats.

Mechanism	Option 1	Option 2
Priming	Y es	N o
Cross reactivity	T ype specific	A ll
Antigenic seniority	Y es	N o
Typed exposures	3 types	6 types
Waning	B iphasic	M onophasic
Titre-dependent boosting	Y es	N o

4.3 Materials & Methods

4.3.1 Study Data

Antibody titre data from a previously published ferret study were provided by Karen Laurie, now at Seqirus, Victoria, Australia. This study was originally designed to reflect different possible human infection and vaccination histories at the time of the 2009 pandemic [420]. Five experimental groups each of three ferrets underwent different combinations of infection with seasonal influenza A and/or vaccination with Northern and Southern Hemisphere TIV, with or without Freund's incomplete adjuvant (IFA), over the course of 70 days (Table 4.3). Serum samples were collected at days 0, 21, 37, 49 and 70 from all ferrets (Fig 4.3). HI titres were used to determine antibody titres to each infection and TIV strain. Dilution plates with 12 wells were used, such that the highest possible recorded dilution was 1:40960, and the lowest detectable titre was 1:20. Undetectable titres were recorded as $<1:20$. All analyses here were carried out using log titres, defined as $k = \log_2(\frac{D}{10})$, where D was the recorded dilution. Observed log titres were therefore assigned values between 0 and 12, where $<1:20 = 0$, $1:20 = 1$ and $\geq 1:40960 = 12$.

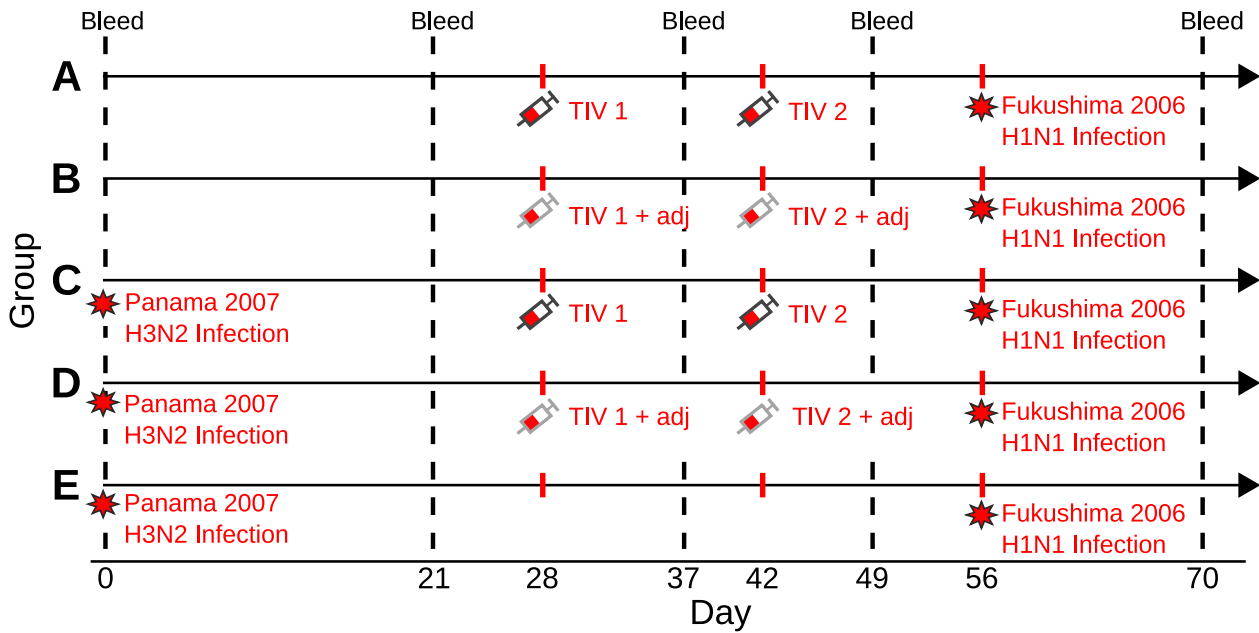


Figure 4.3: Summary of experimental protocol. Days since first event are shown on the x-axis, with the 5 groups shown as rows. Red stars represent infection with either A/Panama/2007/1999 (H3N2) or A/Fukushima/141/2006 (H1N1). Red syringes represent vaccination with either Southern Hemisphere TIV 2008 (TIV 1) or Northern Hemisphere TIV 2007/2008 (TIV 2) with (grey border) or without (black border) adjuvant. Vertical, dashed black lines represent times of blood sample collection, providing HI titres against each of the vaccination and infection strains at that time point.

Full adult doses of human TIV were used in groups A, B, C and D. The first vaccination (Southern

Hemisphere 2008 TIV) contained A/Solomon Islands/3/2006 (H1N1), A/Brisbane/10/2007 (H3N2) and B/Brisbane/3/2007, administered at day 28 (TIV 1). The second vaccination (Northern Hemisphere 2007/2008 TIV) contained A/Solomon Islands/3/2006 (H1N1), A/Wisconsin/67/2005 (H3N2) and B/Malaysia/2506/2004, administered at day 42 (TIV 2). Vaccines used in groups B and D were emulsified in an equal volume of IFA immediately before administration (TIV 1/2 + adjuvant). All vaccines contained $15\mu\text{g}$ of HA of each strain, and were delivered to sedated animals intramuscularly in the quadriceps muscles of both hind legs. Infections were carried out by dropwise intranasal challenges with $10^{3.5}$ 50% tissue culture infectious doses (TCID₅₀) in 0.5 mL with A/Panama/2007/1999 (H3N2) in groups C, D and E, and with A/Fukushima/141/2006 (H1N1) in all groups.

Table 4.3: Description of experimental protocol

Group	Infection with A/Panama/2007/99 (H3N2)	Immunisation with S.H TIV 2008*	Immunisation with N.H TIV 2007/2008**	Infection with A/Fukushima/141/06 (H1N1)	Number of ferrets
Group A	No	Yes (no adjuvant)	Yes (no adjuvant)	Yes	3
Group B	No	Yes (with adjuvant)	Yes (with adjuvant)	Yes	3
Group C	Yes	Yes (no adjuvant)	Yes (no adjuvant)	Yes	3
Group D	Yes	Yes (with adjuvant)	Yes (with adjuvant)	Yes	3
Group E	Yes	No	No	Yes	3

*Southern Hemisphere (S.H.) TIV 2008: A/Solomon Islands/3/2006 (H1N1), A/Brisbane/10/2007 (H3N2), B/Brisbane/3/2007

**Northern Hemisphere (N.H.) TIV 2007/2008: A/Solomon Islands/3/2006 (H1N1), A/Wisconsin/67/2005 (H3N2), B/Malaysia/2506/2004

4.3.2 Model fitting

The model predicts log antibody titres as latent states on a continuous scale for a given set of parameter values. Observed antibody titres were assumed to follow a truncated normal distribution with mean given by the model predicted latent titre. HI titres were taken as the highest 2-fold dilution of sera at which haemagglutination was inhibited, and the observed value was therefore discrete and observed as the lower integer bound (ie. a true log titre of 5.5 would be observed as 5). Furthermore, the limits of the assay meant that only log values between 0 and 12 could be observed. The likelihood of observing a titre, x , given a true titre, y , is given by:

$$L(x|y, \epsilon) = \begin{cases} \int_{-\infty}^1 g(s)ds & \text{if } x = 0 ; \\ \int_x^{x+1} g(s)ds & \text{if } x \in \{1, 11\} ; \\ \int_{12}^{\infty} g(s)ds & \text{if } x = 12 . \end{cases} \quad (4.13)$$

where $g(s) = \frac{1}{\sqrt{2\pi\epsilon}} e^{-\frac{(s-y)^2}{2\epsilon}}$ is the probability density function of the normal distribution with mean y and standard deviation ϵ . This measurement model gives an observation error matrix for the probability

of an observed log HI titre x given a true, underlying log titre y , as shown in Fig 4.4.

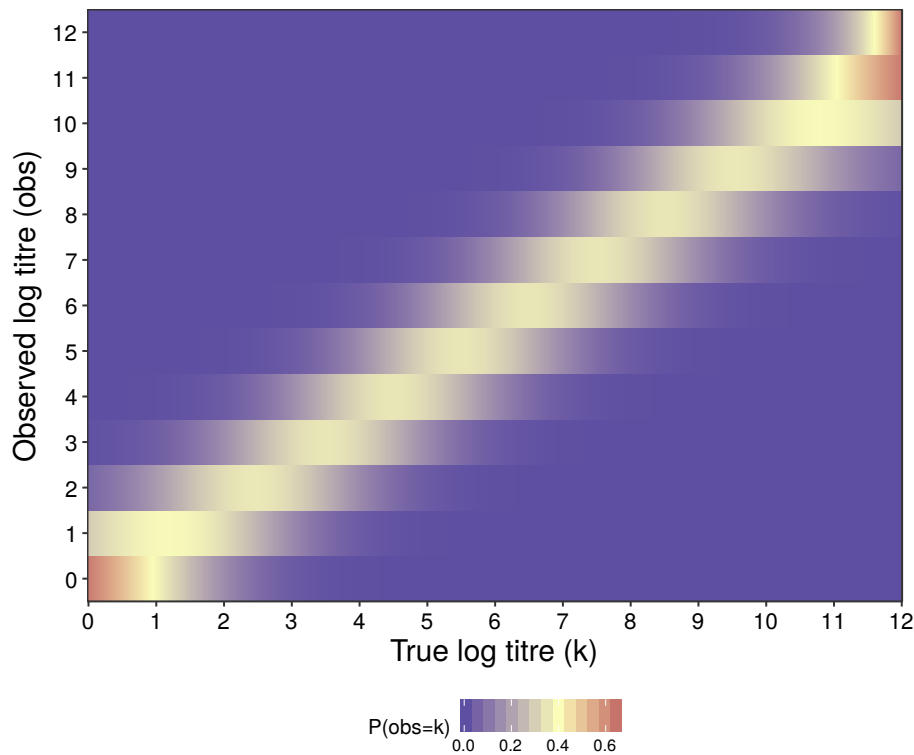


Figure 4.4: Observation error matrix. Probability of observing a particular log titre given an underlying true, latent titre. Note that the true titre is a continuous value, whereas observations are discrete. Furthermore, truncation of the distribution at the upper and lower limit of the assay results in an asymmetrical distribution when the true value is at either of these limits. True values outside of these limits will be observed as a value within the assay limits.

Each of the 64 possible model variants were fit to the HI data using parallel-tempering Markov chain Monte Carlo (PT-MCMC) to achieve an effective sample size (ESS) of at least 200 for all parameters. The models ranged from the most biologically simple with 8 free parameters (monophasic waning; type non-specific cross reactivity; no antigenic seniority, priming effect or titre-dependent boosting; and 3 distinct exposure types) to the most complex with 35 free parameters (biphasic waning; type-specific cross-reactivity; antigenic seniority; titre-dependent boosting; priming; and 6 distinct exposure types).

Uniform priors were assigned to all free model parameters, with upper and lower bounds shown in the Table 4.1. The time to peak titre parameter, t_p , was fixed at 12 days, as estimating this additional parameter allowed the model too much flexibility given the amount of data (though a sensitivity analysis varying t_p was performed to ensure that this assumption did not bias the results). Parameter estimates were obtained for each set of μ , d , t_s and m , as well as σ , β , c , ρ , γ and y_{switch} . For each model variant, 3 MCMC chains were run for 5000000 iterations, and convergence was assessed visually

and based on Gelman-Rubin convergence diagnostics (potential scale reduction factor, PSRF) with the `coda` R package [383]. Where the ESS was < 200 or the upper 95% confidence interval on the PSRF was > 1.1 for any parameter, 5 chains were re-run for 10000000 iterations and with a greater number of temperatures to improve convergence. 1 of the 1296 estimated parameters still had an upper 95% confidence interval for $\hat{R} = 1.103$ and another had an ESS of 197 (only 5 other parameters had an ESS < 400). All analyses were performed with R and C++.

4.3.3 Model comparison

To validate the base model before incorporating additional complexity arising from multiple exposures, I first fit the model for a single exposure to a single virus from group E, where 3 ferrets were only infected with a single A/H3N2 virus (A/Panama/2007/99 (H3N2)). I compared four variants of this base model: (i) boosting followed by no antibody waning ($t_s = d = m = 0$); (ii) boosting followed by monophasic waning ($t_s = d = 0$); (iii) boosting followed by biphasic waning with no longer term waning ($m = 0$); and (iv) boosting followed by biphasic waning (all model parameters free).

A full model comparison analysis was carried out on the 64 main model variants to (i) justify the inclusion of more complex mechanisms in the model and (ii) check for consistency of parameter estimates between model variants. Models were compared based on their expected log pointwise predictive density (ELPD), estimated using Pareto-smoothed importance sampling leave-one-out cross-validation (PSIS-LOO) with the `loo` R package, and the Widely Applicable Information Criterion (WAIC). PSIS-LOO and WAIC serve a similar purpose to the Akaike Information Criterion (AIC) but are more suitable in a Bayesian setting [447–449]. Mechanisms that were more common amongst higher ranked models are better supported by the data, whereas mechanisms that are absent do not provide sufficient additional explanatory power to justify their added complexity to the model. The aim was not to maximize predictive performance but rather to quantify mechanisms of immunological interest and justify interpretation of estimated parameter values. I therefore did not perform a full Bayesian model averaging analysis or formal variable selection, though I did perform Pseudo-Bayesian model averaging (Pseudo-BMA+) to estimate the relative weights of each model variant and therefore each mechanism.

When using PSIS-LOO, the `loo` package calculates the shape parameter, $\hat{k}$, for each data point for each fitted model. For many of the model variants, $\hat{k}$ estimates were > 0.7 for a small number of data points (1%), suggesting that the PSIS-LOO estimated ELPD estimates were not reliable for these fits. Visual inspection suggested that problematic data points were outliers that were not adequately

described by the models or were highly influential. I refit each model excluding each data point with $\hat{k} > 0.7$ (leave one out cross-validation) to directly calculate the ELPD for these data points, and updated the model ELPD estimates accordingly.

4.3.4 Simulation recovery experiments

I performed simulation recovery experiments for the base model and all 13 model variants with a $\delta\text{ELPD} < 20$ to test the accuracy of estimating known parameter values. For the base model, data were simulated that matched the experimental protocol for group E (3 individuals, infection on day 0, HI titres measured at days 0, 21, 37, 49 and 70) and also with increased sampling frequency (daily and weekly HI titres). Group E was chosen as it represents single exposure to a single immunogen. True parameter values used were: $\mu = 10.0$, $d = 0.5$, $t_s = 19$, and $m = 0.04$. For the main model variants, I took the maximum likelihood parameter values from the real model fits and used these to simulate data under each of these 13 models that matched data from the real experimental protocol (5 groups of 3 ferrets, 5 tested strains, 5 blood samples taken). Parameter re-estimation was considered accurate when the 95% or 99% credible intervals (CI) of the inferred posterior distribution encompassed the true parameter value. Simulation-recovery results are shown in Appendix B.

4.4 Results

4.4.1 Antibody kinetics following a single exposure support biphasic waning

To validate the boosting and biphasic waning model for a single exposure, the base model was fit to HI titres against A/Panama/2007/1999 (H3N2) from group E alone (Fig 4.5). Ignoring the later exposure to A/Fukushima/141/06 (H1N1) at day 56, from which no cross-subtypic antibody reactivity is expected, these data in isolation reflect a typical antibody trajectory following exposure to a single immunogen and measurement of antibodies against it [222,408]. The models with biphasic waning (both with estimated long term waning rate, m , and fixed $m = 0$) were better supported than the models with monophasic waning or no waning (ELPD -20.6 (standard error (SE), 3.35) and -20.5 (SE, 3.78) compared to -23.4 (SE, 3.71) and -28.1 (SE, 3.48) respectively), although these differences are small with respect to the standard error of the ELPD estimates. The biphasic waning models with estimated long-term waning m and fixed long-term waning $m = 0$ had a difference in ELPD of <1 , suggesting that both models had similar predictive performance. Overall, these results suggest that the model with monophasic waning is justified over the version with no waning, and that the biphasic waning model is better still than the monophasic waning model. Posterior estimates for model parameters were: $\mu = 9.91$ (posterior median, 95% CI 7.08-12.7); $d = 0.551$ (posterior median, 95% CI 0.183-0.695); $t_s = 19.5$ days (posterior median, 95% CI 6.39-27.4 days); $m = 0.0414$ (posterior median, 95% CI 0.00405-0.103).

4.4.2 Variation in antibody kinetics driven by different exposure histories

Overall, ferrets that received more frequent and immunogenic exposures achieved the highest, most broadly reactive and long-lived antibody titres. The full data show substantial variation in observed antibody titres across the groups driven by different exposure types and combinations. Following two doses of unadjuvanted TIV, ferrets achieved only modest increases in titres against the vaccine strains (Fig 4.6A), with 2 out of 3 ferrets failing to generate H3N2 titres that persisted past day 37. The addition of an adjuvant resulted in increased and persistent titres against the vaccine strains in all ferrets by day 49. Titres against A/Fukushima/141/2006 (H1N1), which is antigenically similar to A/Solomon Islands/3/2006 (H1N1), were also increased at this time point (Fig 4.6B). Similarly, priming infection resulted in higher and long-lived titres to the vaccine strains and A/Fukushima/141/2006 (H1N1) relative to ferrets in the unprimed, unadjuvanted TIV protocol (Fig 4.6C). Observed titres at day 21 against A/Panama/2007/1999 (H3N2) were consistently high following priming infection in

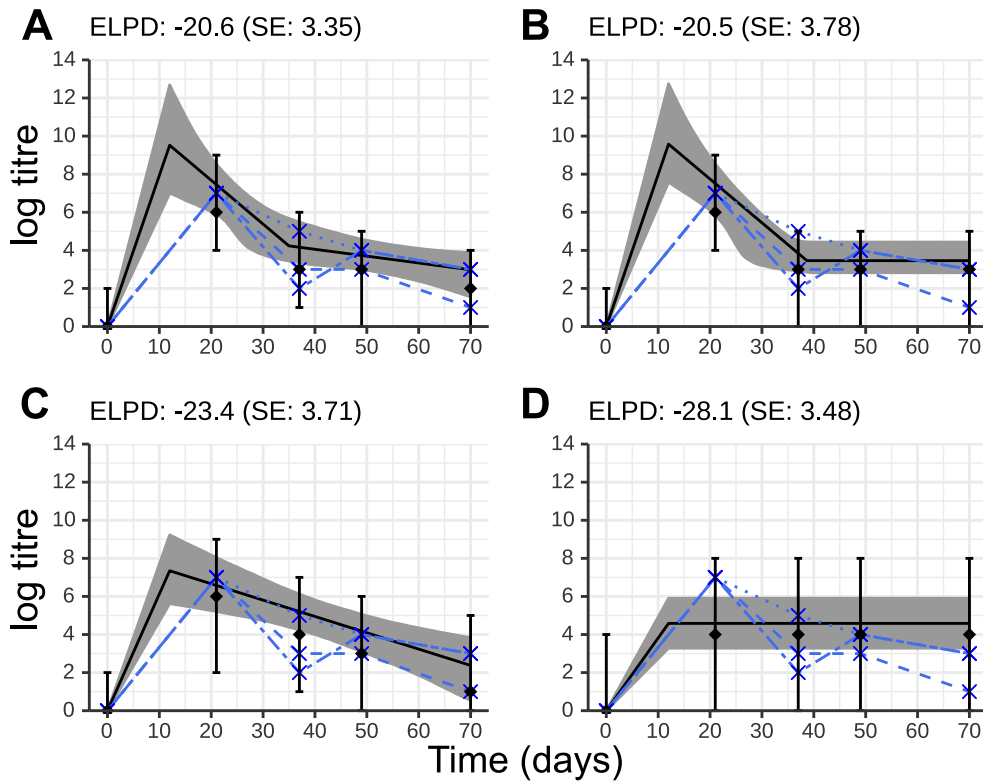


Figure 4.5: Comparison of four base model fits to data from three ferrets following exposure to a single immunogen. All ferrets were infected with A/Panama/2007/99 (H3N2) at day 0. Y-axis shows log HI titre against A/Panama/2007/99 (H3N2). Note that ferrets were also infected with A/Fukushima/141/06 (H1N1) at day 56, but no cross-subtypic antibody reactivity is expected. Solid black line and grey region show best-fit model trajectory and 95% credible intervals (CI) of latent antibody titres. Black diamond and error bars show posterior median and 95% CI of model predicted observations. Blue crosses and dashed lines show observed log HI titre for the three individual ferrets. Subplot titles show estimated expected log-predictive density (ELPD) and corresponding standard error (SE). (A) Biphasic waning. (B) Biphasic waning with a fixed long-term waning rate of $m = 0$. (C) Monophasic waning with $t_s = d = 0$. (D) No short or long term waning with $m = t_s = d = 0$.

groups C-E, with one ferret in each of groups C and E also experiencing some boosting of antibodies against the other H3N2 strains. All ferrets were infected with A/Fukushima/141/2006 (H1N1) at day 56, leading to elevated titres to both H1N1 strains by day 70 in all ferrets.

4.4.3 Model comparison results

The top two model variants had ELPD estimates of -412.1 (SE, 21.4) and -412.6 (SE, 20.7) respectively. Both of these models included: a role for priming infection in increasing subsequent vaccine response; different boosting profiles between vaccination and infection; different boosting profiles with adjuvant versus without adjuvant; and biphasic antibody waning. The model with the lowest ELPD (model ID 21, key NAY6BY, Table 4.4) had 30 free parameters and also included titre-dependent boosting, no antigenic seniority, and no exposure type-specific cross reactivity. The other model (model ID 62, key

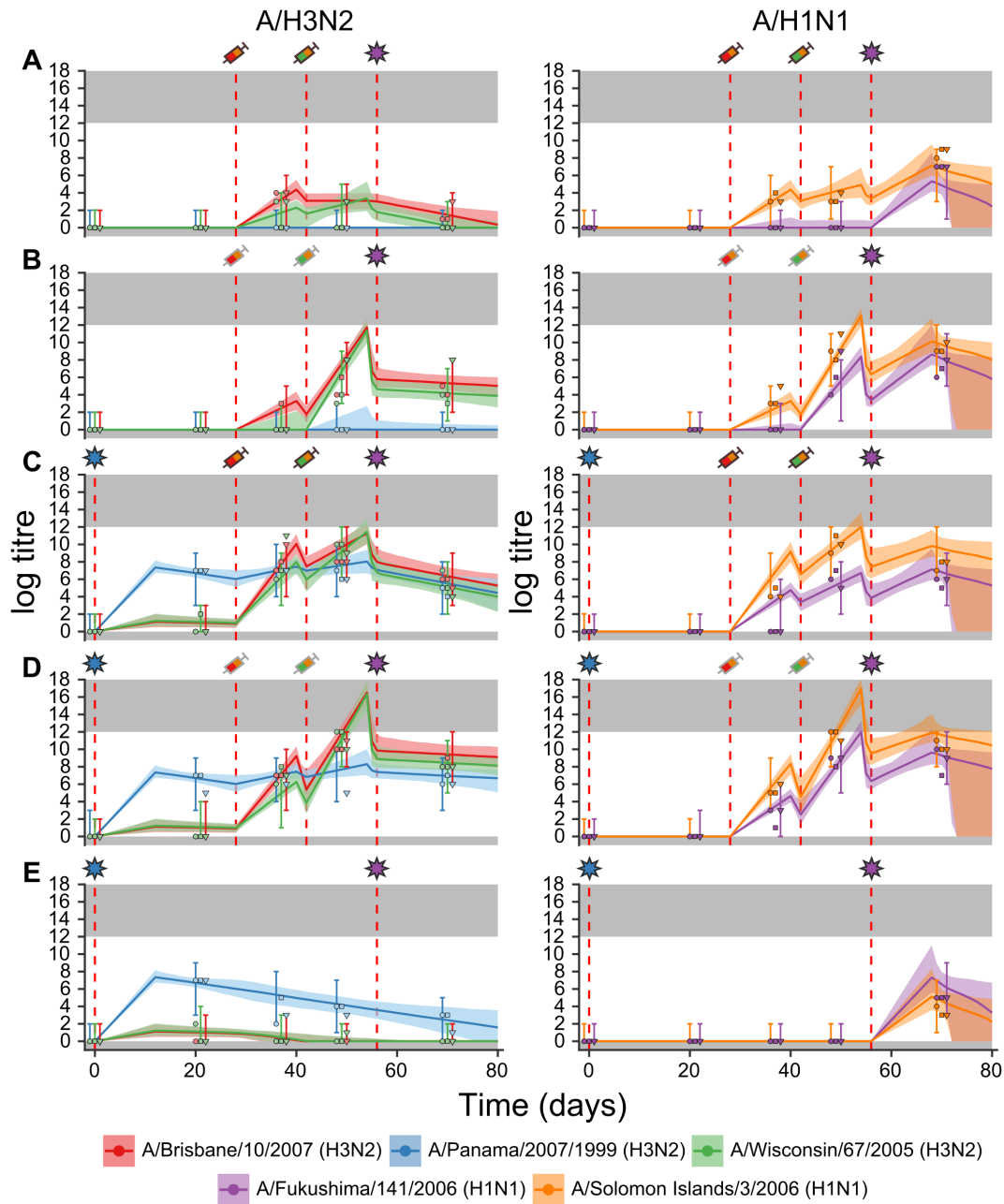


Figure 4.6: Model trajectory fits. Coloured lines show the posterior median latent antibody trajectories following exposure. Coloured shaded regions show 95% credible intervals of the model fit. Coloured points show observed discrete log antibody titres by HI assay for each of the three individual ferrets in each group. Gray shaded regions show the upper and lower limits of detection in the assay. For the same ferrets, titres to A/H3N2 strains are shown in the left column and A/H1N1 strains in the right column. Red dashed lines show times of exposures. Groups A-E correspond to descriptions in Fig 4.3. Exposure events are as described in Table 4.3. Symbols above each subplot: star represents infection; syringe represents TIV; and syringe with gray border represents TIV + adjuvant. Symbols are coloured based on their formulation. TIV 1 contained the following influenza A strains: A/Solomon Islands/3/2006 (H1N1) and A/Brisbane/10/2007 (H3N2). TIV 2 contained: A/Solomon Islands/3/2006 (H1N1) and A/Wisconsin/67/2005 (H3N2).

YTY6BN, Table 4.4) had 33 free parameters and did not include titre-dependent boosting, but did include antigenic seniority and exposure type-specific cross reactivity. Fig 4.6 shows the latter (more complex) model variant fitted to the data. Parameter estimates for model ID 62, key YTY6BN, are shown in Table 4.5 and for model ID 21, key NAY6BY in Table 4.6. The remainder of the results refer to model ID 62.

Overall, ELPD estimates ranged from -412.1 (SE, 21.4) in the highest ranked model (model ID 21, key NAY6BY) to -543.6 (SE, 21.8) in the lowest ranked model (model ID 12, key NTN3MN) (Table 4.5). The simplest model (model ID 11, key NAN3MN) with 8 free parameters was the third lowest ranked model (ELPD -539.0 (SE, 21.9)), whereas the most complex model with 35 free parameters was the 7th highest ranked model (model ID 54, key YTY6BY) (ELPD -417.0 (SE, 21.2)). Some of the simpler model variants may have similar predictive performance to the best fitting model and may therefore be more suitable in a general predictive application. For example, further constraining the antibody trajectories in Fig 4.6E from day 56 is possible by assuming shared kinetics parameters for the A/Panama/2007/1999 (H3N2) and A/Fukushima/141/2006 (H1N1) infections, as we would expect these trajectories to be similar given that they are both primary exposures to that subtype. One of the fitted model variants (model ID 64, key YTY3BN, Table 4.4) that was identical to the one in Fig 4.6 but assumed 3 rather than 6 distinct exposure types produced tighter 95% CIs post A/Fukushima/141/2006 (H1N1) infection (Fig B.1, Appendix B). However, this model variant is less well supported based on the model comparison analysis (ELPD -441.7 (SE, 20.3), δ ELPD=29.6) and provided estimates of post-infection waning that were almost identical to those for A/Panama/2007/1999 (H3N2) infection in the 6 exposure type model; overall, suggesting that the A/Fukushima/141/2006 (H1N1) data did not contribute to the posterior estimates (model ID 64, key YTY3BN, Table 4.5).

Pseudo-BMA+ was performed as a crude measure of mechanism importance. This estimates the relative weights of each model variant and thereby weights of models with a particular mechanism relative to models without [450]. Although comparison of variable importance using information criteria must be interpreted with caution (for example, changing the sample size or experimental protocol may change the results), Pseudo-BMA+ serves as a rough estimate of which mechanisms are most important in explaining these data [451]. Variable weights were: 1.00 for the presence of priming; 0.999 for the presence of 6 exposure types; 0.836 for the presence of biphasic waning; 0.579 for the presence of titre-dependent boosting; 0.572 for the presence of type specific cross reactivity; and 0.406 for the presence of antigenic seniority. The top two models had Pseudo-BMA+ weights of

Table 4.4: Description of models with $\delta\text{ELPD} < 20$. Table is ranked by ELPD score, such that the model best supported by ELPD (lowest) is at the top.

Run Identifier	Antigenic Seniority	Cross Reactivity	Priming	Typed exposures	Waning	Titre-dependent boosting	δELPD	ELPD error	standard
NAY6BY	Absent	Universal	Present	6 types	Biphasic	Present	0.000	21.373	
YTY6BN	Present	Type specific	Present	6 types	Biphasic	Absent	0.421	20.656	
YAY6BY	Present	Universal	Present	6 types	Biphasic	Present	2.059	21.279	
NTTY6BY	Absent	Type specific	Present	6 types	Biphasic	Present	2.636	21.194	
NTTY6BN	Absent	Type specific	Present	6 types	Biphasic	Absent	4.060	20.599	
NTTY6MY	Absent	Type specific	Present	6 types	Monophasic	Present	4.625	20.938	
YTY6BY	Present	Type specific	Present	6 types	Biphasic	Present	4.830	21.172	
YAY6BN	Present	Universal	Present	6 types	Biphasic	Absent	5.185	21.146	
YTY6MY	Present	Type specific	Present	6 types	Monophasic	Present	5.807	20.889	
NAY6MY	Absent	Universal	Present	6 types	Monophasic	Present	7.781	21.135	
YAY6MY	Present	Universal	Present	6 types	Monophasic	Present	7.996	21.131	
NAY6BN	Absent	Universal	Present	6 types	Biphasic	Absent	10.530	21.036	
YAY6MN	Present	Universal	Present	6 types	Monophasic	Absent	17.607	20.841	

Table 4.5: Parameter estimates for model ID 62, key YTY6BN.

Parameter	Exposure type	Mean	Median	Mode	2.5% CI	97.5% CI
Homologous boosting						
μ	Infection 1	7.393	7.372	7.352	6.771	8.134
μ	Infection 2	9.524	9.253	8.726	6.775	13.897
μ	TIV 1	4.354	4.356	4.398	3.278	5.419
μ	TIV 1 + adjuvant	3.296	3.289	3.27	2.21	4.42
μ	TIV 2	2.437	2.326	2.199	0.219	5.425
μ	TIV 2 + adjuvant	14.294	14.464	14.868	12.685	14.978
c	Priming	7.306	7.278	7.144	5.854	8.917
Waning rates						
d	Infection 1	0.226	0.216	0.183	0.020	0.488
d	Infection 2	0.517	0.526	0.809	0.029	0.976
d	TIV 1	0.663	0.701	0.944	0.166	0.988
d	TIV 1 + adjuvant	0.751	0.777	0.954	0.370	0.990
d	TIV 2	0.859	0.882	0.970	0.590	0.995
d	TIV 2 + adjuvant	0.592	0.596	0.600	0.477	0.680
m	Infection 1	0.085	0.085	0.087	0.027	0.142
m	Infection 2	5.969	5.963	0.903	0.293	11.695
m	TIV 1	5.765	5.652	0.921	0.275	11.670
m	TIV 1 + adjuvant	5.894	5.883	0.645	0.217	11.705
m	TIV 2	0.164	0.110	0.104	0.035	0.226
m	0.035	0.026	0.015	0.001	0.103	0.104
Duration of initial waning phase (days)						
ts	Infection 1	19.641	18.875	16.521	10.999	29.260
ts	Infection 2	15.003	14.569	4.962	2.334	29.194
ts	TIV 1	5.605	4.806	4.629	1.226	17.371
ts	TIV 1 + adjuvant	4.002	3.283	2.898	1.963	12.521
ts	TIV 2	1.636	1.396	1.901	0.083	5.146
ts	TIV 2 + adjuvant	1.085	1.073	1.103	0.053	2.164
Antigenic seniority						
τ	All	0.213	0.213	0.213	0.134	0.300
Cross reactivity						
σ	Infection 2	1.777	1.705	1.642	0.338	3.628
σ	TIV 1	4.202	2.967	2.935	1.823	6.067
σ	TIV 1 + adjuvant	46.450	45.941	3.619	1.827	97.235
σ	TIV 2	51.218	51.190	85.220	4.634	97.468
σ	TIV 2 + adjuvant	2.393	2.311	2.188	1.662	3.512
β	Priming	0.907	0.882	0.859	0.531	1.491
Error distribution						
ϵ	All	1.442	1.437	1.422	1.291	1.620

Table 4.6: Parameter estimates for model ID 21, key NAY6BY.

Parameter name	Exposure Type	Mean	Median	Mode	2.5% CI	97.5% CI
Homologous boosting						
μ	Infection 1	9.463	9.207	8.984	7.183	13.779
μ	Infection 2	13.116	13.452	14.65	9.54	14.937
μ	TIV 1	3.619	3.617	3.64	2.765	4.479
μ	TIV 1 + adjuvant	3.165	3.173	3.23	2.106	4.202
μ	TIV 2	1.823	1.746	1.701	0.206	3.935
μ	TIV 2 + adjuvant	12.474	12.507	12.582	10.68	14.1
c	Priming	6.037	5.995	5.885	5.068	7.252
Waning rates						
d	Infection 1	0.391	0.416	0.462	0.037	0.66
d	Infection 2	0.308	0.313	0.337	0.067	0.508
d	TIV 1	0.539	0.554	0.855	0.038	0.979
d	TIV 1 + adjuvant	0.556	0.576	0.919	0.041	0.98
d	TIV 2	0.612	0.646	0.754	0.076	0.981
d	TIV 2 + adjuvant	0.789	0.812	0.96	0.435	0.991
m	Infection 1	0.074	0.076	0.09	0.008	0.139
m	Infection 2	4.706	3.811	1.825	0.999	11.362
m	TIV 1	5.93	5.912	1.248	0.307	11.683
m	TIV 1 + adjuvant	5.974	5.966	2.653	0.286	11.703
m	TIV 2	2.679	1.392	0.388	0.147	10.726
m	TIV 2 + adjuvant	5.432	5.292	0.378	0.077	11.664
Duration of initial waning phase (days)						
ts	Infection 1	18.635	20.822	26.548	1.426	29.545
ts	Infection 2	1.35	1.365	1.658	0.104	1.891
ts	TIV 1	15.155	14.531	9.287	1.92	29.13
ts	TIV 1 + adjuvant	14.404	13.384	5.879	2.709	29.1
ts	TIV 2	12.757	14.364	15.531	2.225	15.825
ts	TIV 2 + adjuvant	19.677	19.972	21.366	7.344	27.067
Titre-dependent boosting						
γ	All	0.09	0.09	0.089	0.079	0.102
y_{limit}	All	10.712	10.864	11.746	8.615	11.948
Cross reactivity						
σ	All	2.334	2.319	2.312	1.743	3.007
β	Priming	0.85	0.67	0.572	0.213	2.153
Error distribution						
ϵ	All	1.489	1.485	1.475	1.342	1.657

0.331 and 0.303, whereas the third model had a weight of 0.0977. The top two models included only titre-dependent boosting and antigenic seniority respectively, suggesting that inclusion of at least one of these mechanisms improved predictive performance. The consistency of parameter estimates across the best fitting model variants is demonstrated in Fig B.6-B.13 in Appendix B.

4.4.4 Comparison of homologous boosting by exposure type

The level of homologous boosting resulting from priming infection (Infection 1) and secondary infection (Infection 2) was similar, shown by similar estimates for μ from both infections (Fig 4.7A). Antibody titres fell only marginally following the initial waning phase ($\mu(1 - d)$, Fig 4.7B). The antibody waning rate was not identifiable for secondary infection due to the lack of observations following this exposure. There was evidence for only low levels of homologous antibody boosting following both initial and secondary doses of unadjuvanted TIV (TIV 1 and TIV 2) that quickly waned to near undetectable levels during the initial waning phase.

The addition of an adjuvant appeared to have no significant impact on the homologous antibody response to the first vaccine dose, but did improve the response to a second dose of vaccine (TIV 1 compared to TIV 1 + adjuvant and TIV 2 compared TIV 2 + adjuvant, Fig 4.7B). Titres against A/Brisbane/10/2007 (H3N2) and A/Solomon Islands/3/2006 (H1N1) were similar following the first unadjuvanted vaccine dose and the first adjuvanted vaccine dose (TIV 1 compared to TIV 1 + adjuvant, Fig 4.6A&B). However, the second adjuvanted TIV dose appeared to elicit a significant persistent boost to the vaccine strains, which resulted in peak titres near the limit of detection of this assay (TIV 2 compared to TIV 2 + adjuvant, Fig 4.6A&B).

4.4.5 Comparison of cross reactivity by exposure type

In models with type-specific cross-reactivity, there were differences in the breadth of cross reactivity elicited by the 6 exposure types shown in Fig 4.7. Secondary infection appeared to elicit a level of cross reactivity in line with that of the priming infection, whereas cross reactivity for both unadjuvanted and adjuvanted vaccination appeared to be narrower and only boosted antibodies that were effective against antigenically similar viruses (Fig 4.8). σ describes the degree by which antibody titre decreases as a function of antigenic distance, where higher values of σ suggest lower cross reactive breadth. When a single cross reactivity gradient was assumed for all exposure types (as in the highest ranked model), the cross reactivity gradient was estimated to be 2.33 (posterior median; 95% CI: 1.74–3.01), suggesting narrower cross reactivity than would be expected given the definition for cross reactivity

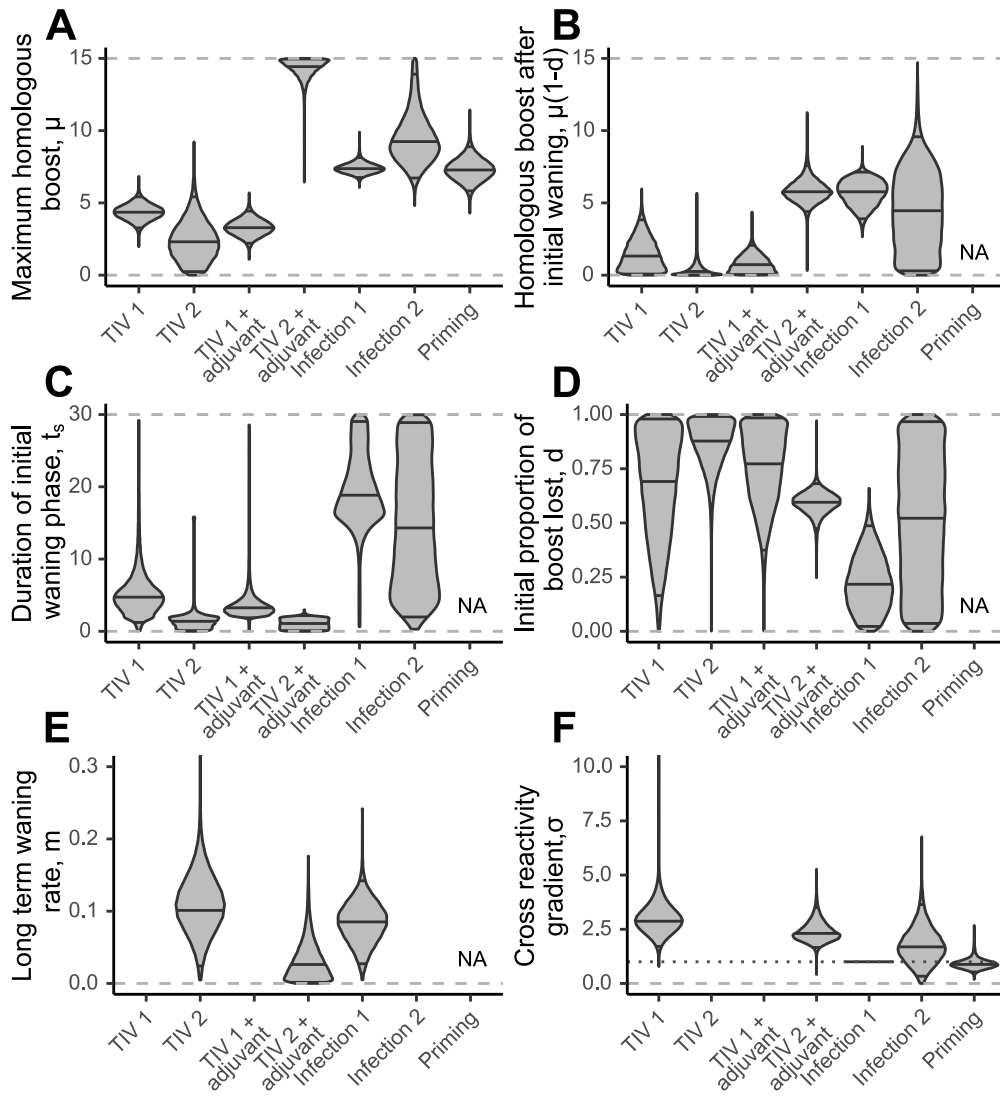


Figure 4.7: Estimated model parameters. Violin plots showing estimated posterior densities with posterior medians and 95% credible intervals marked as horizontal black lines. Violin plots are similar to boxplots, but show the full probability density with some smoothing through a kernel density estimator. Dashed gray lines show bounds on uniform prior. (A) Estimates for homologous boosting parameter, μ . (B) Estimates for homologous boost at the end of the initial waning period, $\mu(1-d)$. (C) Estimates for duration of initial waning phase, t_s . (D) Estimates for proportion of initial boost lost during the initial waning phase, d . (E) Estimates for long term waning rate, m . Estimates for TIV 1, TIV 1 + adjuvant and Infection 2 excluded due to lack of identifiability. (F) Estimates for cross reactivity gradient, σ . Note that this value is fixed at 1 for priming infection (Infection 1), shown by the horizontal dotted line. Values for TIV 2 and TIV 1 + adjuvant excluded due to lack of identifiability.

based on ferret antisera (an antigenic distance of 1 unit should see a reduction in antibody boosting of 1 log titre unit) [82]. Fig 4.8 demonstrates that homologous boosting (the y-intercept) was too small to elicit any measurable cross reactive boosting at these antigenic distances. The cross reactivity gradient parameter, σ , could therefore not be identified for the second dose of unadjuvanted TIV and first dose of adjuvanted TIV. These values were therefore excluded from Fig 4.7F.

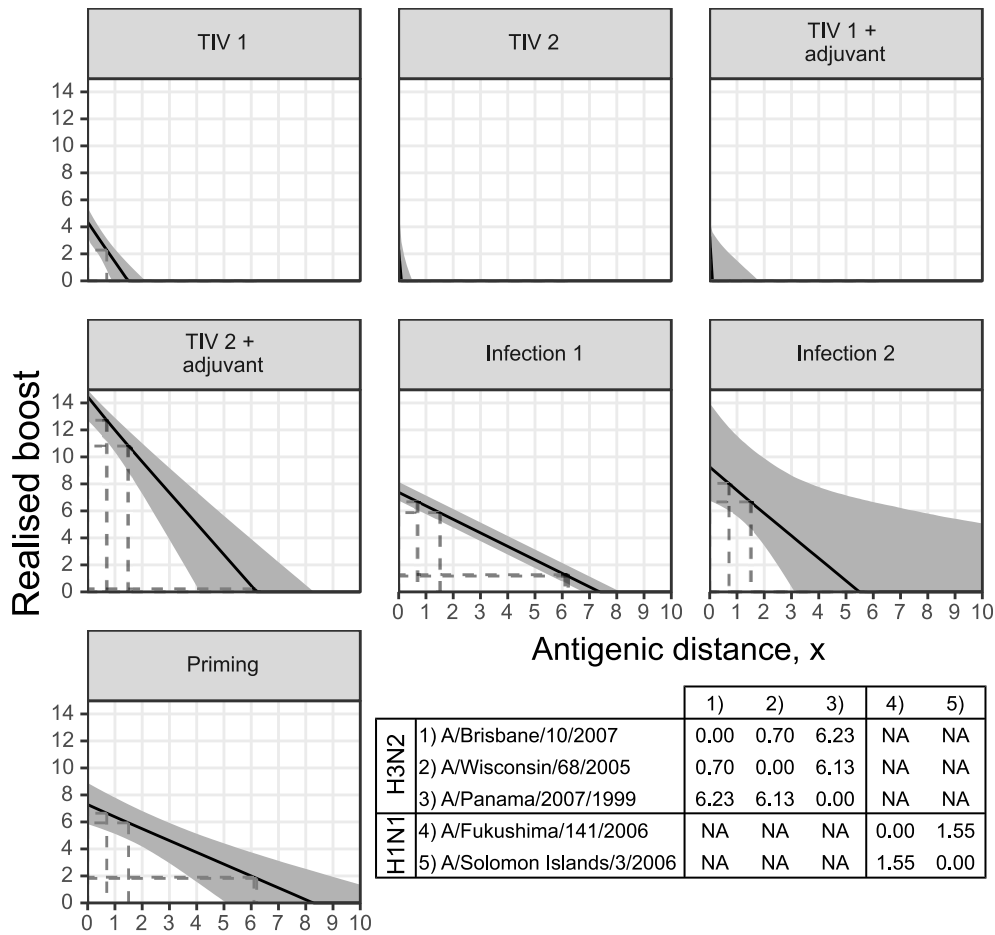


Figure 4.8: Estimated cross reactivity profiles by exposure type. Solid black lines shows posterior means; shaded regions show 95% credible intervals. Dashed black lines show antigenic distances and corresponding cross reactive boosts given the strains used here. Note that the y-intercept shows the degree of homologous boosting for that exposure type. Table shows assumed antigenic distances between each strain, with no cross reactivity between subtypes.

4.4.6 Magnitude and duration of waning phases

The model provided support for the presence of an initial short-term, rapid waning phase followed by a secondary long-term, sustained waning phase. For all vaccine doses, the majority of the antibody boost waned within two weeks of reaching the peak (upper 95% CI 17.3, 5.15, 12.5 and 2.16 days for TIV 1, TIV 2, TIV 1 + adjuvant and TIV 2 + adjuvant respectively, Fig 4.7A&B). Conversely for priming infection, the antibody titre was maintained at near peak levels with an estimated initial waning phase duration of 18.9 days (posterior median; 95% CI 11.0–29.3) and a 21.6% (posterior median; 95% CI: 2.02–48.8%) drop in log titre relative to the peak. Similar long-term waning rates were estimated for second unadjuvanted TIV, second adjuvanted TIV and priming infection (Fig 4.7E).

Estimates for the waning phases that take place following infection with A/Fukushima/141/2006 (H1N1) at day 56 were not well constrained, given that only one subsequent observation was made

at day 70. Although the 95% CI does not exclude biphasic waning rates consistent with the other exposures, any single trajectory in this range that passes through the single observation is similarly likely given these data.

4.4.7 Impact of priming

Prior to receiving non-adjuvanted TIV, experimental group C was infected with H3N2 Panama/2007/1999 at day 0, which represented a host being primed by natural infection prior to vaccination. The model suggested that priming infection added a substantial boost (7.28 log units (posterior median, 95% CI 5.85-8.92)) to antibodies against the A/H1N1 and A/H3N2 vaccine strains at the time of vaccination in addition to that provided by the vaccine itself (Fig 4.7A). Given the assumption that a log titre of 0 represents the true absence of antibodies, it is unclear if the higher titres observed at day 37 are due to a single large boost from primed vaccination rather than an antibody boost below the limit of detection from the priming infection followed by a small subsequent TIV boost. However, previous antibody kinetics results showing higher vaccine-induced antibody boosting following priming from the same detectable starting titre suggest that the former explanation is likely [452].

The cross reactivity of this additional boost was estimated to be broad with a gradient of 0.882 (posterior median; 95% CI: 0.531–1.49), suggesting that priming increases the cross-reactive breadth of the vaccine response. It should be noted that whilst additional priming-induced vaccine boosting is well supported by the model fit, the model overestimates the antibody titre to A/Fukushima/141/2006 (H1N1) at day 37 elicited by initial dose of TIV following priming by H3N2 infection (Fig 4.6C). This may be a result of subtype-specific interactions that are not captured by the model.

4.4.8 Limited evidence for antigenic seniority and titre-dependent boosting

Despite the relatively short duration of these experiments, there was some evidence for a trend of decreasing antibody response with increasing number of prior exposures and/or higher pre-exposure titres. In the best fitting model with antigenic seniority, τ was estimated at 0.213 (posterior median; 95% CI: 0.134-0.300), suggesting that antibody boosting decreased substantially with increasing number exposures after taking into account exposure type and priming. τ measures the proportion of the full boost that is lost with each successive exposure experiences relative to the first (ie. boosting decreases linearly as a function of increasing prior exposures). A higher value of τ therefore indicates more

boosting suppression with an increasing number of prior exposures. Based on these estimates, the amount of antibody boosting would be reduced by over 50% following 4 exposures.

In the best fitting model with titre-dependent boosting, the titre-dependence gradient γ was estimated at 0.0898 (posterior median; 95% CI: 0.0788-0.102) applying to all log titres below 10.9 (posterior median; 95% CI: 8.62-11.95). γ gives the proportion of full boost that is lost per unit increase in log titre at the time of exposure (with no suppression from a starting log titre of 0). The full posterior estimate for the titre-dependent boosting mechanism is shown in Fig B.2 in Appendix B. The inferred titre-dependent boost relationship may be different if the limit of detection of the HI assay was lower. The top two model variants incorporated one of antigenic seniority or titre-dependent boosting, suggesting that either one significantly improves model fit relative to the model variants with neither. The two mechanisms are correlated in these experiments, and antigenic seniority was not well identified for models with both titre-dependent boosting and antigenic seniority. However, all of the top models with antigenic seniority but no titre-dependent boosting give constrained estimates for τ away from 0 (Fig B.13).

4.4.9 Sensitivity analyses

Consistency of parameter estimates across model variants

Parameter estimates for comparable mechanisms were broadly consistent across the 13 best supported model variants with a $\delta\text{ELPD} < 20$ compared to the best fitting model (Table 4.4). Parameter estimates for all 64 model variants are given in Table 4.5. Across the model variants with 6 distinct exposure types and biphasic waning, estimates for boosting and initial waning parameters were similar within each exposure type (Fig B.6, Fig B.7 and Fig B.8. Models with δELPD of less than 20 are shown). None of the best supported model variants included three exposure types (unadjuvanted TIV, adjuvanted TIV and infection), though those that did suggested low levels of transient boosting following TIV and TIV + adjuvant, and high levels of persistent boosting following infection. These results contrast with the consistent estimates of higher levels of persistent boosting from TIV 2 + adjuvant for the rest of the model variants, which were better able to describe the differences in boosting observed between the first and second vaccine doses. Similarly, all of the 13 models included a role for priming infection in increasing subsequent vaccine response. For model variants with identifiable biphasic waning, estimates for the long-term waning rate were consistently low across all exposure types, though generally higher for TIV 1, TIV 1 + adjuvant and Infection 2 (Fig B.9). 5 of the models with a $\delta\text{ELPD} < 20$ included

monophasic waning, suggesting that monophasic waning was able to accurately describe observed antibody titres from these experiments.

All but 2 of the top 13 models included antigenic seniority and/or titre-dependent boosting, suggesting that models accounting for a pattern of decreasing antibody boosting improved predictive power (Fig B.13). Estimates for type specific cross-reactivity parameters were also consistent across model variants; vaccination elicited an antigenically narrower response than priming infection, whereas secondary infection and additional primed-vaccine boosting elicited a cross-reactive response similar to that of priming infection (Fig B.10 and Fig B.11).

Varying time to peak parameter

Varying the time to peak antibody titre parameter, t_p , did not significantly change the inferred parameter estimates. I re-fit the top 3 models (based on ELPD) under two scenarios: (i) the time to peak titre was considered to be unknown, but constrained between 10 and 14 days for primary infection and between 5 and 14 days for all other exposures; (ii) the time to peak titre was fixed at 12 days for primary infection and 8 days for all other exposures. In all cases, the ELPD of each model variant was highest with t_p fixed at 12 days for all exposures, as in the main results. Estimating t_p or fixing t_p at lower values resulted in poor identifiability of parameter t_s . However, the inferred estimates for all other parameters were very similar whether t_p was fixed or estimated, suggesting that this assumption did not bias the results.

4.5 Discussion

In this chapter, I used a mathematical model of antibody kinetics to describe boosting and waning following influenza vaccination or infection in a group of well characterised ferrets. Of the 64 model variants, the two best supported models both included: type-specific antibody boosting; type-specific biphasic waning; 6 distinct exposure types; and a role for priming in increasing subsequent vaccine response. Antigenic seniority, antigenic distance-mediated cross reactivity specific to each exposure type and titre-dependent boosting were also included amongst these top models, suggesting that these mechanisms may be important in accurately describing observed antibody titres following multiple exposures. There were quantitative differences in the level of homologous and cross-reactive antibody boosting between vaccination, infection and adjuvanted vaccination in this ferret model. A single TIV dose with or without adjuvant elicited negligible levels of homologous and cross reactive boosting. A second dose of TIV with adjuvant resulted in significant, broadly reactive antibody boosting, whereas a second dose of TIV without adjuvant did not elicit significant antibody boosting. The profile of boosting for primary infection was consistent across experimental groups, and similar in magnitude to secondary infection. Furthermore, priming infection induced a significantly broader and stronger boosting profile following subsequent vaccination.

Studies in humans have compared homologous and cross reactive antibody responses elicited by different vaccinations and infections with varying conclusions. Fisher *et al.* found no significant differences between in HI titre boosting and waning rates between pandemic H1N1 (pdmH1N1) monovalent vaccination and laboratory confirmed pdmH1N1 infection in pregnant women [453]. In contrast, Kang *et al.* found that geometric mean titres were significantly higher following pdmH1N1 natural infection compared to monovalent pdmH1N1 vaccination in children ≤ 18 , though it is difficult to compare these results given differences in demographic, serum sampling times relative to exposure, and oseltamivir use [442]. Although their analysis was not designed to quantitatively compare antibody kinetics differences by exposure type, Fonville *et al.* compared antibody landscapes pre- and post-vaccination, post-seroconversion, and post PCR confirmed A/H3N2 infection [87]. PCR confirmed A/H3N2 infection elicited the strongest and broadest antibody increases. However, the distribution of titre increases pre- and post- exposure was similar between individuals that were vaccinated and individuals that were presumed to have been infected.

The data presented here included only TIV with and without IFA, and estimates of any boosting

parameters are therefore conditional on the presence of three antigens in a single vaccination. It would be interesting to compare the inferred homologous and cross-reactive boost of different vaccination strategies (eg. a three antigen TIV compared to a monovalent vaccine, or comparison by inoculum dose), and for adjuvants more relevant to human vaccination such as MF59 [454,455]. There may also be underlying heterogeneities in antibody response between and within influenza subtypes as well as between vaccine types [456]. For example, Live Attenuated Influenza Vaccines (LAIV) and newer DNA vaccines may provide different antibody kinetic profiles and may elicit broader antibody responses, or provide different priming effects [437,457]. Statistical models by Freeman *et al.* have also suggested differences and variation in HI titre boosting between subtypes (A/H3N2, A/H1N1, pandemic H1N1, B Yamagata and B Victoria lineages) elicited by TIV and laboratory confirmed infection in children [458,459]. Although children are the best human study population to measure antibody kinetics without confounding from unobserved exposure histories and immunosenescence, data from animal models have the advantage of allowing complete control of each individual's exposure history [437].

Although the model-predicted latent antibody titres were broadly in line with expected immune dynamics, there were some exceptions. The model variant presented in the main text (model ID 62, key YTY6BN) was selected based on PSIS-LOO. Other model variants or designs may provide results more in line with biological expectations, but would require estimation of more parameters or collection of additional data. Given the relative sparsity of samples across time, some aspects of the chosen models were poorly identified and did not match biological expectations at some time points. For example, the predicted latent titres at the time of secondary vaccination (day 42) were unexpectedly lower in group D than group C in model ID 62 (Fig 4.6C&D). Oil-in-water adjuvants are hypothesised to increase recruitment of neutrophils, antibody presenting cells and antigen bearing B cells at draining lymph nodes, suggesting that antibody titres should be higher following adjuvanted than unadjuvanted TIV at all times [460,461]. These unexpected results are likely due to limitations of the flexible model structure, which finds the set of parameter estimates best supported by all of the data, potentially at the cost of some biological realism. A model variant identical to the one used in Fig 4.6 with the addition of titre-dependent boosting provided waning parameter estimates in line with the expectation of adjuvanted vaccination leading to higher antibody titres at all times relative to unadjuvanted vaccination (Fig B.3, model ID 54, Table 4.5).

This model is most useful if observed antibody boosting involved the memory B cell (MBC) repertoire

and de novo B cell response in the same proportions we would expect following exposure in humans. However, in addition to fundamental biological differences between humans and ferrets, the experimental timeline was much shorter than the typical human exposure timescale of months to years [86]. There was a minimum gap of 14 days between the two vaccine doses and 28 days between infection and vaccination. No further increase in antibody titre was detected from 14 days post TIV 1 in group A or post A/Panama/2007/1999 (H3N2) infection in group E, suggesting that serum antibody titres consistently peaked within 14 days and therefore before each subsequent exposure. Furthermore, germinal centre (GC) structures and GC-derived ASCs had likely developed within this time, as it has been shown in other small mammals (mice) that GC B cells are present within 14 days post infection [160, 462, 463]. However, there may not have been sufficient time for MBCs to form and subsequently differentiate into plasma cells at the time of the second vaccine dose, which would be the main contributors to post-vaccination antibody titres in humans [150].

These experiments were also limited by the sparse sampling around the biphasic waning period of the vaccinations and following the final exposure event, which resulted in poor identifiability for some of the waning and timing parameters. Experiments of a similar design with fewer exposures and more frequent sampling would power the model to elucidate these waning phases further and look for differences in response longevity by exposure type.

Another limitation of the model is the assumption that a log titre of 0 (the value for undetectable in the HI assay) represents the true lower bound of latent antibody levels. Relaxing this assumption to allow antibody kinetics to occur below the limit of assay detection may change the inferred kinetics parameters. For example, exposures that elicited lower or undetectable changes in titre (eg. unadjuvanted TIV) may in fact generate substantial homologous and cross-reactive boosting that cannot be detected here. Changes to the model structure could help to relax the impact of this assumption. One approach that I tried was to include the true lower bound on latent antibody titres (ie. the log titre that represents a true absence of antibodies) as a model parameter. However, allowing this parameter to change in the MCMC procedure or setting it to lower negative values resulted in drastically worse model fits and did not generate interpretable results. Another mathematical approach may be to integrate over a range of lower-titre bounds (eg. -10 to 0) when generating model-predicted titres. However, performing this integration at each MCMC iteration would likely be prohibitively slow. Alternatively, assessing how titres change from these potentially hidden, low-magnitude kinetics could be elucidated by using an assay with lower initial dilutions, increased sensitivity or by exposing ferrets at higher pre-existing

titres.

Model comparison analysis supported models with biphasic waning following both primary infection and secondary vaccination. The magnitude and duration of waning differed between exposure types: TIV 1 and 2 and adjuvanted TIV 1 waned very quickly, whereas Infection 2 and TIV 2 + adjuvant were more persistent. Heterogeneity in antibody waning rates between individuals and vaccine types have been shown for other pathogens [228,464]. Although studies of influenza antibody response duration have been carried out in humans, quantifying waning rates independent of subsequent exposures that cause repeated boosting is difficult [199,430,441,465–467]. The model fit to the single exposure ferrets provides an estimation of the waning rate of homologous antibodies in the absence of further exposure, but the cut off of 70 days limits the applicability of this waning rate to a timescale more relevant to humans. Extrapolating the estimated waning rate following primary infection would suggest that antibody titres would wane to non-detectable within a few months, whereas antibody responses against many viruses are known to persist for decades [85,464]. Longer term studies investigating the longevity of the antibody response in the absence of repeated exposure would be useful to quantify a long-term, steady state antibody waning rate [231]. Further mechanisms such as differential waning rates between cross-reactive and homologous antibodies are likely to be important, but were not identifiable here [87,434].

“Prime-boosting” has been described previously as a strategy to induce broadly reactive immune responses that may be rapidly boosted in advance of exposure to an antigenically novel virus [432,433,452]. Models that included a priming mechanism were ranked systematically higher in the model comparison analysis than those that did not, suggesting that this phenomena is important in explaining titres arising from repeated exposures. Vaccine responses to A/H1N1 strains were higher and more broadly reactive in A/H3N2 primed ferrets compared to unprimed ferrets, though the model did not account for subtype specific interactions and subsequently overestimated post vaccination A/H1N1 titres in primed ferrets. Although the phylogenetic relationship between the priming and subsequent boosting strain is likely to be important, heterosubtypic protection has been shown previously in animal models, potentially via cytotoxic T lymphocyte responses [423,424].

Overall, these results suggest that mathematical models of antibody kinetics that explicitly consider immunological mechanisms and exposure-type specific parameters would be valuable for the prediction of antibody landscapes in human populations. Human cohort studies tracking infants from birth as they experience their first few influenza exposures are also now underway [468]. Combining these

studies with single-cell immune profiling and mathematical models of multiple exposure kinetics will help to elucidate the role of these immunological mechanisms in building human antibody profiles. Direct inference from long-term observational data in humans may be difficult, but experimental models, such as the ferret system described here, provide an excellent alternative data source for the inference of short-term immunological mechanisms that may map onto models recovered using human sera [86, 87, 225, 230].

S1 Table csv file containing all posterior distribution estimates for all model variants.
Available at <https://doi.org/10.1371/journal.pcbi.1007294.s006>

S2 Table. csv file containing convergence diagnostics (including minimum effective sample size and $\hat{R}$) and expected log predictive density estimates for all model variants.
Available at <https://doi.org/10.1371/journal.pcbi.1007294.s007>

Chapter 5

A statistical framework to represent influenza infection histories

In the previous chapter, I developed and fit mathematical models describing antibody kinetics following repeated exposures to influenza. The data used in those analyses came from a ferret experimental system where the nature and timing of each infection was known, which is usually not the case when analysing comparable data from human serosurveillance. The aim of this chapter is to develop an inference framework similar to that described in Chapter 4, but with the inclusion of exposure times as unknown parameters. This chapter will extend a model previously published by Kucharski *et al* [1]. After outlining the original method and my own additions, I demonstrate shortcomings in the existing infection history framework – something which only became apparent when fitting the model to a different data set. I develop the underlying statistics to accurately describe the link between latent infection states and the infection generating process, thereby accounting for implicit biases that arise using the original method. I describe the infection history data structure independently of the antibody kinetics model, leading to a general statistical framework for representing multiple latent infection states. I then demonstrate the behaviour of this statistical framework through simulation-recovery experiments and show antibody kinetics and influenza infection history estimates from real serological data.

5.1 Introduction

The statistical problem of inferring unobserved infections can be framed to fall within the remit of Bayesian binary variable selection: a well described area of research in the context of regression models and a notoriously challenging problem as the number of binary variables and resulting model space grows large [469–471]. Regularised regression methods are commonly used for classical regression problems with many variables (here, variables may be thought of as infection states). The aim of regularised regression methods is to penalise complex models, either by shrinking model coefficients by some common factor (L2 regularisation) or through shrinking the model space towards sparser models by penalising inclusion of a larger number of model coefficients (L1 regularisation). For model selection, L1 regularisation using Lasso (Least Absolute Selection and Shrinkage Operator) regression may be used to penalise the inclusion of non-zero coefficients, resulting in the shrinkage of some coefficients to exactly 0. When the Lasso scheme is implemented in a Bayesian framework, Laplace priors (or Spike-and-Slab in a related method) are placed on the model coefficients, placing high *a priori* density near 0 [472, 473]. However, it is difficult to apply existing implementations of these methods to the present problem, as the probability of including each variable (infection state) may not be independent of other variables (eg. through a shared force of infection), and variables and model coefficients do not strictly have a one-to-one relationship (ie. each infection indicates the start time of a time-varying, non-linear immune process that may be the same across individuals).

Methods such as stochastic search variable selection and Reversible Jump (RJ) Markov chain Monte Carlo (MCMC) are also established for binary variable selection, but are insufficient for the present problem, where binary outcomes are the result of complex, unobserved epidemiological processes. RJ-MCMC approaches have been used to represent multiple latent infection states previously [40, 280]. However, these analyses benefited from a number of constraints that limits their generalisability. For example, the data used by Salje *et al.* had many PCR-confirmed dengue virus (DENV) infections, which constrained antibody kinetics immediately following known infection times [40]. Furthermore, their analysis assumed that individuals could only be infected once with each of the four DENV serotypes, which is not the case for influenza.

My original aim was to extend the framework described by Kucharski *et al.* to infer antibody kinetics parameters and influenza infection histories for a new data set with more individuals, less antibody titre data per individual, and at a sub-annual time resolution [1]. I expected this to simply be a case

of re-implementing the exact model and inference framework to deal with the higher computational requirements. However, although I was able to reproduce the results of Kucharski *et al.*, the same framework was not able to produce unbiased parameter and attack rate estimates using a larger data set (1,130 versus 69 individuals) and relatively fewer antibody titres per individual (on average 63 titres per individual versus 168) (Fig 5.4).

This chapter is split into two conceptual parts, though the focus is largely on the first. After describing the existing antibody kinetics model with my additions, the infection history model and the data, I demonstrate that the original framework in Kucharski *et al.* places a hidden prior on the latent infection histories that was not apparent in the original paper. I show that this effect was masked by the rich data set (the Ha Nam cohort, Vietnam) by comparison with simulated data based on an ongoing serological study in Guangzhou, China. I then outline different models for the latent infection histories and infection generating process which lead to unbiased estimates of historical “seroresponse” (a detectable serological response) rates. I consider the fact that not all infection events are independent across individuals. For example, individuals that are infectious at a given time exert a force of infection on other individuals in the same population, and *a priori* we do not know if an individual experienced many or few infections over their lifetime. Note that I distinguish seroresponses from true, clinically relevant infections here and throughout the remainder of the thesis, as inferred events represent all detectable serological responses (infection, vaccination or asymptomatic exposure). Any reference to attack rate or infection therefore has the caveat of this assumption. In Appendix C.3, I also discuss how the assumption of independence of infection probabilities for a given individual between time periods may be relaxed through the inclusion of a titre-mediated immunity term, but show that this does not drastically change estimated antibody kinetics parameters and population attack rates.

In the second conceptual part, I outline extensions to the antibody kinetics and observation process model. A crucial extension is the inclusion of virus-specific measurement bias, which I use in Chapter 6. I also extend the base antibody kinetics model to include titre-dependent and age-dependent antibody boosting, and compare parameter estimates when fit to real data.

5.2 Materials & Methods

5.2.1 Serological data

I used antibody titre data from two previously described cohorts summarised in Table 5.1 [87, 474, 475]. Simulated data were also generated based on the protocols of these studies. The Fluscape study in Guangzhou, China recruited 1,130 individuals each with two paired serum samples taken from two rounds of sampling between 2009 and 2015 inclusive. The month of sampling, age of participant, vaccination history and other socioeconomic covariates were available for all individuals. Haemagglutination inhibition (HI) assays were performed for each sample to measure antibody titres against 20 A/H3N2 strains that circulated between 1968 and 2014 inclusive, with ≈ 2 year spacing between circulation years. Fig 5.1 shows example HI titre measurements for 3 randomly chosen individuals from the Fluscape cohort. The Vietnam data consisted of 69 participants from Ha Nam, Vietnam, each with 6 serum samples taken annually from 2007-2012 as described previously [87]. In this cohort, HI assays were performed against a panel of up to 57 A/H3N2 strains isolated between 1968 and 2008, with greater sampling of titres against more recent strains. No other meta-data were available for the Ha Nam cohort, though unmatched ages were available and used to scale inferred attack rates to only individuals that were alive in a particular year. Fig 5.2 shows the distribution of participant ages and tested influenza A/H3N2 strains across the two studies.

In both datasets, antibody titres were transformed to the log scale, $\log_2(D/10)+1$, where $10 \leq D \leq 1280$ represents the highest dilution of the sera which inhibited haemagglutination. Undetectable titres of <10 were treated as a log titre of 0. An antigenic summary path was calculated to position each virus in antigenic space by fitting a smoothing spline through observed points, as described in [1].

For the simulated data, annual attack rates were simulated from $\phi_j \sim \text{unif}(0, 0.25)$, though the attack rate for 1968 was set to 0.6 to represent the pandemic year. Infection histories were simulated from these attack rates as $z_{i,j} \sim \text{Bern}(\phi_j)$.

	Ha Nam, Vietnam [87]	Guangzhou, China [474]
Individuals	69	1,130
Number of serum samples	Up to 6 per individual (2007-2012)	2 per individual (2009-2015)
Number of strains tested	57	20
Number of titres	11,597	67,683
Strain coverage	Mostly recent	Uniform since 1968

Table 5.1: Comparison of the two HI titre datasets

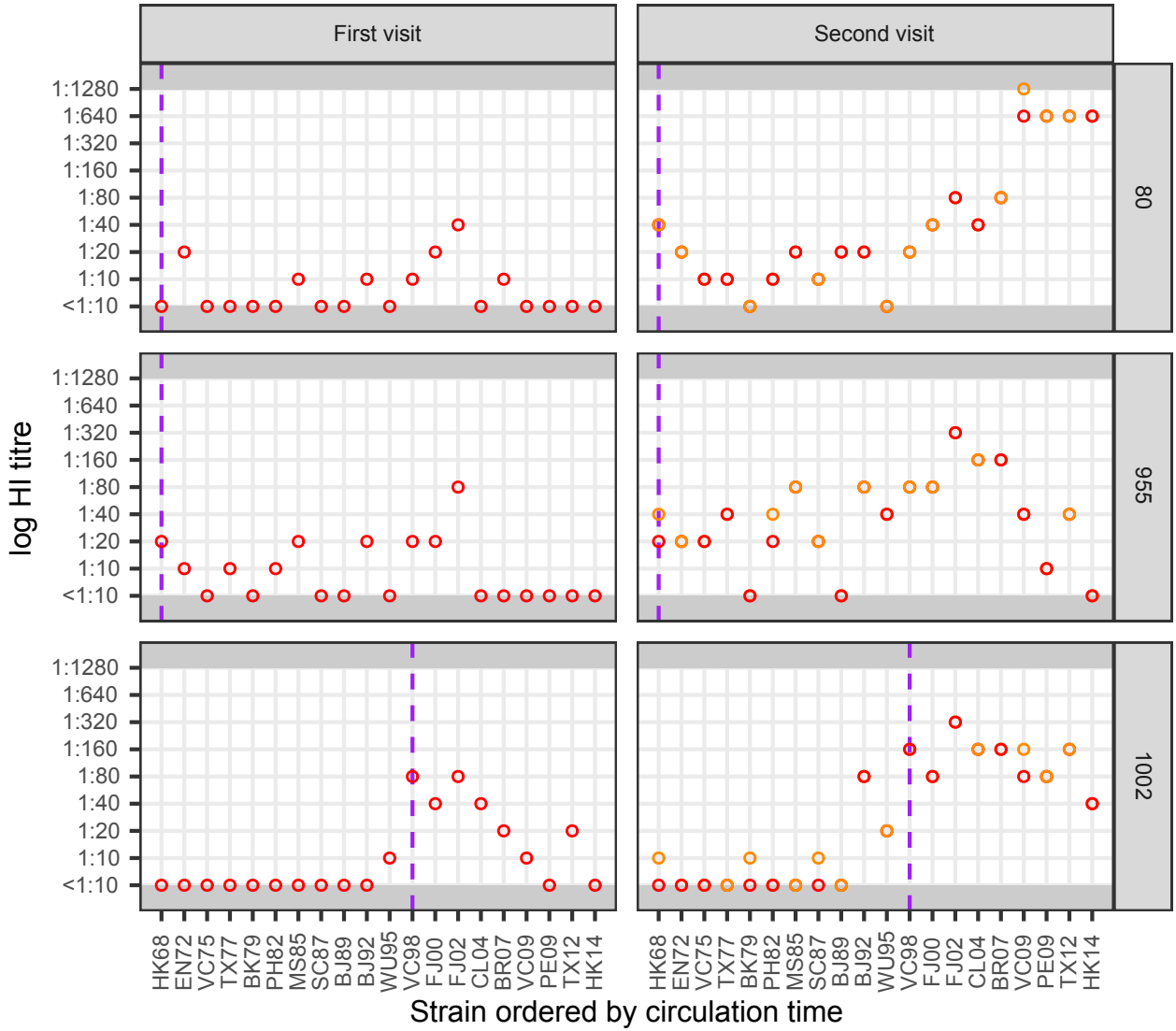


Figure 5.1: HI titres for 3 randomly chosen individuals from the Fluscape cohort. Each row shows one individual. Each column represents a serum sample. Points show log HI titre, with repeats shown in orange. X-axis shows virus against which serum was tested, ordered by time of circulation. Purple dashed line shows strain nearest to year of birth. Grey rectangles represent titres below and above the limit of detection of the assay.

5.2.2 Antibody kinetics model

In this chapter, vectors are represented in bold, capital letters represent random variables, and lower case letters represent values of random variables. An individual's entire infection history is given as a vector of binary variables, $\mathbf{Z}_i = [Z_{i,1}, Z_{i,2}, \dots, Z_{i,j}]$. I assume that each infection event, $Z_{i,j}$, is the outcome of a single Bernoulli trial, where $Z_{i,j} = 1$ indicates that individual i was infected in discrete time period j , $Z_{i,j} = 0$ indicates that they were not.

Infection events are not observed directly. Rather, infections lead to the production of antibodies

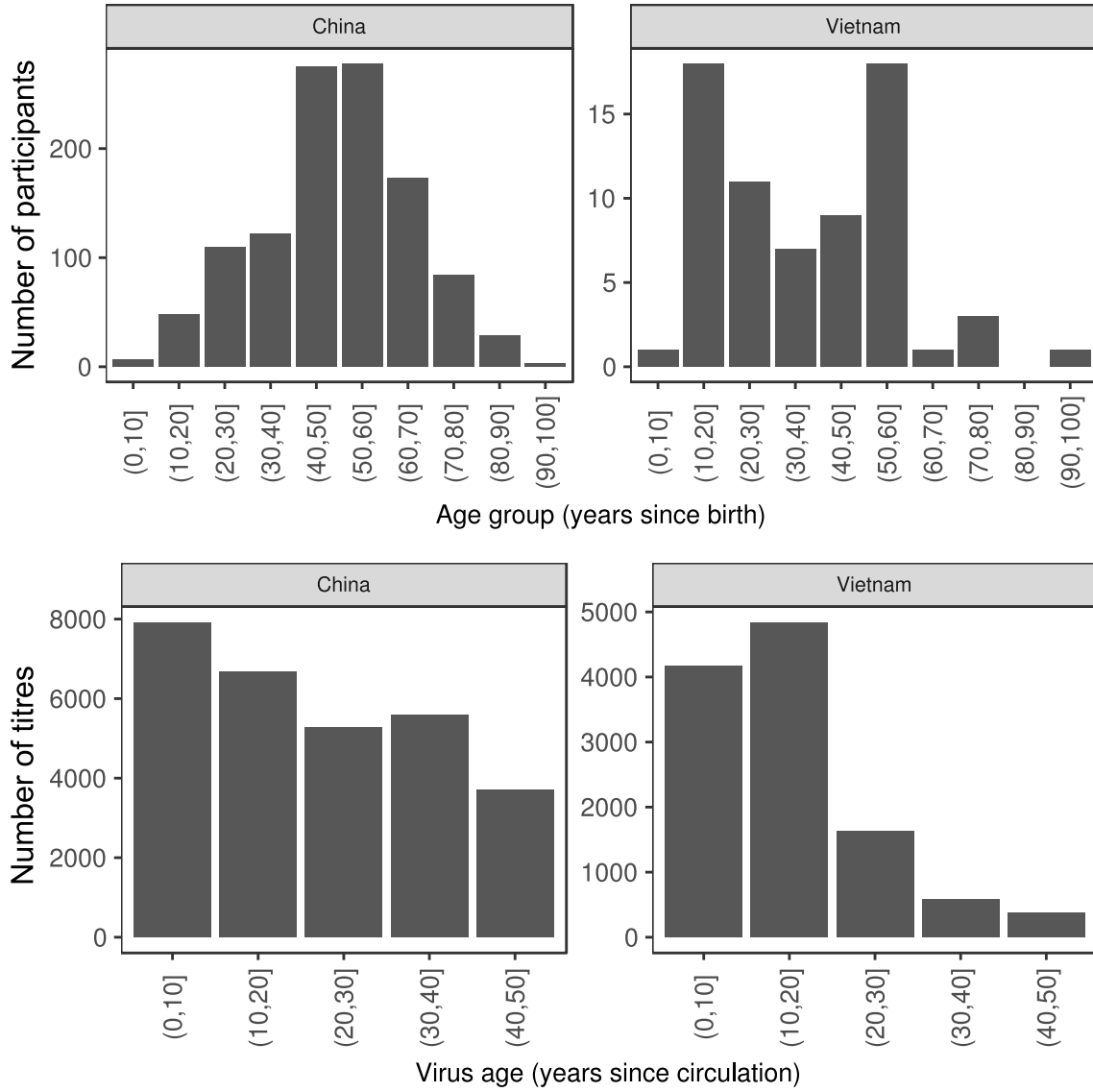


Figure 5.2: Top row: distribution of participant ages. Both cohorts include individuals across all ages, though data from Vietnam has greater representation of younger individuals. Bottom row: distribution of tested strain ages. X axis shows time since tested strain was observed to be circulating in human populations. Data from China includes good coverage of viruses that circulated at all times since A/H3N2 was introduced, whereas data from Vietnam predominantly tested recently circulating strains.

that undergo longitudinal and cross-reactive kinetics. The vector of latent antibody titres across all time periods is given as $\mathbf{Y}_i = [Y_{i,1}, Y_{i,2}, \dots, Y_{i,j}]$, which is generated from an antibody kinetics process with parameters $\boldsymbol{\theta}$. These latent antibody titres also undergo an observation process. The vector of observations is given as $\mathbf{Q}_i = [Q_{i,1}, Q_{i,2}, \dots, Q_{i,t}]$. Note that the time index for $\mathbf{Q}_i$ is different to $\mathbf{Y}_i$ and $\mathbf{Z}_i$, as observations are only made at a subset of t times whereas latent infection states and antibody titres must be represented at all j times.

The antibody kinetics model used here is based on a previously published model, but with novel

extensions [1,476]. In the base model, given an individual with infection history $\mathbf{Z}_i$, expected titres ($\mathbf{Y}_i$) were generated through the interaction of three processes:

1. Persistent, long-term boosting by μ_l log units against the infecting strain and $\mu_l d_l(k, j) = \max\{0, \mu_l(1 - \sigma_l \delta_{k,j})\}$ to all other strains, where $\delta_{k,j}$ is the Euclidean distance between the infecting strain j and the measured strain k on an antigenic map [82].
2. Temporary, short-term boosting by μ_s log units against the infecting strain and $d_s(k, j) = \max\{0, \mu_s(1 - \sigma_s \delta_{k,j})\}$ to all other strains. This boost wanes over time such that the amount of short-term boosting t_j ($t_j = j - k$) years after infection is given by $\mu_s w(j) = \mu_s \max\{0, 1 - \omega t_j\}$.
3. Antigenic seniority resulting from suppression of boosting via antigenic imprinting. Boosting was scaled by $s(Z_{i,j}, j) = \max\{0, (1 - \tau N_j - 1)\}$ after each infection, where $N_j - 1$ is the number of previous infections before strain j .

The full antibody kinetics model to give an expected titre $Y_{i,j,t}$ for individual i against strain j measured at time t as a function of past infections was given as:

$$Y_{i,j,t} = \sum_{k \in \mathbf{Z}_i} Z_{i,k} s(Z_{i,k}, k) [\mu_l d_l(k, j) + \mu_s w(k) d_s(k, j)] \quad (5.1)$$

μ_l , μ_s , σ_l , σ_s , τ , and ω are all parameters to be estimated. Overall, the model captures the short- and long-term arms of the adaptive immune response and preferential recognition of previously encountered epitopes by reactivating pre-existing B-cell populations over naive B-cells [93,147].

5.2.3 Additional immunological mechanisms

I added two new immunological mechanisms to the model. First, a titre-dependent boosting mechanism was included to account for potential antibody ceiling effects, whereby antibody boosting is decreased at higher initial titres [434,446]. The form of this model is the same as in Chapter 4. For each infection with strain j (strain j and only strain j circulates only in one time period (t_j) and so the subscript is used twice), the predicted log antibody titre in Equation 5.1 was multiplied by $b(j)$:

$$b(j) = \begin{cases} \max\{0, 1 - \gamma_t Y_{i,j,t_j}\} & \text{if } Y_{i,j,t_j} \leq y_{switch} ; \\ \max\{0, 1 - \gamma_t y_{switch}\} & \text{else} \end{cases} \quad (5.2)$$

where γ_t is a fitted parameter, y_{switch} is the titre-dependent boosting threshold, and Y_{i,j,t_j} is the titre of individual i against strain j at time of infection t_j . Initial titres above y_{switch} do not experience any further boosting suppression.

Age-dependent antibody boosting was also included and was conceptually similar to boosting suppression via antigenic seniority, but was conditional on the age of the individual rather than the number of prior infections. Boosting from each infection was scaled by $a(Z_{i,j}, A_{i,j}) = \max\{0, (1 - \gamma_a A_{i,j})\}$, where $A_{i,j}$ is the age of individual i in time period strain j circulated, and γ_a is a parameter to be estimated.

The antibody process model can be simplified by setting certain parameter values equal to 0. For example, a model without antigenic suppression can be created by setting $\tau = 0$ or a model with only waning responses by setting $\mu_l = 0$.

5.2.4 Infection history model

Infections are represented by binary variables, where $Z_{i,j} = 1$ indicates that individual i was infected with the strain circulating during time period j . I assume that only one strain circulates per time period. The entire infection history matrix for all n individuals across all m years was therefore represented by a binary matrix, as shown in Table 5.2.

Individual	1968	1969	1970	1971	...	2014
1	0	1	0	0	...	1
2	1	0	0	1	...	0
3	1	0	0	0	...	0
...						

Table 5.2: Representation of infection histories for a population of three individuals from 1968 to 2014.

5.2.5 Observation model

The expected titres $Y_{i,j,t}$ defined in Equation 5.1 feed into the observation model. Whereas latent antibodies are continuous variables, observations are discrete in many antibody assays. I assumed that titres observed at time t were normally distributed with mean $Y_{i,j,t}$ and variance ε as in Kucharski *et al.*, with censoring to account for the upper and lower bounds of the assay [1]. Hence the probability of observing an empirical titre at time t within the limits of a particular assay $Q_{i,j,t} \in \{0, \dots, q_{max}\}$ given

expected titre $Y_{i,j,t}$ is,

$$P(Q_{i,j,t}|Y_{i,j,t}) = f(Q_{i,j,t}|\mathbf{Z}_i, \theta) = \begin{cases} \int_{Q_{i,j,t}}^{Q_{i,j,t}+1} g(s)ds & \text{if } Q_{i,j,t} \in \{1, Q_{max} - 1\} ; \\ \int_{-\infty}^1 g(s)ds & \text{if } Q_{i,j,t} = 0 ; \\ \int_{q_{max}}^{\infty} g(s)ds & \text{if } Q_{i,j,t} = q_{max} . \end{cases} \quad (5.3)$$

where $g(q) = \frac{1}{\sqrt{2\pi\epsilon}} e^{-\frac{(q-y_{i,j})^2}{2\epsilon}}$, the probability density function of the normal distribution.

I extended the measurement model to include virus-specific measurement bias in a similar way to Salje *et al.*, who considered dengue subtype-specific measurement bias in HI assay titres when fitting models of antibody dynamics [40]. An additional observation error is added to the predicted log antibody titre at a given time; this measurement error can be different for each individual strain or can be specified for a group (or cluster) of strains. The predicted titre $Y'_{i,j,t}$ taking into account virus-specific measurement bias is given as:

$$Y'_{i,j,t} = Y_{i,j,t} + \chi_j \quad (5.4)$$

Where χ_j is the measurement offset for strain j , and $Y_{i,j,t}$ is the model predicted latent titre for individual i against strain j observed at time t .

Given that the model predicts one latent titre against the representative circulating strain for each time period j , I assumed that there was one χ_j that applied to all measured strains that circulated during a particular time period j . There is likely further variation in virus avidity between strains that were isolated in the same year. However, in the Fluscape dataset, only one representative virus for each two year period was measured, and this was therefore analogous to estimating one χ_j per measured strain. Furthermore, any within-year variation should be largely captured by the variance of the Gaussian measurement model (ϵ), such that χ_j captures only systematic bias.

I assumed that all χ_j were unknown parameters to be estimated. All χ_j may be estimated as independent parameters, or may be placed within a hierarchical model such that the χ_j are normally distributed:

$$\chi_j \sim \mathcal{N}(\bar{\chi}, \sigma_{\chi}^2) \quad (5.5)$$

where it was assumed that $\bar{\chi} = 0$ and σ_{χ}^2 was unknown. All χ_j were also given fixed lower and upper bounds of -3 and 3 respectively. Bounds were chosen to be roughly in line the range of titre residuals when the model was fit without virus-specific measurement bias.

Fig 5.3 provides a schematic combining the antibody kinetics, infection history and observation models. This example shows the longitudinal and cross-sectional antibody dynamics to three antigenically related strains over a lifetime with two infections. Note that the time axis in this figure relates to consecutive serum samples (ie. the columns in Fig 5.1), whereas each line corresponds to a different point in antigenic space (ie. the x-axis in Fig 5.1).

5.2.6 Inference using MCMC

In all of the following analyses, models were fit using a custom, adaptive MCMC framework to sample from the joint posterior distribution of θ and $\mathbf{Z}$ conditional on the observed antibody titre data $\mathbf{Q}$ (Equation 5.8). The framework I developed was initially based on previous work [1]. The original framework used a Metropolis-Hastings algorithm that alternated between re-sampling antibody kinetics parameters and individual infection histories. Infection history proposals were either the addition of an infection, removal of an infection or moving an infection to a different time. All code was implemented as a series of R scripts (available at <https://github.com/adamkucharski/flu-model/>), but the original code was not flexible nor fast enough to apply to larger datasets.

In the framework I developed, proposals also alternated between sampling values for θ or $\mathbf{Z}$. Proposals for θ were made using an adaptive Metropolis-within-Gibbs algorithm. Uniform positive priors were placed on all parameters in θ . The biggest change from the previous framework is that the full sampling algorithm for $\mathbf{Z}$ is dependent on the assumed infection history prior, and its description forms the basis for most of this chapter. Appendix E.3 describes the full proposal algorithm for each of the assumed infection history priors. Briefly, the algorithms use a random-scan Metropolis-within-Gibbs proposal on infection histories to either propose new infection states or swap the times of existing infection states. These steps were developed to improve MCMC mixing when the infection states in adjacent time periods may be highly correlated. Step sizes for θ proposals and the number of sampled individuals for $\mathbf{Z}$ proposals were automatically tuned to achieve an acceptance rate of 0.44. Chains were run to achieve an effective sample size of >200 for all inferred parameters. Convergence was assessed visually and using the Gelman-Rubin diagnostic criteria ($\hat{R}$) with the coda R-package [383]. All features of the model were implemented in R version 3.5.1 and C++ using the Rcpp package, with the R-package available at <https://github.com/seroanalytics/serosolver> [476–478].

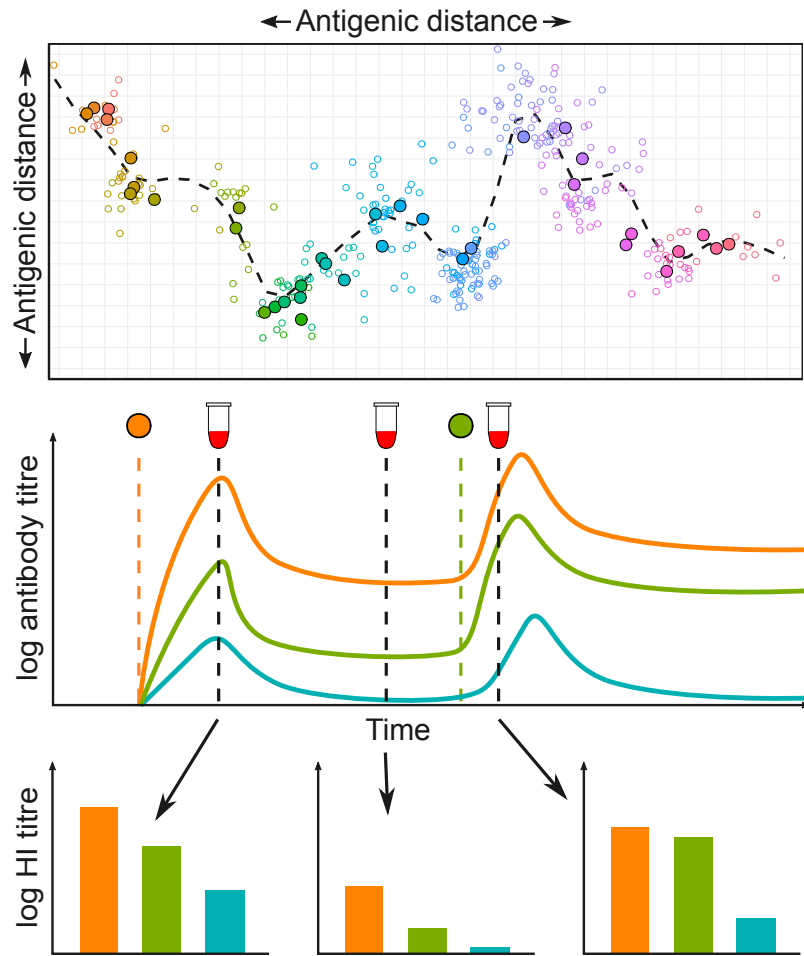


Figure 5.3: Conceptual overview demonstrating how an antibody landscape develops and may be observed over an individual's lifetime. (Top panel) antigenic map for influenza A/H3N2 using coordinates from [82], with different viruses coloured by year of isolation. Solid points show centroids across all strains isolated in a given year, hollow points show individual strains. Dashed line shows an antigenic summary path, generated by fitting a smoothing spline through the observed isolates. Points further apart in space are less cross-reactive. (Middle panel) antibody kinetics against 3 strains from the antigenic map. An individual is infected with the orange virus, which results in boosting and waning of homologous antibody titres. In parallel, antibodies that cross react with viruses at different points in antigenic space are also boosted and wane (green and blue viruses). The individual is later infected by the green virus, which leads to further boosting and waning of antibodies. (Bottom panel) HI titres measured from serum samples taken at different times capture different parts of the homologous and cross reactive antibody kinetics. Different sampling strategies will represent different subsets of these measurements eg. a cross-sectional study might inform a single subplot, whereas a longitudinal study might inform just the orange bars from each of the three subplots. Clearly a sampling strategy with multiple serum samples and many viruses tested per sample will provide the most information.

5.2.7 Model settings key

In this chapter, I run many simulation-recovery experiments and fit different models to simulated serological data sets. Table 5.3 shows an example of parameter values and data protocols to accompany each analysis. This table also serves as a reference for the antibody kinetics parameters.

Data set parameters		Model parameters					
Number of individuals n	1000	Parameter	Description	Value	Prior lower bound	Prior upper bound	Estimated
First infection year	1968	μ_l	Long term antibody boosting	1.8	0	8	Yes
Final infection year	2015	μ_s	Short term antibody boosting	2.7	0	8	Yes
Number of time periods m	48	σ_l	Long term cross reactivity	0.1	0	1	Yes
Time resolution (infection states)	annual (48)	σ_s	Short term cross reactivity	0.03	0	1	Yes
Number of serum samples per individual	2	τ	Suppression	0.05	0	1	Yes
Number of viruses tested	24	ω	Waning	0.8	0	1	Yes
Number of titre measurement repeats	2	ϵ	Measurement error	0.8	0	25	Yes
Total number of measurements	9600	α	Infection history prior	1	NA	NA	No
First sample year	2009	β	Infection history prior	1	NA	NA	No
Final sample year	2015	γ_t	Titre-dependent boosting gradient	0	0	1	No
Minimum age (years)	6	y_{switch}	Extent of titre-ceiling effect	0	0	8	No
Maximum age (years)	75	γ_a	Age-dependent boosting gradient	0	0	1	No
Infection history prior	Explicit ϕ_j	χ_j	Virus-specific measurement offsets	0	-3	3	No

Table 5.3: Example simulation settings table. Data set parameters refer to properties of the simulated cohort and observations. Model parameters refers to the values used in the antibody kinetics model.

5.3 Motivation for understanding prior assumptions

To assess the ability of the original framework to accurately infer antibody kinetics, infection histories and epidemiological dynamics from a data set with different dimensions (number of individuals, number of strains tested etc.), I simulated antibody titre data with the same characteristics of the previously described Fluscape cohort [474]. Fig 5.4A shows the inferred attack rates for each year, revealing the poor accuracy of this framework in fitting the model to simulated data for which the true attack rates are known. These results suggested substantial bias in inferred attack rates despite fitting to simulated antibody titres and re-estimating known individual infection histories (Fig 5.5).

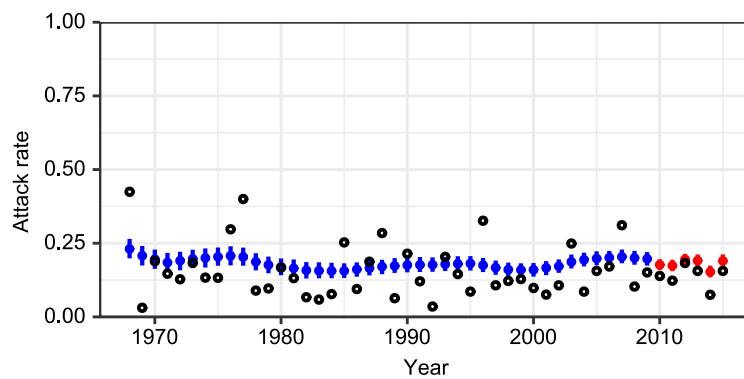


Figure 5.4: Inferred annual A/H3N2 attack rates using simulated Fluscape data with original framework described in Kucharski *et al.* [1]. Points and lines show posterior median and 95% credible intervals of annual attack rate estimates. Red points show years in which serum samples were taken. Black points show true annual attack rates from which infection histories were simulated. Mismatch between model estimates (coloured points and lines) and true values (black points) suggest substantial bias in the fitting framework.

If the contribution of the antibody titre data to the posterior distribution is small relative to the contribution of the infection history prior (ie. the expected number and frequency of infections per individual or unit time before seeing any data), then the inferred posterior distribution may be more a reflection of the prior. For example, an infection history with frequent infections combined with low magnitude antibody boosting may give a similar likelihood to an infection history with infrequent infections with high magnitude antibody boosting (as both situations would generate similar observations). If the implicit infection history prior places higher probability on frequent infections, then the inferred antibody boosting parameters would support lower magnitude boosting. These results motivate the exploration of how the model structure and prior assumptions impact infection history and antibody kinetics parameter estimates.

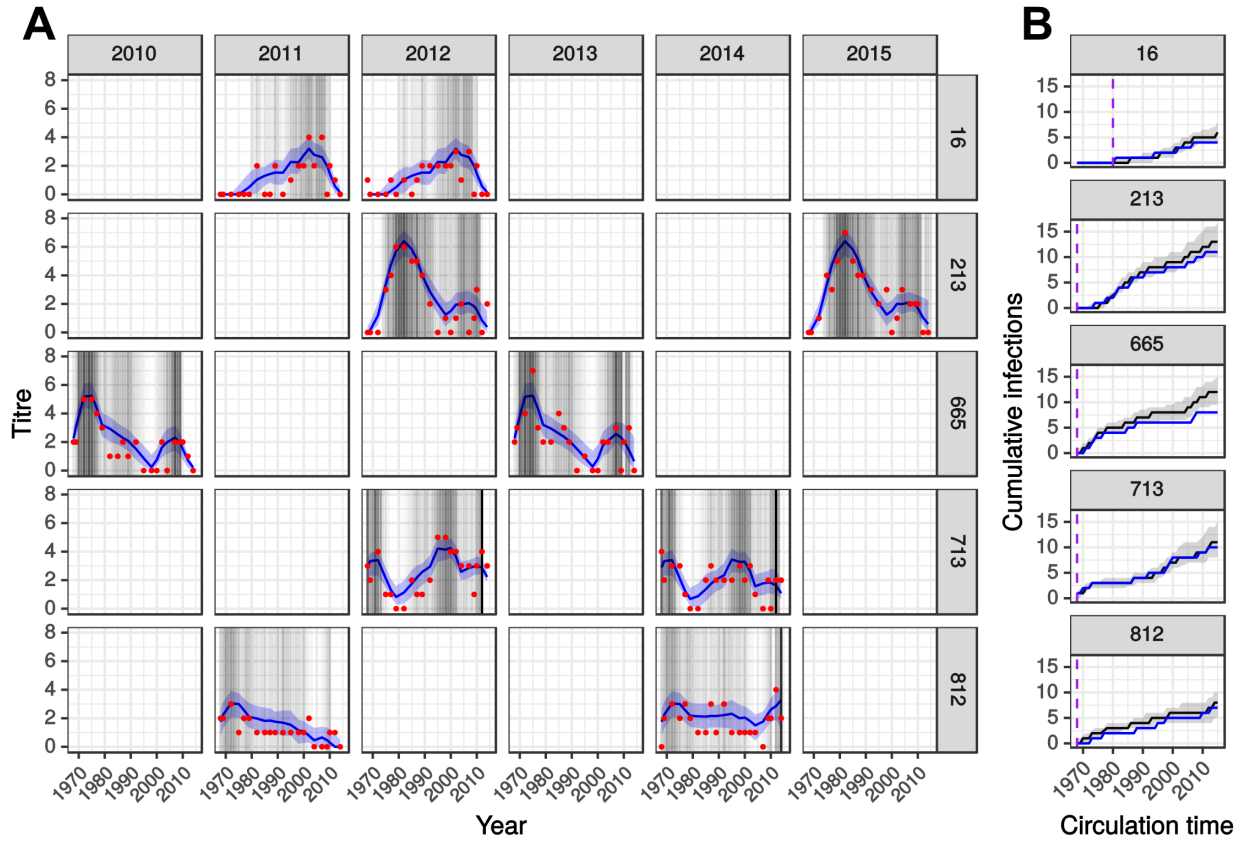


Figure 5.5: Example of individual model fits to simulated Fluscape-like titre data using original framework. Each row represents samples for a single individual, whereas each column represents the time at which a serum sample was taken. At each serum sample, HI titres are measured against a panel of influenza strains that represent points across antigenic space. X-axis represents the year at which each strain circulated. (A) Red points show observed antibody titres; blue lines and shaded region shows model suggested median latent titres and 95% credible intervals; vertical black lines show presence of infection where opacity is proportional to probability of infection (proportion of MCMC sampled that suggested an infection in that year, with white = 0 and black = 1). (B) Inferred cumulative infection histories corresponding to the individuals in (A). Blue line shows true, simulated cumulative infection history. Black line and shaded region shows posterior median and 95% credible interval. Purple dashed line shows year of birth.

5.4 The inference problem

The aim of this inference framework is to obtain the joint posterior distribution of antibody kinetics parameters, individual infection histories and individual latent antibody levels over time given a set of observed serological data. Based on the notation outlined in Section 5.2.2, the matrix of infection histories for all individuals is given as $\mathbf{Z} = [\mathbf{Z}_1, \mathbf{Z}_2, \dots, \mathbf{Z}_i]$, with analogous notation used for the overall matrix of latent antibody levels $\mathbf{Y}$ and observations $\mathbf{Q}$. If we consider a system with n individuals who may be infected once in each of m distinct time periods, then there are nm possible infection events. Although the framework is motivated by and developed for influenza, the antibody kinetics and infection history models are conceptually separate. I therefore discuss the infection history model as a general statistical challenge, and for now assume that the antibody kinetics model may be any arbitrary observation process.

5.4.1 Description of the full model

An individual's latent antibody titres $\mathbf{Y}_{i,t}$ at time t are generated as a deterministic function of all exposures prior to time t and the antibody kinetics parameters $\boldsymbol{\theta}$ which describe antibody boosting, waning, and cross-reactivity rates:

$$\mathbf{Y}_{i,t} = f(Z_{i,1}, Z_{i,2}, \dots, Z_{i,t}, \boldsymbol{\theta}) \quad (5.6)$$

where we considered a number of different functions for f . In the influenza example, $\mathbf{Y}_{i,t} = [Y_{i,1,t}, Y_{i,2,t}, \dots, Y_{i,j,t}]$, representing a vector of titres against each strain j . Observations of these latent antibody levels $\mathbf{Q}_{i,t}$ are also subject to random error in the observation process:

$$\mathbf{q}_{i,t} \sim g(\mathbf{Y}_{i,t}, \boldsymbol{\theta}) \quad (5.7)$$

Where $g(\mathbf{Y}_{i,t}, \boldsymbol{\theta})$ represents the stochastic process of generating observations from latent antibody levels. The likelihood of an observation $\mathbf{Q}_{i,t}$ given the latent antibody levels and antibody kinetics parameters is given as $P(\mathbf{Q}_{i,t}|\mathbf{Y}_{i,t}, \boldsymbol{\theta})$. The inference problem can therefore be framed as estimating the following joint posterior distribution:

$$P(\mathbf{Z}, \mathbf{Y}, \boldsymbol{\theta}|\mathbf{Q}) = \prod_{i=1}^n \left(\prod_{t=t_{1,i}}^{t_{max,i}} P(\mathbf{Q}_{i,t}|\mathbf{Y}_{i,t}, \boldsymbol{\theta}) \right) \left(\prod_{j=1}^{j_{max}} P(\mathbf{Y}_{i,j}|Z_{i,1}, Z_{i,2}, \dots, Z_{i,j}, \boldsymbol{\theta}) P(Z_{i,j}) \right) P(\boldsymbol{\theta}) \quad (5.8)$$

where θ is the vector of antibody kinetics parameters that describes the link between $\mathbf{Z}$ and $\mathbf{Y}$. $P(\mathbf{Z})$ and $P(\theta)$ are independent. Crucially, only $\mathbf{Q}$ is observed, so we must infer (or augment) the values of $\mathbf{Z}$ as latent features. The entire system can be represented as a directed acyclic graph (Fig 5.6) similar to that shown in Fig 2.9 to demonstrate its generalised form.

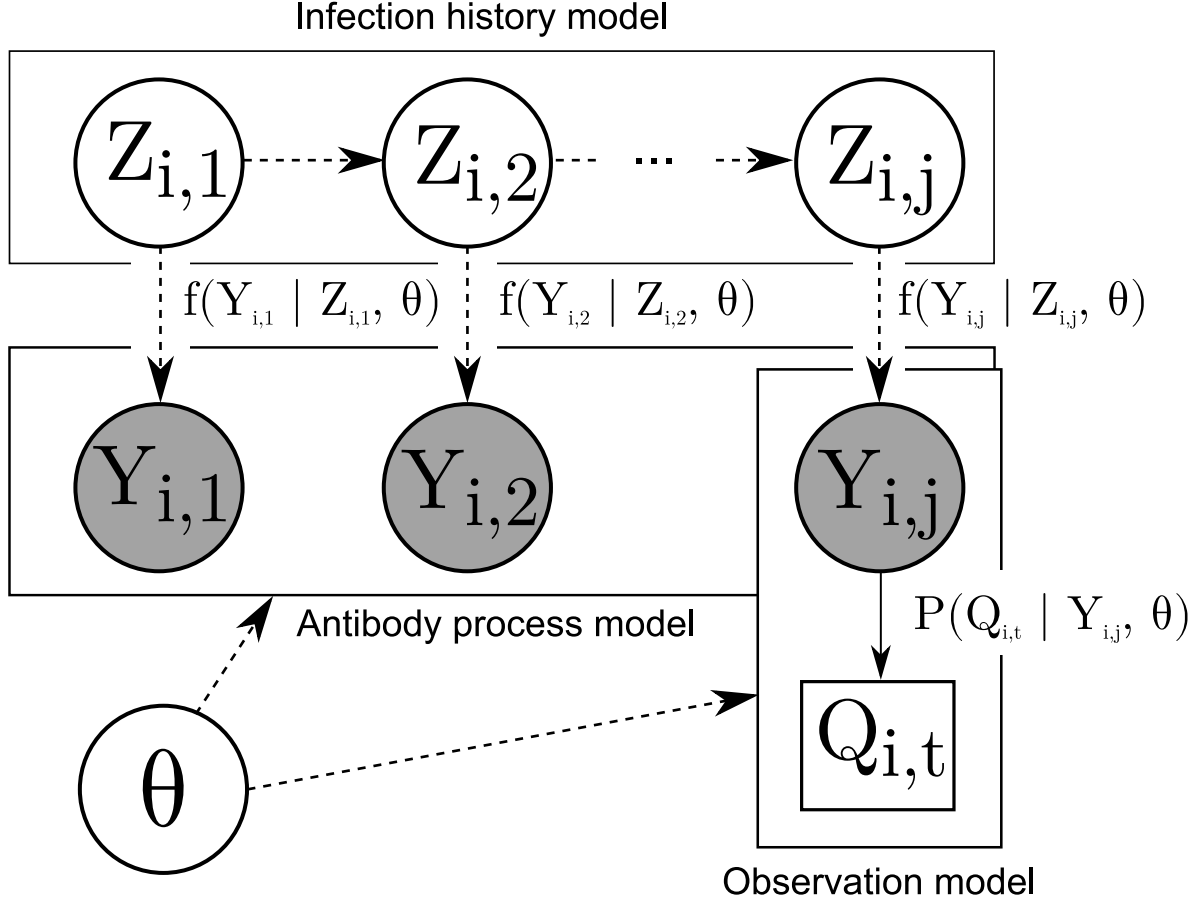


Figure 5.6: Directed acyclic graph representation of the original model. White circles represent parameters/latent states of interest ($Z_{i,j}$ shows the infection states with respect to each virus j , $Y_{i,j}$ shows the set of latent antibody titres at time j (ie. immediately after infection), and $Q_{i,t}$ shows the set of observations at time t), grey circles represent deterministic latent states, the box around $Q_{i,t}$ distinguishes the observations from the latent states, solid arrows represent stochastic dependencies, dashed arrows represent deterministic dependencies. The different model levels are shown within boxes.

5.5 Refinement of methods for inferring latent infection states

I now discuss the importance of defining a valid prior on the infection history matrix $\mathbf{Z}$. After outlining the problems arising from an incomplete approach, I present four new model definitions: (i) a beta prior on the probability of infection for a given individual at any time assuming independence between individuals; (ii) a flexible prior on the per-time probability of infection assuming that individuals are under the same infection generating process at a given time but that infection generating processes are independent across time; (iii) a beta prior on the per-time probability of infection similar to (ii); (iv) a beta prior on the probability of any given infection. These different priors have their uses depending on the distribution of titre data, as demonstrated in the simulation-recovery experiments at the end of the section. I also extended the model to incorporate immunity as a function of prior infection states. However, as this model is not used in Chapter 6, it is described only in Appendix C.3.

5.5.1 Intuitive prior

Intuitively, an uninformative prior on an indicator random variable, $Z_{i,j}$, might be that $z_{i,j} = 1$ occurs with fixed $p_{i,j} = 0.5$ and $z_{i,j} = 0$ occurs with fixed $q_{i,j} = (1 - p_{i,j}) = 0.5$ such that:

$$P(Z_{i,j} = z_{i,j}) = p_{i,j}^{z_{i,j}} (1 - p_{i,j})^{1-z_{i,j}} \quad (5.9)$$

$$P(\mathbf{Z}_i) = \prod_{j=1}^m p_{i,j}^{z_{i,j}} (1 - p_{i,j})^{1-z_{i,j}} \quad (5.10)$$

$$P(\mathbf{Z}) = \prod_{i=1}^n \prod_{j=1}^m p_{i,j}^{z_{i,j}} (1 - p_{i,j})^{1-z_{i,j}} \quad (5.11)$$

If we consider an individual's infection history $\mathbf{Z}_i$ to be a sequence of binary variables, $\mathbf{Z}_i = [Z_{i,1}, Z_{i,2}, \dots, Z_{i,m}]$, then this prior implicitly assumes that the total number of infections experienced by individual i is binomially distributed with mean pm . The total number of infections across all n individuals in a given time period, j , is also binomially distributed with mean pn , where $p = p_{i,j}$ for all individuals and all times. Setting $p_{i,j} = 0.5$ is equivalent to assuming that all infection histories are equally likely: an infection history with all $z_{i,j} = 1$ is as likely as one with all $z_{i,j} = 0$, and as likely as any other individual sequence of 1s and 0s.

Although intuitive, this set of assumptions results in a strong prior on the total number of infections. For example, pm infections are substantially more likely than an infection history with 0 or m total infections. Therefore, in the situation where there is relatively little data, $P(\mathbf{Z})$ would bias the inferred

infection histories towards pm infections per individual and pn infections per unit time. The posterior probability of an infection history with few infections and large amounts of antibody boosting per infection would be far lower than of an infection history with $0.5m$ infections and low antibody boosting.

In reality, influenza infections likely happen less frequently than every other year, though frequency does vary between individuals. Similarly, although the total number of infections in a given influenza season are well described by a binomial distribution, the distribution of infections across multiple outbreaks is likely over-dispersed relative to the binomial distribution due to between-outbreak variation in severity. A prior that allows us to capture these features would therefore be more desirable.

5.5.2 The infection history prior is not defined in the original framework

The inference framework in Kucharski *et al.* did not explicitly define the prior placed on the augmented infection events, $P(Z_{i,j})$ [1]. The prior on both the total number of infections during time j and the total number of infections during individual i 's lifetime was therefore also not defined. Kucharski *et al.* used a Metropolis-Hastings (MH) MCMC algorithm to sample from the posterior distribution of θ and $\mathbf{Z}$ given the observed data $\mathbf{Q}$. In the full MH algorithm, a proposal from the current infection history state $\mathbf{Z}$ to a proposed state $\mathbf{Z}'$ is accepted with the following probability:

$$A(\mathbf{Z}', \mathbf{Z}) = \min\left(1, \frac{P(\mathbf{Q}|\mathbf{Z}')P(\mathbf{Z}')g(\mathbf{Z}|\mathbf{Z}')}{P(\mathbf{Q}|\mathbf{Z})P(\mathbf{Z})g(\mathbf{Z}'|\mathbf{Z})}\right) \quad (5.12)$$

where $P(\mathbf{Q}|\mathbf{Z}')$ is the observation model (ie. the likelihood of observing the antibody titre data $\mathbf{Q}$ given the latent infection histories $\mathbf{Z}$); $P(\mathbf{Z})$ is the infection history prior; and $g(\mathbf{Z}'|\mathbf{Z})$ is the proposal probability or proposal distribution (ie. the conditional probability of moving from $\mathbf{Z}'$ given $\mathbf{Z}$).

The acceptance ratio $A(\mathbf{Z}', \mathbf{Z})$ can be simplified in two ways. First, $\frac{g(\mathbf{Z}|\mathbf{Z}')}{g(\mathbf{Z}'|\mathbf{Z})}$ can be removed when $g(\mathbf{Z}|\mathbf{Z}') = g(\mathbf{Z}'|\mathbf{Z})$, which is a special case of the MH algorithm called the Metropolis algorithm. Second, if the proposal distribution is the same as the prior (ie. propose values $\mathbf{Z}'$ directly from $P(\mathbf{Z}')$ such that $P(\mathbf{Z}') = g(\mathbf{Z}'|\mathbf{Z})$), then the proposal and prior probabilities cancel out in the acceptance ratio and one can sample directly from the prior and simplify the acceptance ratio to:

$$A(\mathbf{Z}', \mathbf{Z}) = \min\left(1, \frac{P(\mathbf{Q}|\mathbf{Z}')}{P(\mathbf{Q}|\mathbf{Z})}\right) \quad (5.13)$$

This was the acceptance ratio used by Kucharski *et al.* The infection history prior $P(Z_{i,j})$ and the

infection history proposal probabilities $g(\mathbf{Z}'|\mathbf{Z})$, which were not symmetric, were not explicitly included in the acceptance ratio. Using Equation 5.13 as the acceptance ratio therefore assumed that the prior $P(Z_{i,j})$ was defined by the proposal distribution, thereby placing a hidden prior on each individual's infection history.

At each MCMC iteration in the original framework, for an individual i , one of the following three proposals are made with equal probability:

1. Choose a random infection event $z_{i,j} = 1$ and change to $z_{i,j} = 0$;
2. Choose a random non-infection event $z_{i,j} = 0$ and change to $z_{i,j} = 1$;
3. Choose two entries $z_{i,j}$ and $z_{i,l}$ and swap their contents, where $j \neq l$.

Option 3's contribution to the prior can be ignored here, as this step generates symmetric proposals (choosing any combination of two entries is equally likely). However, a consequence of steps 1 and 2 is that extreme infection histories (eg. all $z_{i,j} = 0$ or all $z_{i,j} = 1$) were sampled more frequently than intermediate infection histories (eg. equal $z_{i,j} = 0$ and $z_{i,j} = 1$). For example, consider going from $\mathbf{Z}_i = (0, 0, 0)$ to $\mathbf{Z}'_i = (0, 0, 1)$. The forward proposal probability is $g(\mathbf{Z}'_i|\mathbf{Z}_i) = \frac{1}{3} \times \frac{1}{3} = \frac{1}{9}$ ie. probability of choosing the “addition” step and choosing the 3rd 0; whereas the reverse is $g(\mathbf{Z}_i|\mathbf{Z}'_i) = \frac{1}{3} \times 1$ ie. probability of choosing the “removal” step and choosing the only 1. The impact of this algorithm on the posterior can be removed by explicitly including the proposal probability ratio in the acceptance step, which recovers the prior in Equation 5.9 above.

5.5.3 Prior 1: Beta prior on per-individual infection probabilities

The above algorithm assumed that each individual was independent, but that between-time infection probabilities within an individual's infection history were not independent, as the probability of adding a new infection depended on the number of infections already in the infection history. This is not entirely unreasonable: individuals may be effectively independent if they are in different locations, and the development of adaptive immunity means that infections may become less likely as an individual experiences more infections.

This sampling strategy can be modelled with a beta-Bernoulli model by treating the probability of an

individual becoming infected as a random variable Λ_i [479]:

$$z_{i,j} \sim \text{Bernoulli}(\lambda_i) \quad (5.14)$$

$$\lambda_i \sim \text{Beta}(\alpha, \beta) \quad (5.15)$$

This places a beta prior on the per-time probability of infection, assuming that all individuals' infection probabilities are independent and not identically distributed (ie. each individual has a unique Λ_i). The prior probability of a particular infection history for individual i , $\mathbf{Z}_i$, is therefore given by a standard beta-Bernoulli distribution with probability Λ_i . It is possible to marginalise over Λ_i to define $P(\mathbf{Z}_i)$ directly.

If the prior on Λ_i follows the beta distribution:

$$P(\Lambda_i) = \frac{1}{B(\alpha, \beta)} \Lambda_i^{\alpha-1} (1 - \Lambda_i)^{\beta-1} \quad (5.16)$$

where B is the beta function defined as $B(\alpha, \beta) = \int_0^1 t^{\alpha-1} (1-t)^{\beta-1} dt$. The likelihood of $\mathbf{Z}_i$ given $\Lambda_i = \lambda_i$ is:

$$P(\mathbf{Z}_i | \Lambda_i = \lambda_i) = \lambda_i^{k_i} (1 - \lambda_i)^{m_i - k_i} \quad (5.17)$$

then the marginal distribution of $P(\mathbf{Z}_i)$ is:

$$P(\mathbf{Z}_i) = \mathbb{E}_P(P(\mathbf{Z}_i) | \Lambda_i) \quad (5.18)$$

$$= \int_0^1 \lambda_i^{k_i} (1 - \lambda_i)^{m_i - k_i} P(\lambda_i) d\lambda_i \quad (5.19)$$

$$= \frac{B(\alpha + k_i, \beta + m_i - k_i)}{B(\alpha, \beta)} \quad (5.20)$$

$$= \frac{\alpha^{[k_i]} \beta^{[m_i - k_i]}}{(\alpha + \beta)^{[m_i]}} \quad (5.21)$$

where $\mathbf{Z}_i = [Z_{i,1}, Z_{i,2}, \dots, Z_{i,m}]$, k_i is the total number of infections experienced by individual i ($\sum_{j=1}^m Z_{i,j}$), m_i is the number of time periods that individual i could be infected in, and $r^{[x]}$ denotes the ascending power $r(r+1) \dots [r+(x-1)]$. The probability mass function for the total number of infections k_i is therefore given by:

$$P(k_i, m_i | \alpha, \beta) = \binom{m_i}{k_i} \frac{B(\alpha + k_i, \beta + m_i - k_i)}{B(\alpha, \beta)} \quad (5.22)$$

which is the beta-binomial distribution. This prior makes the following assumptions:

1. Each p_i comes from a single draw from the same beta distribution;
2. All λ_i are equal for a given i (ie. all $z_{i,j}$ are drawn from the same Bernoulli distribution);
3. All p_j are independent for a given j (ie. each p_j is drawn from a different distribution for each j).

Formulating the prior in this way allows an explicit prior to be defined through α and β on the total number of infections in a particular infection history $\mathbf{Z}_i$, with $\mathbb{E}(k_i) = m \frac{\alpha}{\alpha+\beta}$ and $\text{Var}(k_i) = m \frac{\alpha\beta}{(\alpha+\beta)^2} [1 + (m-1) \frac{1}{\alpha+\beta+1}]$. An intuitive uniform prior on an infection history would therefore be that any total number of lifetime infections is equally likely, which is the case where $\alpha = \beta = 1$. In addition, as $\lim \alpha = \beta \rightarrow \infty$, $P(k, m | \alpha, \beta) \rightarrow \text{Binom}(k, m)$, where any infection history is equally likely. A more informative prior on $\mathbf{Z}_i$ is also possible by choosing values for α and β that give a desired mean and variance on the total number of infections per individual. I assume that α and β are the same for all individuals.

Appendix E.3 provides an algorithm implementing a Gibbs sampler for the above prior.

Prior on attack rates

The assumption of independent individuals and a beta-Bernoulli prior on the total number of lifetime infections places a binomial prior on the attack rate within a given time period j across n individuals. The marginal likelihood of infection in an individual's infection history is the same across all individuals, such that:

$$P(Z_{i,j} = 1 | P_{i,j} = p_{i,j}) = p_{i,j} \quad (5.23)$$

$$P(P_{i,j} = p_{i,j}) = \frac{p_{i,j}^{\alpha-1} (1 - p_{i,j})^{\beta-1}}{B(\alpha, \beta)} \quad (5.24)$$

$$P(Z_{i,j} = 1) = \int_0^1 P(Z_{i,j} = 1 | P_{i,j} = p_{i,j}) P(P_{i,j} = p_{i,j}) dp_{i,j} \quad (5.25)$$

$$= \int_0^1 p_{i,j} \frac{p_{i,j}^{\alpha-1} (1 - p_{i,j})^{\beta-1}}{B(\alpha, \beta)} dp_{i,j} \quad (5.26)$$

$$= \mathbb{E}(p_{i,j}) \quad (5.27)$$

$$= \frac{\alpha}{\alpha + \beta} \quad (5.28)$$

Individuals are independent and therefore all $p_{i,j}$ are independent across j , and α and β are the same

for all individuals i . It then follows that $P(\sum \mathbf{Z}_j = Z_{1,j} + Z_{2,j} + \dots + Z_{n,j})$ is binomially distributed with probability $\frac{\alpha}{\alpha+\beta}$ and $N = n$, the number of individuals. Importantly, $P(\sum \mathbf{Z}_j)$ is binomially distributed with mean $0.5n$ for all $\alpha = \beta$, even in the case where $\alpha = \beta = 1$.

This prior would suggest that the infection status of individual $n+1$ during time j follows the Bernoulli distribution with $p_j = \frac{\alpha}{\alpha+\beta}$, and the overall number of infections k_j follows the binomial distribution with the same p_j and $N = n_j$. This does fulfil a number of desirable properties: (i) if we know that a proportion $\frac{\alpha}{\alpha+\beta}$ of the population were infected, then the expectation of the attack rate prior would also be $\frac{\alpha}{\alpha+\beta}$; (ii) certainty in the attack rate estimate should increase with increasing n . However, with large n and this binomial prior, the majority of the probability density for the attack rate is in a relatively small region of parameter space, resulting in a prior that strongly influences the posterior, and might therefore swamp the likelihood. Furthermore, given that $\mathbb{E}(p_j) = \frac{\alpha}{\alpha+\beta}$, then necessarily $p_1 = p_2 = \dots = p_m$ for all m .

Comparison of original prior and beta prior on per-individual infection probability

The prior distribution on attack rates and total number of lifetime infections were compared when simulating directly from the analytical form of the beta prior and when using the proposal algorithm described in Appendix E.3. Fig 5.7 shows the results of running a random walk with 100% acceptance using the original proposal function (ie. using the R function directly from Kucharski *et al.*) and the new beta-binomial proposal function compared to simulating directly from a beta-binomial random generation function in R. Performing a random walk in this way with 100% acceptance but no likelihood function samples directly from the prior. These results show that the empirical prior on the total number of lifetime infections is truly beta-binomially distributed under the new proposal function. Using the original proposal function from Kucharski *et al.* gave an empirical prior similar to the beta-binomial, but with bias at the tails of the distribution. The empirical prior on the proportion of individuals infected in a given time period was binomially distributed in both cases, which explains why the attack rate estimates in Fig 5.4 are highly constrained but biased given the large number of individuals ($n = 1,130$).

5.5.4 Prior 2: Prior on per-time infection probability

When individuals are in the same population, their risks of infection are likely correlated in a given time period. The assumption of independence between individuals is therefore unrealistic, and a model that takes this dependence into account is more appropriate.

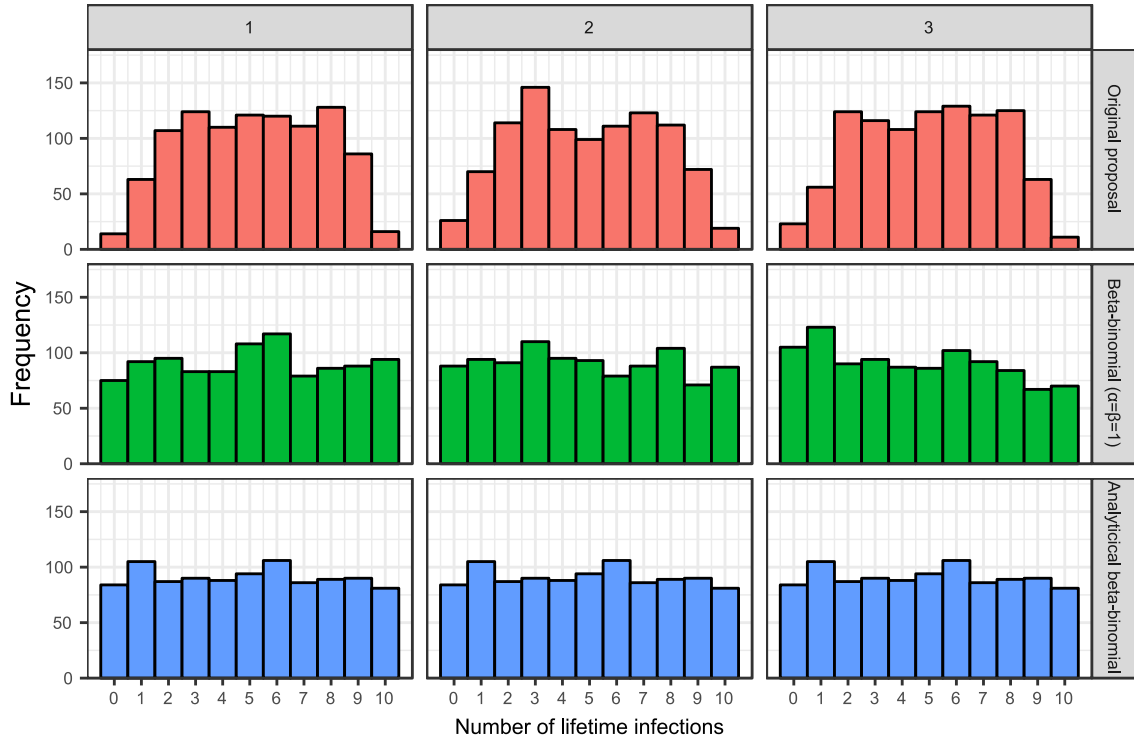


Figure 5.7: Empirical priors on the total number of infections over a 10 year period in the inference framework compared to random samples from the beta-binomial density. Top and middle rows show histogram of total number of lifetime infections for 3 individuals obtained from random walks of 100000 iterations from the original proposal and updated beta proposal function. Bottom row shows histogram of 1000 random samples from a beta-binomial density with parameters $\alpha = \beta = 1$.

An alternative model is therefore to assume that individuals are infected by the same infection process during a given time period (ie. there is a force of infection on the population), and that infection processes are independent across time periods. There is an intuition to this approach which is revealed by the following question: given a sample of n individuals for which we know all n infection states for time j , what is the prior predictive probability that individual $n + 1$ was also infected during time j ? If we know that 80% of the population were infected at time j , then we should have some prior belief that individual $n + 1$ was also infected.

Under this prior, the probability of infection is given as $p = \Phi_j$. Φ is related to the attack rate and therefore gives the probability of any individual in the population becoming infected. Under this prior, the infection generating process is:

$$z_{i,j} \sim \text{Bernoulli}(\phi_j) \quad (5.29)$$

$$\phi_j \sim f(j) \quad (5.30)$$

Where f is any arbitrary function describing the distribution of Φ . The probability mass function for an individual infection event in time period j is therefore given by:

$$P(Z_{i,j}|\Phi_j = \phi_j) = \phi_j^{Z_{i,j}}(1 - \phi_j)^{(1-Z_{i,j})} \quad (5.31)$$

Thus, the likelihood of observing a particular combination of infections at time j is given by a Bernoulli process:

$$\begin{aligned} P([Z_{1,j}, Z_{2,j}, \dots, Z_{n,j}]|\Phi_j = \phi_j) &= \prod_{i=1}^n \phi_j^{Z_{i,j}}(1 - \phi_j)^{(1-Z_{i,j})} \\ &= \phi_j^{k_j}(1 - \phi_j)^{(n_j - k_j)} \end{aligned}$$

Retaining the correlation between individuals and adding m infection times to the system, the likelihood of $\mathbf{Z}$ conditional on Φ becomes:

$$P(\mathbf{Z} | [\Phi_1 = \phi_1, \Phi_2 = \phi_2, \dots, \Phi_m = \phi_m]) = \prod_{j=1}^m \phi_j^{k_j}(1 - \phi_j)^{(n_j - k_j)} \quad (5.32)$$

Where $\mathbf{Z}$ is an n by m matrix representing the outcome of m possible infection events for n individuals. The first example in Section 5.5.1 above makes the strong assumption that Φ_j is fixed at 0.5 for all times j . To avoid this strong assumption of binomially distributed attack rates, we can assume that all Φ are unknown parameters to be estimated by defining a prior on Φ . Equation 5.8 is then modified to give:

$$P(\mathbf{Z}, \mathbf{Y}, \boldsymbol{\theta}, \Phi | \mathbf{Q}) = \frac{P(\mathbf{Z}, \mathbf{Y}, \Phi, \boldsymbol{\theta}, \mathbf{Q})}{P(\mathbf{Q})} \quad (5.33)$$

$$= \frac{P(\mathbf{Q} | \mathbf{Z}, \mathbf{Y}, \Phi, \boldsymbol{\theta}) P(\mathbf{Z}, \mathbf{Y}, \Phi, \boldsymbol{\theta})}{P(\mathbf{Q})} \quad (5.34)$$

$$= \frac{P(\mathbf{Q} | \mathbf{Z}, \mathbf{Y}, \Phi, \boldsymbol{\theta}) P(\mathbf{Z}, \mathbf{Y} | \Phi, \boldsymbol{\theta}) P(\Phi, \boldsymbol{\theta})}{P(\mathbf{Q})} \quad (5.35)$$

$$= \frac{P(\mathbf{Q} | \mathbf{Y}, \boldsymbol{\theta}) P(\mathbf{Y} | \mathbf{Z}, \boldsymbol{\theta}) P(\mathbf{Z} | \Phi) P(\Phi) P(\boldsymbol{\theta})}{P(\mathbf{Q})} \quad (5.36)$$

$$P(\mathbf{Z}, \mathbf{Y}, \boldsymbol{\theta}, \boldsymbol{\Phi} | \mathbf{Q}) \propto \prod_{i=1}^n \left(\prod_{t=t_{1,i}}^{t_{max,i}} P(\mathbf{Q}_{i,t} | \mathbf{Y}_{i,t}, \boldsymbol{\theta}) \right) \left(\prod_{j=1}^{j_{max}} P(\mathbf{Y}_{i,j} | Z_{i,1}, Z_{i,2}, \dots, Z_{i,j}, \boldsymbol{\theta}) P(Z_{i,j} | \Phi_j) P(\Phi_j) \right) P(\boldsymbol{\theta}) \quad (5.37)$$

This structure opens up a number of useful possibilities. For example: $\boldsymbol{\Phi}$ may be defined as a function rather than a variable; different priors may be placed on different times j ; $\boldsymbol{\Phi}$ may be inferred explicitly. Fig 5.8 shows an update of Fig 5.6 taking into account the infection generating process.

Prior on number of lifetime infections

Assuming that all $P(\Phi)$ are independent, the total number of lifetime infections for an individual follows a binomial distribution with $p = \mathbb{E}(\Phi)$. Using a beta prior for $P(\Phi)$ with parameters α and β and assuming that α and β are equal for all j , then $P(\sum_j Z_{i,j} = Z_{i,1} + Z_{i,2} + \dots + Z_{i,m})$ follows a binomial distribution with mean $\frac{\alpha}{\alpha+\beta}$ and $N = m_i$, where m_i is the number of time periods that individual i could be infected in. A binomial prior is intuitive here: if the expectation of the attack rates for all times j is $p = 0.5$, then one would assume *a priori* that individuals are infected in every other time period.

5.5.5 Prior 3: Beta prior on per-time infection probability

The above prior allows for explicit control over the form of $P(\Phi)$, but also results in a large number of additional nuisance parameters that must be estimated (each Φ_j). It is possible to calculate the marginal distribution $P(\mathbf{Z})$ under the above prior by integrating out $\boldsymbol{\Phi}$. In terms of MCMC mixing, integrating over possible all possible Φ_j for each j reduces the number of free parameters to be estimated rather than needing to estimate each Φ_j . This is particularly useful because inferring posterior distributions for $\boldsymbol{\Phi}$ and $\mathbf{Z}$ simultaneously based on Equation 5.37 is practically difficult due to their clear correlation, particularly when m is large. The reader is referred to related work on the Indian Buffet Process: a stochastic process defining a probability distribution over sparse binary matrices with finite rows and infinite columns [480]. This problem is the related case where binary matrices are not necessarily sparse, and the number of columns can be considered finite. However, there is a potential avenue for infection history inference where the number of infection periods (the number of columns) is not necessarily fixed and finite, and therefore I refer to this work here.

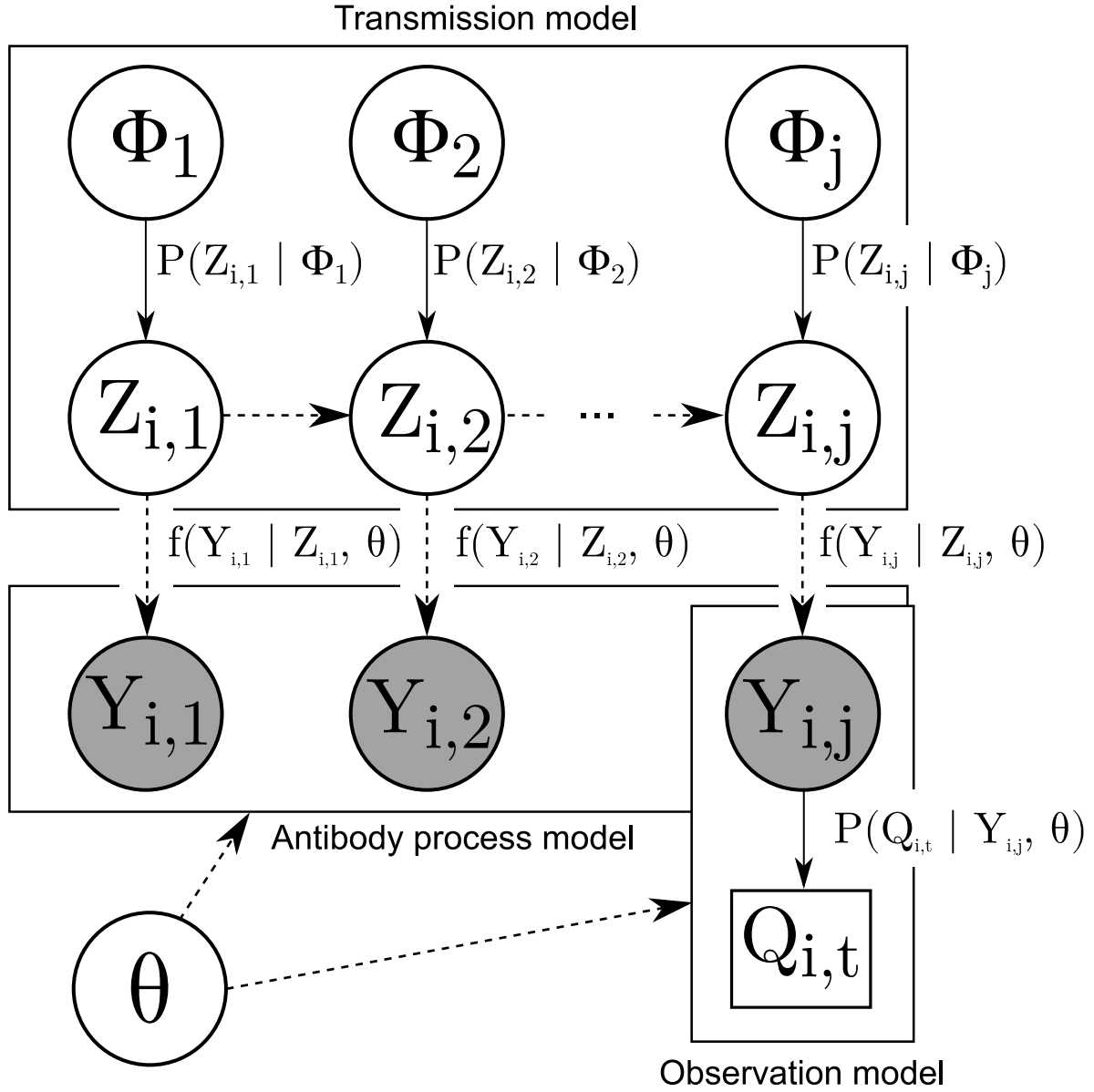


Figure 5.8: Directed acyclic graph representation of the updated model. White circles represent parameters/latent states of interest ($Z_{i,j}$ shows the infection states with respect to each virus j , $Y_{i,j}$ shows the set of latent antibody titres at time j , and $Q_{i,j}$ shows the set of observations at time j , Φ_j shows the probability of any individual becoming infected during time j), grey circles represent deterministic latent states, solid arrows represent stochastic dependencies, dashed arrows represent deterministic dependencies. The different model levels are shown within boxes. Note that $f(Y_{i,j} | Z_{i,1}, Z_{i,2}, \dots, Z_{i,j}, \theta)$ has been simplified for notational convenience.

First, define a beta-Bernoulli process for the generation of $\mathbf{Z}$ as:

$$z_{i,j} \sim \text{Bernoulli}(\phi_j) \quad (5.38)$$

$$\phi_j \sim \text{Beta}(\alpha, \beta) \quad (5.39)$$

The prior probability of $P(\Phi_j = \phi_j)$ is defined as:

$$P(\Phi_j = \phi_j) = \frac{\phi_j^{\alpha-1}(1-\phi_j)^{\beta-1}}{B(\alpha, \beta)} \quad (5.40)$$

where $B(\alpha, \beta)$ is the Beta function:

$$B(\alpha, \beta) = \int_0^1 \phi_j^{\alpha-1}(1-\phi_j)^{\beta-1} d\phi_j \quad (5.41)$$

$$= \frac{\Gamma(\alpha)\Gamma(\beta)}{\Gamma(\alpha + \beta)} \quad (5.42)$$

$Z_{i,j}$ is independent of all other entries in $\mathbf{Z}$, conditional on Φ_j which are also assumed to be independent of all other entries in Φ . $P(Z_{i,j})$ can then be calculated directly without representing Φ by integrating over all Φ , giving the marginal likelihood of the entire infection history matrix $\mathbf{Z}$ as:

$$P(\mathbf{Z}) = \prod_{j=1}^m \int_0^1 \left(\prod_{i=1}^n P(Z_{i,j} | \Phi_j = \phi_j) \right) P(\Phi_j = \phi_j) d\phi_j \quad (5.43)$$

$$= \prod_{j=1}^m \frac{B(k_j + \alpha, \beta + n_j - k_j)}{B(\alpha, \beta)} \quad (5.44)$$

In the MCMC framework, new values for each $Z_{i,j}$ may be sampled directly from this prior as:

$$P(Z_{i,j} = 1 | \mathbf{Z}_{-i,j}, \alpha, \beta) = \frac{P(Z_{i,j} = 1, \mathbf{Z}_{-i,j})}{P(\mathbf{Z}_{-i,j})} \quad (5.45)$$

$$= \frac{\alpha^{[k_j+1]} \beta^{[n_j-k_j]} (\alpha + \beta)^{[n_j]}}{(\alpha + \beta)^{[n_j+1]} \alpha^{[k_j]} \beta^{[n_j-k_j]}} \quad (5.46)$$

$$= \frac{k_j + \alpha}{n_j + \alpha + \beta} \quad (5.47)$$

Giving the proposal probability of $z_{i,j} = 1$ and $z_{i,j} = 0$ otherwise, where k_j is the number of infections during time j less $z_{i,j}$, and n_j is the number of individuals alive during time j less individual i . The acceptance probability then just becomes the ratio of likelihoods in the Metropolis step as in Equation 5.13.

Values for α and β may be chosen to give a prior on the attack rate with known properties: $\mathbb{E}(k_j) = n \frac{\alpha}{\alpha + \beta}$ and $\text{Var}(k_j) = n \frac{\alpha\beta}{(\alpha + \beta)^2} [1 + (n - 1) \frac{1}{\alpha + \beta + 1}]$. When $\alpha = \beta$, the attack rate prior has an expectation of $0.5n$, and the variance may be decreased by increasing α and β . For example, values of α and β that

have a desired mode and certainty may be chosen by solving the following:

$$\alpha = Mo(c - 2) + 1 \quad (5.48)$$

$$\beta = (1 - Mo)(c - 2) + 1 \quad (5.49)$$

Where Mo is the desired mode, and c is analogous to the number of prior observations (ie. $c = 2$ corresponds to having seen two prior outcomes). Values for α and β that have a particular mean with the largest possible variance are found by solving:

$$\alpha = \bar{\phi}^2 \frac{1 - \bar{\phi}}{\sigma_{\phi} - \frac{1}{\bar{\phi}}} \quad (5.50)$$

$$\beta = \alpha \frac{1}{\bar{\phi} - 1} \quad (5.51)$$

where $\bar{\phi}$ is the desired mean attack rate, and σ_{ϕ} is the maximum variance for ϕ that results in a uni-modal distribution of ϕ . Note that values of α and β may be set that lead to a multi-modal distribution of ϕ eg. $\alpha = \beta = \frac{1}{2}$.

Prior on number of lifetime infections

The implicit prior on an individual's number of lifetime infections is the same as in Section 5.5.3: the beta prior on $P(\phi)$ with parameters α and β resulting in a binomial distribution on the total number of lifetime infections with mean $\frac{\alpha}{\alpha+\beta}$ and $N = m_i$, where m_i is the number of time periods that individual i could be infected.

5.5.6 Prior 4: Beta prior on the probability of any infection event

The final and perhaps most truly “uninformative” prior comes from the assumption that *all* infections are independent and identically distributed events; belonging to a common group or time period is considered irrelevant and the order does not matter. In this case:

$$z_{i,j} \sim \text{Bernoulli}(\phi) \quad (5.52)$$

$$\phi \sim \text{Beta}(\alpha, \beta) \quad (5.53)$$

Such that:

$$P(\Phi = \phi) = \frac{\phi^{\alpha-1}(1-\phi)^{\beta-1}}{B(\alpha, \beta)} \quad (5.54)$$

$$P(Z_{i,j}|\Phi = \phi) = \phi^{Z_{i,j}}(1 - \phi)^{1-Z_{i,j}} \quad (5.55)$$

and the marginal likelihood of Z is:

$$P(\mathbf{Z}) = \int_0^1 \left(\prod_{j=1}^m \prod_{i=1}^n P(Z_{i,j}|\Phi = \phi) \right) P(\Phi = \phi) d\phi \quad (5.56)$$

$$= \frac{B(k + \alpha, \beta + nm - k)}{B(\alpha, \beta)} \quad (5.57)$$

where k is the total number of infections across all years and individuals and nm is the total number of possible infection events. This gives the conditional probability that an individual was infected at a given time, and also a distribution to draw new infection states from:

$$P(Z_{i,j} = 1|\mathbf{Z}_{-i,j}, \alpha, \beta) = \int_0^1 P(Z_{i,j}|\Phi = \phi) P(\Phi = \phi|\mathbf{Z}_{-i,j}) d\phi \quad (5.58)$$

$$= \frac{k_{-i} + \alpha}{nm_{-i} + \alpha + \beta} \quad (5.59)$$

This assumption has the desirable property of placing a beta prior on both the total number of infections over a lifetime for a given individual and on the total number of infections during a given time. However, these properties are traded off against the strong and potentially unrealistic assumption that infection events are conditionally independent across both time and individuals.

5.6 Results

5.6.1 Comparison of infection history priors using simulation results

Fig 5.9 compares infection histories simulated from models with (i) a beta prior on the per-individual probability of infection (Section 5.5.3), (ii) a beta prior on per-time infection probability (Section 5.5.5) and (iii) a beta prior on the probability of any infection event (Section 5.5.6) compared to the analytical priors on the total number of infections per unit time j and per lifetime of individual i . These results are intended to highlight the contribution of the different priors on inferred attack rates, total number of lifetime infections and overall number of infections. In all simulations, I used 100 individuals who could each be infected in 60 time periods ($n = 100$ and $m = 60$). Note that in the rightmost panel in all these plots, the distribution for the total across nm is not defined, but resembles an over-dispersed binomial distribution.

Fig 5.10 shows the prior on an individual's cumulative number of lifetime infections by running the full MCMC framework under each prior with the contribution of the likelihood set to 0 (ie. sampling from the priors). A uniform beta prior is used with $\alpha = \beta = 1$, though we see that this leads to a highly constrained binomial prior on the total number of lifetime infections in the top two panels, despite a uniform prior on the probability of infection in any given year as in Fig 5.9. Conversely, in the bottom two panels with the same beta parameters, the prior on the total number of lifetime infections follows the beta-binomial distribution. The different shapes and widths of these distributions demonstrate how different prior forms may lead to strong assumptions about an individual's infection history. Fig D.1 makes the same comparison, but choosing informative beta parameters of $\alpha = 2$ and $\beta = 10$.

5.6.2 Impact of prior choice on model estimates

To illustrate the impact of different prior assumptions on infection history and antibody kinetics parameter inference, I ran simulation-recovery experiments for (i) a beta prior on the per-individual probability of infection (Section 5.5.3), (ii) a beta prior on per-time infection probability (Section 5.5.5) and (iii) a beta prior on the probability of any infection event (Section 5.5.6) with varying amounts of titre data and beta prior parameters. The simulated sero-survey designs are described in Table 5.4. For each prior version, I considered three data scenarios: (i) sparse data, only one blood sample and titres against 9 viruses taken; (ii) full data, one blood sample and titres against each of the 41 viruses (one per year); (iii) additional data, 5 blood samples taken at random intervals between 2000 and

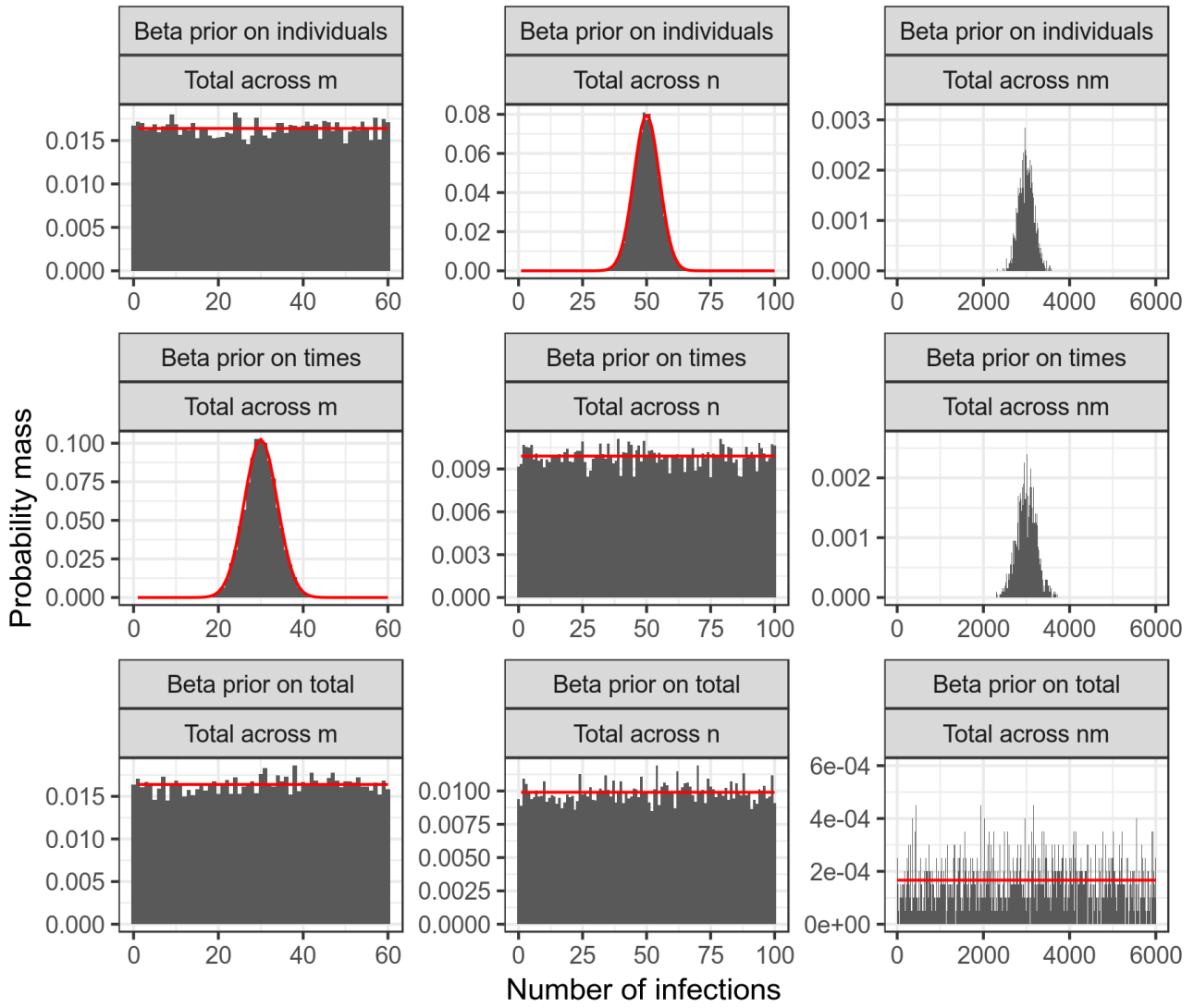


Figure 5.9: Simulated versus analytical infection history priors when $\alpha = \beta = 1$. Bars show a density histogram of infections from 20000 simulated infection histories. The red lines show known probability mass function. Top row refers to the assumption that a beta prior is placed on the probability of an infection within an individual's infection history, but that individuals are independent. Middle row refers to the assumption that a beta prior is placed on the attack rate within a given time period, and that time periods are independent. Bottom row refers to the assumption that a beta prior is placed on the probability of an infection in any time period for any individual. Leftmost column (total across m) refers to the distribution of lifetime infections for one individual. Middle column refers to the distribution on attack rate for a single time period (total across n). Rightmost column refers to the distribution of the total number of infections across all times and individuals (total across nm).

2009, with 41 viruses tested for each blood sample. These three data scenarios represent a range of low data contribution to the posterior up to extremely high data contribution. For each data scenario and prior version, I tested 4 beta prior assumptions: (i) neutral prior with $\alpha = \beta = \frac{1}{3}$; (ii) a uniform prior with $\alpha = \beta = 1$; (iii) weakly informative prior with prior probability of infection mode of 0.15 and high variance, with $\alpha = 1.3$ and $\beta = 2.7$, conceptually similar to a prior informed by 4 previous

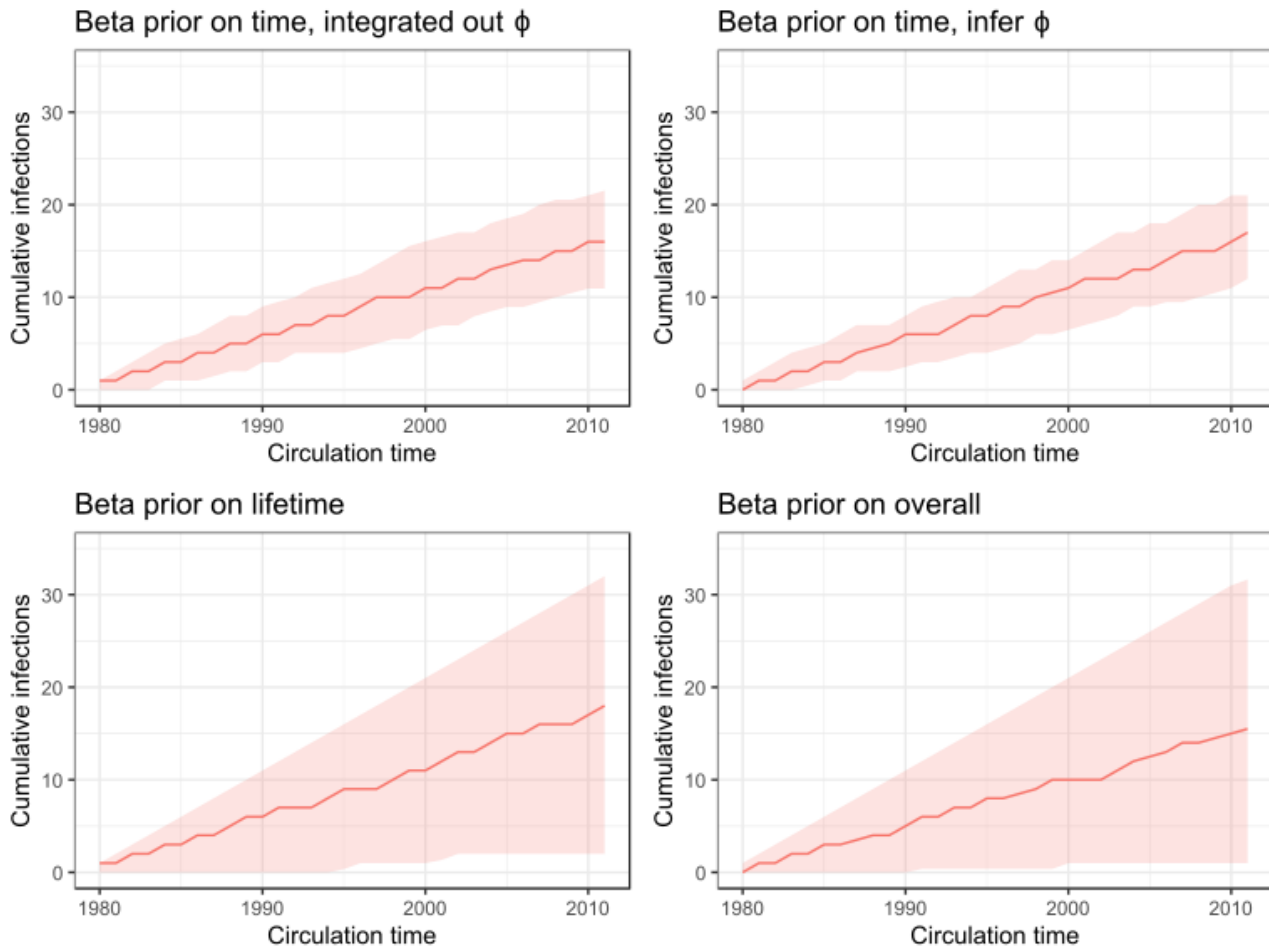


Figure 5.10: Priors on cumulative number of lifetime infections when $\alpha = \beta = 1$. Subplot titles show assumed prior. Red line shows posterior median. Red shaded regions show posterior 95% credible intervals.

infection observations; (iv) strongly informative prior with prior probability of infection mode of 0.15 and low variance, with $\alpha = 15.7$ and $\beta = 84.3$, conceptually similar to a prior informed by 100 previous infection observations. I ran the MCMC framework to generate 5 chains of 1000000 iterations for these scenarios with a 200000 iteration burn in period.

Data set parameters			Model parameters				
n	200	Parameter	Description	Value	Prior lower bound	Prior upper bound	Estimated
Year min	1968	μ_l	Long term antibody boosting	1.8	0	8	Yes
Year max	2009	μ_s	Short term antibody boosting	2.7	0	8	No
m	41	σ_l	Long term cross reactivity	0.1	0	1	Yes
Time resolution (infection states)	annual (48)	σ_s	Short term cross reactivity	0.03	0	1	No
Number of serum samples per individual	1/1/5	τ	Suppression	0.05	0	1	Yes
Number of viruses tested	9/41/41	ω	Waning	0.8	0	1	Yes
Number of titre measurement repeats	1	ϵ	Measurement error	0.8	0	25	Yes
Total number of measurements	Varied	α	Infection history prior	$\frac{1}{3}/1/1.3/15.7$	NA	NA	No
First sample year	2000	β	Infection history prior	1	$\frac{1}{3}/1/2.7/84.3$	NA	No
Final sample year	2009						
Minimum age (years)	10						
Maximum age (years)	75						
Infection history prior	Priors 1, 3 and 4						

Table 5.4: Simulation settings to compare different infection history prior assumptions.

Fig 5.11 shows how placing priors on the per-time probability of infection and the overall probability

of infection recovers unbiased estimates of the long-term boosting parameter μ_l for all data and prior scenarios, whereas the prior on per-individual probability of infection is only unbiased with a large amount of data or strong prior information. Under this survey design, using prior versions 3 (beta prior on the per-time probability of infection) or 4 (beta prior on the overall probability of infection) would be recommended for estimating long-term dynamics and attack rates. This is supported by Fig 5.12, where recovering the true attack rates shows little bias under all scenarios for prior versions 3 and 4, but strong bias in all but the strongest data scenario for prior version 1 (beta prior on per-individual infection probability). Attack rate estimation becomes increasingly precise with increasing data availability.

Figs D.2–D.4 show the ability of these different priors to infer the same individual’s infection history. Prior versions 3 and 4 are able to accurately recover the timing of the individual’s infections even under the neutral and uniform priors. Prior version 1 does not recover constrained posterior estimates for the cumulative infection history under all but the strongest prior (Fig D.2). However, under the more data rich scenarios, prior version 1 is able to recover unbiased estimates of the true cumulative infection history, despite clear bias in the inferred attack rates (not shown). These results highlight that these different priors have their uses depending on the distribution of titre data, the resolution of the model and the particular question under consideration: data sets may be rich in different dimensions (eg. number of individuals versus number of viruses tested), which leads to different levels of inferential accuracy for different quantities. For example, a data set with very few individuals but a large number of tested titres per individual may be well suited to analysis under prior version 1 where the aim is to infer an individual’s lifetime infection history, but not necessarily population-level attack rates. Table D summarises each of these priors and the situations when each one is advised.

5.6.3 Refinement of real historical attack rate estimates

Fig 5.13 demonstrates the impact of using the refined prior version 2 (using a uniform beta prior on per-time infection probability, but not marginalising over each Φ_j with $\alpha = \beta = 1$) compared to prior version 1 (using a uniform beta-Bernoulli process to represent the prior used in the original framework with $\alpha = \beta = 1$) on inferred attack rates. Simulation-recovery of a cohort matching exactly the real Ha Nam cohort (Table 5.5) with known θ and $\mathbf{Z}$ using prior version 1 recovered the general trends in historical attack rates, but demonstrated significant over- or under-estimates for years long before serum samples were taken (Fig 5.13A). Accuracy was far higher in years where data was taken. Fitting the model with prior version 1 to the real data (Fig 5.13B) revealed epidemiological dynamics in line with expectation; attack rates were high in 1968 after the introduction of A/H3N2 and declined steadily

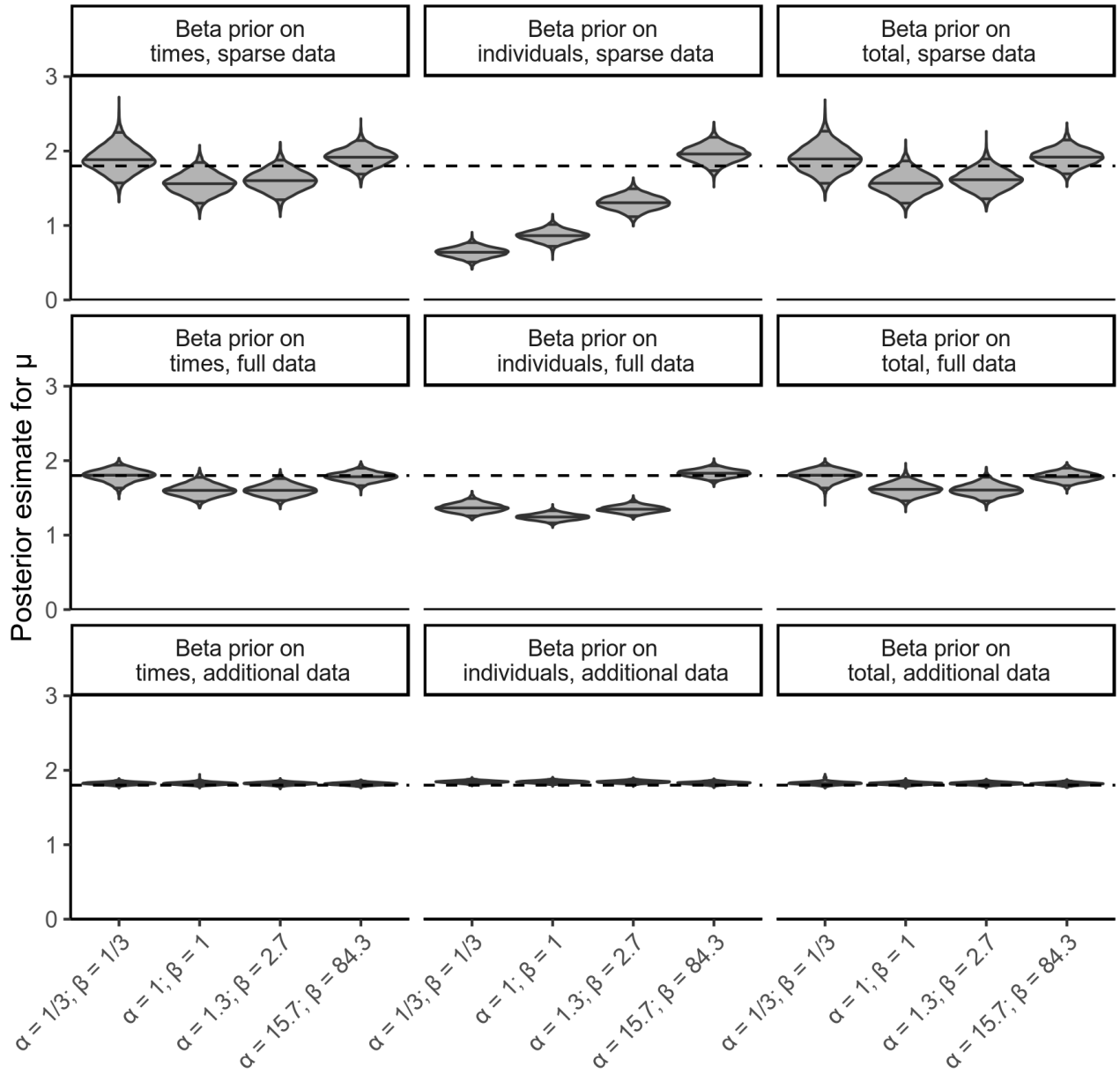


Figure 5.11: Posterior distribution of long-term boosting parameters μ_l under different prior and data scenarios. Horizontal dashed line shows true parameter value $\mu_l = 1.8$, shaded regions show inferred posterior distribution with medians and 95% credible intervals are shown as solid horizontal lines. X-axis shows assumed beta prior parameters. Left-hand column shows results under prior version 3 (beta prior on per-time infection probability, ϕ_j); middle column shows results under prior version 2 (beta prior on per-individual infection probability, Λ_i); right-hand column shows results under prior version 4 (beta prior on overall infection probability, ϕ).

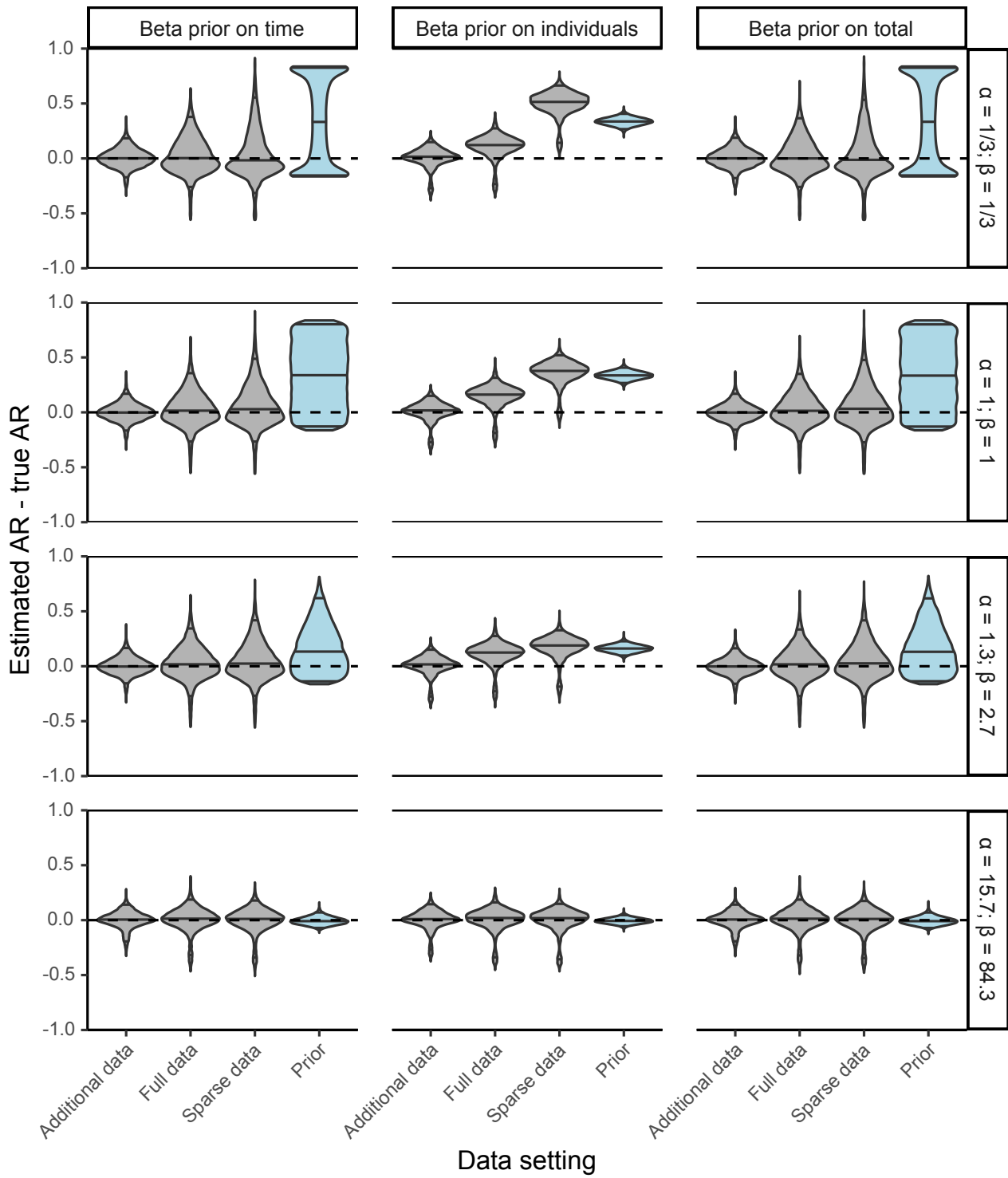


Figure 5.12: Accuracy of estimated attack rates under the various priors, prior strengths and data scenarios. Violin plots show posterior distribution of inferred attack rate minus the true attack rate. Horizontal lines show posterior medians and 95% credible intervals. X-axis shows decreasing contribution of the data relative to the prior (far right). Top labels show assumed infection history prior version. Right labels show prior parameter settings.

to low attack rates during the 1970s and 80s. Attack rates increased drastically in the early 2000s, which corresponds to a cluster transition to Fujian 2002 [481].

Data set parameters			Model parameters				
n	69	Parameter	Description	Value	Prior lower bound	Prior upper bound	Estimated
Year min	1968	μ_l	Long term antibody boosting	1.8	0	8	Yes
Year max	2012	μ_s	Short term antibody boosting	2.7	0	8	Yes
m	44	σ_l	Long term cross reactivity	0.1	0	1	Yes
Time resolution (infection states)	annual (48 years)	σ_s	Short term cross reactivity	0.03	0	1	Yes
Number of serum samples per individual	6	τ	Suppression	0.05	0	1	Yes
Number of viruses tested	22	ω	Waning	0.8	0	1	Yes
Number of titre measurement repeats	up to 6	ϵ	Measurement error	1	0	25	Yes
Total number of measurements	11597	α	Infection history prior	1	NA	NA	Yes
First sample year	2007	β	Infection history prior	1	NA	NA	Yes
Final sample year	2012						
Minimum age (years)	10						
Maximum age (years)	72						
Infection history prior	Priors 1 and 2						

Table 5.5: Settings for simulations matching the Ha Nam cohort.

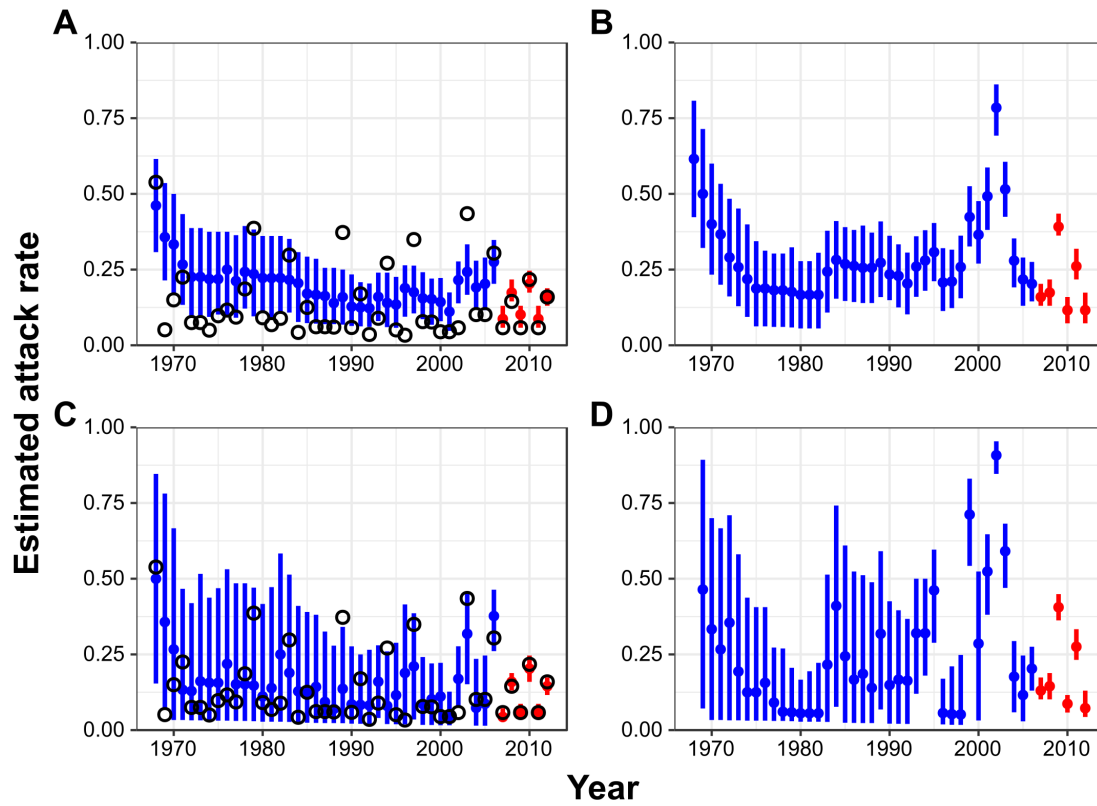


Figure 5.13: Inferred annual A/H3N2 attack rates using real and simulated Ha Nam data comparing original and refined framework with prior version 2 (beta prior on per-time infection probability, ϕ_j). Points and lines show posterior median and 95% credible intervals on attack rate estimates. Red points show years in which serum samples were taken. (A) Inference of attack rates using original framework on simulated serological data from Ha Nam, Vietnam. Hollow black points show true annual attack rates from which infection histories were simulated. Results show substantial bias in some years. (B) Inference of attack rates using original framework on real serological data from Ha Nam, Vietnam. (C) Inference of attack rates using refined framework on simulated serological data from Ha Nam, Vietnam. Hollow black points show true annual attack rates from which infection histories were simulated. Results show increase accuracy relative to (A). (D) Inference of attack rates using refined framework on real serological data from Ha Nam, Vietnam. Inferred attack rates in recent years were similar to those inferred with the original framework due to extensive sampling during this period.

Assuming instead that infections arose from a common beta-Bernoulli process at each point in time under prior version 2 (uniform beta prior on per-time infection probability), attack rate estimate accuracy increased at all times in the simulation-recovery experiments at the expense of constraint at historical times (Fig 5.13C). This reduced accuracy of historical attack rates is likely a result of the lack of individuals alive during this time and lack of antibody titres against strains circulating during these times (Fig 5.2). Fitting the model with prior version 2 to the real data did not significantly change the overall trend in attack rates, though precision did decrease as in the simulated data. Interestingly, inferred attack rates in recent years (where serum samples were available) did not differ depending on the prior used.

Fig 5.14A shows inferred attack rates using real serological data from the Fluscape cohort using the refined prior version 2. Attack rate trends were similar to those in the Ha Nam cohort but with increased precision due to the much larger number of individuals and better representation of historical ages and strains: attack rates were estimated to be very high in 1968/69, 1985, 1992, 2001/2002, 2012 and 2015, and low from 1977-1983 and 1986-1991. Simulation recovery of known attack rates using a simulated cohort matching the age and sampling distribution of the Fluscape cohort (Table 5.6) demonstrates that this refined framework is free of bias and accurately estimated the known simulated attack rates, in contrast to Fig 5.4.

Data set parameters		Model parameters					
n	1000	Parameter	Description	Value	Prior lower bound	Prior upper bound	Estimated
Year min	1968	μ_l	Long term antibody boosting	1.8	0	8	Yes
Year max	2015	μ_s	Short term antibody boosting	2.7	0	8	Yes
m	48	σ_l	Long term cross reactivity	0.1	0	1	Yes
Time resolution (infection states)	annual (48)	σ_s	Short term cross reactivity	0.03	0	1	Yes
Number of serum samples per individual	2	τ	Suppression	0.05	0	1	Yes
Number of viruses tested	21	ω	Waning	0.8	0	1	Yes
Number of titre measurement repeats	1	ϵ	Measurement error	1	0	25	Yes
Total number of measurements	42000	α	Infection history prior	1	NA	NA	Yes
First sample year	2010	β	Infection history prior	1	NA	NA	Yes
Final sample year	2015						
Minimum age (years)	10						
Maximum age (years)	74						
Infection history prior	Priors 1 and 2						

Table 5.6: Settings for simulations matching the Fluscape cohort.

5.6.4 Systematic bias in measurements not captured by the model

Fig 5.15A shows that the model fits observed titres from the real Fluscape data reasonably well, and infers the presence of infection in regions of antigenic space with high titres. Fig 5.15B shows that inferred infection histories are highly constrained.

However, the model systematically over and underestimated titres in some years. Fig 5.16B shows the histogram of differences between observed and model predicted titres across all titres, demonstrating

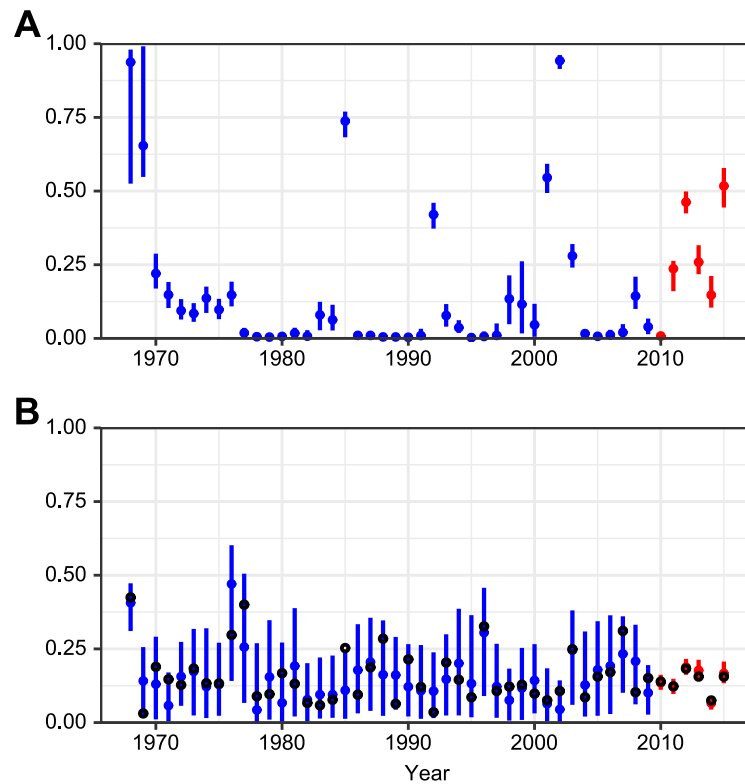


Figure 5.14: Inferred annual A/H3N2 attack rates using real and simulated Fluscape data with refined framework, prior version 2 (beta prior on per-time infection probability, ϕ_j). Points and lines show posterior median and 95% credible intervals on attack rate estimates. Red points show years in which serum samples were taken. (A) Inference of attack rates using real serological data from Guangzhou, China. (B) Inference of attack rates using simulated serological data from Guangzhou, China. Hollow black points show true annual attack rates from which infection histories were simulated. Agreement between model estimates (coloured points and lines) and true values (black points) suggest accurate inference with the refined fitting framework.

that the model fits through the observed data well on average. However, stratifying by virus year shows systematic bias for some years (Fig 5.16A). For example, model predicted titres are systematically lower than observations for the viruses that circulated in years 1985 and 2002, but systematically higher for years 1995 and 2010. Such virus-specific differences in HI titres have been reported previously [84,87,92]. These virus-specific biases are potentially problematic for the attack rate estimates, as the only way the model can account for elevated titres against a particular strain is through adding more infections. If some viruses have systematically higher antibody titres, then the model may incorrectly infer more infections. Fig 5.17 shows that these systematic observation biases likely influenced the attack rate estimates, as attack rates were high for strains with systematically underestimated titres and low for systematically overestimated titres.

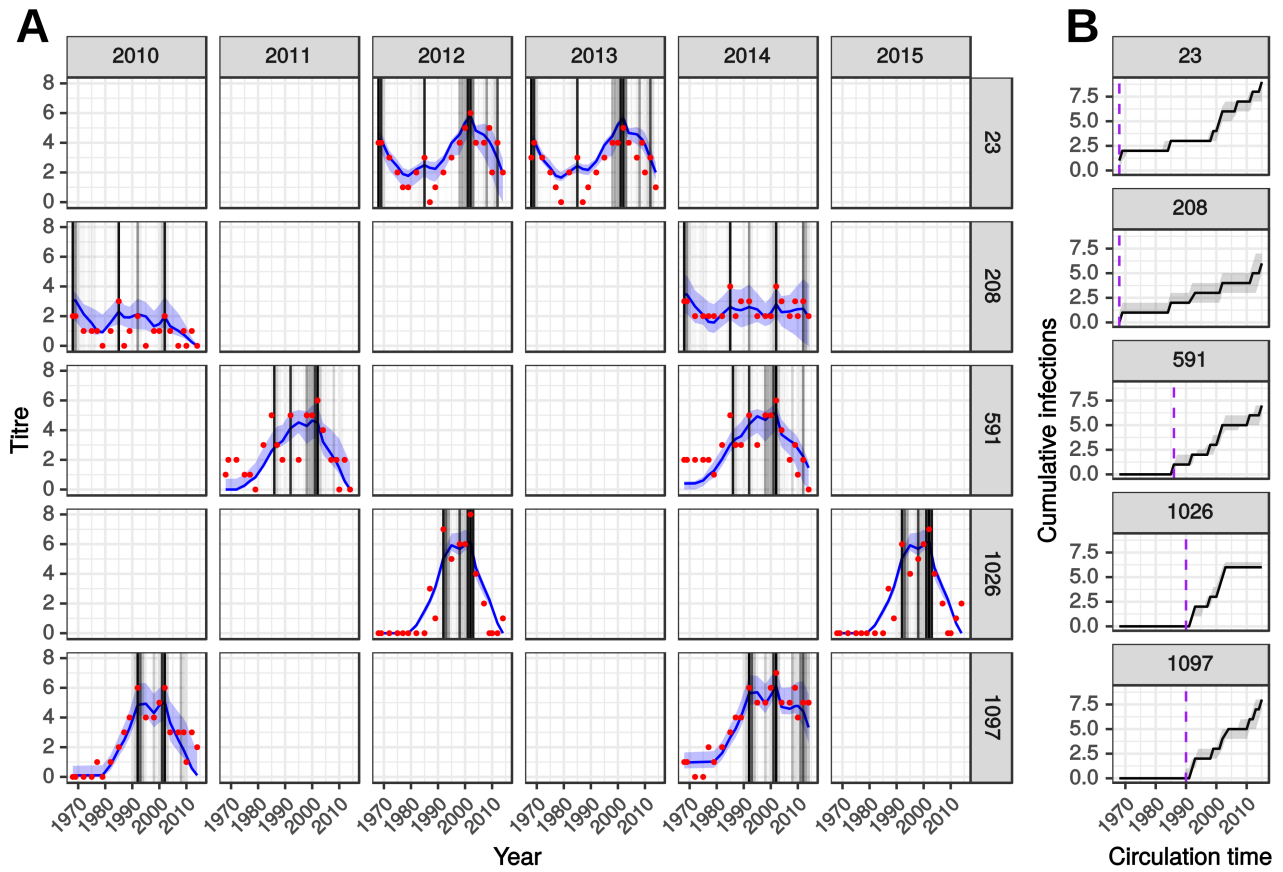


Figure 5.15: Example of individual model fits to real Fluscape titre data using refined framework with prior version 2 (beta prior on per-time infection probability, ϕ_j). Figure description is identical to Fig 5.5

5.6.5 Inference of virus-specific measurement offsets

To account for these virus-specific biases, I re-ran the annual model described above with the addition of measurement offset terms for each of the 20 measured strains under different infection history prior assumptions. I compared the results of fitting 4 model variants to the real Fluscape data. For each model variant, 6 MCMC chains were run each for 10000000 iterations with an additional 2000000 iteration burn in period. These model variants were selected to (i) investigate the impact of the assumed infection history prior on inferred virus-specific offsets and (ii) incorporate similar, prior information from the literature to test for consistency. These variants were:

1. Model with infection history prior version 3 (beta prior on the per-time probability of infection), with $\alpha = \beta = 1$, and assuming that virus-specific offsets are drawn from a normal distribution with $\bar{\chi} = 0$ and estimated σ_{χ}^2 ;
2. Model with infection history prior version 1 (beta prior on the per-individual probability of

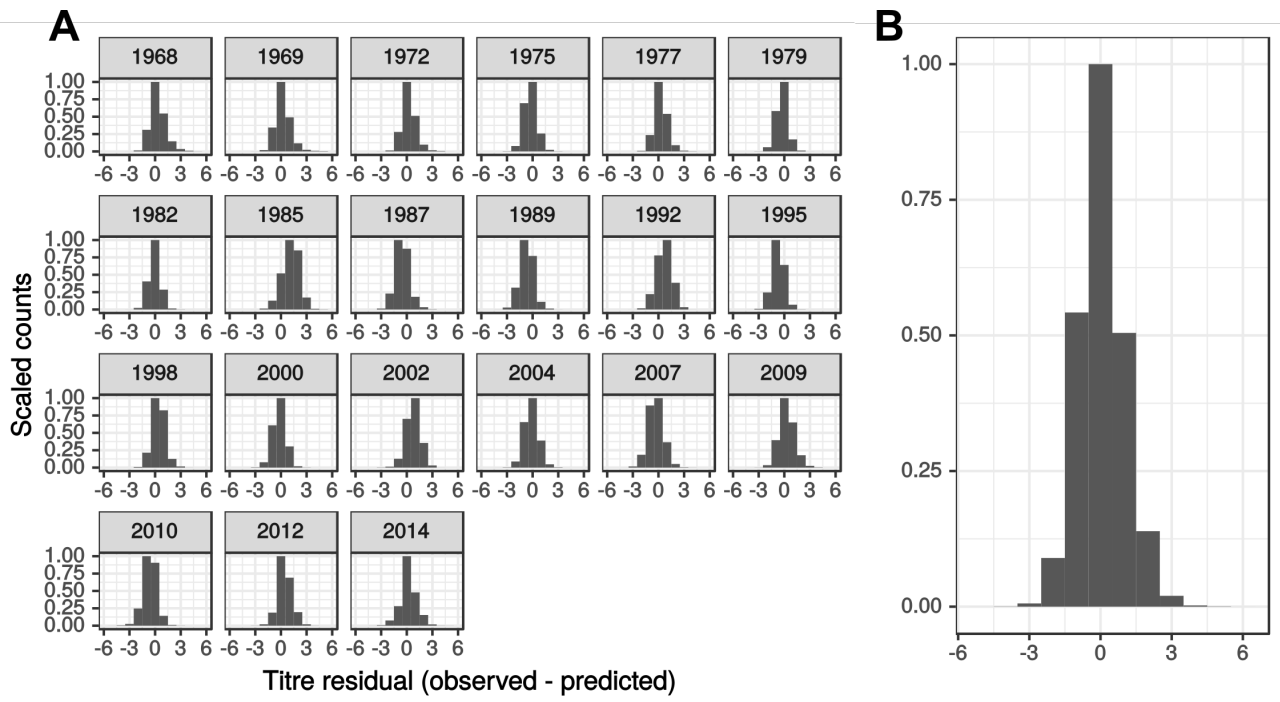


Figure 5.16: Distribution of antibody titre prediction errors (observed - model predicted). (A) Distribution of titre prediction errors stratified by circulation year, where antibody titres were measured against only one strain at each year of potential infection. (B) Overall distribution of titre prediction errors across all measured viruses.

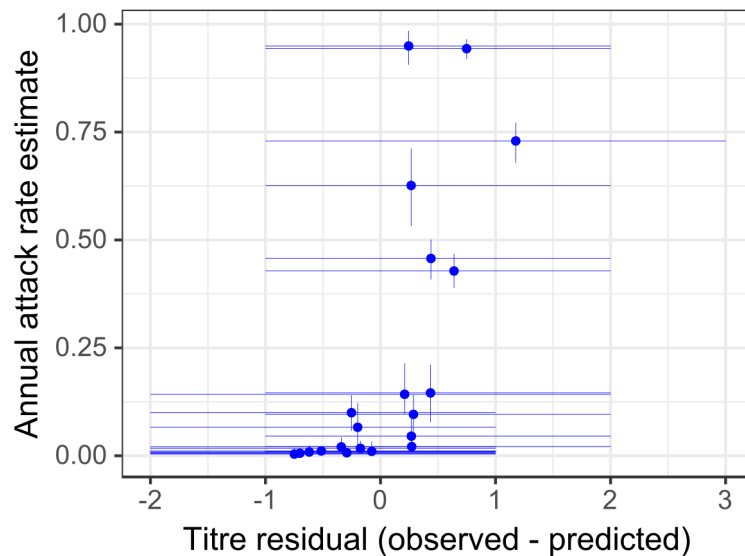


Figure 5.17: Model residuals against estimated attack rates grouped by year of circulation using the Fluscape data. Each point and two-dimensional line range shows posterior median and 95% credible intervals of the attack rate estimate for that year against the titre prediction errors for the strain that circulated in that year. These results suggest that, when fitting the model to data from the Fluscape cohort, years with high attack rates are associated with an underestimation of titres against the strain that circulated in that year.

- infection), $\alpha = \beta = 1$, and assuming that virus-specific offsets are drawn from a normal distribution with $\bar{\chi} = 0$ and estimated σ_{χ}^2 ;
3. Model with infection history prior version 1, $\alpha = 0.80625$ and $\beta = 4.56875$ (chosen to give a mean prior probability of infection of 0.15), and assuming that virus-specific offsets are drawn from a normal distribution with $\bar{\chi} = 0$ and estimated σ_{χ}^2 ;
 4. Model with infection history prior version 1, with $\alpha = 0.80625$ and $\beta = 4.56875$, and assuming that virus-specific offsets are drawn from a normal distribution with $\bar{\chi} = 0$ and estimated σ_{χ}^2 . In this variant, virus-specific offsets were fixed for all but 9 strains using the “virus avidity” estimates from Bedford *et al.* [84]. The original estimates were generated as part of a large-scale Bayesian analysis to infer antigenic maps alongside viral phylogenies. As part of this analysis, Bedford *et al.* fit model-predicted HI titres to observed titres from ferret sera. The “virus avidity” terms provided a virus-specific contribution to predicted titres.

Under prior version 3, I was able to generate well mixed, converged chains that accurately recovered known parameters, virus-specific offset terms and infection histories for simulated data. However, when I fit this model to the real Fluscape data, I was unable to produce converged chains (model variant 1 as described above). Posterior estimates converged to a similar area of high posterior density, but did not mix well. This was likely due to correlation between the virus-specific offset terms and the probability of infection terms for each time period, as each time period had only one representative virus measured.

The remaining 3 model variants did fit the data and generated reasonably well mixed, converged chains, finding evidence of significant measurement bias driven by virus-specific effects. However, the upper 95% confidence intervals on the potential scale reduction factor for some of the model parameters were higher than 1.1, particularly for the model variants using an informative prior 3 and using estimates from Bedford *et al.* I therefore use the estimates from prior 3 with $\alpha = \beta = 1$ in Chapter 6. Fig 5.18 compares the predicted virus offsets for the three model variants.

Fig 5.19 shows the model-predicted titres against the real Fluscape data for 5 randomly selected individuals, demonstrating that the offset terms shift the model-predicted titres to better fit the data. The additional flexibility afforded by the offset terms generated lower log-likelihoods than the base model and typically lower antibody boosting estimates (Table 5.7). The offset term estimates from model variants with no prior input from Bedford *et al.* showed a consistent trend: offsets were highest

and positive for HK68, MS85 and FJ02 (overestimated titres). Offset estimates for many strains were negative, notably for BK79, SC87, BJ89 and WU95 (underestimated titres). Offsets for strains in recent years were slightly higher than for older strains, suggesting that titres against recent strains were systematically underestimated in the model without measurement bias. Some offset estimates were consistent with the virus avidities estimated in Bedford *et al.*, namely HK68, EN72, PH82, and HK14. In contrast, these new estimates for VC75, TX77, SC87 and WU95 were far lower, whereas our estimates for BK79, BJ92, FJ02, PE09 and TX12 were higher.

The impact of including virus-specific measurement bias on attack rate estimates is demonstrated in Chapter 6.

Table 5.7: Comparison of antibody kinetics parameter estimates under different model assumptions for ρ .

Parameter	Description	Prior 3, $\alpha = \beta = 1$	$\hat{R}$	Prior 3, $\alpha = 0.861$ $\beta = 4.57$	$\hat{R}$	ρ from Bedford <i>et al.</i> , prior v1	$\hat{R}$
μ_l	Long term antibody boosting	1.37 (1.34–1.40)	1.12	1.47 (1.44–1.51)	1.14	1.88 (1.84–1.93)	1.46
μ_s	Short term antibody boosting	2.21 (2.12–2.30)	1.03	2.00 (1.88–2.09)	1.24	1.63 (1.58–1.68)	1.25
τ	Suppression	0.0245 (0.0226–0.0263)	1.06	0.0256 (0.0231–0.0283)	1.11	0.0199 (0.0161–0.0228)	1.23
ω	Waning	0.359 (0.344–0.377)	1.12	0.387 (0.373–0.406)	1.22	0.542 (0.518–0.564)	1.1
σ_l	Long term cross reactivity	0.0765 (0.0749–0.0773)	1.04	0.0736 (0.0724–0.0746)	1.20	0.0772 (0.0768–0.0774)	1.15
σ_s	Short term cross reactivity	0.0114 (0.0105–0.0122)	1.02	0.00923 (0.00781–0.0104)	1.09	0.000119 (5.24e-06–0.000545)	1.07
ϵ	Measurement error	0.670 (0.666–0.675)	1.01	0.674 (0.669–0.679)	1.09	0.869 (0.863–0.876)	1.03
$\sum Z$	Total infections	10700 (10600–10900)	1.05	9320 (9050–9470)	1.47	8650 (8480–8770)	1.95
Log likelihood	Log likelihood from posterior	-67700 (-67900–67500)	1.08	-68100 (-68300–67900)	1.25	-80900 (-81000–80700)	1.08
Log posterior	Log posterior	-67700 (-67900–67500)	1.08	-68000 (-68200–67900)	1.25	-80800 (-81000–80700)	1.08
ρ_{sd}	Standard deviation of ρ	0.656 (0.538–0.822)	1.03	0.618 (0.513–0.756)	1.05	1.12 (0.94–1.40)	1.02

5.6.6 Antibody kinetics estimates

Using the refined inference framework with prior version 2, estimated biological parameters using the real Fluscape data were qualitatively similar to those inferred using the Ha Nam data with prior version 1 and in Kucharski *et al* [1]. All estimates were consistent with the observation of an initial, broadly reactive antibody response that decays to leave a narrower, persistent antibody response. However, there were differences in the strength and persistence of the short term antibody response (μ_s and ω) between the data sets (Table 5.8). The Ha Nam data suggested high levels of broadly reactive short-term boosting that waned within 2 years ($\mu_s = 2.74$) log units (posterior mean; 95% CI: 2.52-2.97) and $\omega = 0.706$ log units per year (posterior mean; 95% CI: 0.650-0.766). Estimates using the Fluscape data suggested weaker and more long-lived antibody boosting ($\mu_s = 1.54$ log units (posterior mean; 95% CI: 1.49-1.58) and $\omega = 0.525$ log units per year (posterior mean; 95% CI: 0.502-0.536), corresponding to 2 years from peak short term boosting to full waning). Estimates for the degree of homologous long-term boosting (μ_l) and boosting suppression through antigenic seniority (τ) were similar, though short-term cross reactivity was estimated to be far broader in the Fluscape data. Differences in measurement error are likely a result of inter-lab variation, and a reflection of the variation in measured virus avidity [250]. These estimates must be interpreted with caution: the time between serum samples was not strictly annual in the Fluscape data as in the Ha Nam data, and bucketing sampling times into years is therefore a strong assumption. Refined estimates will be presented in Chapter 6 that consider 3-month long time periods and demonstrate greater consistency with the Ha Nam results.

Parameter	Vietnam, prior 1	Vietnam, prior 2	China, prior 2
Long-term boost (μ_l)	1.93 (1.87-1.99)	1.98 (1.91-2.05)	1.70 (1.67-1.74)
Short-term boost (μ_s)	2.68 (2.45-2.91)	2.74 (2.52-2.97)	1.54 (1.49-1.58)
Antigenic seniority (τ)	0.0401 (0.0359-0.0436)	0.0465 (0.0422-0.0498)	0.0199 (0.0175-0.0224)
Waning (ω)	0.763 (0.689-0.835)	0.706 (0.650-0.766)	0.525 (0.502-0.536)
Long-term cross-reaction (σ_l)	0.112 (0.109-0.114)	0.113 (0.110-0.115)	0.0896 (0.0889-0.0899)
Short-term cross-reaction (σ_s)	0.0273 (0.0234-0.0307)	0.0259 (0.0223-0.0296)	5.21×10^{-5} (1.67×10^{-6} - 2.77×10^{-4})
Observation error (ϵ)	1.300 (1.28-1.32)	1.29 (1.27-1.32)	0.902 (0.896-0.909)

Table 5.8: Parameter estimates from fitting model to data from China and Vietnam using the original and refined framework. Values shown are posterior medians and 95% credible intervals

5.6.7 Titre-dependent and age-dependent boosting

The estimated titre-dependent boosting gradient was extremely close to 0, suggesting limited support for a titre-ceiling effect on antibody boosting in these data. Simulation recovery results were able to accurately identify a gradient of 0.1, suggesting that non-identifiability was either due to features of the real data, or a genuine lack of this mechanism. Estimates for the age-dependent boosting gradient

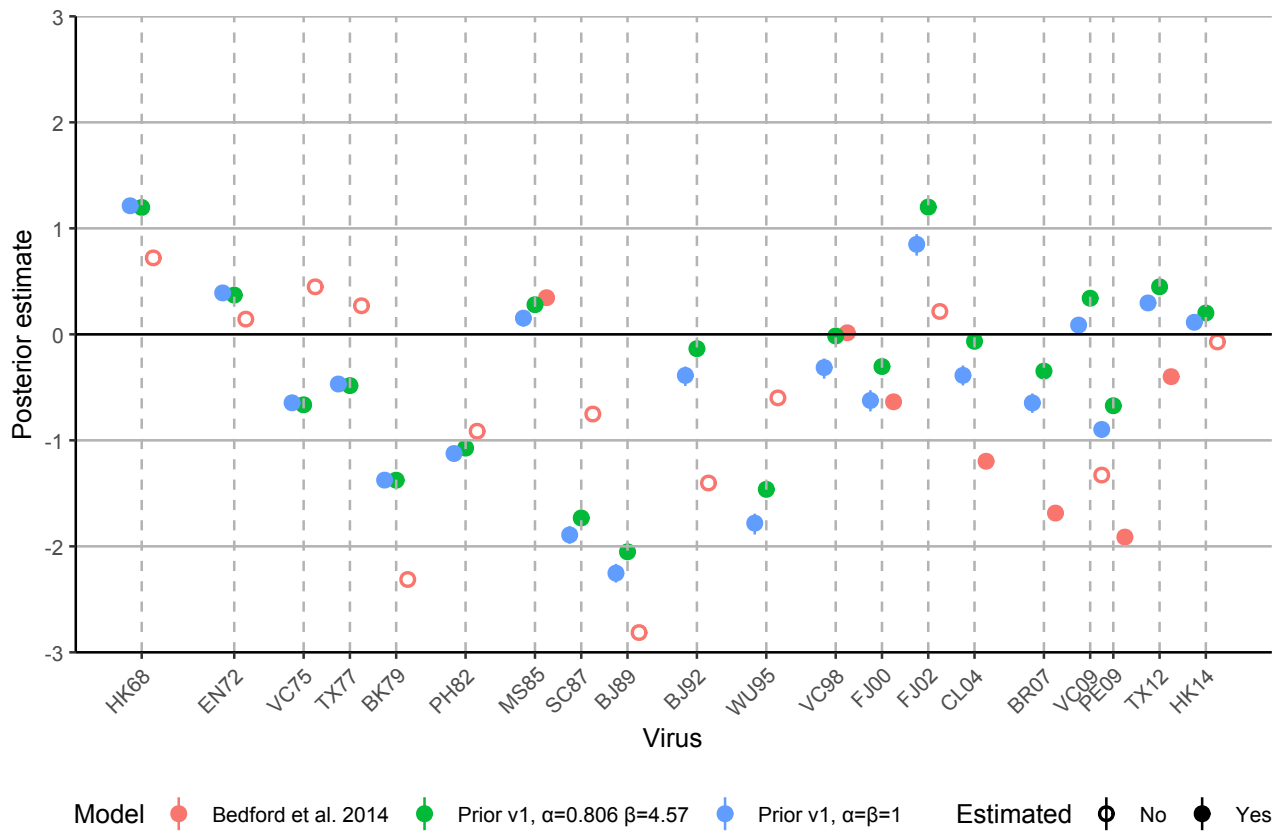


Figure 5.18: Estimates for measurement bias parameters, ρ , by strain and model version. Points show posterior median estimates. Lines are plotted for upper and lower 95% CI, but these are too small to see. Filled points were estimated, hollow points were assumed to be fixed.

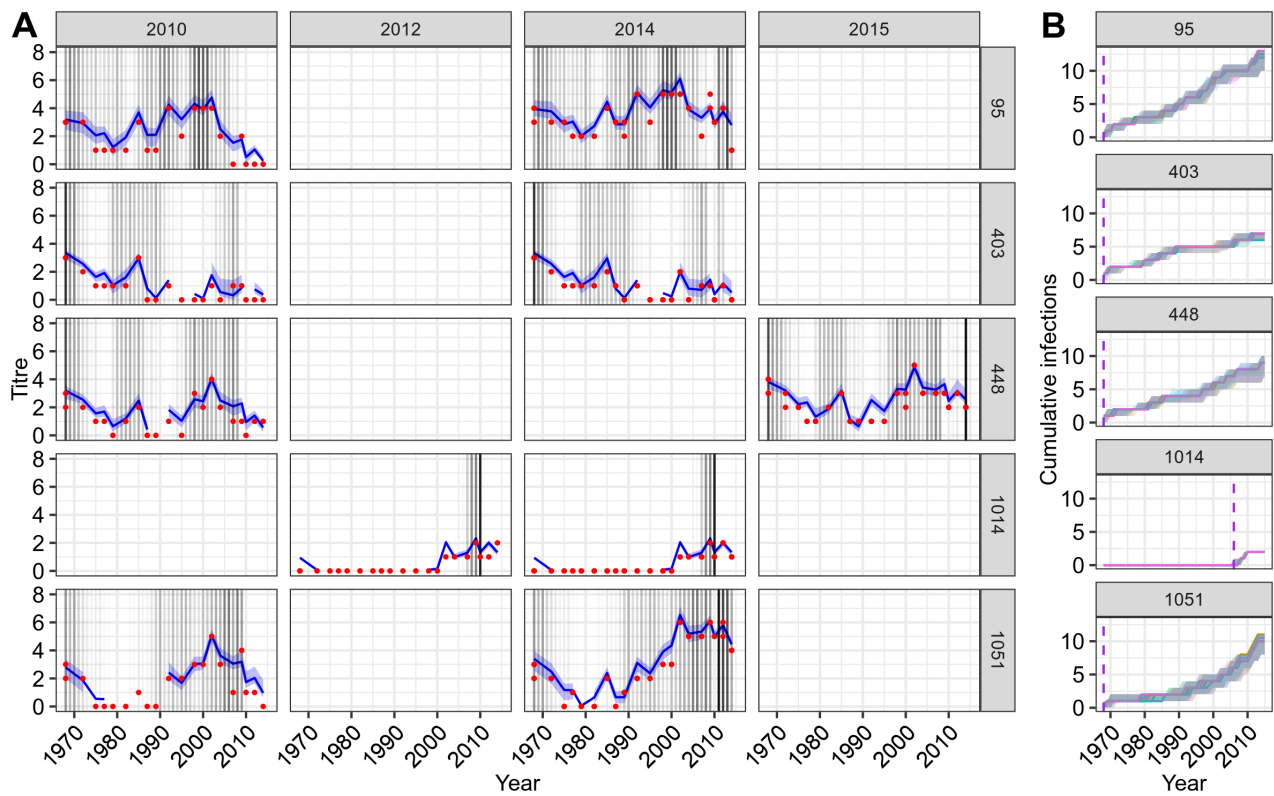


Figure 5.19: Individual model fits to Fluscape-like titre data with virus-specific measurement bias under prior version 3 (beta prior on per-individual probability of infection). Model trajectory is absent where predicted titres are undetectable. Figure description is identical to Fig 5.5

were well constrained. I estimated that each additional year of age elicited a 0.385% reduction in the total boost experienced. For example, if a primary infection at age 0 elicited a boost of 2 log units, a 50 year old would experience a boost of 1.62 log units from a primary infection. However, estimates for the antigenic suppression parameter, τ , decreased drastically in this model, suggesting that the original term already accounted for a substantial amount of the observed trend of decreasing antibody boosting with increasing age. This finding is unsurprising, as both mechanisms would be observed in a similar way (given that the cumulative number of infections increases with age). As the log-likelihoods of these model fits were very similar and other estimates were largely unchanged, the simpler model with just τ is justified.

Table 5.9: Comparison of parameter estimates using the base model, model with titre-dependent boosting and model with age-dependent boosting.

Parameter	Description	Original model	Titre-dependent boosting	Age-dependent boosting
μ_l	Long term antibody boosting	1.7 (1.67-1.74)	1.75 (1.71-1.79)	1.83 (1.8-1.85)
μ_s	Short term antibody boosting	1.53 (1.48-1.59)	1.54 (1.49-1.59)	1.69 (1.64-1.74)
τ	Suppression	0.0199 (0.0172-0.0224)	0.0224 (0.0198-0.0248)	0.00546 (0.00168-0.00912)
ω	Waning	0.518 (0.501-0.537)	0.515 (0.499-0.535)	0.51 (0.499-0.53)
σ_l	Long term cross reactivity	0.0896 (0.0889-0.0899)	0.0887 (0.0884-0.0896)	0.0894 (0.0887-0.0898)
σ_s	Short term cross reactivity	5.02e-05 (1.93e-06-0.00026)	4.9e-05 (1.23e-06-0.000258)	5.44e-05 (2.55e-06-0.000273)
γ_t	Titre-dependent suppression gradient	NA	0.000797 (2.39e-05-0.00963)	NA
y_{switch}	Threshold for titre dependent boosting	NA	2.47 (1.02-7.65)	NA
γ_a	Age-dependent suppression gradient	NA	NA	0.00385 (0.00353-0.00416)
ϵ	Measurement error	0.902 (0.896-0.908)	0.902 (0.896-0.908)	0.901 (0.895-0.907)
$\sum Z$	Total infections	9040 (8900-9170)	8830 (8670-9000)	8940 (8850-9040)
Log likelihood	Log likelihood from posterior	-88400 (-88600-88300)	-88400 (-88600-88300)	-88300 (-88500-88200)
Log posterior	Log posterior	-103000 (-103000-103000)	-103000 (-103000-102000)	-102000 (-103000-102000)

5.7 Discussion

In this chapter, I developed a statistical framework to represent multiple influenza infection states for a sample population. Given the conceptual separation of the transmission, antibody process and observation levels of the model shown in Fig 5.8, this framework could readily be adapted to many infectious disease systems, particularly those that are modelled well by a continuous antigenic space such as DENV. This approach differs to previously described infection state inference in that it models infection states as possible events at fixed discrete times rather than as a continuous time parameter, which enables extension to a larger number of infection states with limited data [40,280,482]. I described 4 different model assumptions regarding the infection generating process, all of which are valid, but have different impacts on inferred epidemiological quantities of interest, including annual attack rates and total number of lifetime infections per individual. Explicitly describing the infection history prior allows the incorporation of prior knowledge on probability of exposure either from surveillance data or temporal climate variables. In the results presented here, I mostly used uniform priors for the probability of infection. However, more complex priors could be imposed by having a different prior distribution for the probability of infection at each time point (ie. different value of α and β) to account for seasonality in transmission dynamics. Alternatively, non-parametric forms for the continuous-time infection generating process (or force of infection) could be defined, for example using a Gaussian process or B-spline, with parameters for these hyperpriors either informed by past observations or estimated [483,484].

If the data being fitted has few individuals, few infection times and a large number of observations, then the assumptions of these priors have relatively little impact on the inferred infection histories. However, if the amount of data and therefore weighting of the likelihood is small, then the infection history prior becomes important. Under prior version 1 (beta prior on per-individual probability of infection), a large number of individuals (large n) places a very strong binomial prior on the attack rate (total number of infections per time period), whereas a high time resolution (large m , eg. monthly infections) has little impact on the *a priori* total number of lifetime infections per individual. Conversely, with prior versions 2 and 3 (priors on the per-time probability of infection), a large n has no impact on the attack rate prior, but a large m does have an impact on the prior for the total number of lifetime infections per individual. The choice of which prior to use therefore depends on the data structure, prior knowledge and scientific aims (Table D). For example, inferring antibody kinetics that describe individual titres is readily achieved using prior version 1 (because the model finds the infection history

estimate that best describes the antibody data), whereas inferring accurate historical attack rates is better suited to prior versions 2 and 3 (because information on infection states is shared between individuals).

Assuming that individual infections are generated from a common process substantially increased the accuracy of estimated historical influenza A/H3N2 seroresponse rates relative to the framework described by Kucharski *et al* [1]. Although estimates for recent seroresponse rates in Ha Nam data were similar under different priors, estimated historical seroresponse rates showed reduced bias when assuming that individual infection probabilities were correlated within a given year. Simulation experiments using data similar to the Fluscape dataset, which included uniform coverage of historical strains and a large number of individuals, suggested that historical seroresponse rates could be accurately estimated back to 1968 when A/H3N2 began circulating in humans. Differences between these data sets are likely driven by the smaller sample size and sparser sampling of historical strains, but more frequent serum sampling and better titre coverage of recent strains in the Ha Nam data. The different accuracy of these two sampling strategies in inferring different processes suggest that serological surveys could be optimised to answer particular epidemiological questions. We (myself and collaborators at the London School of Hygiene and Tropical Medicine) have recently used this framework to explore efficient serological study designs to answer different epidemiological questions (submitted manuscript).

There are a number of potential explanations for the very high seroresponse rate estimates in some years. The first is that these estimates represent genuinely high seroresponse rates. Estimated A/H3N2 seroresponse rates were up to 99% in the Fluscape data, which surpasses estimates of pandemic H1N1 incidence in children (who had little preexisting immunity) during the 2009/10 wave in Hong Kong [485]. A second potential explanation is that the magnitude of antibody boosting is dependent on properties of the infecting strain. It has previously been suggested that different influenza types, subtypes and strains may elicit different levels of homologous boosting [459]. Another possibility is that the Gaussian measurement error assumed here did not take into account systematic bias towards higher titres in particular strains. It is known that the maximum titres achieved by naive ferrets following infection differs between strains and virus [84,87]. Accounting for differences in the measurement process, or clarification of assay specific variability through comparison with microneutralisation titre results may clarify the relative contributions of true antibody presence and measurement bias [241]. Finally, the assumption of a smooth, continuous evolutionary path in antigenic space may limit the ability of the model to account for titres that are very high in a small region of antigenic space relative to viruses

that circulated in adjacent years.

I found a positive correlation between estimated annual influenza A/H3N2 seroresponse rates and systematic bias in predicted antibody titres. Serum potency (some sera are stronger) and virus avidity (some viruses perform better in the HI assay) are known sources of variation when performing antigenic cartography using ferret sera, and a model testing variation in human infection histories should therefore account for these biases. Virus effects are likely due to virus-specific differences in haemagglutinin (HA) reactivity, which has been previously described [51, 486]. When using locally-weighted multiple linear regression to fit antibody landscape surfaces to antibody titre data, Fonville *et al.* found that some viruses had systematically under-predicted values (eg. NL/620/89) whereas others were systematically overestimated (eg. Victoria/361/11) [87]. This phenomenon was also described by Bedford *et al.* when simultaneously performing antigenic cartography alongside phylogenetic tree reconstruction using both genetic and HI titre data. Bedford *et al.* found models that explicitly estimated parameters for “virus avidity” to represent the contribution of virus-specific effects to observed titres were better supported than models that did not.

Including a virus-specific offset term as part of the observation process enabled the antibody titre model to account for these systematic biases. Unrestricted model fitting was not possible under the infection history model of choice (prior 3, placing a beta prior on the per-time infection probability). This is likely because the offset terms and infection probability terms are highly correlated; elevated titres against a strain that circulated in a given year may be explained either by high seroresponse rates or systematic overestimation of titres to that strain. Estimation of the virus-specific offset terms was therefore most successful under the model that assumed independence of infection risk between individuals (prior 1). Despite issues in inferring seroresponse rates, this model is appropriate for describing observed titres (as demonstrated in Fig 5.5). It is therefore possible to use estimates from this model fit as inputs to other analyses with the same data. Ideally, virus-specific offsets would be generated from independent experiments that control for other sources of variation. I attempted to do this using estimates of “virus avidity” from Bedford *et al.*. However, seroresponse rate estimates under a model with fixed values from Bedford *et al.* were nonsensical (not shown, very low for 1968, almost 100% in 1992, above 60% for many years), suggesting that there likely were genuine differences in assay preparation. Generating an independent database of virus-specific effects would be useful, but extremely hard to standardise given variation in assay preparation and virus properties [250]. For example, although the HI assay aims for a fixed amount of virus in each well of the assay plate, this

can only be assessed based on quantify of HA to some degree of precision [487].

I presented extensions to the biological and immunological mechanisms included in the model, but found that these did not significantly affect antibody kinetics and infection history estimates. Both titre-ceiling effects and decreased antibody boosting at older ages are mechanisms found in other studies [225, 434, 488]. In Chapter 4 I found that inclusion of one of either titre-ceilings or boosting suppression improved model fits, suggesting that these mechanisms explain reduced boosting from higher pre-existing titres. The model with age-dependent boosting suggested that decreasing antibody boosting as a function of age may help to explain the data, but generated an almost indistinguishable effect from boosting suppression as a function of increasing number of prior infections. In fact, a study by Höpping *et al.* aiming to investigate age-specific differences in vaccine response found that age effects disappeared once number of prior vaccinations had been taken into account [445].

A methodological advancement of the model that was not presented in the main text (Appendix C.3) was the inclusion of titre-mediated immunity. This involved calculating the probability of an infection event conditional on an individual's pre-existing antibody titre to the infecting strain. Inclusion of this mechanism did not significantly impact parameter or attack rate estimates, though did have the expected effect of slightly reducing the total number of infections experienced by each individual (Fig C.7). Estimating the additional latent exposure states incurs a significant additional computational and statistical burden, and I therefore do not use this mechanism in Chapter 6. However, the posterior function described here may be generalised to consider any type of immunity as a function of prior exposures. These methods will therefore be included in the *serosolver* R-package that accompanies this work, with the hope that it may be used to test additional hypotheses of past infection and other models of immunity.

Waning rate results from the Ha Nam cohort suggest that the antigenically broad, short-term antibody response fully wanes within two years, contrasting with the Fluscape results which suggested waning over a slightly longer period. The former result is in greater agreement with previous studies suggesting substantial antibody waning within a year of infection or vaccination [87, 441, 453, 467, 489]. Serum samples from the Ha Nam data were taken at roughly equal one-year intervals, whereas the times between serum samples were varied in the Fluscape cohort. It is therefore difficult to compare these estimates, and likely that parameter estimates from the Fluscape data are not reliable when inferring infections at an annual resolution. Chapter 6 resolves this issue by inferring infection histories at a sub-annual resolution.

5.8 Conclusion

The overall contribution made here is the development of a computationally efficient model framework to allow the inference of infection histories at an arbitrary time resolution and for any serological data set. Predicting when an influenza epidemic will begin and peak is useful for public health planning and vaccination deployment, particularly in highly seasonal regions [490–492]. Serology is an important source of data with which to predict epidemic dynamics, but gaps remain in linking serology to sub-annual incidence patterns across multiple years [236, 493]. These results suggest that sub-annual seroresponse rate estimates may be achievable with rich serological data. Potential model modifications may take into account climactic factors in temperate, tropical and subtropical regions to estimate common sub-annual patterns across years to improve parameter identifiability [494–496]. Estimating historical, sub-annual seroresponse rates has the potential to greatly improve our understanding and prediction of influenza transmission dynamics.

5.9 Serosolver R-package

The majority of the work in this chapter has been developed into an open source R-package, **serosolver**, that can be used to obtain epidemiological and immunological insights from routinely generated serological data. **serosolver** is available at <https://github.com/seroanalytics/serosolver>, and a submitted pre-print of the accompanying paper is available at <https://doi.org/10.1101/730069>. This package extends previous work [1,86], but makes a number of novel additions to the infection history, antibody kinetics, and observation models. In addition to drastic computational speed ups, the package contains functions and options to allow users to fit and analyse a general model of infection histories and antibody kinetics to an arbitrary data set.

Chapter 6

Patterns of spatial and individual variation in influenza incidence and immunity in a southern Chinese cohort

The previous two chapters described the statistical framework and some biological considerations for inferring multi-strain infection histories based on antibody titre data. Here, I undertake the computationally challenging task of obtaining influenza A/H3N2 infection histories for 1,130 individuals in 3-month windows. Key contributions include: the confirmation of current estimates of A/H3N2 infection frequency; a description of infection patterns with age; demonstrating the importance of virus-specific measurement bias in estimating infection histories; the consistency of antibody kinetics estimates with previous analyses; small-scale spatial variation in seroresponse rates between nearby locations; and the age-specific relationship between antibody titre at the time of exposure and subsequent infection risk.

6.1 Introduction

Patterns of historical infections in humans are highly varied across time, host age and space [497, 498]. It is not just antigenic variation and evolution that contributes to variation in influenza incidence, but also a combination of individual and population level factors [81, 445]. Contact and movement patterns [309, 499–502], climatic variation [494, 503, 504], school terms [505, 506], and household structure and size [507, 508] have all been shown to be associated with variation in influenza incidence. Differences in city structure have also been shown to influence influenza epidemic dynamics [509]. Smaller cities tend to exhibit sharply peaked seasonal epidemics, whereas larger cities exhibit large, drawn out epidemics, driven by differences in population density and socioeconomic factors [510–512]. A better understanding of who, where and when influenza incidence is likely to be highest would be enormously beneficial for public health planning and forecasting [491, 513, 514].

Analyses of serological data suggest that humans are infected with a novel influenza strain approximately every 5 years as immune protection from past exposures wanes and becomes ineffective against circulating strains [86, 225]. Individuals experience their first infection within the first 6–7 years of life, with some potential variation due to local epidemiological patterns [87, 90, 497]. Seroprevalence against circulating strains then tends to decrease into middle age, suggesting that individuals experience less frequent infections, or at least less frequent antibody boosts against the haemagglutinin head as they age [86]. This may be due to decreased exposure through fewer infectious contacts, though may also result from differences in the recruitment of *de novo* versus memory B cell responses and their epitope targets [89, 500]. Nuanced age patterns and cohort effects may therefore be explained by taking into account individual age and which particular epitopes an individual has seen previously [123, 189].

Finding predictors of influenza incidence timing and severity requires reliable data on the incidence of infection rather than just symptoms associated with influenza. Influenza-like-illness (ILI), adjusted for variation in reporting rates and possibly laboratory-confirmed, is often used as a proxy measure for true incidence and to assess the effectiveness of the seasonal vaccine [515–519]. Relying on reporting of symptomatic cases alone is limiting. ILI is a non-specific case definition also caused by other aetiological pathogens, and many influenza infections are asymptomatic or mild and will therefore be missed [520, 521]. Furthermore, variation in surveillance quality in different regions and over time make it difficult to identify individual-level or age-specific effects over a longer period of time.

These issues may be overcome using serological data [261, 269, 271, 522]; however, recent work has revealed

the shortcomings of traditional seroconversion and seropositivity metrics in accurately identifying unobserved infections [1, 225, 274]. The antibody response to an influenza exposure is highly dynamic, driven by the number, timing, route and antigenic properties of all past exposures [488]. Immunological interactions of antigenic drift and the antigenic targets of one's immune memory occur as 'original antigenic sin' and seniority effects, whereby an individual's exposure history influences which influenza epitopes are targeted and the magnitude of an antibody response [93]. Interpreting any given serological measurement as a proxy for infection must therefore account for these phenomena.

In this chapter, I apply the infection history, antibody kinetics and measurement models described in Chapter 5 to data from an ongoing serological study in Guangzhou, China – a region that experiences annual cycles of influenza activity that typically peaks in the spring [523, 524]. This allowed me to obtain individual-level, lifelong infection histories for over 1000 individuals [476]. The study population came from various age groups, social backgrounds and geographical areas, thereby providing an ideal augmented data set to investigate predictors of influenza infection and small-scale spatial variation. In fitting the model, antibody kinetics parameter estimates were obtained based on a large antibody titre data set, providing power to elucidate long- and short-term antibody dynamics. Put together, these results provide an insight into the historical epidemiological and immunological dynamics of influenza A/H3N2 that are only possible using serological data.

6.2 Materials & Methods

6.2.1 Participant data

The Fluscape study is an ongoing serological, contact and demographic survey in and near Guangzhou, China. The study captures 40 locations randomly selected from a 60km cone-shaped transect extending from Guangzhou city centre into the surrounding rural area. Metadata for each location available for these analyses included: the distance from Guangzhou city centre (km); log population density at the 1km and 9km grid cell levels; and whether the location is classified as rural or urban. 60 households within 1km of each chosen location were randomly selected and contacted one-by-one until 20 households with at least one member willing to provide a blood sample and answer the survey questionnaire were contacted [474].

All household members aged 2 or above were eligible to participate. 5ml of blood was taken for each blood sample from each visit [474]. Metadata for each individual available for these analyses were: date of birth; height and weight; presence of influenza-like-illness in the past year or since the previous study visit; vaccination status in the past year or since the previous study visit; smoking status in the previous month; and if the individual had a chronic disease (heart disease, diabetes, hypertension, cancer, lung disease and allergies). Given that the aim of this analysis was to investigate incidence trends, I used data only from the 1,130 individuals that had two serum samples taken from two different rounds of sampling between 2009 and 2015 inclusive.

6.2.2 Serological data

Haemagglutination inhibition (HI) assays were performed for each sample to measure antibody titres against 20 A/H3N2 strains that circulated between 1968 and 2014 inclusive, with ≈ 2 year spacing between circulation years. Repeat titres were available for all of the strains tested from the second serum sample (23,686 repeat measurements in total). Titres were tested in serial 2-fold dilutions from 1:10 to 1:1280, with undetectable titres recorded as $<1:10$. The recorded titre reading was the highest dilution at which haemagglutination was still inhibited. For all analyses, titres were transformed to a $\log_2$ scale, where $y = \log_2(\frac{D}{5})$, giving log titres between 0 and 8 (undetectable titres were treated as a 0 log titre). Seroconversion between study visits was defined as a 4-fold rise in titre, equivalent to a ≥ 2 unit increase on the log scale. Seropositivity was defined as having a titre of $\geq 1:40$ (log titre ≥ 3).

6.2.3 Antibody kinetics and observation model

The antibody kinetics model used here was identical to that in Section 5.2.2. Briefly, the model described linear short- (μ_s) and long-term (μ_l) antibody boosting on the log scale immediately following infection. Short-term boosting waned by a proportion ω over time following infection, whereas long-term boosting persisted for the remainder of time. In addition to boosting of antibodies against the infecting strain, infection also elicited the production of cross-reactive antibodies against antigenically related strains. Cross-reactivity decreased linearly with antigenic distance by a factor of $\min(0, 1 - \sigma x_{i,j})$, where $x_{i,j}$ represents the antigenic distance between the infecting strain i and the measured strain j . Independent parameters were assumed for short-term (σ_s) and long-term (σ_l) boosting. Finally, antigenic seniority by suppression was included, wherein the full amount of boosting decreased linearly by a proportion τ for every infection following the first. I did not include age-dependent boosting nor titre-dependent boosting, as they were not identifiable in Chapter 5 using similar data.

Given the significant impact of virus-specific measurement bias on inferred seroresponse rates, I used fixed values for the virus-specific measurement effect based on estimates from Chapter 5. Values were fixed as preliminary analyses suggested that chain mixing and identifiability were poor when fitting this model alongside estimating virus-specific measurement bias. Specifically, I fit an annual resolution infection history and antibody kinetics model to the full Fluscape data set using the prior described in Section 5.5.3, which assumed that an individual's probability of infection was independent of all other individuals at all times. In contrast, the main results presented here used the prior from Section 5.5.5, which assumed that all individual infections arose from the same infection generating process in each time period. I used the maximum posterior probability estimates for the virus-specific offset terms from the annual model (caveat explained in Section 6.4.1), with exact values shown in Table 6.1. I also fit a variant of the model with no virus-specific measurement bias as a comparison.

6.2.4 Infection history model and assumptions

Infection histories were estimated at a 3-monthly (quarterly) resolution, such that individual i could be infected ($Z_{i,j} = 1$) or not infected ($Z_{i,j} = 0$) in each 3-month period j . In the model, individuals could be infected in the quarter after they were born, but not before, and in the quarter of their latest serum sample, but not after. Independent, uniform beta priors were assumed for the infection probability in each possible infection period that applied to all individuals (Section 5.5.5). This resulted in a uniform prior estimate on the overall attack rate between 0 and 1. Note that as in Chapter 5, I use the

Table 6.1: Virus-specific offset terms used in the main model fits. Values shown are maximum posterior probability estimates from a less flexible version of the model fit to the same data (described in the main text). Values shown to three significant figures. Note caveat explained in Section 6.4.1.

Virus	Offset used
A/HongKong/1968	0.899
A/England/1972	0.0159
A/Victoria/1975	-0.995
A/Texas/1977	-0.736
A/Bangkok/1979	-1.57
A/Philippines/1982	-1.29
A/Mississippi/1985	0.00993
A/Sichuan/1987	-2.03
A/Beijing/1989	-2.38
A/Beijing/1992	-0.535
A/Wuhan/1995	-1.94
A/Victoria/1998	-0.546
A/Fujian/2000	-0.889
A/Fujian/2002	0.554
A/California/2004	-0.700
A/Brisbane/2007	-0.956
A/Victoria/2009	-0.24
A/Perth/2009	-1.20
A/Texas/2012	0.0606
A/HongKong/2014	-0.0494

term “attack rate” to refer to the rate of all observable serological responses, termed “seroresponses” (infection, vaccination and asymptomatic exposure).

Based on the analysis shown in Appendix C.3, I did not include titre-mediated immunity in the model, as this was shown not to qualitatively alter inferred results, but incurred the cost of inferring many more latent states.

6.2.5 Model fitting

All infection states and antibody kinetics parameters were assumed to be unknown parameters to be estimated. I used a Markov Chain Monte Carlo (MCMC) algorithm as described in Chapter 5 to fit the model [476]. 6 chains were run for 35,000,000 iterations, with the first 10,000,000 iterations discarded as burn in. Such long runs were needed to ensure that the potential scale reduction factor (PSRF, a measure of between-chain variability with respect to within-chain variability) was greater than 1.1 ($\hat{R} < 1.1$) and the effective sample size (ESS) > 200 were obtained for all model parameters. MCMC proposals alternated between either proposing new values for one random antibody kinetics parameter or new infection history states for all individuals. Step sizes for all antibody kinetics parameter proposals were also scaled automatically during the burn in to achieve an acceptance rate

of 0.44 for all parameters. Infection history state proposals were randomly chosen between one of two options: (i) select two potential infection times 12 quarters apart and swap infection states for all individuals that could have been infected at these times; (ii) randomly select 20% of individuals and for each individual, with 50% probability, either sample new infection state values for all possible infection times (see Appendix E.3), or choose two potential infection times 12 quarters apart and swap their values. Uniform priors were assumed for all antibody kinetics parameters.

Preliminary analysis and validation on simulated data suggested that chain mixing was poor for infection states in recent and some early years, and I therefore weighted the infection history proposal step to re-sample recent infection states at a higher frequency. Time periods to sample at higher frequency were chosen based on visual assessment of the preliminary MCMC chain trace plots: time periods that showed poor mixing were sampled at increased frequency. Specifically, during the MCMC infection history proposal step, infection times from Q3-1984–Q1-1985 and from Q3-2011 onward were sampled at a ratio of 3:1 to the other time periods.

6.2.6 Validation using simulated data

To validate the choice of infection history time resolution and antibody kinetics model for these data, I simulated titre data and quarterly infection histories for 1,000 individuals with ages ranging between 6 and 75 years old, each with 2 blood samples taken between 2009 and 2015 and titres measured against every other year's circulating strain (24 strains), each with 2 repeats. I then fit the model to these simulated data to ensure that the true antibody kinetics parameters and infection histories could be estimated. 5 MCMC chains were run for 12,000,000 iterations with a 2,000,000 iteration burn in period. Virus-specific measurement bias terms were fixed as in the fits to the real data. Model fits were assessed based on the root-mean-squared error (RMSE) of the model predicted latent antibody titres after converting the continuous model-predicted titres into discrete measurements as they would be observed (negative titres recorded as a titre of 0 and all titres observed as their integer floor value) compared to the observed titres.

6.2.7 Statistical and post-hoc analyses

I fit generalised additive models for all analyses investigating the link between outcomes (raw HI titre at time of second serum sample, change in HI titre between visits, presence of infection etc) and predictor variables (age, age at time of strain circulation etc). All analyses were performed using the

mgvc R-package [477, 525]. Statistical model comparisons were made based on the Akaike information criteria (AIC) and Bayesian information criteria (BIC).

To test for patterns of influenza incidence in space, nonparametric correlation as a function of distance was tested using the *Sncf* function from the *ncf* R-package [526, 527], where observations were the quarterly attack rate estimates stratified by location ID (40 locations, 190 observations per location). I fit these spline correlograms to 100 samples from the posterior distribution, resampling the 40 locations with replacement for each sample to generate posterior medians and 95% credible intervals (CI). This analysis was repeated using estimated attack rates since 1968, since 2008, and pre-2008 with and without virus-specific measurement bias.

I also investigated the relationship between titre at the time of infection and probability of infection. Samples were drawn from the estimated posterior distribution of antibody kinetics parameters and infection histories, and model-predicted pre-infection latent antibody titres against the circulating strain were generated for each individual at each possible infection time. Predicted titres were converted to integers, with titres ≥ 8 assumed to be 8 to match the observation process. I calculated the proportion of time periods where infection occurred ($Z_{i,j} = 1$) (the overall probability of infection) stratified by log titre at the time of infection relative to the overall probability of infection with a log titre of 0. Many of the inferred infection histories had runs of infections in consecutive quarters (eg. (0, 0, 0, 1, 1, 1, 0, 0)). It is possible that these consecutive runs do not represent true reinfections, but rather single infections that elicited higher antibody boosting than allowed by the model (due to variation in boosting magnitude not captured by the fixed effect on μ_l and μ_s). I therefore repeated this analysis after removing consecutive infections (eg. (0, 0, 0, 1, 1, 1, 0, 0) becomes (0, 0, 0, 1, 0, 0, 0, 0)). I repeated this process for 100 posterior samples to generate median and 95% credible interval estimates on the relationship between titre and relative risk of infection.

6.3 Results

In this section, I first describe the raw HI titre data and demographics of the cohort. I then investigate how the raw HI titres vary by age and across space by fitting generalised additive models. Then, after demonstrating the validity of the model fits to simulated and real data, I use the augmented infection histories to investigate epidemiological trends. First, I summarise the inferred attack rates, focusing on recent years and reinfection frequency. I then demonstrate that these attack rates vary significantly across locations. I then move on to describe patterns of A/H3N2 infection incidence with age. Finally, I reconstruct antibody titres for each individual at all times in their life and find an age-specific relationship between homologous HI titre and probability of infection.

6.3.1 Description of Fluscape data

A total of 67,683 HI titres were measured for 1,130 individuals, each with 2 serum samples against 20 A/H3N2 strains. For the individuals used here, 40 unique locations and 651 unique households were included in the analysis. Participant ages in 2015 ranged from 6 years to 97 years, with a median of 50 years. Study visit times with serum samples were between 2009-12-22 and 2015-06-02 (Fig F.1).

The Fluscape cohort participants were similar to the age and household size distribution of the Chinese population for all but the youngest age group, which was underrepresented, and the 40-59 age group, which was over-represented (Table 6.2 and Table F.1) [474]. When asked at the most recent study visit, the vast majority of individuals reported not to have received an influenza vaccination since the last visit (99.55%). When considering all study visits (including those visits for which serum samples were not available), 19.5% of individuals reported to have ever received an influenza vaccination when asked at study visit 1 (N=892), compared to only 2.48% of individuals when asked at study visit 4 (N=1087). Of the 654 participants that answered (at the time of their second visit), 446 (68.20%) of individuals reported to have experienced ILI since the last study visit. The vast majority of households were family homes with running water. The majority of locations were classified as rural (30/40), were between 20 and 80 minutes travel time from Guangzhou city centre, and had a population density between 256 and 367,346 at the 9km grid cell level (Table F.2).

6.3.2 Antibody titres vary by age and in space

Antibody titres followed an age-specific pattern and systematically varied between strains. I describe these trends briefly here, though note that a parallel effort is underway with Fluscape collaborators.

Table 6.2: Demographic characteristics of the 1,130 Fluscape participants.

Variable	Response	N	%
Gender	Male	589	52.12%
	Female	541	47.88%
	Total	1130	
Age group	0-9	7	0.62%
	10-19	48	4.25%
	20-29	110	9.73%
	30-39	122	10.80%
	40-49	275	24.34%
	50-59	276	24.42%
	60+	292	25.84%
	Total	1130	
Occupation status	Full time employment	310	31.60%
	Self employed	181	18.45%
	Employed part time	70	7.14%
	Retired	142	14.48%
	Student	41	4.18%
	Looking after house/family	101	10.30%
	Permanently sick/disabled	7	0.71%
	Unemployed	124	12.64%
	None of the above	5	0.51%
	Total	981	
Smoked in the past month	No	714	63.35%
	Yes	413	36.64%
	Total	1127	
BMI at latest visit	Underweight (BMI <18.5)	114	10.33%
	Healthy ($18.5 \leq \text{BMI} < 25$)	683	61.87%
	Overweight ($25 \leq \text{BMI} < 30$)	268	24.28%
	Obese ($30 \leq \text{BMI} < 40$)	39	3.53%
	Total	1104	
Vaccination since last visit (at latest visit)	No	1114	99.55%
	Yes	5	0.45%
	Total	1119	
ILI since last study visit (at latest visit)	No	208	31.80%
	Yes	446	68.20%
	Total	654	

Fig 6.1 shows the distribution of log HI titres for each individual stratified by tested strain at each individual's first and second visit. Three patterns emerge. First, log titres were mostly low or undetectable against strains that circulated before individuals were born. Second, antibody titres increased against multiple recent strains between study visits (Fig 6.1C), suggesting a high level of recent exposure and possible cross-reactive boosting against antigenically related strains. Third, some strains exhibit systematically higher titres than others, for example, titres against A/Fujian/2002, A/Beijing/1992 and A/Mississippi/1985 are higher than all other strains for all individuals and particularly high for individuals that were young at the time of their circulation.

Stratifying the population by age group and considering seropositivity, defined as having a log HI titre $\geq 1:40$, highlights these findings further (Fig 6.2). The proportion seropositive tends to be highest for the age groups that are youngest at the time of strain circulation, and increased between the first and second serum samples (Fig 6.2A&B). The proportion of individuals that seroconverted between serum samples was also high against recent strains at near 50% across all age groups. Of note is the seroconversion of the youngest age group to strains that circulated prior to their birth (Fig 6.2C, A/California/2004, A/Fujian/2002, A/Fujian/2000 and A/Victoria/1998).

To compare trends in these data to previously published work, I fit generalised additive models to both raw HI titre at the time of the second serum sample (as a measure of overall exposure) and to change in HI titre between visits (as a measure of recent exposure). Models that included strain independent intercepts and basis spline terms on age at sample time and age at time of strain circulation were substantially better supported than models with only strain independent intercepts (Table F.3). The pattern of highest titres occurring among individuals that were within their first decade of life during strain circulation, decreasing into middle age and increasing again at older ages is in agreement with analysis of an earlier pilot study of a similar population, demonstrated in Fig F.2 [92]. Serological trends were qualitatively similar between study locations (Fig 6.3); however, statistical analysis suggested that regression models that included a fixed effect on location ID were better supported by AIC and BIC than models without, and explained a greater proportion of the deviance (Table F.3).

6.3.3 Fitting of the antibody kinetics and infection history model

Using the *serosolver* R-package, I fit a quarterly infection history and antibody kinetics model to the titre data, simultaneously providing infection history estimates at a quarterly resolution and antibody dynamic estimates [476]. Visual assessment of MCMC convergence suggested good fits for all estimated

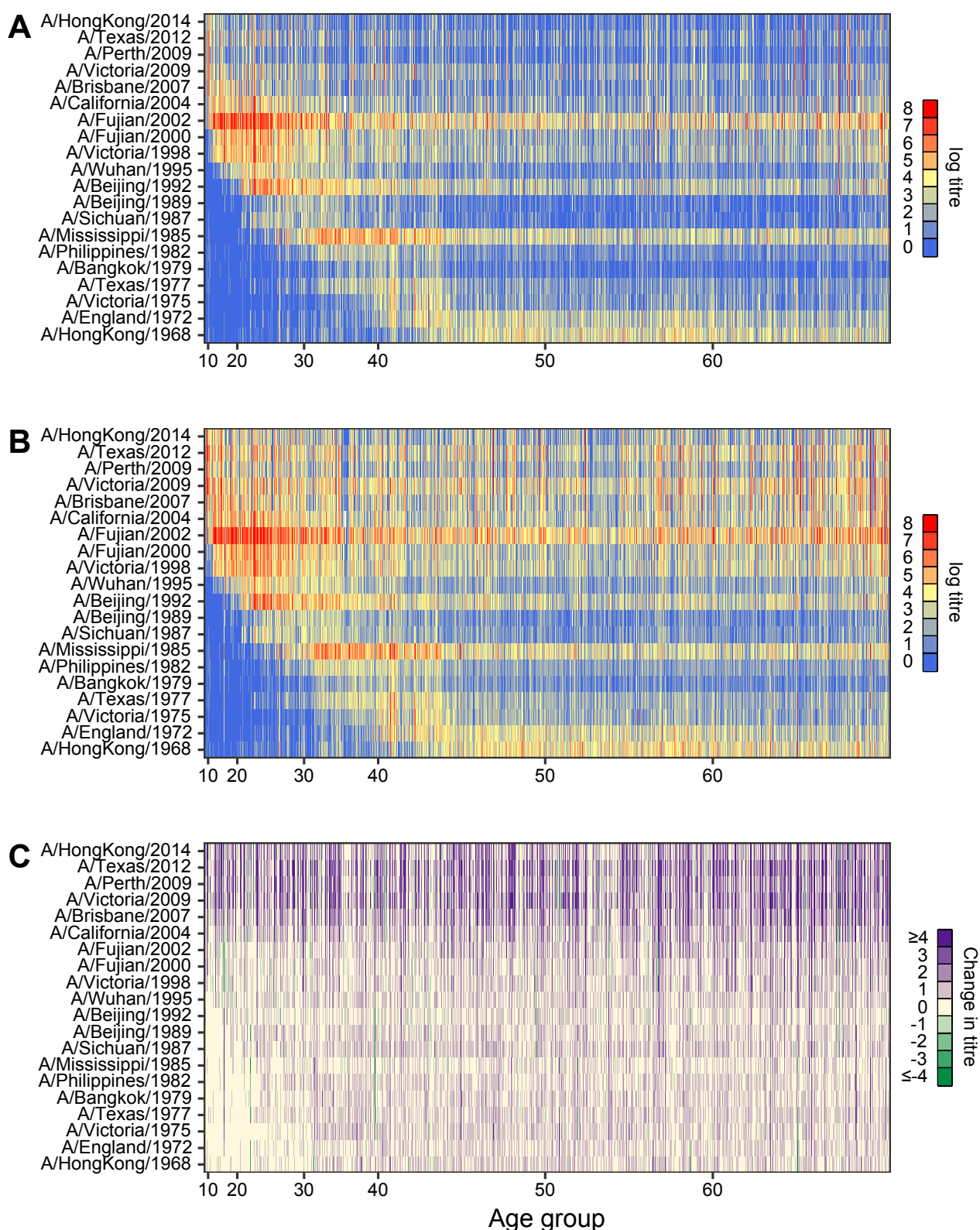


Figure 6.1: Distribution of log HI titres against 20 A/H3N2 strains circulating from 1968 to 2014. Each row corresponds to an influenza strain. Each column corresponds to an individual, grouped and ordered by increasing age. (A) Distribution of titres at the first serum sample. (B) Distribution of titres at the second serum sample. (C) Change in titre between study visits. Adapted from code written by Bingyi Yang and Justin Lessler.

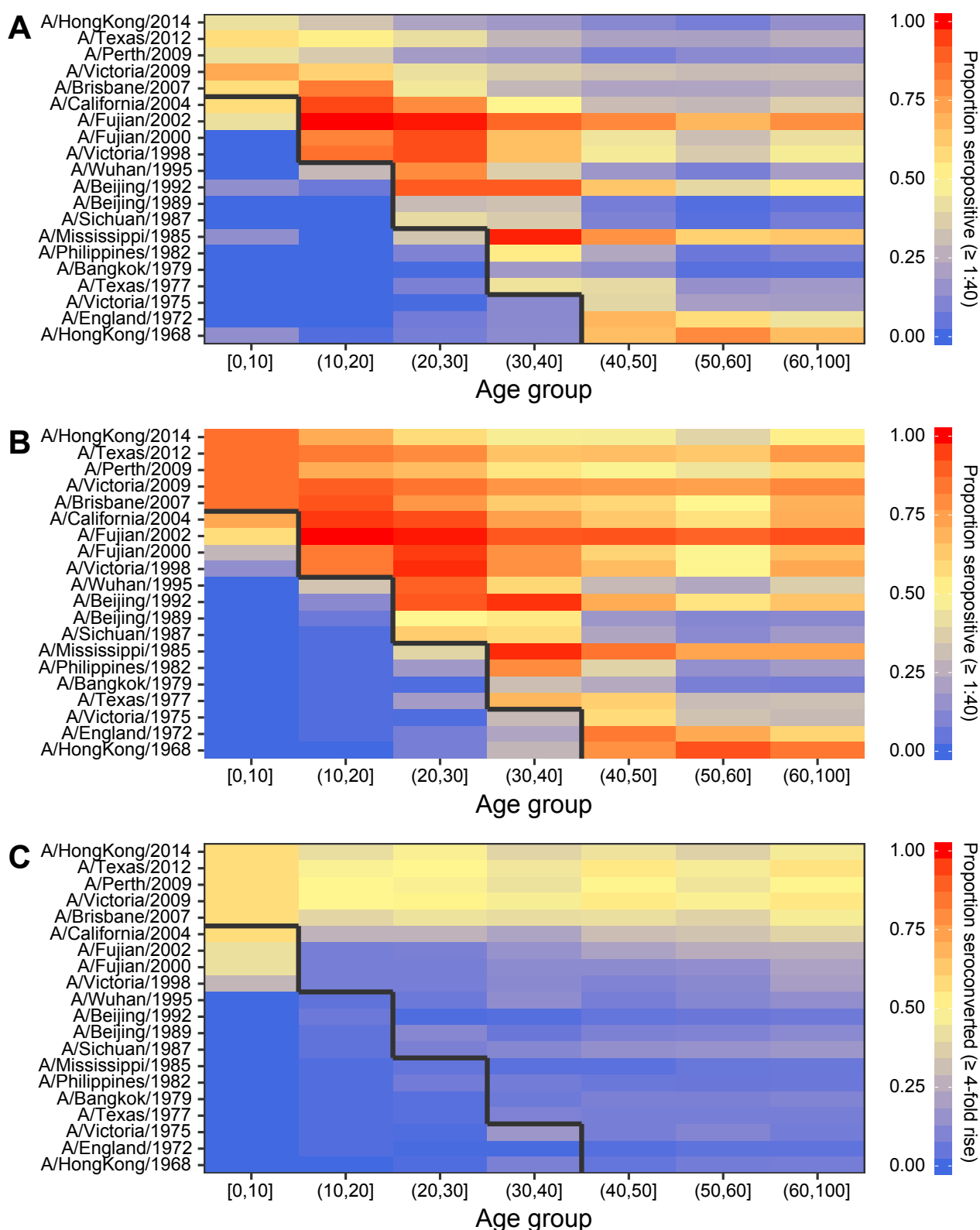


Figure 6.2: Proportion of individuals seropositive and seroconverted to 20 A/H3N2 strains circulating from 1968 to 2014 stratified by age. Solid black line divides age groups that were alive at the time of strain circulation. Seropositivity was defined as having an HI titre of $\geq 1:40$ (a log titre of 3). (A) First serum sample. (B) Second serum sample. (C) Seroconversion between samples, defined as a ≥ 4 -fold increase in HI titre.

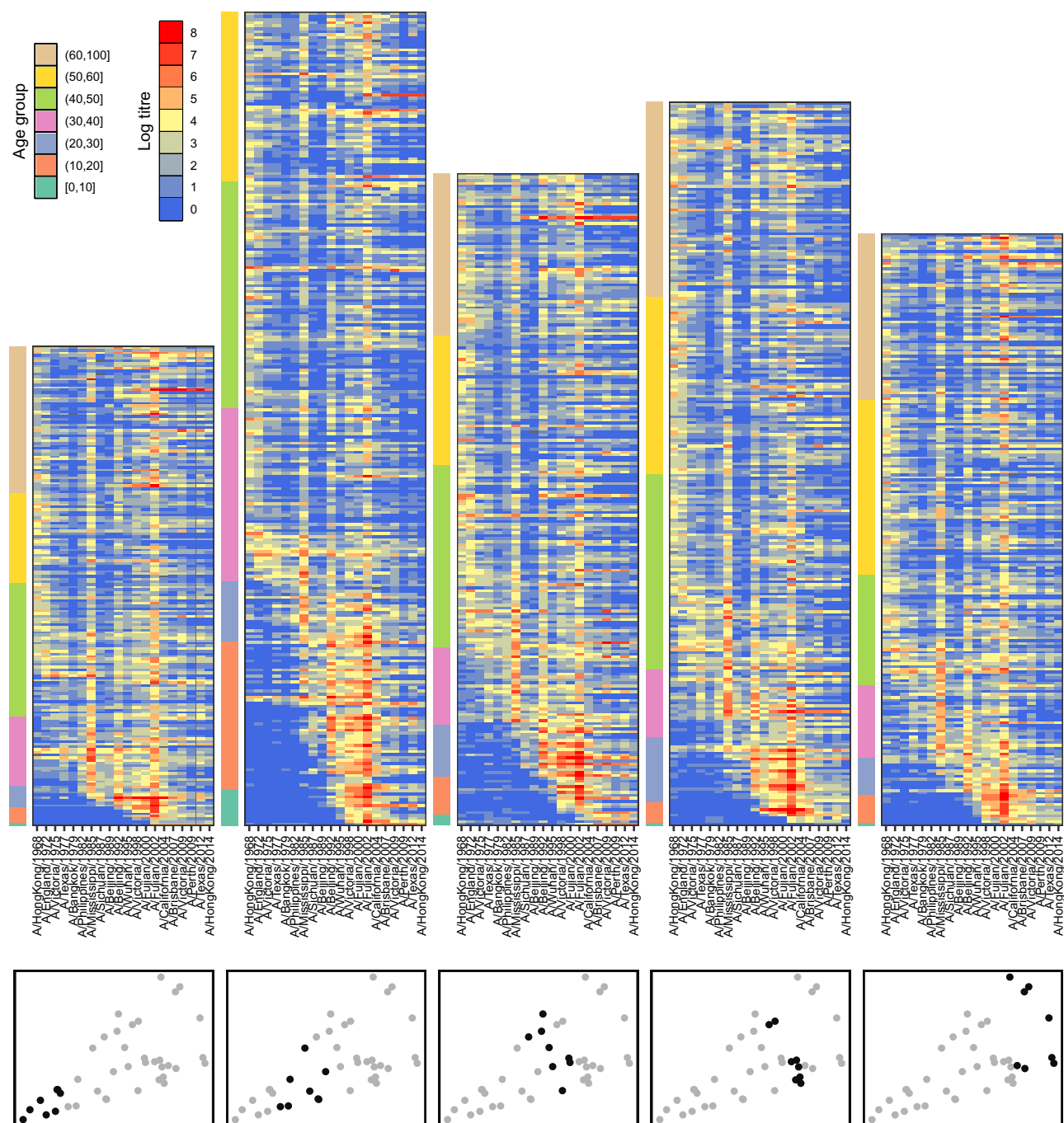


Figure 6.3: Distribution of log HI titres by study location at first serum sample. Locations were grouped into quintiles based on increasing distance from Guangzhou city centre (bottom panels). Individuals were grouped by age and plotted with increasing age. Colours to the left of each subplot show age group. Adapted from code written by Justin Lessler and Derek Cummings.

model parameters and quarterly attack rates (Fig F.3&F.4). Furthermore, effective sample sizes > 200 and upper 95% confidence intervals on the PSRF < 1.1 were obtained for all model parameters (Table 6.4). Model predicted titres and infection histories compared to observed titres are shown for 5 randomly chosen individuals in Fig F.5. The RMSE of the posterior median predicted titres was 0.671 when using the model with virus-specific measurement bias compared to 0.864 using the model without. Infection history and antibody kinetics parameter recovery was accurate when using the simulated dataset, with all 95% CIs capturing the true values (Fig F.6). However, in the simulated dataset, chain mixing was poor for attack rates in some historic and recent times, which motivated longer chains and greater sampling of infection states for these times. RMSE of the posterior median predicted titres was 0.811 for the simulation-recovery data.

Parameter estimates from the model without virus-specific measurement bias were broadly the same (Table F.4), though measurement error variance and long-term antibody boosting were higher. The higher antibody boosting was likely due to the model attempting to explain the artificially high titres for some years that would otherwise be captured by the measurement bias terms.

6.3.4 Inferred historical and contemporary attack rates

Sample attack rates were estimated with high precision using the augmented infection histories and were particularly well constrained for circulation times during the Fluscape study period (Fig 6.4A). The estimated median quarterly sample attack rate was 4.36% (median across all posterior samples; 95% CI: 3.78%-4.94%). Overall, quarterly attack rate estimates varied substantially over time, ranging from a minimum of 0.459% (posterior median; 95% CI: 0.00%–2.81%) in Q2-1977 to a maximum of 68.6% in Q1-1968 (posterior median; 95% CI: 56.2%–75.6%). The attack rate estimate for Q1-1985 was unusually high (58.5% (posterior median; 95% CI: 47.4%–67.7%)), suggesting that there might be residual bias from systematically higher titres for the A/Mississippi/1985 virus not captured by the virus-specific measurement bias term.

Quarterly attack rate estimates were substantially higher since the first Fluscape serum sample in Q4-2009 than the overall average (4.36% (median; 95% CI: 3.78%-4.94%)), with the median quarterly attack rate estimated to be 9.93% (median of all posterior samples; 95% CI: 8.67%–11.5%) in this period. Annual attack rates (defined as the proportion of individuals who were inferred to have been infected at least once per year) fluctuated, with lower attack rates in 2010, 2012 and 2013, and high attack rates in 2011 and 2014 (Table 6.3). These attack rate estimates were of a similar magnitude to

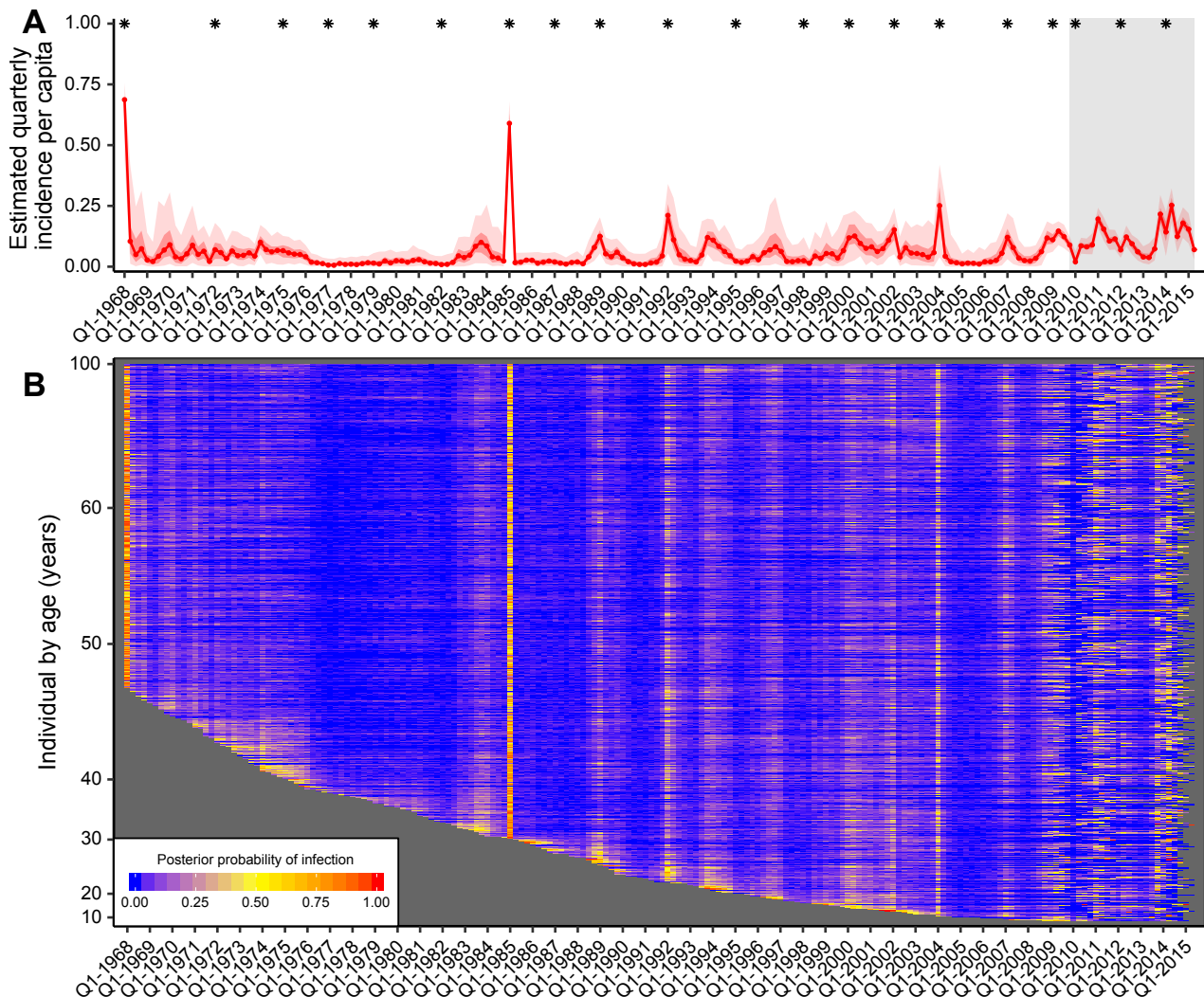


Figure 6.4: Quarterly incidence and individual infection histories from the Fluscape dataset. (A) Model predicted per-capita incidence per quarter. Attack rates were estimated by dividing the number of inferred infections by the number alive in each 3 month period. Red line shows posterior median estimate from 100 posterior samples. Dark and light red shaded regions show 50% and 95% credible intervals respectively from 100 posterior samples. Gray shaded box shows duration of the Fluscape study. Asterisks mark times from which a sample circulating strain was tested. (B) Inferred infection histories for each individual. Each row represents an individual ordered by increasing age. Each column represents the time of a potential infection. Cells are shaded based on the number of the posterior samples with an infection at that time divided by the total number of posterior samples for that infection state.

the proportion of individuals that seroconverted between study visits against strains that circulated during that time.

Table 6.3: Estimated attack rates and infection patterns 2010–2014. Percentages shown are posterior median and 95% credible intervals. Attack rate was defined as the proportion of individuals who were infected at least once in that year. “Reinfected” gives the percentage of people that were infected more than once in a year. “Seroconverted” gives the percentage of individuals that seroconverted to that measured strain, with ranges showing 95% binomial confidence intervals. Bottom row shows percentage of individuals that were infected 0, 1, 2, 3, 4 or 5 times from 2010-2014 inclusive.

Year	Measured strain	Attack rate with virus offsets	Attack rate without virus offsets	Reinfected	Seroconverted (N=1127)
2010	A/Perth/2009	25.0% (21.3%–28.4%)	21.9% (18.7%–25.4%)	3.27% (2.21%–4.42%)	47.7% (44.8%–54.1%)
2011		43.5% (40.6%–46.5%)	46.0% (41.8%–49.9%)	6.09% (4.48%–8.28%)	
2012	A/Texas/2012	27.2% (22.9%–31.5%)	30.6% (24.8%–36.6%)	3.97% (2.35%–6.23%)	50.8% (47.8%–53.7%)
2013		31.7% (25.7%–37.7%)	29.8% (21.4%–37.6%)	7.09% (5.34%–8.93%)	
2014	A/HongKong/2014	54.0% (49.5%–58.6%)	62.9% (57.1%–67.3%)	6.27% (5.17%–7.46%)	42.7% (39.8%–45.6%)
Total number of infections from 2010-2014					
0	1	2	3	4	5
11.9% (10.8%–13.0%)	31.1% (29.2%–32.9%)	32.6% (30.4%–34.8%)	18.1% (16.3%–19.9%)	5.58% (4.42%–7.04%)	0.796% (0.354%–1.24%)

Based on the unmodified infection histories, a significant percentage of people were reinfected within a single year, with higher reinfection rates in the years with higher attack rates. 36.7% (posterior median; 95% CI: 33.5%–40.2%) of all reinfections occurred since 2008, whereas only 16.6% of possible infection states were in this time frame, suggesting that reinfections were disproportionately more likely in recent time periods. Although these reinfections may be real, particularly amongst children [475, 528–532], it is likely that the model is explaining unusually high antibody boosts from single infections through a series of consecutive infections. Annual reinfection rates, defined as having been infected at least once in a year across multiple years, were also high for recent years (Table 6.3).

I fit a logistic regression model to inferred infection or seroconversion versus reported ILI, but found no association between reported ILI and inferred infection or seroconversion between study visits.

6.3.5 Attack rates varied in space but were not predicted by proximity

Attack rates exhibited variation between locations over time (Fig 6.5), though the timing of high attack rate periods was synchronised. Fig F.8 shows snapshots from an animation of attack rates over time across space, demonstrating that the seasons of high influenza incidence are synchronised across the study locations, but that there is some variation in the timing and magnitude of incidence. The overall coefficient of variation across locations for all times was 1.02 (posterior median; 95% CI: 0.950–1.09). To demonstrate the significance of these results, I generated a comparable null model where no significant spatial variation would be expected. First, I drew an attack rate from a uniform distribution between 0 and 1. Then, for each of the 40 locations, I drew a number from a binomial distribution with this mean and $n = 20$. I repeated this process 1000 times to give sample-based attack rate estimates for each study location comparable to those estimated using the augmented infection states. The mean coefficient of variation for these simulations was 0.293 (95% quantiles: 0.0307–1.23), suggesting that there was substantially more variation in the inferred attack rates across space than would be expected by chance. Fig 6.6A demonstrates that this pattern is maintained across time, though may become less pronounced in recent years. However, despite this spatial variation, there appeared to be no correlation in attack rates between locations that were close to each other (Fig 6.6B). This lack of correlation was observed when sub-setting by recent (from 2008 onward) or historic (from 1968 to 2008) times, or when using results from the model without virus-specific measurement bias.

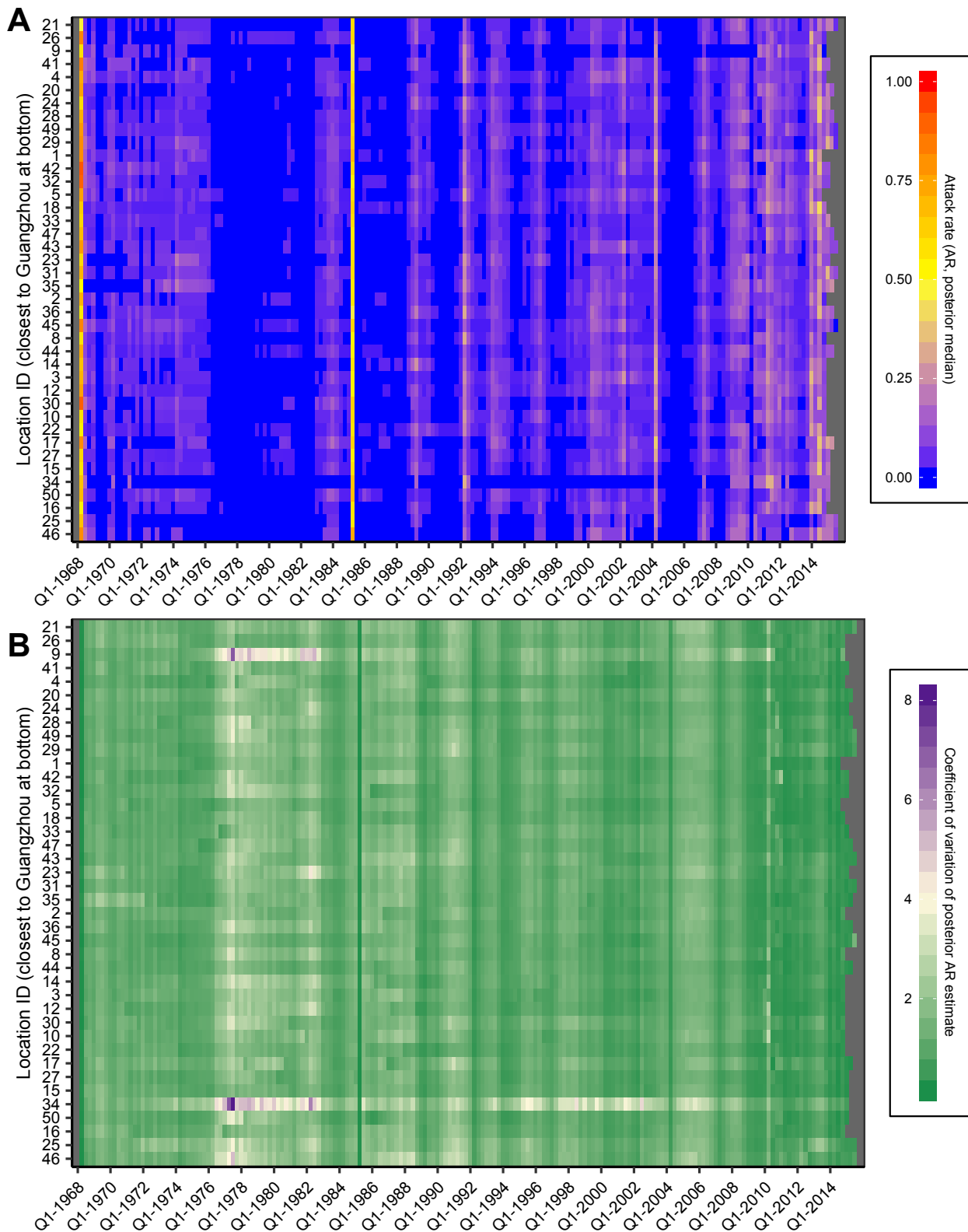


Figure 6.5: Quarterly attack rates across the 40 Fluscape study locations. Each row represents one study location ordered by increasing distance from Guangzhou city center. Each column represents a 3 month period. Cells are shaded by the posterior median inferred attack rate. (B) Coefficient of variation of the posterior quarterly attack rate estimate for each location.

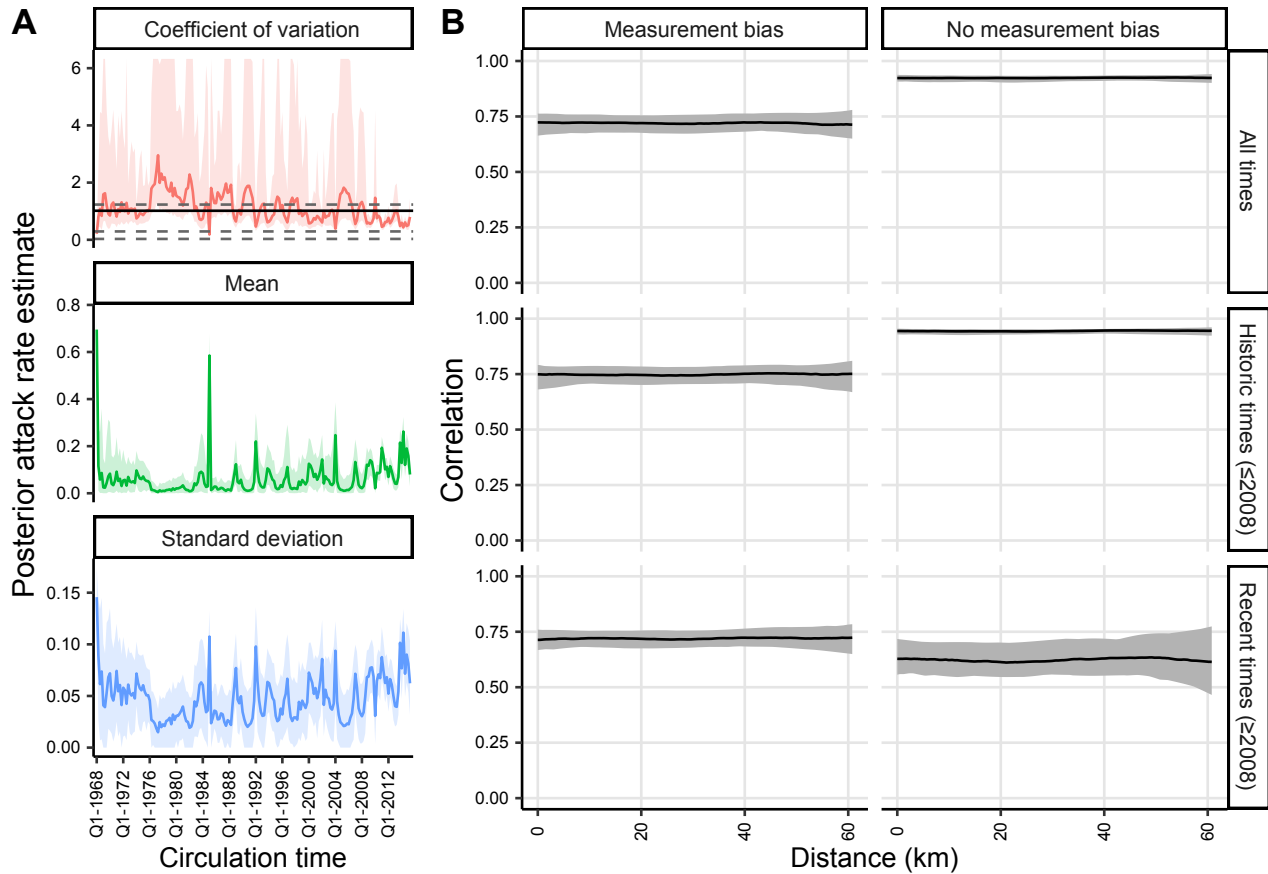


Figure 6.6: Spatial variation in attack rate estimates over time and correlation of nearby locations. (A) Coefficient of variation, overall mean and standard of attack rate estimates across the 40 study locations. Solid lines and shaded regions show posterior medians and 95% credible intervals. Solid black horizontal line shows overall coefficient of variation across all time. Dashed horizontal lines show mean and 95% quantiles on simulated coefficients of variation under the assumption that attack rates are the same across space. (B) Fitted spline correlograms showing lack of correlation between incidence and spatial proximity. 100 samples were drawn from the posterior and 40 locations were re-sampled with replacement. For each sample, the spline correlogram was calculated using the *Scnf* function from the *ncf* R-package. Solid lines and shaded regions show median and 95% quantiles of these 100 samples. Each subplot shows the same relationship under the model with strain-specific measurement bias and without, and for infection states from all times, since 2008 and before 2008.

Table 6.4: Antibody kinetics parameter estimates from the full model fit to the Fluscape data, assuming fixed strain-specific measurement bias. Results shown are posterior medians and 95% credible intervals. $\hat{R}$ value shown is the upper 95% confidence interval on the PSRF.

Parameter	Description	Ha Nam	Fluscape	$\hat{R}$	ESS
μ_l	Long term antibody boosting	1.98 (1.91–2.05)	1.37 (1.35–1.40)	1.09	730
μ_s	Short term antibody boosting	2.74 (2.52–2.97)	2.24 (2.16–2.35)	1.05	516
τ	Suppression	0.0465 (0.0422–0.0498)	0.032 (0.0303–0.034)	1.03	899
ω	Waning	0.706 (0.650–0.766) per year	0.205 (0.199–0.218) per quarter	1.03	430
σ_l	Long term cross reactivity	0.133 (0.110–0.115)	0.0711 (0.0704–0.0718)	1.02	1220
σ_s	Short term cross reactivity	0.0259 (0.0223–0.0296)	5.71e-05 (3.08e-06–0.000297)	1.01	2380
ϵ	Measurement error	1.20 (1.27–1.32)	0.641 (0.636–0.646)	1.02	1920
$\sum X$	Total infections	NA	12400 (12100–12600)	1.07	882

6.3.6 Age-specific infection patterns

Periods of high infection probability were synchronised across all individuals regardless of age (Fig 6.4B). However, two age-specific patterns emerged. First, almost all individuals that were alive in 1968 were almost certainly infected in 1968, demonstrated by the high posterior probability of infection across all individuals. Second, the posterior probability that an individual was infected soon after birth was consistently high, demonstrated by the lower edge of Fig 6.4B. The posterior mean, median and 95% CI on the age of first infection was 1.14, 0.50, and 0.00–5.25 years respectively (estimates using 100 posterior samples for all individuals born since 1968). These results suggest that based on these augmented infection histories, individuals were infected almost immediately after birth, in contrast to previous findings [90].

Based on the inferred infection histories, individuals were estimated to be infected 2.35 times per 10 year period (1.27–9.45) (posterior median; 95% CI) (Fig 6.7D). The number of infections per 10 year period also appeared to decrease with increasing age at time of infection (Fig 6.7C). To test the sensitivity of these estimates to the assumed infection history prior (as discussed in detail in Chapter 5), I compared infection history estimates under the prior assuming independent infection risk across all individuals, rather than a population-wide, time-specific probability of infection applying to all individuals as in the main results (Fig F.9). Infection frequency patterns and trends with respect to age were similar under both priors, suggesting that these findings were driven by features of the data and not artefacts of the assumed infection history prior.

6.3.7 Relationship between titre and probability of infection

Using samples from the estimated posterior distribution of antibody kinetics parameters and infection histories, I calculated the model-predicted true latent titres at each potential time of infection against the strain circulating at that time (Fig 6.8). For each sample from the posterior, I found the proportion of infection states (across all times and individuals) where infection occurred stratified by log titre at the time of infection. Using the unmodified infection histories suggested an unusual relationship between infection probability and titre: as titres increased from 0, the relative risk of infection decreased up to a log titre of 3, and then appeared to increase at higher titres (Fig F.10). I hypothesised that this was due to the abundance of consecutive reinfections as discussed above, where the model attempts to explain very high titres through repeated reinfection with antigenically similar strains. I therefore repeated the analysis after removing repeat infections. The total number of infections (out of 186,925

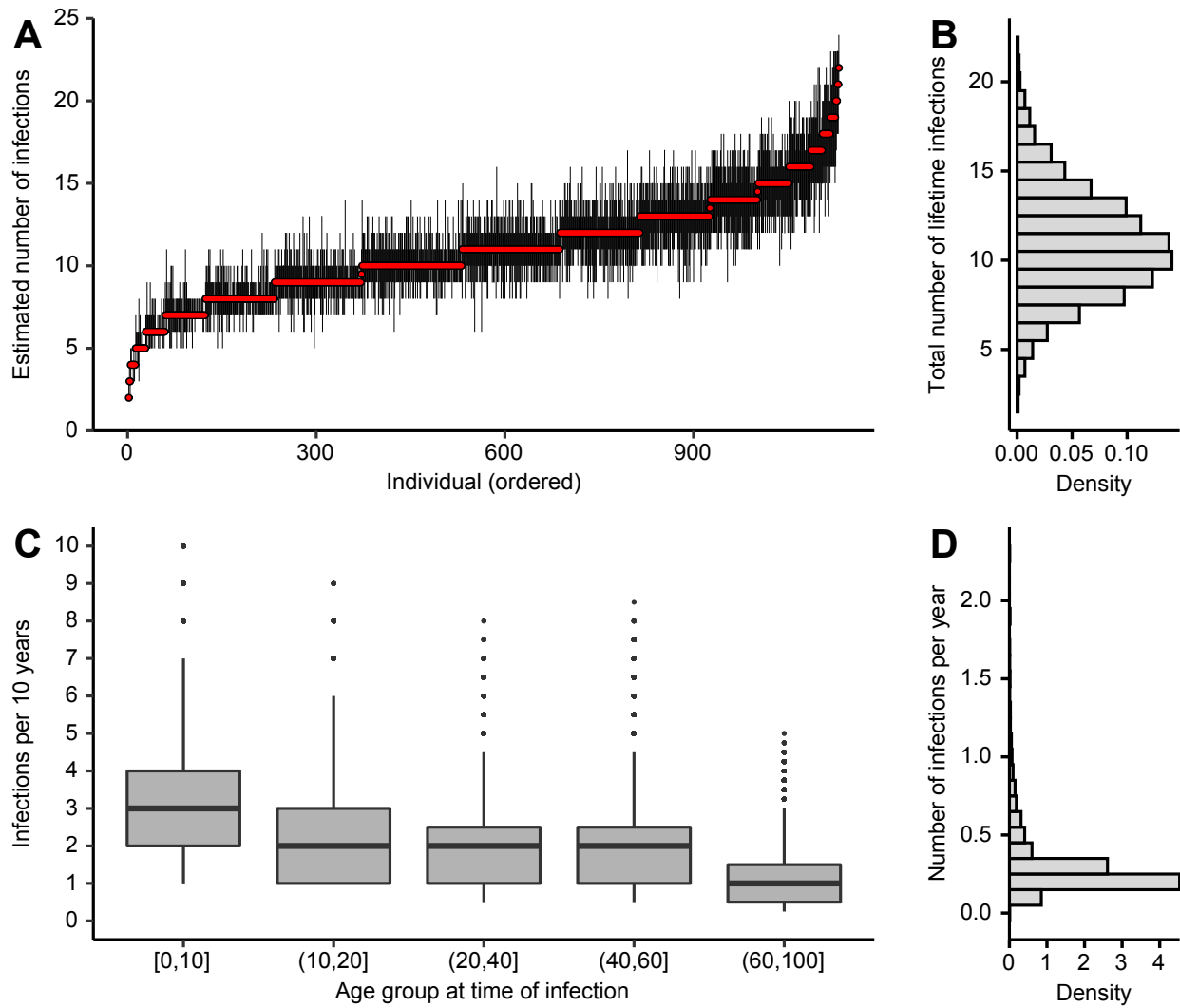


Figure 6.7: Age-specific patterns of infection. (A) Pointrange plot shows median and 95% CI on the total number of lifetime infections for each individual. (B) Distribution of the total number of infections across all individuals based on the posterior median total number of infections. (C) Number of infections per 10 year period stratified by age group at the time of infection. (D) Number of infections per year across all individuals.

possible infections) before removing repeats was 12,350 (posterior median; 95% CI: 12,165–12,525) compared to 11,251 (posterior median; 95% CI: 11,071–11,442) after (for comparison, there were 10,956 (posterior median; 95% CI: 10,699–11,226) total infections in the model with no virus-specific offsets).

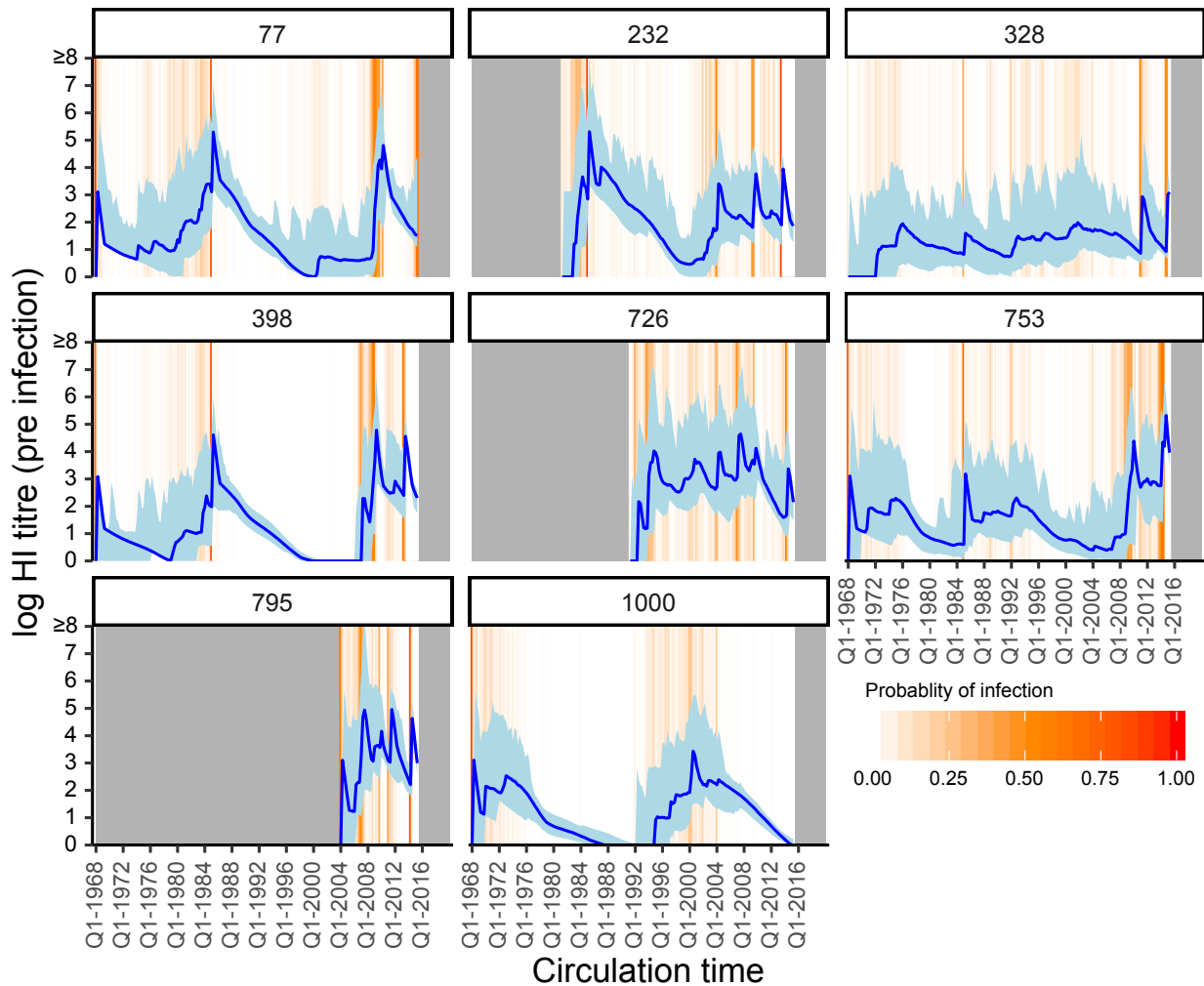


Figure 6.8: Model predicted titres against circulating strains since birth. Each subplot shows one randomly selected individual. X-axis shows time since birth. Blue line and shaded region show model-predicted, true latent titre against the strain assumed to be circulating at each time period (posterior median and 95% credible intervals). Note that titres are continuous and do not include virus-specific measurement bias. Orange lines indicate times of high posterior probability of infection. Grey regions show times before birth and after the last serum sample.

Once consecutive repeated infections were removed from the inferred infection histories, there was a clear pattern of decreasing relative risk of infection as a function of increasing titre (Fig 6.9). An HI titre of 1 : 40 (log titre of 3) corresponded to a relative risk of infection of 0.550 (posterior median; 95% CI: 0.521–0.594). This pattern appeared to vary with age. An HI titre of 1 : 40 gave a relative risk of infection of 0.287 (posterior median; 95% CI: 0.250–0.320) in the 0–10 age group, but only 0.753 (posterior median; 95% CI: 0.627–0.889) in the 60+ age group. This pattern was retained when

repeating the analysis using estimates from the model without virus-specific measurement bias, though the relative risk for the 60+ age group was in line with the other > 10 age groups. Fig F.11 shows the results of fitting a logistic regression model to the inferred infection states versus log latent titre.

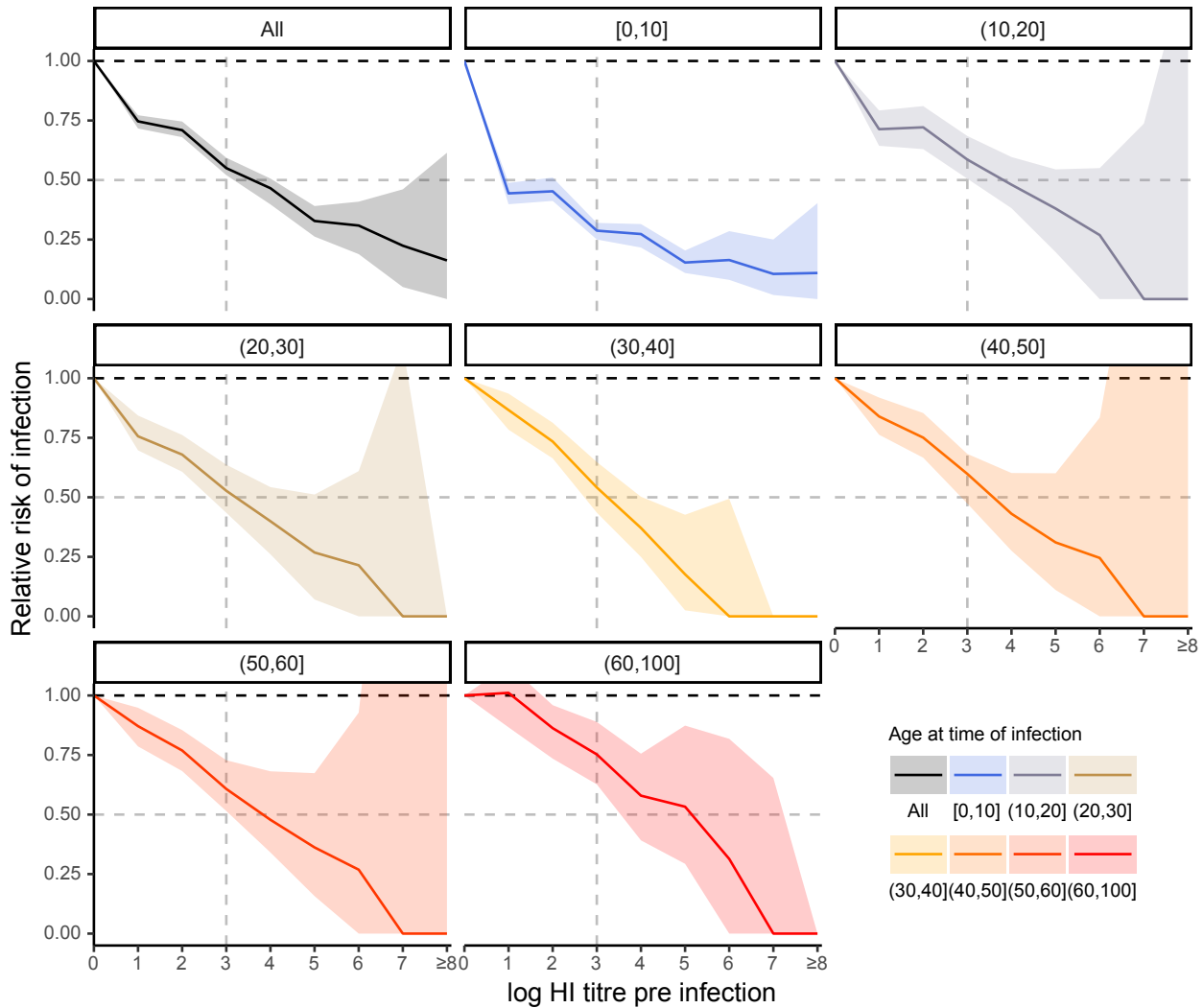


Figure 6.9: Estimated relationship between HI titre and probability of infection. Titres were predicted based on unmodified infection histories, but infection risk was assessed based on infection histories with consecutive reinfections removed as described in the main text. Top left panel shows the relative risk of infection at all time points stratified by model-predicted log titre against the circulating strain just before infection for all age groups. Remaining plots show same relationship but stratified by age group at the time of infection. Solid lines and shaded regions show posterior median and 95% CI. Vertical dashed line shows HI titre 1 : 40. Horizontal grey dashed line shows 50% protective titre.

6.4 Discussion

In this chapter, I have demonstrated that antibody titres against influenza A/H3N2 strains exhibited significant variability across strains, time and locations based on a cohort of 1,130 individuals in Guangzhou, China. Variation in antibody titres across individuals and to different strains may be generated in three ways: (i) exposure to different combinations of antigens at different times; (ii) time-dependent antibody kinetics observed at different times relative to an exposure; (iii) systematic and random variability of different viruses in the assay used. I have shown that accounting for these mechanisms allowed for the reconstruction of each individual's entire infection history for each 3-month window from birth. I reconstructed population-wide and location-specific historical seroresponse rates from these infection histories, finding that influenza exposure incidence may be higher than previously thought. Finally, estimates of each individual's true antibody titre against circulating strains for each 3-month period since birth were generated, showing that elevated antibody titres were associated with a marked reduction in infection risk that became less effective with increasing age.

Influenza A/H3N2 seroresponse rate estimates were higher here than in many previous estimates. 5-15% is an oft-cited figure for the population-wide annual seroresponse rate of influenza [533]. Conversion of quarterly to annual incidence is difficult for these inferred infections, as individuals could be infected multiple times per year. Furthermore, the cutoff of an influenza season is not as clearly defined for this region as for temperate regions [534]. However, a 4.36% quarterly seroresponse rate corresponds to a $\sim 16\%$ annual seroresponse rate, which is at the upper end of the 5-15% estimate. When considering the proportion of individuals who were infected at least once, these annual seroresponse rate estimates were very high from 2010–2014, ranging from 25.0% to 54.0% of individuals infected per year. Although virus-specific bias in the HI assay likely led to overestimates of seroresponse rates in some historic years relative to models that took this bias into account, estimates for recent times were consistent under both models. Previous estimates in Hong Kong for the same time period based on four-fold rises in homologous titres were lower at 7-19% [535, 536].

Conversely, seroresponse rate estimates were near zero from 1977-1983 and 1986-1989. Although these estimates may be biased due to the infection history model (eg. shrinkage through the beta prior on infection probability at these times) the generally low titres against strains circulating at these times (eg. Fig 6.1, A/Bangkok/1979) suggest that exposures may have been genuinely infrequent. Furthermore, seroresponse rates for these years was low despite the use of large, negative measurement bias terms

for A/Victoria/1975, A/Texas/1977, A/Bangkok/1979, A/Philippines/1982, A/Sichuan/1987 and A/Beijing/1989 (Table 5.7). Rather, this assumption should lead to bias for increased seroresponse rates, as the model is able to explain low titres with a higher number of infections than if measurement bias was assumed to be negligible or positive.

These results suggest that a boost in homologous log HI titre of 1.37 units marks long-term persistent antibody boosting, and a four-fold rise in titre (a rise of 2 units) alone would therefore only detect infection should some of the transient short-term homologous boost remain. It has been shown previously that traditional threshold values for seroepidemiology risk reductions in sensitivity, and it may be that taking into account antibody kinetics and sub four-fold rises in titre changes our perception of influenza incidence [274].

These results demonstrate that seroresponse rates varied significantly at a small spatial scale throughout time, though I did not find any correlation between adjacent locations. The spatial dynamics of influenza at a large scale are fairly well understood thanks to molecular data, but explaining spatial dynamics at smaller scales has revealed contrasting results and remains the focus of ongoing work [502, 506, 537]. The results shown here have a clearer interpretation than previous analysis of antibody titres in this population [497], as the model accounts for variation in infection histories, exposure duration by age, time-varying seroresponse rates and strain-specific effects. A key future direction is to use these augmented infection histories as data in further regression analyses, for example investigating the role of population density, commuting patterns, changing movement patterns since 1968 and antigenic drift in explaining small-scale spatial heterogeneity [502, 538–541].

I found that the frequency of influenza A/H3N2 infections decreased with age. This finding is consistent with previous age-stratified epidemiological data [225, 475], regression analyses of randomised control trial data [542], theoretical models incorporating age-specific differences in social behaviour [500, 543–546], and a previous analysis of the Fluscape pilot study [86]. The results presented here have a number of strengths. Here, I considered an individual's lifetime exposure history, whereas most epidemiological data is restricted to inference in a particular window of time. Clear age patterns were also observed when different assumptions were made about infection history model structure and priors, suggesting that these patterns emerge from information in the data rather than through any *a priori* assumptions (Fig F.9). Another strong confirmatory result is the estimate of A/H3N2 influenza infection occurring at a rate of 2.35 (posterior median; 95% CI: 1.27–9.45) times per 10 year period. Individuals could be infected every quarter rather than every year as in previous analyses (Chapter 5 and [1]), which

drastically increases the number of potential infections. The fact that the estimated frequency of infection remained the same despite this increase is reassuring. These analyses also included far more individuals and had good coverage of all age groups, which may explain why we see a continued decrease in infection frequency into very old age that was not observed in previous analyses [86].

I found evidence for both a short-term, antigenically broad antibody boost that waned within 2 years, and a persistent, antigenically narrow antibody boost. These parameter estimates show good qualitative consistency with results using a data set from Ha Nam, Vietnam (Chapter 5, Table 5.7), though with some quantitative differences. Estimates for boosting suppression through antigenic seniority (τ) and waning rate were very similar to estimates from the Ha Nam cohort (Table 6.4). Titres resulting from the transient arm of the response were estimated to return to baseline levels within 1.22 years (posterior median; 95% CI: 1.15–1.26) based on these data compared to 1.31 years (posterior median; 95% CI: 1.20–1.45) using the Ha Nam data. Both of these estimates are in line with previous observations of antibody titre longevity waning to near baseline approximately one year post exposure [87, 441, 453, 467, 489]. However, the fact that these waning estimates include some residual waning past one year post exposure does disagree with previous findings which find negligible changes after one year [87]. This may be a limitation of the model structure; the short-term arm of the antibody response was assumed to wane linearly until only the long-term arm remained, whereas the reality may be that non-linear waning or differential waning as a function of antigenic distance may result in the vast majority of response being lost within one year. The waning parameter presented here may therefore be some average of these two processes.

Overall antibody boosting was inferred to be lower here, and short-term boosting was estimated to extend across the entirety of antigenic space [87, 547]. Direct comparison of estimates is difficult given differences in (i) the strains used in the assays, (ii) assay protocols, (iii) the inclusion of virus-specific measurement bias and (iv) the number and ages of individuals used in the analyses [249, 548]. A potential explanation for the increased cross-reactive breadth of antibody boosting is the relatively higher weighting of the titre data towards historical strains and the inclusion of more elderly individuals. In the Ha Nam dataset, there are very few individuals that were alive in 1968 and most titres were tested against strains that circulated in recent years (Fig 5.2). The model may therefore be assigning greater weight to antigenically narrower short term boosting to explain titres against recent strain in the Ha Nam data, but must give greater weighting to boosting of historical strains (back-boosting) in the Fluscape data.

A future refinement of the antibody kinetics model will be to relax the assumption that all exposures elicit the same degree of antibody boosting and waning. This assumption may have confounded the infection history inference to some degree, as the model inferred consecutive quarters of repeated infections to account for higher titres than could be explained by a single boost. Chapter 4 demonstrated that describing antibody kinetics following multiple, varied exposures is complicated, with significant variation in boosting magnitude by exposure type and number [488]. Variation in antibody kinetics parameters, particularly waning rates, have been demonstrated across age groups and by subtype [230, 445, 459, 467]. Variation in antibody boosting by age is captured somewhat by the suppression term in the model, τ , though this imposed a linear decrease in boosting as a function of increasing number of exposures. Number of repeated exposures may account for some of this age-variability along with antigenic seniority [86, 92], though there may be remaining heterogeneity, for example via immunosenescence [549].

Antibody boosting may also vary in an exposure-dose-dependent manner or as a function of disease severity, whereas I assumed that all exposures elicited the same boost [550]. A large number of influenza infections are asymptomatic [520, 521]. This is often seen as a benefit of using serology, which may detect these asymptomatic exposures. However, ascribing all antibody boosts as “infections” does not necessarily give a true picture of clinically relevant incidence. Indeed, these results have the caveat that “attack rates” or “seroresponse” rates refer to all detectable serological responses from an individual’s antibody titre data, and may represent a variety of exposure types rather than just infections. Relaxing the assumption of a fixed antibody boosting may capture some of this variability [225–227, 230], though doing so would drastically expand the already large parameter space and computational burden in this analysis, and was therefore out of scope for this thesis. Constraining infection times for a subset of the population using PCR-confirmed or symptomatic cases would improve the identifiability of infections to power more complex analyses, though again may bias inferred antibody boosting estimates towards a certain class of infection [40, 225, 230].

I have demonstrated here the importance of considering systematic virus-specific variability in HI assay measurements when interpreting serological data. The magnitude of historical seroresponse rate estimates changed dramatically for certain time periods when measurement bias was included in the model compared to the model without virus-specific measurement bias, though not for recent years (Fig 6.4 compared to Fig F.7). The importance of these biases has been discussed previously in the context of antigenic cartography [51, 82, 84, 495], but has not yet been incorporated as a standard

consideration in serosurveillance. Furthermore, given that these biases may be large, they may have a significant impact on estimated latent titres. Fig F.5 demonstrates how the same model-predicted latent titres differ when systematic measurement bias is included or not.

A remarkable finding is the consistency of the inferred relationship between HI titre at the time of infection and probability of experiencing an antibody boost with the longstanding dogma of 50% protection for an HI titre of 1 : 40 [233,243,551]. Another key finding was the decreasing association between reduction in risk and log HI titre with increasing age. These findings are agreement with recent work by Ranjeva *et al.*, who found that although antibody titre was a good correlate of protection in children to A/H1N1 infection, it was not appropriate for adults [225]. Rather, adult protection was better described as a function of increasing age since last infection. These results do contrast with some previous findings [235,312]. Black *et al.* 2011 found that a higher titre cutoff than 1 : 40 was needed for 50% protection in young children (1 : 85 (95% CI, 35.6–137.9) at the time of exposure for 50% protection) – lower than the threshold generated using data from adults in Hobson *et al* [233,235,552].

6.4.1 Study limitations

A key limitation is that the virus-specific measurement bias terms could not be estimated as part of the main model fitting procedure, but rather had to be fixed in advance. This may limit the reliability of the offsets used here and may also have lead to model over-fitting. For example, the inferred seroresponse rate in Q1-1985 was far higher than in any quarter other than Q1-1968. Validating the offset terms using external data would be useful, but is difficult to do across studies given variability in laboratory protocols and serum potency [84,250,553].

It is important to note that there were some small numerical differences in the virus-offset terms used here and those presented in Chapter 5. The results in Chapter 5 were generated using a more recent iteration of *serosolver* after a few bug fixes and improvements. The main results in this chapter (the 3-monthly resolution infection histories) are also run with the updated code and are therefore reliable. I do not expect that this will change any key results, as the updated offset estimates demonstrated almost identical patterns, but with some small difference in magnitude (Fig F.12).

The lack of correlated seroresponse rates between nearby locations may be real, though there are limitations to the analytical approach that may mask any true spatial patterns. First, the infection history model makes the assumption that there is one probability of infection across all locations in a given period of time. This induces a strong population averaging effect on the probability of

individual infections. Second, although it would be possible to assume location-specific infection probabilities, inference of location-specific seroresponse rates is limited by these data given that there are approximately only 25 individuals per location. Locations may be linked in space, but at this small spatial scale, location-specific differences may be difficult to identify alongside other uncertainties in the inferred infection histories. Third, it may be that other location-specific features play a role, such as urban/rural dynamics, that I did not test here.

There are some caveats to the age-specific infection patterns found here. I estimated that individuals experience their first influenza infection within the first year of life, which is far earlier than previous estimates of a gradual increase in seroprevalence up to 6 years of age [554]. The youngest individual in this cohort was born in 2008, limiting the reliability of infection dynamic estimates in very early life and not allowing maternal antibodies to be taken into account. A prospective birth cohort and improved choice of circulating antigenic variants in those early years would help to clarify the timing of first infection based on antibody kinetics. It is also important to highlight the distinction between observed antibody boosting in HI titres and clinically relevant infection. Many of the inferred boosts in these augmented infection histories may be the result of asymptomatic infection or even unsuccessful exposures. Other studies suggest a possible increase in seropositivity in very old individuals, possibly linked to survivor bias [92,555]. These findings must be taken alongside the observation that elderly individuals experience higher rates of hospitalisations and death [556]. The model currently does not allow waning of long-term antibody titres with age nor a non-linear relationship between boosting and age, both of which may mask further boosting at older ages. The decreased exposure frequency in the elderly inferred here must therefore be interpreted alongside the distinction between seropositivity and unobserved antibody boosting.

A limitation of the antibody kinetics model in explaining antibody back-boosting is that cross-reactivity was assumed to extend linearly across antigenic space. In the short-term antibody boosting arm of the model, cross-reactivity was found to boost the entirety of antigenic space regardless of an individual's age to account for the clear back-boosting of titres against strains encountered early in life. However, this model is inappropriate for younger individuals, whose immune systems may not have encountered any of these historic antigens and would therefore have no targeted memory B-cells to stimulate. Some boosting of antibodies to antigenic variants that circulated long before individuals were born has been observed in children following vaccination [547,557], which may be due to the conservation or recycling of some epitopes over time [141]. However, this observation is currently an exception rather than the

norm, and it is unlikely that individuals generally produce antibodies that cross-react with strains that circulated long before birth. For example, Fonville *et al.* demonstrated that cross-reactivity was detected for strains that circulated up to two antigenic clusters before birth [87]. A model that distinguishes boosting of heterologous antibodies through back-boosting of the memory response as opposed to cross-reactive antibodies from a *de novo* response through targeting shared epitopes would provide a more realistic model of the observed, antigenically broad short term boost. This would capture back-boosting of titres to older strains in older individuals without forcing the model to predict elevated titres to pre-birth strains in younger individuals. A serological data set, such as the Ha Nam data, that has a greater number of titre measurements per individual than the Fluscape data could be used to parameterise this more complex model in the future. The concept of back-boosting in the context of underlying B-cell dynamics is discussed in the general discussion, Chapter 7.

Based on results from Chapter 5, I did not include a mechanism for titre-dependent antibody boosting here. This is because parameters for this mechanism were estimated to have no impact on observed antibody kinetics. Omission of titre-dependent antibody boosting, possibly resulting from “antigen-trapping” as described previously [187], does neglect known biological observations of a diminished increase in antibody titre following exposure when pre-existing titres are already elevated [87, 434]. This mechanism might explain the pattern seen in Figure 4.6A, where the second dose of unadjuvanted vaccine did not elicit any additional antibody response following the first dose. In Chapter 4 I found that inclusion of a titre-ceiling mechanism improved model fits, supporting its inclusion in these antibody kinetics models. The inability to identify titre-dependent boosting using the human data in Chapter 5&6 may be due to a lack of power or the flexibility of the model to capture its effects using other parameters (eg. boosting suppression via antigenic seniority). Future work could use additional data to inform parameters for titre-dependent boosting. For example, the Ha Nam data, which has frequent longitudinal sampling, may be better suited to inform short term kinetics. Model comparison may then be carried out as in Chapter 4 to support the inclusion or exclusion of additional model complexity.

I assumed that there was one antigenic variant circulating in each quarter and that antigenic drift proceeded at a constant rate in time along an antigenic summary path. The assumed antigenic distance matrix does take inter-year variation in antigenic distance into account, though antigenic variants may actually be replaced in a more punctuated fashion [82]. Co-circulation of multiple antigenic variants may also affect these results, as observed titres would vary depending on the antigenic distance

between the representative strain for a given time period and the actual strain that they were infected with [71,81,524]. These assumptions may explain why the inferred seroresponse rates did not strictly align with timings of high incidence based on viral isolate data in Guangdong and Hong Kong for the same time period [535,558]. In particular, high seroresponse rates were estimated for 2011 that are not present in other studies [535,558]. The antigenic coordinates of strains that circulated in recent times were based on extrapolations of the smoothing spline fit to available antigenic coordinates for strains up to 2009, and the choice of antigenic variants for these times may therefore be inaccurate. Relaxing the assumptions around antigenic space, heterologous antibody boosting as a function of antigenic distance, and choice of representative circulating strain for infection history inference would be a significant but challenging advancement of these methods, and is left for future work.

Another limitation that applies to this Chapter and Chapter 4 was the assumption that a log titre of 0 represented the true absence of antibodies. For example, individuals enter the model with a log titre of 0 against all strains (assuming that maternal antibodies have waned by this point). As in Chapter 4, this assumption may mask low-magnitude antibody kinetics acting at or below the limit of detection of the assay. This may lead to the inference of a lack of seroresponses, whereas these may simply not be detectable. Re-running the model with lower values for the “true zero” titre would allow comparison of the inferred infections histories and antibody kinetics parameters when this assumption is relaxed. A mathematically suitable, but computationally infeasible approach within the MCMC framework would be to integrate over values for the “true zero” titre at each iteration. These advances are left for future work.

As stated by Hobson in 1972, care must be taken in assigning causality to the titre-mediated infection risk estimated here. In the present model, titres necessarily decreased over time following infection due to antigenic drift and short-term waning. If protection is governed by non-HI immunity that wanes at a similar rate, than the same association between titre and relative risk would be observed. It is also important to consider that HI titre is a proxy for exposure here and that vaccination coverage is low. Antibody titres achieved through vaccination may not be accompanied by as strong a cellular response, and the titre cutoff for 50% protection following vaccination may therefore need to be higher than for titres elicited by natural infection. Non-HI detectable protection may also explain the findings of decreased infection frequency at older age despite decreasing titre-mediated protection, for example via non-haemagglutinin head targeted antibodies or cellular responses [559,560]. Finally, it is important to clarify that the relationship here describes reduced risk of experiencing a serological response, and not

necessarily an infection.

6.5 Conclusion

The results shown here demonstrate the amount of information inherent in longitudinal serological studies when antibody titres to multiple strains are measured. However, I have also shown how assumptions regarding the underlying biological process and infection generating process can significantly impact the interpretation of results. The ability to reconstruct individual level infection histories at a fine spatial scale and for historical strains provides a new source of augmented epidemiological data, which can be used to answer new scientific questions or to validate previous findings.

Chapter 7

Discussion

Antigenically variable pathogens generate complex disease dynamics, confounding the detailed interpretation of surveillance data. A large part of this complexity is due to repeated exposures to antigenically varied, but still related strains or variants. Because many of these exposures occur long before epidemiological studies are conducted, it is difficult to account for an individual's life course epidemiology with respect to an infectious disease. A common thread of this thesis was to develop Bayesian inference methods to generate probabilistic estimates of past exposures and the dynamical process parameters that sit between exposure and observation. In this final section, I discuss the key findings of each chapter and summarise their implications. I end the thesis by discussing future directions. I pay particular attention to potential methodological advances in linking further immunological complexity to influenza antibody kinetics models and extension of the *serosolver* R-package to incorporate different exposure types and observation models.

7.1 Summary of findings and implications

Congenital Zika syndrome is an ongoing puzzle but data synthesis may provide answers [20]

The Zika virus (ZIKV) outbreak in 2015–2016 generated some seemingly incongruous data across South America. Although a link between infection and congenital outcomes was present in many settings, the magnitude and timing of this link appeared to be very different when investigating different data sources. In Chapter 3, I fit a convolution model describing the link between ZIKV infection incidence and microcephaly outcomes to various data sources from locations across South America, finding different gestational-time-varying risk of ZIKV-associated microcephaly. Some locations, namely Bahia, Brazil, suggested relatively high early-pregnancy risk and little risk in the third trimester in line with clinical observations. When constrained by seroprevalence data as a proxy for true ZIKV attack rates, the magnitude of risk that I estimated was broadly in line with clinical findings: approximately 3% given infection in the first trimester. Other locations, namely Colombia, suggested that the risk could be high throughout pregnancy. I showed that realistic changes in reporting rates and behaviour likely explained some of the discrepancy in microcephaly incidence in Brazil during the first and second waves.

Fortunately, although ZIKV continues to pose a global threat, the incidence of associated adverse congenital outcomes has decreased drastically [561]. This does mean that we may never be able to find the biological reason (if any) for such high microcephaly rates in Brazil. Changes in reporting behaviour are likely the main culprit, but it remains to be seen if any enhancement from prior dengue infection plays a role. Understanding differences in prior flavivirus exposure between areas of high and low microcephaly incidence based on serological analysis would help answer this question. Nonetheless, it is my hope that the methods I developed in this chapter might help motivate the use of data synthesis in model fitting when different data streams suggest different stories. Although the results from a formal data synthesis analyses were not included in the published paper or here due to space constraints, generating a single microcephaly risk curve that was consistent with as much surveillance data as possible through accounting for differences in reporting rates and ZIKV dynamics was a useful exercise. For ZIKV and microcephaly, data synthesis of available clinical data to generate a consistent picture of risk is an ongoing challenge [562].

Repeat-exposure animal models allow a detailed insight into antibody kinetics [488]

Chapter 4 explored antibody kinetics models of increasing complexity to describe longitudinal antibody titres following multiple exposures, demonstrating the breadth of immunological mechanisms involved. I found that infection, vaccination, and adjuvanted vaccination generated quantitatively different boosting, waning and cross-reactivity profiles. Including a mechanism to describe changing dynamics with each repeated exposure was also a consistent feature of the models best supported through model comparison. Although there are limitations in using animal models and the time frame of these experiments, these results provide a guideline for how the antibody response from different exposure types may be quantified in models of human antibody dynamics.

There is an impressive amount of ongoing research revealing complex immunology in the adaptive immune response to influenza, and aligning these findings with recent advances in population immunology is an outstanding challenge [468]. Multiple exposure animal experiments are rare for influenza and are not typically performed with the aim of quantifying longitudinal antibody profile changes. However, they are arguably one of the best model systems for observing how antibody profiles develop from birth. The timing and nature of each exposure is known, and repeated samples can be taken over a short time scale. Using results from animal systems with different exposure histories may be useful when choosing vaccine strains and vaccines with different immunological profiles. Quantitative comparisons of cross-reactivity profiles, as I have demonstrated here, could be a useful tool in comparing the effectiveness of different adjuvants and vaccines, which would provide a measurable benefit to trade-off against safety and immunogenicity concerns [563, 564].

A well described statistical model to represent a life time of influenza exposures [476]

In Chapter 5, I built on a previous model linking influenza infections to observed antibody titres. I demonstrated that the statistics underlying the infection history model were incomplete and could lead to biased inference. I developed a statistical model to represent repeated exposures as a binary vector, and implemented different prior assumptions about the process that generates infections in a population. I also developed a statistical framework for a model including feedback between past infections and the probability of future infection. I validated this model on simulated and previously published data. Another key contribution of this work was to demonstrate the impact of virus-specific measurement effects when inferring influenza infection histories.

Most of the methods in Chapter 5, excluding titre-mediated immunity, have been implemented as an

R-package with an accompanying manuscript under review [476]. Although the model has been tailored to influenza, it is my hope that the code base will be built upon to address questions in different disease systems and with different data sets. In the *serosolver* manuscript, we applied the model to 2 previously published data sets not included in this thesis. Here, I applied the model to published data from Ha Nam, Vietnam, and the ongoing Fluscape cohort [474, 475]. Collaborators at the London School of Hygiene and Tropical Medicine have used the code we developed for simulation-guided serosurvey design (submitted manuscript), and model fitting to antibody titre data from children’s cohort in The Gambia and a household observational cohort in South Africa [565, 566]. As *serosolver* is successfully applied to more data sets, it would be great to see support for a new standard of statistical methods to analyse serological data.

Individual-level infection histories reveal patterns of influenza incidence across age, time and location

The final results chapter in this thesis used the methods developed in Chapter 5 to obtain influenza A/H3N2 infection histories for 1,130 individuals in Guangzhou, China. This amounted to identifying 12,350 infections from 186,925 possible latent infection states using 67,683 haemagglutination inhibition (HI) titre measurements. Based on these estimates, it is possible that influenza infections, or at least detectable antibody boosts, occur at a higher frequency than previously thought. I demonstrated that accounting for virus-specific measurement effects can significantly alter inferred attack rates for some historical but not recent times. With these reconstructed infection states, I corroborated previous findings that individuals experience antibody boosting at a lower frequency as they grow older, and that infections occur roughly twice per 10 year period. Attack rates over time varied across locations within the 60km study area, though I did not find evidence for correlation in space. Finally, I demonstrated that an individual’s risk of infection was reduced as a function of their antibody titre against circulating strains. Having an HI titre of 1 : 40, the traditional threshold for protection, afforded a risk of infection relative to having an undetectable titre of 28.7% in children < 10, 75.3% in adults aged 60+, and 55.0% in the overall population.

There are a number of avenues for using these inferred infection histories as augmented data. First, finding predictors of incidence, aligning periods of high and low incidence with antigenic data, and generating historical population immunity landscapes may generate insights for influenza forecasting. These results might motivate the use of serology as a more integral part of routine influenza surveillance. For example, an in-prep manuscript from Fluscape collaborators has been investigating infection

periodicity based on the raw HI titre data and we aim to corroborate their findings using these augmented infection histories. We have also begun preliminary work fitting generalised additive models to the augmented infections to understand why influenza attack rates vary across locations. Second, it is interesting that age-patterns of titre-mediated immunity emerged as a feature of the data without being explicitly modelled. Because we have infection histories for each individual and reconstructed life-long antibody landscapes, it will be interesting to investigate other factors that predict susceptibility. A long term goal might be to make personalised vaccine recommendations based on the nature of one's pre-existing immunity or serostatus, as is currently done for the dengue vaccine.

7.2 Future directions

7.2.1 Expanding the infection history data structure and use of multiplex assays

A key methodological contribution from this work is the generalisation and extensive exploration of Bayesian inference and priors for infection histories. The detail in this framework is not such an issue for many disease systems with few potential infections, and reversible jump Markov chain Monte Carlo works well without extensive prior considerations [40]. However, for disease systems like influenza, infection with the same pathogen lineage may be repeated many times with no clear upper bound. Multi-strain, multi-exposure statistical models are relatively new, and a rigorous consideration of how data contribute to estimates relative to any implicit assumptions in the model structure is needed.

One of the main additions to the *serosolver* framework I would like to make is the inclusion of alternative exposure types and co-circulation. First, the model does not currently distinguish between vaccination and infection [425, 453]. The data that I used have relatively low vaccination coverage, so vaccination was likely not a confounder here (roughly 20% had ever been vaccinated, so this would only account for only a few hundred antibody boosts). Inclusion of vaccination with different boosting and waning profiles would be a significant advancement of the methods to consider different populations and to investigate the relationship between infection, vaccination and antibody dynamics. Second, many pathogens exhibit co-circulation (eg. dengue), whereas *serosolver* currently allows for only one infection in a time period. Extending the framework to capture co-circulation is conceptually straightforward: the matrix of infection histories could be extended to another dimension representing infection status with respect to each possible exposure type. As a start, vaccination could be modelled by using one matrix for infection and another for vaccination. The antibody kinetics model could then capture contributions from both exposure types to study how these different exposure types interact. The reverse problem could also be tackled: which antigens need to be tested to give the most reliable measure of exposure?

Another assumption I aim to relax is the use of a continuous strain space to represent cross-reactivity. At the moment, *serosolver* requires the input of an antigenic map. An alternative model for multi-strain interactions is to consider loci at discrete epitopes [143]. One's infection history is comprised of multiple exposures to variants with different combinations of those epitopes, and the resulting antibody response is some culmination of those responses. Antigen-specific multiplex assays are under development both for specific diseases and across multiple diseases [229, 567–569]. As these assays become cheaper

and higher throughput, data will become available to inform antibody kinetics following exposure to different epitope sets [570]. In terms of *serosolver*, each infection state could represent exposure to a particular set of epitopes, and the antibody kinetics model could describe stimulation of antigen-specific antibodies against those epitope variants. Such a change would be necessary to apply the framework to other, antigenically complex pathogens such as malaria.

7.2.2 Developing further antibody kinetics models and B-cell dynamics

In influenza, HI assays measure only the aggregated activity of polyclonal antibodies targeting multiple epitopes on the haemagglutinin head. I was therefore unable to investigate epitope- or B cell-specific contributions to serum antibody titres [56]. Understanding the immunological mechanisms of imprinting effects, short-term kinetics due to germinal centre (GC) overlap, and the relative contributions of memory B cell (MBC) derived and *de novo* antibody boosting would require data either on epitope-specific antibodies or single-cell assays [150, 160, 571]. A model that captures the contribution of MBCs and naive B cells targeting a variety of epitopes may explain how immune imprinting contributes to observed antibody titres, and may include additional insights such as decreased cross-reactive breadth and magnitude with each repeated exposure. Work by Sarah Cobey’s lab aims to tackle this problem, and long-term prospective cohorts of children will provide a rich data source [468].

Fig G.1 and G.2 in the final appendix illustrate a prototype antibody kinetics model that explicitly captures naive and memory derived antibody responses. I recently implemented this model in *serosolver*, and it would therefore be possible to fit it to a sufficiently rich data set. This prototype model aims to capture the “antigen trapping” hypothesis described by Fonville *et al* [87]. The idea is that antibodies are produced by antibody secreting (ASC) populations centered around a single point in antigenic space, similar to the “ball of stimulation” described in [187]. This point corresponds to the antigenic location of the infecting strain that bound to and stimulated those B-cells. With each subsequent infection, the amount of antibody boosting could be mediated by immune interference of pre-existing MBC populations. If the infecting strain is antigenically close to pre-existing B-cell receptors, then this memory response sequesters much of the antigen and results in low levels of antibody production from naive B cells. If the infecting strain is antigenically far from any pre-existing memory cells or is the first infecting strain, then antigen clearance requires a full response, similar to a primary infection.

The choice of parameter values in this prototype can give different model behaviour. For example, an interference effect may be included by simply reducing boosting overall when antigenic distance is small,

rather than stimulating expansion of MBC populations. Although the models generate qualitatively similar results to the model in Chapter 5, the prototype explicitly assumes that back-boosting results from antigen trapping. It would be interesting to compare fits to data for different model variants and to quantify the relative contributions of novel and memory-derived antibodies.

Using the *serosolver* framework, a model comparison analysis could be carried out to find support for different immunological mechanisms as in Chapter 4. *Serosolver* currently does not have implemented code for model comparison, and it is not clear how traditional information criteria metrics would work with the large number of augmented infection states. Developing a robust model comparison framework is therefore a key direction for future work. K-fold cross validation would likely be entirely suitable to generate expected log predictive density estimates, but is computationally intensive for the full model in Chapter 6.

7.3 Conclusion

An improved understanding of life course immunology, development of high throughput assays, and advances in inference methodologies have enabled the development of a new generation of analytical tools for serological data. The traditional seroconversion/seropositivity paradigm loses much of the information inherent in the magnitude and dynamics of an antibody response, and a key future direction in the field will be to make these analytical methods a new standard. Of course, there is still much value in using threshold measures, and it will be important to not default to over-engineered solutions.

This thesis has two broad messages for the analysis of serological data: (i) the structure of statistical models must be carefully considered to avoid biased inference, and (ii) it is important to distinguish between clinically relevant infection, antibody signal from different exposure types or unsuccessful exposure, and erroneous features of assay measurements. An ongoing challenge will be to communicate the potential benefit of these powerful but often complex methods to improve public health action. Validating infection history inference models with cohort studies alongside virologically confirmed infections, human challenge studies where appropriate, and animal models will therefore be an important next step.

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Appendix A

Epidemiological dynamics of ZIKV-associated microcephaly risk

Table A.1: Transmission parameter estimates for the primary analyses based on estimated posterior distributions. Columns describe the name of the analysis as shown in Fig 3.6; the name of the parameter; and the posterior means and 95% credible intervals for those parameters.

Analysis	Parameter	Posterior mean (95% CI)
Bahia, Brazil	Attack rate	0.715 (0.711-0.719)
Colombia (confirmed)	Attack rate	0.692 (0.677-0.707)
Colombia (notified)	Attack rate	0.621 (0.618-0.624)
Northeast Brazil	Attack rate	0.703 (0.673-0.734)
Pernambuco, Brazil (confirmed)	Attack rate	0.811 (0.771-0.845)
Rio Grande do Norte, Brazil (confirmed)	Attack rate	0.817 (0.798-0.835)
Rio Grande do Norte, Brazil (notified)	Attack rate	0.817 (0.799-0.836)
Bahia, Brazil	Basic reproductive number, R_0	1.76 (1.75-1.77)
Colombia (confirmed)	Basic reproductive number, R_0	1.7 (1.67-1.74)
Colombia (notified)	Basic reproductive number, R_0	1.56 (1.56-1.57)
Northeast Brazil	Basic reproductive number, R_0	1.73 (1.66-1.8)
Pernambuco, Brazil (confirmed)	Basic reproductive number, R_0	2.06 (1.91-2.2)
Rio Grande do Norte, Brazil (confirmed)	Basic reproductive number, R_0	2.08 (2-2.16)
Rio Grande do Norte, Brazil (notified)	Basic reproductive number, R_0	2.08 (2.01-2.16)
Bahia, Brazil	Epidemic seed time	2014-05-02 (2014-04-28-2014-05-05)
Colombia (confirmed)	Epidemic seed time	2014-11-30 (2014-11-14-2014-12-16)
Colombia (notified)	Epidemic seed time	2014-09-15 (2014-09-11-2014-09-19)
Northeast Brazil	Epidemic seed time	2014-03-04 (2014-01-31-2014-04-04)
Pernambuco, Brazil (confirmed)	Epidemic seed time	2014-04-14 (2014-03-14-2014-05-12)
Rio Grande do Norte, Brazil (confirmed)	Epidemic seed time	2014-08-17 (2014-08-04-2014-08-31)
Rio Grande do Norte, Brazil (notified)	Epidemic seed time	2014-08-18 (2014-08-04-2014-08-31)
Salvador, Brazil	Epidemic seed time	2014-02-05 (2014-02-05-2014-02-05)

Table A.2: Microcephaly parameter estimates for the primary analyses based on estimated posterior distributions. Columns describe the name of the analysis as shown in Fig 3.6; the name of the parameter; and the posterior means and 95% credible intervals for those parameters.

Analysis	Parameter	Posterior mean (95% CI)
Bahia, Brazil	First risk week	1.93 (0.714-4.04)
Colombia (confirmed)	First risk week	0.223 (0.143-1)
Colombia (notified)	First risk week	0.386 (0.143-2.29)
Northeast Brazil	First risk week	8.16 (5.29-10.9)
Pernambuco, Brazil (confirmed)	First risk week	0 (0-0)
Rio Grande do Norte, Brazil (confirmed)	First risk week	5.74 (1.14-11.3)
Rio Grande do Norte, Brazil (notified)	First risk week	0.35 (0.143-1.57)
Salvador, Brazil	First risk week	0.19 (0.143-0.429)
Bahia, Brazil	Last risk week	21.4 (16.3-25.6)
Colombia (confirmed)	Last risk week	39.6 (36.6-39.9)
Colombia (notified)	Last risk week	39.7 (37.7-39.9)
Northeast Brazil	Last risk week	15.1 (11.9-18.6)
Pernambuco, Brazil (confirmed)	Last risk week	7.67 (3.57-12.6)
Rio Grande do Norte, Brazil (confirmed)	Last risk week	29.4 (21.6-39.6)
Rio Grande do Norte, Brazil (notified)	Last risk week	39.7 (38-39.9)
Salvador, Brazil	Last risk week	31.6 (28.6-35.3)
Bahia, Brazil	Mean first trimester risk	0.0281 (0.0251-0.0315)
Colombia (confirmed)	Mean first trimester risk	0.00279 (0.00229-0.00334)
Colombia (notified)	Mean first trimester risk	0.00303 (0.00239-0.00367)
Northeast Brazil	Mean first trimester risk	0.00878 (0.00721-0.01)
Pernambuco, Brazil (confirmed)	Mean first trimester risk	0.013 (0.0115-0.0145)
Rio Grande do Norte, Brazil (confirmed)	Mean first trimester risk	0.00452 (0.00119-0.00743)
Rio Grande do Norte, Brazil (notified)	Mean first trimester risk	0.0147 (0.011-0.0186)
Salvador, Brazil	Mean first trimester risk	0.0306 (0.0266-0.0349)
Bahia, Brazil	Mean second trimester risk	0.00365 (0.000781-0.00597)
Colombia (confirmed)	Mean second trimester risk	0.00224 (0.00193-0.00257)
Colombia (notified)	Mean second trimester risk	0.00268 (0.00228-0.00322)
Northeast Brazil	Mean second trimester risk	0.000588 (5.59e-21-0.00205)
Pernambuco, Brazil (confirmed)	Mean second trimester risk	0.000379 (0.000177-0.000628)
Rio Grande do Norte, Brazil (confirmed)	Mean second trimester risk	0.00726 (0.00452-0.0111)
Rio Grande do Norte, Brazil (notified)	Mean second trimester risk	0.0117 (0.00895-0.0148)
Salvador, Brazil	Mean second trimester risk	0.00805 (0.00649-0.0098)
Bahia, Brazil	Mean third trimester risk	2.71e-05 (4.49e-11-0.000146)
Colombia (confirmed)	Mean third trimester risk	0.00154 (0.00116-0.00189)
Colombia (notified)	Mean third trimester risk	0.00186 (0.00135-0.00232)
Northeast Brazil	Mean third trimester risk	1.7e-08 (0-5.13e-08)
Pernambuco, Brazil (confirmed)	Mean third trimester risk	0.00022 (0.000103-0.000361)
Rio Grande do Norte, Brazil (confirmed)	Mean third trimester risk	0.000586 (4.76e-08-0.00203)
Rio Grande do Norte, Brazil (notified)	Mean third trimester risk	0.00562 (0.00217-0.00919)
Salvador, Brazil	Mean third trimester risk	0.000833 (0.000407-0.00142)
Bahia, Brazil	Number of weeks at risk	19.6 (12.3-24.7)
Colombia (confirmed)	Number of weeks at risk	39.4 (36-39.7)
Colombia (notified)	Number of weeks at risk	39.3 (36-39.7)
Northeast Brazil	Number of weeks at risk	7 (1.71-12.9)
Pernambuco, Brazil (confirmed)	Number of weeks at risk	7.67 (3.57-12.6)
Rio Grande do Norte, Brazil (confirmed)	Number of weeks at risk	23.7 (11.3-37.6)
Rio Grande do Norte, Brazil (notified)	Number of weeks at risk	39.4 (36.7-39.7)
Salvador, Brazil	Number of weeks at risk	31.4 (28.1-35.1)
Bahia, Brazil	Peak risk week	7.83 (6.57-9)
Colombia (confirmed)	Peak risk week	5.1 (0.429-10.9)
Colombia (notified)	Peak risk week	7.3 (0.857-13.9)
Northeast Brazil	Peak risk week	11.2 (10-12.4)
Pernambuco, Brazil (confirmed)	Peak risk week	0 (0-0)
Rio Grande do Norte, Brazil (confirmed)	Peak risk week	14.1 (10.3-16.9)
Rio Grande do Norte, Brazil (notified)	Peak risk week	7.69 (1.14-12.6)
Salvador, Brazil	Peak risk week	5.27 (3.71-6.57)

Table A.3: Parameter estimates for the analysis of the second ZIKV infection incidence wave. Analysis column describes which mechanism was under investigation for that analysis; parameter column describes the parameter being estimated; values shown are the posterior mean and 95% credible intervals.

Analysis	Parameter	Posterior mean (95% CI)
Abortions only	Abortion rate	0.925 (0.898-0.949)
Avoided births only	Abortion rate	NA
Forecast analysis	Abortion rate	0.4 (0.0145-0.921)
Microcephaly reporting change only	Abortion rate	NA
No ZIKV reporting change	Abortion rate	0.699 (0.118-0.903)
ZIKV reporting change only	Abortion rate	NA
Abortions only	Births avoided	NA
Avoided births only	Births avoided	0.92 (0.89-0.944)
Forecast analysis	Births avoided	NA
Microcephaly reporting change only	Births avoided	NA
No ZIKV reporting change	Births avoided	NA
ZIKV reporting change only	Births avoided	NA
Abortions only	Microcephaly reporting rate 2015	NA
Avoided births only	Microcephaly reporting rate 2015	NA
Forecast analysis	Microcephaly reporting rate 2015	1.43 (1.01-1.98)
Microcephaly reporting change only	Microcephaly reporting rate 2015	6.01 (4.92-7.26)
No ZIKV reporting change	Microcephaly reporting rate 2015	1.52 (1.12-2.04)
ZIKV reporting change only	Microcephaly reporting rate 2015	NA
Abortions only	Number of aborted births	1090 (803-1480)
Avoided births only	Number of aborted births	NA
Forecast analysis	Number of aborted births	161 (6-999)
Microcephaly reporting change only	Number of aborted births	NA
No ZIKV reporting change	Number of aborted births	353 (18-805)
ZIKV reporting change only	Number of aborted births	NA
Abortions only	Proportion of pregnant women avoiding infection	NA
Avoided births only	Proportion of pregnant women avoiding infection	NA
Forecast analysis	Proportion of pregnant women avoiding infection	0.315 (0.0112-0.82)
Microcephaly reporting change only	Proportion of pregnant women avoiding infection	NA
No ZIKV reporting change	Proportion of pregnant women avoiding infection	0.464 (0.0233-0.882)
ZIKV reporting change only	Proportion of pregnant women avoiding infection	NA
Abortions only	ZIKV reporting rate change	NA
Avoided births only	ZIKV reporting rate change	NA
Forecast analysis	ZIKV reporting rate change	4.46 (0.336-12.1)
Microcephaly reporting change only	ZIKV reporting rate change	NA
No ZIKV reporting change	ZIKV reporting rate change	NA
ZIKV reporting change only	ZIKV reporting rate change	18.9 (10-59.1)

A.1 Additional data methods

A.1.1 Summary of data

ZIKV and microcephaly incidence data from 2015 were available from publications and epidemiological reports for the Brazilian states of Pernambuco, Rio Grande do Norte and Bahia (at state level and for the city of Salvador), though no useable data sets from 2015 were found for any other state. Monthly microcephaly incidence and births by state were also found online from the SINASC/CGIAE/SVS/MS system as reported previously [19,370]. An additional source of ZIKV incidence for all Brazilian states was also obtained from a publication in 2016 [17]; however, the timing of the epidemic peak in these data suggested that incidence peaked in July 2015, contrasting with state-level reports which suggested an earlier peak. I also considered preliminary and later confirmed ZIKV and microcephaly incidence data published from the Brazilian ministry of health, which suggested a later ZIKV infection peak time compared to early state-level reports [25,361]. Microcephaly and ZIKV incidence data were obtained for Colombia at the national level [362,363]. Further confirmed case reports were available for Colombia from weekly epidemiological bulletins; however, these did not include the date of report of confirmed cases, and incidence data could not be extracted from the reported cumulative cases [394].

Although the timing of peak microcephaly incidence was similar between states and between data sets (around November 2015), the timing of peak ZIKV incidence in the first wave varied between states, as well as between data sources for the same state. For example, for the state of Bahia, Brazil, one source suggested peak ZIKV incidence in May 2015, whereas another reported a peak in July 2015 [17,25].

A.1.2 Detail of additional incidence data extraction

Extraction of incidence data from [361] was slightly more involved, as exact microcephaly case numbers were not provided. However, de Oliveira *et al.* estimated the number of pregnant women from the number of reported live births, which can be reversed to recover the number of live births given number of pregnant women. [361] reported confirmed microcephaly incidence per 10,000 births and notified ZIKV infection incidence per 10,000 pregnant women, as well as the total number of microcephaly cases and monthly number of infected pregnant women. The number of monthly births can be inferred as follows:

$$\begin{aligned}
 \text{pregnant women}(t) &= (\text{births}(t) * 9) + (\text{births} * 0.2) * 1.5 \\
 \text{pregnant women}(t) &= \text{births}(t) * 9.3 \\
 \text{births}(t) &= \text{pregnant women}(t)/9.3
 \end{aligned}
 \tag{A.1}$$

Table A.4: ZIKV and microcephaly incidence data. For each location, the type of incidence data, the time resolution of reports and the data source are provided.

Country	Locations	Incidence type	Reported or Confirmed?	Resolution	Note	Source
Brazil	Northeast states (Alagoas, Bahia, Cear, Maranhão, Paraíba, Pernambuco, Piauí, Rio Grande do Norte and Sergipe)	Microcephaly and ZIKV	Microcephaly confirmed; ZIKV reported	Weekly		[25]
Brazil	Northeast states (Alagoas, Bahia, Cear, Maranhão, Paraíba, Pernambuco, Piauí, Rio Grande do Norte and Sergipe)	Microcephaly and ZIKV	Microcephaly confirmed & predicted; ZIKV reported	Weekly	Extracted from incidence per 10000 births/pregnant women see Appendix A.1.2	[361]
Colombia	Country-wide	Microcephaly	Reported	Weekly	Extracted from cumulative values for under review, discarded and confirmed, so not strictly true incidence. New under review cases are $R_t - R_t(t-1) + \text{new } D_t + \text{new } C_t$ (review, discarded, confirmed)	[362]
Colombia	Country-wide	ZIKV	Reported	Weekly		[362]
Colombia	Country-wide	ZIKV	Confirmed	Weekly		[362]
Colombia	Country-wide	Microcephaly	Reported	Monthly		[363]
Colombia	Country-wide	Microcephaly	Reported	Weekly		[394]
Colombia	Country-wide	Microcephaly	Confirmed	Weekly	Extracted from cumulative values for under review, discarded and confirmed, so not strictly true incidence. Confirmed cases are number of confirmed cases in week t less the number of cases in time $t-1$	[394]
Brazil	Bahia	Microcephaly	Reported	Weekly		[364]
Brazil	Bahia	Microcephaly	Reported	Monthly		[370]
Brazil	Bahia	ZIKV	Reported	Weekly		[365]
Brazil	Rio Grande do Norte	Microcephaly	Reported	Monthly		[367]
Brazil	Rio Grande do Norte	Microcephaly	Confirmed	Monthly		[367]
Brazil	Rio Grande do Norte	Microcephaly	Reported	Monthly		[370]
Brazil	Rio Grande do Norte	ZIKV	Reported	Weekly		[368]
Brazil	Pernambuco	Microcephaly	Confirmed & Reported	Weekly		[366]
Brazil	Pernambuco	ZIKV	Reported	Weekly		[572]
Brazil	Pernambuco	Microcephaly	Reported	Monthly		[370]
Brazil	Salvador, Bahia	ZIKV	Reported	Weekly		[573]
Brazil	Salvador, Bahia	Microcephaly	Reported	Weekly		[573]

Table A.5: Vital statistics and sources.

State/Country	Population size [377, 378]	Life expectancy (years) [375, 376]
Pernambuco	9345173	73.0
Bahia	15203934	73.0
Cear	8904459	73.4
Rio Grande Norte	3442175	75.2
Colombia 2014	47791393	73.95
Colombia 2015	48228704	73.95
Colombia 2016	49067981	73.95
Salvador, Bahia	2922037 (inferred from total incidence)	73
Northeast Brazil (aggregated)	56560081	72.3

where $\text{pregnant women}(t)$ is the number of pregnant women in month t (which is known) and $\text{births}(t)$ is the number of births in month t . The number of microcephaly cases per month is then:

$$\text{microcephaly cases}(t) = \text{births}(t) * \text{microcephaly incidence}(t) \quad (\text{A.2})$$

where $\text{microcephaly cases}(t)$ is the number of microcephaly cases reported in month t and $\text{microcephaly incidence}(t)$ is the per birth incidence of microcephaly in month t .

Note that the total population size for Northeast Brazil is used as the denominator for per capita incidence, as the reporting rate parameter (described below) accounts for the fact that infected pregnant women only represent a fraction of the true infected population.

A.1.3 Birth data

Numbers of live births were usually obtained from the same sources as microcephaly incidence data. However, where accompanying birth data were not available either by source, for a particular time resolution or for a particular time period, I estimated the number of live births by manipulating available birth data as follows:

1. Conversion of monthly births to weekly births by first spreading monthly births uniformly across days in that month and then summing daily births by epidemiological week.
2. Forecasting of live births for which data are not available (end of 2016) by averaging the number of live births for that period in the preceding two years.
3. Using birth data from the same location but from a separate source eg. when fitting to weekly microcephaly data for Bahia from state-level reports, I estimated weekly births from monthly birth data from national-level reports.

Monthly live births were obtained alongside monthly microcephaly incidence data for Brazil [19]. Accompanying birth data were not available for the reports of weekly incidence for Bahia, Pernambuco, Rio Grande do Norte and Colombia. For Bahia, unreported births were forecast using the `stl` (seasonal decomposition of time series data) and `predict` functions in R using monthly birth data from 01/01/2010 to 24/07/2016 to predict births based on previous trends. For Pernambuco and Rio Grande do Norte, I used the monthly birth data as described above [370]. For Colombia, I used the monthly birth data from Cuevas *et al.* 2016 [24]. This publication provided births for 2015 and most of 2016. However, data were missing for the last 2 months of 2016 and January 2016. To estimate the births in this period, I took the the total number of births for Colombia in that year from and assumed that the difference between the total number of 2016 births recorded in Cuevas *et al.* and those recorded on the governmental website were uniformly spread across the remaining days.

A.2 Methods for forecasting the second wave of microcephaly incidence

I added four additional model parameters to quantify potential changes in behaviour and reporting rates across two seasons of microcephaly and ZIKV incidence that would explain the two seasons of observed data, where only one wave of microcephaly incidence was observed despite two waves of ZIKV incidence. I tested four hypotheses as described in Section 3.3.6.

I defined the probability of not developing microcephaly during the first $i - t + 40$ days of pregnancy as $\bar{b}p_i : (1 - bp)^{i-t+40}$. I also made the following notational simplification to give the probability of a fetus developing ZIKV-associated microcephaly at time i , $P_i : P_I(i)P'_m(i - t + 40)$. The expected proportion of observed microcephaly affected live-births was therefore given by:

$$P_{micro}(t) = \phi_m \sum_{i=t-40}^t \begin{cases} \bar{b}p_i(P_i + bpP_i + bp) & t < t_{switch} \\ (1 - a_r)\bar{b}p_i((1 - b_r)P_i + bp(1 - b_r)P_i + bp) & t \geq t_{switch} \text{ \& } (i - t + 40) < t_{abortion} \\ \bar{b}p_i((1 - b_r)P_i + bp(1 - b_r)P_i + bp) & t \geq t_{switch} \text{ \& } (i - t + 40) \geq t_{abortion} \end{cases} \quad (\text{A.3})$$

where $P_I(i)$ is the probability of becoming infected at time i ; $P'_m(i - t + 40)$ is the probability of developing microcephaly given infection in gestational week $i - t + 40$; t_{switch} is the time at which behavioural changes could have occurred (assumed to be 01/02/2016, mechanism 2); b_r is the proportion of potentially affected births that were avoided (mechanism 3); a_r is the proportion of microcephaly-affected births that were aborted (mechanism 2); and $t_{abortion}$ is the gestational time before which abortions could occur, assumed to be 24 weeks (mechanism 2); bp is the baseline daily probability of developing microcephaly during pregnancy (note that this is different to the previous definition of baseline microcephaly, P_b , which was defined as a rate per observed live birth rather than a probability per day during pregnancy).

Overall, this term gives the probability of an individual not developing baseline microcephaly during the first $i - t + 40$ days of pregnancy and either developing ZIKV-associated microcephaly, baseline microcephaly or both on day i . This term is then multiplied by the probability of observing that microcephaly case (ie. the reporting rate), given by ϕ_m , which was assumed to have one value before and one after and including 13/03/2016 (mechanism 1).

As the transmission model described incidence in only a single season, I used reported ZIKV incidence directly to estimate the per capita infection risk across two seasons. The probability of becoming

infected with ZIKV is:

$$P_I(t) = \begin{cases} \frac{I(t)}{\phi_{inc1}} & t < t_1 \\ \frac{I(t)}{\phi_{inc2}} & t \geq t_1 \end{cases} \quad (\text{A.4})$$

where $I(t)$ is the observed per capita ZIKV incidence at time t ; ϕ_{inc1} is the proportion of true ZIKV cases that were reported before t_1 ; ϕ_{inc2} is the proportion of true ZIKV cases that were reported after t_1 ; and t_1 is the time at which reporting behaviour was assumed to have changed, fixed here at 11/11/2015.

Although I did not use the SEIR model generated force of infection to predict infection risk in this analysis, I did include the SEIR model to estimate the proportion of ZIKV cases that were reported in the first wave. In other words, the SEIR-model predicted incidence curve was fit to reported ZIKV incidence for the single wave only as a component of the likelihood function. The other component of the likelihood was the probability of observing the two seasons of microcephaly-affected births given $P_{micro}(t)$. If ZIKV transmission followed SEIR-like dynamics with a mosquito vector, then the shape of the incidence curve would give inferential power regarding the relationship between observed and true incidence dynamics, allowing us to estimate plausible values of t_0 , R_0 and ϕ_{inc1} that might generate observed incidence data for that season. Values for ϕ_{inc2} were therefore estimated relative to the inferred value of ϕ_{inc1} .

The number of aborted births could be calculated by estimating the proportion of microcephaly-affected pregnancies that were aborted ($P_{micro}(t)$ defined above, ignoring the impact of reporting rate), divided by the proportion of microcephaly-affected pregnancies that weren't aborted ($P_{micro}(t)$ defined above, but replacing $1 - a_r$ with a_r , ignoring the impact of reporting rate) multiplied by the observed number of microcephaly cases.

A.3 Sensitivity analyses

A.3.1 Fitting to seroprevalence data from Salvador, Brazil

I performed a sensitivity analysis with better constraint on the true ZIKV attack rate by taking microcephaly and AEI data from Salvador, Brazil for 2015 scaled by recent ZIKV IgG seroprevalence data [369].

I obtained reported acute exanthematous illness (AEI) attributed to Zika virus and microcephaly incidence in Salvador, Brazil during 2015 [573] I assumed that reported AEI was proportional to the true incidence of ZIKV during this time, and scaled the weekly reported incidence to give a final attack rate in line with seroprevalence estimates for Salvador. Scaling was done by dividing reported AEI cases each week by ϕ_I which was calculated as follows:

$$\phi_I = \frac{\sum_t I(t)/N}{AR} \quad (\text{A.5})$$

Where $I(t)$ is the reported incidence at week t , N is the population size of Salvador, and AR is the reported attack rate based on seroprevalence. We placed a uniform prior on AR such that the total attack rate was between 59.4 and 66.8% [398]. N was inferred by dividing the total number of reported AEI cases by the reported total incidence per 1000 persons (giving 2922037); life expectancy was assumed to be the same as Bahia overall at 73.1 years. I calculated weekly number of live births by backtracking from the reported microcephaly incidence in this time period and the total number of microcephaly cases reported. Paploski *et al.* report “367 newborns with suspected microcephaly (15.6 cases/1,000 newborns during July 2015–February 2016, which peaked at 31.4 cases/1,000 newborns in December)”, suggesting that there were 23,526 newborns in this period. I assumed that these births were distributed uniformly across each week such that there were 420 births per week from July 2015 – February 2016. The microcephaly reporting rate, ϕ_m , was assumed to be 100%.

Model fitting was then carried out as in the main results; fixing all model parameters other than ϕ_I ; α ; β ; and c as described in Table 3.1. Note that in this analysis the SEIR model component is not included.

Appendix B

Characterising antibody kinetics of influenza exposure using a ferret model

B.1 Model variants referred to in main text

B.2 Simulation-recovery results

I carried out simulation-recovery experiments to test that the model fitting framework was able to re-estimate known model parameters from simulated data that matched the experimental protocol for group E (3 individuals, infection on day 0, HI titres measured at days 0, 21, 37, 49 and 70) (Fig B.4). 95% credible intervals (CI) for the marginal posteriors of the model parameters all encompassed the true parameter values, though were wide for some parameters. These were: true value $\mu = 10.0$, estimated $\mu = 8.49$ (posterior median, 95% CI 6.36-12.4); true value $d = 0.5$, estimated $m = 0.499$ (posterior median, 95% CI 0.128-0.666); true value $t_s = 19$ days, estimated $t_s = 18.0$ days (posterior median, 95% CI 3.16-27.7 days); true value $m = 0.04$, estimated $m = 0.0609$ (posterior median, 95% CI 0.0207-0.105). Performing simulation-recovery on simulated data with weekly HI titres resulted in more constrained marginal posteriors, suggesting that further experiments with more frequent observations would provide more tightly constrained estimates: true value $\mu = 10.0$, estimated $\mu = 9.47$ (posterior median, 95% CI 8.76-10.2); true value $d = 0.5$, estimated $d = 0.628$ (posterior median, 95% CI 0.506-0.715); true value $t_s = 19$ days, estimated $t_s = 24.5$ days (posterior median, 95% CI 17.2-27.8 days); true value $m = 0.04$, estimated $m = 0.0428$ (posterior median, 95% CI 0.00898-0.0777). There was some bias in the inferred boosting and initial waning proportion towards sharper initial

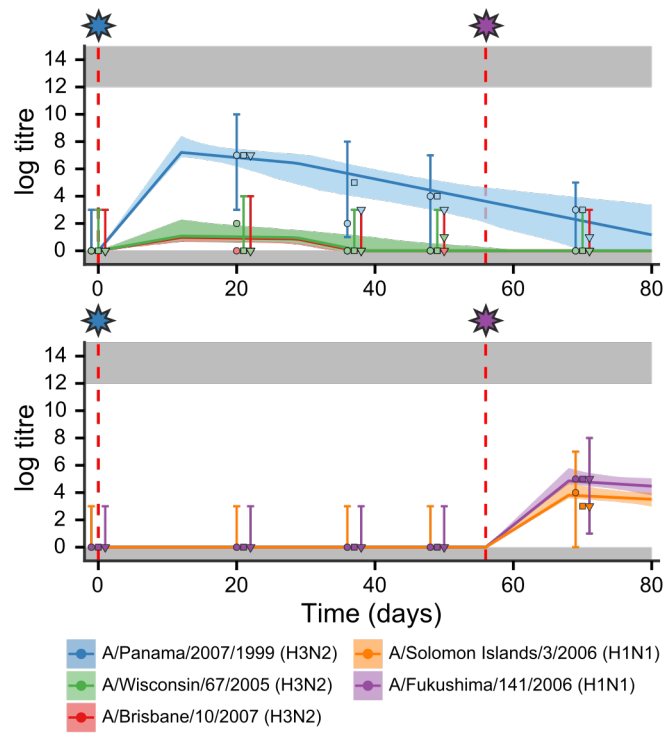


Figure B.1: Antibody trajectories for group E from model variant 64. Equivalent to Fig 4.6E, but using a model assuming shared kinetics parameters between post A/H3N2 and A/H1N1 infection, between both adjuvanted TIVs and between both unadjuvanted TIVs. Solid coloured lines and shaded regions show posterior median and 95% credible intervals of latent titres. Points show observed antibody titres. Bars show 95% prediction intervals on observable titres. Red dashed lines show time of infection with A/Panama/2007/99 (H3N2) and A/Fukushima/141/2006 (H1N1) respectively.

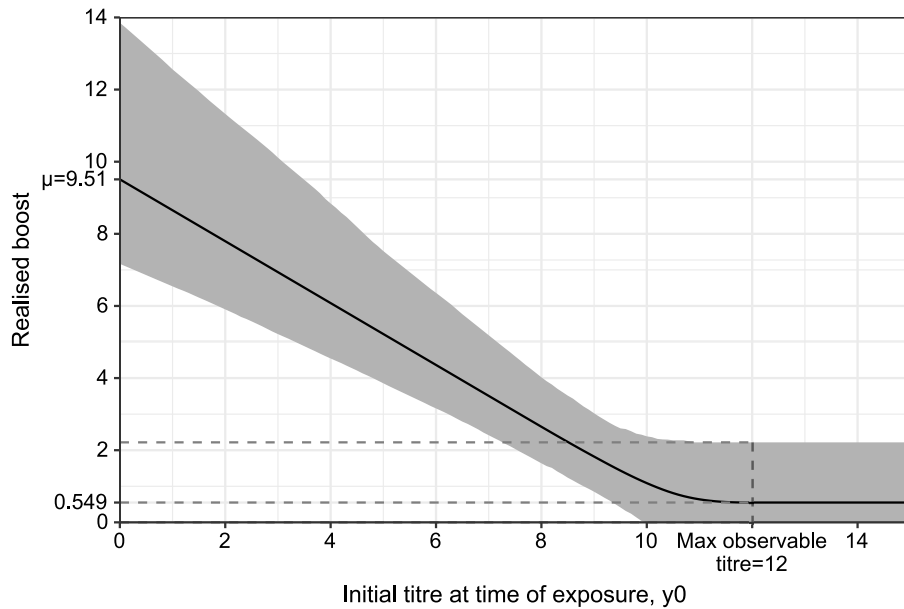


Figure B.2: Posterior estimates for titre-dependent boosting relationship from the best supported model which included titre-dependent boosting. Shaded gray regions shows 95% credible intervals (CI) drawn from the multivariate posterior. Solid black line shows multivariate posterior mean; Dashed gray lines show posterior median and 95% CI for realised antibody boosting from a titre of 12.

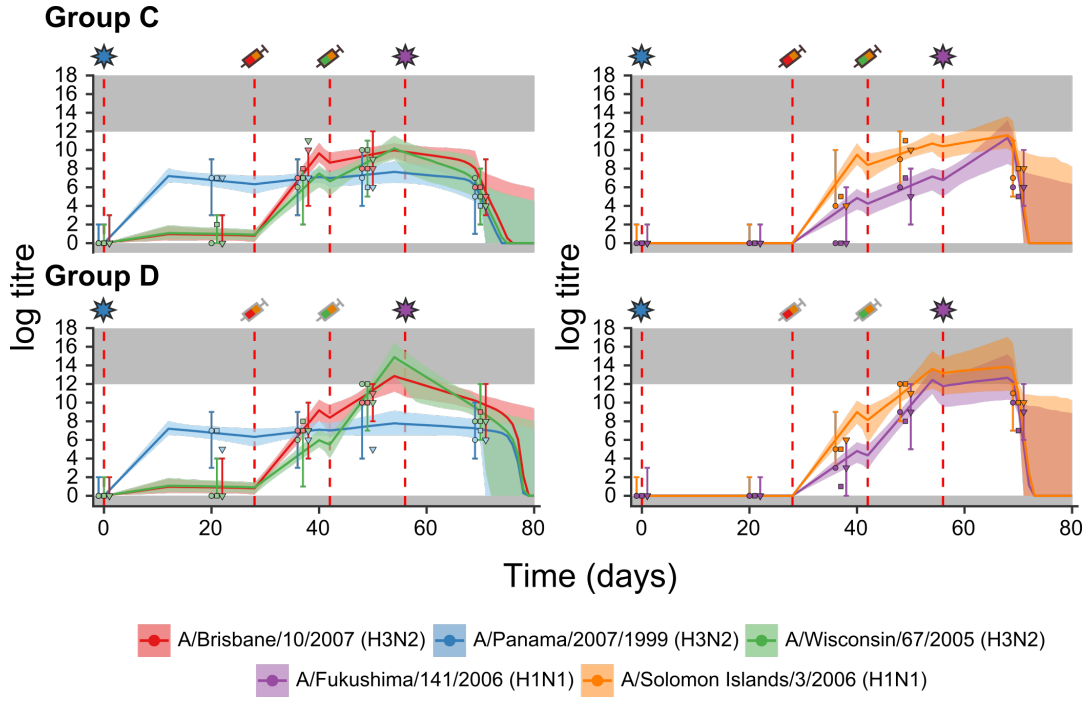


Figure B.3: Antibody trajectories for groups C&D from model variant 54. Equivalent to Fig 4.6C&D, but using a model with titre-dependent boosting. Solid coloured lines and shaded regions show posterior median and 95% credible intervals of latent titres. Points show observed antibody titres. Bars show 95% prediction intervals on observable titres. Red dashed lines show exposures as in Fig 4.3

waning. This may be attributed to the discretised nature of the data, where all observations of the hidden, continuous true antibody titre are observed as the lower integer. For example, all true log titres $5 \leq x < 6$ would be observed as a log titre of 5, therefore the posterior probability for any combined value of $5 \leq d\mu < 6$ would be uniform given one observed log HI titre $k = 5$ at time $t_p + t_s$.

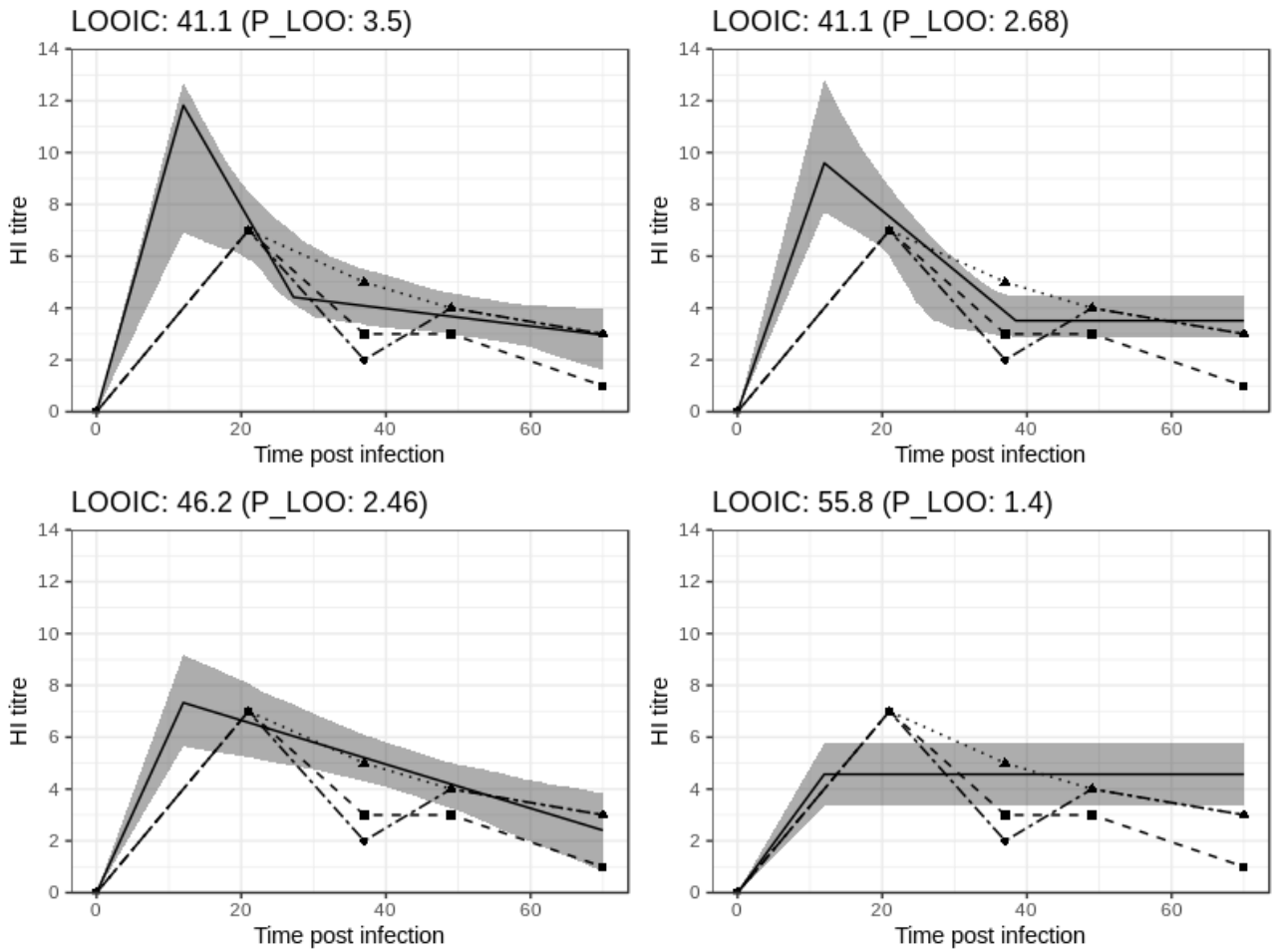


Figure B.4: Simulation-recovery of the four base model fits to data from three ferrets following exposure to a single immunogen matching the real protocol. Y-axis shows log HI titre against the exposure immunogen from day 0. Solid black line and grey region show best-fit model trajectory and 95% credible intervals (CI) of latent antibody titres. Black diamond and error bars show posterior median and 95% CI of model predicted observations. Blue crosses and dashed lines show observed log HI titre for the three individual ferrets. Figure titles show estimated expected log-predictive density (ELPD) and corresponding standard error (SE). (Top left) Biphasic waning; (Top right) biphasic waning with a fixed long-term waning rate of $m = 0$; (Bottom left) monophasic waning with $t_s = d = 0$; (Bottom right) no short or long term waning with $m = t_s = d = 0$.

I also ran simulation recovery experiments for the 13 models with a $\delta\text{ELPD} < 20$ to test that I could estimate parameters using simulated data generated from the experimental protocol. I took the maximum likelihood parameter values from the real model fits and used these to simulate data under each of these 13 models that matched data from the experiments (5 groups of 3 ferrets, 5 tested strains, 5 blood samples taken). I considered a parameter re-estimation as accurate when the 95% or 99% CI of the inferred posterior distribution encompassed the true parameter value. Parameter re-estimation accuracy was 87.5% (posterior median; range 73.3-100%) for the 95% CI case and 90.6% (posterior median; range 83.3%-100%) for the 99% CI case. The vast majority of inaccurately estimated

parameters either narrowly missed the true value or were the result of very weak identification that recovered only the prior for that parameter. Fig B.5 shows the inferred posterior distributions against the true parameter values for the model variant with biphasic waning, 6 exposure types, priming, antigenic seniority, type specific cross reactivity and no titre-dependent boosting. Although posterior estimates were weakly constrained for some parameters as in the real data, the models fit to and explained the simulated observations well.

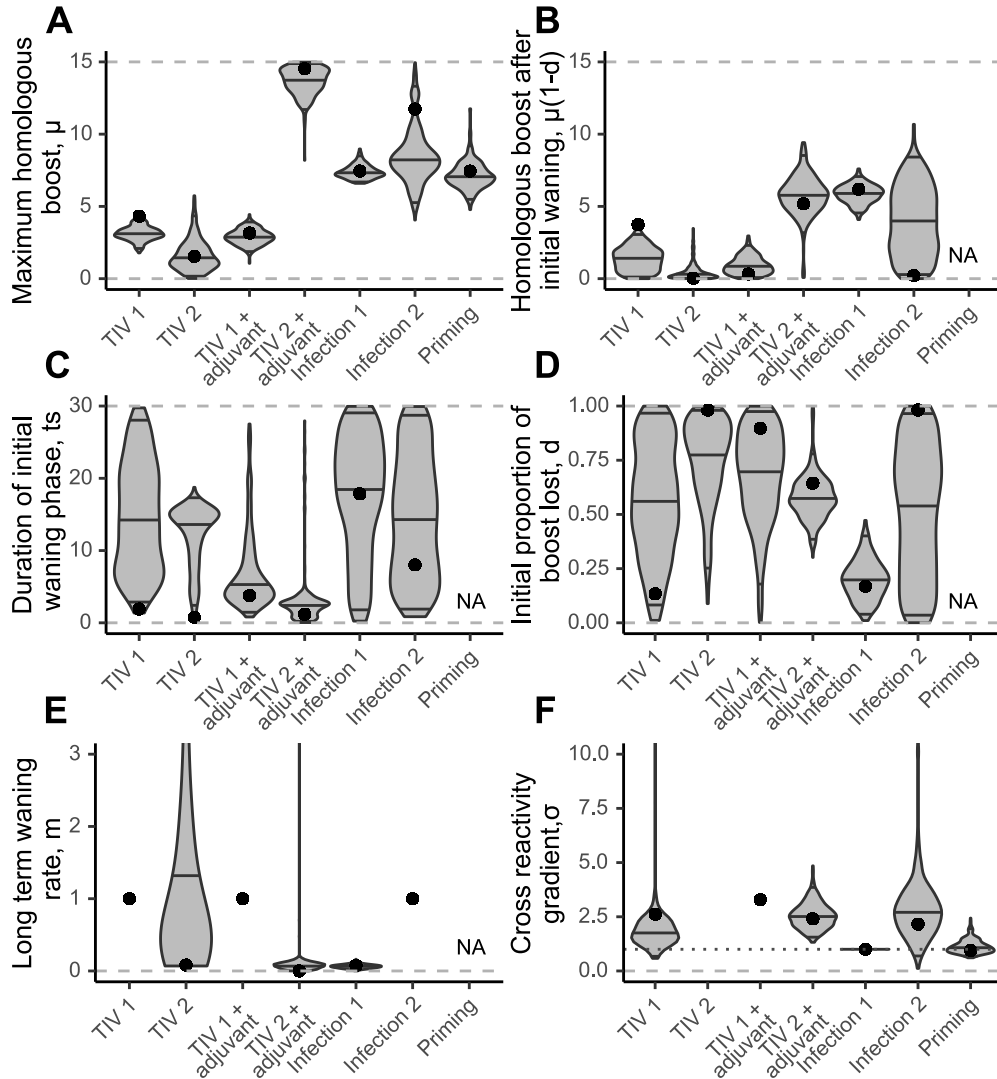


Figure B.5: Re-estimated model parameters from simulated data. Violin plots show estimated posterior densities with posterior medians and 95% credible intervals marked as horizontal black lines. Dashed gray lines show bounds on uniform prior. Black dots show true values. (A) Estimates for homologous boosting parameter, μ . (B) Estimates for homologous boost at the end of the initial waning period, $\mu(1-d)$. (C) Estimates for duration of initial waning phase, t_s . (D) Estimates for proportion of initial boost lost during the initial waning phase, d . (E) Estimates for long term waning rate, m . Estimates for TIV 1, TIV 1 + adjuvant and Infection 2 excluded due to lack of identifiability. (F) Estimates for cross reactivity gradient, σ . Note that this value is fixed at 1 for priming infection (Infection 1), shown by the horizontal dotted line. Values for TIV 2 and TIV 1 + adjuvant excluded due to lack of identifiability.

B.3 Further model comparison results

Performing direct leave-one-out cross validation for the data points with $\hat{k} > 0.7$ resulted in the fitting of a further 375 models. I applied the same convergence diagnostics for these runs as in the main text (5000000 iterations, minimum effective sample size of 200 and $\hat{R} < 1.1$ for all parameters). Where either of these conditions were violated, I re-ran the MCMC chains for 10000000 iterations. After running the chains for 10000000 iterations, the upper 95% confidence interval for $\hat{R}$ was between 1.1 and 1.3 for 26 of the 7891 estimated parameters, suggesting that there was still some between-chain variance in the inferred posterior mean for 0.3% of the parameters. Inspection of each of these marginal posteriors revealed that this lack of convergence was due to poorly identified parameters, where changes in parameter value have no impact on model predicted values. For example, the majority of problematic parameters were long-term waning rates (m) where the majority of probability density was at parameter values $m < 1$, but with some remaining density spread uniformly across $1 < m < 12$. Given that I am interested in comparing the model predicted values to the observed data in this step and not in obtaining accurate parameter estimates, I was confident that these 26 parameters would not impact the ELPD estimates.

A linear regression with ELPD as the outcome variable and included mechanisms as predictors suggested that inclusion of antigenic seniority, priming, 6 exposure types, biphasic waning and titre-dependent boosting were all associated with improved model fits, but inclusion type-specific cross reactivity was not. Regression coefficients were: presence of antigenic seniority, 6.51 (95% CI: 1.05-12.0); priming, 77.7 (95% CI: 72.3-83.2); 6 exposure types compared to 3 exposure types, 21.7 (95% CI: 16.3-27.2); biphasic waning compared to monophasic waning, 11.4 (95% CI: 5.97-16.9); titre-dependent boosting, 13.3 (95% CI: 7.82-18.7); no type-specific cross reactivity, 2.26 (95% CI: -3.19-7.73).

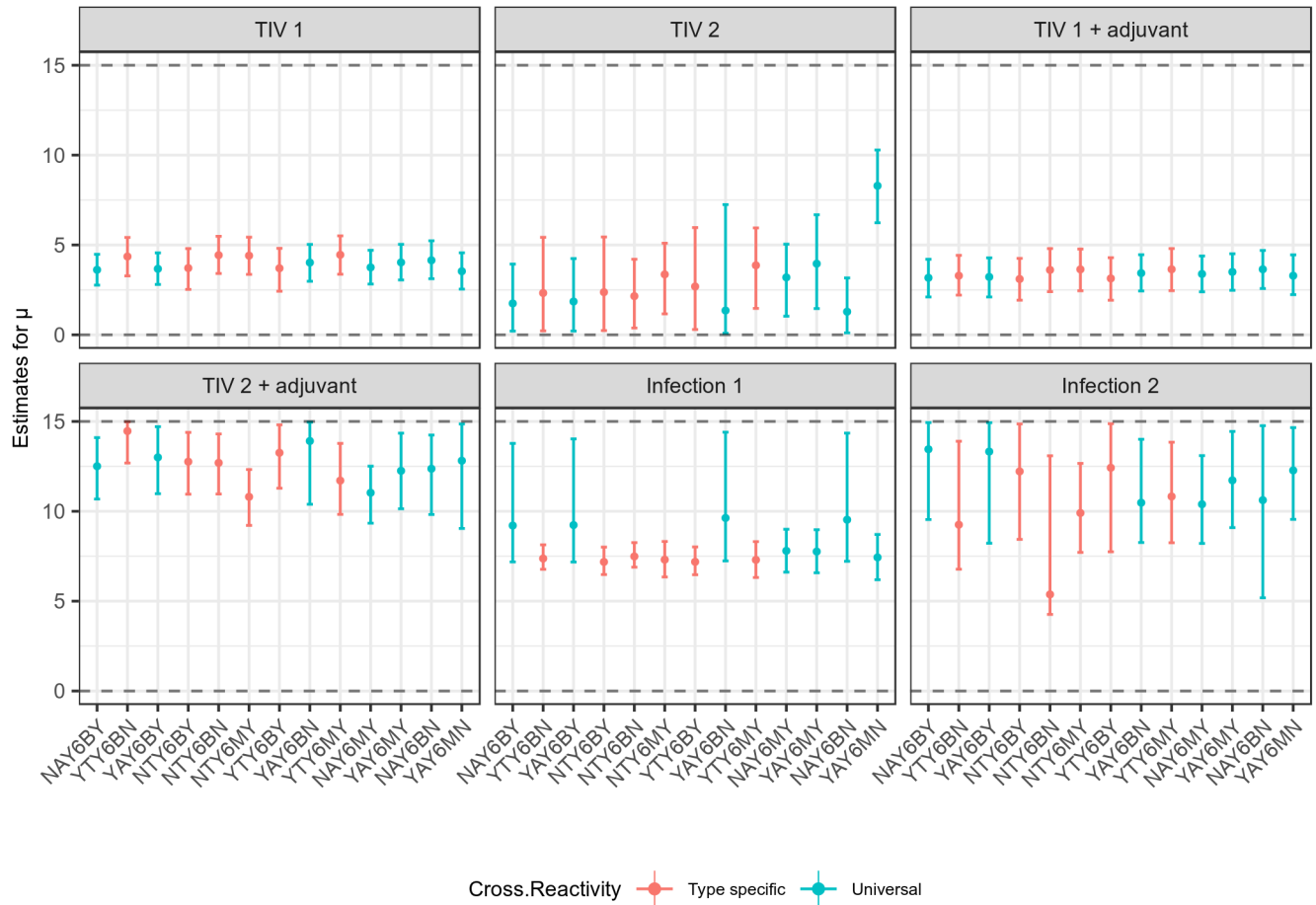


Figure B.6: Summary of posterior distribution estimates for homologous boosting parameter, μ from models with $\delta\text{ELPD} < 20$. Points show posterior median; line ranges show 95% credible intervals. Estimates are stratified by exposure type and ordered in order of increasing ELPD. Estimates are coloured according to whether or not cross reactivity was assumed to be a universal parameter or type-specific. Dashed horizontal lines represent uniform prior range. Model codes on x-axis relate to the first letter of each mechanism as described in Table 4.2.

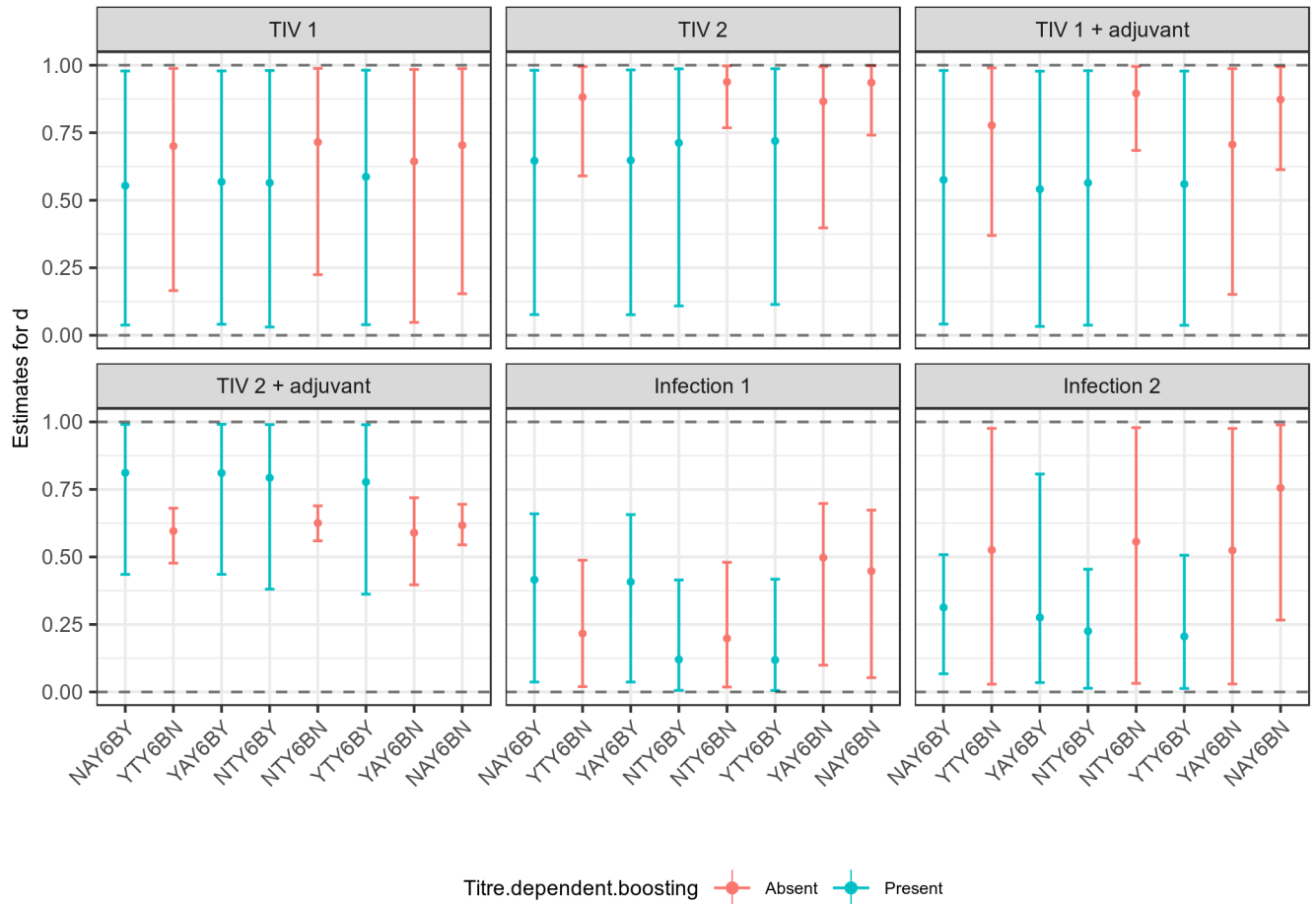


Figure B.7: Summary of posterior distribution estimates for initial waning phase proportion, d from models with $\delta\text{ELPD} < 20$. Points show posterior median; line ranges show 95% credible intervals. Estimates are stratified by exposure type and ordered in order of increasing ELPD. Estimates are coloured according to whether or not titre-dependent boosting was included. Dashed horizontal lines represent uniform prior range. Model codes on x-axis relate to the first letter of each mechanism as described in Table 4.2.

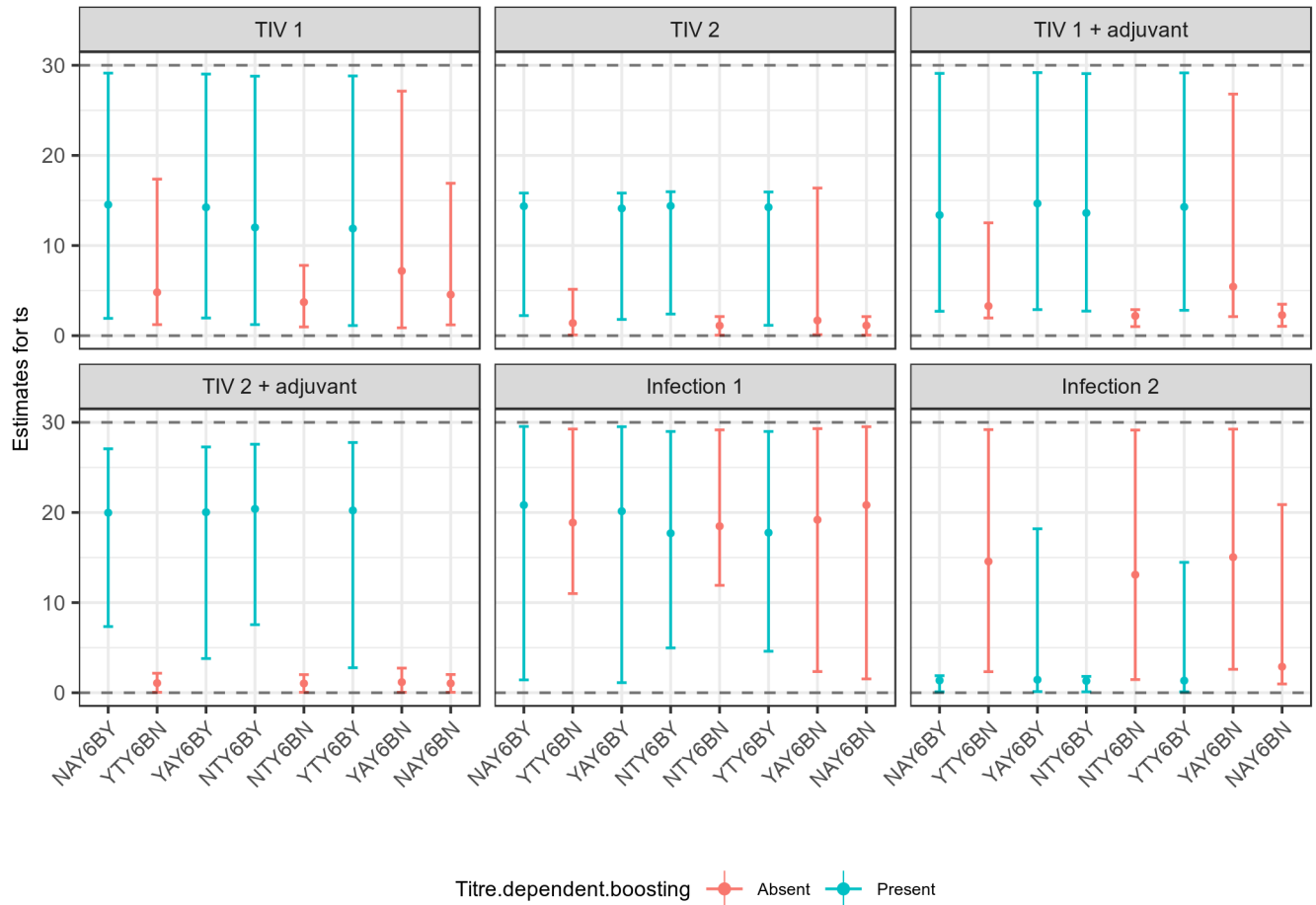


Figure B.8: Summary of posterior distribution estimates for duration of initial waning phase, t_s from models with $\delta\text{ELPD} < 20$. Points show posterior median; line ranges show 95% credible intervals. Estimates are stratified by exposure type and ordered in order of increasing ELPD. Estimates are coloured according to whether or not titre-dependent boosting was included. Dashed horizontal lines represent uniform prior range. Model codes on x-axis relate to the first letter of each mechanism as described in Table 4.2.

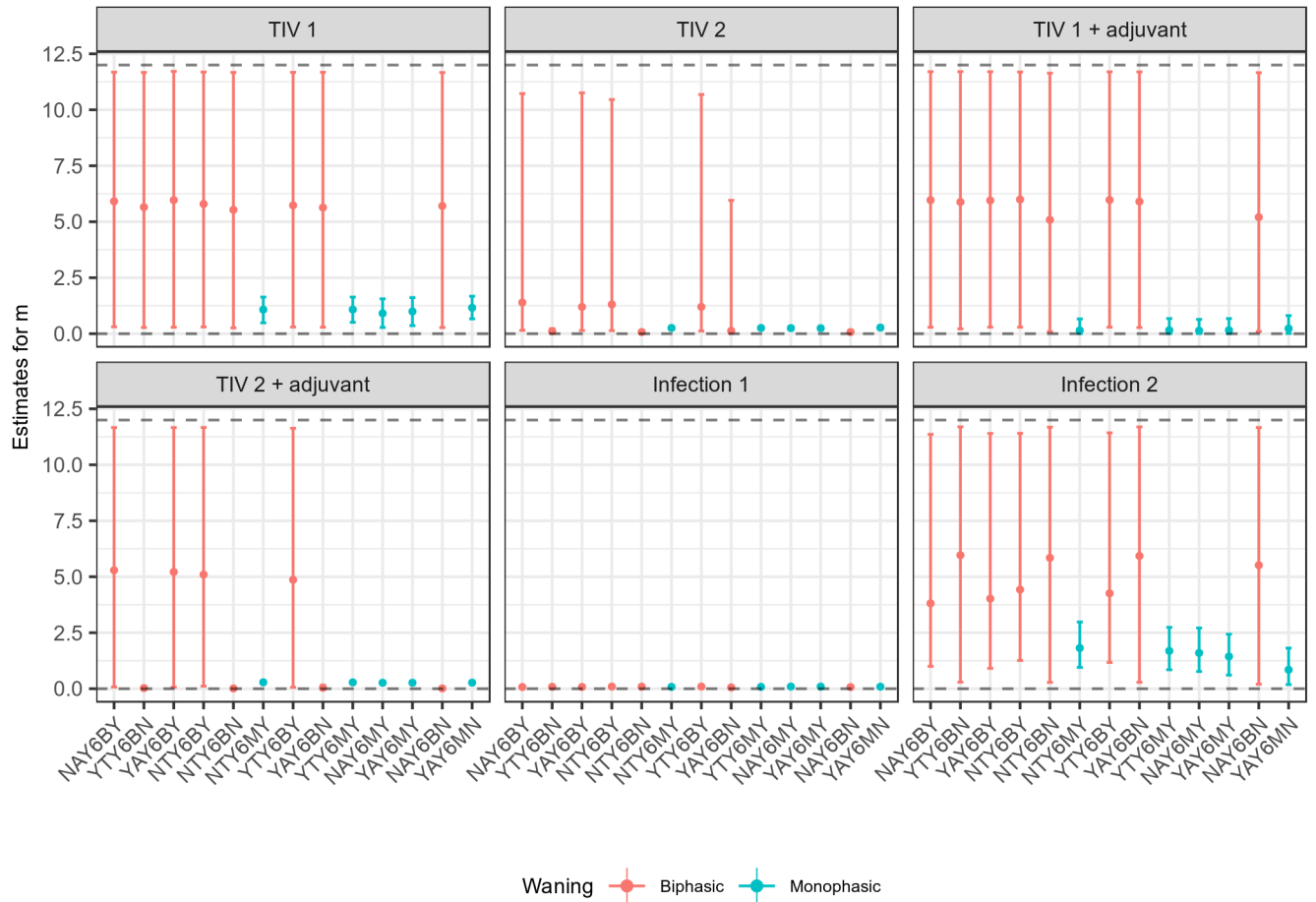


Figure B.9: Summary of posterior distribution estimates for long-term waning rate, m from models with $\delta\text{ELPD} < 20$. Points show posterior median; line ranges show 95% credible intervals. Estimates are stratified by exposure type and ordered in order of increasing ELPD. Estimates are coloured according to whether or not waning was assumed to be biphasic or monophasic. Dashed horizontal lines represent uniform prior range. Model codes on x-axis relate to the first letter of each mechanism as described in Table 4.2.

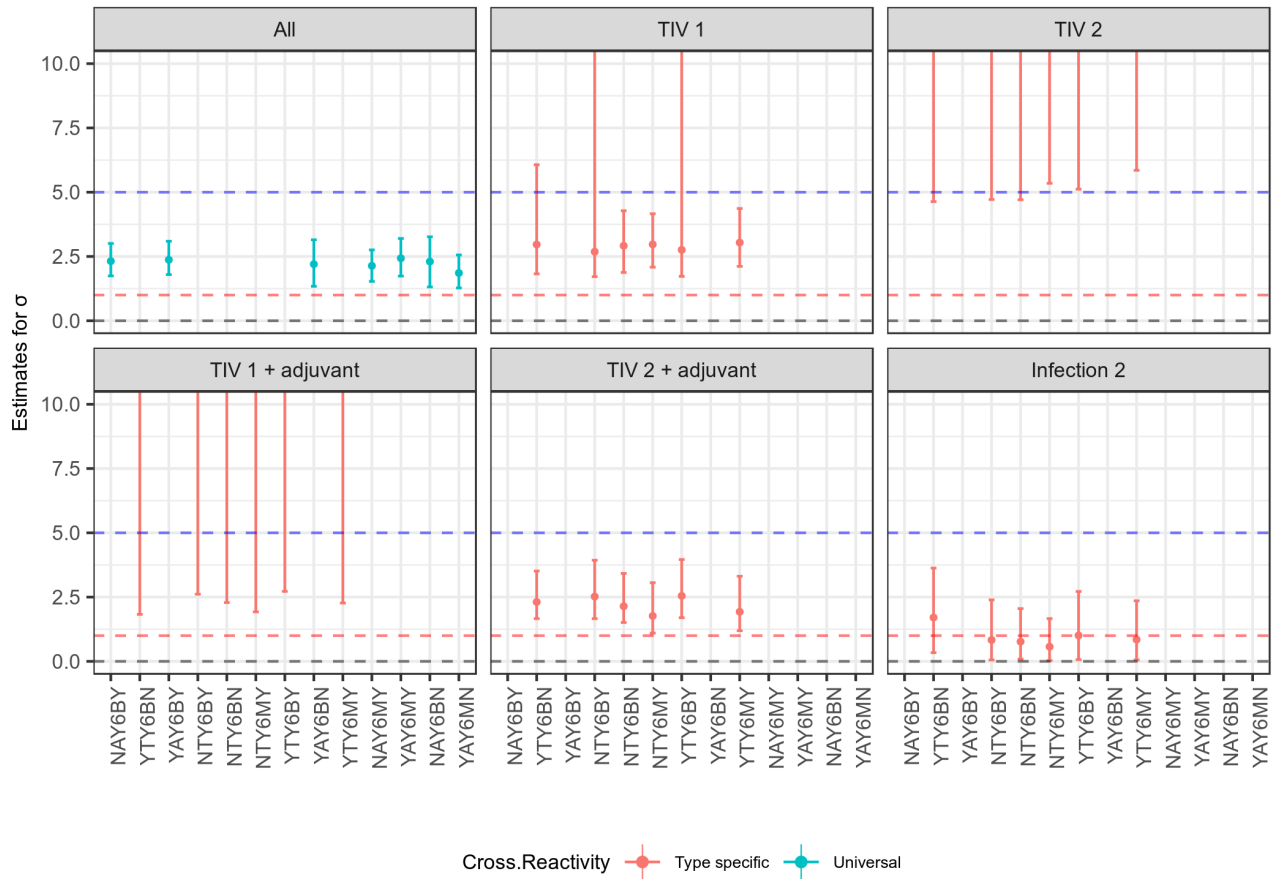


Figure B.10: Summary of posterior distribution estimates for cross reactivity gradient, σ from models with $\delta\text{ELPD} < 20$. Points show posterior median; line ranges show 95% credible intervals. Estimates are stratified by exposure type and ordered in order of increasing ELPD. Estimates are coloured according to whether or not cross reactivity was assumed to be a universal parameter or type-specific. Plots are truncated from above at 10 for clarity, but upper prior bound was 100. Red dashed line shows the fixed value of $\sigma = 1$ for priming infection. Blue dashed line shows value above which a homologous boost of $=5$ would give an observed boost of 0 against a strain with an antigenic distance of 1. Model codes on x-axis relate to the first letter of each mechanism as described in Table 4.2.

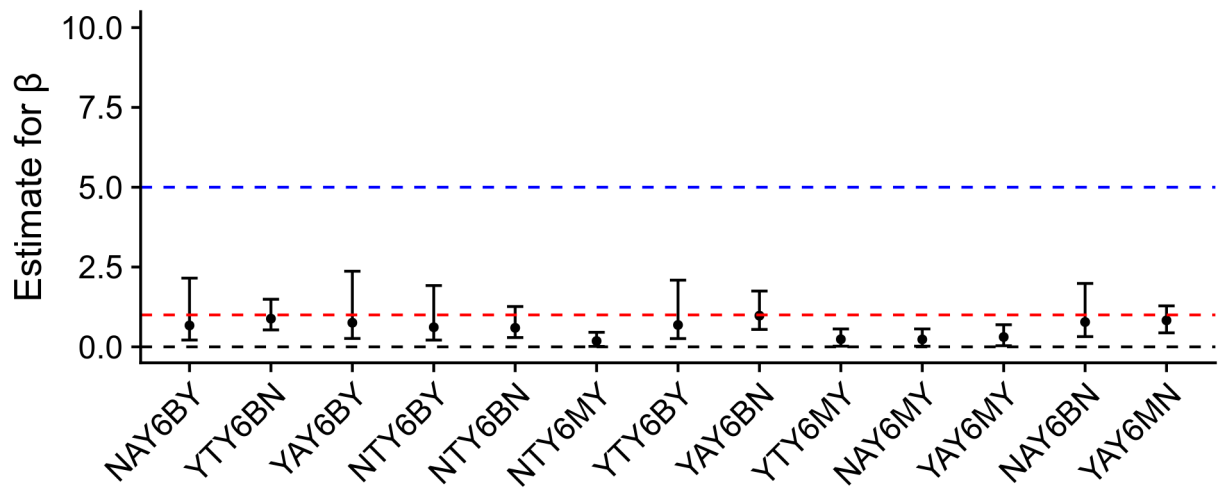


Figure B.11: Summary of posterior distribution estimates for priming cross reactivity gradient, β from models with $\delta\text{ELPD} < 20$. Points show posterior median; line ranges show 95% credible intervals. Red dashed line shows the fixed value of $\sigma = 1$ for priming infection. Blue dashed line shows value above which a homologous boost of $=5$ would give an observed boost of 0 against a strain with an antigenic distance of 1. Estimates are ordered by increasing ELPD. Model codes on x-axis relate to the first letter of each mechanism as described in Table 4.2.

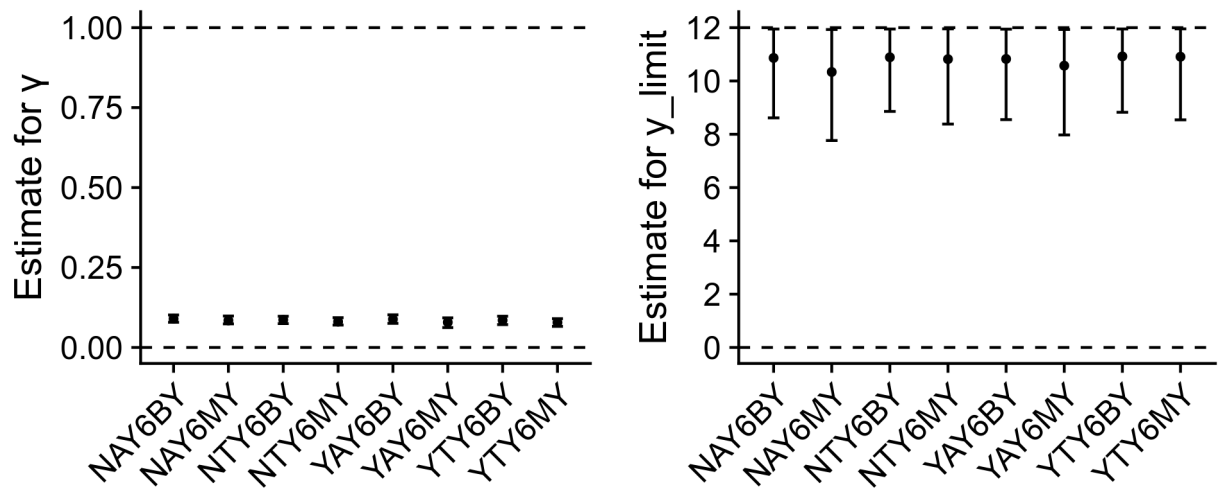


Figure B.12: Summary of posterior distribution estimates for titre dependence gradient, γ and titre-dependent switch point, y_{switch} from models with $\delta\text{ELPD} < 20$. Points show posterior median; line ranges show 95% credible intervals. Estimates are ordered by increasing ELPD. Model codes on x-axis relate to the first letter of each mechanism as described in Table 4.2.

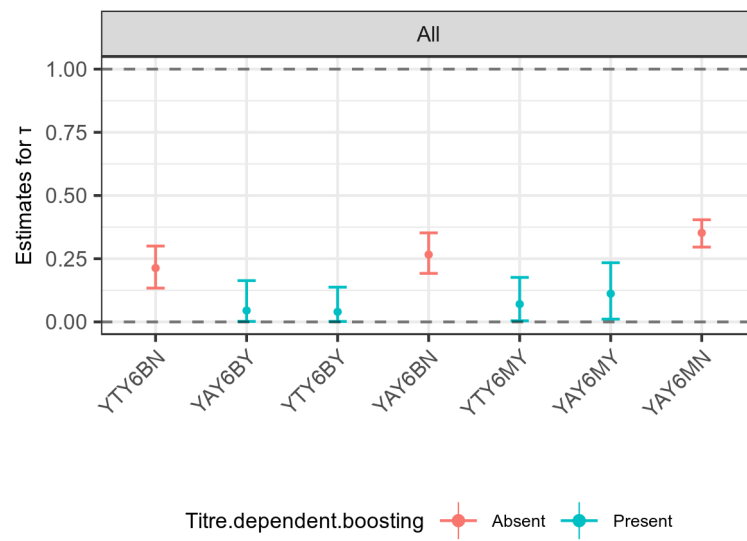


Figure B.13: Summary of posterior distribution estimates for antigenic seniority parameter, τ from models with $\delta\text{ELPD} < 20$. Points show posterior median; line ranges show 95% credible intervals. Estimates are ordered by increasing ELPD. Estimates are coloured according to whether or not titre-dependent boosting was also included in the model. Model codes on x-axis relate to the first letter of each mechanism as described in Table 4.2.

Appendix C

Adding titre-mediated immunity to *serosolver*

C.1 Adding immunity to the infection process

The infection history model with independent infection generating processes for each time period assumes that the outcome of each possible infection event (a Bernoulli trial) is independent of all previous infections. The distribution of times between infection events therefore follows the beta-geometric distribution (a geometric distribution but with success probability drawn from a beta distribution, which corresponds to the attack rate at each time period). The distribution of times between infections, independent of antibody waning and antigenic drift, has a mode near 0. This pattern has been shown previously when inferring the time between dengue virus infections [282]. Fig C.1 top panel shows the distribution of times between infections from simulating quarterly infection histories (a new infection state every 3 months) for 2000 individuals across 48 years using the model described above with a quarterly attack rate of 0.05. Under this simulation, the median time between infections is 2.5 years, and the mode is 0.25 years. Without sufficient antibody titre data to inform when infections actually occurred, this prior risks inferring infections closer together in time than we expect in reality due to the presence of protective immunity [76, 225]. In this section, I incorporate protective immunity following infection into the model. I note that these methods are not included in the submitted *serosolver* paper, though are present in a branch of the R-package and will be merged in the future.

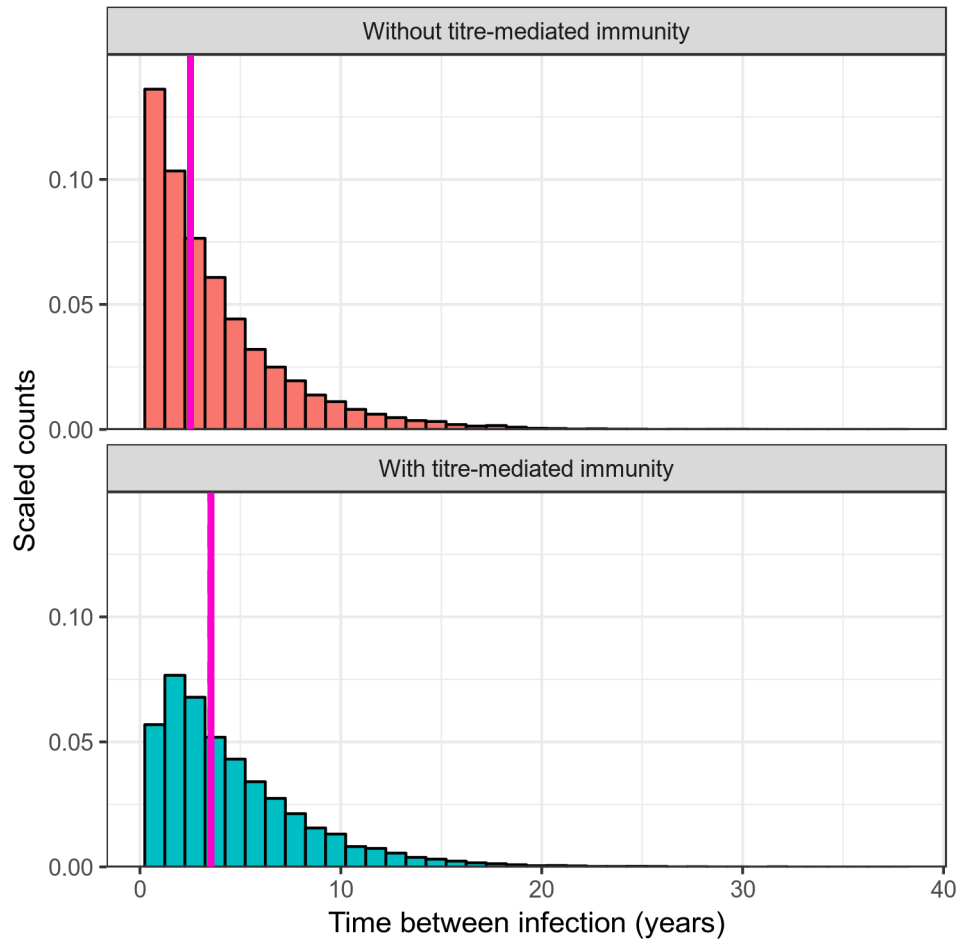


Figure C.1: Distribution of times between infections sampled from the infection history model. Results shown are based on simulating infection histories for 2000 individuals across 48 years, assuming that infections can occur every 3 months with a quarterly attack rate of 0.05. Vertical lines show median. Top panel: simulated infection histories from the model without immunity. Bottom panel: simulated infection histories from the model with titre-mediated immunity based on latent titres at the time of exposure and the relationship described in [243].

C.1.1 Titre-mediated immunity

Coudeville *et al.* described the relationship between an individual's log HI titre at the time of infection, $Y_{i,j-}$, and the probability of protection as [233, 243]:

$$g(Y_{i,j-}) = 1 - \frac{1}{1 + e^{b(Y_{i,j-} - a)}} \quad (\text{C.1})$$

Note that I have abused the notation slightly, using $Y_{i,j-}$ to represent the antibody titre against the strain circulating during time period j , but before the infection has happened ie. any antibody waning from time $j - 1$ to j will have occurred. a is the 50% protective titre. This relationship is shown in Fig C.2. Throughout these analyses, I use fixed values for the titre-mediated immunity function of $a = 2.844$ and $b = 1.299$ based on the mean estimates from Coudeville *et al.* Under this new model, the likelihood of an individual infection event is:

$$P(Z_{i,j}|\Phi_j, Y_{i,j-}) = \left((1 - g(Y_{i,j-}))\Phi_j\right)^{Z_{i,j}} \left((1 - \Phi_j) + g(Y_{i,j-})\Phi_j\right)^{1-Z_{i,j}} \quad (\text{C.2})$$

ie. the likelihood of an infection state is: the probability that the individual was exposed and didn't escape infection via titre-mediated immunity; didn't get exposed; or was exposed but had sufficient prior immunity to avoid infection, where Φ_j is the probability of exposure. Equivalently:

$$P(Z_{i,j}|\Phi_j, Y_{i,j-}) = \Phi_j \left((1 - g(Y_{i,j-}))^{Z_{i,j}} g(Y_{i,j-})^{1-Z_{i,j}}\right) + (1 - \Phi_j) (0^{Z_{i,j}} 1^{1-Z_{i,j}}) \quad (\text{C.3})$$

The likelihood of a set of infection states given the infection generating process Φ and antibody titres $\mathbf{Y}$ is:

$$P(\mathbf{Z}|\Phi, \mathbf{Y}) = \prod_i \prod_j \left((1 - g(Y_{i,j-}))\Phi_j\right)^{Z_{i,j}} \left((1 - \Phi_j) + g(Y_{i,j-})\Phi_j\right)^{1-Z_{i,j}} \quad (\text{C.4})$$

We are more interested in the total joint probability distribution of $\mathbf{Z}$, ϕ and $\mathbf{Y}$, as all of these parameters are unknown quantities of interest. Stepping through infection histories of increasing size for one individual, we can arrive at a simple formulation for the joint probability:

$$P(Y_1, Z_1, \Phi_1, \theta) = P(Z_1|\Phi_1, Y_0, \theta)P(Y_0)P(\Phi_1)P(\theta) \quad (\text{C.5})$$

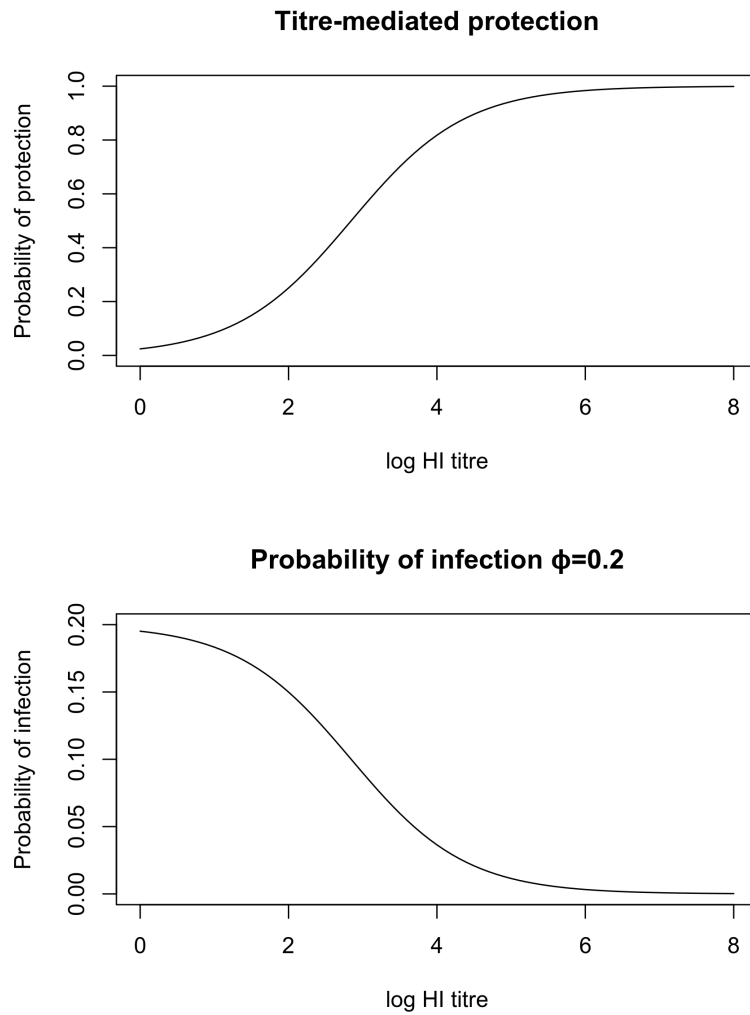


Figure C.2: Relationship between HI titre and immunity. Top panel: probability of protection from infection given exposure as a function of titre using parameter values from [243] ($a = 2.844$, $b = 1.299$). Bottom panel: Overall probability of infection as a function of HI titre given a population-wide probability of exposure of 0.2.

$$\begin{aligned}
P(Y_1, Z_1, Y_2, Z_2, \Phi_1, \Phi_2, \boldsymbol{\theta}) &= P(Z_2 | \Phi_2, Y_1, \boldsymbol{\theta}) P(Y_1 | Z_1, \boldsymbol{\theta}) P(\Phi_2) \\
&\quad P(Z_1 | \Phi_1, Y_0, \boldsymbol{\theta}) P(Y_0) P(\Phi_1) P(\boldsymbol{\theta})
\end{aligned} \tag{C.6}$$

$$\begin{aligned}
P(Y_0, Z_1, Y_1, Z_2, Y_2, Z_3, \Phi_1, \Phi_2, \Phi_3, \boldsymbol{\theta}) &= P(Z_3 | \Phi_3, Y_2) P(Z_2 | \Phi_2, Y_1, \boldsymbol{\theta}) P(Z_1 | \Phi_1, Y_0, \boldsymbol{\theta}) \\
&\quad P(Y_2 | Z_1, Z_2, \boldsymbol{\theta}) P(Y_1 | Z_1, \boldsymbol{\theta}) P(Y_0) \\
&\quad P(\Phi_3) P(\Phi_2) P(\Phi_1) P(\boldsymbol{\theta})
\end{aligned} \tag{C.7}$$

where Y_0 is the initial antibody titre (0 at the time of birth). Finally:

$$P(\mathbf{Y}, \mathbf{Z}, \boldsymbol{\Phi}, \boldsymbol{\theta}) = \prod_{i=1}^n \prod_{j=1}^m P(Z_{i,j} | \Phi_j, Y_{i,j-}) P(Y_{i,j-} | Z_{i,1}, Z_{i,2}, \dots, Z_{i,j-1}, \boldsymbol{\theta}) P(\Phi_j) P(\boldsymbol{\theta}) \tag{C.8}$$

and because the antibody kinetics model generating antibody states from an infection history is deterministic and $P(Y_0 = 0) = 1$:

$$P(\mathbf{Y}, \mathbf{Z}, \boldsymbol{\Phi}, \boldsymbol{\theta}) = \prod_{i=1}^n \prod_{j=1}^m P(Z_{i,j} | \Phi_j, Y_{i,j-}) P(\Phi_j) P(\boldsymbol{\theta}) \tag{C.9}$$

Fig C.3 depicts the full model, and the full posterior distribution can be re-written as:

$$P(\mathbf{Y}, \mathbf{Z}, \boldsymbol{\Phi}, \boldsymbol{\theta} | \mathbf{Q}) = \prod_{i=1}^n \left(\prod_{t=t_{1,i}}^{t_{max,i}} f(Q_{i,t} | Y_{i,t}, \boldsymbol{\theta}) \right) \prod_{j=1}^m P(Z_{i,j} | \Phi_j, Y_{i,j-}) P(\Phi_j) P(\boldsymbol{\theta}) \tag{C.10}$$

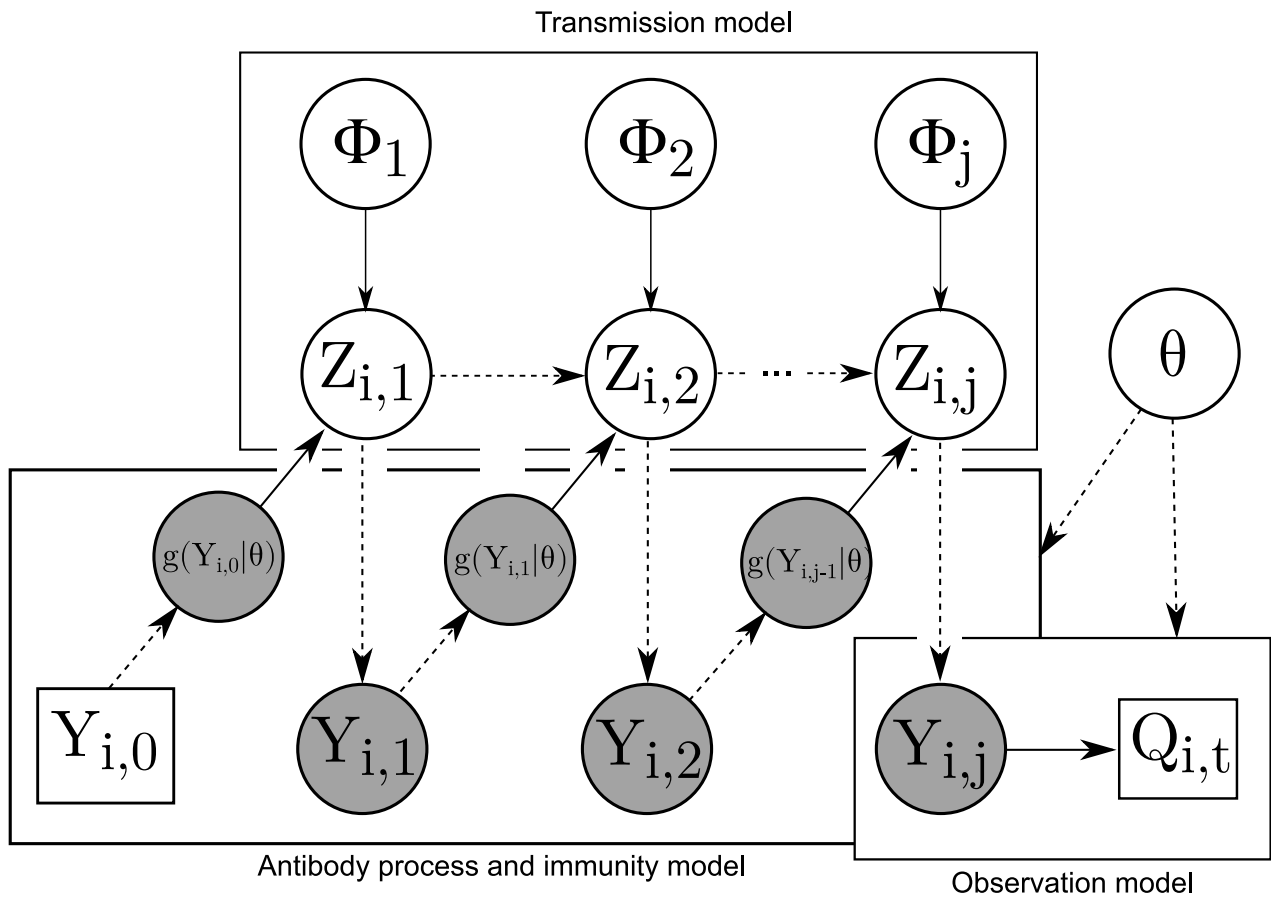


Figure C.3: Directed acyclic graph representation of the modified model with immunity. Square boxes represent observations, white circles represent parameters/latent states of interest, grey circles represent deterministic latent states, solid arrows represent stochastic dependencies, dashed arrows represent deterministic dependencies. The different model levels are shown within boxes. $Q_{i,t}$ and $Y_{i,0}$ are shown in boxes to distinguish them from the latent states. $Z_{i,j}$ represents the overall infection history as of time j .

C.1.2 Implementation of the immunity model

The first step to simplifying this model is to separate out exposure events and infection events. That is, an individual may be exposed to an infectious contact with some probability, and there is some probability that that contact will lead to an infection. Φ_j is the probability that an individual is exposed to an infectious contact, so I augment this whole process by making the current infection state variable an **exposure** state variable Z , and by adding a new infection state variable called X . Let's also consider only one time point for now, and symbolise the titre-mediated immunity $V_i = g(Y_{i,j-})$. A simplified visualisation is then to consider a hierarchical Bernoulli model (visualised in Fig C.4):

$$x_i \sim \text{Bern}(z_i(1 - v_i)) \quad (\text{C.11})$$

$$z_i \sim \text{Bern}(\phi) \quad (\text{C.12})$$

$$\phi \sim \text{Beta}(\alpha, \beta) \quad (\text{C.13})$$

In Sections 5.5.5 and 5.5.6 I gave a closed form expression for the marginal probability of the infection history matrix $\mathbf{Z}$. This formulation has proven useful for inferring $\mathbf{Z}$ without needing to explicitly infer Φ , which drastically improved MCMC convergence. The aim here is to derive a similar expression to give the marginal probability of the exposure history matrix $\mathbf{X}$, $P(\mathbf{X})$. Equation C.14 gives the marginal probabilities for each combination of augmented latent states (ie. the infection and exposure states), antibody kinetics parameters θ and infection generating process parameters Φ . Any parameter

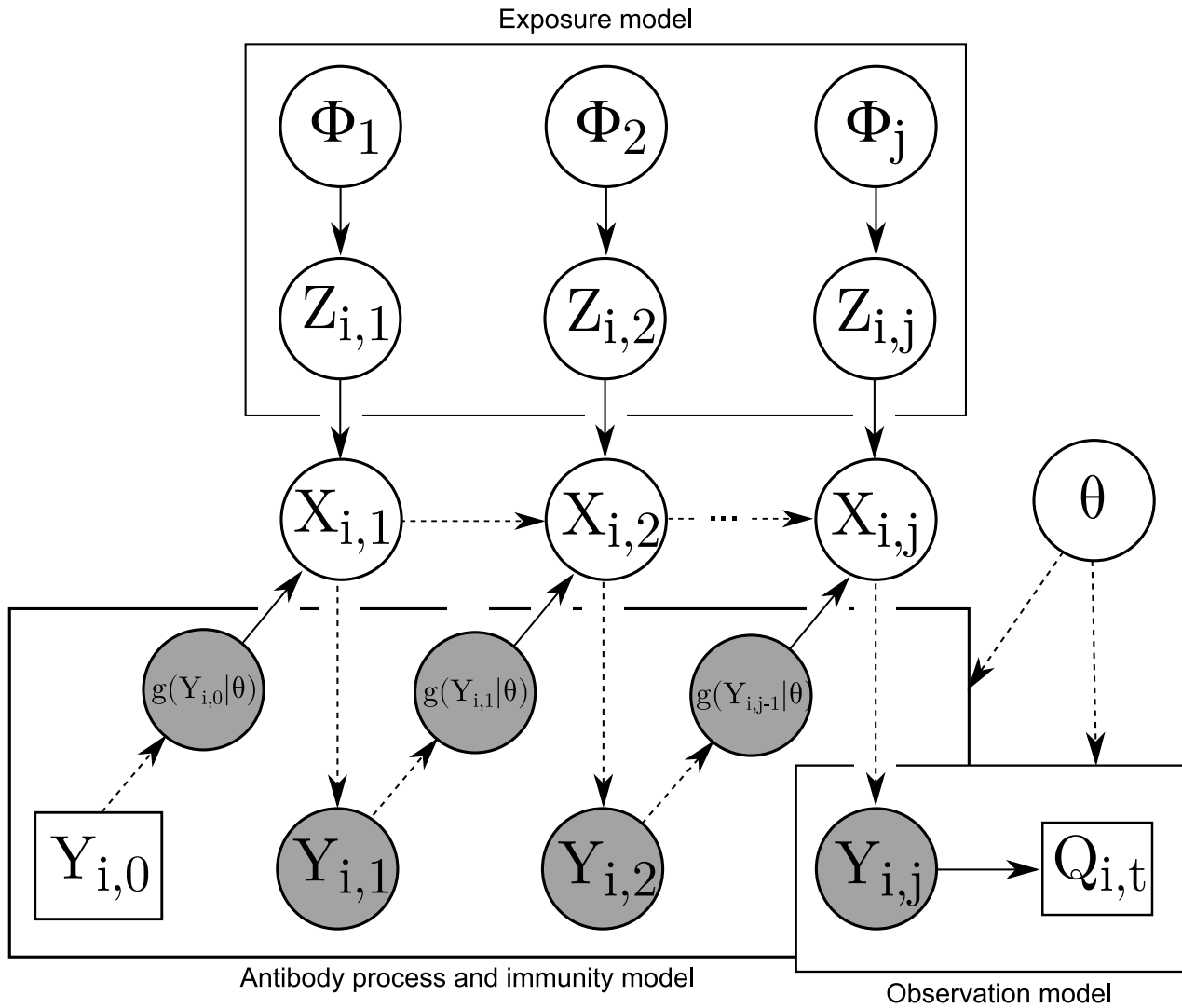


Figure C.4: Directed acyclic graph representation of the modified model with separate exposure and infection states. Square boxes represent observations, white circles represent parameters/latent states of interest, grey circles represent deterministic latent states, solid arrows represent stochastic dependencies, dashed arrows represent deterministic dependencies. The different model levels are shown within boxes. $Z_{i,j}$ represents the overall infection history as of time j .

included in the equation on the left must be explicitly represented in the MCMC procedure.

$$P(\mathbf{X}, \mathbf{V}, \mathbf{Z}, \Phi, \theta) = P(\mathbf{V}|\mathbf{X}, \theta)P(\mathbf{X}|\mathbf{Z})P(\mathbf{Z}|\Phi)P(\Phi)P(\theta) \quad (\text{C.14})$$

$$= \prod_{i=1}^n \prod_{j=1}^m P(X_{i,j}|Z_{i,j}, V_{i,j-}, \theta)P(Z_{i,j}|\Phi_j)P(V_{i,j-}|X_{i,1}, \dots, \quad (\text{C.15})$$

$$X_{i,j-1}, \theta)P(\Phi_j)P(\theta)$$

$$P(\mathbf{X}, \mathbf{V}, \mathbf{Z}, \theta) = \left(\prod_{i=1}^n \prod_{j=1}^m P(X_{i,j}|Z_{i,j}, V_{i,j-}, \theta) \right) P(\mathbf{Z})P(\theta) \quad (\text{C.16})$$

$$P(\mathbf{X}, \mathbf{V}, \Phi, \theta) = \prod_{i=1}^n \prod_{j=1}^m P(X_{i,j}|V_{i,j-}, \Phi_j)P(V_{i,j-}|X_{i,1}, \dots, X_{i,j-}, \theta) \quad (\text{C.17})$$

$$P(\Phi_j)P(\theta)$$

$$P(\mathbf{X}, \mathbf{V}, \theta) = \prod_{j=1}^m \left(\int_0^1 \prod_{i=1}^n P(X_{i,j}|V_{i,j-}, \Phi_j)P(V_{i,j-}|X_{i,1}, \dots, X_{i,j-1}, \theta) \quad (\text{C.18})$$

$$P(\Phi_j)d\Phi_j \right) P(\theta) \quad (\text{C.19})$$

Note that $P(V_{i,j-}|X_{i,1}, \dots, X_{i,j-1}, \theta) = 1$, as the model linking past infections to future susceptibility is deterministic. The ideal result would be to implement an analytical solution for Equation C.18, as we are only interested in the infection states $\mathbf{X}$ (which can be used to generate the estimated attack rates) and antibody kinetics parameters θ . However, unlike in Section 5.5.5 and despite the apparent simplicity of this model, there is no closed form solution for the integral as in Equation 5.40 (as each $V_{i,j-}$ in $P(X_{i,j}|V_{i,j-}, \Phi_j) = \left((1 - V_{i,j-})\Phi_j \right)^{X_{i,j}} \left((1 - \Phi_j) + V_{i,j-}\Phi_j \right)^{1-X_{i,j}}$ is different for each i and j). Any implementation of this equation therefore requires numerical integration or approximation.

C.2 Approximations of $P(X, V, \theta)$

In this section, I implement three approximations for $P(X, V, \theta)$ and compare their accuracy and computation time.

C.2.1 Numerical integration over Φ

Note that all Φ_j are independent, and I therefore seek approximations for the case where $m = 1$. Note also that $P(V_{j-}) = 1$. Numerical integration of:

$$P(X_j, V_{j-}, \theta) = \mathbb{E}_P(P(X_j, V_{j-}, \theta) | \Phi_j) = \int_0^1 P(X_j | V_{j-}, \Phi_j) P(\Phi_j) d\Phi_j P(\theta) \quad (\text{C.20})$$

For example, using the `integrate` function in R, is conceptually simple and guaranteed to give a good approximation of $P(X_j, V_{j-}, \theta)$.

C.2.2 Approximate integration by iterating over Φ

An alternative to calculating the full integral is to simply iterate over values for Φ from 0 to 1:

$$P(X_j, V_{j-}, \theta) \approx \sum_{k=0}^{k_{max}} \frac{1}{k} \left(P(X_j | V_{j-}, \theta, \Phi_j = \frac{k}{k_{max}}) P(\Phi_j = \frac{k}{k_{max}}) P(\theta) \right) \quad (\text{C.21})$$

where k_{max} is chosen to trade-off between computational time and accuracy.

C.2.3 Importance sampling approximation

The final approximation that I test here is importance sampling. Rather than integrating over Φ_j , I instead represent the marginal distribution $P(X_j, V_{j-}, \theta)$ via integration over all possible augmented exposure states Z_j :

$$P(X_j, V_{j-}, \theta) = \int_{Z_j} P(X_j | Z_j, V_{j-}, \theta) P(Z_j) dZ_j P(\theta) \quad (\text{C.22})$$

$P(Z_j = Z_j^k)$ has a closed form solution from Section 5.5.5, and $P(X_j | Z_j, V_{j-}, \theta)$ is defined in Equation C.16. Clearly, iterating over all possible Z_j is infeasible as there are 2^n possible exposure histories for n individuals. In theory it would be possible to approximate $P(X_j, V_{j-}, \theta)$ by sampling Z_j^k from the marginal distribution $P(Z_j)$ such that:

$$P(X_j, V_{j-}, \theta) \approx \frac{1}{N} \sum_{k=1}^N P(X_j | Z_j = Z_j^k, V_{j-}, \theta) \quad (\text{C.23})$$

However, given the vast space of Z_j as n becomes large, and given that many realisations will have probability zero (as $P(X_{i,j} = 1|Z_{i,j} = 0) = 0$), this would require too many realisations to be of practical use. The realisations of Z_j can be made more efficient to better representing the actual distribution of $P(X_j, V_{j-}, \theta)$ using a similar importance sampling-based approach to Cauchemez *et al.* 2008 [505]. Cauchemez *et al.* 2008 achieved good performance with $N = 1$, which would aid computational performance in our case. $P(X_j, V_j, \theta)$ can be re-written as:

$$P(X_j, V_{j-}, \theta) = \int_{Z_j} \frac{P(X_j|Z_j, V_{j-}, \theta)P(Z_j)P(\theta)}{h(Z_j|X_j, V_{j-}, \theta)} h(Z_j|X_j, V_{j-}, \theta) dZ_j \quad (\text{C.24})$$

where $h(Z_j|X_j, V_{j-}, \theta)$ can be chosen to simulate exposure histories Z_j^k that are consistent with the infection histories X_j . N exposure histories are simulated as $Z_j^k \sim h(Z_j|X_j, V_{j-}, \theta)$, and the integral is approximated as:

$$P(X_j, V_{j-}, \theta) = \mathbb{E}_h \left(\frac{P(X_j|Z_j, V_{j-}, \theta)P(Z_j)P(\theta)}{h(Z_j|X_j, V_{j-}, \theta)} \right) \approx \frac{1}{N} \sum_{k=1}^N \frac{P(X_j|Z_j^k, V_{j-}, \theta)P(Z_j^k)P(\theta)}{h(Z_j^k|X_j, V_{j-}, \theta)} \quad (\text{C.25})$$

It is simple to choose a distribution for $h(Z_j|X_j, V_{j-}, \theta)$ that is close to $P(X_j|Z_j, V_{j-}, \theta)$ by sampling a value for each $Z_{i,j}$ independently, as the values for $Z_{i,j}$ are quite constrained given the values of $X_{i,j}$ based on the following terms:

$$P(Z_i|\Phi) = \Phi^{Z_i}(1 - \Phi)^{1-Z_i} \quad (\text{C.26})$$

$$P(x_i|V_i, Z_i) = [(1 - V_i)^{x_i} V_i^{1-x_i}]^{Z_i} [0^{x_i} 1^{1-x_i}]^{1-Z_i} \quad (\text{C.27})$$

$$P(Z_i = 1|X_i = 1, V_i, \mathbf{X}_{-i}, \mathbf{V}_{-i}) = 1 \quad (\text{C.28})$$

$$P(Z_i = 0|X_i = 1, V_i, \mathbf{X}_{-i}, \mathbf{V}_{-i}) = 0 \quad (\text{C.29})$$

$$P(Z_i = 1|X_i = 0, V_i, \mathbf{X}_{-i}, \mathbf{V}_{-i}) = ? \quad (\text{C.30})$$

$$P(Z_i = 0|X_i = 0, V_i, \mathbf{X}_{-i}, \mathbf{V}_{-i}) = ? \quad (\text{C.31})$$

ie. an individual must be exposed to become infected, and cannot be infected if they were not exposed. We therefore need to find good approximations for $P(Z_i = 1|X_i = 0, V_i, \mathbf{X}_{-i}, \mathbf{V}_{-i})$ and $P(Z_i = 0|X_i = 0, V_i, \mathbf{X}_{-i}, \mathbf{V}_{-i})$ which take into account the known V_i . The Z_i are not conditionally independent of $\mathbf{X}_{-i}/V_i$ given X_i/V_i (consider that $P(Z_i = 1|V_i, X_i = 0, \text{all } \mathbf{X}_{-i} = 1, \mathbf{V}_{-i}) > P(Z_i = 1|V_i, X_i = 0, \text{all } \mathbf{X}_{-i} = 0, \mathbf{V}_{-i})$), which means that there is no simple form for these terms. This can

be shown using Bayes' rule:

$$P(Z_i = 1|X_i = 0, V_i, \mathbf{X}_{-i}, \mathbf{V}_{-i}) = \frac{P(X_i = 0|Z_i = 1, V_i, \mathbf{X}_{-i}, \mathbf{V}_{-i})P(Z_i = 1|V_i, \mathbf{X}_{-i}, \mathbf{V}_{-i})}{P(X_i = 0|V_i, \mathbf{X}_{-i}, \mathbf{V}_{-i})} \quad (\text{C.32})$$

The second half of the numerator and denominator cannot be solved without integrating over the latent exposure states $\mathbf{Z}_{-i}$ or the exposure probability Φ , which means that so far we are doing no better than the integration and iteration approximations above. However, a good approximation for $h(Z_j|X_j, V_{j-}, \theta)$ can be found by assuming conditional independence between Z_i and $\mathbf{X}_{-i}/V_i$ given X_i/V_i and using the law of total probability:

$$P(Z_i = 1|X_i = 0, V_i, \alpha, \beta) = \frac{P(X_i = 0|Z_i = 1)P(Z_i = 1)}{P(X_i = 0|Z_i = 1)P(Z_i = 1) + P(X_i = 0|Z_i = 0)P(Z_i = 0)} \quad (\text{C.33})$$

$$= \frac{V_i P(Z_i = 1)}{V_i P(Z_i = 1) + P(Z_i = 0)} \quad (\text{C.34})$$

$$= \frac{V_i \frac{\alpha}{\alpha+\beta}}{V_i \frac{\alpha}{\alpha+\beta} + \frac{\beta}{\alpha+\beta}} \quad (\text{C.35})$$

and $P(Z_i = 0|X_i = 0, V_i, \alpha, \beta) = 1 - P(Z_i = 1|X_i = 0, V_i, \alpha, \beta)$. It is easy to sample from this distribution for all i , and $h(Z_j|X_j, V_{j-}, \theta)$ is the product of these individual probabilities.

C.2.4 Comparison of approximation implementations

To check the accuracy of these approximations and feasibility of implementation, the above 3 approximations for $P(\mathbf{X})$ were calculated and compared to the known joint probabilities. 1000 simulations of the hierarchical Bernoulli model shown in Fig C.4 were made with $n = 1000$, $m = 1$, $\alpha = 1$, $\beta = 1$, $a = 3$ and $b = 5$ (the latter two terms being part of the titre-mediated immunity model), and each of the equations in Equation C.14 were calculated and compared on the log scale. Fig C.5 demonstrates that both the integration method and iterative search approximations were identical to $P(\mathbf{X}, \Phi)$, which is correct given that $P(\Phi)$ is uniform when $\alpha = 1$ and $\beta = 1$. However, the importance sampling method performed poorly at the tails of the distribution, suggesting that $h(Z_j|X_j, V_{j-}, \theta)$ was a poor distribution choice.

C.2.5 Computation time prevents these solutions from being useful

Although the integration and iteration approximations are accurate, implementation in an MCMC algorithm requires re-sampling the entire infection history matrix $\mathbf{X}$ at each MCMC iteration and performing a full accept-reject to move to the proposed infection history matrix $\mathbf{X}'$. This leads to extremely low acceptance rates, as the majority of proposed moves using a simple proposal distribution

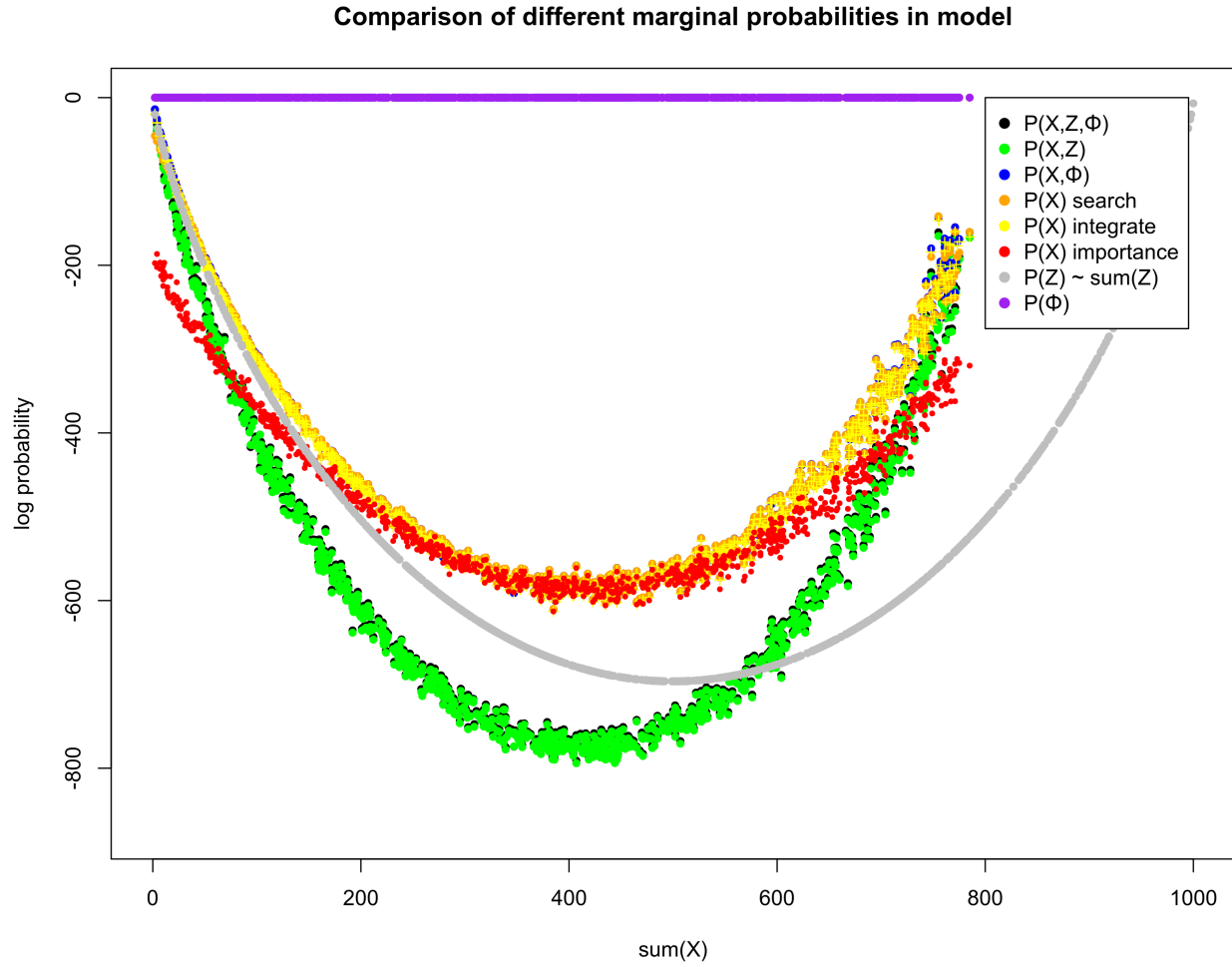


Figure C.5: Comparison of joint distribution approximations to known full joint distributions. Each point of a given colour is the associated log probability calculated of the labelled joint distribution using one simulation from the hierarchical Bernoulli model with $n = 1000$, $m = 1$, $\alpha = 1$, $\beta = 1$, $a = 3$ and $b = 5$. X-axis shows the total number of infections for that simulation, and y-axis shows the log probability.

give very low posterior probabilities. Even tuning the number of individuals i and time periods j re-sampled at each iteration does not lead to improved acceptance rates (results not shown). Chapter 5 resolved this issue by either performing an independent accept/reject step for each individual (which is possible when Φ is explicitly represented and individual infection states are conditionally independent), or by performing a Gibbs step to re-sample individual infection histories independently. Both of these options led to high acceptance rates and an efficient random walk. However, the latter option required calculation of the marginal density $P(\mathbf{Z})$ for each proposed infection state, and there is no equivalent closed form solution for $P(\mathbf{X})$ as discussed above. Although these approximations could be nested within the Gibbs sampler to calculate $P(X_{i,j} = 1 | \mathbf{X}_{-i,j})$, $P(\mathbf{X})$ would need to be approximated separately for each of n individuals, m time periods, and N MCMC iterations. Given that $n \approx 1000$,

$m \approx 50$ and $N \approx 10000000$, even a solving time of 1 microsecond would add an additional 5.8 days of computation time. The actual solving time for any of the above solutions would likely be even longer. For example, both the integration method using `integrate` and the iteration method have a run time of ≈ 2 milliseconds in R for $m = 1$ and $n = 1000$. Even nesting these functions within the C++ code will not improve computation time sufficiently.

C.2.6 No closed form solution for the logit immunity model

A final attempt to solve this problem without explicitly representing Φ or $\mathbf{Z}$ is to focus on the infection history re-sampling step. An efficient MCMC sampler was implemented in Chapter 5 through sampling new infection history states for each individual i and each time j from the conditional distribution $P(X_{i,j}|\mathbf{X}_{-i})$. Here, I attempt to redefine the overall infection probability as a function of an individual's antibody titre $Y_{i,j-}$ and the infection states of all other individuals in the population $\mathbf{X}_{-i}$:

$$P(X_{i,j} = 1|a, b, Y_{i,j-}, \mathbf{X}_{-i,j}) = f(Y_{i,j-}, \mathbf{X}_{-i}, a, b) = \frac{h(\mathbf{X}_{-i,j})}{1 + e^{b(Y_{i,j-} - a)}} \quad (\text{C.36})$$

$h(\mathbf{X}_{-i,j})$ may be considered as the probability that an individual was exposed to another infectious individual in the population at time j (ie. we work with force of infection in the population). However, given the assumption that the time-span of j is large (eg. a year), that individuals may be from different locations, and that we do not model an explicit infectious period, a hazard model does not make sense [223]. The above equation does makes clear the relationship between the infection history model and a logistic regression model, as we wish to model the relationship between the outcome of n Bernoulli trials and some co-variate data, the antibody titres. If we replace $h(\mathbf{X}_{-i,j})$ with Φ , finding the conditional probability $P(X_{i,j} = 1|a, b, Y_{i,j-}, \Phi_j)$ becomes a case of finding the value for Φ that maximises the log likelihood:

$$L = \log\left(\prod_{i=1}^{n-i} \left(\frac{\Phi}{1 + e^{b(Y_{i,j-} - a)}}\right)^{X_{i,j}} \left(1 - \frac{\Phi}{1 + e^{b(Y_{i,j-} - a)}}\right)^{1-X_{i,j}}\right) \quad (\text{C.37})$$

$$= \sum_{i=1}^{n-i} X_{i,j} \log(\Phi) + (1 - X_{i,j}) \log(e^{b(Y_{i,j-} - a)} - \Phi + 1) - \log(1 + e^{b(Y_{i,j-} - a)}) \quad (\text{C.38})$$

where all parameters other than Φ are known. Let $a_i = 1 + e^{b(y_{i,j-} - a)}$ and differentiate L with respect to Φ :

$$\frac{dL}{d\Phi} = \sum_{i=1}^{n-i} \frac{a_i X_{i,j} - \Phi}{\Phi(a_i - \Phi)} \quad (\text{C.39})$$

The maximum likelihood estimate is found by setting $\frac{dL}{d\Phi} = 0$ and solving for Φ ; however, it is clear that for even small n the expansion of this sum is a complex, high order polynomial with no neat closed form solution. A closed form solution for binary logit regression is only available when the predictors are binary or categorical [574], and a more standard 1-dimensional numerical optimisation approach is therefore necessary. I tested the feasibility of this approach by testing the speed of a C++ implementation of the Brent minimisation routine [575], but the computation time was still ≈ 500 microseconds for $n = 1000$ and $m = 1$ for an average of 6 likelihood evaluations. This approach is therefore also not feasible to nest within the MCMC framework.

C.3 Representing $\mathbf{X}$ and Φ explicitly

Although an analytical solution to marginalising out both $\mathbf{X}$ and Φ is not possible, it is still reasonable to fit the full model by representing one or the other explicitly. To test the feasibility of this approach, simulation-recovery experiments were run to assess the if known parameters could be accurately re-estimated, and if MCMC convergence was achievable within a reasonable time whilst explicitly estimating Φ . The model was fit to a simulated data set described in Table C.1 using the posterior distribution defined in Equation C.17 (explicit $\mathbf{X}$ and Φ), running 5 MCMC chains each for 10000000 iterations.

Data set parameters		Model parameters					
n	1000	Parameter	Description	Value	Prior lower bound	Prior upper bound	Estimated
Year min	1968	μ_l	Long term antibody boosting	1.8	0	8	Yes
Year max	2015	μ_s	Short term antibody boosting	2.7	0	8	Yes
m	48	σ_l	Long term cross reactivity	0.1	0	1	Yes
Time resolution (infection states)	annual (48)	σ_s	Short term cross reactivity	0.03	0	1	Yes
Number of serum samples per individual	2	τ	Suppression	0.05	0	1	Yes
Number of viruses tested	21	ω	Waning	0.8	0	1	Yes
Number of titre measurement repeats	2	ϵ	Measurement error	1	0	25	Yes
Total number of measurements	96000	α	Infection history prior	1	NA	1	Yes
First sample year	2010	β	Infection history prior	1	NA	NA	Yes
Final sample year	2015	a	log titre giving 50% protection	2.844	NA	NA	No
Minimum age (years)	6	b	Titre-immunity scaling	1.299	NA	NA	No
Maximum age (years)	75						
Infection history prior	Prior 1						

Table C.1: Settings for simulations matching the Fluscape cohort with titre-mediated immunity.

The true parameter values and individual infection histories were accurately recovered. However, the convergence of the latent infection states and infection probability parameters Φ_j was poor for most of the time periods prior to any serum samples. As a result, estimates for historical attack rates were unreliable and did not match true attack rates for some years. This is likely due to the correlation between Φ_j and $\sum_{i=1}^{n_j} Z_{i,j}$. Because Φ_j and $Z_{i,j}$ are sampled independently, the MCMC algorithm must iteratively move both the latent infection states and Φ into different parts of the parameter space, which is inefficient and leads to low effective sample sizes.

C.4 Representing X and Z explicitly

Given the success of marginalising out Φ in Section 5.5.4, I hypothesised that fitting the model by explicitly inferring the latent exposure states Z in addition to the latent exposure states X would be more efficient than inferring Φ and X . Based on the posterior distribution in Equation C.16, I modified the Gibbs sampling algorithm described in Section E.3 to sample new values for each infection and exposure state directly from $P(\mathbf{X}, \mathbf{V}, \mathbf{Z}, \boldsymbol{\theta})$. Briefly, every other MCMC iteration, a proportion of individuals are selected to have a subset of their infection and exposure histories updated. A random time period j is selected, and a new exposure state is sampled from the conditional distribution $P(Z_{i,j} | \mathbf{Z}_{-i,j})$. A new infection state is then sampled from the conditional distribution $P(X_{i,j} | Z_{i,j}, V_{i,j-})$. After re-sampling a number of exposure and infection states, the likelihood of the observation process is calculated, and the move to the proposed state vectors is either accepted or rejected based on the ratio of likelihoods relative to the old state vectors. Individual infection histories are therefore sampled independently without the need to estimate a probability of exposure parameter.

The model was fit to a simulated data set described in Table C.1 (but with $\epsilon = 0.8$, though this change makes no difference to identifiability) using the posterior distribution defined in Equation C.16 (explicit X and Z), running 5 MCMC chains each for 10000000 iterations and a 2000000 burn in period. Similar to the version with explicit Φ , the true parameter estimates were accurately estimated (with some variation due to the stochastic nature of the simulation). Convergence of infection histories and per-time attack rates ($\sum Z$) was drastically improved compared to the version with explicit Φ whilst maintaining good convergence and high effective sample sizes for the antibody kinetics parameters, suggesting that this version of the model would be suitable to fit to the real data set. Furthermore, the number of iterations required to achieve high effective sample sizes was far lower in the explicit Z version compared to the explicit Φ version, with a mean effective sample size per MCMC iteration of 0.00103 compared to 0.000264 for the antibody kinetics parameters $\boldsymbol{\theta}$ and 0.000798 compared to 0.000215 for the per-time total number of infections $\sum_{i=1}^n Z_{i,j}$ respectively.

C.5 Results

C.5.1 Impact of titre-mediated immunity on model behaviour

Simulating from this modified model using the parameter estimates from the baseline model fits to the real Fluscape data, but with the addition of titre-mediated immunity, reveals interesting dynamics prior to any additional model fitting. Fig C.1 bottom panel shows that the distribution of times between infection is much more in line with expectations, as the presence of a short-term, antigenically broad antibody boost that wanes within a year elicits significant protection. In contrast to the model without titre-mediated immunity, the median and mode time between infections is 3.5 and 1.25 years respectively. Interestingly, the former estimate coincides with the typical duration of one cluster dominating circulation [82]. Incidence rates also follow patterns based on the antigenic evolution of the circulating strain. In Fig C.6 top panel, attack rates are highest in the first year of A/H3N2 circulation and drop in subsequent years as the majority of individuals all develop some immunity. When antigenic drift is assumed to be constant (the antigenic distance between strains is constant with time), attack rates then increase gradually as naive individuals are born, and population dynamics tend towards an endemic equilibrium. However, when antigenic drift is assumed to more closely follow observed cluster transitions, attack rates are highest immediately following relatively larger antigenic changes and lowest in-between (Fig C.6, bottom panel).

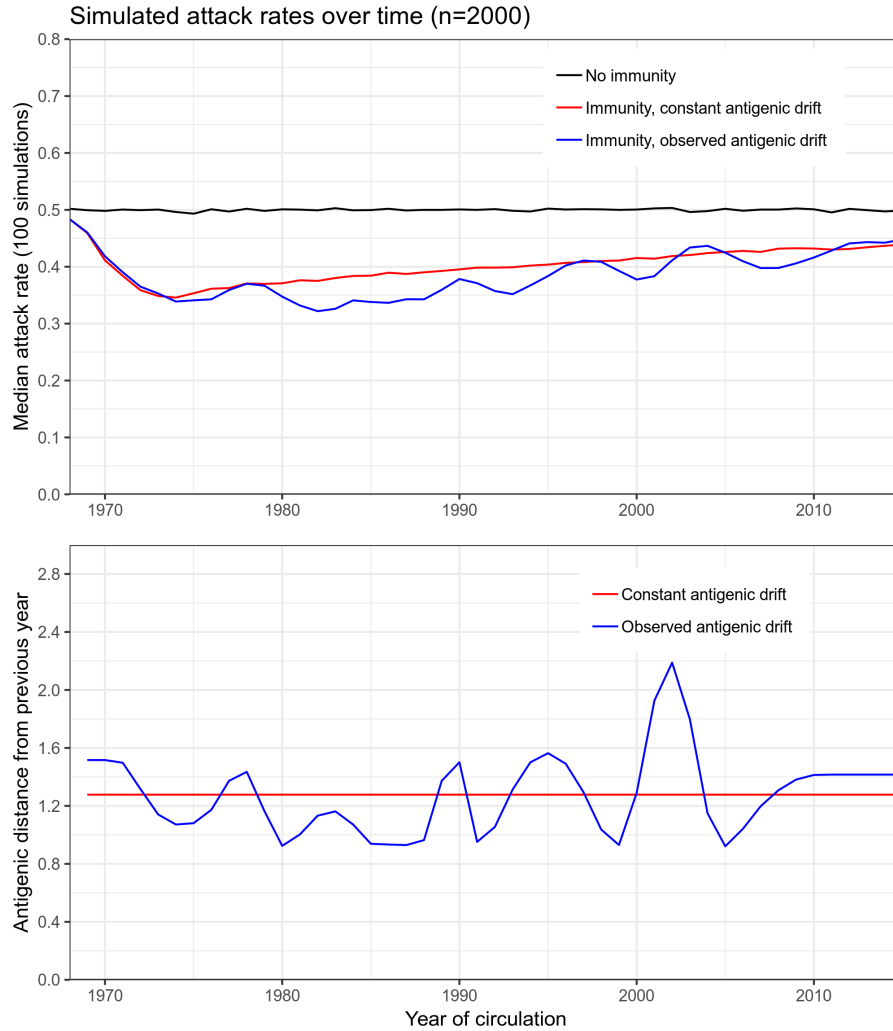


Figure C.6: Comparison of simulated attack rates with and without titre-mediated immunity. Top panel: median annual attack rates for $n = 2000$ individuals across $m = 48$ years, assuming an annual infection probability (probability of an immunological naive individual becoming infected) of 0.5. Black line shows simulated attack rates with no immunity; red line shows simulated attack rates with titre-mediated immunity as shown in Fig C.2 and assuming constant antigenic drift of the circulating virus over time; blue line shows simulated attack rates with titre-mediated immunity as shown in Fig C.2 using observed antigenic distances as in Chapter 5. Bottom panel: antigenic drift of the circulating virus matching times in the top panel.

C.5.2 Comparison of real parameter estimates and attack rates with titre-mediated immunity

The purpose of adding titre-mediated immunity to the transmission level of the model was to capture more realistic infection probabilities over the lifetime of an individual. I therefore fit both the base model without explicit immunity and the modified model with titre-mediated immunity to the real Fluscape data, estimating exposure states and infection states. 6 MCMC chains were run each for 10000000 iterations with the aim of achieving an effective sample size of > 200 and Gelman-Rubin diagnostic criteria ($\hat{R}$) of < 1.1 for all model parameters.

Parameter estimates and infection history estimates were largely unchanged between the two model variants. The models used the same number of free parameters and were therefore comparable based on their log-likelihood, which was almost identical, suggesting that both models fit the data equally well (Table C.2). The only difference in antibody kinetics estimates was that long-term antibody boosting was estimated to be higher with the inclusion of titre-mediated immunity than without (1.84 (median, 95% CI: 1.81-1.86) vs. 1.70 (median, 95% CI: 1.67-1.74)), which coincided with a decrease in the total number of inferred infections (8380 (median, 95% CI: 8280-8480) vs. 9040 (median, 95% CI: 8890-9180)). This is unsurprising, as titre-mediated immunity decreases the probability of repeated infection. The model with titre-mediated immunity therefore more parsimoniously describes the data with fewer infections, each of which lead to slightly higher antibody boosting to give the same observed data.

Trends in attack rate estimates were also largely unchanged between the two models (Fig C.7). The magnitude of extremely high attack rates was slightly lower with the inclusion of titre-mediated immunity, and attack rates immediately following 1968 followed a smoothed pattern of decreasing magnitude as the population accrues short-term protection from re-infection.

Parameter	Description	Original model	$\hat{R}$	ESS	Titre-mediated immunity	$\hat{R}$	ESS
μ_l	Long term antibody boosting	1.70 (1.67-1.74)	1.11	303	1.84(1.81-1.86)	1.12	272
μ_s	Short term antibody boosting	1.54(1.49-1.58)	1.07	654	1.48(1.44-1.53)	1.19	351
τ	Suppression	0.0199(0.0175-0.0224)	1.07	550	0.0191(0.0167-0.0214)	1.01	262
ω	Waning	0.518(0.502-0.536)	1.03	633	0.545(0.528-0.564)	1.07	451
σ_l	Long term cross reactivity	0.0896(0.0889-0.0899)	1.06	707	0.0888(0.0884-0.0895)	1.14	407
σ_s	Short term cross reactivity	5.21e-05(1.67e-06-0.000277)	1.01	804	4.43e-05(1.5e-06-0.000277)	1.03	452
ϵ	Measurement error	0.902(0.896-0.909)	1.00	989	0.907(0.902-0.912)	1.01	411
$\sum Z$	Total infections	9040(8890-9180)	1.14	295	8380(8280-8480)	1.31	195
Log likelihood	Log likelihood from posterior	-88400(-88600-88300)	1.02	710	-88800(-88900-88600)	1.10	371

Table C.2: Comparison of parameter estimates and convergence diagnostics of the model with titre-mediated immunity and without. $\hat{R}$ gives the upper 95% confidence interval on $\hat{R}$, and I note that convergence is poor for some parameters. Visual assessment of these MCMC traces suggests that this would be remedied by running longer chains.

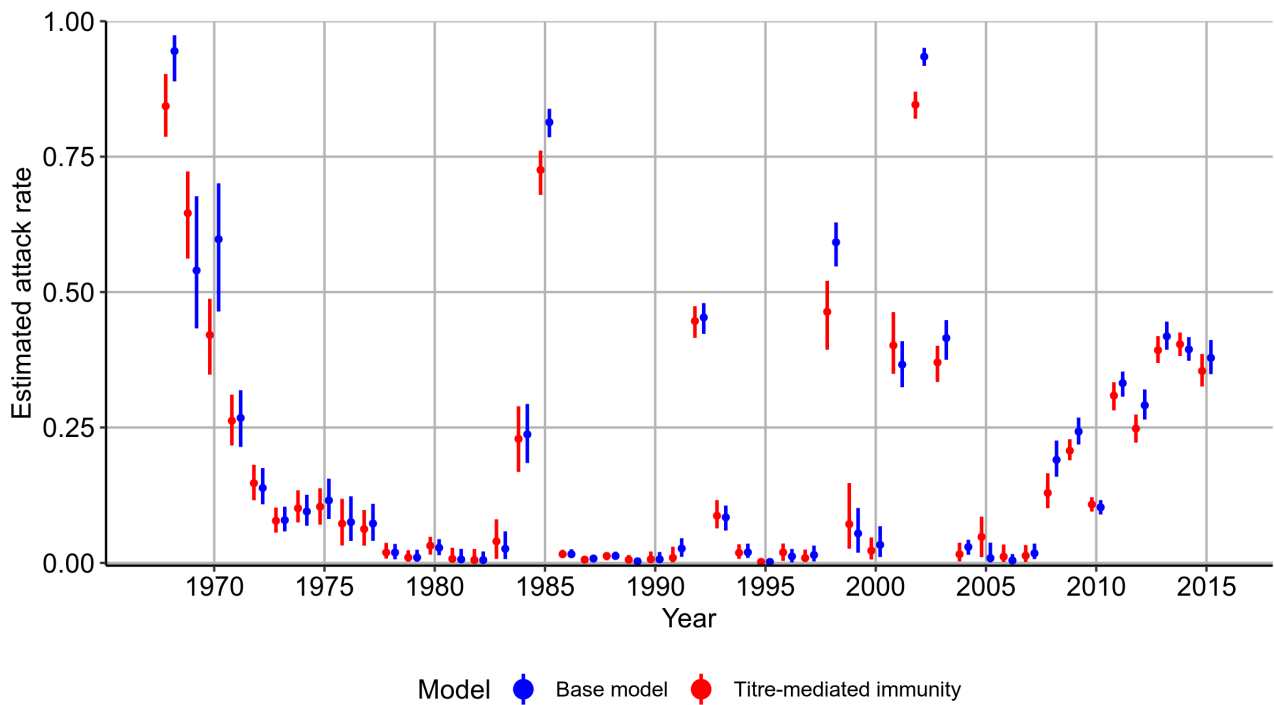


Figure C.7: Comparison of inferred attack rates from the Fluscape data using the base model and the model with titre-mediated immunity. Points and ranges show posterior median and 95% credible intervals. Estimates are coloured by the model used (blue, base model, red, titre-mediated immunity model).

Appendix D

Additional figures for “A statistical framework to represent influenza infection histories”

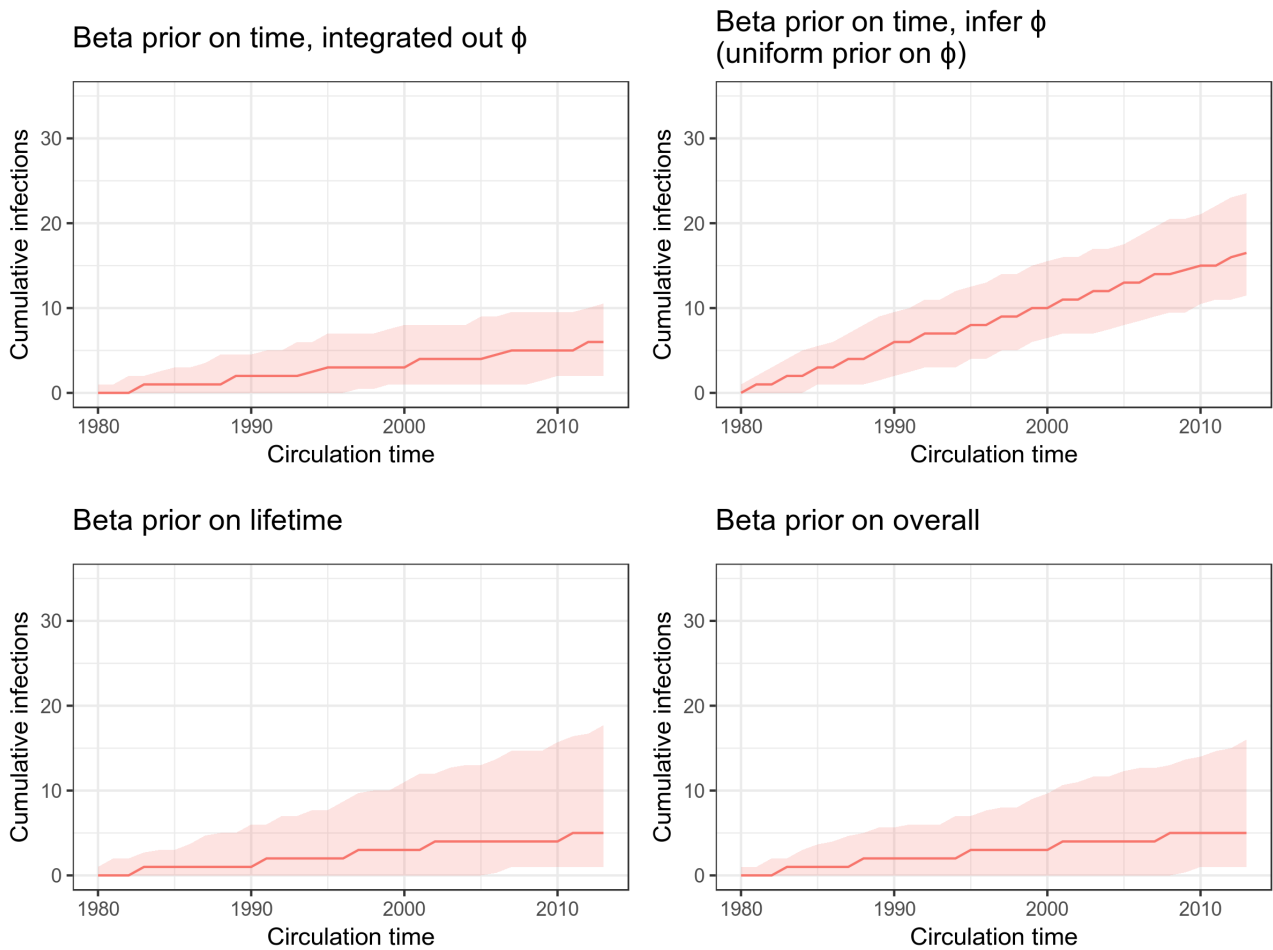


Figure D.1: Priors on cumulative number of lifetime infections when $\alpha = 2$ and $\beta = 10$. Subplot titles show assumed prior. Red line shows posterior median. Red shaded regions show posterior 95% credible intervals. Note that a uniform prior is assumed for ϕ , and this prior therefore matches that in Figure 5.10.

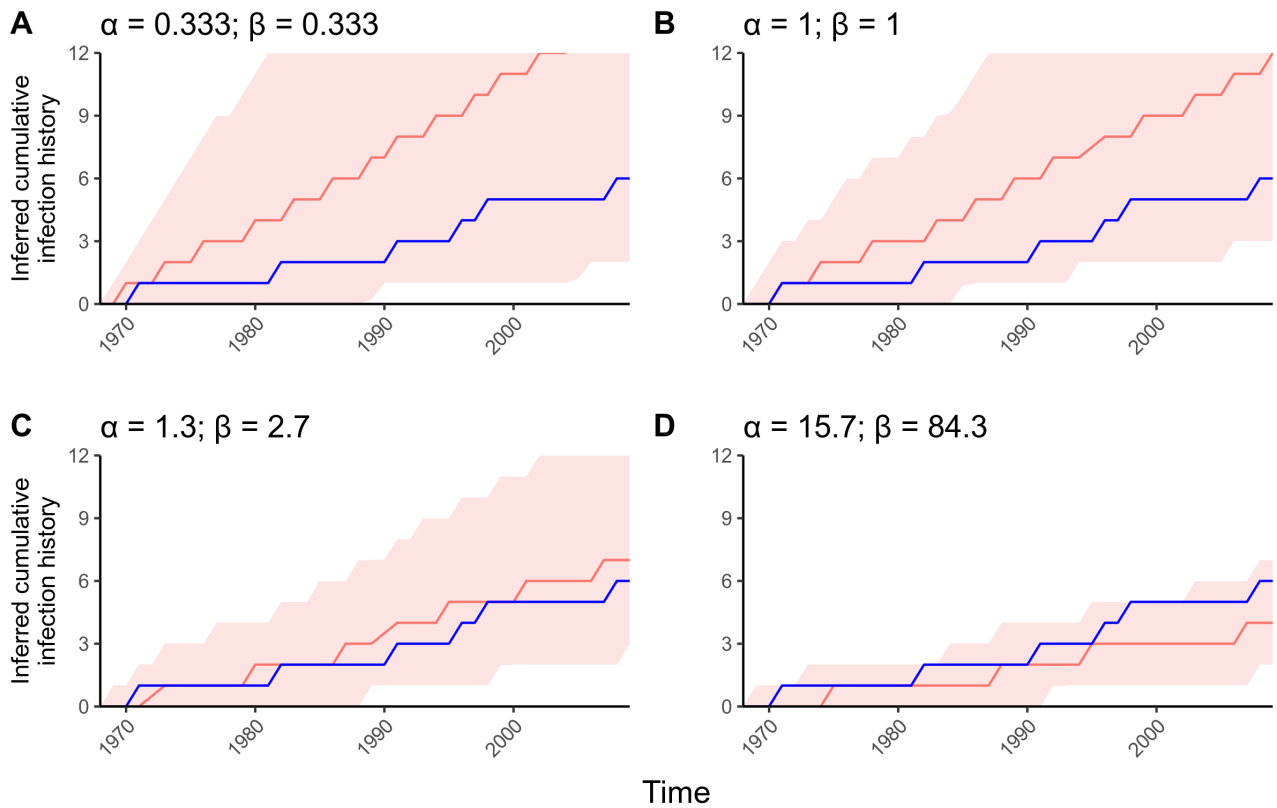


Figure D.2: Simulation-recovery of one individual's infection history using prior version 1 (beta prior on per-individual infection probability, P_i) with various strength priors, sparse data. Simulation with 200 individuals, 9 viruses tested for each individual, one blood sample taken. Y-axis shows the cumulative number of infections for this individual over time. Red line and shaded region shows posterior median and 95% credible intervals. Blue line shows the true cumulative number of infections over time.

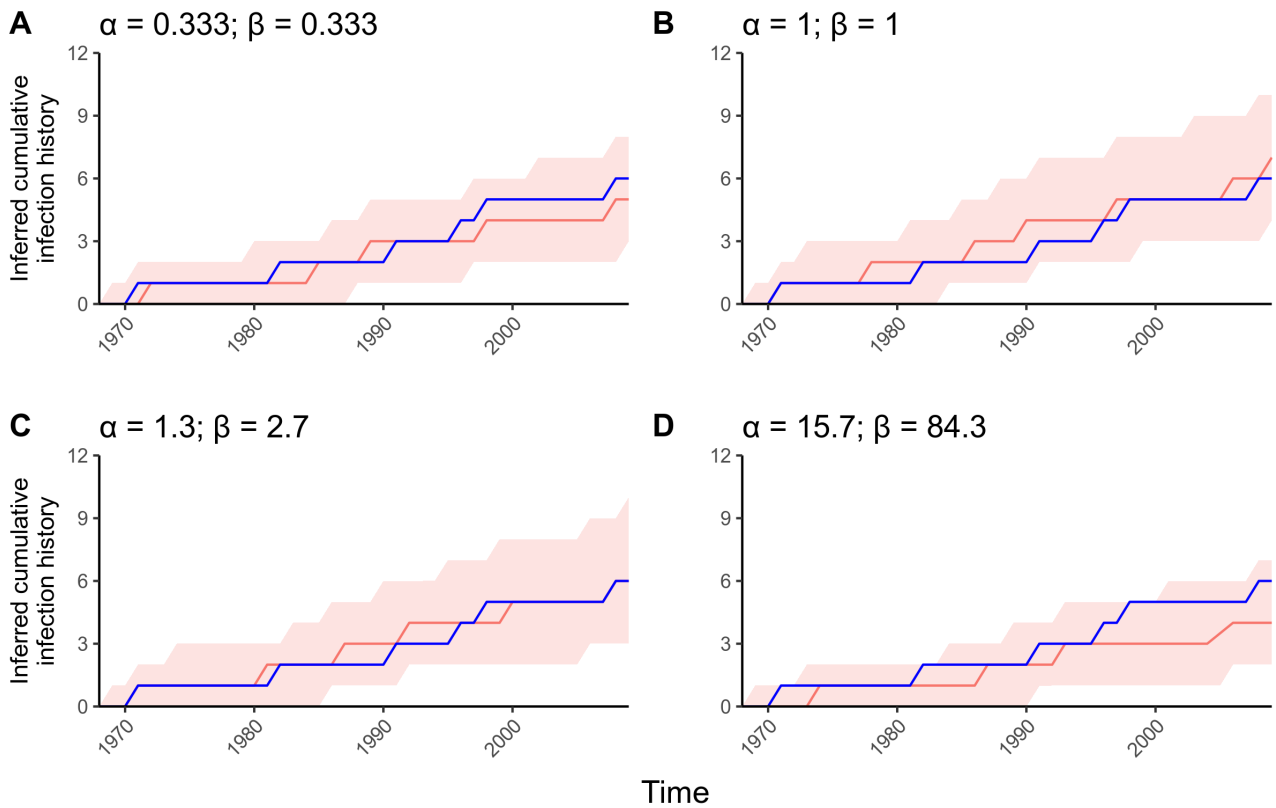


Figure D.3: Simulation-recovery of one individual's infection history using prior version 3 (beta prior on per-time infection probability, ϕ_j) with various strength priors, sparse data. Simulation with 200 individuals, 9 viruses tested for each individual, one blood sample taken. Y-axis shows the cumulative number of infections for this individual over time. Red line and shaded region shows posterior median and 95% credible intervals. Blue line shows the true cumulative number of infections over time.

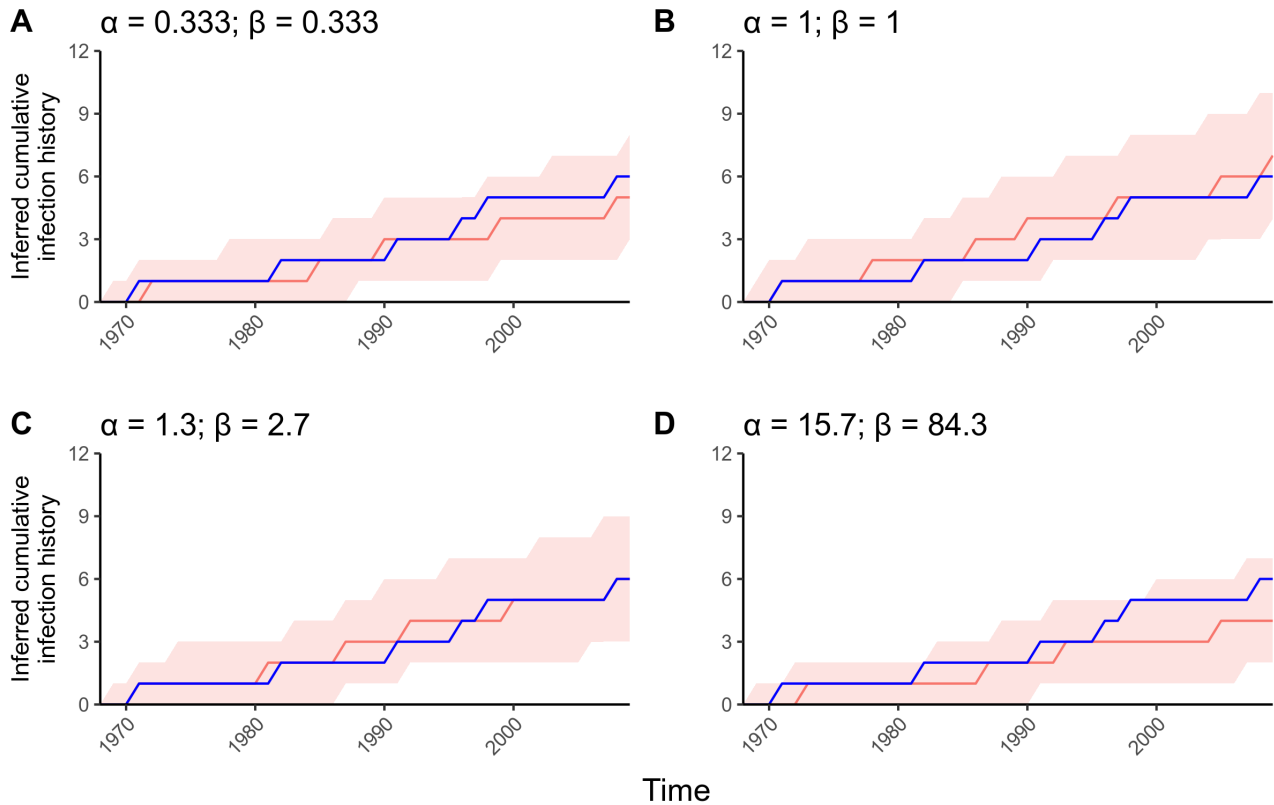


Figure D.4: Simulation-recovery of one individual's infection history using prior version 4 (beta prior on overall infection probability) with various strength priors, sparse data. Simulation with 200 individuals, 9 viruses tested for each individual, one blood sample taken. Y-axis shows the cumulative number of infections for this individual over time. Red line and shaded region shows posterior median and 95% credible intervals. Blue line shows the true cumulative number of infections over time.

Description	Version	Assumption	Summary	Prior on lifetime infections	Prior on augmented attack rates	Use cases
Beta prior on per-individual probability of infection	1	Infection status in a given time is independent of infection status of population, but correlated with infection status of that individual at other times.	A beta prior is placed on the probability of a given individual becoming infected in any time period. However, whereas the above priors assumed independence between times but not individuals, this prior assumes independence between individuals but not between times, but infection probabilities are drawn from a single Beta distribution.	Beta-binomial with parameters α and β	binomial, $p = \frac{\alpha}{\alpha+\beta}$	Unbiased per-individual infection history inference. Reason to assume that individuals are under different infection processes, but share antibody kinetics parameters eg. different locations or populations. Appropriate with a relatively small number of individuals and large amount of antibody data per individual.
Hyper-prior placed on the probability of infection terms, Φ	2	Infection status in a given time correlated with infection status of population at that time, but independent of other times.	The prior probability of any individual i becoming infected during time j is $P_{i,j} = \Phi_j$, and all time periods are independent such that each time period j has a unique parameter Φ_j as in Equation 5.37. These parameters are estimated explicitly and are independently of one another.	Binomial	beta-binomial	Where Φ_j is of interest and a distinct user-specified prior is desired for each j , $P(\Phi_j)$. Appropriate when the number of time periods under consideration j is small. Can otherwise lead to poor convergence when j is large. Allows the user full control over the form of $P(\Phi_j)$, which is not possible with other versions.
Beta prior on per-time probability of infection	3	Infection status in a given time correlated with infection status of population at that time, but independent of other times.	Mathematically equivalent to version 1, but Φ is integrated out by placing a conjugate Beta prior on the probability of infection terms. All infection times j are independent, and users may specify the beta parameters to define the prior distribution.	Binomial, $p = \frac{\alpha}{\alpha+\beta}$	Beta-binomial with parameters α and β .	Unbiased attack rate inference. Reason to assume that individuals are under the same probability of infection process eg. same location, but require better mixing over inferring all Φ_j independently as in version 1. Appropriate when there are a large number of individuals in the sample, but not necessarily a large amount of antibody data per individual.
Beta prior on overall probability of infection	4	Infection status correlated to all other infection events ie. frequent infection in other individuals and in the past suggests more likely to be infected in the future.	A Beta prior is placed on the probability of any infection, assuming that infection events are independent both across individuals and time periods.	Beta-binomial with parameters α and β .	Beta-Binomial with parameters α and β .	Weakly informative priors on both attack rates and lifetime infections are desired, over-dispersed relative to the binomial distribution on all summaries. Appropriate with a small number of individuals and relatively small amount of antibody data per individual, as convergence is slower than under other versions.

Appendix E

Algorithms for “A statistical framework to represent influenza infection histories”

E.1 Prior 1: Further considerations and Gibbs sampling

In practice, assuming that all j are exchangeable, it is possible to sample $Z_{i,j}$ from the prior directly in a Gibbs-like fashion, which leads to far more efficient proposals. Rather than either moving to a proposed location or staying in the previous location, we can think about the proposal steps as offering the algorithm two choices, to either add or remove an infection:

1. For an individual i , choose a random location, j , from the infection history vector, $\mathbf{Z}_i$
2. Remove element j to give $\mathbf{Z}_{i,-j}$
3. There are now two potential moves to get back to a vector with the same dimensions as $\mathbf{Z}_i$. Set $Z_{i,j} = 1$ or $Z_{i,j} = 0$.

Let $\mathbf{Z}'_i$ be the case where $Z_{i,j} = 0$ and $\mathbf{Z}_i$ be the case where $Z_{i,j} = 1$. More generally, the proposals can be drawn from:

$$P(\text{propose } \mathbf{Z}_i) = \frac{g(\mathbf{Z}_i | \mathbf{Z}_{i,-j})}{g(\mathbf{Z}_i | \mathbf{Z}_{i,-j}) + g(\mathbf{Z}'_i | \mathbf{Z}_{i,-j})} \quad (\text{E.1})$$

In the case of the binomial prior on k (where $P(Z_{i,j} = 1) = 0.5$ when $\alpha = \beta = \infty$), we would have a proposal such that $g(\mathbf{Z}_i|\mathbf{Z}'_i) = g(\mathbf{Z}'_i|\mathbf{Z}_i) = g(\mathbf{Z}'_i|\mathbf{Z}'_i) = g(\mathbf{Z}_i|\mathbf{Z}_i)$. In this case, the probability of proposing $\mathbf{Z}'_i$ is the same as the probability of proposing $\mathbf{Z}_i$ (ie. 50/50). However, if we explicitly define $g(\mathbf{Z}_i|\mathbf{Z}_{i,-j})$ and $g(\mathbf{Z}'_i|\mathbf{Z}_{i,-j})$ then we can control the proposal distribution and therefore sample from the prior:

$$g(\mathbf{Z}_i|\mathbf{Z}_{i,-j}, \alpha, \beta) = f(Z_{i,j} = 1|\mathbf{Z}_{i,-j}, \alpha, \beta) \quad (\text{E.2})$$

$$g(\mathbf{Z}'_i|\mathbf{Z}_{i,-j}, \alpha, \beta) = f(Z_{i,j} = 0|\mathbf{Z}_{i,-j}, \alpha, \beta) \quad (\text{E.3})$$

$$f(Z_{i,j} = 1|\mathbf{Z}_{i,-j}, \alpha, \beta) = \frac{P(\mathbf{Z}_i)}{P(\mathbf{Z}_{i,-j})} \quad (\text{E.4})$$

$$= \frac{\alpha^{[k+1]} \beta^{[m-k]} (\alpha + \beta)^{[m]}}{(\alpha + \beta)^{[m+1]} \alpha^{[k]} \beta^{[m-k]}} \quad (\text{E.5})$$

$$= \frac{\alpha + k}{\alpha + \beta + m - 1} \quad (\text{E.6})$$

$$f(Z_{i,j} = 0|\mathbf{Z}_{i,-j}) = 1 - f(Z_{i,j} = 1|\mathbf{Z}_{i,-j}, \alpha, \beta) \quad (\text{E.7})$$

$$= \frac{\beta + m - k - 1}{\alpha + \beta + m - 1} \quad (\text{E.8})$$

where $k = \sum \mathbf{Z}_{i,-j}$, and α and β are the left and right parameters of the beta distribution. Following this proposal (which is equivalent to sampling from the prior for $Z_{i,j}$, the proposal is accepted based on the Metropolis acceptance probability:

$$A(\mathbf{Z}_{new}, \mathbf{Z}_{old}) = \min(1, \frac{P(\mathbf{Y}|\mathbf{Z}_{new}, \boldsymbol{\theta})}{P(\mathbf{Y}|\mathbf{Z}_{old}, \boldsymbol{\theta})}) \quad (\text{E.9})$$

E.2 Proposal algorithm under prior 1: Beta prior on per-individual infection probability

To improve mixing, we extended the logic in Section 5.5.3 to generate a proposal algorithm to add/remove an arbitrary number of infections with each proposal. Italicised terms referring to arguments in *mcmc_pars* of `serosolver::run_MCMC`:

1. Select a random proportion *hist_sample_prob* of n individuals
2. For each individual, i , propose a new infection history $\mathbf{Z}_i$ by performing one of the following steps:
 - (a) Sample new values for $\mathbf{Z}'_i$ with probability $1 - \text{swap_propn}$ as follows:
 - i. Select $\mathbf{z} = \text{inf_propn} * m_i$ random time points, where m_i is the number of time points that individual i could be infected. Remove these from the infection history vector, $\mathbf{Z}_{i,-\mathbf{z}}$
 - ii. Count the length of $\mathbf{Z}_{i,-\mathbf{z}}$ and the number of 1s in $\sum \mathbf{Z}_{i,-\mathbf{z}}$ (this gives m and k as in the beta-Bernoulli respectively)
 - iii. Iterate through 1 to $\mathbf{z}$ with index j :
 - A. Calculate $p = \frac{\alpha+k}{\alpha+\beta+m}$
 - B. Add a 1 at location $\mathbf{Z}_{i,j}$ with probability p , add a 0 otherwise
 - C. If a 1 was added, $k = k + 1$.
 - D. $m = m + 1$
 - E. Go to the next location in $\mathbf{z}$
3. Accept the proposed move with the acceptance ratio, noting that the proposal probability and prior $P(\mathbf{Z}_i)$ cancel out:

$$A(\mathbf{Z}'_i, \mathbf{Z}_i) = \min(1, \frac{f(y_i|\mathbf{Z}'_i, \boldsymbol{\theta})}{f(y_i|\mathbf{Z}_i, \boldsymbol{\theta})}) \quad (\text{E.10})$$

E.3 Proposal algorithm under prior 2: Hyper-prior on probability of infection

In `serosolver`'s MCMC algorithm, all Φ are treated as unknown parameters under this prior and therefore sampled alongside θ . Proposals for $\mathbf{Z}$ are made through a random scan across n and m , with a proposed transition of flipping the binary entry for $Z_{i,j}$ ie. $Z'_{i,j} = 1$ if $Z_{i,j} = 0$, and $Z'_{i,j} = 0$ if $Z_{i,j} = 1$. The sampling algorithm for $\mathbf{Z}$ under the prior model 2 (section 5.5.4) is as follows:

1. With probability *hist_switch_prob*:

- (a) Select a time point j
- (b) Select another time point between 1 and *move_size* time points away from j with uniform probability.
- (c) Select *year_swap_propn** n individuals
- (d) For each of these selected individuals, swap the values of $Z_{i,j}$ and $Z_{i,l}$, adhering to restrictions of birth times (ie. individuals cannot be infected before they are born)
- (e) Increase/decrease Φ_j and Φ_l proportional to the number of infections gained/lost
- (f) Let $\mathbf{Z}$ and Φ represent the infection history matrix and probability of infection terms before this proposal step, and $\mathbf{Z}'$ and Φ' represent these terms after the proposal step. Set $\mathbf{Z} = \mathbf{Z}'$ and $\Phi = \Phi'$ with the following acceptance ratio:

$$A((\mathbf{Z}', \Phi'), (\mathbf{Z}, \Phi)) = \min(1, \frac{P(\mathbf{Z}', \theta, \Phi' | \mathbf{Y})}{P(\mathbf{Z}, \theta, \Phi | \mathbf{Y})}) \quad (\text{E.11})$$

2. Otherwise:

- (a) Select a random proportion *hist_sample_prob* of n individuals
- (b) For each individual, i , propose a new infection history $\mathbf{Z}'_i$ by performing one of the following steps:
 - i. Perform a “flip” step with probability $1 - \text{swap_propn}$:
 - A. Select *inf_propn** m_i time points, where m_i is the number of time points that individual i could be infected
 - B. Perform a binary flip on each of these times, $Z'_{i,j} = 1 - Z_{i,j}$

- ii. Otherwise, perform a “swap” step:
 - A. Select a location j
 - B. Select a location, l , 0 to $move_size$ time steps away with equal probability
 - C. Set $Z_{i,l} = Z_{i,j}$ and $Z_{i,j} = Z_{i,l}$
- (c) For each sampled individual, independently accept or reject the proposed new infection state with the acceptance ratio:

$$A((\mathbf{Z}'_i, \mathbf{\Phi}'), (\mathbf{Z}_i, \mathbf{\Phi})) = \min(1, \frac{P(\mathbf{Z}'_i, \boldsymbol{\theta}, \phi | \mathbf{Y}_i)}{P(\mathbf{Z}_i, \boldsymbol{\theta}, \mathbf{\Phi} | \mathbf{Y})}) \quad (\text{E.12})$$

E.4 Proposal algorithm under prior 3: Beta prior on per-time probability of infection

The proposal algorithm for prior version 3 is similar to that of prior version 2, but rather than performing a “flip” step, infection history entries are proposed in a Gibbs-like fashion conditional on the infection status of all other individuals at that time point.

1. With probability *hist_switch_prob*:

- (a) Select a time point j
- (b) Select another time point between 1 and *move_size* time points away from j with uniform probability, where $j \neq l$
- (c) Select *year_swap_propn** n individuals and filter for individuals that were alive during both time points
- (d) For each of these selected individuals, swap the values of $Z_{i,j}$ and $Z_{i,l}$
- (e) Let $\mathbf{Z}$ represent the infection history matrix before this proposal step, and $\mathbf{Z}'$ represent it after. Set $\mathbf{Z} = \mathbf{Z}'$ with the following acceptance ratio:

$$A(\mathbf{Z}', \mathbf{Z}) = \min(1, \frac{\prod_i f(\mathbf{Y}_i | \mathbf{Z}'_i, \boldsymbol{\theta}) P(\mathbf{Z}'_i)}{\prod_i f(\mathbf{Y}_i | \mathbf{Z}_i, \boldsymbol{\theta}) P(\mathbf{Z}_i)}) \quad (\text{E.13})$$

2. Otherwise:

- (a) Select a random proportion *hist_sample_prob* of n individuals
- (b) For each individual, i , propose a new infection history $\mathbf{Z}'_i$ by performing one of the following steps:
 - i. Sample new values for $\mathbf{Z}_i$ with probability $1 - \text{swap_propn}$ as follows:
 - A. Select *inf_propn** m_i time points, where m_i is the number of time points that individual i could be infected. For each time point, j :
 - B. Calculate the number of infected individuals less the selected individual $k_j = (\sum_x Z_{x,j}) - Z_{i,j}$
 - C. Calculate the number of individuals that could be infected during time j , n_j
 - D. Set $Z_{i,j} = 1$ with probability $\frac{k_j + \alpha}{n_j + \alpha + \beta}$, and $Z_{i,j} = 0$ otherwise

- E. Accept the proposed move with the acceptance ratio, noting that by sampling directly from the prior $P(\mathbf{Z})$ that this cancels out in the Metropolis ratio:

$$A(\mathbf{Z}'_i, \mathbf{Z}_i) = \min(1, \frac{f(\mathbf{Y}_i|\mathbf{Z}'_i, \boldsymbol{\theta})}{f(\mathbf{Y}_i|\mathbf{Z}_i, \boldsymbol{\theta})}) \quad (\text{E.14})$$

- ii. Otherwise, perform a "swap" step:

- A. Select a location j
- B. Select a location, l , 0 to *move_size* time steps away with equal probability
- C. If $Z_{i,l} \neq Z_{i,j}$, set $Z_{i,l} = Z_{i,j}$ and $Z_{i,j} = Z_{i,l}$ with the acceptance ratio:

$$A(\mathbf{Z}'_i, \mathbf{Z}_i) = \min(1, \frac{f(\mathbf{Y}_i|\mathbf{Z}'_i, \boldsymbol{\theta})P(\mathbf{Z}'_i)}{f(\mathbf{Y}_i|\mathbf{Z}_i, \boldsymbol{\theta})P(\mathbf{Z}_i)}) \quad (\text{E.15})$$

E.5 Proposal algorithm under prior 4: Beta prior on overall probability of infection

The proposal algorithm for prior version 4 is identical to prior version 2, with only three changes:

1. In the step 2(b)(i)B, k_j is replaced with $k_{-ij} = (\sum_x \sum_y Z_{x,y}) - Z_{i,j}$
2. In step 2(b)(i)C, n_j is replaced by nm
3. $P(\mathbf{Z})$ is the prior as described in Section 5.5.6

Appendix F

Patterns of spatial and individual
variation in influenza incidence and
immunity in a southern Chinese cohort

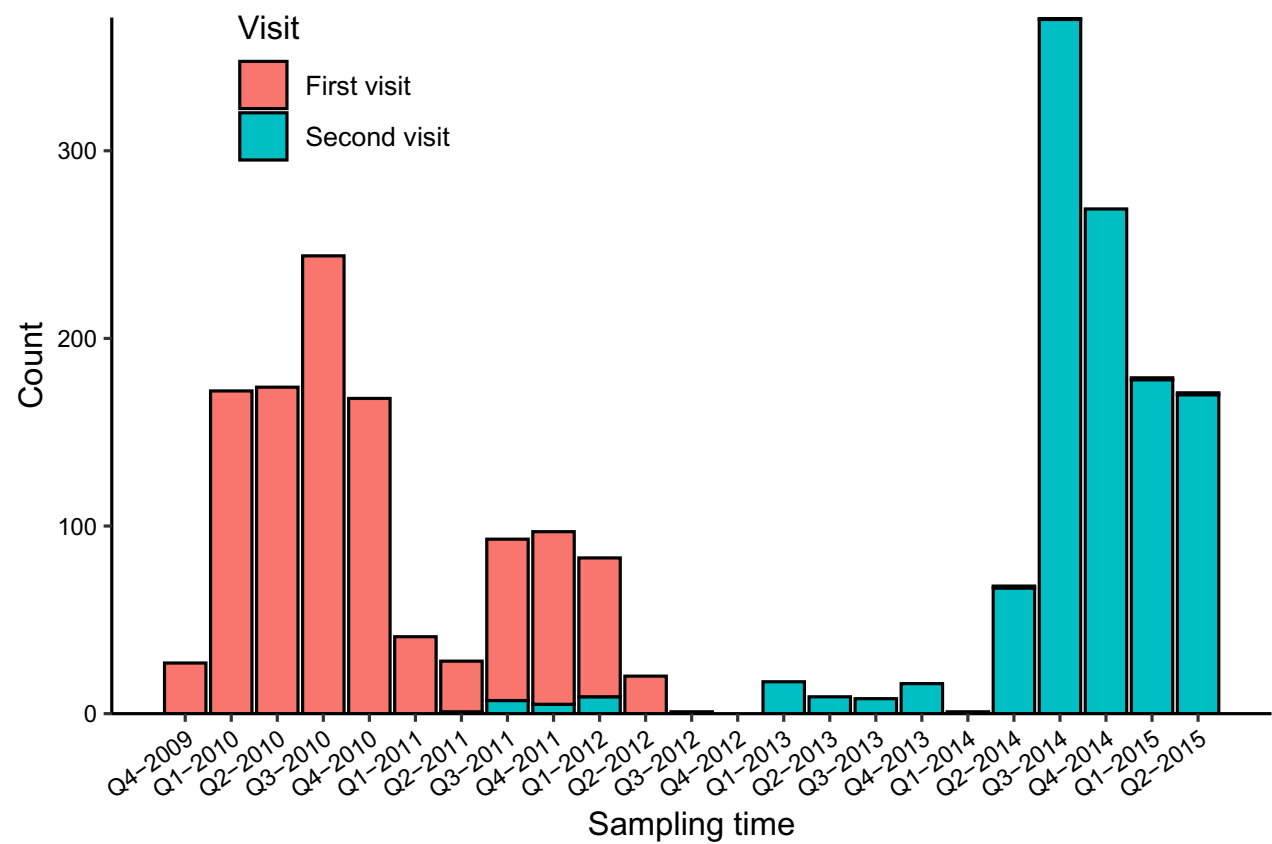


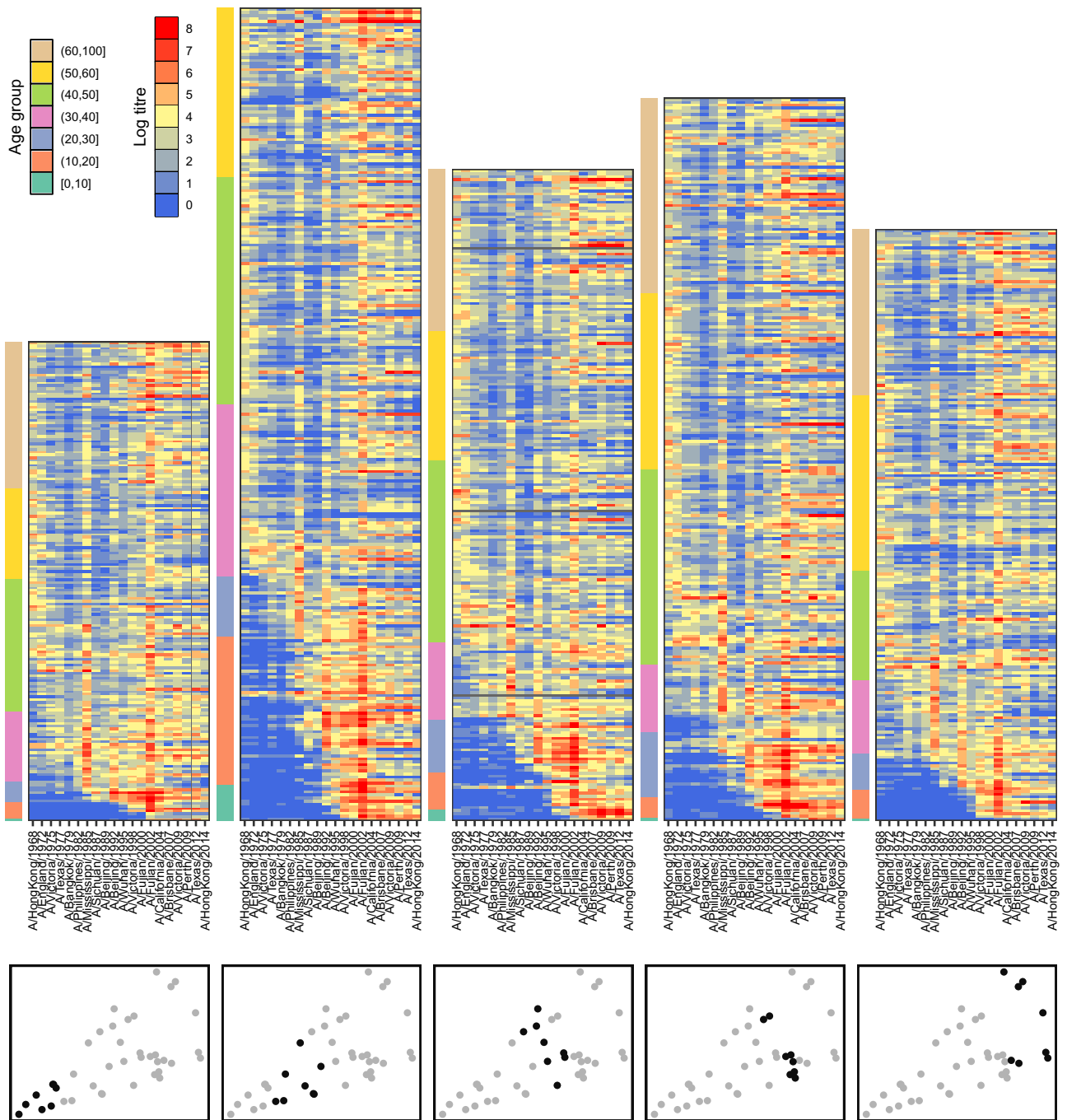
Figure F.1: Distribution of serum sampling times from the Fluscape cohort.

Table F.1: Demographics of the 651 households used.

Variable	Response	N	%
Number of members			
	1	36	5.64%
	2	111	17.40%
	3	107	16.77%
	4	141	22.10%
	5	105	16.46%
	6	63	9.87%
	7	30	4.70%
	8	28	4.39%
	9	7	1.10%
	10+	10	1.57%
	Total	638	
Type			
	Family home	599	92.01%
	Elderly care home	2	0.31%
	Worker's dormitory	0	0.00%
	Multi-family home	50	7.68%
	Total	651	
Running water			
	No	123	18.92%
	Yes	527	81.08%
	Total	650	
Monthly household income			
	<RMB 999	63	9.94%
	RMB 1,000-RMB 1,999	70	11.04%
	RMB 2,000 -RMB 2,999	73	11.51%
	RMB 3,000-RMB 3,999	75	11.83%
	RMB 4,000-RMB 4,999	40	6.31%
	RMB 5,000-RMB 5,999	44	6.94%
	>RMB 6,000	269	42.43%
	Total	634	

Table F.2: Characteristics of the 40 Fluscape study locations.

Variable	Response	N	%
Urban/Rural	Urban	10	25.00%
	Rural	30	75.00%
	Total	40	
Log population density (1km)	[0,2]	0	0.00%
	(2,4]	1	2.50%
	(4,6]	17	42.50%
	(6,8]	9	22.50%
	(8,10]	10	25.00%
	10+	3	7.50%
	Total	40	
Log population density (9km)	[0,2]	0	0.00%
	(2,4]	0	0.00%
	(4,6]	1	2.50%
	(6,8]	10	25.00%
	(8,10]	19	47.50%
	10+	10	25.00%
	Total	40	
Travel distance to Guangzhou (km)	[0,20]	6	15.00%
	(20,40]	10	25.00%
	(40,60]	15	37.50%
	(60,80]	6	15.00%
	(80,100]	3	7.50%
	Total	40	100.00%
Travel time to Guangzhou (minutes)	[0,20]	3	7.50%
	(20,40]	9	22.50%
	(40,60]	12	30.00%
	(60,80]	13	32.50%
	(80,100]	3	7.50%
	Total	40	



Distribution of log HI titres by study location at second serum sample.]Distribution of log HI titres by study location at second serum sample. Locations were grouped into quintiles based on increasing distance from Guangzhou city centre (bottom panels). Individuals were grouped by age and plotted with increasing age. Colours to the left of each subplot show age group. Adapted from code written by Justin Lessler and Derek Cummings.

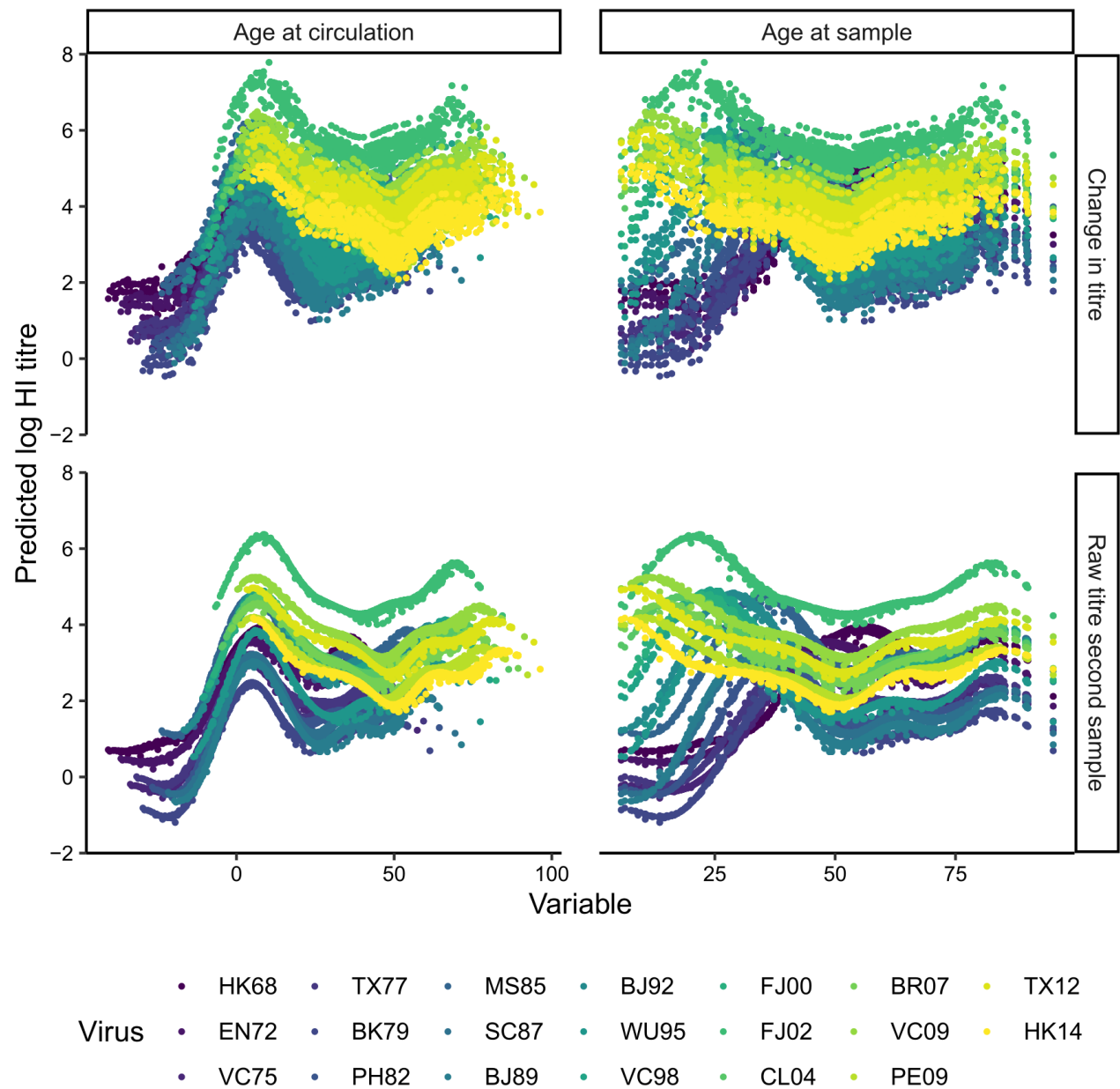


Figure F.2: Relationship between age and titre. Points show model-predicted values based on results from the highest ranked generalised additive model in Table F.3 (spline on age, age at circulation, fixed effect on strain and fixed effect on study location).

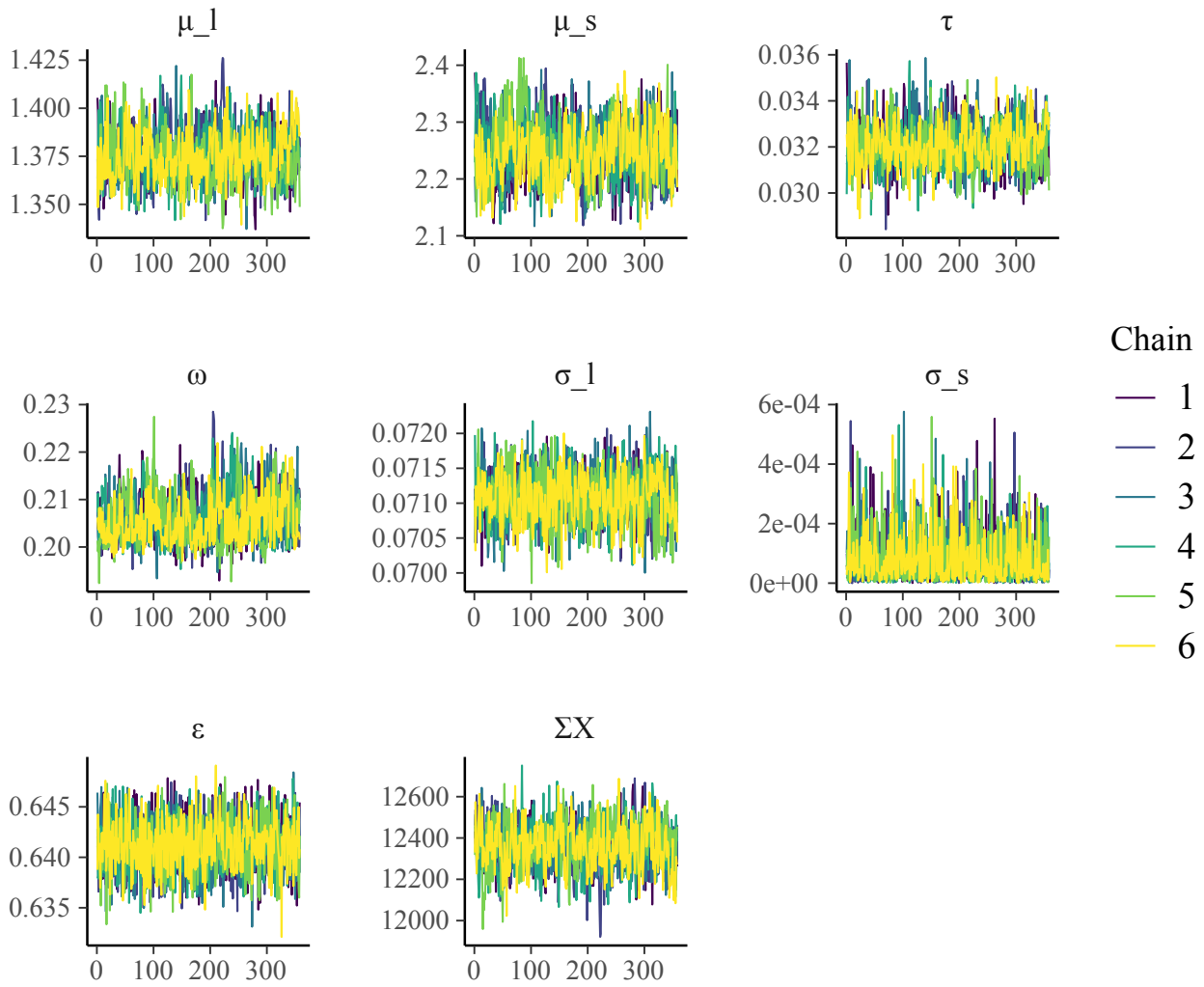


Figure F.3: Convergence diagnostics of antibody kinetics parameters from fitting the full model with virus-specific measurement bias. Plots are MCMC traces for each estimated antibody kinetics parameter, showing good mixing and consistency between 6 independent chains.

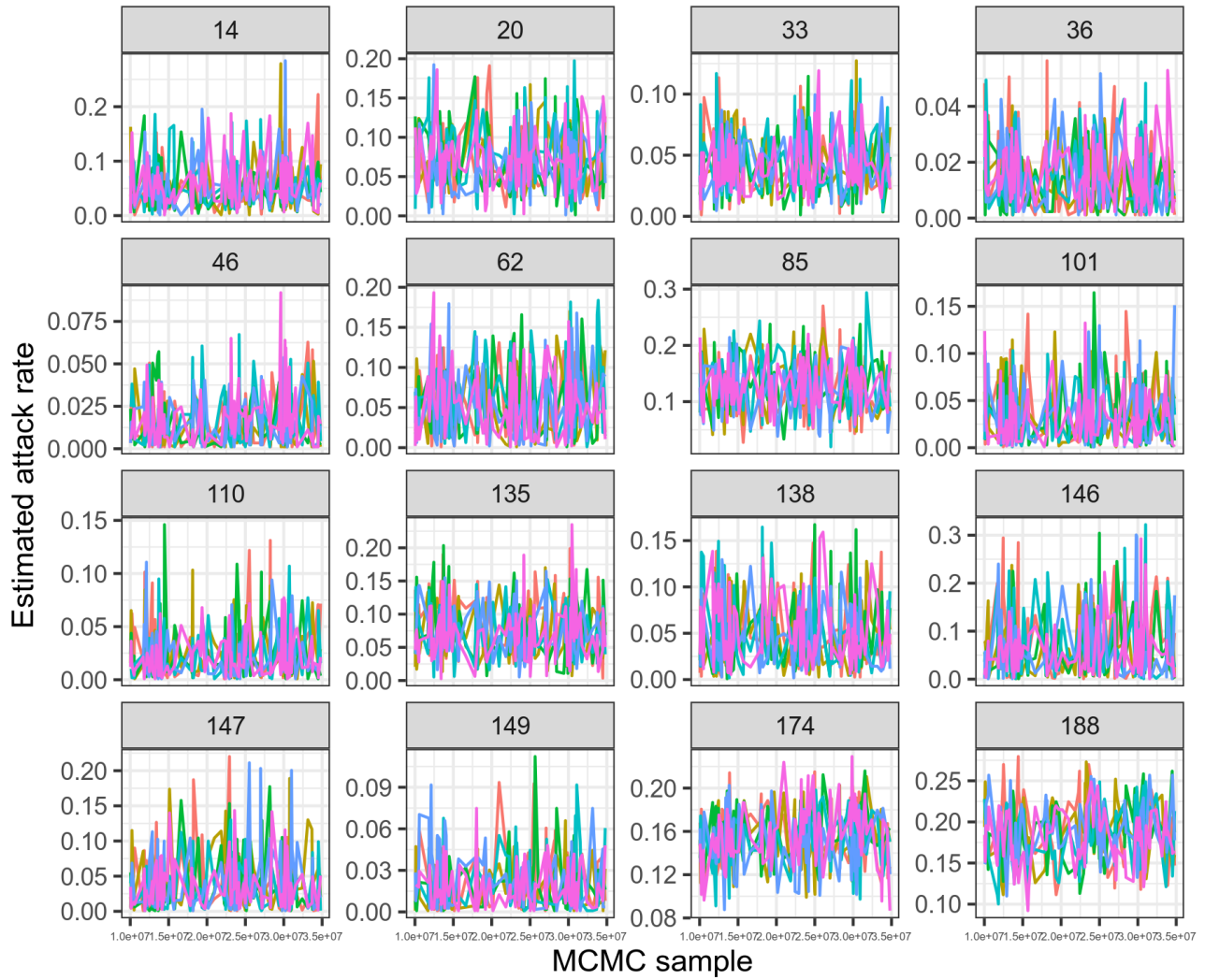


Figure F.4: Convergence diagnostics of quarterly attack rate estimates from fitting the full model with virus-specific measurement bias. Plots are MCMC traces for 16 randomly selected quarters, showing good mixing and consistency between 6 independent chains. Note that chains were extensively thinned due to computer memory and storage constraints.

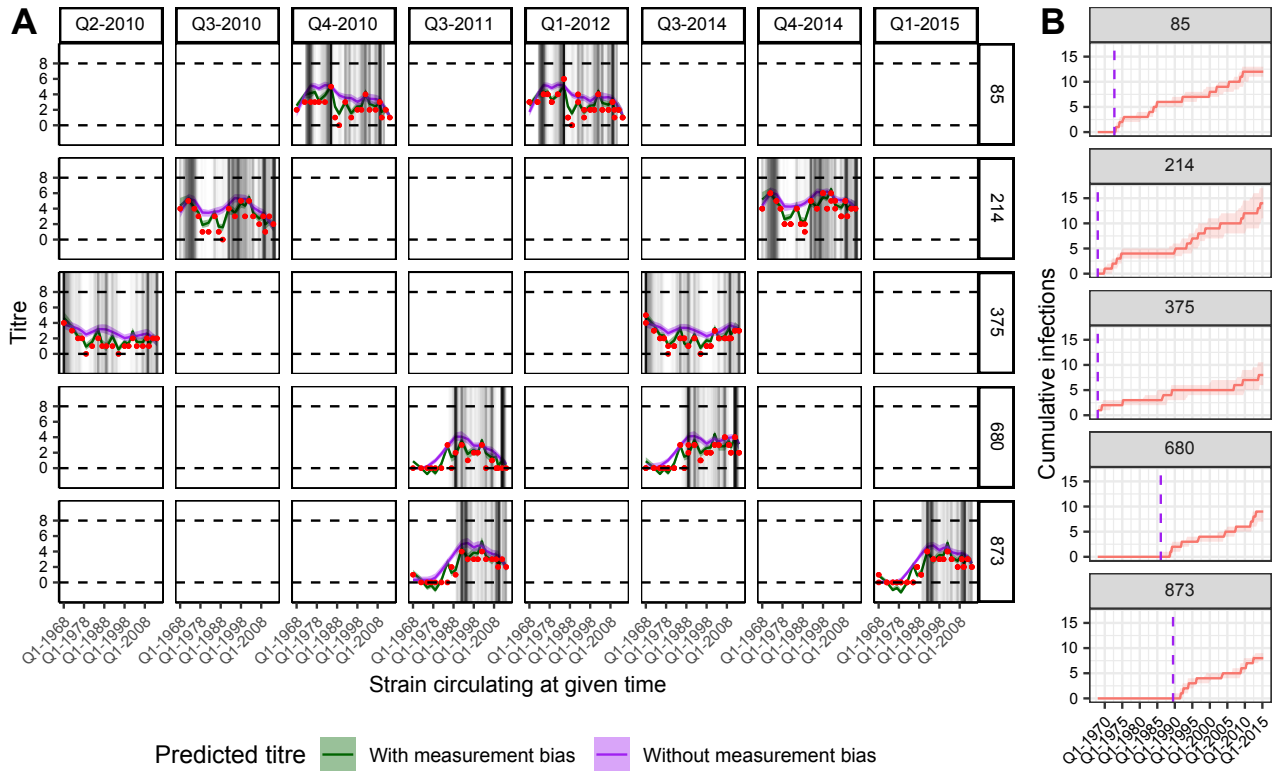


Figure F.5: Example inferred latent antibody titres and infection histories. (A) Model-predicted antibody titres compared to observed HI titres at each sampling time for 5 randomly selected individuals. Row represents an individuals. Each subplot represents the antibody titres based on serum samples taken at that time. X-axis represents a position along the antigenic summary path, with strains circulating from 1968 to 2015 ordered from left to right. Red dots show observed titres. Green line and shaded region shows posterior median and 95% CI on model-predicted latent titres with virus-specific measurement offsets included. Purple line and shaded region shows posterior median and 95% CI on model-predicted latent titres before the addition of these offsets. Dashed horizontal lines show the limit of detection of the HI assay. (B) Posterior median and 95% CI for the cumulative number of infections over time from birth (purple dashed line).

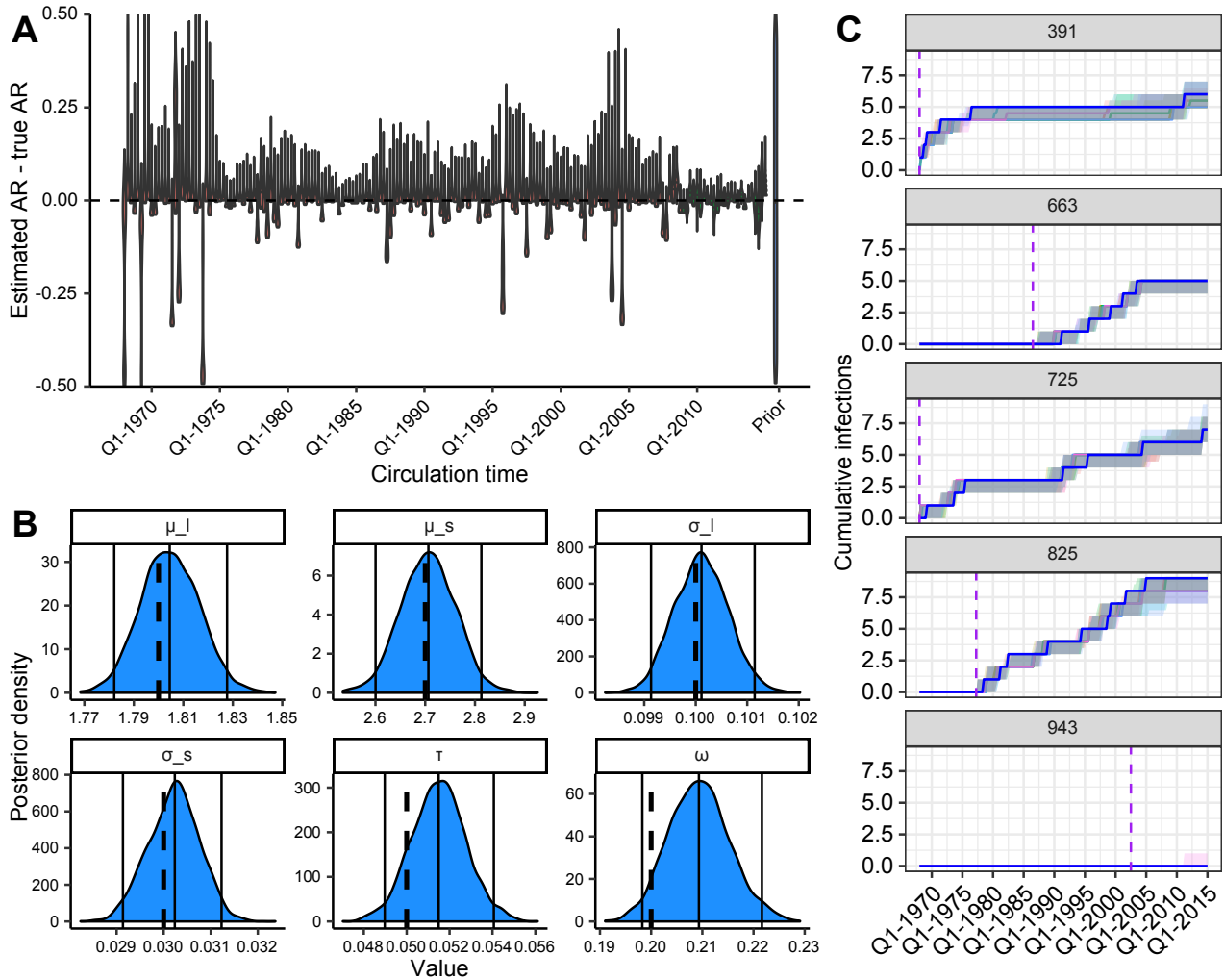


Figure F.6: Assessment of model fitting accuracy based on simulated data. Results shown are from fitting the full model to simulated infection histories and antibody titres with known parameters. (A) Distribution of residuals across 100 MCMC samples between model-predicted attack rate estimates and true, known attack rates. Times in which serum samples were taken are shown in green. The attack rate prior is shown on the right in blue. (B) Posterior distribution of estimated antibody kinetics parameters. Solid lines show posterior median and 95% CI. Dashed line shows true value. (C) Posterior median and 95% CI for the cumulative number of infections over time from birth. Each colour shows results from a separate MCMC chain. Blue solid line shows the true, known cumulative number of infections.

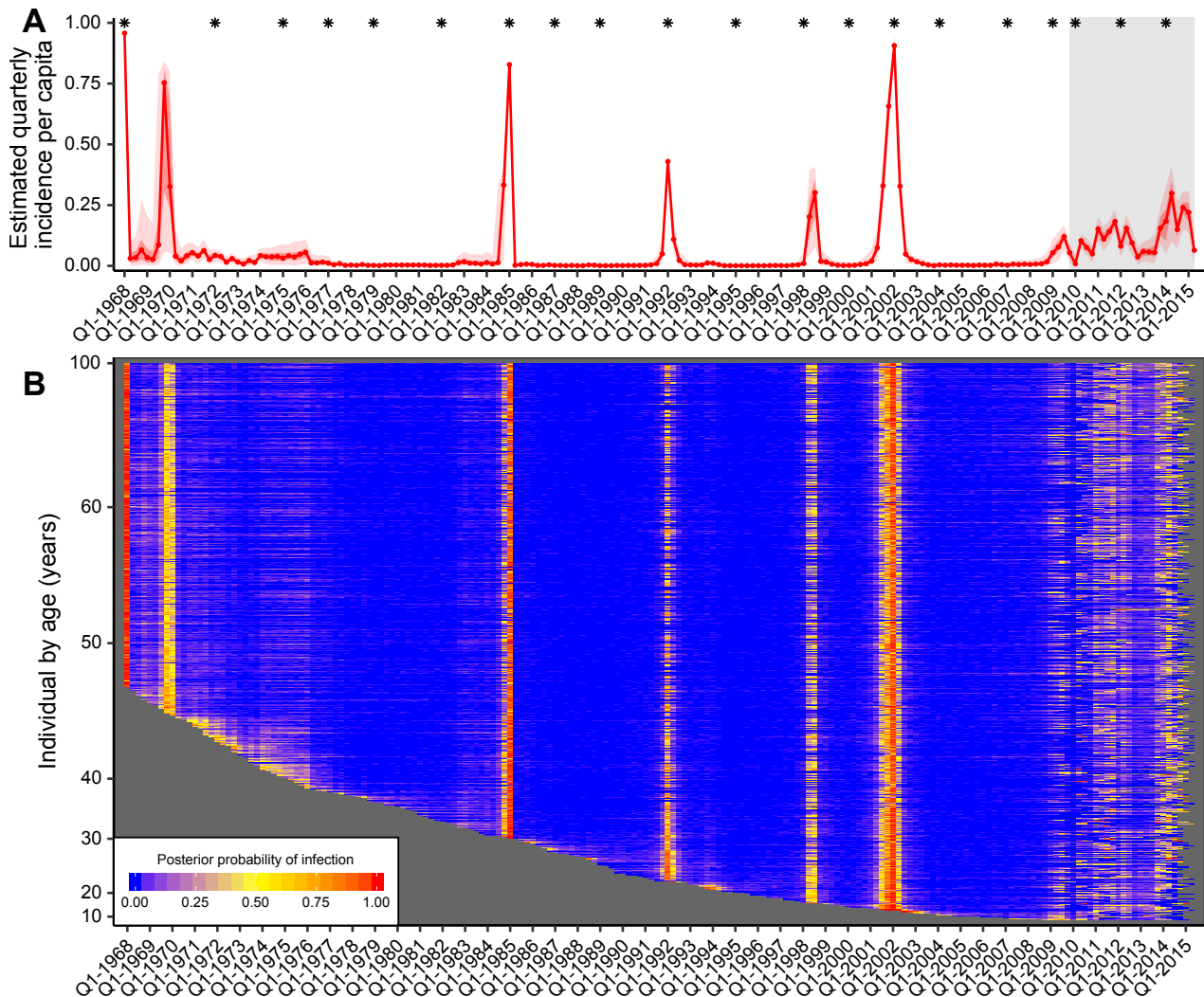


Figure F.7: Quarterly incidence and individual infection histories from the Fluscape dataset using the model without virus-specific measurement bias. (A) Model predicted per-capita incidence per quarter. Attack rates were estimated by dividing the number of inferred infections by the number alive in each 3 month period. Red line shows posterior median estimate from 100 posterior samples. Dark and light red shaded regions show 50% and 95% credible intervals respectively from 100 posterior samples. Gray shaded box shows duration of the Fluscape study. Asterisks mark times from which a sample circulating strain was tested. (B) Inferred infection histories for each individual. Each row represents an individual ordered by increasing age. Each column represents the time of a potential infection. Cells are shaded based on the number of the posterior samples with an infection at that time divided by the total number of posterior samples for that infection state.

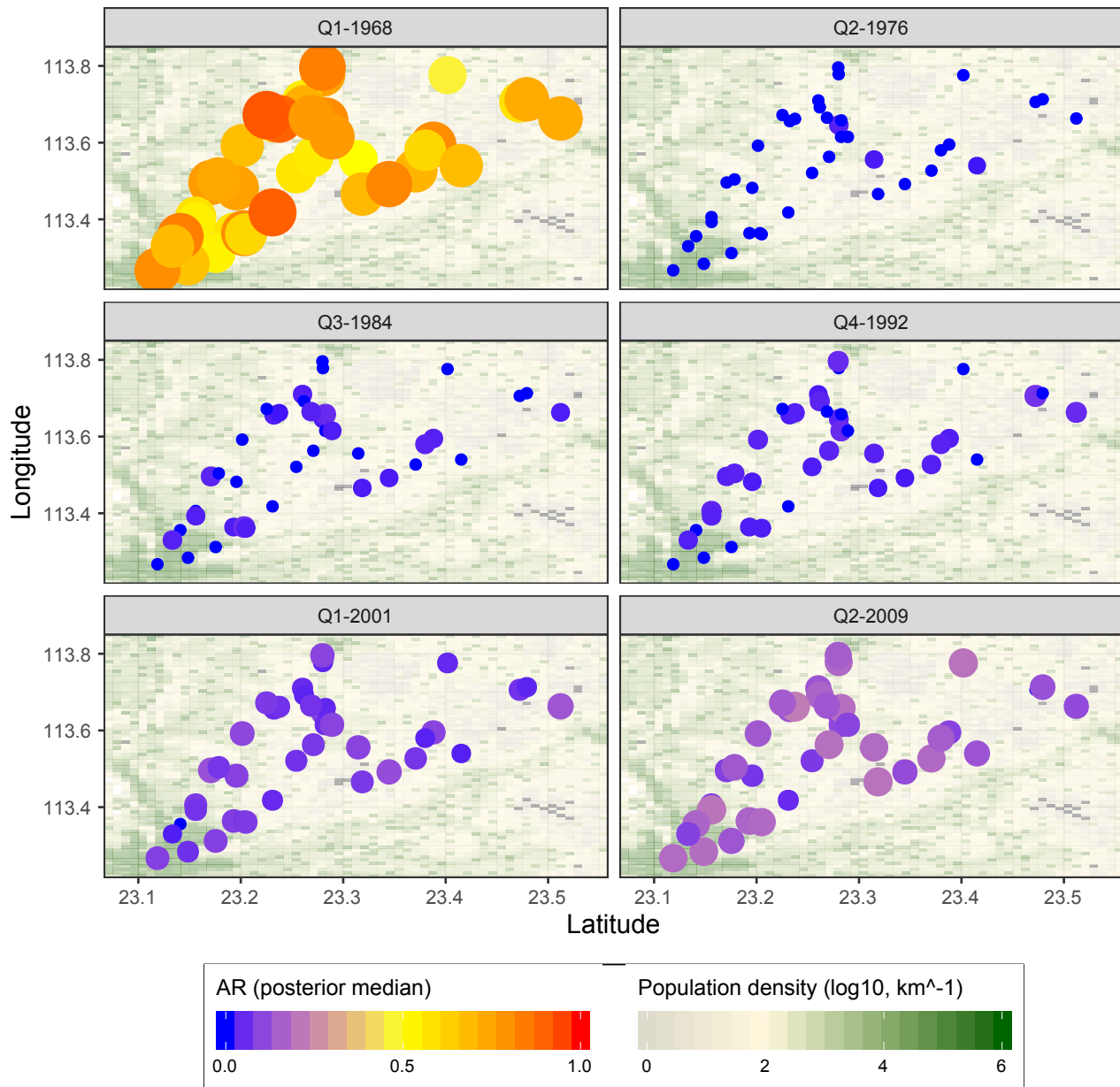


Figure F.8: Distribution of quarterly attack rates by location over time. Each panel is one frame from a full animation, available at: <https://figshare.com/s/1162bae5dedfcbcd9156>. The equivalent plot using the model without virus-specific measurement bias is available at: <https://figshare.com/s/bb9c0d40c295b9aca860>.

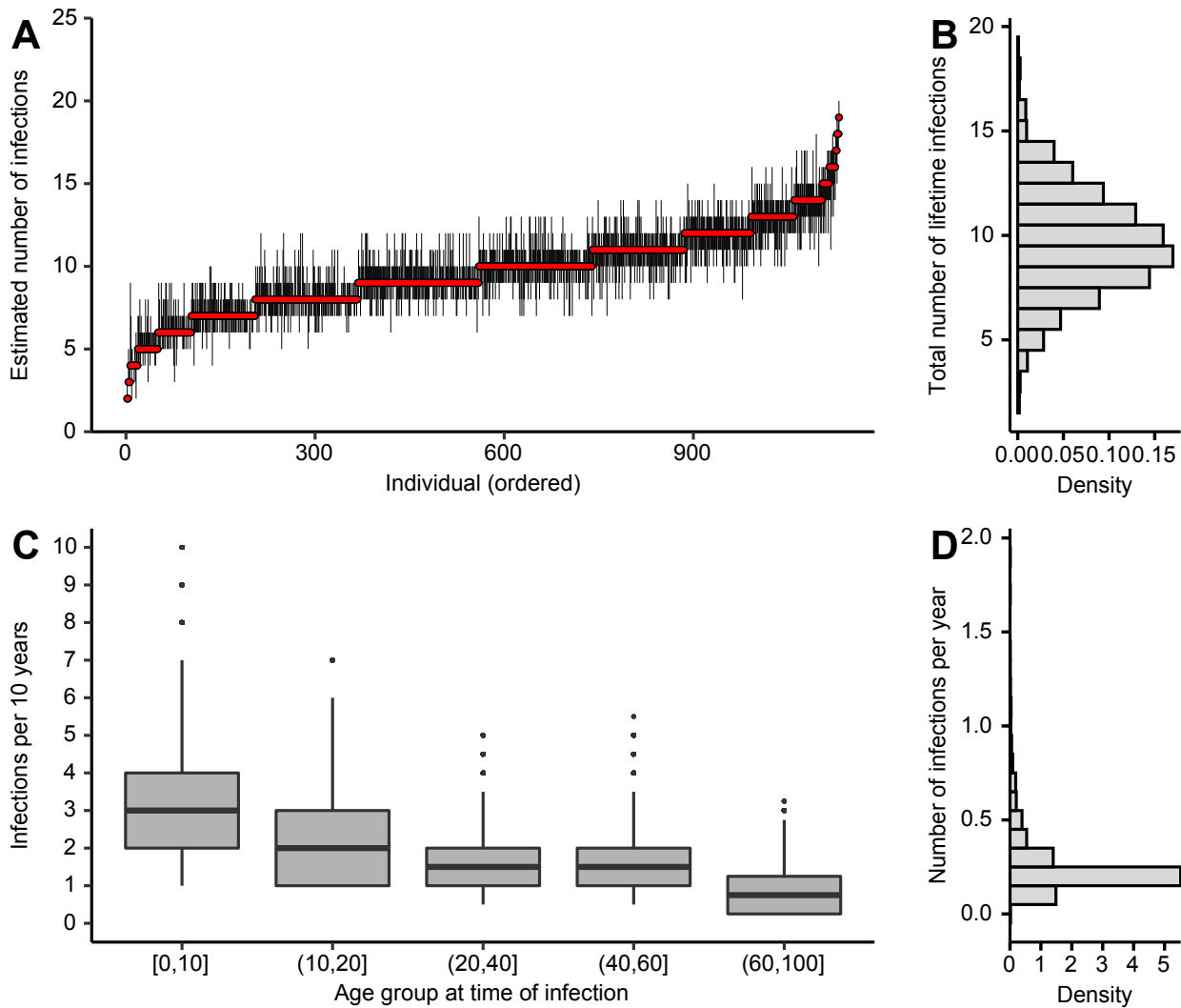


Figure F.9: Age-specific patterns of infection under alternative infection history prior assumptions. Model used is identical to the main text antibody kinetics model, but with infection histories at an annual resolution, virus-specific measurement terms estimated as free parameters, and the assumption of no population-wide risk of infection, as described in Chapter 5. (A) Pointrange plot shows median and 95% CI on the total number of lifetime infections for each individual. (B) Distribution of the total number of infections across all individuals based on the posterior median total number of infections. (C) Number of infections per 10 year period stratified by age group at the time of infection. (D) Number of infections per year across all individuals.

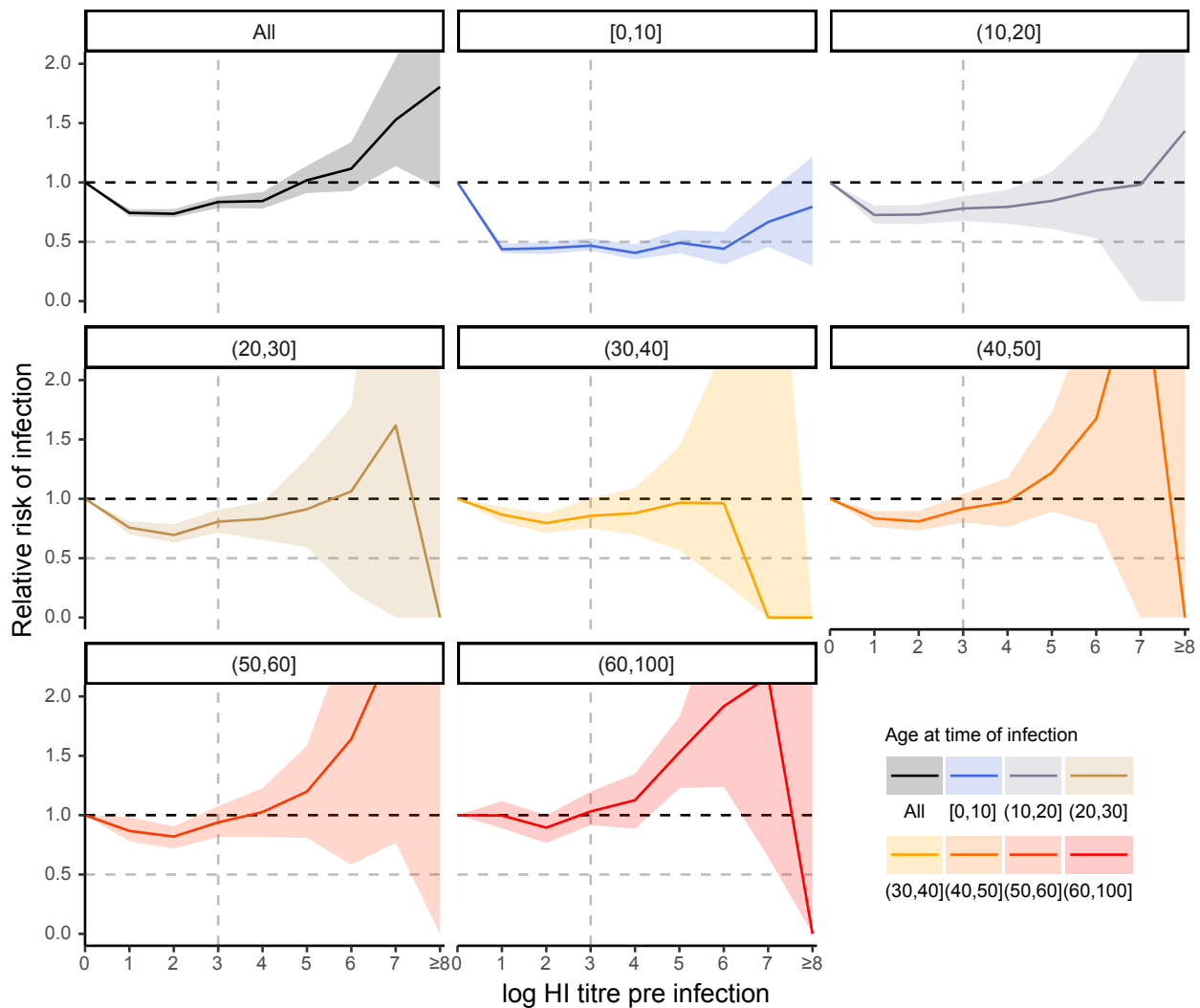


Figure F.10: Estimated relationship between HI titre and probability of infection based on unmodified model-predicted infection histories. Top left panel shows the relative risk of infection at all time points stratified by model-predicted log titre against the circulating strain just before infection for all age groups. Remaining plots show same relationship but stratified by age group at the time of infection. Solid lines and shaded regions show posterior median and 95% CI. Vertical dashed line shows HI titre 1 : 40. Horizontal grey dashed line shows 50% protective titre.

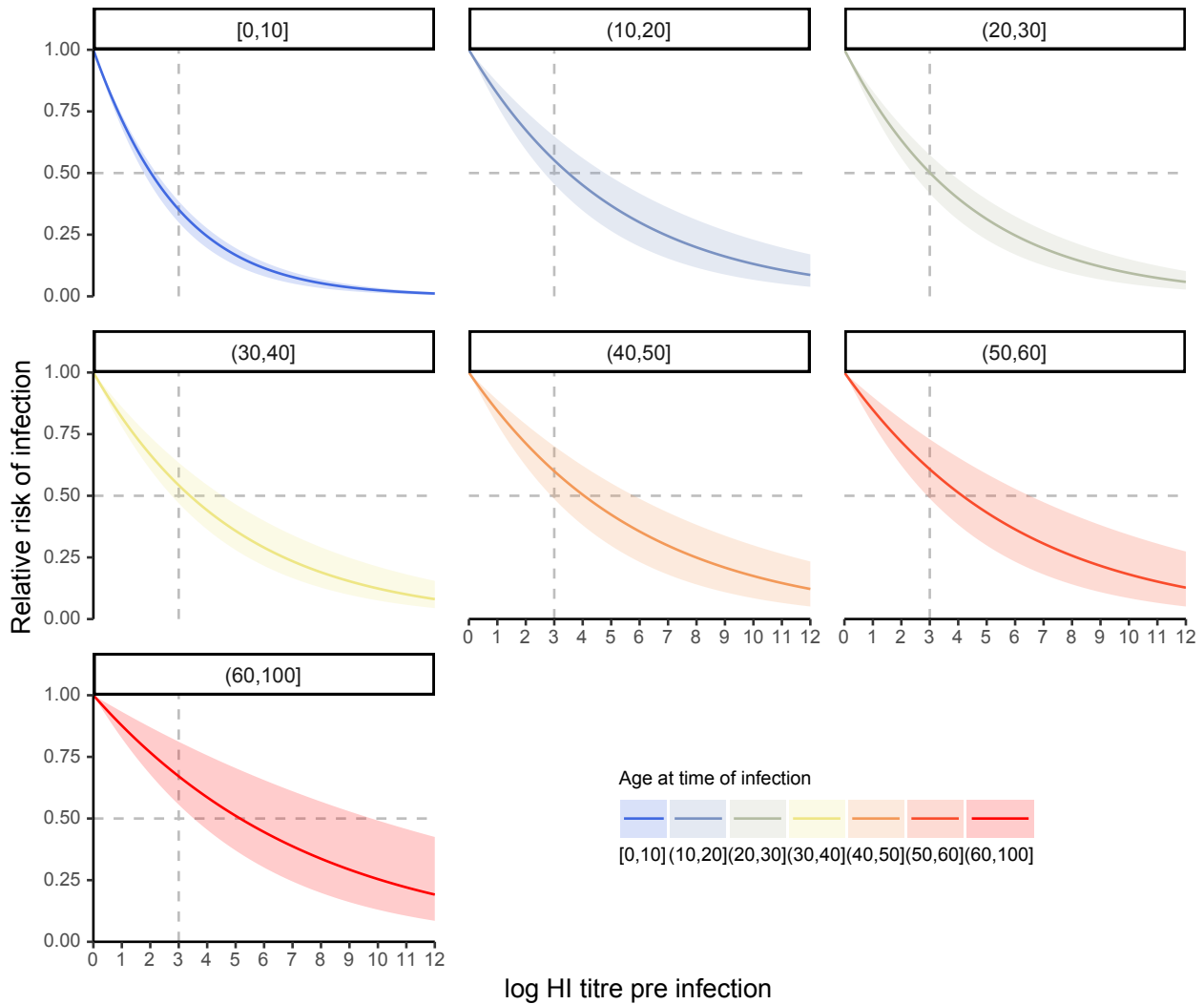


Figure F.11: Logistic regression showing relative risk of infection as a function of titre stratified by age group. For each of 100 draws from the multivariate posterior, the regression model was fit to each age group independently. Solid lines and shaded regions show posterior median and 95% CI across these 100 fits. Vertical dashed line shows HI titre 1 : 40. Horizontal grey dashed line shows 50% protective titre.

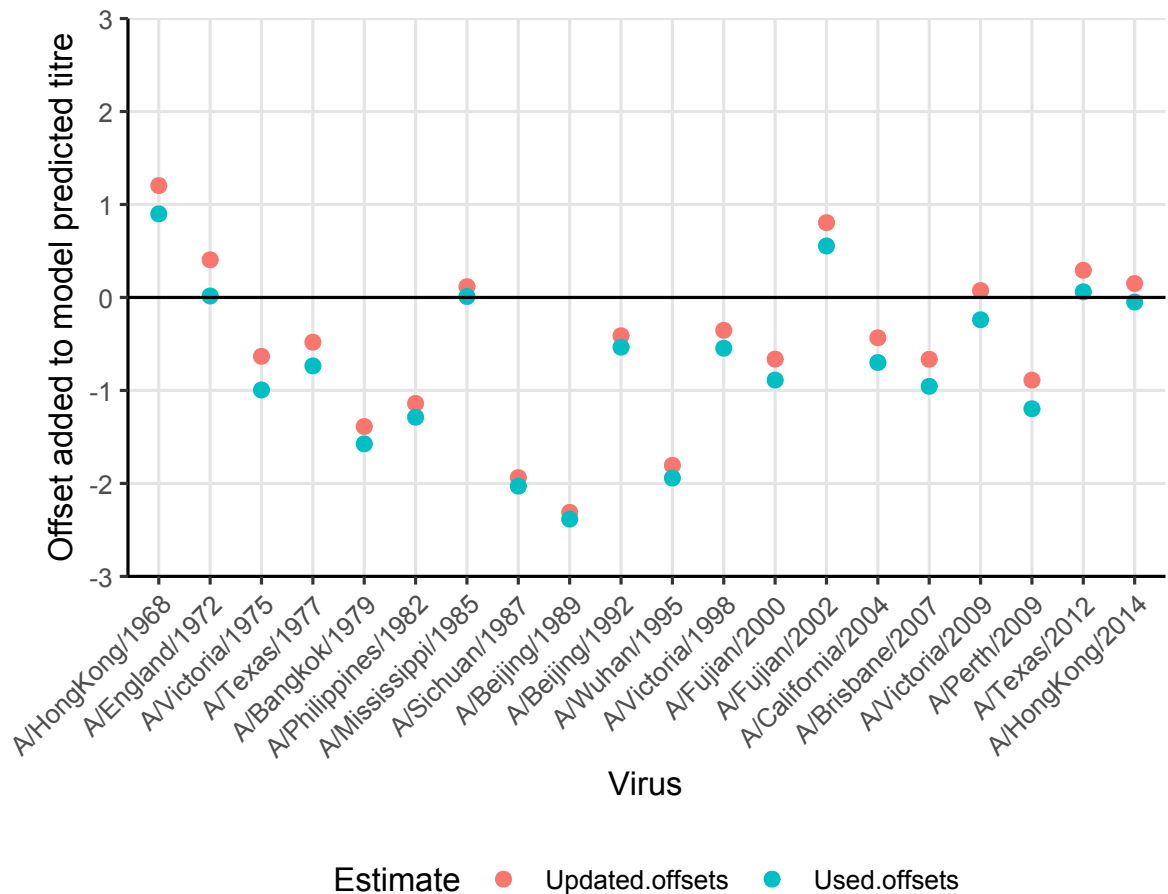


Figure F.12: Comparison of virus-specific offsets used and estimates in Chapter 5. “Used.offsets” refers to values used in Chapter 6, whereas “Updated.offsets” refers to the updated estimates in Chapter 5. Y-axis range shows upper and lower prior bounds.

Table F.3: Comparison of generalised additive model fits using raw HI from second serum sample or change in HI titre as outcome. Models are ordered by increasing AIC. Smoothing splines were placed on terms with an $s()$. Strain and location were included as fixed effects.

Model	Deviance explained (%)	AIC	BIC	Δ AIC	Δ BIC
Raw HI titre at visit 2 as outcome					
strain + s(age) + s(age at circulation) + location	41.3	153711	154390	0	0
strain + s(age) + s(age at circulation) + s(travel time to Gz)	39.9	154690	155107	978	716
strain + s(age) + s(age at circulation) + s(log density 9km)	39.8	154765	155180	1054	790
strain + s(age) + s(age at circulation)	39.4	155045	155385	1334	994
strain + s(age)	25.2	164516	164777	10805	10387
strain	22.8	165915	166098	12203	11707
Change in HI titre as outcome					
strain + s(age) + s(age at circulation) + location	40.1	133383	134051	0	0
strain + s(age) + s(age at circulation) + s(travel time to Gz)	38.5	134354	134764	971	713
strain + s(age) + s(age at circulation) + s(log density 9km)	38.3	134473	134883	1091	832
strain + s(age) + s(age at circulation)	37.7	134806	135140	1423	1089
strain + s(age)	25.0	142047	142303	8664	8253
strain	22.4	143380	143560	9998	9510

Table F.4: Antibody kinetics parameter estimates from the full model fit to the Fluscape data without strain-specific measurement bias.

Parameter	Description	Median (95% CI)	$\hat{R}$ upper 95% CI	ESS
μ_l	Long term antibody boosting	1.55 (1.51.59)	1.06	674
μ_s	Short term antibody boosting	1.94 (1.832.07)	1.05	433
τ	Suppression	0.0329 (0.02960.0361)	1.09	385
ω	Waning	0.217 (0.1940.259)	1.06	145
σ_l	Long term cross reactivity	0.0896 (0.08890.0899)	1.01	1450
σ_s	Short term cross reactivity	6.21e-05 (2.39e-060.000298)	1.00	2560
ϵ	Measurement error	0.875 (0.8690.881)	1.01	2420
$\sum X$	Total infections	11000 (1070011200)	1.03	1030

Appendix G

Discussion figures

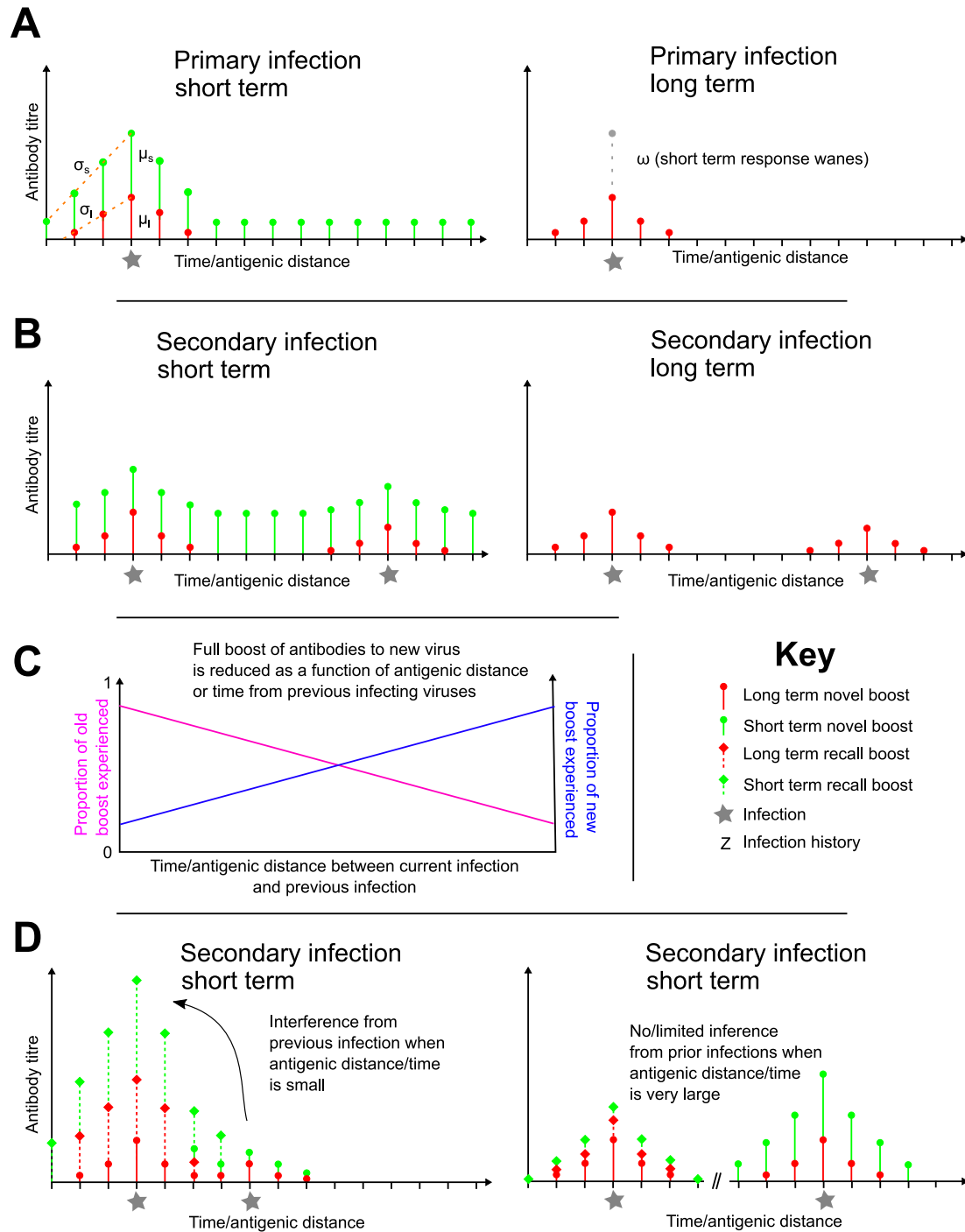


Figure G.1: Schematic comparing the current antibody kinetics model and a prototype with antigen trapping. (A) Schematic of model in Chapter 5 following primary infection. (B) Following secondary infection, short-term antibody boosting extends across antigenic space. Crucially, as cross-reactivity decreases linearly from the position of the infecting strain, the model must generate boosting across all of antigenic space to generate any back-boosting of historic responses. (C) Simple concept of how B-cell responses may be novel or memory based as a function of antigenic distance between infecting and memory strains. (D) Illustration of how this model would lead to different kinetics depending on antigenic distance.

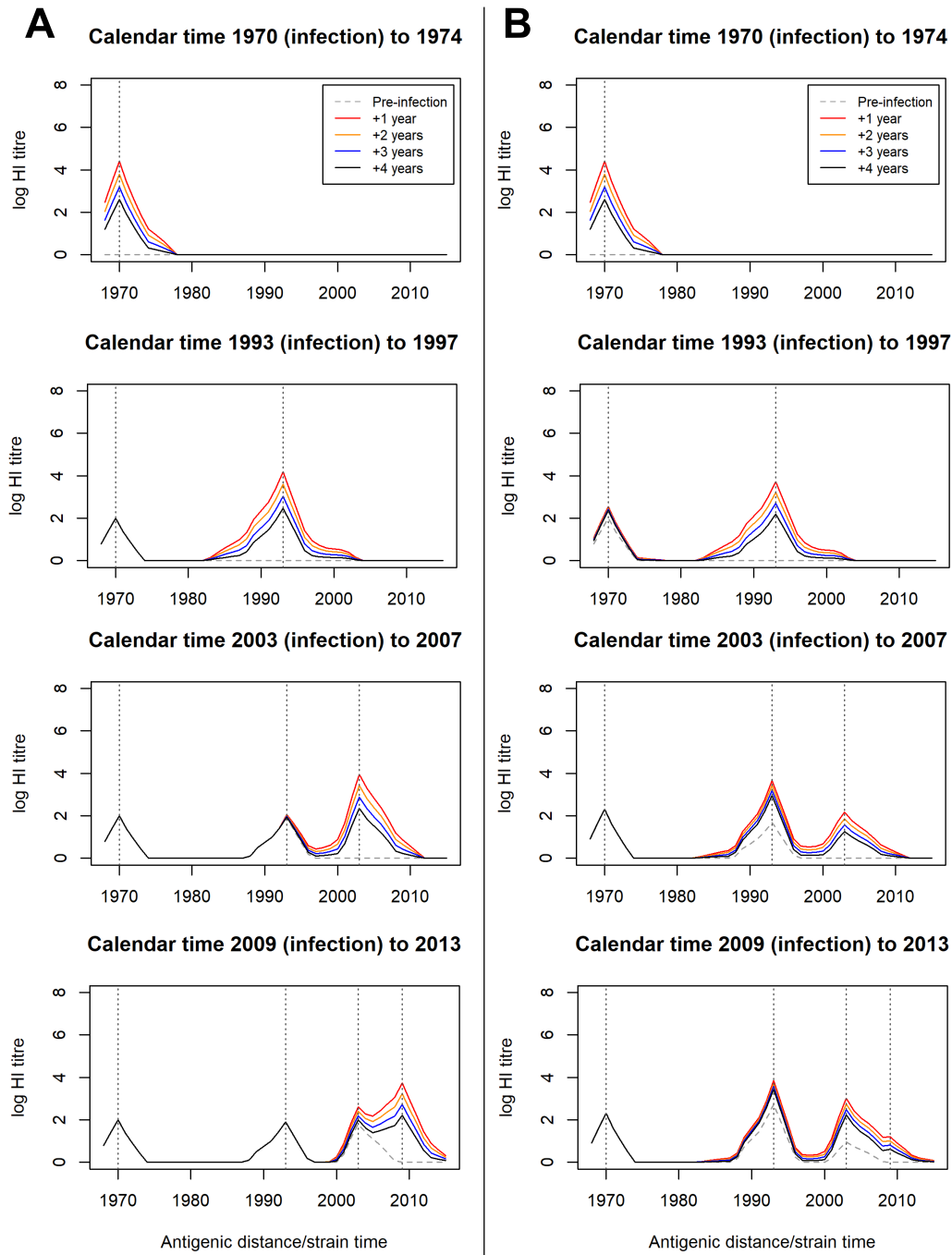


Figure G.2: Comparison of prototype antigen trapping model with current model. Coloured lines show predicted antibody profile at year intervals post infection following short-term waning. (A) Demonstrates how an individual's HI antibody profile builds over their life from 4 infections in 1970, 1993, 2003 and 2009. The x-axis represents the antigenic position of the strain that circulated at that time along a 1-dimensional summary of antigenic space. (B) Demonstrates the same infection history, but using the a prototype of the model described in Fig G.1. Antibody profiles are comparable after the first and second infection, as the antigenic distance between these strains is large. However, at the infection in 2003, the original model predicts a large boost to only the infecting strain, whereas the new model predicts a boost against both the 2003 strain the 1993 strain, though the novel boost is slightly reduced. After the infection in 2009, the original model predicts a strong boost to only a narrow region of antigenic space, whereas the new model redirects much of the boost against the 2003 strain at the cost of a novel response.