Applied Epidemiology of Infectious Diseases

Thesis submitted for the degree of Master of Philosophy (Applied Epidemiology)

of the Australian National University

Elenor Kerr

2019–2020

Field Placements

Communicable Diseases Branch, Queensland Health

Epidemiology and Public Health Unit, Institut Pasteur du Cambodge (IPC)

Peter Doherty Institute for Infection and Immunity

Academic Supervisors

A/Prof Stephen Lambert

Dr Emma Field

Field Supervisors

Dr Jonathan Malo (Queensland Health)

Dr Patrice Piola (IPC)

Prof Jodie McVernon (Doherty Institute)

Originality statement

I hereby declare that this submission is my own work and to the best of my knowledge it contains no materials previously published or written by another person, or substantial portions of material which have been accepted for the award of any other degree or diploma at the Australian National University or any other educational institution, except where acknowledgement is made in the thesis. The work was undertaken from March 2019 to October 2020 as part of the degree of Master of Philosophy in Applied Epidemiology, Australian National University. The research undertaken during my second year of placement at the Institut Pasteur du Cambodge and Peter Doherty Institute for Infection and Immunity was supported by the Department of Foreign Affairs and Trade ASEAN-Australia Health Security Fellowship Program.

Any contribution made to the research by others, with whom I have worked is explicitly acknowledged in this thesis. I also declare that the intellectual content of this thesis is the product of my own work, except to the extent that assistance from others in the project's design and conception or in style, presentation, or linguistic expression is acknowledged.

Elenor Kerr

October 2020

Look for causes, not villains.

Look for systems, not heroes.

— Hans Rosling

Acknowledgements

So many placements, and too many people to thank for their help along the way.

I am so grateful to Stephen Lambert for being my constant supporter and mentor. Despite all the different challenges; from squeezing projects into tight timelines, to an extremely rushed exit from Cambodia, I always felt confident that your guidance would get me through. Jonathan Malo, thank you for all your do-files, passing on your passion for record linkage, and for without fail finding every grammatical and referencing error. I think it's an obligation to mention that between Stephen and yourself, I'll never misuse an en or em dash.

I was also lucky to have a range of supervisors that while the overall time with me was brief, they were nonetheless crucial to developing my epi skills and progressing through the program. Thank you to Patrice Piola, Emma Field, and Jodie McVernon, for your confidence in me and always having the time to listen, support, and advise. I'd also like to specially mention Megan Counahan, who may not have formally been my supervisor, but was an incredible mentor during my time in Cambodia. Although, I never stepped foot into their offices, I'm also grateful to Ivo Mueller and Leanne Robinson for welcoming me into their project and supporting me despite all the incredible challenges of 2020.

Thank you also to Pip and Hayley in the ASEAN program, for all the stress and time spent navigating the new and unexpected territory. I'd have literally been stranded without you both.

Over the 22-months I got the opportunity to work with and get to know brilliant people in Queensland, Phnom Pehn, Melbourne, and Port Moresby. A special thank you goes to Wendy Morotti (Queensland Health) and Srean Chhim (Pasteur Institute) for making me so welcome.

Everyone always told me how important your cohort becomes to you; they are not wrong. Thank you to my cohort buddies for being there with support, coffee dates, and muffins. Laura and Hannah, I am sincerely grateful for you both.

I also owe a lot to those from earlier cohorts, from James Fielding for encouraging me to pursue this, to Dharshi who guided me through pretty much every step (and to every good market in Brisbane). I'm also very grateful for alumni like Kaitlyn Vette, who willingly jumped in when I needed technical guidance for a project.

Thank you to my family and friends for supporting me despite the distance, stress, and uncertainty. Danny for always listening and giving yourself a crash course on almost every topic I was working on and providing unsolicited Excel advice. Mum, for passing on your prolific writing skills and being there with daily coffee and wine deliveries during quarantine. Frank, the best of boys, for providing sanity.

Table of contents

Abbreviations

ACT	Artemisinin-based combination therapy
ART	Antiretroviral treatment
BBV/STI	Blood-borne viruses and sexually transmissible infections
AUSLAB	Queensland electronic pathology system
CDB	Communicable Diseases Branch, Queensland Health
CDNA	Communicable Diseases Network Australia
CD4+	CD4+ T cell
DFAT	Department of Foreign Affairs and Trade
DHHS	Department of Health and Human Services, Victoria
ERU	Epidemiology and Research Unit, Queensland Health
FIF	Food Incident Forum
G6PD	Glucose-6-phosphate dehydrogenase
HHS	Hospital and Health Service, Queensland
HIV	Human immunodeficiency virus
HPHT	HIV public health team, Queensland Health
IDU	Injecting drug user
IPC	Institut Pasteur Cambodia
LLIN	Long-lasting insecticidal nets
LM	Light microscopy
MJOI	Multi-jurisdictional outbreak investigation
MSM	Men who have sex with men
NNDSS	National Notifiable Disease Surveillance System
NoCS	Queensland Notifiable Conditions System
RDT	Rapid diagnostic test
PHU	Public health unit
QHPHML	Public Health Microbiology Reference Laboratory Queensland
UNAIDS	Joint United Nations Programme on HIV/AIDS
VFFS	Victorian Food Frequency Survey
WGS	Whole genome sequencing
WHO	World Health Organization

Abstract

This thesis details projects undertaken during my 22-month field placement in Australia and Cambodia. From February to December 2019, I undertook my MAE field placement at the Queensland Health Communicable Diseases Branch, which is responsible for the surveillance, prevention, and control of communicable diseases in Queensland. From January until late March 2020 I was based in Phnom Pehn, Cambodia, and undertook field placement with the Institut Pasteur Cambodia (IPC). IPC is a not-for-profit French foundation and manages one of only two laboratories in Cambodia. During this time, I was also seconded to support the Cambodian Ministry of Health COVID-19 response. On return to Australia in late March 2020, I was placed at the Doherty Institute in Melbourne and from July onwards was subsequently seconded on a part-time basis to the Victorian Department of Health and Human Services (DHHS) COVID-19 Public Health Intelligence team.

My projects comprised: a multi-jurisdictional outbreak investigation of *Salmonella* Heidelberg, 2018–2019; investigating trends in the epidemiology of inter-seasonal influenza in Queensland, 2009–2019; exploring the feasibility of a surveillance mechanism for rapid viral suppression among those newly diagnosed with HIV in Queensland; and analysis of a malaria re-infection in a child cohort in Madang Province, Papua New Guinea, 2013. This thesis also describes other experiences and public health activities undertaken during my placements: involvement in trachoma screening and a sero-prevalence research project in Doomadgee, Northern Queensland; involvement in measles outbreak support activities in South Brisbane; data analysis support for a human leptospirosis case-control study in the Yangon Region, Myanmar; field epidemiological support to the Cambodian Ministry of Health in response to COVID-19; and involvement in COVID-19 outbreaks at the Victorian DHHS.

These projects and experiences fulfil the core requirements of the Australian National University Master of Philosophy (Applied Epidemiology) program.

Chapter 1

Introduction to field placements and summary of experience

Overview

I commenced as an MAE scholar at the Queensland Health Communicable Diseases Branch (CDB) in Brisbane on 18 March 2019. I was based at the CDB for 10 months until January 2020 when I relocated to the Institut Pasteur Cambodia (IPC) in Phnom Penh as part of ASEAN-Australia Health Security Fellowship Program. Unfortunately, due to COVID-19 related travel restrictions, I was repatriated to Australia in late March 2020. I completed my final six months of the program placed at the Peter Doherty Institute for Infection and Immunity (Doherty Institute). From late July onwards, I was also seconded part-time to the Victorian Department of Health and Human Services (DHHS) COVID-19 response.

Field placements

Communicable Diseases Branch, Queensland Health

The CDB sits within the Prevention Division of Queensland Health, which is one of the five divisions within the Department of Health. The CDB is responsible for the surveillance, prevention, and control of communicable diseases in Queensland, which includes:

- Overseeing legislation, policy, and operational management of communicable diseases
- Advice and guidelines for preventing disease spreading from person to person and from animals to people
- Protecting the public health of Queenslanders
- Coordinating Queensland's vaccination program
- Monitoring disease outbreaks
- Planning for emerging pandemics and biosecurity threats
- Surveillance

Under the supervision of Dr Stephen Lambert and Dr Jonathan Malo, my primary role and responsibility was to gain real life experience as a public health epidemiologist by conducting epidemiological research projects and taking part in outbreak control and other public health activities occurring within the CDB. I attended weekly epidemiologist's notifications meetings, supported OzFoodNet Queensland in a range of *Salmonella* outbreaks, and attended OzFoodNet teleconferences. I also had the opportunity to spend a week in remote Northern Queensland supporting the Trachoma screening program and in late 2019 provided support to the Metro South public health unit and Queensland Health Immunisation Branch for measles outbreak activities (Chapter 7).

Epidemiology and Public Health Unit, Institut Pasteur Cambodia

The Institut Pasteur is a not-for-profit French foundation with primary focuses of research, public health, education, and the identification of research applications. As the provider of one of only two laboratories in Cambodia, IPC plays a critical role in Cambodia's health system by providing laboratory services and supports to the Ministry of Health. The IPC Epidemiology and Public Health Unit (EPHU) is focused on operational research projects with current projects in forest malaria, leptospirosis, and antimicrobial resistance.

My second placement was at the EPHU and under the supervision of Dr Patrice Piola. The plan was for me to undertake a case-control analysis of leptospirosis in Myanmar. However, as I returned to Australia in March 2020 this work was not completed as planned. Rather, I provided statistical analysis support to the Yangon-based Data Manager for this project (Chapter 7). During my time in Phnom Penh, I also had the opportunity to assist the Cambodian Ministry of Health's Communicable Disease Control Department (Cambodian CDC) with COVID-19 outbreak activities, including a cruise ship screening activity and establishing surveillance and reporting mechanisms during the initial stages of the pandemic in Cambodia (Chapter 7).

Peter Doherty Institute for Infection and Immunity

Following my return to Australia I was placed at the Doherty Institute in Melbourne. The Doherty Institute is a joint venture between the University of Melbourne and Royal Melbourne Hospital which implements a variety of activities related to infection and immunity including research, diagnosis, and treatment of viral infectious diseases, host-pathogen interactions, and translational and clinical research. The Doherty works both domestically and internationally and collaborates with a broad range of partners, including the Walter and Eliza Hall Institute of Medical Research (WEHI) and the Burnet Institute.

My role was to complete a survival analysis for a time-to-reinfection cohort study in Madang Papua New Guinea, 2013 (Chapter 5). I was supervised by Jodie McVernon, with additional supports provided by study investigators based at WEHI and the Burnet Institute. In late July 2020 I was seconded to the Victorian DHHS COVID-19 Public Health Intelligence team. In this capacity I worked in team managed by Hazel Clothier (MAE cohort 2002) and Kylie Carville (MAE cohort 2003) as a field epidemiologist embedded within contact tracing and medical support teams (Chapter 7).

Summary of core activity requirements

Investigation of an acute health problem or threat

A national multi-jurisdictional outbreak of *Salmonella* Heidelberg, 2018–2019

I provided epidemiological support to OzFoodNet Queensland who was the lead agency investigating a national outbreak of *Salmonella* Heidelberg. As part of this multi-jurisdictional outbreak investigation (MJOI), I collected, entered, and analysed case data, including undertaking binomial probability analysis of food risk factor data, undertook a literature review, and drafted internal and external reports, including the OzFoodNet final report. I also presented the investigation on behalf of Queensland Health at the OzFoodNet Structured Audit Process at the December 2019 OzFoodNet face-to-face meeting and was lead author on a draft manuscript prepared for submission to the *Emerging Infectious Diseases* Journal.

Analysis of a public health dataset

Analysis of malaria re-infection in a child cohort, Madang Province, Papua New Guinea, 2013

In Papua New Guinea (PNG) malaria transmission substantially reduced in the decade following the introduction of improved diagnosis and treatment. To better understand the epidemiology of malaria infection among school-aged children in northern coastal PNG, the Mugil II study was undertaken in 2013. Using the Mugil II study database, I undertook a time to event analysis, Cox regression, and Negative Binomial modelling. Results from these analyses demonstrated substantially lower prevalence and incidence of re-infection as compared to a similar study conducted in 2004 and highlighted the importance of targeting control measures to residual reservoirs of infection, including incorporating *P. vivax* interventions.

Establish/evaluate a surveillance or other health information system

Assessing a new, clinically based HIV indicator in Queensland: surveillance of time to viral suppression after diagnosis

Indicators for HIV care have typically failed to recognise the importance of newly diagnosed people quickly achieving viral suppression. A new indicator has been put forward, the proportion of newly diagnosed individuals achieving viral suppression three months after diagnosis. We aimed to explore the usefulness of a rapid viral suppression indicator and evaluate its potential as a surveillance mechanism in Queensland. As part of this project I conducted a pilot study, assessed the proposed system against core attributes, and interviewed key stakeholders. We found that a surveillance mechanism for time to viral suppression in Queensland would have

clear contributions to the assessment of HIV care quality and was considered to be both acceptable and useful by key stakeholders.

Design/conduct an epidemiological study

Evidence for an increase in the intensity of inter-seasonal influenza, Queensland, 2009–2019

Studies of influenza seasonality typically focus on seasonal transmission in temperate regions, leaving our understanding of interseasonal epidemiology limited. I analysed Queensland state-wide influenza notifications and laboratory testing data between 2009 and 2019 by demographics, time period, region, and strain type. I also compared influenza intensity over time using the WHO Average Curve method to provide thresholds for seasonal and inter-seasonal periods. We found a gradual increase in interseasonal influenza over time and suggested that this increase likely results from an interplay between testing, activity and intensity, and strain circulation. A manuscript detailing our findings was submitted to *Influenza and Other Respiratory Viruses* in October 2020.

Additional requirements

Literature review

A targeted literature search and synthesis of relevant information was undertaken for each project. The search strategy conducted as part of *Salmonella* Heidelberg MJOI is detailed in Chapter 2.

Report to a non-scientific audience

I developed a factsheet for an intervention to be implemented as part of two IPC Forest Malaria projects (Chapter 7). The factsheet was to educate people working in the forests about their risk of malaria infection and about intermittent presumptive treatment of malaria (IPTM). In addition to providing information about IPTM at a level of comprehension accounting for low levels of literacy, the factsheet doubled as a treatment consent form.

Peer-reviewed publication

A manuscript was submitted to *Influenza and Other Respiratory Viruses* for my epidemiological project; the epidemiological analysis of inter-seasonal influenza in Queensland. A late stage draft has also been completed for the investigation of the national *Salmonella* Heidelberg outbreak 2018–2019 for submission to *Emerging Infectious Diseases*.

Conference/oral presentation

- A national outbreak of *Salmonella* Heidelberg, 2019. National Center for Epidemiology and Population Health (NCEPH) Lecture Series, Australian National University, Canberra, 28 August 2019.
- Presented a summary of the *Salmonella* Heidelberg Multijurisdictional outbreak at the OzFoodNet Face to Face Meeting in Canberra, December 2019.
- Presented initial results of the HIV feasibility study to the Queensland Sexual Health Ministerial Advisory Committee meeting, November 2019.
- Presented a summary of the proposed analytical methods for the ECOMORE 2 leptospirosis project in Myanmar to senior Cambodia-based study team members, March 2020.

Teaching of topics in field epidemiology

I prepared and conducted a teaching exercised called "Probabilistic record linkage in Stata using reclink2" and attended Lessons from the Field exercises prepared by MAE scholars in my cohort (Chapter 6). I also participated in teaching to first years and mentored a Yangon-based Data Manager to complete a case-control analysis of laboratory-confirmed leptospirosis in Myanmar.

Coursework

I passed the following coursework subjects of the MAE program:

POPH8916 Outbreak Investigation

POPH8917 Public Health Surveillance

POPH8913 Analysis of Public Health Data

POPH8915 Research Design and Methods

POPH8914 Issues in Applied Epidemiology

Additional field placement activities

In addition to activities related to the core program requirements, I also took part in the following activities and projects. These activities are also detailed in Chapter 7:

- Attended weekly CDB notification meetings at Queensland Health.
- OzFoodNet Outbreak Control Team meetings and MJOI teleconferences.
- Epidemiological support to Trachoma screening in Northern Queensland.
- Supported measles outbreak response activities in South Brisbane in late 2019.

- Provided epidemiological support to the Cambodian CDC in response to the initial stages of the COVID-19 pandemic.
- Analytical support to a leptospirosis case-control study based in Yangon, Myanmar.
- Provided epidemiological support to Victorian DHHS in response to COVID-19 outbreaks.

Summary of MAE requirements

A summary of my projects and activities and how they meet the core MAE requirements is detailed in the table below.

Table 1:Summary of MAE competencies and core requirements

Requirements	Chapter					
	2	3	4	5	6	7
Outbreak investigation	✓					✓
Epidemiological study		✓				
Data analysis				✓		
Establish/evaluate a surveillance system			✓			
Literature review	✓					
Teaching activities					✓	
Conference presentation	✓					
Report to non-scientific audience						✓
Peer-reviewed publication	✓	✓				

Chapter 2

A national multi-jurisdictional outbreak investigation of *Salmonella* Heidelberg, 2018–2019

Prologue

Rationale

This chapter represents my first core component of the Master of Philosophy (Applied Epidemiology) (MAE) Program, in which I provided epidemiological support to OzFoodNet Queensland as the lead agency in a multi-jurisdictional outbreak investigation (MJOI) of *Salmonella* Heidelberg.

In mid-December 2018, OzFoodNet Central noted that *Salmonella* Heidelberg cases with diagnosis dates in November 2018 were above the national historical 5-year mean for November. Later that month, OzFoodNet Victoria, Queensland, and New South Wales (NSW) noted increases in cases with onset in November and December. By the time I arrived in the Queensland Health Communicable Disease Branch (CDB) in March 2019, OzFoodNet was seeking endorsement from the Communicable Diseases Network of Australia (CDNA) for an MJOI. When OzFoodNet Queensland was asked to lead the investigation, Russell Stafford, Consultant Epidemiologist for OzFoodNet Queensland, asked if I would be willing to take an active role in the epidemiological aspects of the investigation. The overall aim of this investigation was to find an association between outbreak cases and a food source, and to provide this evidence to outbreak stakeholders to coordinate control measures.[1]

This chapter fulfils the requirement to undertake a literature review and present at a conference. A targeted literature search and synthesis was conducted to identify epidemiological information on *Salmonella* Heidelberg outbreaks in Australia and globally. Literature obtained informed understandings of potential outbreak sources and the search strategy is detailed in the methods section. I presented a summary of the investigation at the National Centre for Epidemiology and Population Health lecture series, August 2019, and at the 59th OzFoodNet Face-to-Face Meeting, December 2019.

My role

OzFoodNet MJOIs have three phases;[1]

1. Alert: detection and declaration
2. Investigation
3. Stand down: review and finalise investigation

I was involved in the Investigation and Stand Down phases of this investigation. The epidemiological investigation was led by Russell Stafford with support from myself. I also

undertook the literature review with assistance from Russell. The laboratory investigation was primarily undertaken by the Public Health Microbiology Reference Laboratory Queensland, with some analysis undertaken by public health laboratories in Victoria, NSW, and South Australia (SA). Health protection NSW led engagement with food industry. My involvement included:

- Adapting an Excel-based data collection template for the collection and descriptive analysis of national case and risk factor information.
- Entering Queensland case, laboratory, and risk factor data from hard copy questionnaires provided by Public Health Units (PHU) and Forensic and Scientific Services (FFS) reports into the national data collection template.
- Communicating with OzFoodNet sites regarding case and risk factor data, and with OzFoodNet Central regarding national notification data.
- Collating national case, laboratory, and risk factor data from all OzFoodNet jurisdictions reporting cases and maintaining the database to ensure accuracy and consistency of data and timeliness of reporting.
- Ensuring that newly reported cases met the probable case definition.
- Interviewing potential outbreak cases reported to OzFoodNet Queensland.
- Undertaking descriptive analysis, including generating epidemiological curves and other charts, for epidemiological and situation reports distributed to OzFoodNet network (Appendix 1).
- Contributing to OzFoodNet *S.* Heidelberg MJOI teleconferences.
- Contributing to briefing papers shared with stakeholders.
- Applying binomial probability calculations to compare 7-day food exposures between interviewed *S.* Heidelberg outbreak cases and those of the general population using data from a recent food frequency survey conducted in Victoria.
- Undertaking a targeted literature review of *S.* Heidelberg outbreaks.
- Drafted the OzFoodNet Final Report.
- Presented a summary of the outbreak at the OzFoodNet Structured Audit for MJOI 2019-002 *Salmonella* Heidelberg in December 2019 (Appendix 2).
- Lead author for a manuscript currently in development for submission to the *Emerging Infectious Diseases* journal.

Lessons learnt

This was my first experience of an MJOI and first experience providing epidemiological support in response to an outbreak. There were, therefore, a great many lessons for me to learn. Firstly,

the laboratory component of the investigation provided a crash course in interpreting whole genome sequencing (WGS) reports and gave me a firsthand experience in the pros and cons of using WGS as an outbreak investigation tool. During the investigation I also assisted in interviewing cases for a concurrent outbreak of a new genotype of *Salmonella* Typhimurium in Queensland April–June 2019. Undertaking interviews using similar questionnaires to those used in this MJOI, provided greater insight into the process of obtaining hypothesis generating data.

As an investigation that involved five jurisdictions, I gained an appreciation for the challenges associated with conducting MJOIs. During data entry and initial analysis, I also learned the pitfalls of using a data collection tool developed for a different purpose. The Excel template was created by Health Protection NSW for the concurrent *Salmonella* Enteritidis MJOI and focused on egg purchase and consumption over a different time-period, and range of jurisdictions. Amending formula and tweaking date ranges was time consuming, required high attention to detail, and ultimately resulted in some errors when slight formula differences were not initially identified.

Whilst pinpointing the source of infection would have been very satisfying, particularly on my first exposure to an outbreak, I am appreciative to have been involved in an investigation where the source of infection was not clear. This enabled me to go through the process of analysing food frequencies and to be involved as the laboratory drew on its capacity to analyse collections of non-human samples and access global sequences. It also caused me to be wildly suspicious of everything in the supermarket. Overall, it demonstrated the challenge and importance of examining all sources of data and provided a great example of how challenging it can be to determine a source, if there is no clear transmission vehicle identified early in the outbreak, when case numbers are larger and ongoing. Attending the *S.* Enteritidis MJOI teleconferences, where the source of transmission was clear, was a useful contrast and highlighted the variety of forms that *Salmonella* investigations can take.

Working on the manuscript for this outbreak was a lesson in succinct writing and the challenges and benefits of involving a large range of stakeholders across jurisdictions and settings. I embarked on writing a short dispatch thinking it would be a quick process, easily achievable within the time frame of this program, but the number of co-authors naturally grew and has taken longer than anticipated. Despite this, I'm proud of the result so far and grateful for the contributions.

Public health impact

Whilst we were unable to identify the vehicle of transmission, this investigation provides further evidence towards the impact of *S.* Heidelberg in Australia. As a relatively uncommon serotype nationally with no published outbreak accounts in Australia, further evidence is important. As an OzFoodNet MJOI, the investigation underwent a structured audit process; during which several issues highlighted by the investigation were discussed with public health action agreed upon. Namely, ensuring that future investigation of *S.* Heidelberg use the extended trawler questionnaire in the first instance, pursuing a national food frequency survey to improve the representativeness of data for multijurisdictional analyses, and clarifying the process for declaring an MJOI in the context of pending WGS. This investigation was also the first instance where a briefing paper was sent to the Food Incident Forum (FIF) and whilst no additional information was provided by FIF members, the investigation was highlighted as successful example of engagement.

MAE core activity requirements addressed

- Investigation of an acute public health event
- Targeted literature review
- Presentation at a national conference

Acknowledgements

I would like to acknowledge the contributions of jurisdictional OzFoodNet epidemiologists, PHU staff, and public health laboratories to this investigation, in particular Joy Greggory for providing the VFFS data and Health Protection NSW for leading food industry engagement. The work of the Queensland Public Health Microbiology Reference Laboratory forms a large part of this investigation, and special thanks is due to Amy Jennison for her assistance guiding the laboratory investigation and coordinating laboratory involvement in the resulting manuscript. Thank you to Rose Wright, OzFoodNet Coordinating Epidemiologist for their assistance with multiple aspects of the investigation. As always, thank you to my supervisors. In particular, Stephen Lambert for his assistance supervising and guiding throughout the investigation and for his support editing the resulting manuscript.

Last but absolutely not least, I would like to thank Russell Stafford for being a brilliant mentor, and to both Russell and Robert Bell for giving me the opportunity to be involved in so many different foodborne outbreak investigations.

Abstract

Background

A multi-jurisdictional outbreak investigation (MJOI) was conducted to determine the source of *Salmonella* Heidelberg infection across five Australian jurisdictions (New South Wales (NSW), South Australia, Victoria, Queensland, and Western Australia) between November 2018 and May 2019.

Methods

Demographic, clinical, and risk factor data were collected and analysed for cases linked by whole genome sequencing. The binomial distribution of outbreak risk factor exposure was compared with background probabilities, as per Victorian Food Frequency Survey data. A targeted literature review and analysis of international sequences and non-human isolates from a Queensland laboratory collection explored potential transmission sources. Resistance mechanisms were analysed to better understand strain characteristics.

Results

Fifty-nine cases (58 confirmed, 1 probable) were identified with illness onset between 05 November 2018 and 25 May 2019. Most cases were reported collectively from NSW (31%), Victoria (24%), and Queensland (22%) and only three cases travelled interstate during their exposure periods. Thirty-six percent (13/45) of cases were hospitalised. Whilst ten food exposures were statistically higher for outbreak cases as compared to healthy Victorians, there was insufficient evidence to generate a strong hypothesis on any single food item. None of the non-human isolates analysed were closely related to the outbreak genotype, and there was no clustering among international sequences analysed. No drug resistance was detected but isolates harboured the antibiotic resistance gene *fosA7* and *saf* fimbrial genes.

Conclusion

Whilst the source of the outbreak was not conclusively identified, the outbreak most likely involved multi-state distribution of a food product. The laboratory investigation suggests a new or novel source of infection. The combined potential for future drug resistance, propensity for severe infection, and past evidence of invasiveness, suggests that *S.* Heidelberg may be of future public health concern in Australia.

Introduction

Salmonellosis is a bacterial disease caused by non-typhoidal *Salmonella* organisms and is commonly manifested by a sudden onset of diarrhoea, fever, abdominal pain, and vomiting.[2] *Salmonella* is among the most common bacterial foodborne pathogens in the world and non-typhoidal *Salmonella* is a major contributor to the global burden of foodborne diseases.[3,4] In Australia, salmonellosis is estimated to be responsible for 39,600 domestically acquired episodes of foodborne gastroenteritis, 2,100 hospitalisations, and between 15 and 19 deaths per year.[5,6] In addition to causing gastroenteritis symptoms, non-typhoidal *Salmonella* can cause severe illness through invasive infection, such as bacteraemia and infection of other normally sterile sites.[4]

From a public health perspective, cases of *Salmonella* infection occur both sporadically and associated with epidemiologically-related cases as part of recognised outbreaks.[7] *Salmonella'*s propensity to cause outbreaks makes it an important cause of foodborne gastroenteritis in Australia.[4,8] Between 2001 and 2009, *Salmonella* was associated with 33% of all foodborne disease outbreaks investigated in Australia and, in 2018, there were 14,154 notifications of Salmonellosis with the largest proportion of these occurring in Queensland (25%), New South Wales (NSW) (24%) and Victoria (22%).[7,9] *Salmonella* infections are predominantly foodborne with an estimated 72% of salmonellosis in Australia transmitted via contaminated food.[2] Risk factors for sporadic salmonellosis, as indicated by case-control studies globally, include consumption of eggs, poultry, raw fruit and vegetables, and domestic preparation of food.[10-14] Outbreak-associated food vehicles typically include eggs, poultry, seafood, meats, nuts, and fresh produce.[7,8]

Among the more than 2,500 different *Salmonella* serotypes, *Salmonella enterica serovar* Heidelberg is a frequently identified serotype among human infections and between 2000 and 2002 was the second most common non-human serotype globally.[3] It is most common in North America, East Africa, and Asia, whilst in Europe and Australia *S.* Heidelberg has been relatively uncommon to date.[15] Between 2014 and 2018, there was an annual average of 45 *S.* Heidelberg notifications in Australia and most states and territories average fewer than 10 notification per year (Table 1). The majority of Australian *S.* Heidelberg cases are notified in Queensland (45%) with an annual average over the last five years of 19.4 notifications. Apart from Queensland, the majority of *S.* Heidelberg infections are acquired overseas.

Table 1: Mean Australian annual *Salmonella* Heidelberg notifications by jurisdiction, 2014–2018

State/Territory	ACT	NSW	NT	QLD	SA	TAS	VIC	WA	Total
Mean annual no. notifications	0.2	9.0	0.8	19.4	2.6	0.4	6.4	4.4	**45.0**
Proportion of national annual notifications (%)	0.5	20.8	1.9	44.9	6.0	0.9	14.8	10.2	**100.0**

Outbreaks of *S.* Heidelberg are relatively infrequent in Australia. Globally, outbreaks of *S.* Heidelberg have been associated with foods of animal origin, including raw chicken, turkey, eggs, beef, and raw milk.[16,17] Whilst predominantly foodborne, outbreaks have also been associated with contact with dairy calves.[18] The most common cause of outbreaks and sporadic infection due to *S.* Heidelberg in North America is poultry and egg-containing products.[12,19] Analysis of Queensland serotypes by source 2000–2011 indicate that most *S.* Heidelberg cases are attributed to chicken and pork products.[20] This serotype has also been detected in other foods, such as nuts, and the plant-based dietary supplement, kratom.[20,21]

S. Heidelberg commonly produces more severe disease compared to other serotypes and, with an estimated 13% of cases developing systemic infections, it is also considered to be one of the most invasive.[22,23] A recent Australian study of invasive non-typhoidal *Salmonella* infection in Queensland between 2007 and 2016 found that amongst the most common serotypes, *S.* Heidelberg had the highest proportion of invasive isolates (9.43%).[4] When adjusted for age groups and gender, the odds of invasiveness in *S.* Heidelberg as compared to *S.* Typhimurium is also increased five-fold (Odds Ratio 5.24, 95% CI: 3.00–9.16).[4] *S.* Heidelberg is also currently regarded as one of the five most common serotypes with resistance to antibiotics used to treat *Salmonella* infections globally, thus increasing risk of severe infection, hospitalisation, and death.[24-26]

Multi-jurisdictional outbreak of *Salmonella* Heidelberg

In mid-December 2018, OzFoodNet Central noted that *Salmonella* Heidelberg cases with diagnosis dates in November 2018 (15 cases) were above the national historical 5-year mean for November (2.4 cases) (Figure 1). Shortly thereafter, OzFoodNet Victoria commenced a cluster investigation. Queensland noted a slight increase in cases of *S.* Heidelberg with onsets in November and early December 2018, yet as the serotype was more common in Queensland compared to other jurisdictions, investigations did not commence. Health Protection NSW began investigating cases in early January.

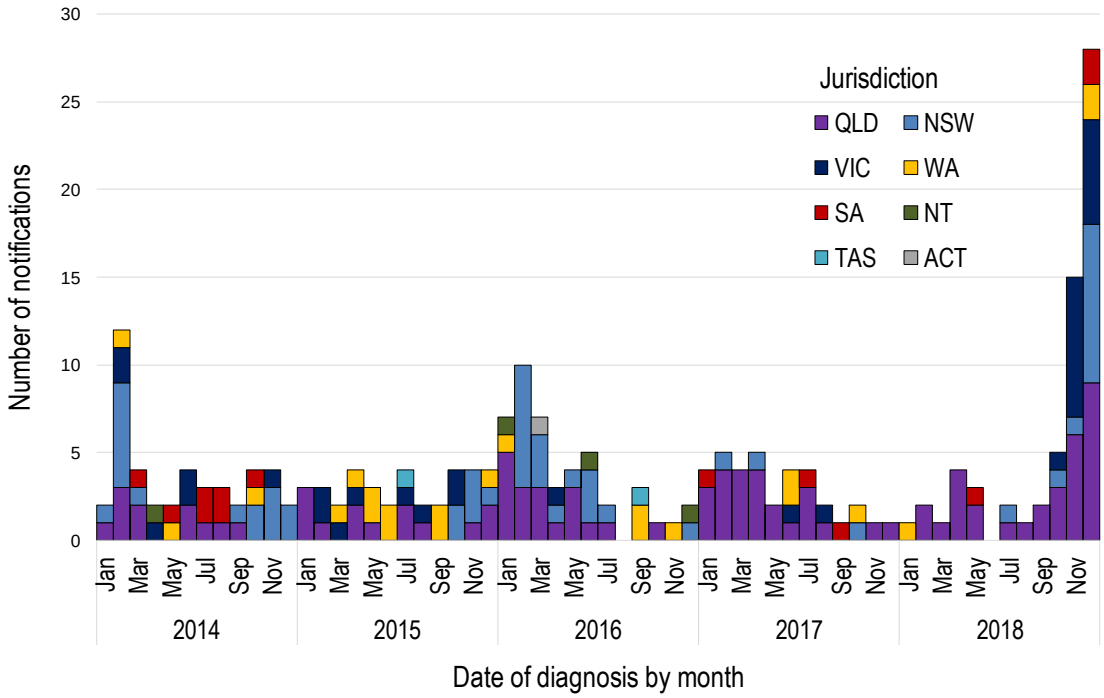


Figure 1: *Salmonella* Heidelberg cases notified to the National Notifiable Disease Surveillance System by month in Australian states and territories, 2014–2018

Methods

The outbreak investigation comprised an epidemiological investigation, literature review, laboratory investigation, and engagement with food industry stakeholders. The aim was to identify the outbreak source and provide evidence to relevant agencies to coordinate public health interventions.[1] OzFoodNet Queensland was appointed the lead agency with Russell Stafford (OzFoodNet Queensland) the Lead Epidemiologist. Rose Wright (OzFoodNet Central) was designated the National Coordinator and Siobhan St George (Victoria Department of Health and Human Services (DHHS)) was appointed the Human Laboratory Liaison Officer.

Epidemiological investigation

The aim of the epidemiological investigation was to determine the potential food vehicle or source of outbreak associated *S.* Heidelberg cases. This involved developing a case definition, conducting hypothesis generating interviews to collect clinical and risk factor data, and analysing and reporting on this information to relevant stakeholders on a regular basis throughout the investigation.

Case definition

Case definitions (Table 2) were agreed in consultation with OzFoodNet jurisdictions and the human laboratory liaison. Definitions were reviewed at each MJOI teleconference, but remained unchanged throughout the investigation.

Table 2: Case definitions used during *Salmonella* Heidelberg multi-jurisdictional outbreak investigation, 2019

Confirmed Case
Any notified* case of *Salmonella* Heidelberg infection genotyped as cgMLST 2561 and/or highly related on phylogenetic analysis to the outbreak sequence (ENA Accession ERS 3357031), with an illness onset since 1 November 2018 and resided in Australia for any period during the 10 days prior to onset.
Probable Case
Any notified case of *Salmonella* Heidelberg infection with an epidemiological link to a confirmed outbreak case, in the absence of whole genome sequencing.
Possible Case
Any notified case of *Salmonella* Heidelberg infection with no epidemiological link to a confirmed case and no reported overseas travel during the 10 days prior to onset, with whole genome sequencing pending.

* Non-typhoidal *Salmonella* is nationally notifiable when confirmed with laboratory definitive evidence (Isolation or detection of *Salmonella* species).

Data collection and management

De-identified data were collected for all notified *S.* Heidelberg cases who met the outbreak case definitions. Basic demographic information was collected via routine notifications to state/territory-based notifiable disease surveillance systems and laboratory records. Additional data were collected through telephone interviews conducted by respective jurisdictions using hypothesis generating questionnaires.

OzFoodNet has standard *Salmonella* hypothesis generating questionnaires. However, as interviews were conducted across several jurisdictions prior to the MJOI, a variety of questionnaires were used (Table 3). The standard OzFoodNet *Salmonella* questionnaire, known as the 'trawler', was used for South Australian (SA) and Western Australian (WA) cases, except for one five-month-old child on limited solids. An abbreviated version of the trawler with a focus on poultry and egg exposure was used for NSW and Victorian cases interviewed between December 2018 and February 2019. Two *Salmonella* questionnaires were used in Queensland. After the establishment of the MJOI, a standardised *S.* Heidelberg questionnaire was used for Queensland cases from February 2019 onwards and was distributed to all jurisdictions.

Table 3: Summary of questionnaire content used by OzFoodNet jurisdictions during *Salmonella* Heidelberg multi-jurisdictional outbreak investigation, December 2018–May 2019

Section/Topic	OzFoodNet *Salmonella* 'Trawler'	OzFoodNet *Salmonella* Abbreviated 'Trawler'	*Salmonella* Hypothesis Generating (QLD)	*Salmonella* Heidelberg (QLD)
Case details *(Name, date of birth, contact details, Aboriginal/Torres Strait Islander identification)*	✓	✓	✓	✓
Occupation *(Including handling of food/drink, contact with sick people/children/elderly, institutional contact)*	✓	✓	✓	×
Clinical symptoms *(Symptoms, onset, duration of illness, hospitalisation)*	✓	✓	✓	✓
Laboratory *(Serotype/sub-type, specimen collection)*	✓	✓	✓	×
Contact exposures *(Family members, friends, colleagues with similar illness)*	✓	✓	✓	✓
Travel exposures *(Overseas, inter-state, within state)*	✓	✓	✓	✓
Social events/functions	✓	✓	✓	✓
Home food purchases *(Poultry, eggs, meats, fruit & vegetables, fish & seafood)*	✓	✓	✓	✓
Special diets *(Diets, allergies, foods never consumed)*	✓	✓	✓	✓
Open ended food history *(Breakfast, lunch, dinner, snacks)*	✓ 5 day*	✓ 5 day	✓ 3 day	✓ 3 day
Eating outside of the home *(Cafes/restaurants/bars, bakeries, takeaway, continental/speciality grocer)*	✓ 7 day	✓ 7 day	✓ 5 day	×
Egg/egg containing foods *(Eaten at home, out of home, desserts, sauces, and other foods containing egg)*	✓ 7 day	✓ 7 day	✓ 5 day	✓5 day
Poultry *(Raw, cooked)*	✓ 7 day	✓ 7 day	✓ 5 day	✓ 5 day
Meat products *(includes seafood, fish, and deli-meats)*	✓ 7 day	×	✓ 5 day	✓ 5 day

Section/Topic	OzFoodNet *Salmonella* 'Trawler'	OzFoodNet *Salmonella* Abbreviated 'Trawler'	*Salmonella* Hypothesis Generating (QLD)	*Salmonella* Heidelberg (QLD)
Vegetable/salad *(Includes salad mixes)*	✓ 7 day	×	†	†
Fruit *(Includes pre-cut, pre-packaged or home-grown)*	✓ 7 day	×	†	†
Convenience and snack food *(Packaged salads, nuts/seeds, commercial dips, frozen meals/berries/products, noodles, coconut, commercial baby food, pate/meat paste)*	✓ 7 day	×	†	†
Drinks *(Juices, smoothies)*	✓ 7 day	×	×	×
Commercial salad dressing & sauces *(Mayonnaise, marinades, sauces)*	✓ 7 day	×	×	×
Herbs & spices *(Bulk orders, fresh, dried)*	✓ 7 day	×	×	×
Takeaway food *(Burgers, kebabs, takeaway pizza/pasta, sandwiches/filled rolls, spring rolls/dim sims, satay sticks, pies/pasties/sausage rolls, fresh pre-made meals)*	✓ 7 day	×	†	†
Water consumption/contact *(Untreated or bottled water, recreational activities)*	✓ 7 day	×	✓ 5 day	×
Environmental and animal exposures *(Rural properties, contact with animals, animal food/treats)*	✓ 7 day	×	✓ 5 day	×

* In the days prior to illness onset.

† Items were included in an abbreviated 'Other food' category for the five days prior to illness. This included: meats (beef/veal, lamb, pork, game meat), sprouts (alfalfa, bean), salad mix (sealed bag, loose), snow peas, cantaloupe/rockmelon, paw paw/papaya, berries (strawberry, blueberry, raspberry, frozen), macadamia nuts, halva, tahini, pate, salami/pepperoni, frozen meals, soft cheese, and yoghurt.

Data were collated and stored in a national data collection template in Microsoft Excel. The national data collection template was adapted from a document used by Health Protection NSW for the *S.* Enteritidis MJOI. The template consisted of a case list, detailed risk factor template, and summary worksheet which produced charts, graphs, and summary statistics. The template focused on foodborne transmission and did not include environmental exposures. The template was distributed to all jurisdictions for the collection of case demographic, clinical, and risk factor data. Risk factor data were recorded for individuals with responses coded as either; Yes, No, Unsure/Missing. Where a food item was consumed, details of brand and place of purchase were recorded.

Analysis

Demographic and clinical information was analysed for all confirmed and probable cases, including sex, age, jurisdiction, symptoms, hospitalisation, and date of specimen collection. Given that 32% (19/59) of cases had no onset date available, specimen collection date which was documented for all cases was used as a proxy to analyse and present the distribution of cases over time.

Risk factor analysis was undertaken for all interviewed confirmed cases. Responses that were missing or "don't know" were excluded from denominators. An initial list of high frequency risk factors was compiled assessing items where greater than 50% of cases reported consumption within five days prior to illness onset. The binomial distribution of outbreak risk factor exposure was compared with background probabilities, as per the Victorian Food Frequency Survey (VFFS) data. The VFFS consists of food frequency information from healthy Victorian residents whose demographics reflect that of *Salmonella* cases notified to the Victorian DHHS between 2008 and 2013. The survey was conducted from November 2014 to October 2016 and was designed to reflect the normal dietary habits of healthy people and capture information routinely collected by the OzFoodNet 'trawler' questionnaire. VFFS data are stratified by season (summer, autumn, winter, spring).

Binomial probability analysis was first completed for high frequency risk factor items only, then subsequently reanalysed for all items. Binomial analysis of all items is recommended when there is potential that the exposure is rare, and calculations can therefore provide suggestive evidence of an outbreak vehicle. To account for the potential influence of seasonality, analysis was undertaken for VFFS data overall and restricted to summer data. Variation in the proportions of cases and VFFS controls and *p*-values corresponding to the binomial probability of cases being at least as high as observed were calculated. A positive increase in outbreak case exposure compared to VFFS controls and $p<0.05$ were considered to represent significantly higher rates.

The VFFS dataset was provided by OzFoodNet Victoria and composite variables were created to reflect the structure of outbreak case data. A new variable "Nuts of any kind"[a] was also created and analysed. All analyses were undertaken in Microsoft Excel and Stata/IC v. 15 (Stata Corp, USA).

Targeted literature review

The aim of the literature review was to inform understandings of potential outbreak sources for *S.* Heidelberg. Three methods were used to obtain literature on *S.* Heidelberg outbreaks in Australia and globally. A database search for peer reviewed papers, a grey literature search to find relevant reports, and a snowball literature search.

Due to the relatively limited number of published papers specifically regarding *Salmonella* Heidelberg, several databases were used. These included the Australian National University (ANU) SuperSearch, which provides access to the full ANU library collection, Scopus, ProMed, and PubMed. No restriction was placed on date ranges but databases filters for 'Scholarly & Peer Reviewed' articles and English language were applied. Articles regarding animal infection of *S.* Heidelberg were checked and excluded if they did not involve human cases. The search strategy used key word search terms consisting of terms for *S.* Heidelberg and key word phrases (Figure 2). Phrases were selected to obtain as broad a scope of relevant articles as possible, whilst minimising the number of references for other more common *Salmonella* serotypes. Each phrase was repeated for all *S.* Heidelberg terms below to account for the variety of ways in which this serotype is described.

S. Heidelberg Terms	**Phrases**
Salmonella AND Heidelberg	AND Australia
	AND Australia AND outbreak
"*Salmonella* Heidelberg"	AND Australia AND outbreak NOT "*Salmonella* Typhimurium"
	AND outbreak NOT "*Salmonella* Typhimurium"
"*Salmonella enterica* serovar Heidelberg"	AND outbreak AND risk NOT "*Salmonella* Typhimurium"

Figure 2: Search terms and phrases used in *Salmonella* Heidelberg outbreak literature search

[a] Brazil, hazelnut, and 'mixed nuts' were not included in the VFFS and were excluded from outbreak case analysis.

Due to the low numbers of published *S.* Heidelberg outbreaks accounts obtained, the search was broadened to include a grey literature. Websites for the Australian Department of Health, Centers for Disease Control and Prevention, Government of Canada, and World Health Organization were searched for *S.* Heidelberg outbreak information or reports. OzFoodNet Queensland records were also searched. Additional papers were also found by manually searching reference lists from already identified relevant literature.

Laboratory investigation

The aim of the laboratory investigation was to identify cases of *S.* Heidelberg, confirm relatedness of cases through WGS, and explore potential links or relatedness to a source of transmission. The Public Health Microbiology Reference Laboratory Queensland (QHPHML) assessed genetic relatedness among *S.* Heidelberg cases using whole genome phylogenetic sequence analysis. Sequences for isolates from Queensland and WA were generated using the Illumina NextSeq genome sequencing platform in the QHPHML. Sequences for isolates from NSW, Victoria, and SA were generated by their respective public health laboratories on Illumina sequence platforms and forwarded to the QHPHML for analysis.

All sequences were analysed by the QHPHML with trimming undertaken using Trimmonmatic v0.36. Sequences were mapped to the reference genome *S.* Heidelberg SL476 (Genbank accession number NC_011083.1). Single-nucleotide polymorphisms (SNPs) were identified using Snippy pipeline V4.3.6 using default settings. Core SNPs from each sample were aligned using a package within Snippy and a Maximum Likelihood tree was generated from the SNP alignment using the RaxML plgin in Geneious R11. cgMLST complex types were generated using Ridom SeqSphere+ based on the Enterobase *S.* enterica scheme and a neighbour joining tree was generated from the cgMLST results of 2,806 genes. Isolates with five or fewer allele differences were considered to belong to the same cluster. The presence of the *saf* operon was investigated using Ridom SeqSphere+ and the *saf* operon sequences from NZ_LS483494.

To further characterise strains, in silico WGS analysis for antibiotic resistance genes was performed by the abricate program using the resfinder database. To explore potential links or relatedness to a source, 65 international sequences collected during 2019, and eight non-human *S.* Heidelberg isolates from Queensland Health Forensic and Scientific Services' (QHFSS) collection were assessed for genetic relatedness to outbreak cases. International sequences sourced from the United Kingdom, the United States, and South Africa, including *S*. Heidelberg strains isolated from humans, foods, poultry, livestock, and the environment were downloaded from EnteroBase.

Food industry engagement

A request for information was sent in May 2019 to the Food Incident Forum (FIF), a government-industry group established in 2016 to discuss food incidents/issues. Members include Australian food industry and Australian government food agencies, as well as the New Zealand Ministry for Primary Industries. The FIF was asked to provide information relating to routine industry testing for/detection of *S.* Heidelberg, any positive samples for *S.* Heidelberg, and share any information related to suspected brands or products which fit the distribution pattern and food consumption data of cases. For this purpose, a briefing report containing outbreak details and the request for information was compiled.

Ethics

Ethics approval for this work was obtained from the Australian National University Human Research Ethics Committee (Protocol 2017/909).

Results

Alert phase investigation

After the increase in *S.* Heidelberg cases nationally was identified, WGS was undertaken on Victorian, NSW, and Queensland isolates (Figure 3). Analyses found that 36 cases (12 from each of Queensland, NSW, and Victoria), each belonging to cgMLST complex type 2561, were highly related by phylogenetic analysis. A small number of *S.* Heidelberg infections were identified in SA and WA. However, prior to the establishment of the MJOI, samples had not yet undergone WGS to determine if they were part of the outbreak. No cases were identified in the Australian Capital Territory, Northern Territory, or Tasmania, and no potential source of transmission had been identified from initial jurisdictional outbreak investigations. At an initial meeting on 19 March 2019, OzFoodNet recommended a MJOI be commenced. The Communicable Diseases Network of Australia (CDNA) were advised of this potential multi-jurisdictional outbreak and MJOI endorsement was received from CDNA on 29 March 2019.

Multi-jurisdictional epidemiological investigation

The MJOI was in place over approximately four months. As there were no new cases identified after May 2019, the investigation was stood down on 10 July 2019.

2018	08 Nov	First case notified (Victoria)
	12 Nov	First South Australian (SA) case notified
	15 Nov	First Queensland case notified
	04 Dec	Onset of first locally acquired New South Wales (NSW) case
	13 Dec	First West Australian (WA) case notified
	14 Dec	Victoria commences a cluster investigation
	20 Dec	National epi-curves are shared with OzFoodNet sites, and Victoria and Queensland discuss increases at OzFoodNet teleconference
2019	15 Jan	NSW issues state-level situation report analysing case increases
	15 Feb	WGS confirms relatedness of NSW, Victoria, and Queensland cases
	19 Mar	Initial MJOI meeting
	29 Mar	CDNA endorses commencement of MJOI (2019/002)
	09 Apr	Case definitions shared and data collation and descriptive analysis commences
	16 Apr	WGS confirms relatedness of WA (8) and SA (4) cases with onsets Dec 2018—Mar 2019
	26 Apr	Binomial probability analysis completed (high frequency risk factor items)
	10 May	Binomial probability analysis completed (all items)
	15 May	Briefing paper submitted to the Food Incident Forum
	16 May	Literature review commenced and antimicrobial susceptibilities reviewed
	27 May	WGS confirms relatedness of additional Victorian case with onset in Mar 2019 Final MJOI case notified in SA
	10 Jun	Results of non-human and international sequences reported WGS identifies additional SA case with onset in Nov 2018 (no risk factor data)
	20 Jun	WGS confirms relatedness of final MJOI case (SA) with onset in May 2019 Binomial probability analysis re-run with additional case data
	10 Jul	CDNA recommends stand down of MJOI

Multi-Jurisdictional Outbreak Investigation

Figure 3: Timeline of *Salmonella* Heidelberg multi-jurisdictional outbreak investigation, November 2018–July 2019

There were 59 outbreak cases identified across five jurisdictions (58 confirmed, 1 probable). A majority of cases were reported collectively from NSW (31%), Victoria (24%), and Queensland (22%) (Table 4). Specimen collection dates among cases ranged from 05 November 2018 to 25 May 2019 with onset dates of illness among the 40/59 cases with onset data available ranging from 03 November 2018 and 20 May 2019. Cases peaked in December 2018, driven predominantly by Victorian cases and a large proportion of NSW cases occurring in the week prior to Christmas (Figure 4). Case frequency declined in March 2019, no cases were reported in April, and one SA case was reported in late May 2019.

Table 4: Counts of national *Salmonella* Heidelberg outbreak cases by jurisdiction and rates per 100,000 population, 01 November 2018–10 July 2019

State/Territory	NSW	Victoria	Queensland	WA	SA	Total*
Confirmed	18	14	12	8	6	**58**
Probable	-	-	1	-	-	**1**
Total Cases	18	14	13	8	6	**59**
Rate per 100,000 population†	0.22	0.21	0.26	0.46	0.23	**0.25**

NSW: New South Wales; WA: Western Australia; SA: South Australia

*Total rate was calculated using population estimates for the five jurisdictions

†Rates per 100,000 population were calculated using total case numbers and Quarterly ABS National, state and territory population estimates for December 2018

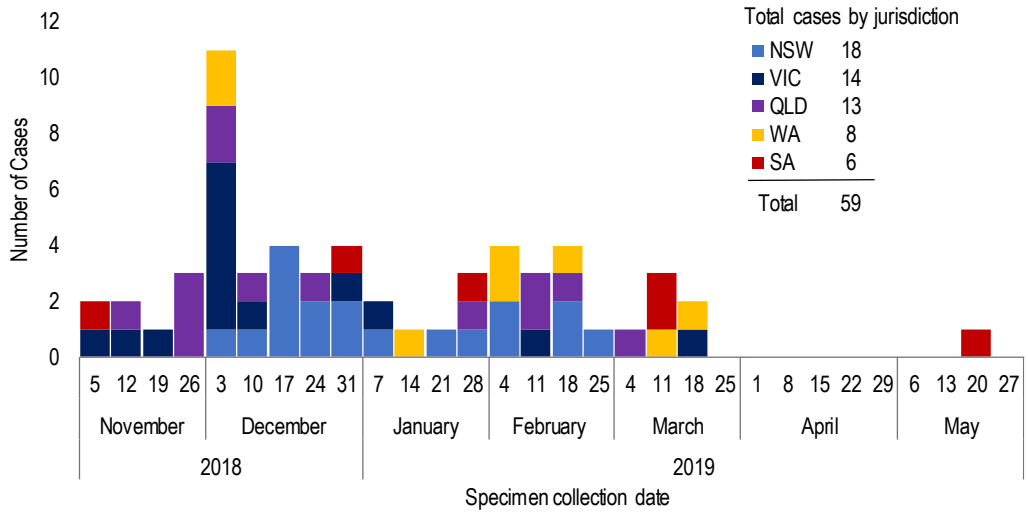


Figure 4: *Salmonella* Heidelberg outbreak cases by week of specimen collection and jurisdiction, November 2018–May 2019 (n=59)

Cases ranged in age between 2 months and 95 years with a median of 43 years, and were predominantly male (56%) (Table 5). Only three cases had a history of inter-state travel during

their exposure period. The median duration of symptoms reported for 28 cases was 7.5 days (range 5 to 40 days). Diarrhoea (90%) and abdominal cramps (81%) were the most common symptoms and 36% (16/45) of outbreak cases with information available were hospitalised (Table 5). The median duration of hospitalisation was 4 days (range 1 to 18 days), with a total of 71 cumulative days.

Table 5: National *Salmonella* Heidelberg outbreak cases by demographic and clinical characteristics, 01 November 2018–10 July 2019

Demographic		No. (%)	Clinical		No. (%)
Sex *(N=59)*	**Male**	33 (56%)	**Symptom**	**Diarrhoea** (N=42)	38 (90%)
	Female	26 (44%)		**Abdominal Cramps** (N=37)	30 (81%)
Age (years) *(N=59)*	**00–04**	10 (17%)	*Median duration (N=28): 7.5 days*		
	05–09	02 (03%)	*Min: 5 days*	**Fever** (N=38)	22 (58%)
	10–19	05 (08%)	*Max: 40 days*	**Vomiting** (N=38)	11 (30%)
Median: 43 years	**20–29**	02 (03%)		**Bloody Diarhoea** (N=34)	07 (21%)
Min: 2 months	**30–39**	07 (12%)	**Hospitalisation** *(N=45)*	**Yes**	16 (36%)
Max: 95 years	**40–49**	05 (08%)		**No**	29 (64%)
	50–59	12 (20%)		**Emergency Dept. Presentation only** (Among hospitalised cases, N=16)	03 (19%)
	60–69	06 (10%)	*Total bed days: 71 days*		
	70–79	03 (05%)	*Median duration: 4 days*		
	80–89	05 (08%)			
	≥90	02 (03%)			

Thirty-nine cases were interviewed between December 2018 and June 2019; NSW (14), Victoria (13), Queensland (5), WA (4), SA (3). Eight Queensland cases with specimen collection dates in 2018 were not interviewed as they were confirmed as outbreak cases several weeks after illness and recall was considered too poor. Twelve cases from NSW (3), WA (4), SA (2), and Victoria (1) either declined to be interviewed or were lost to follow up. One case from NSW provided limited exposure information via text message but was not interviewed.

Among interviewed cases, no place of home food purchase was consistently high (greater or equal to 75% of cases purchasing home food) across all jurisdictions (Table 6). Adjusting for seasonality, binomial probability calculations among food exposures showed that consumption rates by interviewed cases was significantly higher than the VFFS population for ten items (Table 7). However, there was little consistency in the description of items or place of purchase for these foods, and consequently there was insufficient evidence to develop a strong hypothesis on any single food item.

Table 6: Proportion of nationally interviewed *Salmonella* Heidelberg outbreak cases reporting 7-day shopping exposures by jurisdiction, 01 November 2018–10 July 2019

Shopping exposure	NSW	QLD	SA	VIC	WA	Total
Woolworths	06/11 (55%)	03/05 (60%)	02/03 (67%)	06/10 (60%)	03/04 (75%)	**20/33 (61%)**
Coles	06/12 (50%)	02/05 (40%)	01/03 (33%)	05/10 (50%)	00/04 (00%)	**14/34 (41%)**
IGA	09/12 (75%)	01/50 (20%)	02/03 (67%)	02/10 (40%)	02/04 (50%)	**16/34 (47%)**
Aldi	00/11 (00%)	03/05 (60%)	02/03 (67%)	04/10 (40%)	00/04 (00%)	**09/33 (27%)**
Foodworks	01/11 (09%)	00/05 (00%)	00/03 (0%)	01/10 (10%)	00/04 (00%)	**02/33 (06%)**

Table 7: Comparison of 7-day food exposures between nationally interviewed *Salmonella* Heidelberg outbreak cases and background Victorian populations rates, 01 November 2018–10 July 2019

Food exposure within 7 days*	Proportion cases exposed	Background probability†	% var‡	*p*-value
Any cooked chicken	20/35 (59%)	16%	42%	<0.001
Macadamia nuts	06/19 (32%)	07%	25%	0.001
Frozen vegetable products	03/12 (25%)	03%	22%	0.005
Lamb	13/26 (50%)	35%	15%	0.005
Sausages	16/27 (59%)	34%	25%	0.006
Sweet potatoes	09/14 (64%)	31%	33%	0.010
Sauce/chutney	14/17 (82%)	56%	27%	0.022
Salad mix in a sealed bag	10/21 (48%)	26%	22%	0.027
Avocado	10/15 (67%)	42%	25%	0.048

* Table presents food exposures found to be statistically significant ($p<0.05$)
† Based on the VFFS 2014–2016.
‡ Difference in proportion cases exposed compared to the background probability.

Literature review

Reports of thirty-eight *S.* Heidelberg outbreaks between 1961 and 2018 were identified through the literature review (Table 8). A large majority of published outbreaks occurred in the US (66%) and five involved multi-state distribution of food products. Excluding the seven outbreaks with evidence of a co-infection with another enteric pathogen, the 11 *S.* Heidelberg outbreak accounts which reported hospitalisation had hospitalisation rates between 3% and 38%. Outbreaks were predominantly foodborne with only two outbreaks associated with zoonotic transmission from dairy calves and one involving person-to-person spread. Likely outbreak sources were largely associated with poultry or eggs (53%), and almost a quarter of studies (21%) were unable to implicate specific food vehicles.[16,18,27,28]

There were no published accounts involving humans in Australia. However, OzFoodNet records document three outbreaks in Queensland, all associated with eggs, between 1996 and 2001. Reports and communications also indicated three clusters in NSW since 1995 (2005, 2009, 2016) with one in 2009 associated with a brand of raw chicken.

Table 8: Summary of reported *Salmonella* Heidelberg outbreak investigations globally, 1961–2018

Reference	Year	Country	Setting	No. cases	Hospitalised	Demographics	Likely source
Knox et al[29]	1961	United Kingdom	Community	77	n.r.	n.r.	Raw milk
Edgar and Lacey[30]	1962	United Kingdom	Hospital	9	n.r.	n.r.*	No source identified
Hutchinson RI[31]	1963	United Kingdom	Community	28	14%	Male: 61%	Raw milk
William et al[32]	1963	United Kingdom	Hospital	19	n.r.	n.r.	No source identified
Todd and Pivnic[33]	1968	US (Rhode Island)	Supermarket	3	n.r.	n.r.	Undercooked chicken
Todd and Pivnic[33]	1968	US (New Jersey)	Restaurant	100	n.r.	n.r.	Pork sandwiches
Rice et al‡[27]	1972	US (Puerto Rico)	Hospital	56	84%†	Infants	Person-to-person spread
Fontaine et al[34]	1976	US (Colorado)	Community	339	n.r.	n.r.	Cheddar cheese
Lyons et al[28]	1976	US (Connecticut)	Community & Hospital	5	n.r.	Male: 60%	Contact with calves and person-to-person spread
Lintz et al[35]	1976	US (New York)	Hospital	10	n.r.	n.r.	No source identified
French and Lowry[36]	1977	Jamaica	Community	23	n.r.	n.r.	n.r.
Weisse et al[37]	1986	US (New Mexico)	Convention	91	3%	n.r.	Eggs
Grady et al‡[38]	1986	US (multi-state)	Community	n.r.	n.r.	n.r.	Commercial frozen pasta
Carr et al‡[39]	1986	US (Oklahoma)	School Canteen	202	11%	n.r.	Chicken
O'Mahony et al[40]	1987	United Kingdom	Catered event	75	n.r.	n.r.	Raw chicken
Barnass et al[41]	1987	United Kingdom	Hospital	19	n.r.	n.r.	No source identified
Layton et al‡[42]	1993	US (New York)	Aged Care Facility	59	26%	n.r.	Raw chicken liver
Sivapalasingam et al[43]	1993	US (Minnesota)	n.r.	18	n.r.	n.r.	Lettuce
OzFoodNet§	1996	Australia (Queensland)	Airline	> 500	11%	n.r.	Chocolate parfait/Anglaise sauce
OzFoodNet§	1999	Australia (Queensland)	Aged Care Facility	7	n.r.	n.r.	Eggnog drink
Medus et al[44]	1999	US (Minnesota)	Restaurant	25	n.r.	n.r.	No source identified

Reference	Year	Country	Setting	No. cases	Hospitalised	Demographics	Likely source
OzFoodNet§	2001	Australia (Queensland)	Aged Care Facility	12	n.r.	n.r.	Egg flip (drink)
Medus et al[44]	2003	US (Minnesota)	Restaurant	36	n.r.	n.r.	Eggs or pancakes
Hornish et al[45]	2004	Canada	Restaurant	32	19%	Median age: 29 years	No source identified
Smith et al[46]	2005	US (Minnesota)	Community	4	25%	Median age: 30 years Male: 100%	Raw microwavable chicken products
OzFoodNet[47]	2009	Australia (NSW)	n.r.	5	~14%	n.r.	No source identified
Hoffmann et al[48] **CDC**[49]	2011	US (multi-state)	Community	190	19%	Median age: 14 years Male: 51%	Kosher broiled chicken liver
Routh et al[25]	2011	US (multi-state)	Community	136	36%	Median age: 23 years Male: 54%	Ground turkey
Rebolledo et al[15]	2011	International (Tanzania)	Airline	25	20%	Median age: 24 years	Milk tart/egg dish
Gicquelais et al‡[50]	2012	US (Arkansas)	Correctional Facility	51	n.r.	n.r.	Eggs
Gieraltowski et al[24] **AMA**[51] **CDC**[52]	2013	US (multi-state)	Community	634	38%	Mean age: 18 years Male: 50%	Chicken products**
Taylor et al[26]	2013	US (Tennessee)	Correctional Facility	23	13%	Mean age: 35 years	Mechanically separated chicken
Nakao et al‡[53]	2013	US (Alabama)	Catered event	80	79%	Median age: 35 years Male: 37%	Poor handling of raw chicken
Nakao et al‡[53]	2013	US (Colorado)	Catered event	7	71%	Median age: 45 years Male: 43%	No source identified
CDC[18] **Antony et al**[54]	2015–2017	US (multi-state)	Community	56	~35%	n.r.	Contact with dairy calves
Bedard et al[55]	2017	US (New York)	Catered event	28	n.r.	n.r.	Cross contamination with raw chicken
CDC‡[21]	2017–2018	US (multi-state)	Community	199	38%	Median age: 38 years Male: 52%	Kratom (herbal supplement)

n.r. Not Reported; AMA: American Medical Association; CDC: Centers of Disease Control and Prevention

* Cases were reported as infants.

† 44.5% (25/56) of cases had positive blood cultures, indicating invasive infection.

‡ Involved co-infection with another enteric pathogen: Rice at al. *Staphylococcus aureus* and Enterococcus, Grady et al. *Salmonella* Enteriditis, Carr et al. *Salmonella* Stanley, Layton et al. *Campylobacter jejuni*, Gicquelais et al. multiple *Salmonella* strains, Nakao et al. *Staphylococcus aureus*, Kratom: multiple *Salmonella* strains

§ Unpublished OzFoodNet Queensland records

** Included rotisserie chickens, chicken soup, chicken salad, and chicken pieces.

Laboratory investigation

The QHPHML identified 58 outbreak cases, all of which belonged to cgMLST complex type 2561. Isolates differed by 0–3 SNPs. The Phylogenetic Maximum Likelihood Tree (Figure 5) illustrates the relatedness of cases, whereby the length of branches represents distance between groups. Thirteen of the 58 outbreak isolates were phage typed and were all phage type 1 (7- Queensland, 6-SA). There were an additional 26 human isolates source from other notified *S.* Heidelberg cases during the outbreak period that were not closely related genetically to the outbreak strain.

The review of the phenotypic antimicrobial susceptibilities for 9/13 outbreak isolates in Queensland found the outbreak strain to be susceptible to a range of antibiotics including cephalosporins, fluoroquinolones, aminoglycosides, broad-spectrum penicillins, and trimethoprim-sulfamethoxazole. However, according to the *in sillico* WGS analysis, isolates harboured the antibiotic resistance gene *fosA7*, which if expressed could contribute to fosfomycin resistance. *fosA7* was also detected in 91% of international isolates included in the analysis. The *saf* operon was also present in all but one of the isolates included in this study, as well as 12% of international isolates

Non-human sources sequenced from Queensland Health's culture collection were animals (caprine, poultry, equine, reptile), food (macadamia nut), livestock, and environmental. There were no non-human isolates closely related to the current outbreak genotype, and none of the 65 international sequences clustered with the Australian outbreak strain. An additional descriptive review of historical QHFSS environmental/animal isolates from 1991 to 2017 indicated that *S.* Heidelberg is primarily detected in Queensland from ruminant sources (65%) and pork (24%). *S.* Heidelberg was only detected five times in macadamia nuts, accounting for less than 1% of total *Salmonella*-positive macadamia nuts isolates. Most environmental isolates were phage type 1, sources include ruminants (14), poultry (2), pork (1), and macadamia nuts (5). Foods testing and non-human samples submitted to QHFSS from June 2018 were also reviewed, with no *S.* Heidelberg-positive samples identified.

Food industry engagement

Details of the *S.* Heidelberg MJOI was circulated to FIF members. Following engagement, there were no enquires from, or information provided by any FIF members. Health Protection NSW contacted a chicken company implicated in a 2016 *S.* Heidelberg outbreak in NSW, who reported continued routine testing and that the most recent detection of *S.* Heidelberg was in 2017.

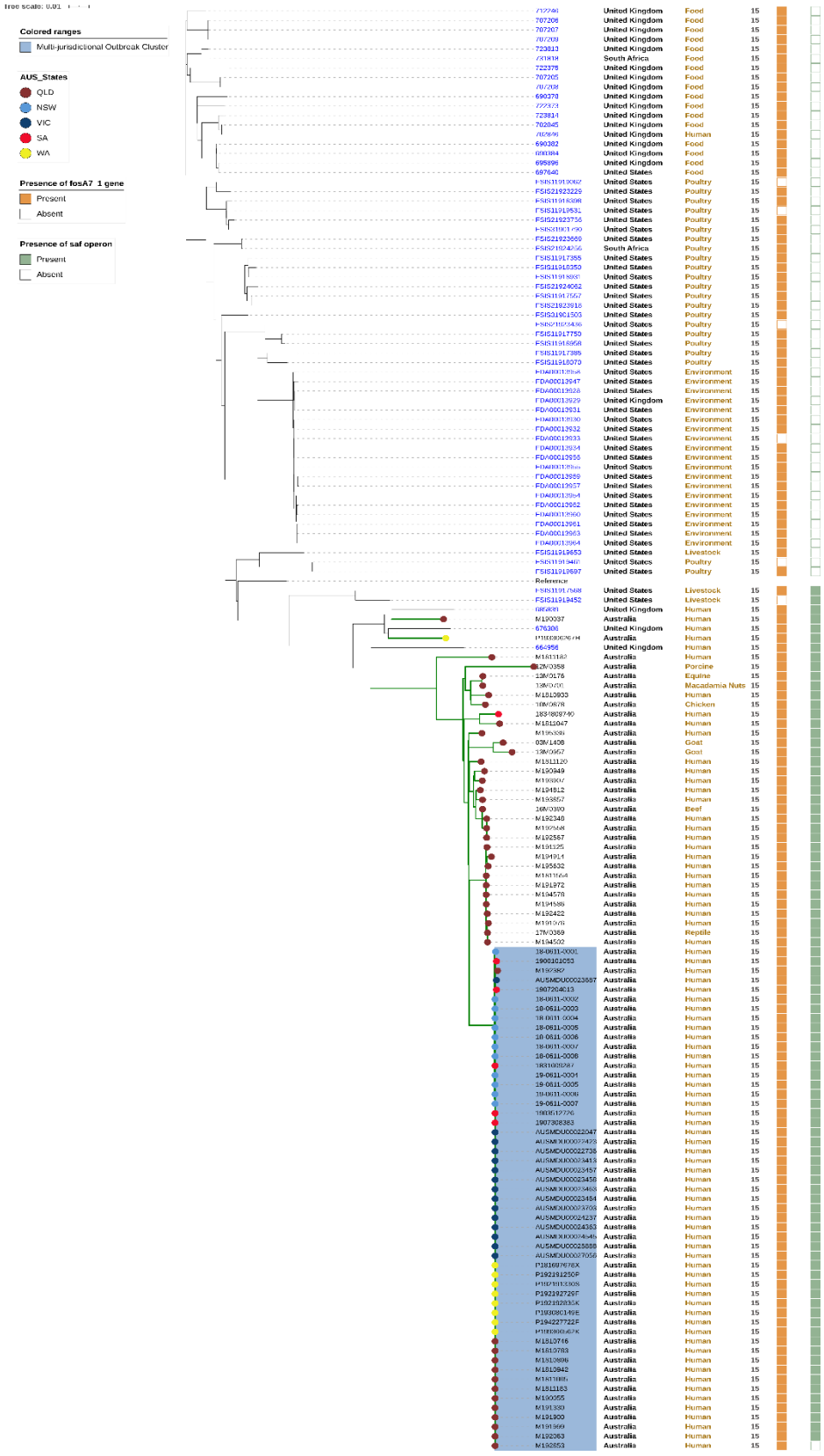


Figure 5: Phylogenetic Maximum Likelihood Tree built using SNP differences between isolates. Green colour branches and black isolate ID represent the Australian isolates whereas black colour branches and blue isolate ID represent the international isolates. Blue coloured range indicates the isolates belong to the multijurisdictional outbreak. Country of isolation, Source, Sequence type, presence of *fosA7* gene and presence of *saf* operon are also included in the tree from left to right after the isolate ID. The length of branches represents distance between groups as indicated by the scale bar.

Discussion

Here we describe an outbreak of a relatively uncommon serotype of *Salmonella* across five jurisdictions over a seven-month period. The outbreak involved 59 cases, yet there are likely more infections than were notified. It is estimated that for every notification of salmonellosis in Australia an additional 7 (95% CI: 4–16) cases occurred in the community, and the probability that a case is reported varies considerably by illness severity.[56] The investigation did not conclusively identify the source of infection. However, the distribution of cases across jurisdictions and low rates of inter-state travel during case exposure periods, indicates multi-state distribution of an outbreak vehicle, most likely a food product. The degree of relatedness between outbreak isolates (0-3 SNPs) is higher than previous reported outbreaks of *S.* Heidelberg; a 2011 outbreak associated with ground turkey had up to 17 SNPs difference across 44 isolates and there were 134 SNPs difference in a 2013 US multi-state outbreak associated with commercial poultry distribution.[24,25,48,57] Although the range of difference in the 2013 US outbreak was attributed to the involvement of multiple processing establishments and farms, it is not possible to conclude that this was either a common source outbreak, or involved multiple producers or farms.[57]

Whilst evidence globally suggests that *S.* Heidelberg outbreaks are most likely to be associated with poultry or eggs products, there was no evidence to suggest that the source of this outbreak was poultry-related. When compared to the Victorian population background consumption rates, egg exposure was not statistically increased among cases and whilst there was a statistically significant increase in the probability of exposure to 'Any cooked chicken' among cases, case reporting of poultry products consumed or place of purchase did not identify a common source. There was also no evidence of clustering between outbreak isolates and poultry isolates. Early in the investigation food purchased from IGA was considered a possible risk factor due to the high frequency among NSW cases. However, once national data were compiled the overall rate of purchases from IGA among cases decreased. Raw macadamia nuts were also considered due to their popularity during summer months, particularly over the Christmas period, and previous detections of *Salmonella* Heidelberg in Queensland.[20,58-60] However, nut contamination is more common among other serotypes such as Aberdeen, Birkenhead, and Hivttingfoss in Australia, and less than 1% of *Salmonella*-positive nut samples in Queensland between 1991 and 2017 detected *S.* Heidelberg.[20] There was therefore no compelling microbiological evidence to support hypotheses of a nut, poultry, or other specific source. The cgMLST complex type was also not identified among international or domestic isolates, suggesting a new or novel source of infection.

The median age of outbreak cases was higher than those reported in published outbreak accounts and the hospitalisation rate of 36% indicates comparatively high severity, particularly when compared to the global *salmonella* average of 22% and *S.* Heidelberg average of 12%.[16,22] Whilst drug resistance was not identified amongst outbreak isolates, severity was particularly consistent to those outbreaks with evidence of multi-drug resistance. Such as, Routh et al (2015) and Gieraltowski et al (2016), who reported 36% and 38% respectively. Drug-resistance is considered a contributor to increased hospitalisation.[54,61,62] However, there is dispute as to whether the association between multi-drug resistant strains and increased hospitalisation is related to increased virulence or other mechanisms, such as an increased risk of developing antimicrobial-resistant *salmonella* infection among persons taking antimicrobial drugs.[57,62] Hospitalisation may also be affected by factors such as patient age, climate, or poor hydration.[57]

Evidence based on US and Dutch *S.* Heidelberg isolates indicate an overall increase in antibiotic resistance profiles in recent *S.* Heidelberg isolates as compared to those from the 1980s and 1990s.[63-65] The emergence and spread of cephalosporin-resistant strains has been of particular concern.[66] Whilst outbreak isolates were pan-susceptible, an antibiotic resistance gene, which may contribute to fosfomycin resistance was identified in both outbreak isolates and 91% of international isolates analysed. The gene is reportedly limited to a few serotypes and was only identified in *S.* Heidelberg in Canada in 2017.[67] However, there is evidence to suggest that *fosA7* is widespread among *S.* Heidelberg isolates. Among isolates analysed in the 2013 US multi-state outbreak associated with commercial poultry, 100% (9/9) possessed the *fosA7* gene.[57] Although most non-typhoidal *Salmonella* infections do not require antibiotic treatment, the potential emergence of isolates with decreased susceptibility increases the risk of treatment failure and limits effective treatment options for severe infections.[63,64,66] Fosfomycin is noteworthy as it is increasingly used in many clinical and veterinary settings.[67] Furthermore, *fosA7* in *S.* Heidelberg isolates may confer high resistance levels and can be transferable. Recent genomic analysis has also highlighted the potential contributing role of *saf* fimbrial genes, which were present in all Australian isolates analysed, to increased disease severity in *S.* Heidelberg infections.[54] The combined potential for future drug resistance, propensity for severe infection, and past evidence of invasiveness, suggests that *S.* Heidelberg may be of particular public health concern in Australia.[66] While the association between *S.* Heidelberg and severe infection is relatively well documented outside of Australia, this investigation provides further evidence towards the potential for severe *S.* Heidelberg infection in Australia.

Study strengths

A key strength of this investigation was the use of WGS to confirm relatedness of cases and exclude sporadic infections. Core genome SNP analysis of *S.* Heidelberg isolates has become the recommended approach to distinguish outbreak strains.[68] WGS offers higher discriminatory power than previous traditional typing methods, providing more confidence that cases are outbreak related.[68-72] *S.* Heidelberg is often highly clonal and previous molecular typing such as pulse-field gel electrophoresis were often unable to distinguish between outbreak and sporadic *S.* Heidelberg strains.[66,68] In the context of an MJOI, WGS was crucial as it enabled geographically and demographically heterogeneous cases to be linked and sporadic cases, particularly those in Queensland, to be confidently excluded.

Attributing infection to a single food item can be challenging as *Salmonella* is a contaminant of many foods and food recall among cases can be poor.[73] As such, the investigation methodically went through stages of analysis and combined available laboratory evidence. The investigation used a recent Australian population-based survey to compare food consumption frequency and leveraged all available WGS resources, such as laboratory collections and international sequences. WGS is increasingly used combined with traditional methods, such as epidemiological analyses to help explore and identify the source of outbreaks and has successfully been used in many multi-state outbreaks to determine how closely clinical, environmental, and food sample isolates are to one another genetically.[68,70-73]

Study limitations

There were several limitations to this investigation. Whilst not logistically possible to actively case find, case ascertainment through laboratory confirmed notifications will not have detected all cases with cases who experienced mild symptoms most likely to be missed.[56] As the outbreak was detected several weeks after infection began, there were also diminished opportunities to collect risk factor data from all cases. Prior to the identification of an MJOI, interviews were completed for cases in jurisdictions where *S.* Heidelberg was unusual. However, as Queensland has a higher rates of *S.* Heidelberg infection, as well as a large burden of *Salmonella* infection overall, *S.* Heidelberg cases were not routinely interviewed. As the accuracy of food history recall often deteriorates over time, collecting risk factor data on Queensland cases was not feasible. Future cases of *S.* Heidelberg infection should be routinely interviewed by jurisdictions to improve case ascertainment for future outbreak investigations. The overall pace of the investigation was also slowed by the lengthy turnaround times for WGS, particularly given that some isolates and sequences were sent inter-state.[69,70,72,73]

The large number of cases interviewed with the abbreviated trawler early in the investigation may also be a limitation. The standard OzFoodNet approach, which considers both resourcing and the likelihood of association with eggs or poultry, is to undertake the abbreviated trawler first. Then where a common food item is not identified, the full trawler is used. As such, most cases early in the outbreak were interviewed using the abbreviated trawler which led to low sample sizes for many risk factor items. Given that prior jurisdictional investigations had not been able to develop a hypothesis regarding potential sources of infection, once the MJOI was established in March 2019 a specific questionnaire was designed by OzFoodNet Queensland for *S.* Heidelberg. However, the decline in case numbers soon after the establishment of the MJOI prevented greater use of this questionnaire.

VFFS data are also based on a Victorian population, which may not accurately represent food consumption patterns of all states. As the Victorian outbreak sample size was low, it was not possible to restrict analysis to test the impact of this potential bias. Alternative sources of food consumption data, such as supermarket loyalty dataset, may have provided an alternative to the VFFS. However, such datasets were not available to investigators. Whilst *S.* Heidelberg has shown to have high levels of invasiveness in Queensland, invasiveness of this outbreak strain was not measured and cannot be inferred from the hospitalisation rate. Lastly, as epidemiological evidence was insufficient to develop a strong hypothesis on any single food item, sampling was not considered feasible and with decreasing cases numbers it was not possible to undertake an analytical study.

Conclusion

Whilst this MJOI did not identify the source of *S.* Heidelberg infection, it was most likely associated with multi-state distribution of a food product. This investigation highlights that whilst poultry or eggs are most likely to be associated with *S.* Heidelberg outbreaks, new or novel sources of *S.* Heidelberg infection in Australia need to be considered. Despite some downsides to WGS as an outbreak investigation tool, WGS played a crucial role to this investigation, both in linking geographically and demographically heterogeneous cases and exploring potential links to transmission sources. A platform for real-time exchange of sequencing data in Australia and potential future implementation of routine WGS for investigation of notified salmonellosis cases, including comparison to local and international strain data, may enable more timely detection and investigation of outbreaks and adds dimension to investigations by assisting hypothesis development. Although *S.* Heidelberg outbreaks are relatively uncommon in

Australia, given the high rate of hospitalization in this outbreak and the near universal presence of *saf* fimbrial genes in the implicated strain, future cases warrant prompt investigation to assess severity and invasiveness.

References

1. OzFoodNet. Guidelines for the epidemiological investigation of multi-jurisdictional outbreaks that are potentially foodborne. [Internet]. Canberra: Department of Health; 2017. [cited 2019 Mar 29]. Available from: https://www.health.gov.au/internet/main/publishing.nsf/Content/cdna-ozfoodnet.htm.

2. Heymann DL, editor. Control of communicable diseases manual. 20th ed. Washington: American Public Health Association Press; 2015.

3. Galanis E, Lo Fo Wong DM, Patrick ME, Binsztein N, Cieslik A, Chalermchikit T, et al. Web-based surveillance and global *Salmonella* distribution, 2000–2002. Emerging Infectious Diseases. 2006;12(3):381-8. DOI: 10.3201/eid1205.050854

4. Parisi A, Crump JA, Stafford R, Glass K, Howden BP, Kirk MD. Increasing incidence of invasive nontyphoidal *Salmonella* infections in Queensland, Australia, 2007-2016. PLoS Neglected Tropical Diseases. 2019;13(3):e0007187. DOI: 10.1371/journal.pntd.0007187

5. Kirk M, Glass K, Ford L, Brown K, Hall G. Foodborne illness in Australia: annual incidence circa 2010. Canberra: Commonwealth of Australia; 2014.

6. Ford L, Haywood P, Kirk MD, Lancsar E, Williamson DA, Glass K. Cost of *Salmonella* infections in Australia, 2015. Journal of Food Protection. 2019;82(9):1607-14. DOI: 10.4315/0362-028x.Jfp-19-105

7. Astridge K, McPherson M, Kirk M, Knope K, Kardamanidis K, Bell R. Foodborne disease outbreaks in Australia 2001–2009. Food Australia. 2011;64(12):44-50.

8. Ford L, Glass K, Veitch M, Wardell R, Polkinghorne B, Dobbins T, et al. Increasing incidence of *Salmonell*a in Australia, 2000–2013. PLoS One. 2016;11(10):e0163989. DOI: 10.1371/journal.pone.0163989

9. National notifiable diseases surveillance system report [Internet]. Commonwealth of Australia. 2019 [cited 6 June 2019]. Available from: http://www9.health.gov.au/cda/source/cda-index.cfm.

10. Beard F, Stafford R, Terry J, Morgan G, Ayyar A, Birrell F. Risk factors for sporadic *Salmonella* Birkenhead infection in Queensland and northern New South Wales: a case control study. New South Wales Public Health Bulletin. 2004;15(9-10):172-7. DOI: 10.1071/NB04037

11. Ziehm D, Dreesman J, Campe A, Kreienbrock L, Pulz M. Risk factors associated with sporadic salmonellosis in adults: a case-control study. Epidemiology and Infection. 2013;141(2):284-92. DOI: 10.1017/s0950268812000684

12. Hennessy TW, Cheng LH, Kassenborg H, Ahuja SD, Mohle-Boetani J, Marcus R, et al. Egg consumption is the principal risk factor for sporadic *Salmonella* serotype Heidelberg infections: a case-control study in FoodNet sites. Clinical Infectious Diseases. 2004;38 Suppl 3:S237-43. DOI: 10.1086/381593

13. Middleton D, Savage R, Tighe MK, Vrbova L, Walton R, Whitfield Y, et al. Risk factors for sporadic domestically acquired *Salmonella serova*r Enteritidis infections: a case-control study in Ontario, Canada, 2011. Epidemiology and Infection. 2014;142(7):1411-21. DOI: 10.1017/s0950268813001945

14. Patrick ME, Adcock PM, Gomez TM, Altekruse SF, Holland BH, Tauxe RV, et al. *Salmonella* enteritidis infections, United States, 1985–1999. Emerging Infectious Diseases. 2004;10(1):1-7. DOI: 10.3201/eid1001.020572

15. Rebolledo J, Garvey P, Ryan A, O'Donnell J, Cormican M, Jackson S, et al. International outbreak investigation of *Salmonella* Heidelberg associated with in-flight catering. Epidemiology and Infection. 2014;142(4):833-42. DOI: 10.1017/s0950268813001714

16. Chittick P, Sulka A, Tauxe RV, Fry AM. A summary of national reports of foodborne outbreaks of *Salmonella* Heidelberg infections in the United States: clues for disease prevention. Journal of Food Protection. 2006;69(5):1150-3. DOI: 10.4315/0362-028x-69.5.1150

17. Chai SJ, Cole D, Nisler A, Mahon BE. Poultry: the most common food in outbreaks with known pathogens, United States, 1998–2012. Epidemiology and Infection. 2017;145(2):316-25. DOI: 10.1017/s0950268816002375

18. Centers for Disease Control and Prevention. Multistate outbreak of multi-drug resistant *Salmonella* Heidelberg infections linked to contact with dairy calves (Final update) Atlanta2018 [cited 2019 31 May 2019]. Available from: https://www.cdc.gov/salmonella/heidelberg-11-16/index.html.

19. Currie A, MacDougall L, Aramini J, Gaulin C, Ahmed R, Isaacs S. Frozen chicken nuggets and strips and eggs are leading risk factors for *Salmonella* Heidelberg infections in Canada. Epidemiology and Infection. 2005;133(5):809-16. DOI: 10.1017/s0950268805004383

20. Fearnley EJ, Lal A, Bates J, Stafford R, Kirk MD, Glass K. *Salmonella* source attribution in a subtropical state of Australia: capturing environmental reservoirs of infection. Epidemiology and Infection. 2018;146(15):1903-8. DOI: 10.1017/s0950268818002224

21. Centers for Disease Control and Prevention. Multistate outbreak of *Salmonella* infections linked to Kartom (Final update) [Internet]. Atlanta: Centers for Disease Control and Prevention; 2018 [cited 2019 May 31]. Available from: https://www.cdc.gov/salmonella/kratom-02-18/.

22. Jones TF, Ingram LA, Cieslak PR, Vugia DJ, Tobin-D'Angelo M, Hurd S, et al. Salmonellosis outcomes differ substantially by serotype. Journal Infectious Diseases. 2008;198(1):109-14. DOI: 10.1086/588823

23. Green A, Defibaugh-Chavez S, Douris A, Vetter D, Atkinson R, Kissler B, et al. Intensified sampling in response to a *Salmonella* Heidelberg outbreak associated with multiple establishments within a single poultry corporation. Foodborne Pathogens and Disease. 2018;15(3):153-60. DOI: 10.1089/fpd.2017.2340

24. Gieraltowski L, Higa J, Peralta V, Green A, Schwensohn C, Rosen H, et al. National outbreak of multidrug resistant *Salmonella* Heidelberg infections linked to a single poultry company. PLoS One. 2016;11(9):e0162369. DOI: 10.1371/journal.pone.0162369

25. Routh JA, Pringle J, Mohr M, Bidol S, Arends K, Adams-Cameron M, et al. Nationwide outbreak of multidrug-resistant *Salmonella* Heidelberg infections associated with ground turkey: United States, 2011. Epidemiology and Infection. 2015;143(15):3227-34. DOI: 10.1017/s0950268815000497

26. Taylor AL, Murphree R, Ingram LA, Garman K, Solomon D, Coffey E, et al. Multidrug-resistant *Salmonella* Heidelberg associated with mechanically separated chicken at a correctional facility. Foodborne Pathogens and Disease. 2015;12(12):950-2. DOI: 10.1089/fpd.2015.2008

27. Rice PA, Craven C, Wells JG. *Salmonella* Heidelberg enteritis and bacteremia. an epidemic on two pediatric wards. American Journal of Medicine. 1976;60(4):509-16. DOI: 10.1016/0002-9343(76)90717-8

28. Lyons RW, Samples CL, DeSilva HN, Ross KA, Julian EM, Checko PJ. An epidemic of resistant *Salmonella* in a nursery. Animal-to-human spread. Journal of the American Medical Association. 1980;243(6):546-7. DOI: 10.1001/jama.1980.03300320038021

29. Knox WA, Galbraith NS, Lewis MJ, Hickie GC, Johnston HH. A milk-borne outbreak of food poisoning due to *Salmonella* Heidelberg. Journal of Hygiene (Lond). 1963;61:175-85. DOI: 10.1017/s002217240002088x

30. Edgar WM, Lacey BW. Infection with *Salmonella* Heidelberg: an outbreak presumptively not foodborne. The Lancet. 1963;281(7273):161-3. DOI: 10.1016/S0140-6736(63)91040-7

31. Hutchinson RI. Milk-borne outbreak of *Salmonella* Heidelberg. British Medical Journal. 1964;1(5381):479-80. DOI: 10.1136/bmj.1.5381.479

32. Wallace WF, Wilson TS. An outbreak of *Salmonella* Heidelberg infection in a general medical unit. Treatment with ampicillin and neomycin. Ulster Medical Journal. 1964;33(2):101-9.

33. Todd E, Pivnick H. Public health problems associated with barbecued food. A review. Journal of Milk and Food Technology. 1973;36(1):1-18. DOI: 10.4315/0022-2747-36.1.1

34. Fontaine RE, Cohen ML, Martin WT, Vernon TM. Epidemic salmonellosis from cheddar cheese: surveillance and prevention. American Journal of Epidemiology. 1980;111(2):247-53. DOI: 10.1093/oxfordjournals.aje.a112892

35. Lintz D, Kapila R, Pilgrim E, Tecson F, Dorn R, Louria D. Nosocomial *Salmonella* epidemic. Archives of Internal Medicine. 1976;136(9):968-73. DOI: 10.1001/archinte.1976.03630090008004

36. French GL, Lowry MF. An outbreak of *Salmonella* Heidelberg infection in Jamaica. West Indian Medical Journal. 1979;28(1):40-4.

37. Weisse P, Libbey E, Nims L, Gutierrez P, Weber N, Voorhees C. *Salmonella* Heidelberg outbreak at a convention--New Mexico. Morbidity and Mortality Weekly Report. 1986;35(6):91.

38. Grady G, Parkin WE, Morse DL. Epidemiological notes and reports Salmonellosis outbreaks associated with commercial frozen pasta – Massachusetts, New Jersey, New York. Morbidity and Mortality Weekly Report. 1986;35(23):387.

39. Carr R, Brown S, Goodall A, Head D, Stacy B, Bryce R. Epidemiological notes and reports salmonellosis in a school system - Oklahoma. Morbidity and Mortality Weekly Report. 1987;36(5):74-5.

40. O'Mahony M, Barnes H, Stanwell-Smith R, Dickens T, Jephcott A. An outbreak of *Salmonella* Heidelberg infection associated with a long incubation period. Journal of Public Health Medicine. 1990;12(1):19-21. DOI: 10.1093/oxfordjournals.pubmed.a042499

41. Barnass S, O'Mahony M, Sockett PN, Garner J, Franklin J, Tabaqchali S. The tangible cost implications of a hospital outbreak of multiply-resistant *Salmonella*. Epidemiology and Infection. 1989;103(2):227-34. DOI: 10.1017/s0950268800030570

42. Layton MC, Calliste SG, Gomez TM, Patton C, Brooks S. A mixed foodborne outbreak with *Salmonella* Heidelberg and Campylobacter jejuni in a nursing home. Infection Control and Hospital Epidemiology. 1997;18(2):115-21. DOI: 10.2307/30142400

43. Sivapalasingam S, Friedman CR, Cohen L, Tauxe RV. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. Journal of Food Protection. 2004;67(10):2342-53. DOI: 10.4315/0362-028X-67.10.2342

44. Medus C, Smith KE, Bender JB, Leano F, Hedberg CW. *Salmonella* infections in food workers identified through routine public health Surveillance in Minnesota: impact on outbreak recognition. Journal of Food Protection. 2010;73(11):2053-8. DOI: 10.4315/0362-028X-73.11.2053

45. Hornish L, Hislop N, Chui L, Tyrrell G. Restaurant foodhandler-associated outbreak of *Salmonella* Heidelberg gastroenteritis identified by calls to a local telehealth service, Edmonton, Alberta, 2004. Canada Communicable Disease Report. 2005;31(10):105-10.

46. Smith KE, Medus C, Meyer SD, Boxrud DJ, Leano F, Hedberg CW, et al. Outbreaks of salmonellosis in Minnesota (1998 through 2006) associated with frozen, microwaveable, breaded, stuffed chicken products. Journal of Food Protection. 2008;71(10):2153-60. DOI: 10.4315/0362-028X-71.10.2153

47. OzFoodNet Working Group. Monitoring the incidence and causes of disease potentially transmitted by food in Australia: annual report of the OzFoodNet network, 2009. Communicable Diseases Intelligence. 2010;34(4):396-426.

48. Hoffmann M, Zhao S, Luo Y, Li C, Folster JP, Whichard J, et al. Genome sequences of five *Salmonella enterica* serovar Heidelberg isolates associated with a 2011 multistate outbreak in the United States. Journal of Bacteriology. 2012;194(12):3274-5. DOI: 10.1128/jb.00419-12

49. Centers for Disease Control and Prevention. Multistate outbreak of human *Salmonella* Heidelberg infections linked to "Kosher Broiled Chicken Livers" from Schreiber Processing Corporation (Final update) Atlanta: Centers of Disease; 2012 [cited 2019 30 May]. Available from: https://www.cdc.gov/salmonella/2011/chicken-liver-1-11-2012.html.

50. Gicquelais RE, Morris JF, Matthew SH, Gladden L, Safi H, Grayson C. Multi-serotype *Salmonella* outbreaks in two prisons - Arkansas, August 2012. Morbidity and Mortality Weekly Report. 2014;63(8):167-73.

51. American Medical Association. Nearly 400 cases reported in *Salmonella* Heidelberg OutbreakNews from the Centers for Disease Control and Prevention. Journal of the American Medical Association. 2013;310(24):2608-. DOI: 10.1001/jama.2013.283915

52. Grinnell M, Provo G, Marsden-Haug N, Stigi K, De Bess E, Kissler B, et al. Outbreak of *Salmonella* Heidelberg infections linked to a single poultry producer -- 13 states, 2012–2013. Morbidity and Mortality Weekly Report. 2013;62(27):553-6.

53. Nakao JH, Talkington D, Bopp CA, Besser J, Sanchez ML, Guarisco J, et al. Unusually high illness severity and short incubation periods in two foodborne outbreaks of *Salmonella* Heidelberg infections with potential coincident Staphylococcus aureus intoxication. Epidemiology and Infection. 2018;146(1):19-27. DOI: 10.1017/s0950268817002655

54. Antony L, Behr M, Sockett D, Miskimins D, Aulik N, Christopher-Hennings J, et al. Genome divergence and increased virulence of outbreak associated *Salmonella* enterica subspecies enterica serovar Heidelberg. Gut Pathogens. 2018;10:53. DOI: 10.1186/s13099-018-0279-0

55. Bedard B, Voos K, Pettit P, Ferris C, Balduf S. A salmonellosis Heidelberg outbreak traced to roast beef served by caterer. American Journal of Epidemiology and Public Health. 2018;2(1):010-3.

56. Hall G, Yohannes K, Raupach J, Becker N, Kirk M. Estimating community incidence of *Salmonella*, Campylobacter, and Shiga toxin-producing Escherichia coli infections, Australia. Emerging Infectious Diseases. 2008;14(10):1601-9. DOI: 10.3201/eid1410.071042

57. Etter AJ, West AM, Burnett JL, Wu ST, Veenhuizen DR, Ogas RA, et al. *Salmonella* Heidelberg food isolates associated with a salmonellosis outbreak have enhanced stress tolerance capabilities. Applied and Environmental Microbiology. 2019. DOI: 10.1128/aem.01065-19

58. Wareing PW, Nicolaides L, Twiddy DR. Nuts and nut products. In: Lund BM, Baird-Parker TC, Gould GW, editors. The microbiological safety and quality of food. 1st ed. Maryland: Aspen Publishers; 2000. p. 919-40.

59. Zhang G, Hu L, Melka D, Wang H, Laasri A, Brown EW, et al. Prevalence of *Salmonella* in cashews, hazelnuts, macadamia nuts, pecans, pine nuts, and walnuts in the United States. Journal of Food Protection. 2017;80(3):459-66. DOI: 10.4315/0362-028x.Jfp-16-396

60. Munck N, Smith J, Bates J, Glass K, Hald T, Kirk MD. Source attribution of *Salmonella* in macadamia nuts to animal and environmental reservoirs in Queensland, Australia. Foodborne Pathogens and Disease. 2020;17(5):357-64. DOI: 10.1089/fpd.2019.2706

61. Hoffmann M, Zhao S, Pettengill J, Luo Y, Monday SR, Abbott J, et al. Comparative genomic analysis and virulence differences in closely related *salmonella enterica* serotype Heidelberg isolates from humans, retail meats, and animals. Genome Biology and Evolution. 2014;6(5):1046-68. DOI: 10.1093/gbe/evu079

62. Varma JK, Greene KD, Ovitt J, Barrett TJ, Medalla F, Angulo FJ. Hospitalization and antimicrobial resistance in *Salmonella* outbreaks, 1984–2002. Emerging Infectious Diseases. 2005;11(6):943. DOI: 10.3201/eid1106.041231

63. Patchanee P, Zewde BM, Tadesse DA, Hoet A, Gebreyes WA. Characterization of multidrug-resistant *Salmonella enterica* serovar Heidelberg isolated from humans and animals. Foodborne Pathogens and Disease. 2008;5(6):839-51. DOI: 10.1089/fpd.2008.0149

64. Liakopoulos A, Geurts Y, Dierikx CM, Brouwer MS, Kant A, Wit B, et al. Extended-spectrum cephalosporin-resistant *Salmonella enterica* serovar Heidelberg strains, the Netherlands. Emerging Infectious Diseases. 2016;22(7):1257-61. DOI: 10.3201/eid2207.151377

65. Medalla F, Gu W, Mahon BE, Judd M, Folster J, Griffin PM, et al. Estimated incidence of antimicrobial drug-resistant nontyphoidal *Salmonella* infections, United States, 2004–2012. Emerging Infectious Diseases. 2016;23(1):29-37. DOI: 10.3201/eid2301.160771

66. Han J, Lynne AM, David DE, Tang H, Xu J, Nayak R, et al. DNA sequence analysis of plasmids from multidrug resistant *Salmonella enterica* serotype Heidelberg isolates. PLoS One. 2012;7(12):e51160. DOI: 10.1371/journal.pone.0051160

67. Rehman MA, Yin X, Persaud-Lachhman MG, Diarra MS. First detection of a fosfomycin resistance gene, fosA7, in *Salmonella enterica* serovar Heidelberg isolated from broiler chickens. Antimicrobial Agents and Chemotherapy. 2017;61(8):e00410-17. DOI: 10.1128/AAC.00410-17

68. Bekal S, Berry C, Reimer AR, Van Domselaar G, Beaudry G, Fournier E, et al. Usefulness of high-quality core genome single-nucleotide variant analysis for subtyping the highly clonal and

the most prevalent *Salmonella enterica* serovar Heidelberg clone in the context of outbreak investigations. Journal of Clinical Microbiology. 2016;54(2):289-95. DOI: 10.1128/jcm.02200-15

69. Ford L, Carter GP, Wang Q, Seemann T, Sintchenko V, Glass K, et al. Incorporating whole-genome sequencing into public health surveillance: lessons from prospective sequencing of *Salmonella* Typhimurium in Australia. Foodborne Pathogens and Disease. 2018;15(3):161-7. DOI: 10.1089/fpd.2017.2352

70. Vincent C, Usongo V, Berry C, Tremblay DM, Moineau S, Yousfi K, et al. Comparison of advanced whole genome sequence-based methods to distinguish strains of *Salmonella enteric*a serovar Heidelberg involved in foodborne outbreaks in Quebec. Food Microbiology. 2018;73:99-110. DOI: 10.1016/j.fm.2018.01.004

71. Phillips A, Sotomayor C, Wang Q, Holmes N, Furlong C, Ward K, et al. Whole genome sequencing of *Salmonella* Typhimurium illuminates distinct outbreaks caused by an endemic multi-locus variable number tandem repeat analysis type in Australia, 2014. BMC Microbiology. 2016;16:211. DOI: 10.1186/s12866-016-0831-3

72. Shariat N, Sandt CH, DiMarzio MJ, Barrangou R, Dudley EG. CRISPR-MVLST subtyping of *Salmonella enterica* subsp. enterica serovars Typhimurium and Heidelberg and application in identifying outbreak isolates. BMC Microbiology. 2013;13:254. DOI: 10.1186/1471-2180-13-254

73. Crowe SJ, Green A, Hernandez K, Peralta V, Bottichio L, Defibaugh-Chavez S, et al. Utility of combining whole genome sequencing with traditional investigational methods to solve foodborne outbreaks of *salmonella* infections associated with chicken: a new tool for tackling this challenging food vehicle. Journal of Food Protection. 2017;80(4):654-60. DOI: 10.4315/0362-028x.Jfp-16-364

Appendix 1: Situation Report

MJOI 2019-002
SITUATION REPORT

Outbreak name	Locally acquired *Salmonella* Heidelberg	**Report Number**	1
Start Date	05/04/2019	**Date of report**	3/5/2019
Lead Agency	Queensland Health	**Lead Epidemiologist**	Russell Stafford / Elenor Kerr

Current Outbreak Case Definitions

Confirmed outbreak case:

Any notified case of *Salmonella* Heidelberg infection genotyped as cgMLST 2561 and/or highly related on phylogenetic analysis to the outbreak sequence (ENA Accession ERS3357031), with an illness onset since 1 November 2018 and resided in Australia for any period during the 10 days prior to onset.

Probable outbreak case:

Any notified case of *Salmonella* Heidelberg infection with an epidemiological link to a confirmed outbreak case, in the absence of whole genome sequencing.

Summary of Investigation

Descriptive epidemiological evidence:

To date (03 May 2019), there have been 57 cases of *S*. Heidelberg associated with this outbreak; 56 confirmed cases and 1 probable case.

Onset dates of illness among interviewed cases (n=36): 3/11/2018 – 14/03/2019

Cases have been reported across five jurisdictions to date (Table 1).

Table 1: Counts of S. *Heidelberg* outbreak cases since 1 Nov 2018, by jurisdiction

Jurisdiction	Confirmed	Probable	Total	Hospital admissions
NSW	18		18	8
VIC	14	-	13	3
QLD	12	1	13	2
WA	8	-	8	1
SA	4	-	4	1
Total	**56**	**1**	**57**	**15**

- Case numbers have declined but still ongoing following a peak in December 2018 (Figure 1).
- Specimen collection dates among cases by week and jurisdiction (Figure 2).
- Outbreak cases include 32 males (56%) and 25 females (44%).
- Age range of cases is 2 months to 95 years old (median 45 years).
- Frequency of cases by age group:
  - 0-4 years (10 cases), 5-19 years (7), 20-59 years (24), 60+ years (16)
- Current hospitalisation rate is 34% (15 of 43 known).

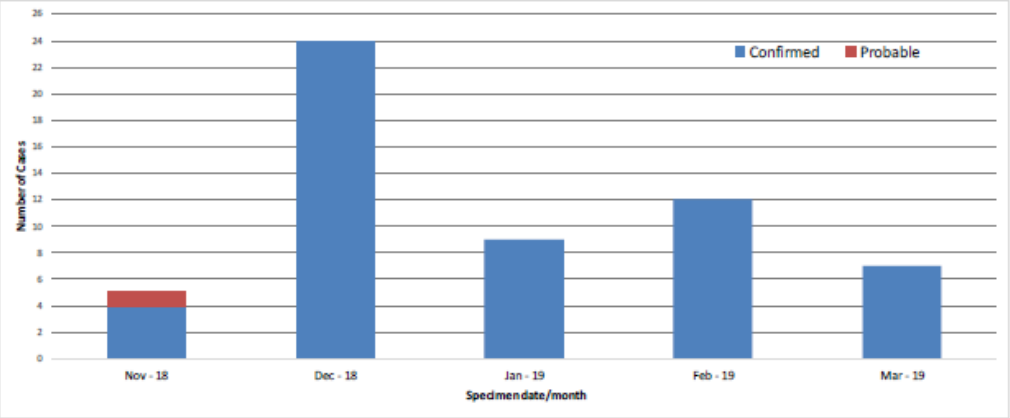


Figure 1: *Salmonella* Heidelberg outbreak cases by month of specimen collection (n=57).

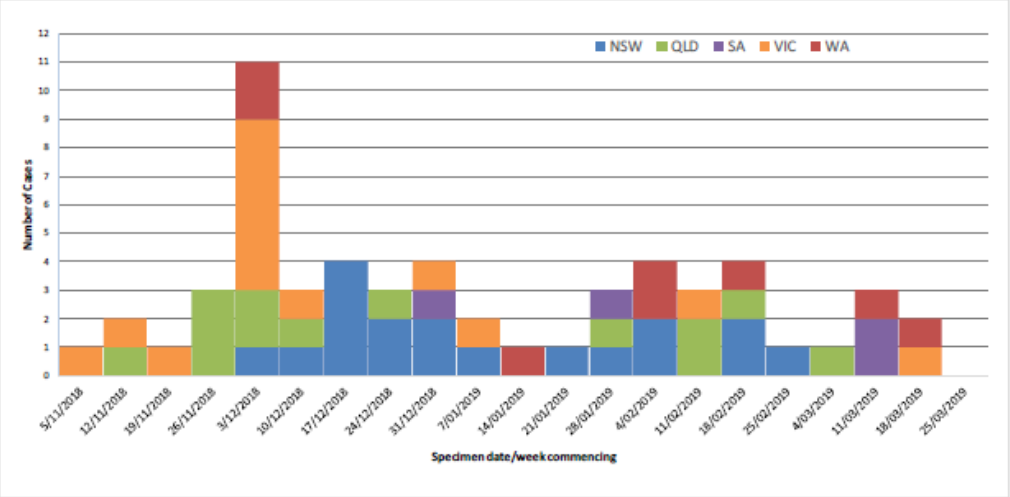


Figure 2: *Salmonella* Heidelberg outbreak cases by week of specimen collection and jurisdiction (n=57).

Food frequencies from hypothesis-generating questionnaires:

To date, 36 confirmed cases of *S*. Heidelberg infection have been interviewed across 5 jurisdictions. This includes 14 from NSW, 11 from Victoria, 5 from QLD, 4 from WA, and 2 from SA.

Based on information collected from these case investigations, consumption rates for selected foods among outbreak cases were compared with those of the general population using data from a population food frequency survey conducted in Victoria between 2014 and 2016.

Although binomial probability calculations highlighted some potential foods of interest, there was insufficient evidence to develop a strong hypothesis on any single food item. Ongoing analyses using data collected from newly notified cases is required.

Environmental investigation

Nil to date.

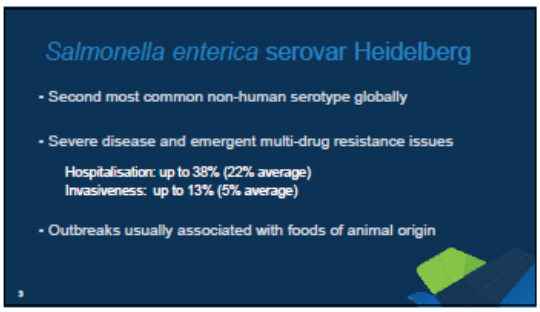
Control Measures to date

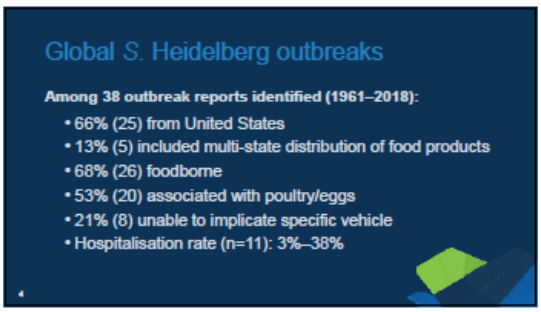
Nil to date.

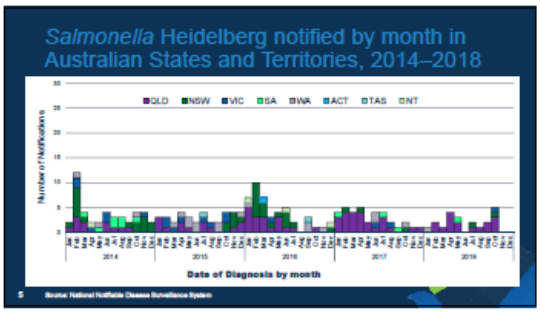
Actions

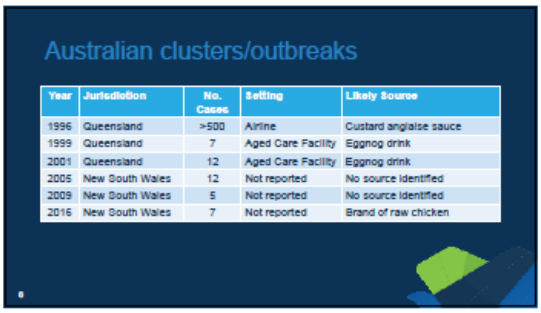
- Public Health Microbiology Reference Laboratory Queensland to report all isolates of *Salmonella* Heidelberg genotyped as cgMLST 2561 and/or highly related on phylogenetic analysis to the outbreak sequence (ENA Accession ERS3357031) to OzFoodNet.
- OzFoodNet sites to continue the epidemiological investigation of confirmed outbreak cases.

Appendix 2: Outbreak summary presentation, OzFoodNet face-to-face meeting, December 2019

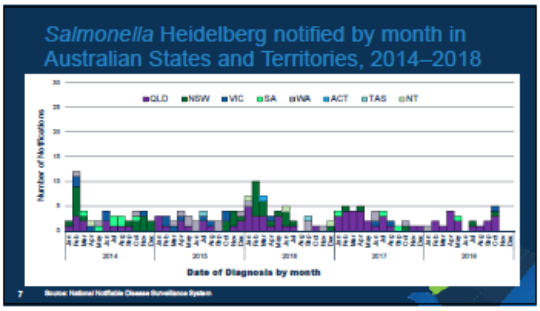


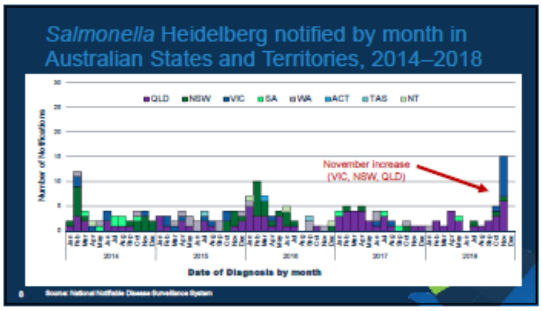


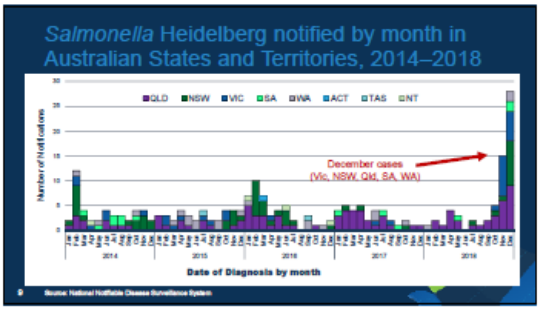


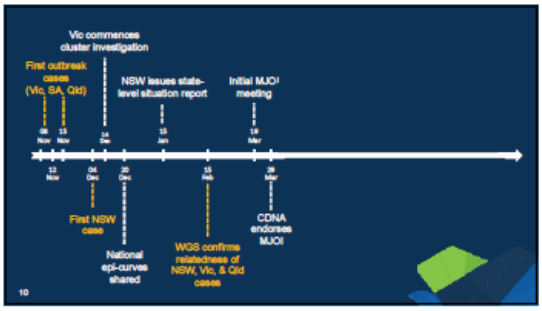


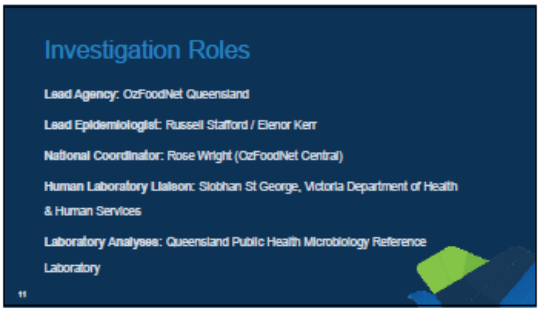
Year	Jurisdiction	No. Cases	Setting	Likely Source
1996	Queensland	>500	Airline	Custard anglaise sauce
1999	Queensland	7	Aged Care Facility	Eggnog drink
2001	Queensland	12	Aged Care Facility	Eggnog drink
2005	New South Wales	12	Not reported	No source identified
2009	New South Wales	5	Not reported	No source identified
2016	New South Wales	7	Not reported	Brand of raw chicken

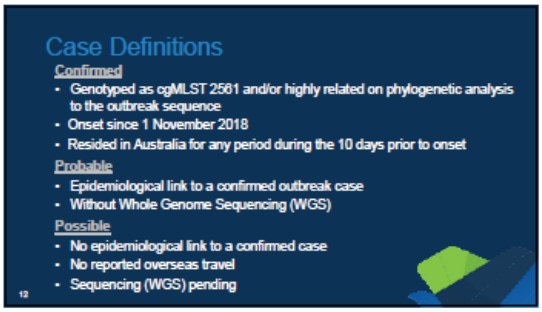

Salmonella Heidelberg notified by month in Australian States and Territories, 2014–2018
QLD NSW VIC SA WA ACT TAS NT
Number of Notifications
Date of Diagnosis by month
7


Salmonella Heidelberg notified by month in Australian States and Territories, 2014–2018
QLD NSW VIC SA WA ACT TAS NT
November increase (VIC, NSW, QLD)
Number of Notifications
Date of Diagnosis by month
8

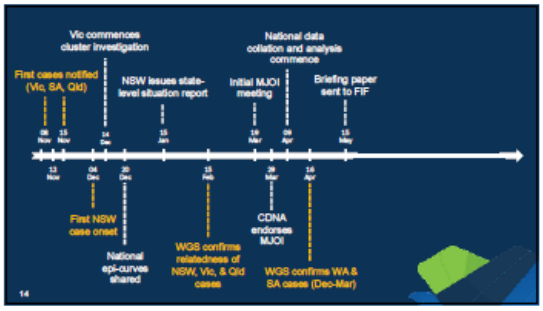

Salmonella Heidelberg notified by month in Australian States and Territories, 2014–2018
QLD NSW VIC SA WA ACT TAS NT
December cases (Vic, NSW, Qld, SA, WA)
Number of Notifications
Date of Diagnosis by month
9

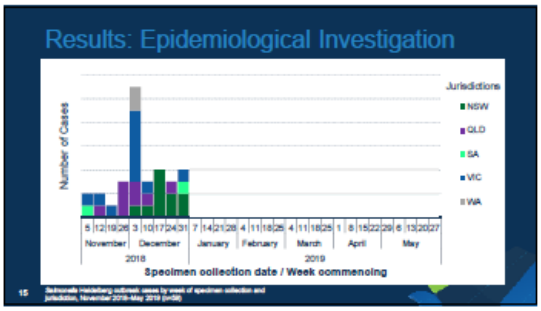

Vic commences cluster investigation
First outbreak cases (Vic, SA, Qld)
NSW issues state-level situation report
Initial MJO! meeting
First NSW case
National epi-curves shared
WGS confirms relatedness of NSW, Vic, & Qld cases
CDNA endorses MJOI
10

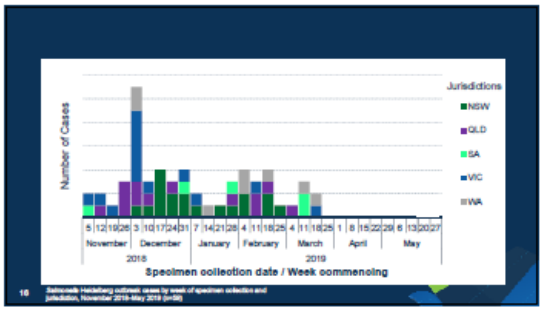

Investigation Roles
Lead Agency: OzFoodNet Queensland
Lead Epidemiologist: Russell Stafford / Elenor Kerr
National Coordinator: Rose Wright (OzFoodNet Central)
Human Laboratory Liaison: Siobhan St George, Victoria Department of Health & Human Services
Laboratory Analyses: Queensland Public Health Microbiology Reference Laboratory
11

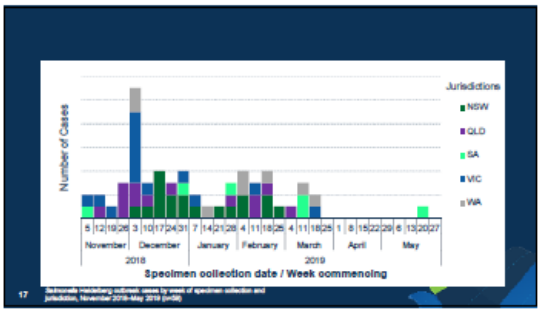

Case Definitions
Confirmed
Genotyped as cgMLST 2561 and/or highly related on phylogenetic analysis to the outbreak sequence
Onset since 1 November 2018
Resided in Australia for any period during the 10 days prior to onset
Probable
Epidemiological link to a confirmed outbreak case
Without Whole Genome Sequencing (WGS)
Possible
No epidemiological link to a confirmed case
No reported overseas travel
Sequencing (WGS) pending
12

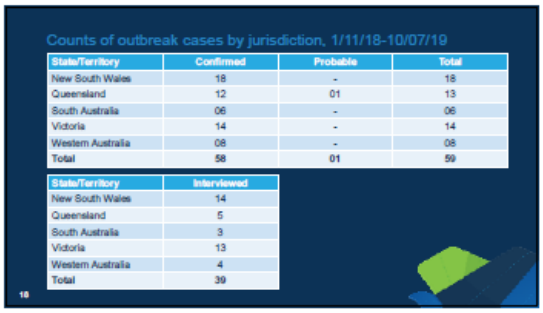

Methods
- Epidemiological Investigation
  - Descriptive
  - Binomial probability analysis
- Laboratory Investigation
  - Whole Genome Sequencing (WGS) of human and non-human isolates
- Literature Review
  - All S. Heidelberg outbreaks globally
- Food Industry Engagement
13


Vic commences cluster investigation
First cases notified (Vic, SA, Qld)
NSW issues state-level situation report
National data collation and analysis commence
Initial MJOI meeting
Briefing paper sent to FIF
First NSW case onset
National epi-curves shared
WGS confirms relatedness of NSW, Vic, & Qld cases
CDNA endorses MJOI
WGS confirms WA & SA cases (Dec-Mar)
14


Results: Epidemiological Investigation
Number of Cases
Jurisdictions
NSW
QLD
SA
VIC
WA
November
December
January
February
March
April
May
2018
2019
Specimen collection date / Week commencing
15


Number of Cases
Jurisdictions
NSW
QLD
SA
VIC
WA
November
December
January
February
March
April
May
2018
2019
Specimen collection date / Week commencing
16


Number of Cases
Jurisdictions
NSW
QLD
SA
VIC
WA
November
December
January
February
March
April
May
2018
2019
Specimen collection date / Week commencing
17


Counts of outbreak cases by jurisdiction, 1/11/18-10/07/19
State/Territory | Confirmed | Probable | Total
New South Wales | 18 | - | 18
Queensland | 12 | 01 | 13
South Australia | 06 | - | 06
Victoria | 14 | - | 14
Western Australia | 08 | - | 08
Total | 58 | 01 | 59
State/Territory | Interviewed
New South Wales | 14
Queensland | 5
South Australia | 3
Victoria | 13
Western Australia | 4
Total | 39
18

Demographic		No. (%)
Sex (N=59)	Male	33 (56%)
	Female	26 (44%)
Age (years) (N=59)	0–4	10 (17%)
	5–19	07 (12%)
Median: 43 years Min: 2 months Max: 95 years	20–59	26 (44%)
	≥ 60	16 (27%)

Clinical		No. (%)
Symptom	Diarrhoea (N=42)	38 (90%)
Median duration (N=28): 7.5 days Min: 5 days Max: 40 days	Cramps (N=37)	30 (81%)
	Fever (N=38)	22 (58%)
	Vomiting (N=38)	11 (30%)
	Bloody Diarrhoea (N=34)	07 (21%)
Hospitalisation (N=45)	Yes	16 (36%)
	No	29 (64%)
Total bed days: 67 days Median duration (N=13): 4 days	Emergency Dept. Presentation only (Among hospitalised cases, N=16)	03 (19%)

19

Food Exposure within 7 days	Proportion cases exposed (%)	Background Probability	p-value
Any cooked chicken	20/35 (57%)	16%	<0.001
Macadamia nuts	06/19 (32%)	07%	0.001
Frozen vegetable products	03/12 (25%)	03%	0.005
Lamb	13/26 (50%)	35%	0.005
Sausages	16/27 (59%)	34%	0.006
Sweet potatoes	09/14 (64%)	31%	0.010
Sauce/Chutney	14/17 (82%)	56%	0.022
Salad in a mix bag	10/21 (48%)	22%	0.027
Avocado	10/15 (67%)	42%	0.048

20

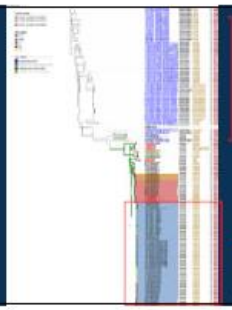
Laboratory Results

- All confirmed cases cgMLST 2561 (0–3 SNPs)
- Among 13 isolates phage typed, 100% were phage type 1
- No clustering with:
  - 8 non-human isolates
  - 65 human and non-human international sequence
- No drug resistance but potential for future resistance

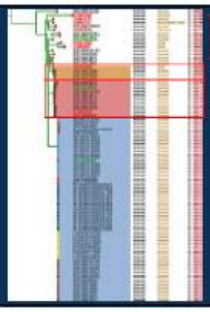
21

Phylogenetic Maximum Likelihood Tree built using SNP differences between isolates

(extracted with permission from QHFHML report, Laboratory Reference 20190619_Bhe)



22

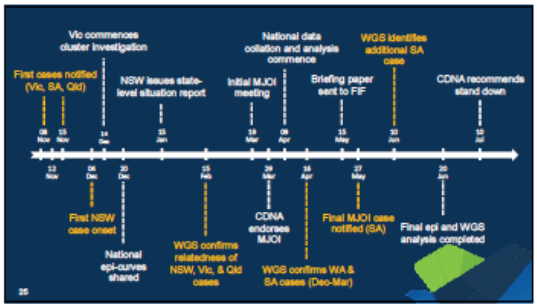


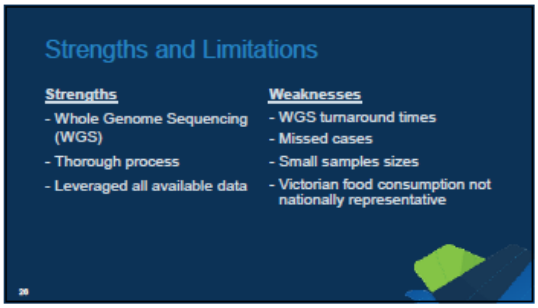
23

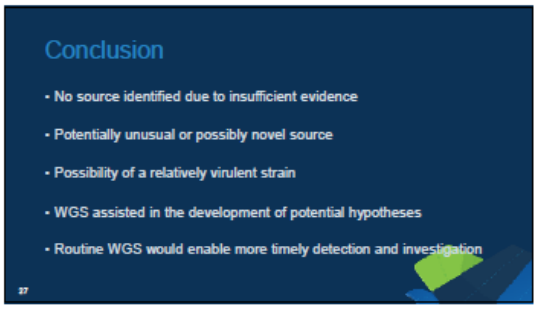
Food Industry Engagement

- No information following the request to FIF members
- Contact with previously implicated poultry company indicated continued routine testing and no recent detections.

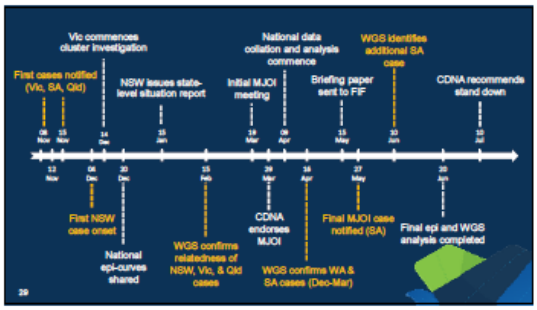
24


Vic commences cluster investigation
First cases notified (Vic, SA, Qld)
NSW issues state-level situation report
National data collation and analysis commence
Initial MJOI meeting
Briefing paper sent to FF
WGS identifies additional SA case
CDNA recommends stand down
First NSW case onset
National epi-curves shared
WGS confirms relatedness of NSW, Vic, & Qld cases
CDNA endorses MJOI
WGS confirms WA & SA cases (Dec-Mar)
Final MJOI case notified (SA)
Final epi and WGS analysis completed


Strengths and Limitations
Strengths
- Whole Genome Sequencing (WGS)
- Thorough process
- Leveraged all available data
Weaknesses
- WGS turnaround times
- Missed cases
- Small samples sizes
- Victorian food consumption not nationally representative


Conclusion
- No source identified due to insufficient evidence
- Potentially unusual or possibly novel source
- Possibility of a relatively virulent strain
- WGS assisted in the development of potential hypotheses
- Routine WGS would enable more timely detection and investigation


Acknowledgements
Queensland Health
Dr Russell Stafford (Lead Epidemiologist)
Dr Stephen Lambert (Supervisor)
Dr Jonathan Malo (Supervisor)
Dr Amy Jennison (Public Health Microbiology Reference Laboratory)
OzFoodNet
Rose Wright (National Coordinator)
Joy Gregory & Siobhan St George (VIC)
OzFoodNet epidemiologists (NSW, Victoria, SA, & WA).
Australian National University
Emma Field (Supervisor)


Vic commences cluster investigation
First cases notified (Vic, SA, Qld)
NSW issues state-level situation report
National data collation and analysis commence
Initial MJOI meeting
Briefing paper sent to FF
WGS identifies additional SA case
CDNA recommends stand down
First NSW case onset
National epi-curves shared
WGS confirms relatedness of NSW, Vic, & Qld cases
CDNA endorses MJOI
WGS confirms WA & SA cases (Dec-Mar)
Final MJOI case notified (SA)
Final epi and WGS analysis completed

Appendix 3: Published manuscript, *Emerging Infectious Diseases*

DISPATCHES

Multistate Outbreak of *Salmonella enterica* Serovar Heidelberg with Unidentified Source, Australia, 2018–2019

Elenor J. Kerr, Russell Stafford, Irani U. Rathnayake, Rikki M.A. Graham, Emily Fearnley, Joy Gregory, Keira Glasgow, Rose Wright, Vitali Sintchenko, Qinning Wang, Peter Howard, Lex E.X. Leong, Mary Valcanis, William Pitchers, Stephen B. Lambert, Amy V. Jennison

We report a multistate *Salmonella enterica* serovar Heidelberg outbreak in Australia during 2018–2019. Laboratory investigation of cases reported across 5 jurisdictions over a 7-month period could not identify a source of infection but detected indicators of severity and invasiveness. The hospitalization rate of 36% suggested a moderately severe clinical picture.

Salmonella enterica serovar Heidelberg is a frequently identified serotype among infections in humans in North America, East Africa, and Asia but is uncommon in Australia. An average of 37 cases of *Salmonella* Heidelberg were notified in Australia annually in 2009–2017, predominantly overseas acquired (*1*). Six outbreaks have been reported nationally since 1995; 1 outbreak in 1996 had >500 cases, but most have <7 cases (R. Bell, pers. comm. [email], 2020 Jun 16). We report a national outbreak of *Salmonella* Heidelberg infection across 5 jurisdictions over 7 months.

Author affiliations: Queensland Health, Brisbane, Queensland, Australia (E.J. Kerr, R. Stafford, I.U. Rathnayake, R.M.A. Graham, S.B. Lambert, A.V. Jennison); Australian National University, Canberra, Australian Capital Territory, Australia (E.J. Kerr, S.B. Lambert); South Australia Health, Adelaide, South Australia, Australia (E. Fearnley); Department of Health and Human Services, Melbourne, Victoria, Australia (J. Gregory); Health Protection New South Wales, St. Leonard's, New South Wales, Australia (K. Glasgow); Department of Health, Canberra (R. Wright); Institute of Clinical Pathology and Medical Research, Westmead, New South Wales, Australia (V. Sintchenko, Q. Wang, P. Howard); South Australia Pathology, Adelaide (L.E.X. Leong); University of Melbourne, Parkville, Victoria, Australia (M. Valcanis, W. Pitchers)

DOI: https://doi.org/10.3201/eid2801.211482

The Study

In December 2018, OzFoodNet, Australia's government-based network for enhanced foodborne disease surveillance, noted that *Salmonella* Heidelberg cases diagnosed in November (15 cases) were above the national historical 5-year mean (2.4 cases). New South Wales (NSW) and Victoria initiated separate investigations during December 2018–February 2019; neither developed a hypothesis regarding potential sources of infection. In February–March 2019, whole-genome sequencing (WGS) analysis of available isolates identified 36 highly related cases, 12 each from Queensland, NSW, and Victoria. Concurrent *Salmonella* Heidelberg infections with WGS pending were subsequently identified in other states: South Australia (SA) (N = 4) and Western Australia (WA) (N = 3). Queensland cases were not initially investigated because *Salmonella* Heidelberg is more common in this state; Queensland contributed 43% of cases in Australia during 2009–2017 (*1*). Outbreak cases were reported across multiple jurisdictions peaking in early December 2018 and continuing through late March 2019, with an outlying case reported in May 2019 (Figure). After confirmation of phylogenetic relatedness and previous jurisdictional inability to identify a common source, OzFoodNet commenced a multijurisdictional outbreak investigation in March 2019. However, case numbers declined soon after, preventing more rigorous, prospective epidemiologic investigation.

We identified 59 outbreak cases in 5 jurisdictions (58 laboratory-confirmed, 1 epidemiologically-linked): NSW (18/59, 31%), Victoria (14/59, 24%), Queensland (13/59, 22%), WA (8/59, 14%), and SA (6/59, 10%) (Table). Case-patients were 2 months–95 (median 43) years of age. None had a history of

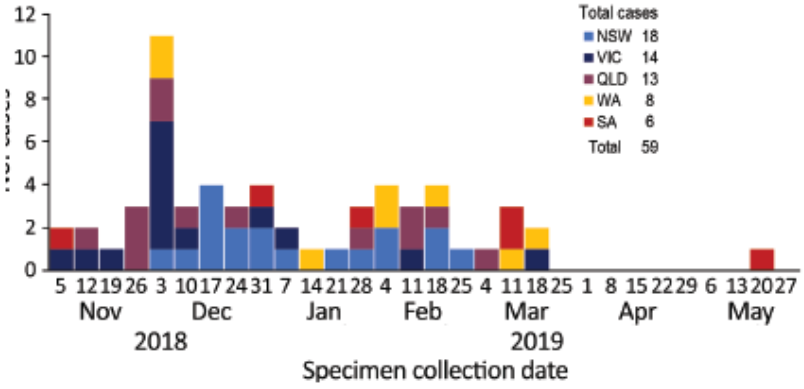


Figure. *Salmonella enterica* serovar Heidelberg outbreak cases by week of specimen collection and jurisdiction, Australia, November 2018–May 2019 (n = 59). NSW, New South Wales; QLD, Queensland; SA, South Australia; VIC, Victoria; WA, Western Australia.

international travel; 3 case-patients reported interstate travel during their exposure period.

Thirty-nine case-patients completed interviews using telephone-administered hypothesis-generating questionnaires. The remaining 20 patients were not investigated because they refused interviews, they could not be contacted, or their diagnosis dates precluded obtaining a reliable food history. Binomial probability analysis compared case-patient food exposures to background rates estimated from a Victoria population survey, accounting for seasonality, from November 2014–October 2016 (*2*).

Probability calculations highlighted potential foods of interest, including cooked chicken ($p<0.001$), macadamia nuts ($p = 0.001$), frozen vegetable products ($p = 0.005$), and lamb ($p = 0.005$). Global epidemiology suggests *Salmonella* Heidelberg outbreaks are most likely associated with poultry or eggs (*3*–*5*), yet case reporting of poultry products consumed and place of purchase did not identify a common source. Raw macadamia nuts were considered because of their popularity during the Christmas period, along with previous detections of *Salmonella* Heidelberg in Queensland (*6*). Because epidemiologic evidence was insufficient to develop a strong hypothesis for any single food item, sampling was not considered feasible; decreasing case numbers precluded an analytic study.

We consolidated sequence data, including isolates sequenced in jurisdictional laboratories. We assessed genetic relatedness among isolates of *Salmonella* Heidelberg cases at the Queensland Health Forensic and Scientific Services laboratory by generating core genome multilocus sequence typing (cgMLST) complex types (<7 allele differences) using Ridom SeqSphere+ version 5.1.0 (https://www.ridom.de) based on the EnteroBase *Salmonella* enterica scheme version 2.0 (https://enterobase.warwick.ac.uk). We determined single-nucleotide polymorphism (SNP) differences using Snippy package version 4.3.6 (https://github.com/tseemann/snippy) using *Salmonella* Heidelberg SL476 (GenBank accession no. NC_011083.1) as a reference. We conducted in silico WGS analysis for antimicrobial-resistance genes by the abricate program version 0.8.10 (https://github.com/tseemann/abricate), using the Resfinder database October 18, 2018, version (https://cge.cbs.dtu.dk/services/ResFinder) to further characterize strains. We investigated the presence of the *saf* operon using Ridom SeqSphere+ against the *saf* operon sequence from GenBank (accession no NZ_LS483494). We generated sequences for isolates on the Illumina NextSeq genome sequencing platform (https://www.illumina.com) using the Nextera XT library preparation kit; the WGS read files were deposited in the US National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov) Sequence Read Archive (BioProject ID PRJNA663036 [NSW] and PRJNA660800 [Queensland, Victoria, SA, WA]). We developed our hypothesis using a range of local and international sequence data sources to assess genetic relatedness to outbreak cases; we used 78 human sequences from 5 jurisdictional laboratories, 8 nonhuman *Salmonella* Heidelberg isolates from a Queensland culture collection from food and animals (macadamia nuts and poultry, caprine, porcine, bovine, equine, and reptile sources), and 86 international sequences downloaded from EnteroBase and the National Center for Biotechnology Information.

The 58 *Salmonella* Heidelberg isolates available from this outbreak were all multilocus sequence type (ST) 15 and belonged to the same cgMLST complex type (2561); isolates differed within the complex type by 0–3 SNPs/cgMLST alleles, whereas the nearest nonoutbreak genomes had ≥34 SNP and ≥16 SNP al-

lele differences (Appendix Figure, https://wwwnc.cdc.gov/EID/article/28/1/21-1462-App1.pdf). This complex type was not identified among a range of international sequences or Australia nonhuman or historical human isolates chosen to inform possible outbreak sources; thus, we could not develop our hypothesis using WGS. No compelling microbiological evidence supported hypotheses of a nut, poultry, or other specific source.

All Australia isolates and 91% of international isolates harbored the antimicrobial-resistance gene *fosA7* for fosfomycin (*7*). Phenotypic analysis of 9 isolates revealed the outbreak strains to be susceptible to antimicrobial drugs including cephalosporins, fluoroquinolones, aminoglycosides, broad-spectrum penicillins, and trimethoprim/sulfamethoxazole.

Of 45 case-patients for whom data were available, 16 (36%) were hospitalized with a median duration of 4 (range 1–18) days, reported by 13 case-patients. Although direct comparison is difficult because of potential confounding by age, the hospitalization rate of 36% was high compared with the rate of 11.6% among 149 US outbreaks of *Salmonella* Heidelberg from 1973–1997 (*3*). Hospitalization rates in that outbreak were also higher than that for *Salmonella enterica* outbreaks in Australia with similar age distributions during 2001–2016 (*8*).

Although this investigation was unable to capture invasiveness of the outbreak strain, *Salmonella* Heidelberg has frequently been associated with greater risk for invasive disease than have other commonly reported nontyphoidal *Salmonella* serotypes, including Typhimurium and Enteritidis (*9*–*14*). In the United States, *Salmonella* Heidelberg is among the 4 most common serotypes isolated from blood; 12%–13% of *Salmonella* Heidelberg infections resulted in invasive disease in North America, higher than the US *Salmonella* average of 7% (*10*–*12*). A study of invasive nontyphoidal *Salmonella* infection in Australia similarly found that almost 10% of *Salmonella* Heidelberg gastrointestinal infections during 2007–2016 were invasive disease, ≈5× higher than *Salmonella* Typhimurium infections (*15*). Concern has grown regarding the virulence of a US bovine-related *Salmonella* Heidelberg outbreak; recent genomic analyses indicated that most identified *Salmonella* virulence genes are present in most *Salmonella* Heidelberg strains. These studies highlighted potential contributions of *saf* fimbrial genes to increased severity via their role in bacterial aggregation, colonization, and biofilm formation (*9*). The *saf* operon has been reported generally absent from the *Salmonella* Heidelberg serovar but was present in a previous outbreak associated with increased severity (*9*). The *saf* operon was present in all Australia isolates in this study (Appendix Figure).

Table. National *Salmonella enterica* serovar Heidelberg outbreak cases by demographic and clinical characteristics, Australia, November 1, 2018–July 10, 2019

Feature	No. (%)
Demographic	N = 59
Sex	
M	33 (56)
F	26 (44)
Age group, y	
0–4	10 (17)
5–9	2 (3)
10–19	5 (8)
20–29	2 (3)
30–39	7 (12)
40–49	5 (08)
50–59	12 (20)
60–69	6 (10)
70–79	3 (5)
80–89	5 (8)
≥90	2 (3)
Symptom	
Diarrhea	38/42 (90)
Abdominal cramps	30/37 (81)
Fever	22/38 (58)
Vomiting	11/38 (30)
Bloody diarrhea	7/34 (21)
Hospitalization	
Yes	16/45 (36)
No	29/45 (64)
Emergency department visit only	3/16 (19)

Conclusions

We report a national outbreak investigation of a locally uncommon *S. enterica* serovar of unknown origins in Australia. Although *Salmonella* Heidelberg outbreaks are relatively uncommon in Australia, given this outbreak's comparatively high hospitalization rate and the presence of *saf* fimbrial genes in the implicated strain, future cases warrant prompt investigation to assess severity and invasiveness. A platform for real-time exchange of sequence data in Australia and use of routine WGS for salmonellosis cases, including comparison with local and international strain data, may enable more timely detection of outbreaks.

Acknowledgments

We thank all referring pathology laboratories for isolation and referral of *Salmonella* isolates and the *Salmonella* reference sections in Public Health Reference laboratories for serotyping of *Salmonella* isolates. We would also like to thank the OzFoodNet Network, which is funded by the Australian Government Department of Health.

Ethics approval for this work was obtained from the Australian National University Human Research Ethics Committee (protocol 2017/909).

While doing this work, E.K. was supported by the Australian National University Master of Philosophy (Applied mEpidemiology) Scholarship, which was funded by Queensland Health.

About the Author

Ms. Kerr is a Master of Applied Epidemiology Scholar (Australia's only Field Epidemiology Training Program, Australian National University) and ASEAN-Australia Health Security Fellow. Her research interests focus on infectious disease and public health.

References

1. Department of Health. *Salmonella* National Notifiable Disease Surveillance System notifications in Australia 2009–2017. Canberra, ACT: Commonwealth of Australia; 2019 [updated 2018 Jul 31; cited 2020 May 6]. http://www9.health.gov.au/cda/source/pub_salmo.cfm
2. St. George SC. Applied epidemiology in communicable diseases, Victoria, 2016–2017. Canberra, ACT: Australian National University; 2018 [updated 2020 May 19; cited 2020 Jul 9]. https://openresearch-repository.anu.edu.au/handle/1885/154282
3. Chittick P, Sulka A, Tauxe RV, Fry AM. A summary of national reports of foodborne outbreaks of *Salmonella* Heidelberg infections in the United States: clues for disease prevention. J Food Prot. 2006;69:1150–3. https://doi.org/10.4315/0362-028X-69.5.1150
4. Chai SJ, Cole D, Nisler A, Mahon BE. Poultry: the most common food in outbreaks with known pathogens, United States, 1998–2012. Epidemiol Infect. 2017;145:316–25. https://doi.org/10.1017/S0950268816002375
5. Gieraltowski L, Higa J, Peralta V, Green A, Schwensohn C, Rosen H, et al.; Salmonella Heidelberg Investigation Team. National outbreak of multidrug-resistant *Salmonella* Heidelberg infections linked to a single poultry company. PLoS One. 2016;11:e0162369. https://doi.org/10.1371/journal.pone.0162369
6. Munck N, Smith J, Bates J, Glass K, Hald T, Kirk MD. Source Attribution of *Salmonella* in macadamia nuts to animal and environmental reservoirs in Queensland, Australia. Foodborne Pathog Dis. 2020;17:357–64. https://doi.org/10.1089/fpd.2019.2706
7. Rehman MA, Yin X, Persaud-Lachhman MG, Diarra MS. First detection of a fosfomycin resistance gene, *fosA7*, in *Salmonella enterica* serovar Heidelberg isolated from broiler chickens. Antimicrob Agents Chemother. 2017;61:e00410–7. https://doi.org/10.1128/AAC.00410-17
8. Ford L, Moffatt CRM, Fearnley E, Miller M, Gregory J, Sloan-Gardner TS, et al. The epidemiology of *Salmonella enterica* outbreaks in Australia, 2001–2016. Front Sustain Food Syst. 2018;2:1–8. https://doi.org/10.3389/fsufs.2018.00086
9. Antony L, Behr M, Sockett D, Miskimins D, Aulik N, Christopher-Hennings J, et al. Genome divergence and increased virulence of outbreak associated *Salmonella enterica* subspecies *enterica* serovar *Heidelberg*. Gut Pathog. 2018;10:53. https://doi.org/10.1186/s13099-018-0279-0
10. Crump JA, Medalla FM, Joyce KW, Krueger AL, Hoekstra RM, Whichard JM, et al.; Emerging Infections Program NARMS Working Group. Antimicrobial resistance among invasive nontyphoidal *Salmonella enterica* isolates in the United States: National Antimicrobial Resistance Monitoring System, 1996 to 2007. Antimicrob Agents Chemother. 2011;55:1148–54. https://doi.org/10.1128/AAC.01333-10
11. Otto SJ, Carson CA, Finley RL, Thomas MK, Reid-Smith RJ, McEwen SA. Estimating the number of human cases of ceftiofur-resistant *Salmonella enterica* serovar Heidelberg in Québec and Ontario, Canada. Clin Infect Dis. 2014;59:1281–90. https://doi.org/10.1093/cid/ciu496
12. Dutil L, Irwin R, Finley R, Ng LK, Avery B, Boerlin P, et al. Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. Emerg Infect Dis. 2010;16:48–54. https://doi.org/10.3201/eid1601.090729
13. Jones TF, Ingram LA, Cieslak PR, Vugia DJ, Tobin-D'Angelo M, Hurd S, et al. Salmonellosis outcomes differ substantially by serotype. J Infect Dis. 2008;198:109–14. https://doi.org/10.1086/588823
14. Williamson DA, Lane CR, Easton M, Valcanis M, Strachan J, Veitch MG, et al. Increasing antimicrobial resistance in nontyphoidal *Salmonella* isolates in Australia from 1979 to 2015. Antimicrob Agents Chemother. 2018;62:e02012-17. https://doi.org/10.1128/AAC.02012-17
15. Parisi A, Crump JA, Stafford R, Glass K, Howden BP, Kirk MD. Increasing incidence of invasive nontyphoidal *Salmonella* infections in Queensland, Australia, 2007–2016. PLoS Negl Trop Dis. 2019;13:e0007187. https://doi.org/10.1371/journal.pntd.0007187

Address for correspondence: Elenor Kerr, National Centre for Epidemiology and Population Health, Australian National University, Bldg 62 Mills Rd, Canberra, ACT 2601, Australia; email: elenor.kerr@anu.edu.au

Chapter 3

Evidence for an increase in the intensity of inter-seasonal influenza, Queensland, 2009–2019

Prologue

Rationale

This chapter contains the publication summarising my epidemiology project; a mixed ecological study of inter-seasonal influenza in Queensland, 2009–2019. Influenza is an acute viral infection which in temperate regions has a strong seasonal cycle. Typically human influenza cases increase between late autumn and early spring, and transmission levels are believed to return to a baseline of activity during warmer summer months.[1,2] Numerous mechanisms have been put forward to account for influenza's seasonality, including changes in population contact patterns, environmental factors, such as humidity or temperature, seasonal variations in immunity and introductions from returned travellers/tourists and bird populations.[1,3] However, no conclusive relationship has been identified.

As the onset, duration, and severity of each influenza season varies, monitoring and understanding influenza's epidemiology is crucial to recognising the potential population impacts, identifying new trends, and detecting and controlling outbreaks.[4-8] Yet, the period between influenza seasons, the inter-seasonal period, is often not considered in influenza studies or surveillance. Outbreaks of influenza have been reported outside of the typical season globally and in Australia. Recent evidence suggests that inter-seasonal influenza cases are increasing in Australia.[9,10] Over the past decade only four Australian studies have examined inter-seasonal influenza; two examined increases in national notifications during discrete inter-seasonal periods and two assessed national trends over a longer timeframe.[2,9-11] Whilst these studies provide insights into Australian inter-seasonal activity, transmission, and testing practices, our understanding of the epidemiology of inter-seasonal periods across different climatic regions over time remains limited.

My interest in inter-seasonal influenza stemmed from my Masters of Public Health research project in early 2018 where I came across a clear gap in literature on inter-seasonal influenza. Coincidentally, due to substantially heightened influenza activity during the 2018/19 period, inter-seasonal influenza became a hot topic shortly before I began the MAE program.[12,13] Queensland's geography and rich surveillance data provided an opportunity to undertake an in-depth epidemiological analysis inter-seasonal influenza over time with respect to the potential differences in climatic regions.

My roles

As the lead investigator of this project I was responsible for the project proposal, epidemiological study design, ethics review application, data analysis plan development, and data cleaning and analysis. Notifications and laboratory data were extracted with assistance from the Epidemiology and Research Unit at the Queensland Health Communicable Diseases Branch (CDB). To find a meaningful and consistent way of classifying seasonal/inter-seasonal intensity, I reached out to Kaitlyn Vette, an MAE alumnus with experience with influenza thresholds. Together we worked through methods that could be applied to available data to calculate inter-seasonal intensity thresholds. Following Kaitlyn's advice and with feedback from CDB epidemiologists, I developed and refined a method for calculating the seasonal and inter-seasonal thresholds and undertook threshold calculations. I was also the lead author for the publication summarising the project's results.

Lessons learned

This project provided a wealth of experience and lessons learned regarding data analysis and publication writing. The laboratory dataset was the largest that I had worked with and required a significant amount of merging, cleaning, and re-shaping. This was a challenge that taught me the importance of talking through your Stata issues with colleagues, having clear and well documented cleaning and analysis strategies, efficient Stata code, and a lot of patience. Unfortunately, I also learned the importance of backing up do. files. We ran the analysis for this project several times between piloting analysis methods, including the 2019 season, and re-fining the project scope. After the first analysis, I misplaced my re-shaping do file, resulting in many avoidable hours trying to recreate my re-shaping strategy and code. I'm appreciative that I learnt this lesson early, as it meant that I became much more conscious of backing up and documenting each stage of analysis. Each subsequent analysis was therefore easier and faster, and this diligence was a benefit to all subsequent project analyses.

This project provided an opportunity to delve deeply into methods for calculating influenza thresholds. In search of a method that could apply to both seasons and inter-seasonal periods, I spent several months working through how to select an appropriate overall method, then trialled and tweaked the specific methodology (smoothing vs no smoothing; composite measurement vs proportion positive). This was also my first manuscript submitted for publication and the process further developed my confidence in writing and my creativity and attention to detail when developing visual representations of the data.

Public health impact

This project provides relevant epidemiological information about a complex and little studied, but increasingly important period of influenza activity in Queensland. The project increases our previously limited understanding of the epidemiology of influenza during inter-seasonal periods with respect to time, population groups, geography, and circulating strains. In particular, the project highlights that the collection and analysis of high-quality surveillance data should not be limited to seasonal periods, and consideration should be given to tropical areas, which bear the largest burden of inter-seasonal influenza.

Intensity thresholds have not previously been calculated for inter-seasonal periods. In this project we demonstrate that the WHO Average Curve method can be adapted and applied to these periods, and provide classifications which consistently characterise the intensity of inter-seasonal periods. The study also explored the influence of seasonal intensity and strain dominance on subsequent seasonal and inter-seasonal activity. This has not previously been undertaken in studies regarding influenza seasonality and our findings suggest further research into this concept over a longer time period may be of benefit. The manuscript for the project was submitted to *Influenza and Other Respiratory Viruses* on 01 October 2020 and at the time of submitting this thesis was still under consideration for publication.

MAE core activity requirements addressed

- Plan and conduct an epidemiological study
- Peer-reviewed publication

Acknowledgements

I would like to acknowledge those who contributed to this project, as well as the great support and guidance that I received. Kaitlyn Vette for the expert technical advice regarding threshold setting methods, providing me with resources to learn about influenza thresholds, and contributing greatly to the manuscript. I also thank you for your kind words of encouragement throughout. Nicole Burt and Mohana Rajmokan (CDB Epidemiology and Research Unit) for providing assistance with data extraction from NoCS and AUSLAB and helping solve all my data access issues. Graeme Nimmo at Pathology Queensland for sharing laboratory insights and contributing to the manuscript. My supervisors, Jonathan and Stephen, for letting me follow my interest (and stopping me when I started to go too far down the rabbit hole) and constantly helping me through the analysis and manuscript.

Abstract

Background

Interseasonal influenza cases have been increasing in Australia. Studies of influenza seasonality typically focus on seasonal transmission in temperate regions, leaving our understanding of interseasonal epidemiology limited. The aim of this project was to improve understanding of influenza epidemiology during interseasonal periods across climate zones, and explored influenza intensity and strain dominance patterns over time.

Methods

Queensland state-wide laboratory-confirmed influenza notifications and public laboratory influenza test data from 2009–2019 were described by demographics, time period, region, and strain type. We compared influenza intensity over time using the World Health Organization (WHO) Average Curve method to provide thresholds for seasonal and interseasonal periods.

Results

Among the 243,830 influenza notifications and 490,772 laboratory tests reported in Queensland between 2009 and 2019, 15% of notifications and 40% of tests occurred during interseasonal periods, with 6.3% of interseasonal tests positive. Interseasonal notifications and tests substantially increased over time and weekly proportions positive and intensity classifications suggested gradually increased over this 10-year period. Tropical interseasonal activity was higher with periods of marked increase. Influenza A was dominant, although influenza B represented up to 72% and 42% of notifications during some seasonal and interseasonal periods, respectively.

Conclusion

Using notification and laboratory testing data, we have demonstrated a gradual increase in interseasonal influenza over time. Our findings suggest this increase results from an interplay between testing, a real increase in virus activity and intensity, and strain circulation. Seasonal intensity and strain circulation appeared to modify subsequent period intensity. Routine year-round surveillance data would provide a better understanding of influenza epidemiology during this infrequently studied interseasonal time period.

Background

In temperate regions human influenza typically has a strong seasonal cycle where influenza activity increases between late autumn and early spring, before returning to baseline activity during warmer months.[1,2] Influenza cases during the summer inter-seasonal period are often considered to be sporadic imported cases unlikely to cause substantial ongoing transmission.[10,14,15] However, recent evidence suggests that inter-seasonal transmission patterns are more complex than previously thought.[10] In tropical and sub-tropical regions, inter-seasonal influenza patterns are variable with some areas experiencing increased activity during rainy seasons, and others not experiencing a well-defined season.[1,16] Regardless of the climate zone, outbreaks of influenza may also occur outside of the typical season as evidenced by the large and widespread influenza outbreaks across Australia during the 2018/19 inter-seasonal period.[9]

Changes to influenza reporting, testing capacity, and testing behaviour likely play a role in increased recognition of influenza infections during inter-seasonal periods, both globally and in Australia.[1] Nonetheless, higher than average inter-seasonal influenza notifications, beyond what could be accounted for by increased testing and reporting, have been observed previously in Australia during the 2010/11 and 2018/19 inter-seasonal periods.[9,11]

Recent Australian analyses provide insights into Australian inter-seasonal activity, transmission, and testing practices, yet previous studies of influenza seasonality typically focus on transmission within seasonal periods in temperate regions.[2,9,10,17] Our understanding of the epidemiology of inter-seasonal periods across different climatic regions over time therefore remains limited.

Here we use routinely collected surveillance data from 2009 to 2019 to summarise the epidemiology of influenza during inter-seasonal periods in Queensland over a 10-year period and explore influenza intensity and strain dominance patterns over time. We focus our analyses on the Australian state of Queensland, which by spanning temperate, sub-tropical, and tropical climates provides insights into patterns of seasonality across different climatic regions.

Methods

Study setting and data sources

Queensland is a state with a population of approximately 5 million people located in Australia's northeast. It has a varied climate including temperate, sub-tropical, and tropical zones. Queensland has well-established influenza surveillance systems which monitor activity using

laboratory-confirmed influenza notifications, public laboratory influenza testing data, and hospital admissions for influenza to Queensland public hospitals. Laboratory-confirmed influenza is a nationally notifiable condition in Australia and has been notifiable on pathological diagnosis in Queensland since 2001. Confirmed cases are notified on laboratory definitive evidence; virus isolation by culture, detection by nucleic acid testing, or antigen detection in appropriate respiratory specimens or seroconversion or a fourfold or greater rise in an antibody titre to influenza virus. Notification is also made on the detection of a single high titre of IgA antibody.

Notifications of laboratory-confirmed influenza from both public and private laboratories are recorded in Queensland's Notifiable Condition System (NoCS). The Queensland public laboratory information system (AUSLAB), holds laboratory test request and result records of all public hospital inpatients and outpatients, as well as testing records for community clinics and prisons in Queensland. Routinely collected data from the beginning of the 2009 season (01 May) to the end of the 2019 season (03 November) were used for this analysis. Laboratory-confirmed influenza notifications and data for all individuals tested for influenza using PCR were extracted from NoCS and AUSLAB, respectively. As serology was infrequent during this study period, representing only 2.45% of total testing and 1.90% of positive tests, only notifications and testing data from PCR results, including GeneXpert data, were used. All influenza PCR tests in this study were performed as part of a multiplex PCR.

Analysis

Notification and testing data were analysed as both overall period (seasonal/inter-seasonal), individual season/inter-seasonal period, and weekly totals. Weeks were defined using International Organisation for Standardisation (ISO) 8601 standard weeks. Consistent with historical Queensland influenza activity, seasonal and inter-seasonal periods were fixed; seasons: weeks 22–44, inter-seasonal periods: weeks 45–21.

Inter-seasonal epidemiology

We examined the distribution of both inter-seasonal and seasonal influenza periods by age group (<5, 5–<10, 10–<20, 20–<30, 30–<40, 40–<50, 50–<60, 60–<65, ≥65 years), sex, region, and influenza strain type (Influenza A: H1, H3, untyped; Influenza B: Yamagata, Victoria, untyped). Geographical regions (Southern, Central, Tropical) align to those used for state-level influenza surveillance and Bureau of Meteorology climate zones. A ratio of seasonal:inter-seasonal influenza notifications, tests, and proportion of tests positive were calculated. The 2009 pandemic season was excluded from ratio calculations due to changes in laboratory testing practices and extreme counts during the H1N1pdm09 pandemic period.[18]

Intensity and strain dominance

To characterise and compare influenza intensity over time, thresholds were set for seasons and inter-seasonal periods separately using the World Health Organization (WHO) average curve method.[19] Historic data from 31 May 2010 (beginning of the 2010 season) to 03 November 2019 were aligned on the median week of peak activity and assigned thresholds of 'no activity' (below annual median value), 'low' (between the annual median value and the upper 40% confidence interval (CI) of the mean peak value of the average curve), 'moderate' (between the upper limit of the 40% CI and 90% CI of the mean peak value of the average curve), 'high' (between the upper limit of the 90% and 97.5% CI of the mean peak value of the average curve), and 'extraordinary' (above the upper limit of the 97.5% CI of the mean peak value of the average curve). Due to large variance in peak values, CIs were calculated using a geometric mean.

Data from the 2010–2019 seasons were used to calculate the seasonal thresholds and data from the 2010/11–2018/19 inter-seasonal periods were used to calculate the inter-seasonal thresholds with 53rd weeks excluded from analysis. Data from the 2009 season and 2009/10 inter-seasonal period were excluded. Thresholds used a weekly composite measurement, the product of laboratory-confirmed notification rates (per 1,000 population per year) and proportion of laboratory tests positive for influenza, which were then smoothed using a 3-week moving average to reduce short-term fluctuations in values. Such composite influenza measurements are considered a better proxy indicator of influenza incidence than either notification or laboratory data alone as they improve representativeness and account for testing practices and behaviours.[20,21] Strain dominance was defined as the largest proportion of strain type among all notifications during that period. Influenza B co-circulation was defined as where influenza B accounted for ≥20% of all notifications for the period.

Ethics statement

This study was approved by the ANU Human Research Ethics Committee (Protocol 2019/442) and approval for access to Queensland Health information was granted under section 284 of the Queensland *Public Health Act 2005*. All data were de-identified.

Results

A total of 243,830 influenza notifications and 490,772 laboratory tests were reported in Queensland. Among which, 190,332 (78%) notifications occurred during seasonal periods (excluding the 2009 season), 17,147 (7%) notifications occurred during the 2009 pandemic season, and 36,351 (15%) notifications occurred during inter-seasonal periods (Table 1).

Table 1: Inter-seasonal laboratory-confirmed influenza notifications, influenza testing, and proportion positive inter-seasonal to seasonal ratio, Queensland 2009/10 to 2018/19

Characteristic	Notifications			Tests			Proportion Positive (%)		
	IS	S†	Ratio‡	IS	S	Ratio	IS	S	Ratio
Total	36,351	190,332	0.19	198,137	267,071	0.74	06.3	18.2	0.35
Age Group (years)									
<5	3,066	21,891	0.14	68,144	73,084	0.93	02.3	11.8	0.20
5–<10	2,825	23,339	0.12	12,468	18,004	0.69	07.0	24.9	0.28
10–<20	3,834	28,699	0.13	9,394	13,746	0.68	10.8	30.1	0.36
20–<30	3,821	20,366	0.19	10,529	16,113	0.65	11.6	27.3	0.43
30–<40	4,343	22,990	0.19	11,048	16,448	0.67	10.6	24.7	0.43
40–<50	4,176	18,964	0.22	12,214	17,245	0.71	09.4	19.8	0.48
50–<60	4,557	16,616	0.27	16,365	23,109	0.71	08.7	17.7	0.49
60–<65	2,187	7,866	0.28	9,940	13,819	0.72	07.8	17.4	0.45
≥65	7,242	29,601	0.24	48,035	75,503	0.64	06.9	17.3	0.40
Sex									
Female	19,641	102,662	0.19	95,148	133,092	0.71	06.8	19.0	0.36
Male	16,710	87,670	0.19	102,989	133,092	0.77	05.9	17.4	0.34
Region									
Tropical	9,637	19,912	0.48	39,649	42,351	0.94	11.4	18.2	0.63
Central	12,866	75,518	0.17	70,287	101,176	0.70	05.3	18.1	0.29
Southern	13,848	94,902	0.15	82,505	116,304	0.71	04.7	18.2	0.26
Strain Type									
Influenza A	29,608	134,972	0.22						
A H1	3,288	7,268	0.45						
A H3	3,915	10,380	0.38						
Untyped	22,405	117,324	0.19						
Influenza B	6,740	55,354	0.12						
Yamagata	205	300	0.68						
Victoria	167	642	0.26						
Untyped	6,368	54,412	0.12						

IS: Inter-seasonal period (ISO week 45–21); S: Seasonal period (ISO week 22–44)

† Excludes the 2009 pandemic season

‡ Ratio of inter-seasonal-to-seasonal values

Among laboratory tests, 267,071 (54.4%) were performed during seasonal periods, 25,564 (5.2%) during the 2009 pandemic season, and 198,137 (40.4%) during inter-seasonal periods. Inter-seasonal notifications increased from 71 (2009/10) to 15,529 (2018/19) over the study period and tests increased from 9,240 to 41,430 (Table 2). Overall 6.3% of tests during inter-seasonal periods between 2009/10 to 2018/19 were positive (12,549/198,137). The inter-seasonal period proportion positive ranged between 0.5% (2009/10) and 12.6% (2018/19). There was no clear overall increasing trend across inter-seasonal periods over time.

GeneXpert testing (103,867 tests, 21.1%) increased from 751 tests in 2015 to 64,285 in 2019. Until 2018 GeneXpert was primarily run in parallel to other PCR testing. Between 2017 and 2019, 11,135 notifications (2.3%) were based on GeneXpert positivity alone.

Table 2: Inter-seasonal laboratory-confirmed influenza notifications, testing, and proportion positive by period, Queensland 2009/10 to 2018/19

Inter-seasonal Period†	Notifications		Tests		Proportion Positive‡ (%)
	N	Weekly Median (IQR)	N	Weekly Median (IQR)	
2009/10	71	02 (01–03)	9,240	299 (229–390)	0.5
2010/11	1,805	67 (46–78)	13,174	460 (388–520)	6.7
2011/12	647	18 (13–27)	9,902	331 (302–369)	2.2
2012/13	796	23 (15–40)	11,850	424 (346–475)	1.9
2013/14	1,975	70 (55–84)	13,459	456 (392–545)	5.0
2014/15	2,138	73 (60–92)	17,482	562 (521–693)	4.1
2015/16	3,091	84.5 (49–167)	26,316	893.5 (753–983)	4.5
2016/17	4,750	162 (144–185)	25,930	916 (836–948)	6.1
2017/18	5,549	196 (137–246)	29,444	1,060 (918–1,109)	6.2
2018/19	15,529	508 (444–569)	41,340	1,322 (1,241–1,643)	12.6
Total	36,351		198,137		6.3

IQR: Interquartile range

† ISO week 45 to 21

‡ Proportion of public laboratory influenza tests influenza positive

Inter-seasonal epidemiology

Increases in notifications and influenza tests over the study period were most evident among the ≥65-year age group; notifications and tests increased each inter-seasonal period on average by 61% and 59%, respectively. Thirty-four percent of all tests during inter-seasonal periods were for children younger than 5-years of age and testing in this age group was high across all inter-seasonal periods, ranging between 5,410 tests (2009/10) and 9,539 (2015/16). However, the

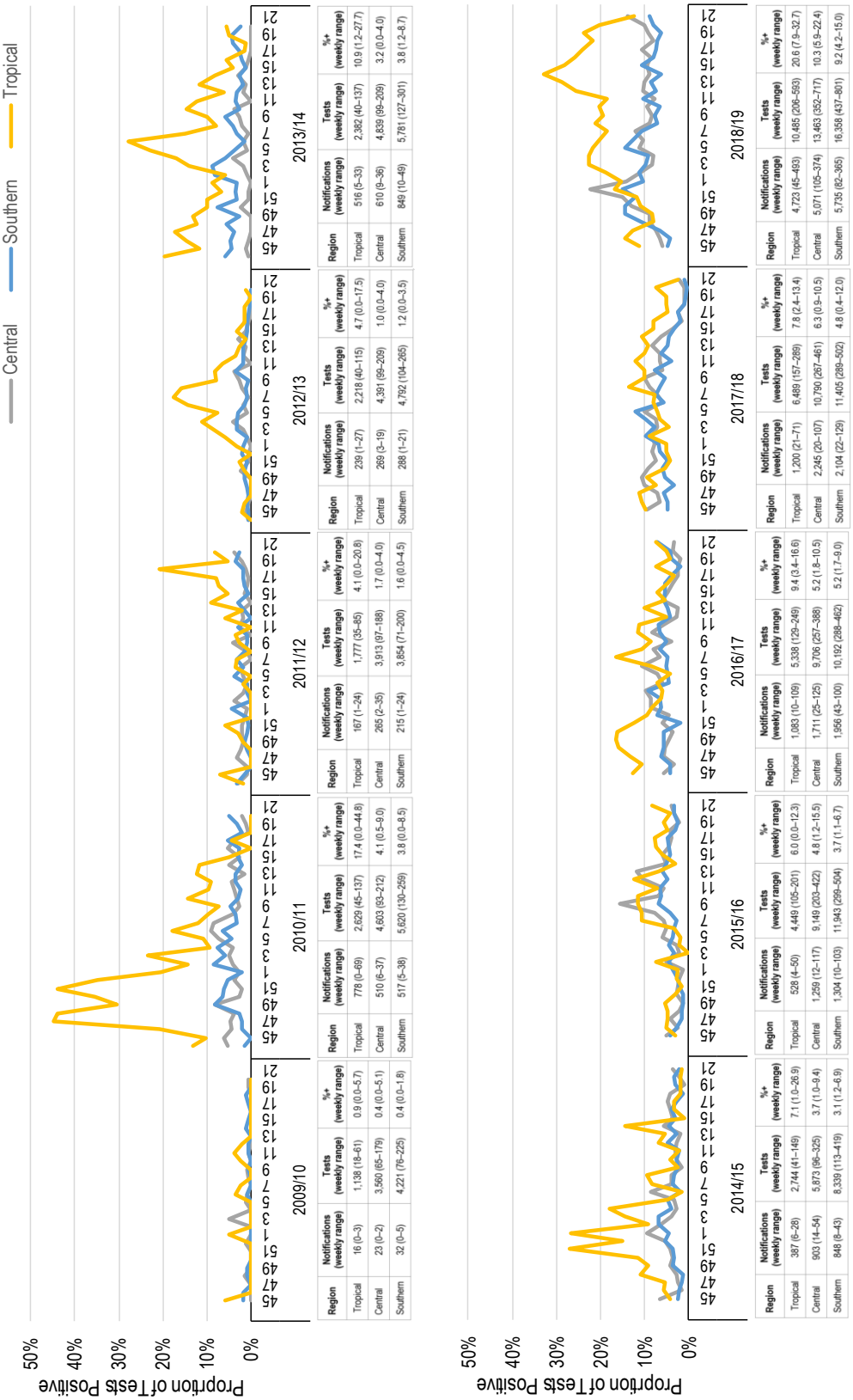
proportion positive for children younger than 5 years of age was the lowest of all age groups (1,592/68,144, 2.3%) and increases in notifications over the study period were consistent across all age groups, apart from the ≥65-year age group.

The Southern and Central regions accounted for 38.1% and 35.4% of inter-seasonal notifications, respectively, and typically had the highest number of tests and notifications each inter-seasonal period. However, the Tropical region had the highest overall inter-seasonal proportion positive (Figure 1). The Tropical region's proportion positive each inter-seasonal period ranged from 0.9% (2009/10) to 20.6% (2018/19), while the Southern and Central regions had lower and narrower proportion positive ranges (Southern: 0.4%–9.2%; Central: 0.4%–10.3%). Plots of weekly proportions positive by region also indicate periods of markedly increased activity within inter-seasonal periods in the Tropical region. The weekly proportion positive exceeded 20% in the Tropical region in 5/11 inter-seasonal periods (2010/11, 2011/12, 2013/14, 2014/15, 2018/19). During the 2010/11 period, 778 notifications were reported in the Tropical region (exceeding Central (510) and Southern (517) region notifications) and the overall proportion positive reached 17.4%.

The overall ratio for inter-seasonal to seasonal periods was 0.19 for notifications and 0.74 for testing. When assessed with other categories, higher testing ratios, comparing inter-seasonal to seasonal periods, were observed among children younger than 5-years of age (0.93), males (0.77), and the tropical region (0.94) (Table 1). The proportion positive ratio was also highest for the Tropical region (0.63) as compared to all other regions and demographic groups. Notification count ratios were lowest among children in the 5–<10-year age group (0.12) and for influenza B (0.12). Among influenza strains, the notification count ratio was highest for the Yamagata Lineage (0.68). However, counts of subtyped influenza B notifications were low.

Intensity and strain dominance

Using the intensity threshold levels, the majority of inter-seasonal periods were categorised as either 'no activity' (2009/10, 2011/12, 2012/13) or 'moderate' (2010/11, 2015/16, 2016/17) (Figure 2a). Composite values (product of notification rate and proportion positive) during the 2016/17 and 2017/18 periods were above the seasonal threshold for a total of 31 and 40 weeks, respectively. Only the 2018/19 period was classified as 'extraordinary'. Three seasons were classified as 'no activity' (2010, 2013, 2018), whilst four exceeded the 'extraordinary' threshold (2009, 2015, 2017, 2019) (Figure 2b).



2009/10

Region	Notifications (weekly range)	Tests (weekly range)	%+ (weekly range)
Tropical	16 (0–3)	1,138 (18–61)	0.9 (0.0–5.7)
Central	23 (0–2)	3,560 (65–179)	0.4 (0.0–5.1)
Southern	32 (0–5)	4,221 (76–225)	0.4 (0.0–1.8)

2010/11

Region	Notifications (weekly range)	Tests (weekly range)	%+ (weekly range)
Tropical	778 (0–69)	2,629 (45–137)	17.4 (0.0–44.8)
Central	510 (6–37)	4,603 (93–212)	4.1 (0.5–9.0)
Southern	517 (5–38)	5,620 (130–259)	3.8 (0.0–8.5)

2011/12

Region	Notifications (weekly range)	Tests (weekly range)	%+ (weekly range)
Tropical	167 (1–24)	1,777 (35–85)	4.1 (0.0–20.8)
Central	265 (2–35)	3,913 (97–188)	1.7 (0.0–4.0)
Southern	215 (1–24)	3,854 (71–200)	1.6 (0.0–4.5)

2012/13

Region	Notifications (weekly range)	Tests (weekly range)	%+ (weekly range)
Tropical	239 (1–27)	2,216 (40–115)	4.7 (0.0–17.5)
Central	269 (3–19)	4,391 (99–209)	1.0 (0.0–4.0)
Southern	288 (1–21)	4,792 (104–265)	1.2 (0.0–3.5)

2013/14

Region	Notifications (weekly range)	Tests (weekly range)	%+ (weekly range)
Tropical	516 (5–33)	2,382 (40–137)	10.9 (1.2–27.7)
Central	610 (9–36)	4,839 (99–209)	3.2 (0.0–4.0)
Southern	849 (10–49)	5,781 (127–301)	3.8 (1.2–8.7)

2014/15

Region	Notifications (weekly range)	Tests (weekly range)	%+ (weekly range)
Tropical	387 (6–28)	2,744 (41–149)	7.1 (1.0–26.9)
Central	903 (14–54)	5,873 (96–325)	3.7 (1.0–9.4)
Southern	848 (8–43)	8,339 (113–419)	3.1 (1.2–6.9)

2015/16

Region	Notifications (weekly range)	Tests (weekly range)	%+ (weekly range)
Tropical	528 (4–50)	4,449 (105–201)	6.0 (0.0–12.3)
Central	1,259 (12–117)	9,149 (203–422)	4.8 (1.2–15.5)
Southern	1,304 (10–103)	11,943 (299–504)	3.7 (1.1–6.7)

2016/17

Region	Notifications (weekly range)	Tests (weekly range)	%+ (weekly range)
Tropical	1,083 (10–109)	5,338 (129–249)	9.4 (3.4–16.6)
Central	1,711 (25–125)	9,706 (257–388)	5.2 (1.8–10.5)
Southern	1,956 (43–100)	10,192 (288–462)	5.2 (1.7–9.0)

2017/18

Region	Notifications (weekly range)	Tests (weekly range)	%+ (weekly range)
Tropical	1,200 (21–71)	6,489 (157–289)	7.8 (2.4–13.4)
Central	2,245 (20–107)	10,790 (267–461)	6.3 (0.9–10.5)
Southern	2,104 (22–129)	11,405 (289–502)	4.8 (0.4–12.0)

2018/19

Region	Notifications (weekly range)	Tests (weekly range)	%+ (weekly range)
Tropical	4,723 (45–493)	10,485 (206–593)	20.6 (7.9–32.7)
Central	5,071 (105–374)	13,463 (352–717)	10.3 (5.9–22.4)
Southern	5,735 (82–365)	16,356 (437–801)	9.2 (4.2–15.0)

Figure 1: Inter-seasonal proportion of influenza test positive by region and International Organisation for Standardisation (ISO) weeks of laboratory test, Queensland, 2009/10 to 2018/19

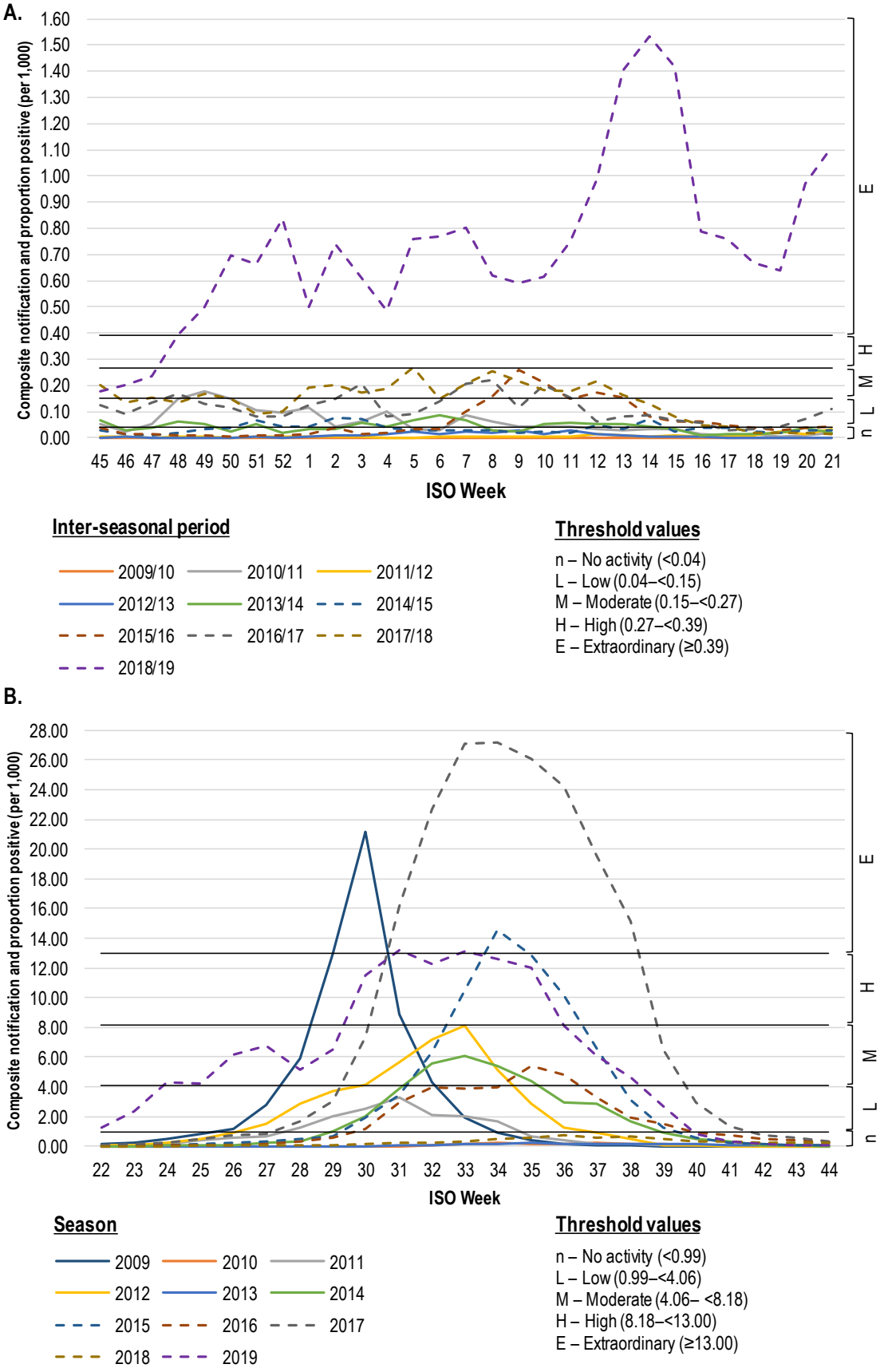


Figure 2: Weekly composite laboratory-confirmed influenza rate (per 1,000 population) and proportion positive and influenza intensity thresholds, Queensland 2009–2019 A) Inter-seasonal periods, 2009/10 to 2018/19 B) Seasonal periods, 2009 to 2019

Influenza A was the dominant strain across all periods, both inter-seasonal and seasonal, apart from the 2015 season where 72.4% of notifications were influenza B (Untyped: 98.7%, Victoria: 0.8%, Yamagata: 0.5%) (Figure 3). Overall 25.5% of notifications were influenza B (including 2009 season); 27% of seasonal notifications (including 2009 season), and 18.5% of inter-seasonal notifications. During the 2017 season and 2017/18 inter-seasonal period, influenza B accounted for 35% and 42% of notifications, respectively. Co-circulation of influenza B (≥20% of notifications) occurred in 45% (5/11) of seasons and 50% (5/10) of inter-seasonal periods. During seasons (excluding the 2009 pandemic) 87% (117,324/134,972) of influenza A notifications were untyped and 91% (43,260/55,354) of influenza B notifications were untyped. Proportions untyped increased to 76% (22,405/29,608) for influenza A and 94.5% (6,368/6,740) for influenza B during inter-seasonal periods.

Seasonal activity, as defined by the weekly composite and threshold classifications, following the influenza A dominant 'extraordinary' seasons (2009, 2017) was classified as 'no activity'. These 'no activity' seasons were then followed by high inter-seasonal activity; the 'extraordinary' 2018/19 period and 'moderate' 2010/11 period with an elevated proportion positive (Figure 3–4). The 2015 influenza B 'extraordinary' season was followed by a moderate season (2016) and moderate inter-seasonal period (2016/17).

Discussion

By examining Queensland notification and laboratory testing data we found markedly increased inter-seasonal influenza notifications and testing over the 10-year period. Gradual increases in weekly inter-seasonal proportions positive and intensity classifications over time were also apparent and provide evidence to suggest a real increase in inter-seasonal influenza in Queensland.

In contrast to earlier inter-seasonal periods where weekly proportions positive exceeding 5% were relatively uncommon, from 2016/17 onwards weekly proportions positive generally exceeded 5%. Similarly, only one of six earlier inter-seasonal periods were classified above 'low' intensity, whilst four consecutive periods between 2015/16 to 2018/19 were classified as 'moderate', 'high', or 'extraordinary'. The 2017/18 inter-seasonal period is noteworthy as intensity did not reach the extraordinary levels evident in the 2018/19 period, yet there was sustained intensity with 15 of 29 weeks within the moderate or high intensity range and 17 of 29 weeks with a proportion positive between 7–10%. The anticipated baseline of influenza activity during inter-seasonal periods appears to have shifted to now include sustained moderately intense activity. It is unlikely that increases in weekly proportions positive and

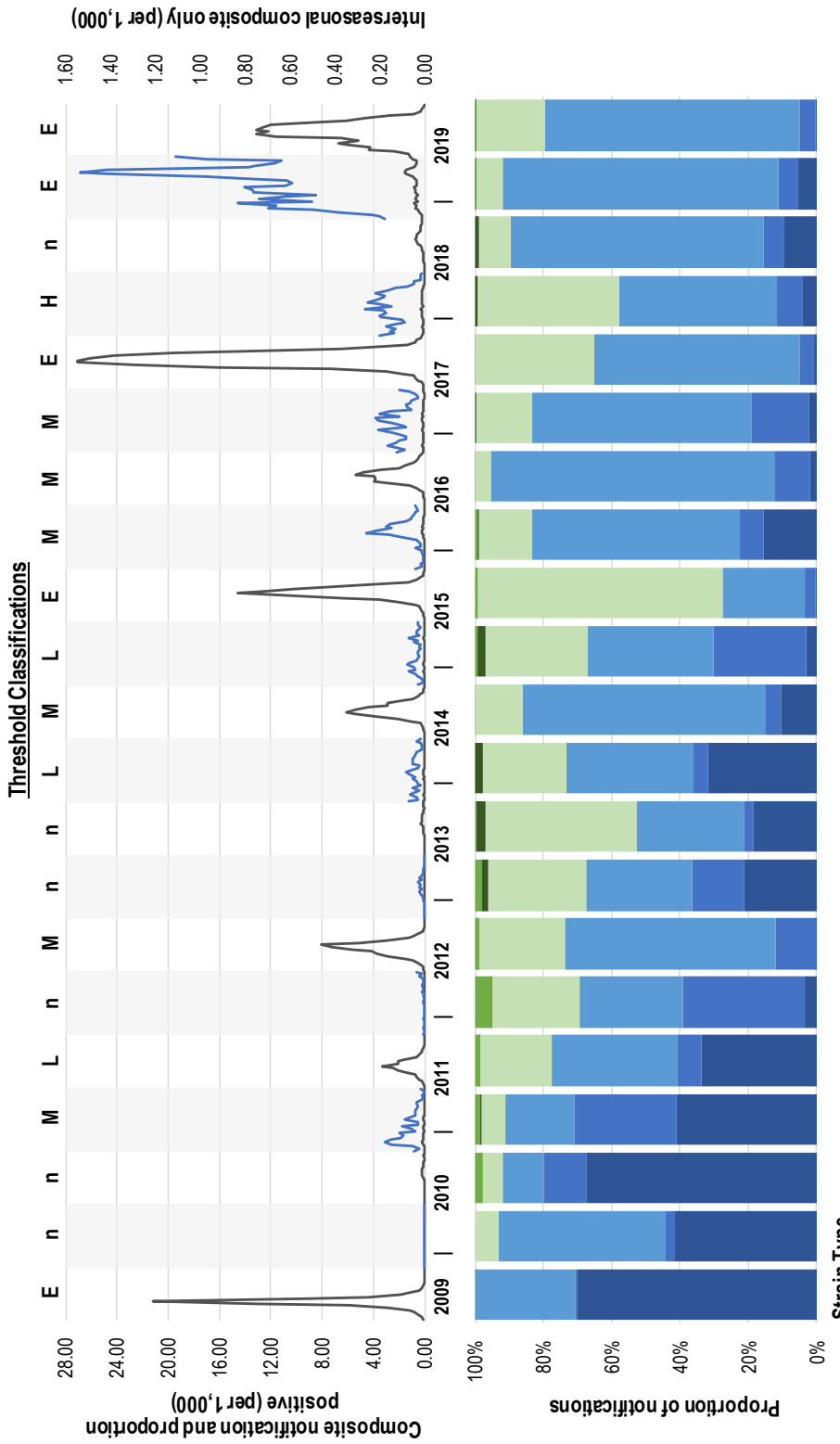


Figure 3: Weekly composite laboratory-confirmed notification rate and proportion positive, and threshold classification and strain circulation by seasonal and inter-seasonal periods, Queensland 2009–2019

Week #	2009	2009–2010	2010–2011	2011–2012	2012–2013	2013–2014	2014–2015	2015–2016	2016–2017	2017–2018	2018–2019
45		0%	5%	3%	0%	7%	4%	4%	6%	8%	6%
46		1%	4%	2%	1%	5%	2%	3%	5%	7%	7%
47		1%	7%	0%	1%	5%	2%	3%	8%	7%	8%
48		0%	14%	1%	0%	6%	4%	2%	8%	7%	10%
49		1%	14%	1%	0%	5%	5%	2%	7%	8%	11%
50		0%	13%	2%	1%	4%	5%	2%	7%	7%	13%
51		1%	12%	2%	2%	6%	8%	2%	6%	6%	13%
52		1%	14%	2%	1%	5%	7%	2%	7%	7%	18%
1		0%	12%	1%	1%	4%	10%	3%	8%	7%	13%
2		1%	8%	2%	2%	5%	8%	5%	8%	9%	13%
3		0%	9%	2%	2%	7%	7%	2%	9%	7%	13%
4		2%	9%	1%	4%	7%	6%	3%	5%	8%	12%
5		0%	7%	1%	5%	8%	4%	4%	6%	10%	15%
6		1%	7%	2%	3%	10%	4%	4%	8%	6%	14%
7		1%	10%	2%	4%	9%	4%	6%	9%	8%	14%
8		0%	7%	2%	5%	5%	4%	7%	8%	9%	12%
9		0%	5%	1%	5%	4%	2%	10%	6%	8%	12%
10		1%	5%	3%	3%	6%	3%	8%	9%	8%	11%
11		0%	5%	1%	5%	6%	2%	6%	8%	7%	12%
12		1%	4%	3%	2%	6%	5%	8%	4%	7%	14%
13		1%	5%	2%	2%	5%	3%	8%	5%	7%	16%
14		0%	4%	2%	2%	4%	6%	5%	6%	7%	16%
15		1%	5%	3%	2%	4%	3%	5%	5%	5%	16%
16		0%	3%	2%	1%	2%	4%	5%	4%	3%	13%
17		0%	3%	2%	1%	3%	4%	5%	3%	3%	13%
18		0%	4%	3%	1%	2%	3%	4%	3%	2%	12%
19		1%	1%	5%	0%	3%	2%	3%	3%	2%	11%
20		0%	2%	3%	0%	4%	3%	3%	4%	2%	12%
21		0%	3%	4%	0%	3%	3%	4%	6%	2%	12%
22	6%	0%	4%	5%	1%	3%	4%	2%	5%	1%	11%
23	6%	0%	6%	6%	1%	2%	3%	3%	6%	1%	15%
24	9%	1%	8%	11%	1%	5%	6%	4%	7%	2%	19%
25	12%	1%	12%	14%	1%	5%	6%	4%	10%	3%	18%
26	17%	1%	16%	18%	1%	6%	7%	5%	14%	3%	21%
27	23%	2%	18%	23%	2%	10%	8%	7%	14%	5%	23%
28	32%	3%	23%	30%	3%	11%	10%	9%	18%	3%	21%
29	36%	2%	28%	29%	2%	19%	13%	11%	24%	4%	23%
30	39%	3%	30%	31%	2%	25%	20%	14%	31%	4%	27%
31	35%	4%	30%	35%	5%	31%	24%	23%	40%	6%	28%
32	29%	5%	24%	36%	5%	35%	31%	25%	45%	6%	27%
33	24%	10%	25%	38%	8%	34%	38%	24%	46%	8%	28%
34	18%	13%	23%	30%	6%	32%	41%	24%	48%	9%	27%
35	14%	12%	16%	23%	9%	29%	39%	28%	49%	9%	27%
36	9%	11%	13%	16%	8%	24%	38%	27%	46%	10%	25%
37	7%	13%	8%	17%	8%	24%	29%	23%	44%	8%	21%
38	4%	10%	9%	14%	8%	21%	21%	21%	42%	11%	19%
39	2%	9%	6%	5%	7%	16%	17%	19%	31%	10%	17%
40	2%	9%	4%	6%	9%	13%	12%	15%	25%	9%	11%
41	2%	6%	6%	4%	8%	12%	8%	14%	16%	8%	8%
42	3%	5%	2%	3%	6%	6%	7%	11%	13%	8%	6%
43	0%	6%	2%	1%	6%	5%	5%	12%	11%	7%	4%
44	0%	5%	1%	1%	7%	3%	4%	9%	10%	7%	3%

Season
Interseason
Gradient Value Minimum values 50th Percentile Maximum values

Figure 3: Heat map of weekly proportions of influenza tests positive by seasonal and inter-seasonal periods, Queensland 2009–2019

intensity classifications are driven by increased notifications alone. Testing and the proportion positive values have also increased over time. Factors such as changing climatic patterns, stepwise increases in vaccine uptake, and increased global interconnectedness and tourist arrival numbers may play a role in increasing inter-seasonal influenza activity.[9,22] Increased vaccination uptake with a well matched vaccine may mimic immunity levels established by a strong influenza season, limiting seasonal activity and thus potentially pushing activity into the inter-seasonal period. A high proportion of influenza cases among children attending a sentinel hospital in temperate Australia during 2019 were also found to be travel related.[17] Importing diverse viruses from northern hemisphere seasonal epidemics or other tropical regions into Australian summers is highly relevant for Queensland which has four of the top ten Australian tourism regions for international visitors.[9,14,23,24] A shift of peak seasonal activity into the latter parts of the seasonal period may also affect the distribution of activity in inter-seasonal periods.

In Queensland it also appeared that seasons with extraordinary levels of influenza transmission intensity, such as the 2009 pandemic and 2017 season, had an impact on the intensity of the subsequent seasons and interseasons. Subsequent seasonal activity is reduced and shifted into the following inter-seasonal period. Seasons subsequent to those characterised as 'extraordinary' in intensity, registered on intensity thresholds as 'no activity'. Yet their subsequent inter-seasonal periods (2010/11, 2018/19) are well established by previous national studies and our findings as having heightened transmission; increased notifications associated with higher proportions positive.[7,9-11] However, this intensity pattern was only evident in 'extraordinary' seasons which were influenza A dominant. This effect may be replicated or reinforced by increasing vaccination use in Australia. Increasing coverage with a well-matched vaccine may mimic immunity levels established by a strong influenza season. This may have the effect of limiting activity in the subsequent season, pushing up intensity in the following inter-seasonal period. Modelling could be used to explore the impact season size, vaccine match, and natural versus vaccine-induced immunity has on future seasonal and inter-seasonal intensity.

Consistent with previous literature, we found that the Tropical region contributed considerably to increased inter-seasonal influenza activity during 2010/11 and 2018/19 periods.[9-11] Additionally, our analysis highlights divergent trends in tropical activity timing and strain circulation. During both periods, we found different weekly peak activity and strain circulation between the tropical, sub-tropical, and temperate regions of Queensland. For example, during the 2018/19 period, whilst influenza activity the Central and Southern region began to return to a baseline inter-seasonal level in January 2019, notifications and the proportion positive

continued increasing in the Tropical region until a peak in April 2019. Similar discrete trends of activity and strain circulation in the Tropical region was evident throughout the 10-year period.

The Tropical region appears to have a typically higher level of influenza circulation during inter-seasonal periods compared to sub-tropical and temperate regions. Higher notification and test counts in Southern and Central regions were most likely associated with higher population density, whilst the overall higher proportion positive in the Tropical region suggests increased activity. Characterising the Tropical region as having higher influenza circulation during inter-seasonal periods is consistent with recent Australian literature and research on seasonality more broadly.[1,9-11] Rather than fitting into seasonality models of other tropical regions, the overall seasonality of the Tropical region still appears to align to that of temperate Australian regions. Unlike regions nearer to the equator, the Queensland Tropical region did not have stable circulation levels throughout the year, and bi-modal peaks were not observed, as would be expected in many South Asian countries.[7,16,25] Tropical influenza activity, as indicated by notifications and the proportion positive, increased by almost 50% during seasonal periods and at a similar time to the sub-tropical and temperate regions. As well as this the same relatively heightened circulation and sporadic outbreaks should be anticipated in Tropical regions during inter-seasonal periods.

Evaluation of the ratio of inter-seasonal-to-seasonal tests also indicated that influenza testing remains at relative constant levels regardless of season/inter-seasonal period for the Tropical region and among children younger than five years of age. This potentially reflects awareness of influenza risk regardless of time point among health practitioners. For the tropical region, health practitioners likely recognise influenza as a cause of respiratory illness during inter-seasonal periods, whilst for children younger than 5 years of age it is likely related to both clinical presentations of influenza and severity. Respiratory symptoms in children lack pathogen specificity, and along with the availability of multi-respiratory virus PCR panels in Queensland including the GeneXpert influenza A/B/RSV test, infants and children with even moderate respiratory illness are likely to be tested for a number of pathogens, including influenza.[26] In this context, influenza seasonality may be less likely to have an impact on a practitioner's decision to test for influenza. Use of multi-virus PCR panels also may create additional test pressures due seasonal circulation of other respiratory viruses.

Influenza A was the dominant strain across the 10-year period, yet there was frequent co-circulation of influenza B, particularly in inter-seasonal periods. Overall 18.5% of inter-seasonal notifications were influenza B and per-period inter-seasonal proportions of influenza B ranged up to 42.2% (2017/18). The proportion of inter-seasonal notifications due to influenza B are

rarely presented: this proportion in Okinawa, Japan (2007–2014) was considerably lower than Queensland values at 4.5%.[27] Our inter-seasonal influenza B proportion was most similar to the Australian seasonal average (17.1%) and global seasonal average (22.6%).[5,6] The majority of influenza B notifications were not characterised, particularly during inter-seasonal periods where overall less typing was observed. The proportion of seasonal and inter-seasonal influenza A notifications subtyped also appears to have reduced over the 10-year period. A similar diminishing trend in influenza A subtyping is nationally evident.[28,29] Influenza B has been considered to play an important role in inter-seasonal influenza and summer epidemics both in Australia and globally, particularly related to viral persistence.[6,10,14,27] A recent Australian analysis found strong evidence for viral persistence in influenza B Victoria Lineage.[10] Influenza B can represent a large proportion of seasonal and inter-seasonal period notifications with potentially differential impacts of severity, timing, and demographic distribution. Therefore, greater resources for typing, particularly during inter-seasonal periods, are required.

Our study has several limitations. Laboratory-confirmed influenza notifications and testing data have limited representativeness as many community influenza cases are not laboratory confirmed and data are subject to presentation and testing biases. The study was also restricted to the use of public laboratory data and therefore may not be fully representative of Queensland testing activity, with gaps most likely in capturing community-managed illness. As such, our understanding of overall influenza activity involved a combination of testing, notifications, and proportion positive. Improvements to PCR testing sensitivity over the study-period may have occurred and could not be controlled for in our analyses. Intensity thresholds have also not been previously calculated for inter-seasonal periods.

Inter-seasonal influenza represents a complex period of influenza activity and transmission, which may result in a considerable burden of disease in tropical and other climates. By understanding the epidemiological trends of influenza over climatic regions over time a clearer understanding of the interplay between testing, activity and intensity, and strain circulation can be achieved. In particular, our study illustrates that the Tropical region of Queensland has a higher baseline of activity during inter-seasonal periods with periods of divergent activity and strain circulation as compared to temperate regions. Inter-seasonal periods have recently gained attention among Australian influenza researchers, yet there are reported increases in inter-seasonal or summer influenza across a number of countries.[22,27,30-33] This study gives further weight to recommendations for year-round influenza surveillance to better understand its epidemiology during this infrequently studied time period. Whilst the largest burden of inter-seasonal influenza is in tropical regions, heightened inter-seasonal influenza activity occurs

across climatic zones, and as demonstrated by the 2018/19 transmission intensity, activity can reach extraordinary levels. Collection and analysis of high-quality surveillance data should not be limited to seasonal periods. Furthermore, strain circulation, timing, and transmission intensity varies between climatic regions, and even among tropical regions, seasonal trends vary. Therefore, countries and regions, specifically those with tropical or varied climates, should look to undertake routine year-round influenza surveillance stratified to account for climatic zones and strain circulation. With potential interconnectedness between seasonal and inter-seasonal influenza activity, surveillance of inter-seasonal influenza may also have value in contextualising or anticipating seasonality and intensity over time. Further research into the potential flow on impacts of seasonal intensity and strain circulation on inter-seasonal and seasonal periods over a longer timeframe may provide greater insights into drivers of influenza seasonality.

References

1. Tamerius JD, Shaman J, Alonso WJ, Bloom-Feshbach K, Uejio CK, Comrie A, et al. Environmental predictors of seasonal influenza epidemics across temperate and tropical climates. PLoS Pathogens. 2013;9(3):e1003194. DOI: 10.1371/journal.ppat.1003194

2. Moa AM, Adam DC, MacIntyre CR. Inter-seasonality of influenza in Australia. Influenza and Other Respiratory Viruses. 2019. DOI: 10.1111/irv.12642

3. Tamerius J, Nelson MI, Zhou SZ, Viboud C, Miller MA, Alonso WJ. Global influenza seasonality: reconciling patterns across temperate and tropical regions. Environmental Health Perspectives. 2011;119(4):439-45. DOI: 10.1289/ehp.1002383

4. Yap J, Tan CH, Cook AR, Loh JP, Tambyah PA, Tan BH, et al. Differing clinical characteristics between influenza strains among young healthy adults in the tropics. BMC Infectious Diseases. 2012;12:12. DOI: 10.1186/1471-2334-12-12

5. Caini S, Huang QS, Ciblak MA, Kusznierz G, Owen R, Wangchuk S, et al. Epidemiological and virological characteristics of influenza B: results of the Global Influenza B Study. Influenza and Other Respiratory Viruses. 2015;9 Suppl 1:3-12. DOI: 10.1111/irv.12319

6. Moa AM, Muscatello DJ, Turner RM, MacIntyre CR. Epidemiology of influenza B in Australia: 2001–2014 influenza seasons. Influenza and Other Respiratory Viruses. 2017;11(2):102-9. DOI: 10.1111/irv.12432

7. Caini S, Andrade W, Badur S, Balmaseda A, Barakat A, Bella A, et al. Temporal patterns of influenza A and B in tropical and temperate countries: what are the lessons for influenza vaccination? PLoS One. 2016;11(3):e0152310. DOI: 10.1371/journal.pone.0152310

8. Sullivan SG, Pennington K, Raupach J, Franklin LJ, Bareja C, de Kluyver R, et al. Summary of influenza surveillance systems in Australia, 2015. [Internet]; 2016. [cited 2019 June 14]. Available from: http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-surveil-ozflu-flucurr.htm/$File/Influenza-Surveillance-Systems-Paper.pdf.

9. Barr IG, Deng YM, Grau ML, Han AX, Gilmour R, Irwin M, et al. Intense interseasonal influenza outbreaks, Australia, 2018/19. Eurosurveillance. 2019;24(33):1900421. DOI: 10.2807/1560-7917.ES.2019.24.33.1900421

10. Patterson Ross Z, Komadina N, Deng YM, Spirason N, Kelly HA, Sullivan SG, et al. Inter-seasonal influenza is characterized by extended virus transmission and persistence. PLoS Pathogens. 2015;11(6):e1004991. DOI: 10.1371/journal.ppat.1004991

11. Kelly HA, Grant KA, Tay EL, Franklin L, Hurt AC. The significance of increased influenza notifications during spring and summer of 2010–11 in Australia. Influenza and Other Respiratory Viruses. 2013;7(6):1136-41. DOI: 10.1111/irv.12057

12. Queensland Health. Statewide weekly influneza surveillance report, ISO weeks 1–22. [Internet]. Brisbane: Department of Health; 2019. [cited 2019 Jun 06]. Available from: https://www.health.qld.gov.au/clinical-practice/guidelines-procedures/diseases-infection/surveillance/reports/flu.

13. Australian Government Department of Health. Australian influenza surveillance report, No. 1, 2019. [Internet]. Canberra: Department of Health; 2019. [cited 2019 Jul 15]. Available from: https://www1.health.gov.au/internet/main/publishing.nsf/Content/E992F2FDA65E0038CA2583FA001951BD/$File/flu-01-2019.pdf.

14. Rambaut A, Pybus OG, Nelson MI, Viboud C, Taubenberger JK, Holmes EC. The genomic and epidemiological dynamics of human influenza A virus. Nature. 2008;453(7195):615-9. DOI: 10.1038/nature06945

15. Nelson MI, Simonsen L, Viboud C, Miller MA, Taylor J, George KS, et al. Stochastic processes are key determinants of short-term evolution in influenza A virus. PLoS Pathogens. 2006;2(12):e125. DOI: 10.1371/journal.ppat.0020125

16. Hirve S, Newman LP, Paget J, Azziz-Baumgartner E, Fitzner J, Bhat N, et al. Influenza seasonality in the tropics and subtropics - when to vaccinate? PLoS One. 2016;11(4):e0153003. DOI: 10.1371/journal.pone.0153003

17. Deng L, Mazzocato P, Saravanos G, Leder K, Britton P. A high proportion of interseasonal childhood influenza cases in 2019 were travel related. Public Health Research and Practice. 2020;30(2):e3022012. DOI: 10.17061/phrp3022012

18. Appuhamy RD, Beard FH, Phung HN, Selvey CE, Birrell FA, Culleton TH. The changing phases of pandemic (H1N1) 2009 in Queensland: an overview of public health actions and epidemiology. Medical Journal of Australia. 2010;192(2):94-7. DOI: 10.5694/j.1326-5377.2010.tb03427.x

19. World Health Organization. Pandemic influenza severity assessment (PISA). [Internet]. Geneva: World Health Organization; 2017. [cited 2019 Jun 10]. Available from: https://www.who.int/influenza/surveillance_monitoring/pisa/guidance/en/.

20. Goldstein E, Viboud C, Charu V, Lipsitch M. Improving the estimation of influenza-related mortality over a seasonal baseline. Epidemiology. 2012;23(6):829-38. DOI: 10.1097/EDE.0b013e31826c2dda

21. Tay EL, Grant K, Kirk M, Mounts A, Kelly H. Exploring a proposed WHO method to determine thresholds for seasonal influenza surveillance. PLoS One. 2013;8(10):e77244. DOI: 10.1371/journal.pone.0077244

22. Towers S, Chowell G, Hameed R, Jastrebski M, Khan M, Meeks J, et al. Climate change and influenza: the likelihood of early and severe influenza seasons following warmer than average winters. PLoS Currents. 2013;5. DOI: 10.1371/currents.flu.3679b56a3a5313dc7c043fb944c6f138

23. Uyeki TM, Zane SB, Bodnar UR, Fielding KL, Buxton JA, Miller JM, et al. Large summertime influenza A outbreak among tourists in Alaska and the Yukon Territory. Clinical Infectious Diseases. 2003;36(9):1095-102. DOI: 10.1086/374053

24. Tourism Research Australia. International visitor survey: YE December 2019: Austrade; 2020 [cited 2020 Apr 17]. Available from: https://www.tra.gov.au/ArticleDocuments/185/IVS_TOURISM_RESULTS_YE_DEC_2019.xlsx.aspx

25. El Guerche-Seblain C, Caini S, Paget J, Vanhems P, Schellevis F. Epidemiology and timing of seasonal influenza epidemics in the Asia-Pacific region, 2010–2017: implications for influenza vaccination programs. BMC Public Health. 2019;19(1):331. DOI: 10.1186/s12889-019-6647-y

26. Kaczmarek MC, Schlebusch S, Ware RS, Coulthard MG, McEniery JA, Lambert SB. Diagnostic testing in influenza and pertussis related paediatric intensive care unit admissions, Queensland, Australia, 1997–2013. Communicable Diseases Intelligence. 2017;41(4):E308-E17.

27. Sunagawa S, Iha Y, Taira K, Okano S, Kinjo T, Higa F, et al. An epidemiological analysis of summer influenza epidemics in Okinawa. Internal medicine (Tokyo, Japan). 2016;55(24):3579-84. DOI: 10.2169/internalmedicine.55.7107

28. Australian Government Department of Health. Influenza (laboratory-confirmed) national notifiable disease surveillance notifications in Australia 2008–2017. [cited 2020 Apr 17]. Available from: http://www9.health.gov.au/cda/source/pub_influ.cfm

29. WHO Collaborating Centre for Reference and Research on Influenza (VIDRL). 2018 Annual report. [Internet]. Melbourne; 2019. [cited 2020 Apr 17]. Available from: http://www.influenzacentre.org/documents/publications_reports/Annual%20Report%202018.pdf.

30. Ghedin E, Wentworth DE, Halpin RA, Lin X, Bera J, DePasse J, et al. Unseasonal transmission of H3N2 influenza A virus during the swine-origin H1N1 pandemic. Journal of Virology. 2010;84(11):5715-8. DOI: 10.1128/jvi.00018-10

31. Gurav YK, Chadha MS, Tandale BV, Potdar VA, Pawar SD, Shil P, et al. Influenza A(H1N1)pdm09 outbreak detected in inter-seasonal months during the surveillance of influenza-like illness in Pune, India, 2012-2015. Epidemiology and Infection. 2017;145(9):1898-909. DOI: 10.1017/S0950268817000553

32. Tsou TP, Su CP, Huang WT, Yang JR, Liu MT. Influenza A(H3N2) virus variants and patient characteristics during a summer influenza epidemic in Taiwan, 2017. Eurosurveillance. 2017;22(50). DOI: 10.2807/1560-7917.ES.2017.22.50.17-00767

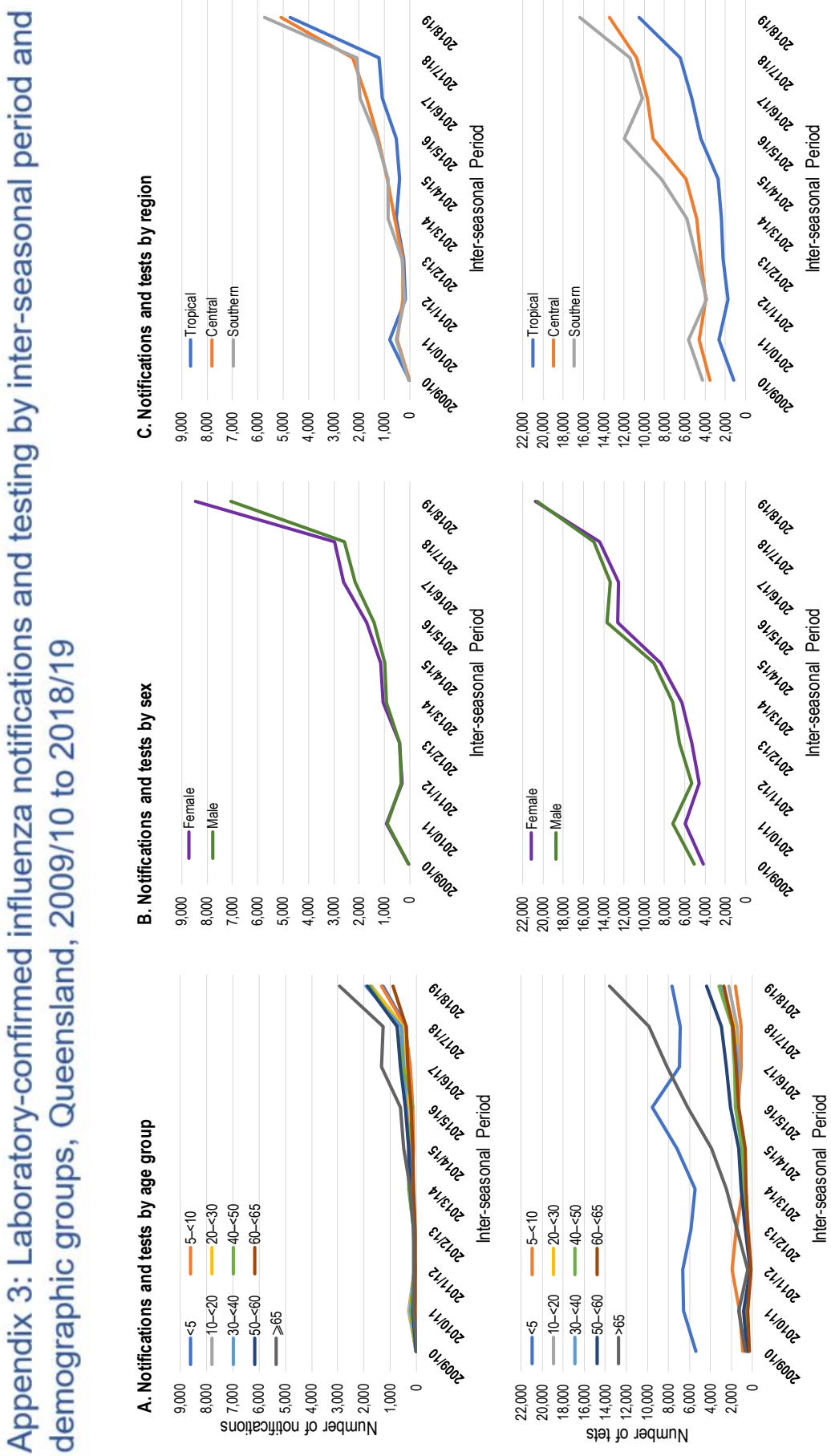
33. Wolf DG, David R, Eitan K, Hay A, Mador N, Evgenia G, et al. A summer outbreak of influenza A virus infection among young children. Clinical Infectious Diseases. 2004;39(4):595-7. DOI: 10.1086/422457

Appendix 1: Summary of geographic regions and Hospital and Health Services regions

Tropical	Central	Southern
Torres and Cape	Central Queensland	Metro South
Cairns and Hinterland	Central West	Darling Downs
North West	Wide Bay	West Moreton
Townsville	Sunshine Coast	South West
Mackay	Metro North	Gold Coast

Appendix 2: Proportion of influenza tests positive by inter-seasonal period and demographic groups, Queensland, 2009/10 to 2018/19

Period	2009/10 (%)	2010/11 (%)	2011/12 (%)	2012/13 (%)	2013/14 (%)	2014/15 (%)	2015/16 (%)	2016/17 (%)	2017/18 (%)	2018/19 (%)	Total (%)
Age Group (years)											
<5 years	0.3	3.1	1.9	0.8	1.7	1.4	1.9	2.4	2.6	6.2	**2.3**
5–<10	0.1	6.7	2.7	1.8	4.6	5.8	4.0	6.1	10.6	23.7	**7.0**
10–<20	2.4	13.8	2.4	3.2	10.7	7.0	5.9	7.8	10.1	19.6	**10.8**
20–<30	0.9	16.8	4.8	5.0	10.4	6.0	8.7	8.1	9.3	19.3	**11.6**
30–<40	1.6	12.9	0.7	3.4	9.6	8.6	8.1	8.2	7.7	17.6	**10.6**
40–<50	1.0	15.3	3.1	4.1	9.4	6.0	7.4	6.1	7.7	15.2	**9.4**
50–<60	1.0	9.8	2.0	4.1	9.2	5.8	6.7	6.8	7.0	14.3	**8.7**
60–<65	1.0	6.3	2.5	4.0	6.3	4.2	6.1	7.2	6.4	13.0	**7.8**
⩾65	0.1	5.7	2.5	2.0	4.5	5.3	4.9	8.0	6.1	9.9	**6.9**
Sex											
Female	0.6	7.5	2.5	2.0	5.5	4.3	4.8	6.4	6.4	13.0	**6.8**
Male	0.4	6.0	1.9	1.8	4.6	3.8	4.3	5.8	6.0	12.2	**5.9**
Region											
Tropical	0.9	17.4	4.1	4.7	10.9	7.1	6.0	9.4	7.8	20.6	**11.4**
Central	0.4	4.1	1.7	1.0	3.2	3.7	4.8	5.2	6.3	10.3	**5.3**
Southern	0.4	3.8	1.6	1.2	3.8	3.1	3.7	5.2	4.8	9.2	**4.7**


Appendix 3: Laboratory-confirmed influenza notifications and testing by inter-seasonal period and demographic groups, Queensland, 2009/10 to 2018/19
A. Notifications and tests by age group
B. Notifications and tests by sex
C. Notifications and tests by region
Number of notifications
Number of tests
Inter-seasonal Period
<5
5–<10
10–<20
20–<30
30–<40
40–<50
50–<60
60–<65
≥65
>65
Female
Male
Tropical
Central
Southern
2009/10
2010/11
2011/12
2012/13
2013/14
2014/15
2015/16
2016/17
2017/18
2018/19

Chapter 4

Assessing a new, clinically based HIV indicator in Queensland: surveillance of time to viral suppression after diagnosis

Prologue

Rationale

This chapter satisfies the MAE requirement to establish or evaluate a surveillance system. Since the benefits of initiating antiretroviral treatment early have been firmly established, public health priorities have shifted towards ensuring that recently diagnosed people are not only on treatment but achieving rapid viral suppression. When I began my placement at Queensland Health in March 2019, two recently published papers (Xia et al. 2019; McManus et al. 2019) clearly identified the limitations of treatment commencement indicators used to monitor progress in HIV care in the United States and Australia.[1,2] A new monitoring indicator was also proposed by Xia et al. as an improved measurement of treatment quality, the proportion of newly diagnosed people achieving viral suppression. Data improvement activities were already well underway at Queensland Health which enabled viral load testing data to be accessed for more individuals within their notification system. This provided an opportunity to explore the feasibility and usefulness of the proposed viral suppression indicator in the Queensland context.

Public health impact

This analysis has not been performed using Queensland or Australian data previously, and provides information about an important metric of HIV control in the era of widely available prevention and treatment options. The newly proposed indicator represents a mechanism for a more data based approach to respond to the 90:90:90 targets. This project also brings focus to data improvement activities regarding HIV testing data within the Queensland Health Communicable Diseases Branch (CDB). Although a process for ensuring testing data from the public laboratory system is complete, the project highlighted some existing limitations, as well as ways in which the data can be routinely used to prompt public health actions. As part of this project I had the opportunity to engage with community members and public health practitioners. This engagement process brought consensus among crucial players in the HIV field about the key populations for HIV surveillance. This is a timely exercise given Queensland Health's transition to a new notifications platform in 2020. There are opportunities to further explore and consolidate the usefulness of the proposed mechanism for surveillance of rapid viral suppression.

Preliminary results from the pilot study were presented to the Queensland Sexual Health Ministerial Advisory Committee and HIV Public Health Team and were accepted as a poster presentation at the 2020 ISID Conference in Malaysia (Appendix 1). Although the conference

was ultimately cancelled due to COVID-19 pandemic, the abstract will be published in the International Journal of Infectious Diseases.

My roles

I worked within the Epidemiology and Research Unit (ERU) to support ongoing data improvement activities, designed and completed a pilot study using notification data for all individuals diagnosed between 2014 and 2018, and undertook stakeholder engagement to explore a process for embedding an analysis of rapid viral suppression after diagnosis into the routine epidemiological work of the CDB. I worked closely with the CDB Data Services Team (DST) to undertake initial scoping activities in May 2019 which indicated that viral load testing data available in the Queensland Notifiable Conditions System (NoCS) was limited prior to 2017. To address this a contractor was employed to identify and enter data from 2014 to 2017. I supported this activity by creating a master list of viral load testing data by extracting AUSLAB records and undertaking probabilistic matching to HIV notification data from 2014 to 2016 using date of birth, sex, Medicare number (first 9 digits), and a 2x2 code for individuals first and surname. Records (N=19,008) were prioritised for data entry based on the likelihood of a new diagnosis and match quality.

In regard to the pilot study, I lead the design, obtained ethical approvals, and completed the data cleaning and analysis. Extraction of the study data was undertaken by the DST. To complete the stakeholder engagement process, I developed the interview guide, coordinated, facilitated and transcribed the interviews. I was also responsible for the writing of the conference abstract that was submitted and accepted by the International Society of Infectious Diseases (Poster presentation).

Lessons learnt

This project first and foremost expanded my capacity to be flexible and adapt to new challenges. This project started out as my epidemiological project but at the point in 2019 where I had completed the study, it morphed into a surveillance project due to some challenges with my split placement. I worked through with my supervisors an appropriate and feasible way of adapting the project scope and activities. I am grateful for how the project evolved, both in terms of the outcome, as well as giving me the opportunity to engage with stakeholders. I learnt a lot from speaking to stakeholders, both about the surveillance system but also the issues and challenges of HIV treatment and care. From a technical stand point this was also my first foray into record linkage which served me very well in a number of subsequent activities within the MAE and became the basis for my Lesson from the Field.

Acknowledgements

I am very grateful to the large number of people who gave me their support and time. Firstly, to my supervisors Stephen and Jonathan for proposing the study and seeing a way for it to go forward as a surveillance system project and to Damin Si and Data Services (Mayet and Belinda) for making the data happen. This project also would not have been possible without the data improvement works pursued by Sonya Bennett. Thank you also to Rob Ware for the crucial statistical analysis support and for the stakeholders who used their valuable time to share their knowledge and experiences.

Abstract

Background

Shortening the time to viral suppression after HIV diagnosis reduces morbidity and transmission. Yet, treatment commencement indicators commonly used to monitor HIV care are limited measures of activity implementation and do not include the more relevant clinical and public health indicator of viral suppression. The primary objectives of this project were to explore a recently proposed outcome indicator, the percentage of newly diagnosed individuals achieving viral suppression within 3 and 6 months, and investigate the feasibility of establishing a system for the surveillance of viral suppression among people newly diagnosed with HIV in Queensland.

Methods

This project consisted of a pilot study to estimate viral suppression trends, an assessment of key surveillance system attributes, and semi-structured interviews with a range of stakeholders engaged in HIV prevention and control activities in Queensland. The potential surveillance mechanism was assessed in regards to usefulness, data quality, representativeness, and acceptability.

Results

From 2014 to 2018, 1,006 individuals were diagnosed with HIV in Queensland and the annual number of HIV diagnoses decreased by 36% (2014: 245; 2018: 180). The likelihood of achieving viral suppression increased year-on-year ($p<0.001$); within three months of diagnosis from 19.6% in 2014 to 58.8% in 2018, and within six months of diagnosis from 46.9% to 82.2%. Ninety-five percent of newly diagnosed people had at least one viral load test at or following diagnosis, yet only 73.3% had treatment commencement data available. Notable limitations to the validity of treatment commencement data were identified by the pilot study and internal stakeholders. There was clear agreement among stakeholders that viral suppression was a more objective and practical measurement of HIV care and were supportive of the implementation of a surveillance mechanism measuring viral suppression trends in people newly diagnosed.

Conclusion

Rapid viral suppression after diagnosis is an important metric for HIV control and its measurement improves our understanding of year-to-year progress for population groups. Monitoring indicators and surveillance systems for HIV care should include viral suppression among those newly diagnosed. In Queensland a surveillance mechanism for viral suppression is both acceptable and useful.

Introduction

Human immunodeficiency virus (HIV) infection impairs the body's immune system. If left untreated, the damage to the immune system can lead to serious infections and cancers over time.[3] Australia's national HIV incidence is lower than many other comparable high-income countries.[4] In 2017, it was estimated that there are between 4,800 and 6,200 people living with HIV (PLWH) in Queensland.[3,5] For this population, receiving treatment through antiretroviral therapy (ART) has important individual and public health benefits.[6,7] ART suppresses the virus replication, preventing disease progression and significantly improving the length and quality of the lives of those living with HIV.[3] Achieving viral suppression also eliminates the risk of transmitting the infection to others and saves money through medical and other costs averted.[6-9] Treatment as prevention (TasP) as an effective HIV prevention strategy is underpinned by evidence from cohorts of serodiscordant couples from the PARTNER (Partners of People on ART—A New Evaluation of the Risks) and Opposites Attract studies and is endorsed by UNAIDS as "Undetectable equals Untransmittable (U=U)".[10-12]

Shortening the time between diagnosis and viral suppression by initiating ART early among all people diagnosed with HIV, regardless of CD4+ (CD4+ T cell) count, is also now recognised as an important component of achieving reductions in morbidity and preventing new HIV infections.[3,6-8] Until 2012, global guidelines recommended delaying ART until a person's CD4+ count indicated immunological decline. However, evidence from studies such as the 2011 HPTN 052 multicentre randomised controlled trial, demonstrated that clinical benefits and transmission reductions are maximised with early ART initiation.[7] Guidelines and strategic focus have shifted towards initiating treatment rapidly after diagnosis, including Australian treatment guidelines which were updated in June 2015, and had promoted treatment as a prevention stategy.[1,7,13] Thus prompting increases in the proportion of Australian people newly diagnosed with HIV receiving treatment within six months of diagnosis.[1]

Whilst treatment is widely available in Australia and most people can physiologically achieve viral suppression within six months of beginning treatment, there are numerous barriers to HIV care.[9,14] These can relate to the individual diagnosed with HIV, as well as clinical and structural barriers to engagement in HIV care.[15-17] In particular, people newly diagnosed must navigate health systems, whilst simultaneously facing stigma and other negative personal and social reactions.[18] Poor retention in care and inconsistent ART adherence within the first year post-diagnosis has been documented by numerous studies.[16] Timely monitoring of engagement in HIV care, in particular viral load monitoring, among those newly diagnosed is therefore

important to maximise the use of resources and optimise individual and population-level HIV health outcomes through disease control and service delivery activites.[8,16,19]

Monitoring of treatment among those newly diagnosed with HIV

Relatively few countries have surveillance indicators specifically relating to newly diagnosed people. Global treatment targets established by the Joint United Nations Programme on HIV/AIDS (UNAIDS) focus on three elements of the cascade of care (diagnosis, treatment, and viral suppression) and World Health Organization (WHO) ART guidelines discuss the importance of ensuring that newly diagnosed persons commence treatment promptly (Figure 1).[8,20] Newly diagnosed people are not specifically mentioned in any current WHO or UNAID targets or monitoring indicators. Indeed few countries, including Australia, have established systems for monitoring early initiation of treatment for those newly diagnosed.[1] The current *Australian National Blood-borne Viruses and Sexually Transmissible Infections (BBV/STI) Surveillance and Monitoring Plan (2018–2022)* does not have any indicators which specifically relate to people newly diagnosed.[21]

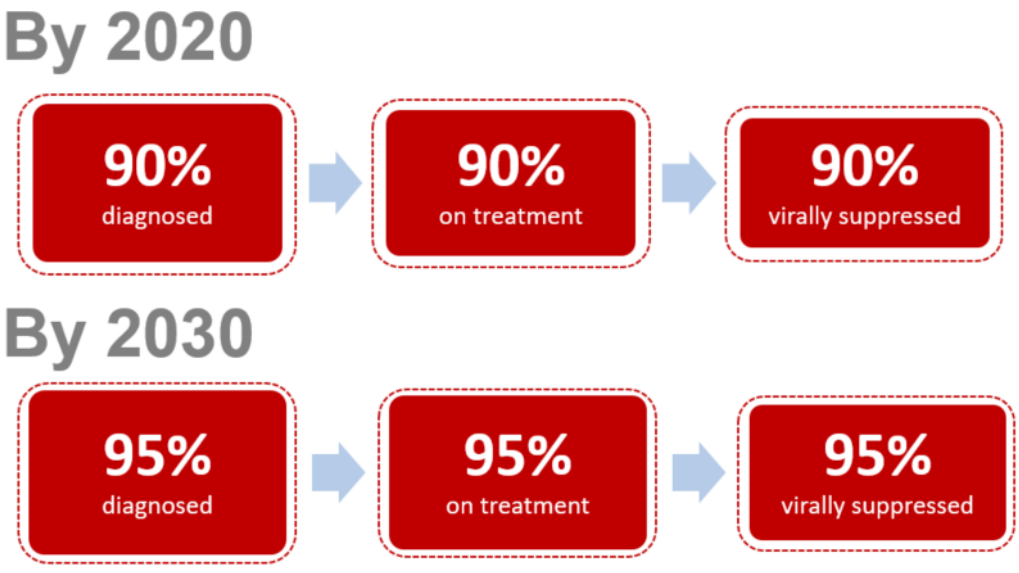


Figure 1: UNAID global cascade of care targets

Process indicators, such as percentage of newly diagnosed persons linked to HIV medical care, to monitor HIV care among persons newly diagnosed with HIV, are used by both the United States (US) and some available Australian strategies (Table 1).[3,22] Yet, the limitations of these indicators have been highlighted in recent Australian and American literature.[1,2] Treatment commencement indicators are process indicators which measure activities and their direct products/deliverables (Table 2).[23] As such, they can indicate whether a program/activity is being implemented as planned, but cannot provide insights as to whether programs are achieving the expected population-level impacts.[23] For HIV treatment programs, a process indicator alone

cannot determine whether those who are recorded as having commenced treatment are achieving the more relevant clinical and public health outcome of viral suppression.[24]

A new outcome indicator has therefore been proposed: the proportion of newly diagnosed persons achieving viral suppression within three months of diagnosis.[2] This proposed outcome indictor is considered better able to measure changes over time and provides greater insights into the effectiveness of the health system providing HIV care.[8] This outcome indicator also reflects a shift in focus away from counting ART activities towards measuring the quality of ART service provision.

Table 1: Summary of selected available targets and treatment uptake indicators related to people newly diagnosed with HIV*

Location	Source	Period	Indicator/Target	Type
Australia[3]	Eighth National HIV Strategy	2018–2022	Improve early uptake and sustained treatment to improve quality of life for people with HIV and prevent transmission.	Target
Western Australia[25] †	WA HIV Strategy Monitoring and Evaluation Framework	2019–2023	Increase the % of people diagnosed with HIV on treatment to 95% within six weeks of diagnosis for those newly diagnosed, reducing this timeframe further over the life of the strategy.	Target
New South Wales[26]	NSW HIV strategy	2016–2020	% people newly diagnosed with HIV on ART within 6 weeks of diagnosis	Process indicator
Queensland[27]	Queensland HIV Action Plan	2016–2021	% people who commence treatment within 6 weeks, 3 months, 6 weeks of diagnosis	Process indicator
United States[22]	National HIV/AIDS Strategy for the US	2015–2020	% of newly diagnosed persons linked to HIV medical care within one month of their HIV diagnosis	Process indicator

* Several Australian jurisdictions either do not have monitoring strategies (Tasmania), only monitor treatment uptake and viral suppression among all people diagnosed (South Australia), or monitor the epidemiology of new diagnoses only (Victoria, Northern Territory).[28-30]

† Although the WA strategy target relates to a six-week period between diagnosis and treatment, the corresponding indicator measures the estimated proportion of people living with HIV dispensed treatment for HIV infection without a timeframe.

Table 2: Summary of selected Centers for Disease Control and Prevention monitoring indicator categories

Indicator	Measurement
Process	Program's activities and outputs (direct products/deliverables of the activities)
Outcome	Whether the program is achieving the expected effects/changes in the short, intermediate, and long term.

Methods

Aims and objectives

The aim of this project was to explore the recently proposed outcome indicator of rapid viral suppression as measurement of service quality and impact of disease control activities and assess the feasibility of establishing a surveillance system for viral suppression among people newly diagnosed with HIV in Queensland.

The key project objectives were to:

- Conduct a pilot study to establish baseline trends of rapid viral suppression in Queensland among people newly diagnosed with HIV infection between 2014 and 2018.
- Assess the usefulness of the rapid viral suppression indicator as compared to the current treatment initiation indicator and its potential application in HIV surveillance.
- Provide recommendations for the establishment and embedding of a surveillance process to produce timely and useful analyses of viral suppression trends among people newly diagnosed with HIV.

There were three components to the project: a pilot study, an assessment of the proposed system against attributes, and key stakeholder interviews.

Pilot study

The study was a retrospective cohort design comprising state-wide de-identified notification data reported to the Queensland Notifiable Conditions System (NoCS) between 1 January 2014 to 31 December 2018. The proportion of people newly diagnosed with HIV who achieve viral suppression at any time within two time points following diagnosis (three month, six months) were calculated overall and annually by demographic, clinical, and exposure risk groups (Table 3). The primary outcome of viral suppression was defined as a viral load measurement of <200 copies/mL at any time within three and six months after diagnosis with HIV infection, consistent with Australian national surveillance and existing national and international literature regarding viral suppression.[6,9,11,14,31]

Table 3: Summary of study demographic, clinical, and exposure risk category covariates for people newly diagnosed with HIV

Demographic
Sex: Male, female
Age at diagnosis (years): <15, 15–24, 25–44, 45–64, ≥65
First Nations status: Aboriginal and/or Torres Strait Islander person, neither Aboriginal nor Torres Strait Islander person
Location at diagnosis: Major city, regional, remote according to Australian Bureau of Statistics Remoteness Areas, 2017.
Clinical
HIV progression at diagnosis: CD4+ count ≥350, 349–200, <200 cells/μL
Evidence of recent acquisition: a new notification with either a negative or indeterminate HIV antibody test in the previous 12 months or a clinically diagnosed primary HIV infection (seroconversion illness).
Blood-borne virus (BBV) infection: Notification of Hepatitis B or C infection prior to/at diagnosis*, within 6-months post diagnosis†, at all-time points, never.
Sexually transmitted infection (STI): Notification of chlamydia, gonorrhoea or syphilis infection prior to/at diagnosis*, within 6-months post diagnosis†, at all-time points, never.
Exposure risk category
Men who have sex with Men (MSM): Includes men who have sex with both men and women. Excludes injecting drug use.
Injecting Drug Use (IDU): Excludes MSM.
MSM and IDU: MSM who are also IDU.
Heterosexual: Includes persons/partner from a high prevalence country (an estimated HIV prevalence of more than 1% as per UNAIDS), partner who is an IDU, partner's risk not further specified.
Other: Includes maternal transmission.
No risk group identified: Not reported/unknown.

*Prior to/at diagnosis refers to a date of onset prior to diagnosis of HIV or within 28 days of diagnosis.

† Within six months of diagnosis excludes a period of 28 days following notification.

To compare the usefulness of Queensland's current treatment commencement indicator and inform on the quality of data recorded on treatment commencement, the proportion of people recorded as commencing treatment at any time within six weeks, three months, and six months of diagnosis was calculated and compared. Treatment commencement was measured as the earliest recorded date of treatment commencement within the study period. A Wald test was used to calculate 95% confidence intervals for proportions.

Univariate logistic regression was undertaken to identify risk factors associated with non-suppression at three and six months. Models for demographic, clinical, and exposure risk category factors, and year of diagnosis were fitted with odds ratios, 95% confidence intervals

and *p*-values calculated. Due to limitations in sample size, the <15-year age group was excluded from univariate analysis and four exposure risk categories were consolidated into an 'Other/Unknown' category (injecting drug users (IDU), men who have sex with men and who are IDU, Other, and No risk group identified). Age at diagnosis was fitted both as a categorical and continuous variable.

De-identified electronic data were provided and stored on secured computers with password access required. Data were imported from a Microsoft Excel file into Stata/Ic v.15.0 (Stata Corp. USA) for cleaning and analysis.

Assessment of the proposed system

Details of Queensland Health HIV surveillance processes and recent data improvement activities were obtained through review of relevant documents and discussions with the Communicable Diseases Branch (CDB) HIV public health team (HPHT) and ERU staff, both epidemiologists and data services team (DST) members. Assessment of the indicator and its application as a surveillance system was guided by the Centers for Disease Control and Prevention (CDC) guidelines for evaluating surveillance systems.[32] Key attributes for assessment were determined to be data quality, usefulness, representativeness, and acceptability. Appropriate methods for their evaluation were developed (Table 4).

Stakeholder engagement

Six key stakeholder groups were identified and relevant individuals were invited to participate in a semi-structured interview. Interview questions consisted of their opinions and experience in HIV surveillance regarding the current treatment commencement indicator, the need for viral suppression monitoring, and the described model of viral suppression monitoring (Appendix 5). Individuals were selected as experts of HIV treatment and surveillance and to provide a breadth of opinion and experience. Individuals interviewed included internal Queensland Health stakeholders (Medical Director, BBV/STI, a HPHT team member, an epidemiologist responsible for HIV surveillance within the CDB) and relevant external stakeholders (a senior sexual health physician, a member of the Sexual Health Ministerial Advisory Community, and the CEO and Life+ Program Manager of Queensland Positive People (QPP)). Interviews were conducted over the phone or by teleconferencing platform and were recorded and transcribed.

Table 4: Methods used to evaluate proposed monitoring system and against selected attributes

Attribute	Evaluation Definition	Evaluation Method
Usefulness	Contribution to the prevention and control of adverse health-related events.	Pilot study to demonstrate the analytic outputs that could be provided by the proposed monitoring indicator and established viral suppression trends. Stakeholders were asked to describe how the current treatment commencement indicator and the proposed monitoring of viral suppression could contribute to their roles and how outputs could be best used to respond to HIV policy, service delivery, and control improvement activities.
Data Quality	Completeness and validity of data recorded.	Pilot study to assess the number of notified individuals with data available (test results, treatment commencement records) and identified any limitations in data validity. Stakeholders were asked to identify any barriers to data completeness and validity for both treatment commencement and viral load testing data.
Representativeness	Accuracy of the described health-related event over time and its distribution in the population by place and person.	Pilot study results were compared to as Australian national-level surveillance trends and available literature regarding epidemiological characteristics of people newly diagnosed and viral suppression.
Acceptability	Willingness of stakeholders to support possible surveillance mechanism and participate in its implementation.	Stakeholders were asked to describe the perceived importance of monitoring indicators (treatment commencement and viral suppression) and identify any concerns regarding privacy and confidentiality which may act as a barrier to community acceptance of monitoring.

Ethical considerations

Ethics approval for this project was granted through the Australian National University Human Ethics Research Committee (Protocol 2019/249). Approval for access to Queensland Health information was granted under section 284 of the *Public Health Act 2005* by the Director-General's delegate.

Results

HIV is a nationally notifiable disease, with three distinct categories; [33,34]

- Newly acquired
- Unspecified
- Individuals less than 18 months of age

Unspecified HIV infection relates to individuals who do not have evidence of acquisition in the previous 12 months. Both unspecified and newly acquired HIV infections relate to individuals aged 18 months or older at the time of blood sample collection. Confirmed and probable newly acquired cases, as defined by Communicable Disease Network Australia (CDNA) national case definitions, are notified by all states and territories to the National HIV Registry at the Kirby Institute (Table 5).[14] In Queensland, under the *Public Health Act 2005* and *Public Health Regulations 2018*, HIV infection is notifiable on pathological diagnosis by laboratories to the Queensland chief executive and is a controlled notifiable condition.[35,36] As such, pathology laboratories must notify the Department of Health when examination of a specimen indicates the individual has the pathological diagnosis.

HIV testing and notifications

The majority of laboratory HIV diagnosis testing in Queensland is performed by the state public pathology laboratory, Pathology Queensland (PQ). PQ provides services to all Queensland public hospitals, prisons, and community health services, including sexual health clinics. Whilst both public and private laboratories can undertake initial screening testing, confirmatory testing is undertaken by PQ's reference laboratory. All positive initial screening samples are forwarded to the reference laboratory for confirmation. PQ test results are recorded in the state-wide electronic pathology system, AUSLAB. Private laboratories are not required to send HIV test results to the CDB and all test records are marked as 'confidential' in their respective databases. Laboratory notifications of HIV are transmitted electronically to the CDB and are recorded in NoCS. Reflecting the importance of protecting confidentiality and privacy, NoCS records all HIV notifications using a 2x2 code in place of a full name and access to HIV data is restricted to the HPHT and CDB ERU.

Table 5: Communicable Disease Network Australia national case definition for human immunodeficiency virus (HIV) (newly acquired)

Laboratory definitive evidence	
1	Repeatedly reactive result on a screening test for HIV antibody followed by a positive result on a western blot AND laboratory evidence of a negative or indeterminate HIV antibody result in the 12 months prior to blood sample collection.
	OR
2	A group IV indeterminate western blot AND detection of HIV by at least one of the following virologic assays (nucleic acid testing for proviral DNA; HIV p24 antigen, with neutralisation; virus isolation). A group IV indeterminate western blot is defined by the presence of a glycoprotein band (gp41, gp120 or gp160) and one or two other HIV specific bands.
Laboratory suggestive evidence	
1	Detection of HIV by at least one of the following virologic assays (nucleic acid testing for proviral DNA; HIV p24 antigen, with neutralisation; virus isolation).
	OR
2	A group IV indeterminate western blot AND detection of HIV by at least one of the following virologic assays (nucleic acid testing for proviral DNA; HIV p24 antigen, with neutralisation; virus isolation). A group IV indeterminate western blot is defined by the presence of a glycoprotein band (gp41, gp120 or gp160) and one or two other HIV specific bands.
Clinical evidence	
1	HIV seroconversion illness within the 12 months prior to blood sample collection.

HIV public health team

The HPHT consists of HIV public health nurses and is based centrally at the CDB. They are responsible for reviewing HIV positive laboratory results and notifications to determine whether cases are newly acquired, or previously diagnosed cases newly arrived in Queensland and for follow-up and enhanced surveillance of notifiable cases of HIV infection. The HPHT communicates with notified individuals and their clinicians. Clinicians are required to complete contact tracing forms and enhanced surveillance forms capturing data related to HIV exposure category, reasons for testing, diagnosis test data and HIV testing history, likely place of acquisition (Australia, Unknown, Overseas), clinical status, and treatment commencement. These enhanced surveillance data are manually entered by the HPHT who work closely with the ERU DST to ensure data quality and completeness (Figure 2Figure 2).

Data improvement activities

Monitoring viral suppression among notified HIV cases in Queensland was proposed in 2018 in response to evidence of viral suppression as a transmission reduction strategy. Activities which would facilitate such monitoring were initiated from May 2018 with the aim of having complete HIV testing data (viral load, CD4+ count, and drug sensitivity) for all notified individuals both historically and prospectively. Processes were developed to extract HIV testing data from AUSLAB and manually enter the relevant data into NoCS. To complete historical data, DST began entering 2018 and 2017 data and a contractor was brought into DST in June 2019 to enter data from 2014 to 2016. The prospective system is based on a weekly extraction of AUSLAB data which are entered into NoCS by a DST member. Individuals without any laboratory records on AUSLAB are flagged with the HPHT for additional follow up, including searching available private laboratory records.

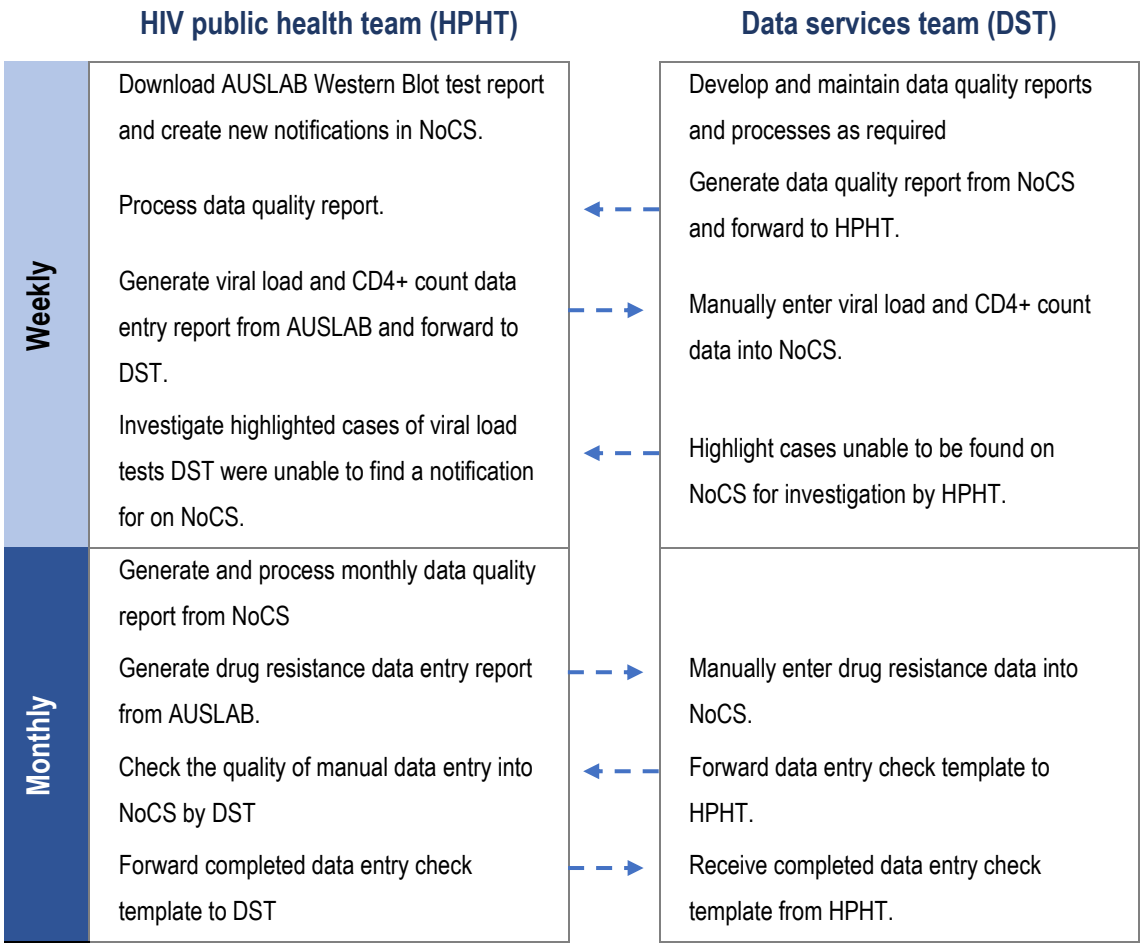


Figure 2: Communicable Diseases Brach HIV data activities

Pilot study and assessment against key attributes

Between 2014 and 2018, 1,006 people were newly diagnosed with HIV in Queensland. Over that time, the annual number of new HIV diagnoses decreased by 36% from 245 cases in 2014 to 180 cases in 2018 (Appendix 2). Newly diagnosed people were predominantly male (89.6%) and resided in the South East Queensland (72.9%) metropolitan area (defined as major cities) at the time of diagnosis. The median age was 35 years (interquartile range (IQR), 28–48 years). Approximately 7.2% of persons newly diagnosed were recorded as Aboriginal and/or Torres Strait Islander.

At the time of diagnosis most people had a CD4+ count of ≥350 cells/µL (61.5%) and almost 70% (688/1,006) had no evidence of recent acquisition. More than 26% of individuals (263) had an STI notification prior to or at diagnosis, and 2.8% of individuals (28) had an STI notification in the six months following their HIV diagnosis. Chlamydia was the most common STI with 53.6% (141/263) of participants with a STI notification prior to or at diagnosis recorded as having a notification of chlamydia. The two most commonly identified exposure risk categories were MSM (660, 65.6%) and heterosexual (215, 21.4%). However, annual new diagnoses who identify as MSM declined from 165 (67.3%) in 2014 to 108 (60%) in 2018. The least common exposure risk categories were IDU (19, 1.9%) and the Other group, which included 5 individuals (0.5%) diagnosed under 15 years of age.

Data completeness and validity

Among all newly diagnosed people between 2014 and 2018, 955 (94.9%) had at least one viral load test at or within 6 months following diagnosis (853, 84.8% ≤30 days of diagnosis, 102, 10.1% between 30 days and ≤6 months of diagnosis), 2 (0.2%) had at least one test after 6 months following diagnosis, and 49 (4.9%) had no viral data available. Individuals with testing data available averaged 1.8 (range: 1–5) tests within 3-months and 2.5 tests (range: 1–8) within 6 months of diagnosis (Table 6). Over the study period the median duration between diagnosis and follow up viral load testing decreased from 100 days for those diagnosed in 2014 to 82 days for those diagnosed in 2018. A date of treatment commencement was available for 737 individuals (73.3%) at any time subsequent to their diagnosis (Table 6Table 6Table 6). Over the study period, there was no noticeable change in the median number of days between diagnosis and commencement of treatment.

Table 6: Mean number of tests and median days between HIV diagnosis and subsequent viral load test or treatment commencement among those newly diagnosed in Queensland, 2014–2018

Year of Diagnosis	2014	2015	2016	2017	2018	Total
% at least one VL test ≤6 months*	90.2	97.0	95.9	94.1	98.9	**94.9**
Mean no. VL tests (s.d) per person ≤ 6 months*	2.7 (1.4)	2.4 (1.7)	2.6 (1.3)	2.4 (1.1)	2.6 (1.1)	**2.5 (1.3)**
Mean no. VL tests (s.d) per person ≤ 3 months*	1.8 (0.8)	1.7 (0.8)	1.8 (0.8)	1.7 (0.8)	1.9 (0.7)	**1.8 (0.8)**
Median no. days between diagnosis and subsequent VL testing (IQR)	100.0 (58–138)	90.0 (58–133)	93.0 (60–135)	86.0 (57–129)	82.0 (49–129)	**91.0 (56–134)**
Median no. days between diagnosis and treatment commencement (IQR)	41.5 (17.5–74)	38.5 (15–70)	35.0 (16–69)	34.0 (16–64)	41.5 (14–80)	**38.0 (15–72)**

VL: Viral load; s.d: standard deviation; IQR: Inter-quartile range

* of diagnosis

Among the 4,164 viral load tests results assessed, data entry errors were identified in only 32 records (0.8%). Errors included incorrect use of "<" or ">" in the result field. A small proportion of individuals (56/1,006, 5.6%) were identified as having no viral load test or CD4+ count data available. These individuals were subsequently identified as having records available on private laboratory systems. Private laboratories were contacted with a request to share viral load testing results.

Over the study period, 384 (38.2%) newly diagnosed people achieved viral suppression within 3 months and 622 (61.8%) achieved viral suppression within 6 months of diagnosis (Figure 33, Table 7). The annual proportions virally suppressed within 3 and 6 months of diagnosis increased over the study period. Over the same period, 344 (34.2%) newly diagnosed individuals commenced treatment within 6 weeks of diagnosis, 525 (52.2%) within 3 months of diagnosis, and 631 (62.7%) within 6 months of diagnosis. Annual proportions of newly diagnosed people commencing treatment increased between 2014 and 2018. However, increases were not linear. All three treatment commencement indicators decreased between 2016 and 2017 by between 9.0–19.1 percentage-points.

Using the 3-month indicators, the overall proportion commencing treatment was 14 percentage-points higher than the proportion virally suppressed (52.2% vs. 38.2%). However, the annual proportions of virally suppressed increased 3-fold over the study period, as compared to a 1.6-fold increase in treatment commencement. In comparison, the overall proportion virally suppressed within 6 months was higher than that for treatment commencement (61.8% vs

52.2%) and from 2017 the annual proportions virally suppressed within 6 months exceeded that of treatment commencement.

Table 7: Annual proportion of newly diagnosed people achieving viral suppression and commencing treatment within 6 months after HIV diagnosis, Queensland 2014–2018

	Indicator (%, 95% confidence interval) by year of diagnosis					
Time point	2014	2015	2016	2017	2018	Total
Viral Suppression						
≤3 months	19.6 (14.6–24.5)	35.3 (28.7–41.9)	38.5 (31.6–45.3)	45.9 (38.7–53.1)	58.3 (51.1–65.5)	**38.2 (29.7–41.2)**
≤6 months	46.9 (40.7–53.2)	53.7 (46.8–60.6)	64.1 (57.3–70.8)	68.1 (61.4–74.9)	82.2 (76.6–87.8)	**61.8 (58.8–64.8)**
Treatment Commencement						
≤6 weeks	24.1 (18.7–29.5)	32.8 (26.3–39.4)	43.1 (36.1–50.0)	34.1 (27.2–40.9)	40.0 (32.8–47.2)	**34.2 (31.3–37.1)**
≤3 months	38.0 (31.9–44.1)	49.8 (42.8–56.7)	66.2 (59.5–72.8)	49.7 (42.5–57.0)	61.7 (54.5–68.8)	**52.2 (49.1–55.3)**
≤6 months	47.3 (41.1–53.6)	58.7 (51.9–65.5)	76.4 (70.4–82.4)	57.3 (50.1–64.5)	78.9 (72.9–84.5)	**62.7 (59.7–65.7)**

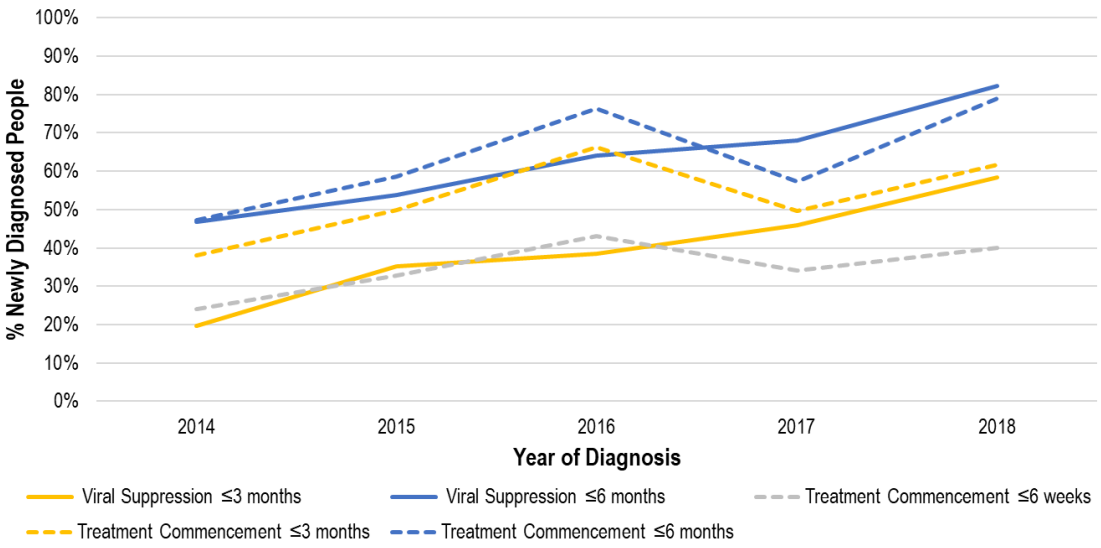


Figure 3: Annual proportion of newly diagnosed people virally suppressed within 3 and 6 months and commencing treatment within 6 weeks, 3 months, and 6 months after HIV diagnosis, Queensland 2014–2018

Comparing viral suppression and treatment commencement indicators by demographic, clinical, and exposure risk categories there was minimal agreement between overall and annual proportions for both 3- and 6-month time points. Variation between proportions of virally

suppressed versus commencing treatment per demographic, clinical, or exposure risk category was higher at the 3-month time point (14.3 percentage points, range: 6.7 to 60.0) than at 6 months (0.9 percentage points, range: 2.9 to 33.3). At both time points annual proportions of virally suppressed exceeded proportions commencing treatment across several demographic, clinical, and exposure risk categories. This occurred six times using the 3-month indicators, predominantly in 2018 (4/6) and affected females, ≥65-year age group, 45–64-year age group, <200 CD4+ count group, and evidence of recent acquisition variables. At the 6-month time point 33.6% of results (52/155) indicated higher viral suppression than treatment commencement. This was concentrated in 2017 (23) and 2018 (20), and affected all demographic, clinical, and exposure risk categories. There were also seven variables where the overall proportion of viral suppression exceeded that of treatment commencement (Table 8Table 8).

Table 8: Comparison of overall proportions commencing treatment and achieving viral suppression within 6 months of HIV diagnosis, Queensland 2014–2018

Variable	Treatment Commencement (TC)	Viral Suppression (VS)	Variance (VS-TC)
Sex: Female	57.1	60.0	**+2.9**
Age Group: 15–24	58.4	61.3	**+2.9**
Age Group: ≥65	70.0	72.0	**+2.0**
Location at diagnosis: Regional	73.0	73.6	**+0.6**
CD4+ Count at diagnosis: ≥350 cells/µ	58.3	59.5	**+1.2**
Exposure risk category: MSM-IDU	60.3	65.5	**+5.2**
Exposure risk category: Heterosexual	64.2	65.1	**+0.9**

Usefulness

We estimated overall and annual proportions of people newly diagnosed who are virally suppressed by a variety of demographic, clinical, and exposure risk categories with corresponding 95% confidence intervals (Appendix 3–4) and undertook univariate logistic regression to identify non-suppression risk factors. The main findings were:

- The likelihood of newly diagnosed persons achieving viral suppression increased year-on-year ($p<0.001$).
- Viral suppression was more common among older age groups and individuals with lower CD4+ counts at diagnosis and lower among those with another BBV or STI pre/at diagnosis and injecting drug users (Figure 4).

- The largest year-on-year improvements were among older age groups and MSM-IDU (Figure 5).
- Differences between diagnosis CD4+ count groups decreased from 2015 onward, consistent with Australia treatment guideline changes.
- A CD4+ count of ≥350 cells/µL or evidence of recent acquisition at diagnosis were risk factors for non-suppression at 3 and 6-month time points (Figure 6).
- An earlier year of diagnosis and younger age at diagnosis were risk factors for non-suppression at 3 months.

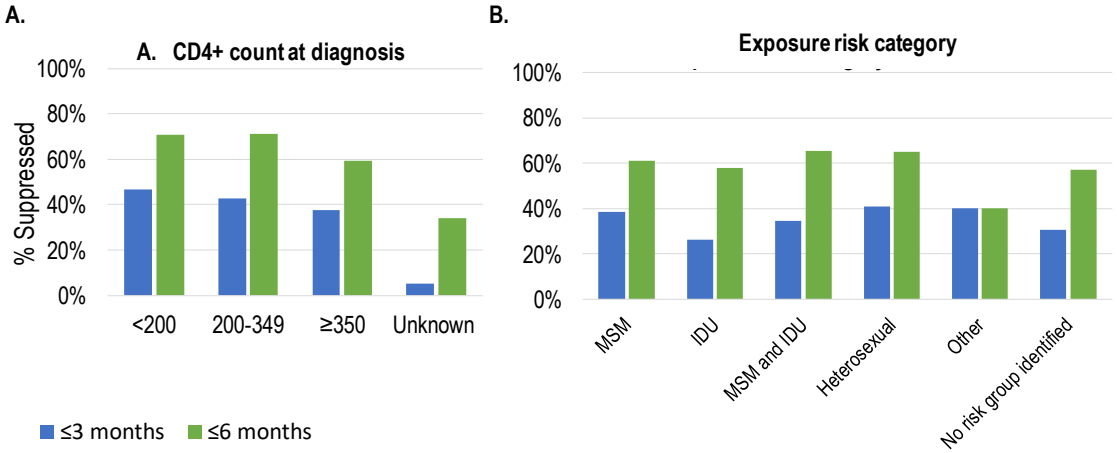


Figure 4: Overall proportion of people newly diagnosed with HIV achieving viral suppression (<200 copies/mL) within three and six months of diagnosis, Queensland 2014–2018 A) by CD4+ count (cells/µL) B) by exposure risk category

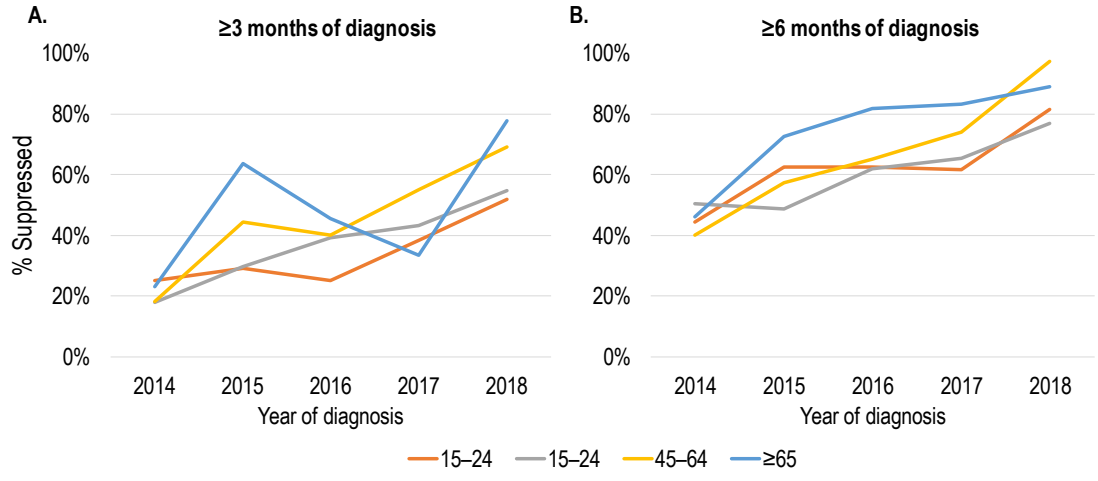
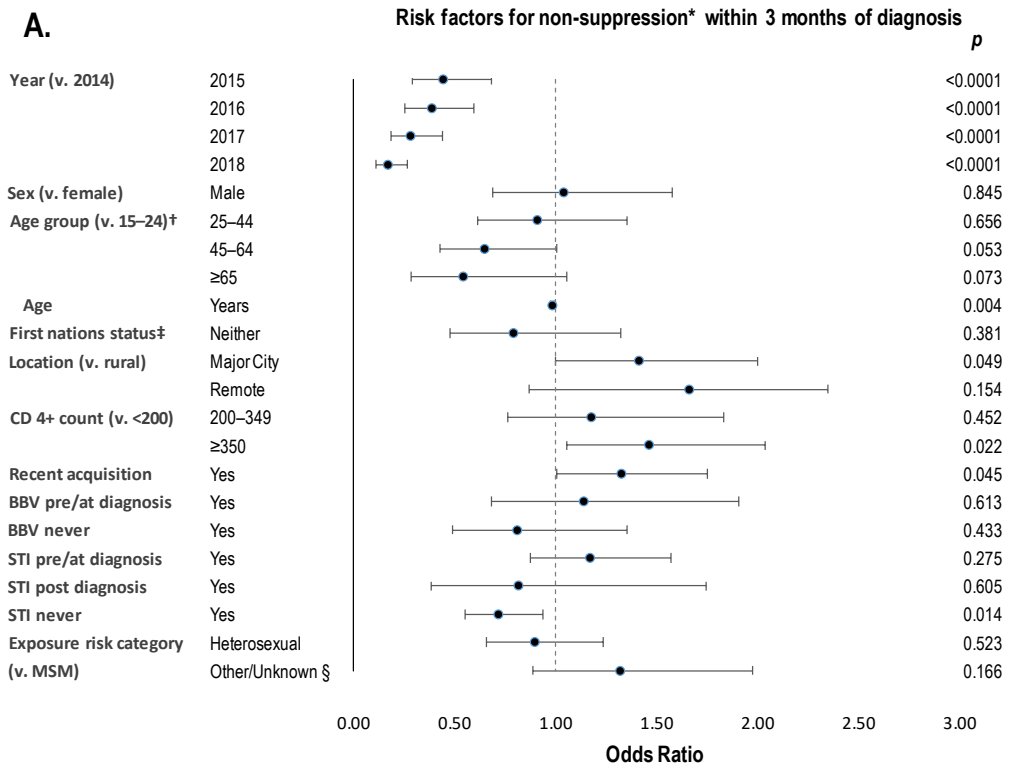
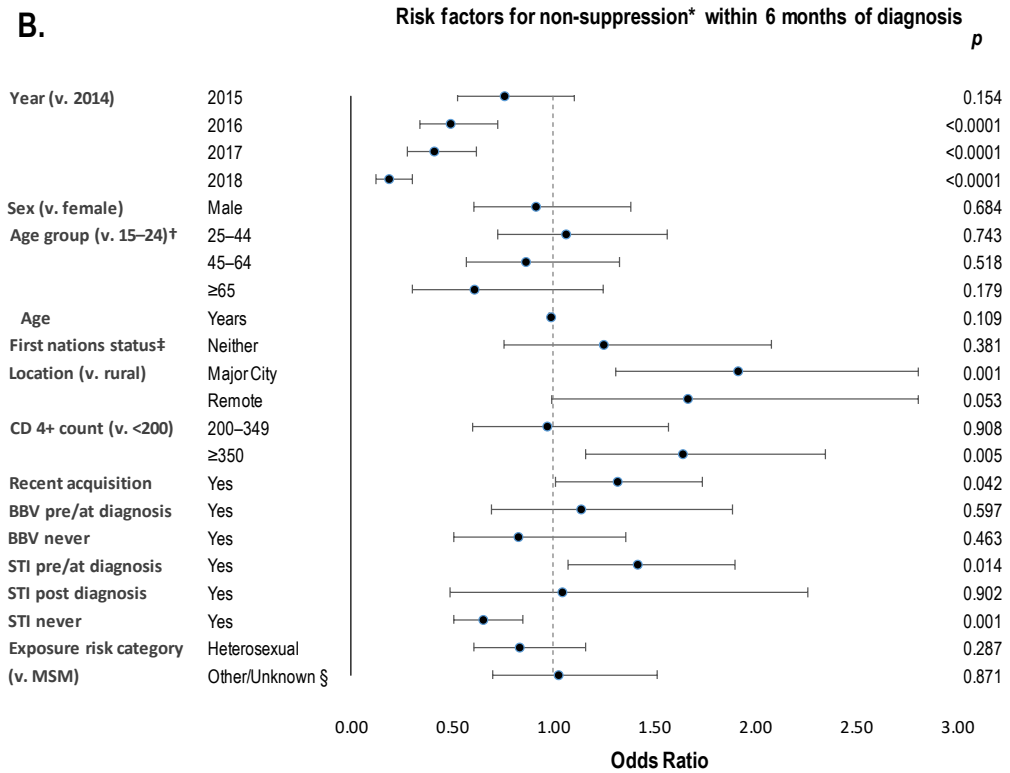


Figure 5: Annual proportion of people newly diagnosed with HIV achieving viral suppression (<200 copies/mL) by age group at diagnosis, Queensland 2014–2018 A) within 3 months B) within 6 months of diagnosis





* Viral load <200 copies/mL

† Due to small sample sizes the <15 years' age group was excluded from analysis.

‡ Reference group: Aboriginal and/or Torres Strait Islander.

§ MSM-IDU, IDU, Other, and Unknown exposure risk categories were combined due to small sample size.

Figure 6: Univariate risk factors for non-suppression in persons newly diagnosed with HIV within (A) 3 months and (B) 6 months of diagnosis, Queensland, 2014–2018

The viral suppression analysis was able to detect trends and provided outputs allowing assessment of prevention and control programs. However, due to small sample sizes some categories had wide confidence intervals and substantial year-to-year variation, making results problematic to interpret. This was particularly evident using the three-month indicator on the following categories; female sex, age groups (<15, 15–24, ≥65 years), Aboriginal and/or Torres strait Islander persons, remote location, BBV infection, and several exposure risk categories (IDU, MSM-IDU, other and no risk group identified). Similarly, due to small sample sizes the <15-year age group was excluded and exposure risk categories were consolidated in regression. Univariate results identifying risk factors may also be confounded by year of diagnosis and age group due to differences in collection of risk factor data over time or across age groups. Multivariate analysis could be undertaken to identify and adjust for confounding.

Stakeholder engagement

Current treatment commencement indicator

The treatment commencement indicator was believed to be useful as a measurement of initiation of care and is aligned to the 90:90:90 targets. Stakeholders representing clinician and community-based organisations responsible for peer HIV treatment support programs also expressed that a specific indicator related to treatment commencement was important as it reflected a key time point on the pathway from diagnosis. These stakeholders also highlighted that whilst treatment guidelines outlined that treatment should be initiated immediately after diagnosis, there are several factors still delaying treatment initiation. These mostly relate to an individual's readiness to engage in care, or clinician's decision to delay care due to the individual's personal circumstances. It was also acknowledged that there can be differences in wait times for first appointments between services, for example, among those diagnosed by a general practitioner (GP) who then require follow-up appointments with a specialist.

All stakeholders agreed that the treatment commencement indicator has not previously been well used at the state-level. Individuals with direct experience entering or using the treatment commencement field also believed that using data from the field was not appropriate as the treatment commencement date is often derived from achievement of viral suppression. Such that the HPHT estimates a date one month prior to a test results with a negligible viral load. Whilst more accurate data could be collected by calling the individual's clinician, this was not routinely undertaken as it was considered time consuming. Although stakeholders with direct experience believed that the treatment commencement data were relatively complete, the quality related issues negated the data's usefulness for monitoring trends.

"I think this is more about the quality of the data, rather than completeness. Because even if you look at a series of dates, you may still be able to find the earliest date that this patient is on treatment. Maybe everyone, or most of them, will have a date but that may not reflect the real commencement date."

The structure of the treatment commencement fields in NoCS was also identified as a hindrance to using the data. The data are collected in two fields; date and treatment status, and there may be multiple dates for each notified individual.

Perceived need for viral suppression monitoring

All stakeholders expressed a clear desire for the outcome indictor to be used for monitoring population-level trends in Queensland. The outcome indicator was perceived as useful in informing service delivery and policy, as well as reporting both internally and externally. Many stakeholders identified clear applications for the monitoring systems outputs. Its capacity to directly inform HIV service delivery, strategy and responses to clusters was highlighted. In particular, stakeholders discussed the role of viral suppression analyses in informing public health responses to a cluster in Cairns. Within the HPHT, monitoring of viral suppression would also better align to team priorities and targets. Internal stakeholders also noted requests from Hospital and Health Service (HHS) teams to provide viral suppression analyses.

Viral suppression was considered to be a more objective measurement of the desired outcome for people diagnosed with HIV, as it was directly informed by test results rather than estimated using an individual's judgement. Measuring viral suppression was also considered to be practical as the process in place for the CDB DST to enter viral load test results into NoCS was considered efficient and timely. Difficulties accessing laboratory records from private laboratories was raised by internal stakeholders as problematic. However, the transition to a new notification system which will facilitate improved data transfer from private laboratories was put forward as an opportunity to address this limitation.

All external stakeholders felt strongly that the viral suppression indicator should be implemented in conjunction with the existing treatment commencement indicators. This would provide a clearer understanding of the cascade of care and allow any leakages from the cascade to be targeted.

The ideal model

The proposed model of quarterly analysis by the CDB ERU was broadly acceptable to internal stakeholders. Stakeholders were highly supportive of a regular report based on the

demographic, clinical, and exposure risk categories included in the pilot study. In particular, there was affirmation of the approach to STIs, location at diagnosis, and age groupings. Although there was a strong expressed desire to maintain consistency with national and/or other jurisdictional indicators, additional categories were suggested as priorities for viral suppression surveillance. These include; transgender, Medicare ineligibility, non-English speaking background (NESB), and individuals in custodial settings. These groups were considered to be either emerging transmission risk groups or those facing particular barriers to access treatment and/or adherence.

The transgender demographic was highlighted by almost all stakeholders and was considered significant, both in terms of acquisition risk and as having potential barriers to access or adherence. Internal stakeholders identified that transgender individuals are currently not well captured in NoCS. The HPHT noted that finding information on whether an individual was transgender using current enhanced surveillance forms was challenging and there is evidence that clinicians report gender subjectively. As such, a transgender person may be reported in either category. This issue will be addressed on the new notifications platform with the inclusion of a field for Gender at time of notification which will include Transgender female and male options.

Individuals ineligible for Medicare were also identified by the majority of stakeholders as facing particularly challenging treatment access barriers as only individuals eligible for Medicare can obtain subsidised treatment. This predominantly affects temporary residents. Socio-economic status or income were similarly suggested as an important indicator for economic barriers to treatment. The co-payment for those with Medicare eligibility is often unaffordable to those on Centrelink or pension support. International students, predominantly those originating from Asian countries, were also suggested to be less likely to be engaged in care. Medicare ineligibility was suggested as a practical proxy for this population. More generally NESB communities were identified as a group that should be included in viral suppression monitoring. Although some concern was raised regarding the potential to generate stigma against particular ethnic or language groups. Alternative approaches to including this population group were region of birth and emigration from high prevalence countries. Additional sub-analyses were suggested for HHS and for populations of particular interest regarding viral suppression, these included heterosexual males and MSM First Nations peoples.

There was some diversity of opinion as to what were the most appropriate and useful time points for measuring viral suppression. All stakeholders agreed on the 6-month time point. However, the 3–month time point was believed by some to be unfeasibly short, whilst others

believed that in consideration of modern treatments and time frames for drug resistance and human leukocyte antigen screening tests, an individual should be able to achieve viral suppression within three months and failure to achieve this milestone should provide impetus to investigate the barriers. Most stakeholders also felt that viral suppression should also be assessed at 12 months. This reflected the experience of those working with HIV positive people who identify the six to 12-month period after diagnosis as being crucial in retention and adherence. Support and motivation are often intensive in the initial first months after diagnosis, and the six-month time point is often when individuals come to terms with the reality of their diagnosis.

All stakeholders agreed there were robust internal processes protecting confidentiality, privacy, and reducing the risk of data misuse. The main concern raised was in regard to individuals being identifiable within small numbers. Although sub-analyses were requested and believed to be useful, drilling analysis down into small cohorts was considered a key risk to individuals, as well as a risk to acceptability of the surveillance mechanism. It was therefore suggested that any results published externally, particularly those published online, should not include sub-analyses, HHS-level analysis, and omit/consolidate groups where the sample size is fewer than five. Stakeholders recommended that resulting analyses should be available to key treatment and care stakeholders to direct the data into public health action. Identified stakeholders included HHS teams, sexual health clinicians, S100 prescriber GPs, and QPP's Life+ program team.

Discussion

With this project I used a mixed methods approach to establish the structure for a proposed surveillance mechanism to measure a key, clinically relevant HIV outcome. The proposed system not only captures an end point relevant to individual clinical care, but in the era of treatment as prevention by reduction in transmission, it also measures a key public health metric.

The methods used in the study included a pilot analysis of available data in the notifiable conditions system, assessment of key system attributes, and key stakeholder engagement to assess feasibility and acceptability of the proposed system. My findings identified some strengths of the proposed system and the need to continue to engage with stakeholders and the relevant community groups during the process.

Usefulness

Routine surveillance of viral suppression would enable timely data based responses to HIV policy, service delivery, and control improvement activities. Stakeholders discussed the usefulness of viral suppression data to improve our understanding of HIV transmission risks and identify priorities for service improvements in Queensland. Stakeholders were engaged and interested to see available laboratory testing and notification data applied to direct public health action. All external stakeholders identified applications for the monitoring system's outputs, including informing changes to HIV peer support programs and service delivery, as well as an advocacy tool to influence policy and funding. Reporting of viral suppression currently through the Kirby Institute using Australian HIV Observational Database and Australian Collaboration for Coordinated Enhanced Sentinel Surveillance (ACCESS) network data is provided at a national level with limited insights into priority populations, and cascade of care results are national-level estimates based on mathematical modelling.[14] A routine analysis of Queensland surveillance data would provide an opportunity to use routinely gathered notification data to provide insights specific to Queensland. It also allows for sub-analyses of geographic and exposure risk groups which identify areas of greatest need of public health action. The analysis was able to provide data commonly limited in its availability which provides valuable insights into the importance of rapid viral suppression, such STI notifications prior to or following viral suppression. Seventeen of the 28 individuals who had an STI notification in the six months following their diagnosis (60.7%) were virally suppressed within that 6-month period. Among these seventeen individuals 53% (9/17) had STI notifications subsequent to viral suppression, 29% had STI notifications prior to viral suppression, and 3 had STI notifications concurrent with viral suppression. The remaining 11 individuals (39.3%) were not virally suppressed within the 6 months (9) or did not have viral load data available (2). A recent time to viral suppression analysis in Alabama has similarly demonstrated how understandings of substantial temporal and geographic variation could be used for targeted public health action.[37]

The incorporation of a relevant function of time into the viral suppression indicator increases its utility in providing actionable feedback. The 90:90:90 targets and other broad evaluations of the cascade of care, including current national-level reporting, address the overall population as a snapshot in time, offering an incomplete picture. The duration between diagnosis and viral suppression is essential to identify specific barriers that delay desired individual and public health outcomes and thereby allows for changes to be determined in a timely manner.[38,39] For example, by incorporating an evaluation of the time between diagnosis and viral suppression a French study was able to identify recent gaps in entry to care for injecting drug users.[38] Although

the median time to treatment is a more commonly used indicator internationally, our analysis demonstrated the sensitivity to change that can be achieved using the proportion virally suppressed within a specified time period. Embedding this process into routine epidemiological work would facilitate timely responses to changing trends and appropriately reflect the Australian context where national ART guidelines are updated annually resulting in changes to recommended drug combinations.[14] Furthermore, the regular real-time analyses facilitate iterative evaluation of program effectiveness thereby maximising the use of resources, improving and refining education, service delivery, and control activities.[16]

At the same time, surveillance of rapid viral suppression as per the pilot study cannot provide a complete picture of HIV care as it does not address service delivery-level factors which may impact rapid viral suppression. Given there is no information regarding ART prescription, the surveillance system also cannot provide an understanding of the mechanisms by which persons were able to achieve suppression.[40] Although there may be differences in clinical management, the data also did not disaggregate newly acquired infections into who had evidence of seroconversion versus those with a negative test in the prior 12 months. The analysis as per the pilot study also did not address the emerging priority populations identified by stakeholders; transgender people, Medicare ineligible people, or people of NESB. These populations are considered priority populations with potentially lower levels of viral suppression and additional support requirements for HIV care.[14,41,42] Medicare ineligibility is also identified in the current Queensland HIV strategy and action plan as a target population for comprehensive prevention approaches and increased treatment uptake.[15,27] Existing data captured in NoCS that would enable analysis of these groups were either not available or not appropriate for analysis. Although there is sufficient flexibility in the systems methodology to incorporate these groups, additional exploration into methods of obtaining or appropriately categorising these data would be required. Country of birth, language spoken at home, and Medicare Number are data fields in NoCS which could be used as proxy measures. Yet additional consideration around country/region/language groupings would be required to make this a meaningful analysis and additional evaluations of the completeness and validity of the Medicare Number field would be required. Additional data collection to obtain direct measurement of Medicare Ineligibility are potentially sensitive for individuals and any changes to enhanced surveillance would need to be carefully explored. Inclusion of transgender as a separate category in addition to Sex can be accommodated by including the currently captured data regarding ‘indeterminate’ sex as per the notification form and going forward should be addressed by the new NoCS platform. Reflecting the importance of viral suppression among all PLHIV and the relative lack of data

internationally, using the method established by the pilot study to assess current viral load results of current and past newly diagnosed individuals may also provide a useful indicator of community viraemia.

Data quality

Viral load testing data was sufficiently complete and valid for use as a measure of population-level viral suppression trends among newly diagnosed people in Queensland. Viral load testing data were widely available with 98.9% of individuals diagnosed in 2018 having a test result available either at or within 6 months of diagnosis. Data cleaning undertaken as part of the pilot study, also identified few data errors. Challenges getting access to private laboratory test results is a limitation to the completeness of viral load testing data at present. However, most testing is completed through public laboratories and a process to access private laboratory records has been established.

Clear limitations to the quality of treatment commencement data were also identified. Whilst the proportions commencing treatment in 2014 (47.3%) and 2015 (58.7%) were consistent to that of the ACCESS network estimate for 2013–2015 (53%), the higher proportions of notified cases who were virally suppressed than initiating treatment indicates that treatment commencement is not completely ascertained or is of lower quality than viral suppression.[1] Although internal stakeholders believed that the treatment commencement data are likely complete, treatment commencement data are currently derived based on a manual review of individual cases and estimation of a feasible commencement date related to viral load testing data. Therefore, use of the treatment commencement data is not appropriate for the accurate monitoring of population-level treatment commencement trends.

Representativeness

The characteristics of the study population were consistent with previously reported characteristics of people newly diagnosed with HIV in Queensland and the observed population-level trends were consistent with existing viral suppression literature and with available Australian national-level and jurisdictional surveillance trends.[5] The overall proportion virally suppressed among all people diagnosed nationally in 2017 was reported to be between 91%–95%.[14] Based on the time point constraint of 6 months post diagnosis and reported overall increasing trend of treatment initiation and viral suppression, the pilot study's proportion virally suppressed at the 6 month time point in 2017 (68.1%) is reasonable. Similarly, the proportional virally suppressed at the 6-month time point in 2018 (82.2%) is consistent with NSW where among the 84.1% of people newly diagnosed (233/277) were found to be virally suppressed at

follow up six months' post diagnosis.[43] The overall increasing trend in viral suppression, both within 3 and 6 months, is similar to trends in treatment initiation within 6 months of diagnosis observed nationally within the ACCESS network between 2004 and 2015.[44] The proportion virally suppressed within 3 months in 2016 (38.5%) was also consistent to that observed in New York (37%).[2]

In regards to demographic, clinical, and exposure risk category differences, findings that younger age groups and MSM-IDU are less likely to achieve viral suppression rapidly after diagnosis are consistent with US and French viral suppression studies and were affirmed by stakeholder insights.[2,38,45,46] Indeed, the associations between these characteristics and poor linkage or retention in HIV care is well established.[45,47-50] Delays in treatment initiation, adherence, and viral suppression among younger people have been linked to factors such as limited financial stability or knowledge and skills to access support services, behaviours associated with adherence, and developmental maturity, as well as overarching factors such as HIV-related stigma, discrimination, and fear of familial and/or social rejection, particularly among young MSM who may be experiencing emerging recognition of sexual identity.[51,52] Lower viral suppression among newly diagnosed people identifying as MSM-IDU is also consistent with Australian national-level findings regarding viral suppression and early treatment initiation among gay and bi-sexual men attending sexual health clinics and high case load GP clinics within the ACCESS network.[14] The identified increasing trend for rapid viral suppression among people diagnosed with higher CD4+ counts and diminishing difference in CD4+ count groups in line with treatment guideline changes has similarly been reported in relation to treatment initiation trends in Australia and the US.[1,46,48,49,53,54] Yet, not all predictors of rapid viral suppression identified in US-based studies were observed in the pilot study, in particular we did not find statistically significant gender-based differences.[2,54]

Acceptability

There was a clear recognition of the public health importance of rapid viral suppression after diagnosis, both in terms of individual and community benefits. The stakeholder engagement process highlighted the willingness of a wide range of stakeholders to participate in a potential surveillance system for rapid viral suppression in Queensland. Viral suppression, as measured through viral load test results entered into NoCS, were considered by internal stakeholders to be objective and of higher quality than current treatment commencement data. As the analysis uses routinely collected data and leverages an established process for prospectively entering available laboratory testing records the mechanism of analysis was considered sufficiently simple and would not require significant manual processes. As discussed, data entry errors were

relatively rare and easily addressed, and the Stata code developed for the pilot study can be easily adapted. However, this will require some familiarity to Stata code and data manipulation.

At a policy level, there were concerns that the monitoring indicator was inconsistent with national-level reporting and could ultimately make comparing progress between jurisdictions challenging. Similar concerns have been raised globally in relation to the challenges created by varied methodological approaches to monitoring of 90:90:90 targets and recommendations have been made to standardised methods.[55,56] Although the surveillance and monitoring plan for the Eighth National HIV Strategy does not include rapid viral suppression indicator, current strategy targets are for an increased focus on treatment among newly diagnosed individuals, and a similar rapid treatment initiation target has been included in the WA Health HIV strategy. Data provided by the surveillance mechanism may also represent a means of corroborating or further contextualising national-level continuum of care target results. Within the analyses, the definition of viral suppression of <200 copies/mL was consistent with current Australian national surveillance, allowing for comparability.

Although stakeholders proposed additional analyses that would require collection of sensitive information such as residency status or income, collection of this information may increase privacy and confidentiality concerns among the community, particularly among already marginalised populations. Therefore, their initial inclusion may be a risk to the acceptability of system. Collection of these data could be re-considered following a period of initial implementation. External stakeholders reflected on the potential benefits of notification data being actively used to inform and improve HIV service delivery. They highlighted that a system which demonstrably targeted service and care improvements would further strengthen community participation in HIV surveillance. Stakeholders from the Queensland Aboriginal and Islander Health Council were not engaged as part of this study, prior to implementation further engagement with the Indigenous sector should be considered to determine the acceptability of this system for Aboriginal and Torres Strait Islander community members.

Conclusion and recommendations

Rapid viral suppression is an important metric for linkage to HIV care and treatment quality and improves our understanding of year-to-year progress for population groups and enables non-suppression risk factors to be understood and addressed. Unlike treatment commencement indicators, the percentage of newly diagnosed people rapidly achieving viral suppression measures beyond one-time point and provides insights into the effectiveness of the systems of care for newly diagnosed people. As such, as a performance measurement it provides a clear

contribution to the assessment of HIV care quality and is better able to detect population-level changes that occur over time than current treatment commencement indicators.

Treatment commencement indicators provide important additional context to understand both treatment initiation and adherence and reflect 90:90:90 targets. Yet, issues identified in the quality of treatment commencement data limit its current application for population-level surveillance in Queensland. Using available viral load testing data to measure rapid viral suppression was acceptable to both internal and external stakeholders and pilot study results were well aligned with recent viral suppression literature and with known changes to treatment guidelines.

Recommendations

Targets, monitoring indicators, and surveillance systems for HIV care should include viral suppression among those newly diagnosed with HIV. Based on the findings of the pilot study, assessment against core surveillance system characteristics, and the stakeholder engagement process, I make the following recommendations:

1. A quarterly analysis of viral suppression within 3 and 6 months of new HIV diagnosis should be embedded into the routine reporting undertaken by the Epidemiology and Research Unit. The definition of viral suppression of <200 copies/mL should be retained to ensure comparability to current Australian surveillance reporting.

2. Although current treatment commencement data are inadequate for use, stakeholders identified a need for treatment commencement to be included in analyses. Improvements to the collection of these data should be explored, particularly in light of the transition to a new notifications platform in 2020.

3. Including viral suppression at 12 months after diagnosis should be explored. From a reporting perspective this is a useful contextual measure and acknowledges that viral suppression is not a fixed state and can rapidly change if adherence, following initial treatment commencement, is poor.[24] Estimations of the proportion of persons with a suppressed viral load at the end of an analysis period has been demonstrated to be a straightforward indicator that approximates well weighted indicators accounting for duration of viral suppression.[57]

4. A quarterly reporting frequency was considered appropriate but may not be feasible in terms of available resources. To establish an appropriate frequency of analysis, variation

in output between each reporting periods should be assessed within the first year. The frequency of reporting should then be adjusted as required.

5. Additional analyses of identified priority populations and risk factors should be explored for inclusion, in particular transgender, Medicare Ineligible, NESB, and income. This may include

    a. Exploring the appropriateness of using existing NoCS fields (country of birth, language spoken at home, Medicare Number)

    b. Exploring improvements to the new NoCS platform (Gender at time of notification)

6. Resulting analyses should be shared with relevant stakeholders including HHS, sexual health clinicians, and community-based organisations involved in treatment support programs. Data reported outside Queensland Health should be appropriately presented to ensure that individuals are not identifiable—categories with <5 individuals should be consolidated or excluded from reporting. These reports should not be published online.

7. An HHS-level analysis should be included and shared with appropriate internal HHS stakeholders. However, due to privacy and confidentiality issues any resulting reports should not be shared externally. Similarly, any additional sub analyses (heterosexual males, MSM first nations status) should be undertaken for internal purposes only.

8. To improve the completeness of viral load testing data, private laboratory stakeholders should continue to be engaged.

References

1. McManus H, Callander D, Donovan B, Russell DB, O'Connor CC, Davies SC, et al. Early initiation of antiretroviral therapy for people newly diagnosed with HIV infection in Australia: trends and predictors, 2004–2015. Medical Journal of Australia. 2019;210(6):269-75. DOI: 10.5694/mja2.50006

2. Xia Q, Coeytaux K, Braunstein SL, Torian LV, Daskalakis DC. Proposing a new indicator for the National Human Immunodeficiency Virus/AIDS Strategy: percentage of newly diagnosed persons achieving viral suppression within 3 months of diagnosis. Journal of Infectious Diseases. 2019;219(6):851-5. DOI: 10.1093/infdis/jiy538

3. Australian Government Department of Health. Eighth national HIV strategy 2018–2020. [Internet]. Canberra: Commonwealth of Australia; 2018. [cited 2019 Mar 27]. Available from: https://www1.health.gov.au/internet/main/publishing.nsf/Content/ohp-bbvs-1//$File/HIV-Eight-Nat-Strategy-2018-22.pdf.

4. UNAIDS. UNAIDS data 2020. [Internet]. Geneva: Joint United Nations Programme on HIV/AIDS; 2020. [cited 2020 Sep 16]. Available from: https://www.unaids.org/sites/default/files/media_asset/2020_aids-data-book_en.pdf.

5. Queensland Health. HIV in Queensland 2017. [Internet]. Brisbane: Queensland Health; 2018. [cited 2019 Mar 28]. Available from: https://www.health.qld.gov.au/clinical-practice/guidelines-procedures/sex-health/reports-surveillance

6. Lundgren JD, Babiker AG, Gordin F, Emery S, Grund B, Sharma S, et al. Initiation of antiretroviral therapy in early asymptomatic HIV infection. New England Journal of Medicine. 2015;373(9):795-807. DOI: 10.1056/NEJMoa1506816

7. Cohen MS, Chen YQ, McCauley M, Gamble T, Hosseinipour MC, Kumarasamy N, et al. Prevention of HIV-1 infection with early antiretroviral therapy. New England Journal of Medicine. 2011;365(6):493-505. DOI: 10.1056/NEJMoa1105243

8. UNAIDS. 90-90-90: an ambitious treatment targets to help end the AIDS epidemic. [Internet]. Geneva: UNAIDS; 2014. [cited 2019 Mar 21]. Available from: http://www.unaids.org/en/resources/documents/2017/90-90-90

9. Li Z, Purcell DW, Sansom SL, Hayes D, Hall HI. Vital signs: HIV transmission along the continuum of care, United States, 2016. MMWR Morbidity and Mortality Weekly Report. 2019;68(11):267-72. DOI: 10.15585/mmwr.mm6811e1

10. Rodger AJ, Cambiano V, Bruun T, Vernazza P, Collins S, van Lunzen J, et al. Sexual activity without condoms and risk of HIV transmission in serodifferent couples when the HIV-positive partner is using suppressive antiretroviral therapy. JAMA. 2016;316(2):171-81. DOI: 10.1001/jama.2016.5148

11. Bavinton BR, Pinto AN, Phanuphak N, Grinsztejn B, Prestage GP, Zablotska-Manos IB, et al. Viral suppression and HIV transmission in serodiscordant male couples: an international, prospective, observational, cohort study. Lancet HIV. 2018;5(8):e438-e47. DOI: 10.1016/s2352-3018(18)30132-2

12. UNAIDS. Undetectable = untransmittable - public health and HIV viral load suppression. [Internet]. Geneva: UNAIDS; 2018. [cited 2021 Oct 14]. Available from: https://www.unaids.org/sites/default/files/media_asset/undetectable-untransmittable_en.pdf.

13. Australasian Society for HIV Medicine (ASHM). HIV management in Australasia: a guide for clinical care 2016 [cited 2019 Apr 10]. Available from: http://hivmanagement.ashm.org.au/

14. The Kirby Institute. HIV, viral hepatitis and sexually transmissible infections in Australia: annual surveillance report 2018. [Internet]. Sydney: The Kirby Institute; 2018. [cited 2019 Mar 25]. Available from: https://kirby.unsw.edu.au/report/hiv-viral-hepatitis-and-sexually-transmissible-infections-australia-annual-surveillance

15. Queensland Health. Queensland sexual health strategy 2016. [Internet]. Brisbane: Queensland Health; 2016. [cited 2019 Mar 25]. Available from: https://www.health.qld.gov.au/public-health/topics/sexual-health/strategy

16. Mugavero MJ, Norton WE, Saag MS. Health care system and policy factors influencing engagement in HIV medical care: piecing together the fragments of a fractured health care delivery system. Clinical Infectious Diseases. 2011;52:S238-S46.

17. Lam Y, Westergaard R, Kirk G, Ahmadi A, Genz A, Keruly J, et al. Provider-level and other health systems factors influencing engagement in HIV care: a qualitative study of a vulnerable population. PLoS One. 2016;11(7):e0158759. DOI: 10.1371/journal.pone.0158759

18. Hoenigl M, Chaillon A, Moore DJ, Morris SR, Mehta SR, Gianella S, et al. Rapid HIV viral load suppression in those initiating antiretroviral therapy at first visit after HIV diagnosis. Scientific Reports. 2016;6:32947. DOI: 10.1038/srep32947

19. Nance RM, Delaney JAC, Simoni JM, Wilson IB, Mayer KH, Whitney BM, et al. HIV viral suppression trends over time among HIV-infected patients receiving care in the United States, 1997 to 2015: a cohort study. Annals of Internal Medicine. 2018;169(6):376-84. DOI: 10.7326/m17-2242

20. UNAIDS. Understanding fast-track: accelerating action to end the AIDS epidemic by 2030. [Internet]. Geneva: UNAIDS; 2015. [cited 2020 Jul 14]. Available from: https://www.unaids.org/sites/default/files/media_asset/201506_JC2743_Understanding_FastTrack_en.pdf.

21. Department of Health. National blood-borne viruses and sexually transmissible infections surveillance and monitoring Plan 2018–2022. [Internet]. Canberra: Commonwealth of Australia; 2020. [cited 2020 Sep 15]. Available from: https://www1.health.gov.au/internet/main/publishing.nsf/Content/AE05C032DDCB7533CA257BF00020AAC4/$File/Surveil-Monit-Plan-2018-2022-Nat-BBV-STI.pdf.

22. White House Office of National AIDS Policy. National HIV/AIDS Strategy for the United States: updated to 2020. [Internet]. Washington: White House Office of National AIDS Policy; 2015. [cited 2019 Mar 26]. Available from: https://www.hiv.gov/federal-response/national-hiv-aids-strategy/overview

23. Centers for Disease Control and Prevention. CDC approach to evaluation: indicators [Internet]. Atlanta: Program Performance and Evaluation Office (PPEO); 2016 [updated 2016 Dec 2; cited 2019 Nov 11]. Available from: https://www.cdc.gov/eval/indicators/index.htm.

24. Dombrowski JC, Baeten JM. It's time to make the time to viral suppression after HIV diagnosis a metric of HIV care success. Journal of Infectious Diseases. 2019;219(6):845-7. DOI: 10.1093/infdis/jiy539

25. Department of Health. Western Australia HIV strategy 2019–2023. [Internet]: Government of Western Australia; 2020. [cited 2020 Sep 19]. Available from: https://ww2.health.wa.gov.au/~/media/Files/Corporate/general%20documents/Sexual%20Health/PDF/strategy/2019-2023/HIV-Strategy.pdf.

26. NSW Ministry of Health. NSW HIV strategy 2016–2020. [Internet]. North Sydney: NSW Ministry of Health; 2015. [cited 2019 July 11]. Available from: https://www.health.nsw.gov.au/endinghiv/Pages/strategy-and-resources.aspx

27. Queensland Health. HIV action plan 2016-2021. [Internet]. Brisbane: Queensland Health; 2016. [cited 2019 Mar 25]. Available from: https://www.health.qld.gov.au/public-health/topics/sexual-health/strategy

28. Department of Health and Human Services. Victorian public health and wellbeing outcomes framework data dictionary. [Internet]. Melbourne: Victorian Government; 2017. [cited 2020 Jul 17]. Available from: https://www2.health.vic.gov.au/about/publications/policiesandguidelines/victorian-public-health-and-wellbeing-outcomes-framework.

29. Northern Territory Health. Surveillance update for notifiable sexually transmitted infections and blood-borne viruses in the Northern Territory: july to december 2019.Report No.: 20(2) [Internet]: Northern Territory Government; 2019. [cited 17 Jul 2020]. Available from: https://digitallibrary.health.nt.gov.au/prodjspui/handle/10137/237.

30. Government of South Australia. Metadata for the South Australian public health indicator framework 2018. [Internet]. Adelaide: SA Health; 2018. [cited 2020 Jul 14]. Available from: https://www.sahealth.sa.gov.au/wps/wcm/connect/5b54938041be864997849fbde9b79454/SAPublicHealthIndicatorsMetadata2018v2.pdf?MOD=AJPERES&CACHEID=ROOTWORKSPACE-5b54938041be864997849fbde9b79454-n5i1GpW.

31. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach, 2016. [Internet]. Geneva: WHO; 2016. [cited 2019 Apr 04]. Available from: https://apps.who.int/iris/bitstream/handle/10665/208825/9789241549684_eng.pdf?sequence=1.

32. German RR, Lee LM, Horan JM, Milstein RL, Pertowski CA, Waller MN. Updated guidelines for evaluating public health surveillance systems: recommendations from the Guidelines Working Group. MMWR Recommendations and Reports. 2001;50(Rr-13):1-35; quiz CE1-7.

33. Australian Government Department of Health. Human immunodeficiency virus (HIV) (newly acquired) case definition Canberra: Commonwealth of Australia; 2004 [updated 2004 Mar 12; cited 2020 Sep 15]. Available from: https://www1.health.gov.au/internet/main/publishing.nsf/Content/cda-surveil-nndss-casedefs-cd_hivnew.htm.

34. Communicable Diseases Network Australia. Human Immunodeficiency virus (HIV): CDNA national guidelines for public health units. [Internet]. Canberra, Australia: Communicable Diseases Network Australia; 2014. [cited 2020 Jan 08]. Available from: https://www1.health.gov.au/internet/main/publishing.nsf/Content/4F6EBB18C84D9E65CA257DA300155DE5/$File/HIV-SoNG.pdf.

35. Queensland Government. *Public Health Regulation 2018*. Brisbane: Queensland Govenment. 2018. Queensland Government

36. Queensland Government. *Public Health Act 2005*. Brisbane: Queensland Government. 2019. Queensland Government

37. Batey DS, Dong X, Rogers RP, Merriweather A, Elopre L, Rana AI, et al. Time from HIV diagnosis to viral suppression: survival analysis of statewide surveillance data in Alabama, 2012 to 2014. JMIR Public Health and Surveillance. 2020;6(2):e17217. DOI: 10.2196/17217

38. Supervie V, Marty L, Lacombe JM, Dray-Spira R, Costagliola D, group F-ACs. Looking beyond the cascade of HIV care to end the AIDS epidemic: estimation of the time interval from HIV infection to viral suppression. Journal of Acquired Immune Deficiency Syndrome. 2016;73(3):348-55. DOI: 10.1097/QAI.0000000000001120

39. Kowalska JD, Ankiersztejn-Bartczak M, Shepherd L, Mocroft A. Cascade of care and factors associated with virological suppression among HIV-positive persons linked to care in the Test and Keep in Care (TAK) project. Infection. 2018;46(4):533-40. DOI: 10.1007/s15010-018-1154-0

40. Monroe AK, Happ LP, Rayeed N, Ma Y, Jaurretche MJ, Terzian AS, et al. Clinic-level factors associated with time to antiretroviral initiation and viral suppression in a large urban cohort. Clinical Infectious Diseases. 2019. DOI: 10.1093/cid/ciz1098

41. Xia Q, Seyoum S, Wiewel EW, Torian LV, Braunstein SL. Reduction in gaps in high CD4 count and viral suppression between transgender and cisgender persons living With HIV in New York City, 2007–2016. American Journal of Public Health. 2019;109(1):126-31. DOI: 10.2105/AJPH.2018.304748

42. Petoumenos K, Watson J, Whittaker B, Hoy J, Smith D, Bastian L, et al. Subsidized optimal ART for HIV-positive temporary residents of Australia improves virological outcomes: results from the Australian HIV Observational Database Temporary Residents Access Study. Journal of the International AIDS Society. 2015;18:19392. DOI: 10.7448/IAS.18.1.19392

43. NSW Ministry of Health. NSW HIV strategy 2016–2020: data report, 2019 quarter 2. [Internet]. Sydney: Health Protection NSW; 2019. [cited 2022 Feb 02]. Available from: https://www.health.nsw.gov.au/endinghiv/Publications/q2-2019-nsw-hiv-data-report.pdf.

44. Dharan NJ, Radovich T, Che S, Petoumenos K, Juneja P, Law M, et al. HIV treatment regimens and adherence to national guidelines in Australia: an analysis of dispensing data from the Australian pharmaceutical benefits scheme. BMC Public Health. 2019;19(1):13. DOI: 10.1186/s12889-018-6325-5

45. Muthulingam D, Chin J, Hsu L, Scheer S, Schwarcz S. Disparities in engagement in care and viral suppression among persons with HIV. Journal of Acquired Immune Deficiency Syndrome. 2013;63(1):112-9. DOI: 10.1097/QAI.0b013e3182894555

46. Toren KG, Buskin SE, Dombrowski JC, Cassels SL, Golden MR. Time from HIV diagnosis to viral load suppression: 2007–2013. Sexually Transmitted Diseases. 2016;43(1):34-40. DOI: 10.1097/OLQ.0000000000000376

47. Richey LE, Halperin J, Pathmanathan I, Van Sickels N, Seal PS. From diagnosis to engagement in HIV care: assessment and predictors of linkage and retention in care among patients diagnosed by emergency department based testing in an urban public hospital. AIDS Patient Care STDS. 2014;28(6):277-9. DOI: 10.1089/apc.2014.0052

48. Bulsara SM, Wainberg ML, Newton-John TRO. Predictors of adult retention in HIV care: a systematic review. AIDS Behav. 2018;22(3):752-64. DOI: 10.1007/s10461-016-1644-y

49. Giordano TP, Hartman C, Gifford AL, Backus LI, Morgan RO. Predictors of retention in HIV care among a national cohort of US veterans. HIV Clinical Trials. 2009;10(5):299-305. DOI: 10.1310/hct1005-299

50. Xia Q, Zhong Y, Wiewel EW, Braunstein SL, Torian LV. Linkage to care after HIV diagnosis in New York City: better than we thought. Journal of Acquired Immune Deficiency Syndrome. 2017;76(1):e18-e21. DOI: 10.1097/qai.0000000000001419

51. Reisner SL, Mimiaga MJ, Skeer M, Perkovich B, Johnson CV, Safren SA. A review of HIV antiretroviral adherence and intervention studies among HIV-infected youth. Top HIV Med. 2009;17(1):14-25.

52. Panel on antiretroviral guidelines for adults and adolescents. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents

with HIV. [Internet]: Department of Health and Human Services; 2021. [cited 2022 Feb 02]. Available from: https://clinicalinfo.hiv.gov/sites/default/files/guidelines/documents/AdultandAdolescentGL.pdf.

53. Crepaz N, Song R, Lyss S, Hall HI. Trends in time from HIV diagnosis to first viral suppression following revised U.S. HIV treatment guidelines, 2012–2017. Journal of Acquired Immune Deficiency Syndrome. 2020. DOI: 10.1097/QAI.0000000000002398

54. Torian LV, Xia Q. Achievement and maintenance of viral suppression in persons newly diagnosed with HIV, New York City, 2006–2009: using population surveillance data to measure the treatment part of "test and treat". Journal of Acquired Immune Deficiency Syndrome. 2013;63(3):379-86. DOI: 10.1097/QAI.0b013e3182926b02

55. Ho ZJM, Huang F, Wong CS, Chua L, Ma S, Chen MIC, et al. Using a HIV registry to develop accurate estimates for the HIV care cascade: the Singapore experience. Journal of the International AIDS Society. 2019;22(7):e25356. DOI: 10.1002/jia2.25356

56. Granich R, Gupta S, Hall I, Aberle-Grasse J, Hader S, Mermin J. Status and methodology of publicly available national HIV care continua and 90-90-90 targets: a systematic review. PLoS Medicine. 2017;14(4):e1002253. DOI: 10.1371/journal.pmed.1002253

57. Xia Q, Wiewel EW, Braunstein SL, Kersanske LS, Torian LV. Comparison of indicators measuring the proportion of human immunodeficiency virus–infected persons with a suppressed viral load. Annals of Epidemiology. 2015;25(4):226-30. DOI: 10.1016/j.annepidem.2015.01.014

Appendix 1: What can viral suppression indicators tell us? Poster presentation for the International Congress on Infectious Diseases, 2020

What can viral suppression indicators tell us?

Monitoring progress towards improvement in HIV care for those newly diagnosed in Queensland, Australia

Authors: Elenor Kerr[1,2], Jonathan Malo[2], Emma Field[1], Damin Si[2], Robert Ware[3], Sonya Bennett[2], Stephen Lambert[1,2]

(1) National Centre for Epidemiology and Population Health, Australian National University
(2) Communicable Diseases Branch, Queensland Health
(3) Menzies Health Institute Queensland

Background

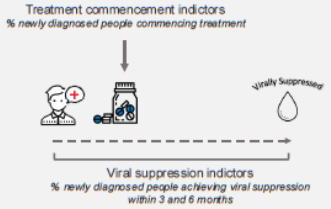
Shortening the time to viral suppression after HIV diagnosis reduces morbidity and transmission. Yet treatment commencement indicators commonly used to monitor HIV care among persons newly diagnosed with HIV do not include the more relevant clinical and public health outcome of viral suppression.[1] Treatment commencement indicators are limited measures of activity implementation and cannot determine the quality or impact of HIV care programs.[2]

With this study, we explore a recently proposed process indicator for HIV care quality, the percentage of newly diagnosed individuals achieving early viral suppression, and investigated risk factors for non-suppression in Queensland, Australia.[1]

Methods

We conducted a retrospective cohort study using Queensland state-wide surveillance data for newly diagnosed people with HIV, 2014–2018. HIV is a nationally notified condition with notification, diagnosis, and testing data captured by the Queensland Notifiable Conditions system.

Percentage of newly diagnosed people achieving viral suppression, viral load <200 copies/mL, at any time within three and six months of diagnosis was calculated overall and annually by demographic, clinical, and population risk groups. Risk factors associated with non-suppression at both the three- and six-month time points were identified using univariable logistic regression.



Results

Of 1,006 individuals diagnosed with HIV in Queensland: 90% were male, 73% were diagnosed in major South-East Queensland cities, and 66% were identified as men who have sex with men (MSM). Over the study period, the annual number of HIV diagnoses decreased by 36% from 245 cases in 2014 to 180 cases in 2018.

Ninety-five percent of newly diagnosed people had at least one viral load test at or following diagnosis. Individuals averaged 1.8 (range: 1–5) and 2.5 (1–8) tests within three and six months of diagnosis, respectively.

The likelihood of newly diagnosed persons achieving viral suppression increased year-on-year (p<0.001); within three months from 19.6% in 2014 to 58.8% in 2018, and six months from 46.9% to 82.2% (Figure 1).

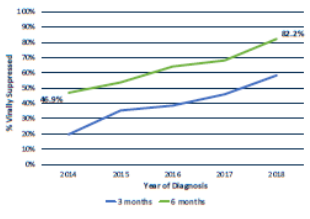


Figure 1: Annual proportion of people newly diagnosed with HIV achieving viral suppression (<200 copies/mL) within three and six months of diagnosis, Queensland 2014–2018

Viral suppression was more common among older age groups and individuals with lower CD4 counts at diagnosis, and lower among those with another blood-borne virus or STI prelat diagnosis, and injecting drug users (IDUs) (Figure 2, 3).

The largest year-on-year improvements were among older age groups and MSM who were also IDUs. Differences between people diagnosed with CD4 count ≥350 cells/µL and <350 cells/ µL decreased from 2015 onward, consistent with Australia treatment guideline changes.

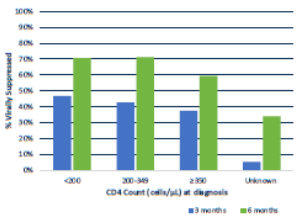


Figure 2: Overall proportion of people newly diagnosed with HIV achieving viral suppression (<200 copies/mL) within three and six months of diagnosis by CD4 count (cells/µL), Queensland 2014–2018

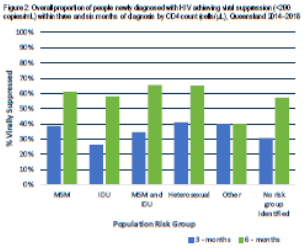


Figure 3: Overall proportion of people newly diagnosed with HIV achieving viral suppression (<200 copies/mL) within three and six months of diagnosis by population risk groups, Queensland 2014–2018

Risk factors for non-suppression at both timepoints included: CD4+ count ≥350 cells/µL and recent acquisition (Table 1). Earlier year of diagnosis and younger age at diagnosis were risk factors at three months and STI infection prelat diagnosis and diagnosis whilst in a major city were risk factors at six months.

Table 1: Risk factors for non-suppression (viral load ≥200 copies/mL) within three and six months of HIV diagnosis for newly diagnosed persons in Queensland, 2014–2018

Risk factor at diagnosis	Within 3 months		Within 6 months	
	OR (95% CI)	p	OR (95% CI)	p
Year (ref 2014)				
2015	0.45 (0.29–0.68)	<0.001	0.76 (0.52–1.11)	0.154
2016	0.39 (0.25–0.59)	<0.001	0.49 (0.34–0.73)	<0.001
2017	0.29 (0.19–0.44)	<0.001	0.41 (0.28–0.62)	<0.001
2018	0.17 (0.11–0.27)	<0.001	0.19 (0.12–0.30)	<0.001
Age (years)	0.99 (0.98–0.99)	<0.001	0.84 (0.56–1.24)	0.374
Location (ref Regional)				
Major City	1.42 (1.00–2.00)	0.049	1.92 (1.31–2.81)	0.001
Remote	1.67 (0.87–2.35)	0.154	1.67 (0.99–2.81)	0.053
CD4 count (cells/µL) (ref <200)				
200–349	1.18 (0.76–1.82)	0.452	0.97 (0.60–1.57)	0.908
≥350	1.47 (1.06–2.04)	0.022	1.65 (1.16–2.35)	0.005
Unknown	15.55 (4.69–51.51)	<0.001	4.71 (2.49–8.90)	<0.001
Recent acquisition (ref No)				
Yes	1.33 (1.01–1.76)	0.045	1.33 (1.01–1.74)	0.042
STI prelat diagnosis (ref No)				
Yes	1.17 (0.88–1.58)	0.275	1.42 (1.07–1.90)	0.014
Ever STI infected (ref Yes)				
No	0.72 (0.56–0.94)	0.014	0.66 (0.51–0.85)	0.001

Conclusion

We found increasing viral suppression following HIV diagnosis between 2014 and 2018 in our cohort. This is likely to be related to health service and monitoring improvements. Age and population risk group differences identified by this study are consistent with recent viral suppression literature.[1] Although it is evident that increasing proportions of notified individuals diagnosed are receiving early treatment regardless of their CD4+ count or evidence of recent acquisition, a high CD4+count or evidence of recent acquisition remains a potential a risk factor for non-suppression.[3] However, the potential for confounding by age group and year of diagnosis will be further explored through multivariable analysis.

These are important metrics for HIV control, improving our understanding of year-to-year progress for population groups and enabling non-suppression risk factors to be understood and addressed. Unlike treatment commencement indicators, the percentage of newly diagnosed people rapidly achieving viral suppression measures beyond one timepoint and provides insights into the effectiveness of the systems of care for newly diagnosed people. Targets, monitoring indicators, and systems for monitoring HIV care should include viral suppression among those newly diagnosed with HIV.

Citations:

1. Xia Q, Coeytaux K, Braunstein SL, Torian LV, Daskalakis DC. Proposing a New Indicator for the National Human Immunodeficiency Virus/AIDS Strategy: Percentage of Newly Diagnosed Persons Achieving Viral Suppression Within 3 Months of Diagnosis. Journal of Infectious Diseases. 2019;219(6):851-5.
2. Dombrowski JC, Baeten JM. It's Time to Make the Time to Viral Suppression After HIV Diagnosis a Metric of HIV Care Success. Journal of Infectious Diseases. 2019;219(6):845-7.
3. McManus H, Callander D, Donovan B, Russell DB, O'Connor CC, Davies SC, et al. Early initiation of antiretroviral therapy for people newly diagnosed with HIV infection in Australia: trends and predictors, 2004-2015. Medical Journal of Australia. 2019;210(9):269-75.

Acknowledgements:

The authors thank the Data Services and the HIV Public Health teams at the Communicable Diseases Brach, Queensland Health for their role in data collection and maintaining data quality.

For further information, please contact Elenor Kerr, Master of Applied Epidemiology (MAE) Scholar (elenor.kerr@anu.edu.au)

Queensland Government

Australian National University

Appendix 2: Demographic, clinical, and exposure risk characteristics of people newly diagnosed with HIV in Queensland, 2014–2018 (n=1,006)

Year of diagnosis	2014 (N=245)		2015 (N=201)		2016 (N=195)		2017 (N=185)		2018 (N=180)		Total (N=1,006)	
	N	%	N	%	N	%	N	%	N	%	N	%
Sex												
Male	220	89.8	180	89.6	174	89.2	162	87.6	159	91.7	**901**	89.6
Female	25	10.2	21	10.4	21	10.8	23	12.4	15	08.3	**105**	10.4
Age at diagnosis												
<15	1	00.4	1	00.5	3	01.5	-	-	1	00.6	**6**	00.6
15–24	36	14.7	24	11.9	24	12.3	26	14.1	27	15.0	**137**	13.6
25–44	135	55.1	111	55.2	97	49.7	95	51.4	104	57.8	**542**	53.9
45–64	60	24.5	54	26.9	60	30.8	58	31.4	39	21.7	**271**	26.9
≥65	13	05.3	11	05.5	11	05.6	6	03.2	9	05.5	**50**	05.0
First Nations status												
Aboriginal and/or Torres Strait Islander person	14	05.7	13	06.5	20	10.3	11	05.9	14	07.8	**72**	07.2
Neither	231	94.3	188	93.5	175	89.7	174	94.1	166	92.2	**934**	92.8
Location												
Major city	188	76.7	141	70.1	135	69.2	145	78.9	124	69.4	**733**	73.1
Regional	32	13.1	37	16.4	35	17.9	26	14.1	33	18.3	**159**	15.8
Remote	25	10.2	27	13.4	25	12.8	13	07.0	22	12.2	**112**	11.1
CD4+ count at diagnosis (cells/μL)												
<200	56	22.9	33	16.4	38	19.5	26	14.4	35	19.4	**188**	18.7
200–349	32	13.1	23	11.4	33	16.9	30	16.2	25	13.9	**143**	14.2
≥350	145	59.2	137	68.2	115	59.0	115	62.2	107	59.4	**619**	61.5
Unknown	12	04.9	8	04.0	9	04.6	14	07.6	13	07.2	**56**	05.6
Evidence of recent acquisition												
Yes	85	34.7	69	34.3	56	28.7	51	27.6	57	31.7	**318**	31.6
No	160	65.3	132	65.7	139	71.3	134	72.4	123	68.3	**688**	68.4
BBV infection												
Pre/at diagnosis	15	06.1	16	08.0	13	06.7	14	07.6	10	05.6	**68**	6.8
Post diagnosis	3	01.2	-	-	-	-	-	-	-	-	**3**	02.7
Never	227	92.7	185	92.0	182	93.3	171	92.4	170	94.4	**935**	92.9
STI Infection												
Pre/at diagnosis	58	23.7	54	26.9	49	25.1	67	36.2	35	19.4	**263**	26.1
Post diagnosis	7	02.9	8	04.0	3	01.5	6	03.2	4	02.2	**28**	02.8
Pre & post diagnosis	1	00.4	-	-	-	-	1	00.5	1	00.6	**3**	00.3
Never	135	55.1	101	50.2	116	59.5	99	53.5	134	74.4	**585**	58.2
Exposure risk category												
MSM	165	67.3	136	67.7	129	66.2	122	65.9	108	60.0	**660**	65.6
IDU	10	04.1	4	02.0	2	01.0	1	00.5	2	01.1	**19**	01.9
MSM and IDU	11	04.5	10	05.0	8	04.1	11	05.9	18	10.0	**58**	05.8
Heterosexual	41	16.7	41	20.4	44	22.6	48	25.9	41	22.8	**215**	21.4
Other	-	-	1	00.5	3	01.5	-	-	1	00.6	**5**	00.5
No risk group identified	18	07.3	9	04.5	9	04.6	3	01.6	10	05.6	**49**	04.9

Appendix 3: Percentage of newly diagnosed persons achieving viral suppression within 3 months of HIV diagnosis in Queensland by demographic, clinical, and exposure risk category, 2014–2018 (n=1,006)

Year of Diagnosis	% Achieving viral suppression within 3 months (95% Confidence Interval)											
	2014		2015		2016		2017		2018		TOTAL	
	%	CI	%	CI	%	CI	%	CI	%	CI	%	CI
Total	**19.6**	14.6–24.5	**35.3**	28.7–41.9	**38.5**	31.6–45.3	**45.9**	38.7–53.1	**58.3**	51.1–65.5	**38.2**	35.2–41.2
Sex												
Male	20.0	14.7–25.3	36.1	29.1–43.1	35.6	28.5–42.7	46.3	38.6–54.0	58.8	51.3–66.3	**38.1**	34.9–41.2
Female	16.0	01.5–30.5	28.6	09.0–48.1	61.9	40.8–82.9	43.5	23.0–64.0	53.3	27.8–78.9	**39.0**	29.7–48.4
Age at diagnosis (years)												
<15	100.0		0.00		66.7		-		00.0		**50.0**	06.1–93.9
15–24	25.0	10.5–39.5	29.2	10.4–47.9	25.0	07.1–42.9	38.5	19.2–57.7	51.9	32.5–71.2	**33.6**	25.6–41.5
25–44	17.8	11.3–24.3	29.7	21.1–38.3	39.2	29.4–49.0	43.2	33.1–53.2	54.8	45.2–64.4	**35.6**	31.6–39.6
45–64	18.3	08.4–28.3	44.4	31.0–28.3	40.0	27.4–52.6	55.2	42.2–68.1	69.2	54.5–84.0	**43.5**	37.6–49.5
≥65	23.1	00.0–47.5	63.6	33.1–94.2	45.5	13.8–77.1	33.3	00.0–75.0	77.8	48.2–100.0	**48.0**	34.0–62.0
First Nations status												
Aboriginal and/or Torres Strait Islander	07.1	0.00–21.4	38.5	10.5–66.5	20.0	01.7–38.3	54.5	23.1–85.9	57.1	29.8–84.5	**33.3**	22.3–44.3
Neither	20.3	15.1–25.6	35.1	28.2–42.0	40.6	33.3–47.9	45.5	38.0–52.8	58.4	50.9–66.0	**38.5**	35.4–41.7
Location												
Major city	19.7	14.0–25.4	33.3	25.5–41.2	36.3	28.1–44.5	45.5	37.1–53.3	58.1	48.9–66.3	**37.0**	33.4–40.4
Regional	18.8	04.9–32.6	48.5	31.0–65.9	42.9	26.1–59.6	53.8	34.1–73.6	63.6	46.8–80.4	**45.3**	37.5–53.1
Remote	20.0	03.8–36.2	29.6	11.9–47.4	44.0	23.9–64.1	38.5	10.6–66.3	54.5	33.0–76.1	**36.6**	27.6–45.6

Year of Diagnosis	% Achieving viral suppression within 3 months (95% Confidence Interval)											
	2014		2015		2016		2017		2018		TOTAL	
	%	CI	%	CI	%	CI	%	CI	%	CI	%	CI
CD4+ count at diagnosis (cells/µL)												
<200	28.6	16.6–40.6	51.5	34.1–68.9	50.0	33.8–66.2	57.7	38.2–77.2	60.0	43.4–76.6	**46.8**	39.6–54.0
200–349	28.1	12.2–44.1	30.4	11.0–49.8	39.4	22.3–56.5	50.0	31.6–68.4	68.0	49.2–86.8	**42.7**	34.5–50.8
≥350	15.9	09.9–21.8	34.3	26.3–42.3	37.4	28.5–46.3	47.8	38.6–57.0	59.8	50.5–69.2	**37.5**	33.7–41.3
Unknown	00.0		00.0		00.0		00.0		23.1	00.0–47.5	**05.4**	00.0–11.3
Evidence of recent acquisition												
Yes	11.8	04.8–41.3	33.3	22.1–44.6	41.1	28.0–54.1	49.0	35.1–62.9	45.6	32.5–58.7	**33.6**	28.4–38.9
No	23.8	17.1–30.4	36.4	28.1–44.6	37.4	29.3–45.5	44.8	36.3–53.2	64.2	55.7–72.7	**40.3**	36.6–43.9
BBV infection												
Pre/at diagnosis	20.0	00.0–41.3	18.8	00.0–38.9	53.8	25.1–82.6	42.9	15.5–70.3	50.0	16.7–83.3	**35.3**	23.8–46.8
Post diagnosis	00.0		-		-		-		-		**00.0**	
Never	19.8	14.6–25.0	36.8	29.8–43.7	37.4	30.3–44.4	46.2	38.7–53.7	58.8	51.4–66.3	**38.5**	35.4–41.6
STI Infection												
Pre/at diagnosis	17.2	07.4–27.1	27.8	15.7–39.9	38.8	24.8–52.6	44.8	32.7–56.8	54.3	37.5–71.1	**35.4**	29.6–41.6
Post diagnosis	14.3	00.0–43.6	50.0	11.2–88.8	00.0		83.3	49.1–100.0	50.0		**42.9**	24.2–61.5
Pre & post diagnosis	00.0		-		-		00.0		00.0		**00.0**	
Never	18.5	13.3–28.6	38.6	22.8–41.2	42.2	22.5–43.4	48.5	49.1–100.0	60.4	37.5–66.8	**41.4**	37.4–45.4
Exposure risk category												
MSM	22.4	16.0–28.8	36.8	28.6–44.9	30.2	22.3–43.3	49.2	40.2–58.1	63.0	53.8–72.1	**38.5**	34.7–42.2
IDU	20.0	00.0–48.0	00.0		50.0		100.0		50.0		**26.3**	05.9–46.7
MSM-IDU	00.0		20.0	00.0–46.7	25.0	00.0–57.8	54.5	23.0–86.1	55.6	31.4–79.7	**34.5**	22.1–46.8
Heterosexual	14.6	03.6–25.6	39.0	23.8–54.2	63.6	49.2–78.1	33.3	19.8–46.9	53.7	38.1–69.1	**40.9**	34.3–47.5
Other	-		00.0		66.7		-		00.0		**40.0**	00.0–88.1
No risk group identified	16.7	00.0–34.8	33.3	00.0–66.8	33.3	00.0–66.8	66.7		40.0	07.2–72.8	**30.6**	17.6–43.7

Appendix 4: Percentage of newly diagnosed persons achieving viral suppression within 6 months of HIV diagnosis in Queensland by demographic, clinical, and exposure risk category, 2014–2018 (n=1,006)

Year of Diagnosis	% Achieving viral suppression within 6 months (95% Confidence Interval)											
	2014		2015		2016		2017		2018		TOTAL	
	%	CI	%	CI	%	CI	%	CI	%	CI	%	CI
Total	**46.9**	40.7–53.2	**53.7**	46.8–60.6	**64.1**	57.3–70.8	**68.1**	61.4–74.9	**82.2**	76.6–87.8	**61.8**	58.8–64.8
Sex												
Male	46.8	40.2–53.4	55.0	47.7–62.3	62.1	54.8–69.3	69.8	62.6–76.9	82.4	76.6–88.3	**62.0**	58.9–65.2
Female	48.0	27.8–68.2	42.9	20.9–64.8	81.0	63.5–98.4	56.5	35.6–77.5	80.0	58.8–100.0	**60.0**	50.6–69.4
Age at diagnosis (years)												
<15	100.0		00.0		66.7		-		00.0		**50.0**	06.1–93.9
15–24	44.4	27.8–61.1	62.5	42.5–82.5	62.5	42.5–82.5	61.5	42.3–80.8	81.5	66.4–96.5	**61.3**	53.1–69.5
25–44	50.4	41.9–58.9	48.6	39.3–58.0	61.9	52.1–71.6	65.3	55.6–74.9	76.9	68.8–85.1	**59.8**	55.6–63.9
45–64	40.0	27.4–52.5	57.4	44.0–70.8	65.0	52.8–77.2	74.1	62.7–85.6	97.4	92.4–100.0	**64.6**	58.9–70.3
≥65	46.2	17.2–75.1	72.7	44.4–100.0	81.8	57.3–100.0	83.3	49.8–100.0	88.9	66.6–100.0	**72.0**	59.4–84.6
First Nations status												
Aboriginal and/or Torres Strait Islander	50.0	22.3–77.6	61.5	33.5–89.5	50.0	27.1–72.9	90.9	72.8–100.0	92.9	78.6–100.0	**66.7**	55.7–77.6
Neither	46.8	40.3–53.2	53.2	44.0–60.3	65.7	58.6–72.8	66.7	59.6–73.7	81.3	75.4–87.3	**61.5**	58.3–64.6
Location												
Major city	45.2	38.1–52.4	50.4	42.1–58.7	62.2	54.0–70.4	65.5	58.0–73.5	79.8	72.0–86.4	**59.3**	55.6–62.7
Regional	56.3	38.6–73.8	63.6	46.7–80.4	77.1	62.9–91.4	80.8	65.2–96.3	90.9	80.9–100.0	**73.6**	66.7–80.5
Remote	48.0	27.8–68.2	59.3	40.2–78.4	56.0	35.9–76.1	69.2	42.9–95.6	86.4	71.5–100.0	**62.5**	53.5–71.5

Year of Diagnosis	% Achieving viral suppression within 6 months (95% Confidence Interval)											
	2014		2015		2016		2017		2018		TOTAL	
	%	CI	%	CI	%	CI	%	CI	%	CI	%	CI
CD4+ count at diagnosis (cells/μL)												
<200	58.9	45.8–72.0	60.6	43.6–77.6	78.9	65.7–92.2	80.8	65.2–96.3	82.9	70.1–95.6	**70.7**	64.2–77.3
200–349	71.9	55.9–87.8	52.2	31.1–73.2	72.7	57.2–88.3	70.0	53.2–86.8	88.0	74.9–100.0	**71.3**	63.9–78.8
≥350	40.0	32.0–48.0	54.0	45.6–62.4	60.0	51.0–69.0	68.7	60.2–77.2	82.2	75.0–89.5	**59.5**	55.6–63.3
Unknown	08.3	00.0–25.0	25.0	00.0–57.8	22.2	00.0–51.7	35.7	09.1–62.3	69.2	42.5–96.9	**33.9**	21.4–46.5
Evidence of recent acquisition												
Yes	32.9	22.9–43.0	63.8	52.3–75.2	60.7	47.8–73.7	64.7	51.4–78.0	75.4	64.1–86.8	**57.2**	51.8–62.7
No	54.4	46.6–62.1	48.5	39.9–57.1	65.5	57.5–73.4	69.4	61.6–77.2	85.4	79.1–91.6	**64.0**	60.4–67.3
BBV infection												
Pre/at diagnosis	60.0	33.9–86.1	37.5	12.5–62.4	53.8	25.1–82.6	78.6	55.9–100.0	70.0	39.5–100.0	**58.8**	47.0–70.6
Post diagnosis	33.3		-		-		-		-		**33.3**	
Never	46.3	39.7–52.8	55.1	47.9–62.3	64.8	57.9–71.8	67.3	60.2–74.3	82.9	77.3–88.6	**62.1**	59.0–65.3
STI infection												
Pre/at diagnosis	43.1	7.4–27.1	37.0	15.7–39.9	61.2	24.9–52.6	62.7	32.7–56.8	82.9	70.3–95.4	**55.5**	29.6–41.6
Post diagnosis	28.6	00.0–64.4	62.5	24.9–100.0	66.7		100.0		50.0		**60.7**	
Pre & post diagnosis	00.0		-		-		00.0		100.0		**33.3**	
Never	48.1	39.7–56.6	57.4	47.7–67.1	68.1	59.6–76.6	72.7	63.9–81.6	83.6	77.3–89.9	**66.0**	62.1–69.8
Exposure risk category												
MSM	47.3	39.6–54.9	55.9	47.5–64.3	59.7	51.2–68.2	68.9	60.6–77.1	81.5	74.1–88.9	**61.1**	57.3–64.8
IDU	80.0	52.0–100.0	00.0		50.0		100.0		50.0		**57.9**	35.1–80.7
MSM-IDU	18.2	00.0–42.6	50.0	16.6–83.4	62.5	25.9–99.1	81.8	57.4–100.0	94.4	83.3–100.0	**65.5**	53.2–77.9
Heterosexual	46.3	30.8–61.9	53.7	38.1–69.2	79.5	67.4–91.7	62.5	48.6–76.4	82.9	71.2–94.7	**65.1**	58.7–71.5
Other	-		00.0		66.7		-		00.0		**40.0**	00.0–88.1
No risk group identified	44.4	20.2–68.7	55.6	20.2–90.9	55.6	20.2–90.9	66.7		80.0	53.2–100.0	**57.1**	43.1–71.2

Appendix 5: Stakeholder engagement semi-structured interview questions

Section 1: Your role

1	Please describe briefly your role(s) in HIV treatment policy, service delivery or disease surveillance/monitoring in Queensland.

Section 2: Current treatment commencement indicator

Among available Australian national indicators, Queensland treatment uptake indicators are more comprehensive and use a process indicator, proportion of people who commence treatment within six weeks, three months, and six months of HIV diagnosis, to measure progress among persons newly diagnosed with HIV infection.

2	In your experience, how often is the current treatment indicator…? i. Calculated ii. Used to inform HIV treatment policy and service delivery
3	In your opinion, how useful is the current treatment commencement indicator for monitoring trends in HIV care among newly diagnosed people?
4	If you've been involved data collection or analysis using NoCs' treatment commencement field, how would you rate the data collected on treatment commencement in regards to: i. completeness ii. timeliness iii. representativeness of population-level treatment commencement trends Please rate each item on a scale from 1–5 (1:Very Poor, 2:Poor, 3:Fair, 4:Good, 5:Excellent)

Section 3: Need for monitoring viral suppression

5	Would you consider viral suppression to be a useful indicator for monitoring population-level impacts of HIV care?
6	How might a routine analysis of viral suppression after diagnosis be beneficial to your role(s).
7	In your opinion, is viral suppression among newly diagnosed people a preferable monitoring indicator as compared to treatment commencement?
8	Do you have any concerns about an analysis of viral suppression, particularly in regards to process or data use?

Section 4: The described model

9	Is the proposed list of population groups sufficiently comprehensive? Does it miss any priority population groups?
10	In your experience and opinion, is it appropriate to assess viral suppression at the three and six-month time points? Would you consider limiting to only one time point or expanding it?
11	Is the proposed quarterly frequency of analysis appropriate?
12	What would be your key concerns for the Epidemiology and Research Unit undertaking this analysis in regards to privacy and how the data might be used?

13	Do you have any concerns about confidentiality related to the proposed design of this system?
14	If you've been involved in HIV surveillance, do you have any concerns related to this proposed system regarding the way in which viral load testing data are captured in NoCS ?
15	If you've been involved in HIV surveillance, do you have any concerns regarding the following attributes of the proposed system: i. Simplicity ii. Flexibility iii. Data Quality/Completeness iv. Representativeness v. Timeliness

Chapter 5

Analysis of malaria re-infection in a child cohort, Madang Province, Papua New Guinea, 2013

Prologue

Rationale

This chapter contains my data analysis project; survival analysis of a time to re-infection study in a school-aged children cohort in Madang, Papua New Guinea (PNG). The Mugil II study was undertaken in the northern coastal province of Madang in 2013. An analysis of the study dataset was proposed by Ivo Mueller (Walter and Eliza Hall Institute of Medical Research) and Leanne Robinson (Burnet Institute) after my return from Cambodia in late March 2020. Undertaking a malaria project during this program seemed destined for me. Several malaria projects were put forward prior to and during my time in Cambodia and I also designed a malaria factsheet on intermittent presumptive treatment for the Institut Pasteur Cambodia (Chapter 7). The Mugil II study dataset had not yet been analysed for time to re-infection and represented an opportunity for me to continue to contribute to international Department of Foreign Affairs and Trade (DFAT) funded health security initiatives despite no longer being placed in the region.

My roles

My role in the Mugil II study was to complete a survival analysis of the study dataset, which was a main outcome for this study. To complete the analysis, I wrote a comprehensive data analysis plan and obtained the necessary ethics approval from the Australian National University (ANU) Human Research Ethics Committee. I then worked with Melbourne and Port Moresby-based study investigators to undertake data cleaning and manage with other data issues. I was responsible for re-shaping and analysing the data using survival analysis methods, and identifying additional approaches to complete the analysis, as required. One such example was that I identified the need to undertake negative binomial modelling to calculate the symptomatic incidence rate due to the over-dispersion of data. A key component of the analysis for study investigators was to interpret results in comparison to a study undertaken in the same area in 2004, prior to malaria control/elimination interventions and the availability of rapid diagnostic tests and artemisinin-based combination therapy. As such, I analysed, presented, and interpreted the results with consideration to this prior study.

Lessons learned

This project represented a big learning curve for me as neither vector-borne diseases, nor survival analysis were strengths of mine. I gave myself a crash course in survival analysis via course notes from a friend and a biostatistician from the Doherty Institute, and spent many hours working through other online resources. I was initially hesitant to take on such a complex

analysis in my second year after a chaotic beginning to the year. However, I appreciate that I now have survival analysis in my epi tool box, as well as the other analytic methods I picked up along the way, including negative binomial regression and statistical approaches to the analysis of parasite densities and other skewed data. The full complexities of malaria epidemiology and the microbiology involved I am sure still evade me, but I feel that my vector-borne disease blind spot has been substantially reduced.

Public health impact

PNG has the highest malaria transmission rates in the Westerns Pacific region, with malaria one of the key causes of early childhood morbidity and mortality. This data analysis provides insights into the time to re-infection for school-aged children in a setting endemic with both *Plasmodium vivax* and *Plasmodium falciparum* species following high use of long-lasting insecticide treated bed nets and availability of improved diagnosis and treatment options. I analysed the data from this study and presented my findings in comparison to a study prior to the implementation of these malaria control interventions. My analysis therefore contributes important information about the epidemiological changes to malaria transmission and re-infection risks among school-aged children in co-endemic settings transitioning to pre-elimination. Findings from this analysis have identified opportunities to improve prevention and control activities and defer re-infection in these children.

MAE core activity requirements addressed

- Analysis of a public health dataset

Acknowledgements

I would like first and foremost to acknowledge that this project relied entirely on the participation of children and their families, as well as the study team responsible for coordinating, collecting, and managing study data. A particular thank you to Maria Kaius and Desmond Gul (Burnet Institute) for sharing their insights into the study cohort, as well as the geographical and malaria context of Madang. Thank you to Ivo Mueller and Leanne Robinson for entrusting me with the analysis and providing crucial support throughout. I would also like to thank my supervisors Jodie and Stephen for their contributions and support, and to Philippa Binns (ANU) for setting up the placement with the Doherty. Also I would have genuinely been lost trying to complete a survival analysis without the support of Niamh Meagher (Doherty Institute) and Laura Goddard (MAE 2019 cohort).

Abstract

Background

Over the past decade, malaria control interventions and the introduction of improved diagnosis and treatment have substantially reduced transmission of malaria in Papua New Guinea (PNG). However, there remain major challenges to further progress towards elimination. This analysis aimed to provide a better understand of the epidemiologic patterns of malarial infections among school-aged children in northern coastal PNG in 2013.

Methods

The Mugil II study was a longitudinal cohort study of school-aged children in Madang conducted in 2013. Children were treated with anti-malarial drugs at enrolment and followed up for 36 weeks for malarial re-infection and symptomatic episodes (febrile episodes with concurrent parasitaemia) by *Plasmodium* spp. using two diagnostic methods (qPCR and light microscopy (LM)). Time to event analysis was undertaken using standard survival analysis methods. Symptomatic incidence was estimated using negative binomial regression and Cox regression was used to test for re-infection risk factors.

Results

Among the 456 children enrolled, 35.7% (163) were *Plasmodium* spp. positive (by qPCR) at enrolment. At baseline (~4 weeks post enrolment and treatment) 16 children had infections detected and did not contribute to time at risk and were excluded from time-to-event analyses. For those children included in time-to-event analyses, the median time to re-infection for any species qPCR detectable infection was 157 days, with 62.5% (275/440) acquiring at least one qPCR detectable re-infection within the 36 weeks. Only 36.3% (161/440) of participants had at least one LM detectable re-infection. Median times to event could not be estimated for LM diagnosed or *P. falciparum* re-infections due to low re-infection rates (<50% of children re-infected). Only 47 symptomatic episodes were observed in 456 children. Prevalence of re-infection was highest for *P. vivax* infections (qPCR or LM) compared to *P. falciparum*. Species specific heterogeneity in re-infection risks were identified across villages and children with qPCR detectable *P. vivax* infections at enrolment were at increased risk of same species re-infection.

Conclusion

The implementation of several distribution rounds of universal long-lasting insecticide treated bed-nets and availability of improved diagnosis and treatment achieved substantial reductions

in malaria transmission among this 2013 cohort compared to ten years earlier. This is exemplified by the reduction in the proportion of children with qPCR detected *P. falciparum* re-infections from over 95% in 2004, to less than 20% in 2013. Epidemiological patterns of re-infection identified in this study were consistent with that of other co-endemic low transmission settings. Relatively smaller reductions in *P. vivax* transmission, demonstrated by re-infection risk, highlight the challenges inherent to controlling this species. Malaria control and elimination in pre-elimination settings should include interventions which target residual reservoirs of infection, including hypnozoite reservoirs.

Introduction

Malaria is an acute febrile illness caused by infection with *Plasmodium* parasites. Parasites are transmitted between humans through the bite of an infective female *Anopheles spp.* mosquito. Globally over 200 million cases of malaria and more than 400,000 deaths are estimated to occur annually.[1] Malaria has historically been endemic across the majority of Papua New Guinea (PNG). However, over the past decade, malaria control interventions and the introduction of improved diagnosis and treatment have substantially reduced transmission in PNG.[2]

Beginning from 2004, the national malaria control program supported by grants from the Global Fund to Fight AIDS, Tuberculosis, and Malaria (GFATM), initiated country-wide free distribution of long-lasting insecticidal nets (LLIN) at the household level.[3] From 2012, rapid diagnostic tests (RDTs) and artemisinin-based combination therapy (ACT) were also made available.[2] Between 2006 and 2014 along the north coast of PNG, prevalence of *Plasmodium falciparum* and *Plasmodium vivax* detected by light microscopy (LM) decreased 12- and 6-fold, respectively.[4] However, there remains major challenges to further progress towards elimination.

As transmissions declines, infections are increasingly likely to cluster among high-risk populations and areas, and more targeted and intensive interventions may be required to impact infections and illness burden in previously hyper-endemic areas. Infections are also more likely to be asymptomatic, low-density, or sub-microscopic, which presents challenges for detection and treatment.[4] Asymptomatic and sub-microscopic infections can carry gametocytes, the transmissible parasite form, which can be infective to mosquitos. They are also less likely to be diagnosed by LM or RDT and will therefore remain untreated and potentially transmissable.[4]

A key complexity to further elimination and control efforts is the biological differences between *P. falciparum* and *P. vivax;* two species which constitute the largest burden of infections and illness in PNG. Whilst the prevalence of both species has decreased over the past decade, *P. vivax* can remain dormant in liver cells as hypnozoites representing a continuing source of relapse at the individual level and reservoir of continuation or re-establishment of infection at the community level. Relapse from long-lasting liver stages account for four out of five *P. vivax* infections in PNG children aged 5–<11 years living in an area with hyperendemic transmission.[5]

Existing control and elimination efforts are primarily directed against *P. falciparum* blood stages. Dormant *P. vivax* hypnozoite stages in the liver cannot be detected with currently available diagnostic tools and requires specific anti-liver stage treatment, primaquine. However, routine treatment with primaquine is not widely implemented as the treatment may cause dangerous

side effects in individuals deficient in the G6PD enzyme, and therefore requires G6PD screening prior to safe use. Primaquine also has a long dosing schedule of up to two weeks.[5]

Mugil II study

The Mugil II study is part of a larger project "Impact of vector control on human immunity to malaria and genetic complexity of *P. falciparum* and *P. vivax* in two holoendemic areas of Papua New Guinea", which was conducted over a 7-year period in two northern PNG provinces; East Sepik and Madang. The project's primary objective was to evaluate the changing pattern of malaria infection and disease that results from progressive deployment of public health interventions to control and eventually eliminate malaria.

The Mugil II study was designed to assess the impact of LLIN and improved diagnosis and treatment with ACT on blood stage immunity to *P. falciparum* and *P. vivax* infection and mild malaria. At enrolment into the study, children from the Mugil area were treated with anti-malarial drugs (Artemether-lumefantrine) and were observed for a period of 36 weeks. Study implementation occurred in the same area and with comparable age groups and protocol to a treatment re-infection study in 2004 (Michon et al 2007).[6] This project therefore represents an opportunity to better understand the epidemiologic patterns of malarial infections among a cohort of school-aged children as compared to a baseline established prior to the implementation of vector control activities and availability of RDT and ACT. An improved understanding of the epidemiology of infection in the context of reduced malaria transmission will help guide continuation of malaria interventions. In particular, informing strategies aimed at the control and elimination of *P. vivax*.

Methods

Setting and participants

Mugil is a coastal lowland area of the northern mainland in Madang province, Momase region. Mugil is located in a rainforest with both coastal and inland villages and is an area of perennial malaria transmission.[3] During peak mosquito biting times, populations tend to spend substantial time outdoors or in structures which offer little protection against mosquitos. The study was conducted in 2013 and the study population was school-aged children between 5–<13 years from Mugil, Madang Province, PNG. The population was drawn from eight schools within the study area; Nom Barikas, Megair, Mirap, Karem (Sarang 2), Nuds Kind, Brias, Mugil, and Sarang. One or more study exclusion criteria were sufficient to exclude participants (Table 1).

Table 1. Mugil II study inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
• Age 5–<13 years (± 6 months for children without a known birth date) • Consent of parent(s)/guardian for child's participation • Attending school in local community • No known chronic health problems	• Parent(s)/guardian failure to provide permission for child to be in the study • Chronic illness such as diabetes, heart disease • Severe malnutrition • Severe anaemia (Haemoglobin concentration <5 g/dL) • Disability which prevents or impedes participation

Data sources

Participant data were actively collected at enrolment (-4 week) and at bi-weekly active follow ups and passively through case detection at local health centres until the end of the 9-month follow up period (Figure 1).

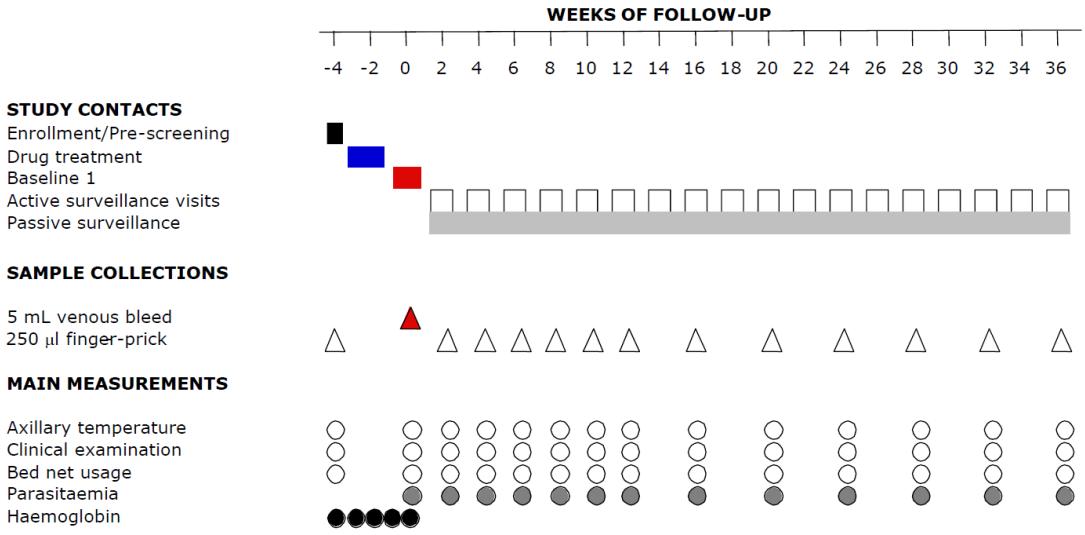


Figure 1: Overview of Mugil II study data collection and measurement

Analysis

The analysis outcome measures were:

Re-infection with *P. falciparum* and/or *P. vivax:* as detected by qPCR or LM after administration of anti-malarial drugs (ACT) at the beginning of the 36-week follow up period.

Symptomatic *P. falciparum* and/or *P. vivax* episode: *Plasmodium* positive by LM with evidence of auxiliary temperature ≥37.5 (within 2 days of presentation) after administration of ACT at the beginning of the 36-week follow up period. LM Parasite density thresholds (*P. falciparum*: >500

parasites/µL or >5,000 parasites/µL, *P. vivax:* >150 parasites/µL) were based on analyses of clinical tolerance to parastaemia in northern coastal PNG.[7]

The cohort was analysed descriptively by demographic and clinical characteristics and *Plasmodium* infection status at enrolment, as well as by passive morbidity events during follow up. Differences between participants at enrolment were tested using appropriate statistical tests. The proportion of children with ≥1 positive sample while at risk was calculated by *Plasmodium* spp. and diagnostic method by demographic group and enrolment infection status and species-specific prevalence of infection was calculated at enrolment and at each bi-weekly follow up. Counts of children with symptomatic episodes (febrile with concurrent parasitaemia by LM) were calculated by species, number of episodes, and incidence rates of symptomatic episodes (per child/year) were calculated using negative binomial regression models due to the distribution of unconditional means. Time at risk was calculated for individuals based on the number of follow up events attended.

The median time to first re-infection or symptomatic episode was calculated using the time between the date of first treatment (enrolment) and an infection-positive result or symptomatic malaria episode. This was calculated by species, diagnostic method (qPCR and LM), and LM parasite density thresholds (*P. falciparum*: >500 and >5,000 parasites/µl; *P. vivax*: >150 parasites/µl). All participants for time-to-event analyses were required to be free of the outcome at baseline (~4 weeks post enrolment and treatment). As such, all participants with infections detected at baseline were excluded from these analyses. Participants were censored at last follow-up if they withdrew, were lost to follow up, or where two active follow up time points were missed. Differences between survival curves were tested using Log Rank tests.

Cox regression was used to test univariate and multivariate risk factors for re-infection with backwards selection and likelihood ratio tests used to identify multivariate model fit. The assumption of proportionality was tested using plots of hazard functions and residuals. Due to smaller sample sizes, some village groups were consolidated appropriate to their geographic location and proximity to the coast. Results of these analyses will be compared to the available 2004 study data published by Michon et al 2007.[6]

Ethical considerations

The project protocol was approved by the Medical Research Advisory Committee of PNG (MRAC No. 11.21) and PNG Institute of Medical Research Institutional Review Board (IRB No. 1116). Ethics approval was obtained for this data analysis from the Australian National University Human Research Ethics Committee (Protocol 2020/325).

Results

Participant characteristics

Between 18 February and 14 March 2013, 456 children were enrolled into the study with a mean age of 8.9 years (s.d. 1.75). Of these children, 52.0% (237/456) were female and 77.0% (351/456) reported using a LLIN the night previous to enrolment (Table 2). Six children (1.3%) had deficient G6PD tests (BinaxNow), all of whom were male. Children belonged to ten village groups with the majority residing in Mirap (17.5%), Biranis (17.1%), Megiar (14.5%), and Karem (13.8%). Seven children had an auxiliary temperature ≥37.5 °C and 16 children had an enlarged spleen at enrolment.

At the time of enrolment, 163 children (35.7%) were *Plasmodium* spp. positive by qPCR, 100 children (21.9%) were *Plasmodium* positive by light microscopy (LM), and one child did not have diagnostic data available. *P. vivax* was the most common infection detected by qPCR (110/163, 67.5%) followed by *P. falciparum* (73/163, 44.8%) (Table 3). Among children with infection detected by LM, 36.7% (18/49) of *P. falciparum* infections were gametocyte positive by LM and 9.8% (5/51) of *P. vivax* infections were gametocyte positive by LM. Statistically significant differences were observed between qPCR infection status at enrolment and geographic locations (school (p<0.001), village (p=0.001)) and mean haemoglobin levels (g/dL) were lower among qPCR *Plasmodium* spp. positive children (p=0.007) (Table 3).

Initial antimalarial treatment

At baseline (~4 weeks post enrolment and treatment), infection was detected in 16 children (qPCR: 13; LM: 2; both qPCR and LM: 1). Among these children, ten were *P. falciparum*-positive and six were *P. vivax*-positive. These children did not contribute to time at risk and were excluded for time to first re-infection analyses.

Passive morbidity

Among the 456 participants, 11.8% (54/456) had at least one passive morbidity event. The majority of children only had one event during follow up (85.1%, 46/54), seven children (13.0%) had two events, and one child (1.9%) had three events. Children with passive morbidity events were predominantly located in Biranis (15/54, 27.8%), Karem (12/54, 22.2%), and Mirap (10/54, 18.5%). A statistically significant difference was observed between children with and without passive morbidity events in relation to village of residence (p=0.015).

There were 63 passive morbidity events, 39 (61.9%) of which were accompanied by a positive RDT. RDTs were predominantly positive for *P. falciparum* (or mixed) (31/39, 79.5%), whilst five

RDTs were positive for *P. vivax/malariae/ovale*, and three were positive for *P. falciparum* (single). Among the 39 positive RDTs, 35 were qPCR positive (*P.f*: 30; *P.v.*: 5). There were no statistically significant differences observed in relation to the characteristics of children with qPCR positive and negative events. Among the 24 non-malaria related events, the majority of diagnoses were urinary tract infections (12/24, 50.0%) or respiratory illnesses (5/24, 20.8%).

Table 2: Participant characteristics at enrolment (% overall) and characteristic differences by *Plasmodium* positivity* status (% by row), Mugil, Papua New Guinea 2013 (N=456)

Characteristic	Total cohort (N=456)† N (*% overall)*	*Plasmodium* negative (N=292) N *(%)*	*Plasmodium* positive (N=163) N *(%)*	*p*-value
Sex				
Female	237 (52.0)	159 (67.4)	77 (32.6)	0.140
Male	219 (48.0)	133 (60.7)	86 (39.3)	
Age group (years)				
<9	242 (53.1)	159 (66.0)	82 (34.0)	0.396
≥9	214 (46.9)	133 (62.1)	81 (37.9)	
School				
Nom Barikas	22 (4.8)	17 (77.3)	05 (22.7)	<0.001
Megiar	83 (18.2)	55 (66.3)	28 (33.3)	
Mirap	106 (23.2)	59 (55.7)	47 (44.3)	
Karem (Sarang 2)	56 (12.3)	30 (53.6)	26 (46.4)	
Nuds Kind	13 (2.9)	09 (69.2)	04 (30.8)	
Biras	91 (20.0)	67 (73.6)	24 (26.4)	
Mugil	61 (13.4)	47 (78.3)	13 (21.7)	
Sarang	24 (5.3)	08 (33.3)	16 (66.7)	
Village				
Biranis	78 (17.1)	56 (71.8)	22 (28.2)	0.001
Bulal	17 (03.7)	12 (70.6)	05 (29.4)	
Karem	63 (13.8)	37 (58.7)	26 (41.3)	
Karkum	38 (08.3)	21 (55.3)	17 (44.7)	
Lixal	21 (4.6)	17 (81.0)	04 (19.0)	
Megiar	66 (14.5)	46 (69.7)	20 (30.3)	
Mirap	80 (17.5)	45 (56.3)	35 (43.8)	
Nom Birikas	22 (4.8)	17 (77.3)	05 (22.7)	
Sarang 1	30 (6.6)	10 (33.3)	20 (66.7)	
Udisis	41 (09.0)	31 (77.5)	09 (22.5)	
Reported use of health center				
Always	442 (96.6)	281 (63.7)	160 (36.3)	0.254
Not always	14 (3.1)	11 (78.6)	03 (21.4)	
Days since last malaria treatment‡	791.5 (312–1,580.5)	790 (329–1,652)	799 (284–1,530)	0.378
Last malaria treatment				
≤6 months	43 (9.4)	26 (60.5)	17 (39.5)	0.594
>6 months	413 (90.6)	266 (64.6)	146 (35.4)	

Characteristic	Total cohort (N=456)† N (*% overall)*	*Plasmodium* negative (N=292) N *(%)*	*Plasmodium* positive (N=163) N *(%)*	*p*-value
Bed net use last night				
Yes	101 (22.1)	64 (63.4)	36 (35.6)	0.836
No	351 (77.0)	226 (64.6)	125 (35.7)	
Unknown	04 (0.9)	02 (50.0)	02 (50.0)	
Sickness status				
Currently sick	26 (5.7)	14 (53.8)	12 (46.2)	0.254
Sick within last 2 weeks	57 (12.5)	40 (70.2)	17 (29.8)	0.704
Temperature (°C)§	36.4 (0.4)	36.4 (0.4)	36.4 (0.4)	0.4845
<37.5	448 (98.2)	288 (64.4)	159 (35.6)	0.427
≥37.5	07 (1.5)	04 (57.1)	03 (42.9)	
Unknown	01 (0.2)	-	01 (100)	
Weight (kg) §	22.8 (4.2)	22.7 (4.0)	23.1 (4.4)	0.374
Height (cm) §	121.0 (9.0)	121.0 (9.0)	121.0 (9.0)	0.952
Enlarged spleenǀ				
Yes	16 (03.5)	08 (50.0)	08 (50.0)	0.397
No	436 (95.6)	282 (64.8)	153 (35.2)	
Unknown	04 (0.9)	02 (50.0)	02 (50.0)	
Hemoglobin level (g/dL)§	10.8 (1.1)	10.9 (1.1)	10.6 (1.0)	0.007
<11	262 (57.5)	159 (60.9)	103 (39.5)	0.111
≥11	186 (40.8)	126 (68.1)	59 (31.9)	
Unknown	08 (1.8)	07 (87.5)	01 (12.5)	
G6PD test¶				
Normal	450 (98.7)	290 (64.6)	159 (35.4)	0.194
Deficient	06 (01.3)	02 (33.3)	04 (66.7)	
Pale appearance**				
Yes	20 (4.4)	08 (40.0)	12 (60.0)	0.054
No	435 (95.4)	283 (65.2)	151 (34.8)	
Unknown	01 (0.2)	01 (100)	-	

G6PD: glucose-6-phosphate dehydrogenase.

* As detected by qPCR.

† Total includes one participant with a missing qPCR result at enrolment. Participant was excluded from *p*-value calculations.

‡ Median (interquartile range), p-value calculated with Mann-Whitney test.

§ Mean (standard deviation), p-value calculated with two-sample t-test.

ǀ Measured using Hackett's grading system.

¶ Test measures the amount of G6PD enzyme in red blood cells. Deficiency in G6PD is a contraindication to some malaria treatments.

** Pale appearance of at least one of the following; conjunctiva, tongue, or palms.

Table 3: Characteristics of children *Plasmodium*-positive (by qPCR) at enrolment by *Plasmodium* species of infection, Mugil, Papua New Guinea, 2013 (N=163)*

Characteristic	*P.f* (N=73)	*P.v* (N=110)	*P.m* (N=02)	*P.o* (N=01)	Mixed† (N=23)
Sex	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**
Female	37 (50.7)	51 (46.4)	01 (50.0)	-	12 (52.2)
Male	36 (49.3)	59 (53.6)	01 (50.0)	01 (100)	11 (47.8)
Age Group (years)					
<9	35 (47.9)	58 (52.7)	01 (50.0)		12 (52.2)
≥9	38 (52.1)	52 (47.3)	01 (50.0)	01 (100)	11 (47.8)
School					
Nom Barikas	04 (5.5)	03 (2.7)	-	-	02 (8.7)
Megiar	15 (20.5)	18 (16.4)	-	-	05 (21.7)
Mirap	22 (30.1)	29 (26.4)	-	-	05 (21.7)
Karem (Sarang 2)	07 (9.6)	22 (20.0)	-	-	03 (13.0)
Nuds Kind	-	04 (3.6)	-	-	-
Biras	14 (19.2)	11 (10.0)	-	-	01 (4.3)
Mugil	04 (5.5)	09 (8.2)	2 (100)	01 (100)	02 (8.7)
Sarang	07 (9.6)	14 (12.7)	-	-	05 (21.7)
Village					
Biranis	12 (16.4)	11 (10.8)	-	-	01 (4.3)
Bulal	02 (2.7)	05 (4.5)	-	-	02 (8.7)
Karem	05 (6.8)	23 (20.9)	-	-	02 (8.7)
Karkum	07 (9.6)	12 (10.9)	-	-	02 (8.7)
Lixal	01 (1.4)	03 (2.7)	01 (50.0)	-	01 (4.3)
Megiar	14 (19.2)	09 (8.2)	-	-	03 (13.0)
Mirap	16 (21.9)	21 (19.1)	-	01 (100)	03 (13.0)
Nom Birikas	04 (5.5)	03 (2.7)	-	-	02 (8.7)
Sarang 1	09 (12.3)	17 (15.5)	-	-	06 (26.1)
Udisis	03 (4.1)	06 (5.5)	01 (50.0)	-	01 (4.3)
Infection status‡					
Asexual stage positive	40 (81.6)	46 (90.2)	01 (50.0)	-	-
Asexual stage density§	4.9 (4.4–5.5)	4.2 (3.9–4.6)	-	-	-
Gametocyte positive	18 (36.7)	05 (9.8)	-	-	-
Gametocyte density§	4.1 (2.4–4.6)	3.3 (2.4–4.6)	-	-	-

P.f: Plasmodium falciparum P.v: P. vivax P.m: P. malariae P.o: P. ovale

* Children with multiple species detected are reported both in the single species and mixed species column.

† Evidence of species combinations.

‡ Proportion calculated using LM detected infections only.

§ Geometric mean (95% confidence interval): log-transformed due to left-skewed distribution.

Proportions re-infected

Of the 440 children included in the time to re-infection analysis, 62.5% (275/440) had at least one re-infection by any *Plasmodium* spp. as detected by qPCR and 36.6% (161/440) as detected by LM (Table 4). Re-infection by *P. vivax* was higher than other species with 51.8% of children experiencing at least one *P. vivax* infection by qPCR and 28.6% by LM. qPCR detected 204 *P. falciparum* infections and 757 *P. vivax* infections. There were only two *P. malariae* (qPCR:1, LM: 1) infections detected and no *P. ovale* infections were detected. Overall proportions of children re-infected (qPCR or LM) were highest among those living in Mirap, Sarang 1, and Karem. However, there was heterogeneity in proportions re-infected across schools and villages and by species of infection. Only 15% (66/440) of children had at least one re-infection above a LM density threshold (*P. falciparum* <500 parasites/µL or *P. vivax* <150 parasites/µL).

The prevalence of qPCR detectable *P. vivax* re-infections gradually increased over the follow up period with the largest increase observed between week 4 (5%) and week 6 (12%) post treatment (Figure 2A). Pre-treatment levels of *P. vivax* were only reached and exceeded once at week 32 post-treatment (24.6% as compared to 24.1%). Prevalence of LM detectable *P. vivax* re-infection was substantially lower than those detected by qPCR, reaching only 7.4%, and pre-treatment levels were not reached. Prevalence of *P. falciparum* re-infections (qPCR and LM) remained low, reaching only 7.2% for qPCR and 4.9% for LM and pre-treatment levels were not reached for either diagnostic method.

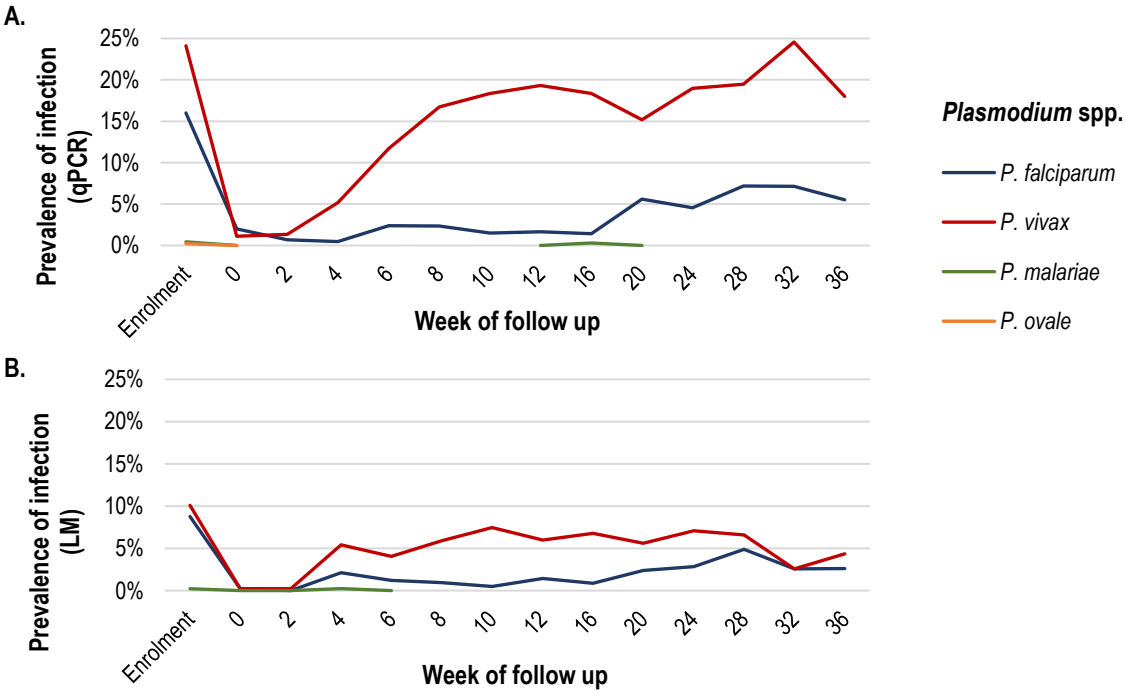


Figure 2: Prevalence of malarial re-infection at enrolment and bi-weekly follow up obtaining finger prick blood samples, Mugil, Papua New Guinea, 2013. A) qPCR diagnosis B) LM diagnosis

Table 4: Proportion of children re-infected by *Plasmodium* species and method of diagnosis, Mugil, Papua New Guinea, 2013 (N=440)

Characteristic	*P.f*		*P.v*		*P.m*		Mixed†		Any spp.	
	qPCR	LM	qPCR	LM	qPCR	LM	qPCR	LM	qPCR	LM
Total	19.8	15.7	51.8	28.6	0.2	0.2	5.7	1.6	62.5	36.6
Sex										
Female	17.0	13.0	47.0	26.5	0.4	0.4	4.8	1.7	55.5	33.9
Male	22.9	18.6	57.1	31.0	-	-	6.7	1.4	65.3	39.5
Age (years)										
<9	17.4	12.6	50.0	27.8	-	0.4	2.2	0.9	57.0	34.8
≥9	22.4	19.0	53.8	29.5	0.5	-	9.5	2.4	58.6	38.6
School										
Nom Barikas	25.0	15.0	50.0	5.0	-	-	5.0	-	60.0	20.0
Megiar	15.9	17.1	51.2	28.0	-	-	6.1	1.2	58.5	35.4
Mirap	20.8	18.8	66.3	38.6	1.0	-	7.9	3.0	69.3	45.5
Karem	21.1	14.0	68.4	45.6	-	-	10.5	-	71.9	52.6
Nuds Kind	8.3	-	41.7	16.7	-	-	-	-	41.7	16.7
Biras	25.8	19.1	31.5	19.1	-	-	3.4	1.1	46.1	31.5
Mugil	5.3	3.5	40.4	15.8	-	1.8	-	-	43.9	19.3
Sarang	40.9	27.3	63.6	40.9	-	-	9.1	9.1	68.2	50.0
Village										
Biranis	30.3	22.4	34.2	21.1	-	-	3.9	1.9	51.3	35.5
Bulal	-	5.9	35.3	17.6	-	-	-	-	35.3	17.6
Karem	19.0	12.7	63.5	38.1	-	-	7.9	-	66.7	44.4
Karkum	8.3	8.3	66.7	27.8	-	-	2.8	-	66.7	30.6
Lixal	5.3	5.3	42.1	15.8	-	-	-	-	42.1	21.1
Megiar	20.0	20.0	43.1	27.7	-	-	7.7	1.5	52.3	36.9
Mirap	23.4	20.8	68.8	41.6	1.3	-	9.1	3.9	72.7	49.4
Nom Birikas	25.0	15.0	50.0	5.0	-	-	5.0	-	60.0	20.0
Sarang 1	35.7	21.4	64.3	46.4	-	-	10.7	7.1	67.9	53.6
Udisis	5.1	2.6	38.5	15.4	-	2.6	-	-	43.6	17.9
Infection status at baseline										
Plasmodium positive	25.0	20.5	72.4	47.4	0.6	0.6	9.0	1.3	76.3	55.1
Asexual stage positive	25.9	27.2	71.6	48.1	-	-	7.4	1.2	77.8	56.8
Gametocyte positive	38.9	27.8	61.1	27.8	-	-	0.1	-	72.2	44.4

*Proportion of children with at least one positive sample while at risk.

† Mixed species infections include *P. falciparum, P. vivax,* and *P. malariae.*

‡ Either *P. falciparum or P. vivax* gametocyte positive.

Time to first re-infection

The median time to first re-infection by any *Plasmodium* spp. was 157 days (qPCR) and by *P. vivax was* 210 days (qPCR) (Figure 3). Median times to re-infection could not be estimated for LM detection, symptomatic episodes, or re-infection/symptomatic episodes above a LM parasite density thresholds due to less than 50% of children becoming re-infected during the study period. The incidence risk of first re-infection for any *Plasmodium* spp. was 1.4 per year by qPCR and 0.74 per year for LM detection (Table 5). Among *Plasmodium s*pp., the highest incidence of first re-infection was in *P. vivax* infections (both qPCR or LM detection).

Table 5: Incidence of new re-infections (per child/year) by *Plasmodium* species and diagnostic method*, Mugil, Papua New Guinea, 2013 (N=440)

	Incidence rate per child/year (95% CI)	
Species of re-infection	***qPCR***	***LM***
P. falciparum	*0.34 (0.27–0.42)*	0.27 (0.21–0.34)
P. vivax	*1.18 (1.03–1.34)*	*0.55 (0.46–0.66)*
P. malariae	*0.004 (0.001–0.026)*	*0.004 (0.001–0.026)*
Mixed†	*0.09 (0.06–0.14)*	*0.03 (0.01–0.05)*
***Any Plasmodium* spp.**	*1.40 (1.24–1.58)*	*0.74 (0.64–0.87)*

CI: Confidence interval; qPCR: Polymerase chain reaction; LM: Light microscopy

† Mixed infections included P. falciparum, P. vivax, and P. malariae

There was statistically significant geographical heterogeneity in the risks of qPCR detectable *P. vivax* re-infection (p=0.0038). Compared to children living in Biranis, children living in the northern coastal area had higher risks of *P. vivax* re-infection (by qPCR). These villages included Mirap (AHR: 2.63 [95% CI 1.61, 4.27] p<0.0001), Karkum (AHR: 2.14 [95% CI 1.21, 3.78] p=0.0009), Karem (AHR: 1.86 [95% CI 1.12, 3.10] p=0.017), and Sarang 1 (AHR: 1.91 [95% CI 1.03, 3.56] p=0.041) (Figure 4). When *P. vivax* re-infection risk was assessed by LM detection, re-infection risk did not significantly vary among study villages overall (p=0.021). However, compared to children in Biranis, children living in Mirap had increased risk of a LM detectable *P. vivax* re-infection (AHR: 1.89 [95% CI 1.03, 3.49] p=0.041).

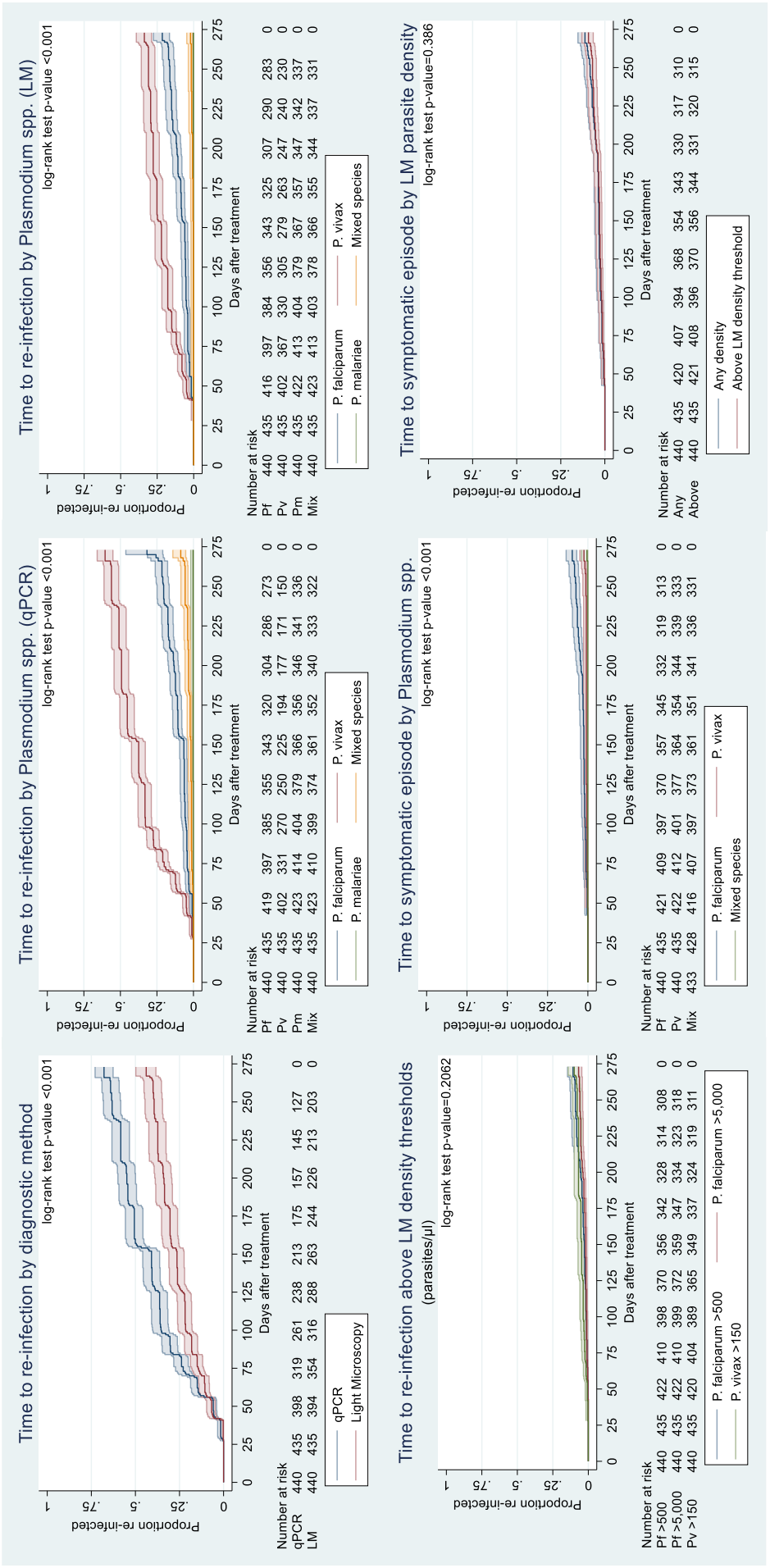
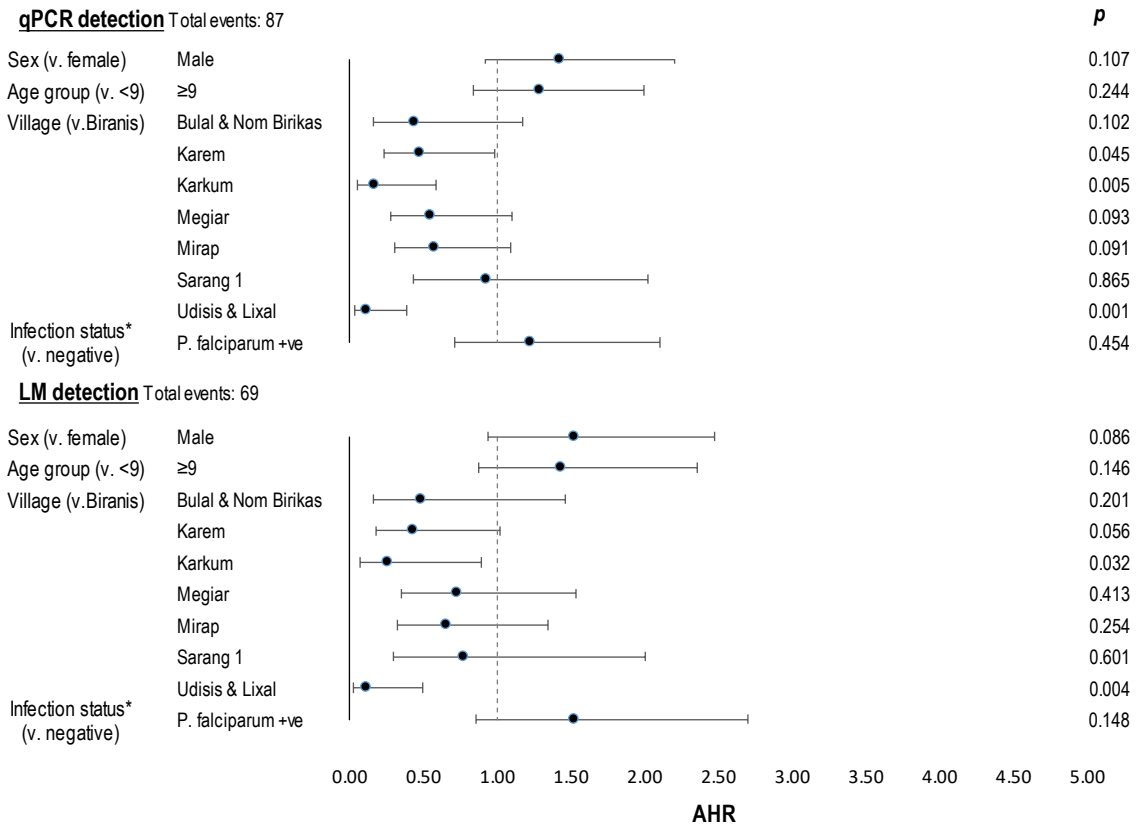
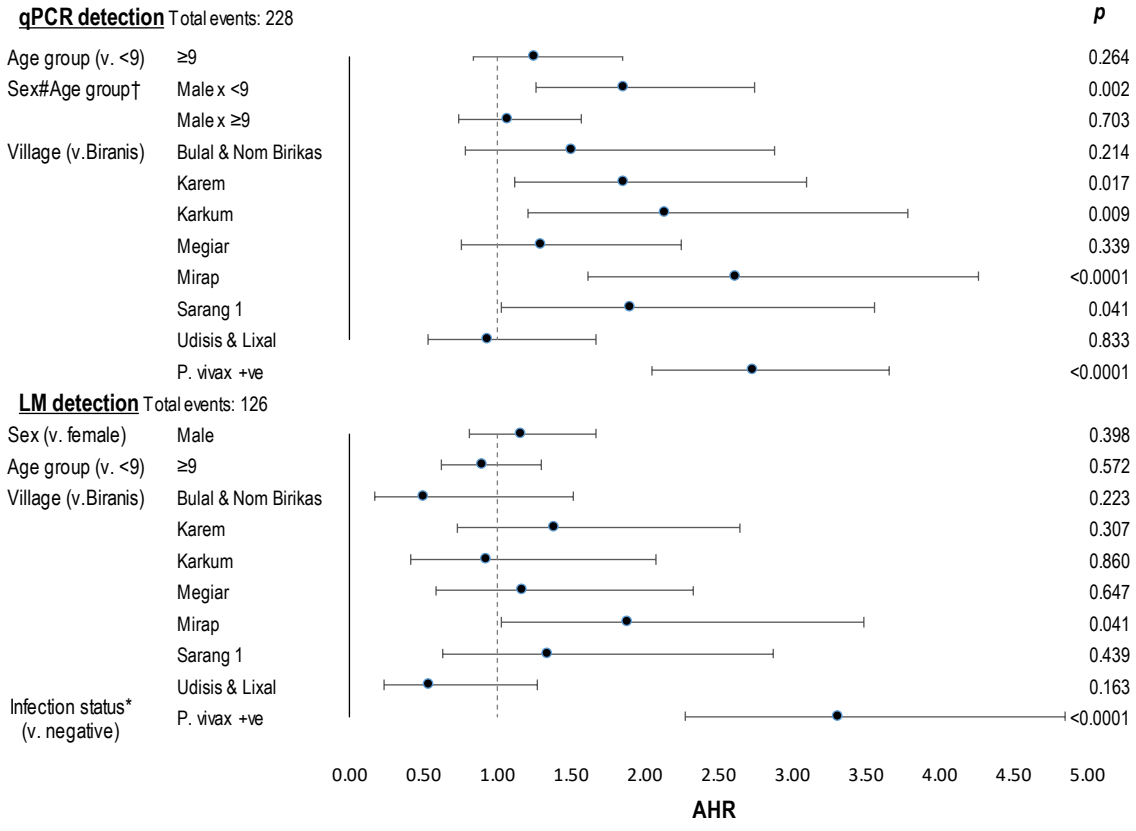


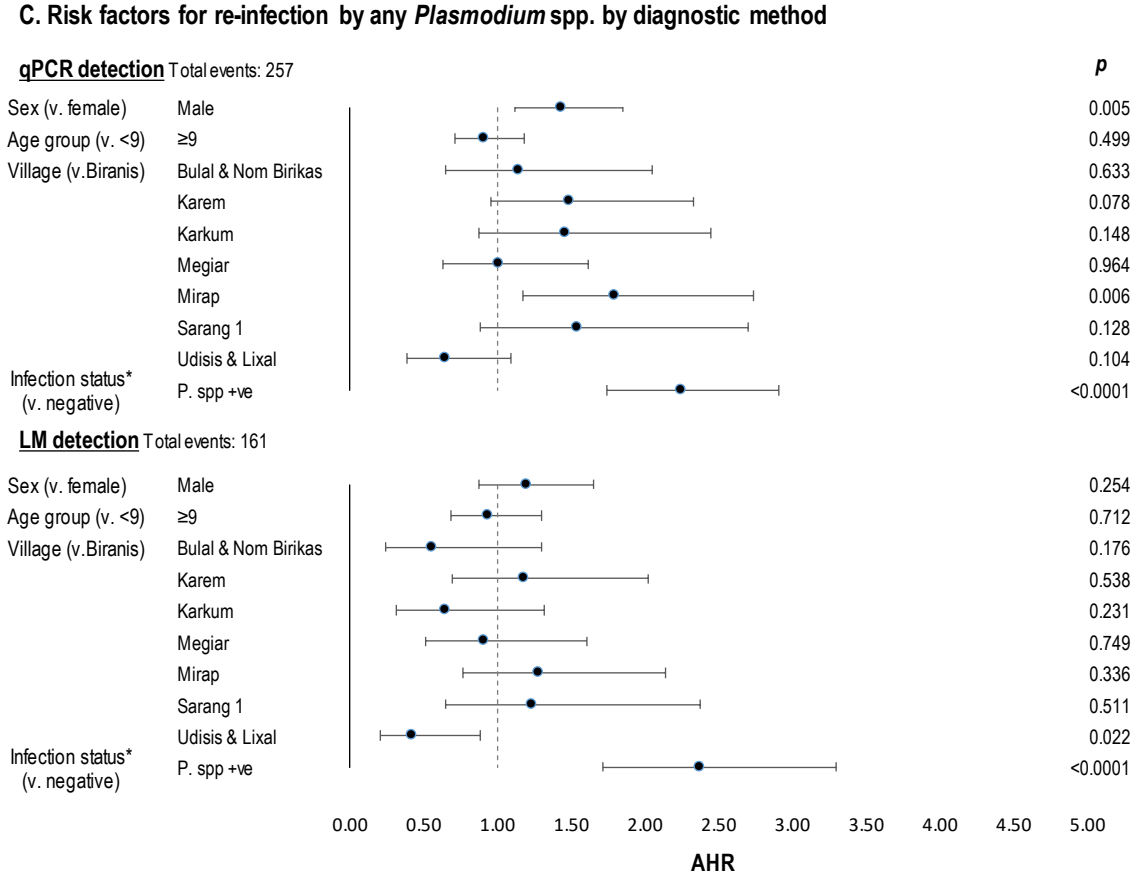
Figure 3: Kaplan-Meier plots of time to first re-infection, Mugil, Papua New Guinea, 2013 (N=440). A) Any *Plasmodium* spp. by diagnostic method B) *Plasmodium* spp. by qPCR detection C) *Plasmodium* spp. by LM detection D) *Plasmodium* spp. by LM parasite density threshold E) Symptomatic episodes by *Plasmodium* spp. F) Symptomatic episodes by LM parasite density threshold. Shaded areas represent the respective 95% confidence intervals.

A. Risk factors for re-infection by *P. facliparum* by diagnostic method



B. Risk factors for re-infection by *P. vivax* by diagnostic method





+ve: positive. AHR: Adjusted Hazard Ratio

* Infection detected by qPCR at enrolment.

† Interaction between sex and age group. Measure of interaction on multiplicative scale: AHR (95% CI) 0.58 (0.34, 0.99) p=0.046

‡ All multivariate Cox regression analyses were adjusted for sex, age, village, and enrolment *Plasmodium*-positivity status (by qPCR).

Figure 4: Multivariate risk factors for re-infection by *Plasmodium* spp. and diagnostic method, Mugil, Papua New Guinea, 2013 A) *P. falciparum* B). *P. vivax C)* Any *Plasmodium* spp.

Geographical heterogeneity was not overall statistically significant for re-infection risk by any *Plasmodium* spp. or *P. falciparum* (qPCR and LM). Yet when compared to children in Biranis, there were some differences in re-infection risks across village. Children living in Megiar had an increased risk of any *Plasmodium* spp. qPCR detectable re-infection (AHR: 1.80 [95% CI 1.18, 2.74] p=0.006), whilst children living in Udisis and Lixal had lower LM detectable re-infection risk (any *Plasmodium* spp.) (AHR: 0.42 [95% CI 0.20, 0.88] p<0.022). Reductions in the risk of *P. falciparum* re-infection (both qPCR and LM) were associated with two villages; Karkum (qPCR AHR: 0.17 [95% CI 0.05, 0.58] p=0.005; LM AHR: 0.26 [95% CI 0.07, 0.89] p=0.032), and Udissis and Lixal (qPCR AHR: 0.12 [95% CI 0.03, 0.39] p=0.001; LM AHR: 0.11 [95% CI 0.03,0.49] p=0.004). Living in Karem was also associated with a reduced risk of qPCR detectable *P. falciparum* re-infection (AHR: 0.48 [95% CI 0.23, 0.98] p=0.045).

There was some heterogeneity in re-infection risks related to sex. Male children were at higher risk of a qPCR detectable *Plasmodium* spp. re-infection (AHR: 1.44 [95% CI 1.12, 1.86] *p*=0.005) and there was evidence of an interaction between sex and age group in the risk of qPCR detectable *P. vivax* re-infection, whereby male children younger than 9 years of age had an increased risk of re-infection (AHR: 1.87 [95% CI 1.27, 2.75] *p*=0.002). No associations between sex and LM detectable *P. vivax* re-infection or *P. falciparum* re-infection detected by either method were identified. Age group was also not associated with re-infection risk for any species or method of detection. Evidence of non-proportionality in re-infection risk related to age group over the study period was identified in the LM detectable *P. falciparum* model with LM detected re-infections more frequent among older children in the second half of follow up. As no plausible biological rationale for age group variation was identified, the model is presented unadjusted.

Children *P. vivax* positive (by qPCR) at enrolment were at higher risk of *P. vivax* re-infection regardless of diagnostic method, with highest re-infection risk for LM (qPCR AHR: 2.74 [95% CI 2.05, 3.66] *p*<0.0001; LM AHR: 3.32 [95% CI 2.27, 4.85] *p*<0.0001). Similarly, the risk of re-infection by any *Plasmodium* spp. was associated with *Plasmodium*-positivity at enrolment (both qPCR and LM). However, *P. falciparum* re-infection risk was not associated with *P. falciparum* infection at enrolment.

Symptomatic malaria episodes

Overall, 47 symptomatic episodes (febrile episodes with concurrent parasitaemia by LM) were observed in 456 children over the 9-month duration of the study (Table 6). The maximum number of symptomatic malaria episodes was two per child. Symptomatic episodes were predominantly caused by *P. falciparum* (38/47, 80.8%) with only 10 episodes related to *P. vivax*, including one infection which was mixed *P. falciparum* and *P. vivax*. Only 9.4% (43/456) of children experienced at least one symptomatic episode while at risk and only 7.9% (36/456) of children experienced a symptomatic episode with a LM parasite density exceeding the species-specific threshold. Among *P. falciparum* symptomatic episodes, 68.4% exceeded the 5,000 parasite/µL density threshold.

Table 6: Number of symptomatic malaria episodes* by *Plasmodium* species and LM parasite density threshold, Mugil, Papua New Guinea, 2013 (N=456)

	No. episodes	*% with ≥1 episode*	*Incidence rate‡*	No children by episode count		
P. falciparum				**0**	**1**	**2**
Any	38	7.9	0.13 (0.10–0.19)	420	34	02
>*500/µL*	32	6.6	0.11 (0.08–0.16)	426	28	02
>5,000/µL	26	5.3	0.09 (0.06–0.14)	432	22	02
P. vivax						
Any	10	2.0	0.04 (0.02–0.07)	447	08	01
>150/µL	08	1.5	0.03 (0.01–0.07)	449	06	01
Mixed†						
Any	01	0.2	§	455	01	-
>Threshold	01	0.2	§	455	01	-
Any *Plasmodium.* spp.						
Any	47	9.4	0.17 (0.12–0.22)	413	39	04
>Threshold¶	39	7.9	0.14 (0.10–0.19)	420	33	03

* *Plasmodium* positive by LM with evidence of auxiliary temperature ≥37.5 within 2 days of presentation.

† LM parasite density >500 parasites/µL for *P. falciparum* infections or >150 parasites/µL for *P. vivax* infections.

‡ Expressed as per child/year. Estimated using negative binomial regression model. Total time at risk = 103,464 person days.

§ Could not be estimated due to low rates of mixed species symptomatic episodes.

¶ Threshold as per lowest corresponding species-specific LM parasite density threshold.

Discussion

The Mugil II study shows substantially reduced prevalence of *P. vivax* and *P. falciparum* re-infection and symptomatic episodes among school-aged children in Madang Province in 2013, as compared to the findings of Michon et al in 2004 prior to the introduction of RDTs and ACT.[6] Whilst in 2004 almost all children had been infected at least once with both parasites over six months of follow up (qPCR detectable *P. falciparum*: 95.3%; qPCR detectable *P. vivax:* 82.0%), only 62.5% of children in this study had one or more re-infections by any *Plasmodium* spp. (qPCR) during the 9-month follow up.[6] The median number of days to first re-infection by *P. vivax* (qPCR) in 2013 was also more than 2.5 times longer than in 2004. Prevalence of infection was also substantially lower than 2004. Prevalence during follow up in 2013 only reached pre-

treatment levels for *P. vivax* (qPCR only) on one occasion 32 weeks' post-treatment; whereas the pre-treatment prevalence for both *P. vivax* and *P. falciparum* were reached during the 2004 study as early as week 6 of follow up. These findings reflect the downward trend in malaria prevalence in PNG between 2009 and 2014. National cross-sectional surveys observed a decrease in all species prevalence in the northern Momase region (which includes Madang Province) from 19.8% in 2008/2009 to 3.3% in 2013/2014.[8,9] Implementation of this study occurred during the lowest time point for malaria transmission in PNG – nationally, prevalence has increased from 2016.[10] Consistent with previous studies, infection and symptomatic episodes with *P. malariae and P. ovale* were rare with only four *P. malariae* and one *P. ovale* infections detected during enrolment or follow up.[5,6,9,11]

Differences in epidemiologic patterns of re-infection and symptomatic episodes between *P. vivax* and *P. falciparum* were evident in this cohort. Regardless of diagnostic method, the prevalence of *P. vivax* re-infection and proportion of children with at least one *P. vivax* re-infection was higher than that of *P. falciparum*. Unlike findings in 2004, where children had similar numbers of *P. falciparum* and *P. vivax* infections during follow up, children in this study had almost four times as many *P. vivax* infections compared to *P. falciparum*. Predominance of *P. vivax* as a primary source of re-infection in children is consistent with other studies over this time period in PNG.[12-14] Differential species patterns in prevalence were also evident in 2004, with qPCR detected *P. vivax* infection prevalence increasing more rapidly after treatment than that of *P. falciparum*. This faster increase in *P. vivax* was similarly evident in 2013. Yet in 2004, *P. falciparum* prevalence gradually overtook *P. vivax*, whilst throughout this follow up *P. vivax* maintained higher prevalence.

Overall, there was a decrease in the prevalence of both species between 2004 and 2013. However, the reductions in the proportion of children re-infected, incidence rates of first re-infection, and symptomatic episodes were larger for *P. falciparum* than *P. vivax*. The proportion of children with at least one *P. falciparum* re-infection decreased 4.8-fold and 5.6-fold for qPCR detection and LM detection, respectively. For *P. vivax* corresponding reductions were only 1.6-fold and 1.7-fold, respectively. The number of symptomatic episodes related to *P. falciparum* also decreased over this time period. The incidence of high density (>5,000/µL) symptomatic episodes of *P. falciparum* infections decreased from 1.17 per child/year in 2004 to 0.09 per child/year in 2013. Whilst for *P. vivax* (any LM density), incidence of symptomatic episodes decreased from 0.11 to 0.04 per child/year between 2004 and 2013. The differential decrease between these two species over time is consistent with cross-sectional surveys in the Momase region between 2008/2009 and 2013/2014 and is consistent with the observations of many

countries co-endemic with these species.[8,9,15-17] Strengthening of control measures typically has larger impacts on *P. falciparum* transmission, whilst *P. vivax* presents a more stubborn challenge. Elimination and control strategies often do not address the specific biological characteristics of *P. vivax* which enable the species to be adaptive to intensive malaria control activities. Thus both increasing control challenges and the tendency towards resurgence, particularly when control efforts cannot be sustained.[16,18,19] The ability of *P. vivax* to remain dormant as hypnozoites and its transmissibility prior to clinical symptom onset presents a range of challenges for diagnosis and treatment. From a diagnostic perspective, these challenges include the inability of point of care tests to identify hypnozoite carriage and additional sensitive diagnostic requirements to detect low parasite density infections.[16,18] To clear both blood stage and hypnozoite infections and therefore prevent relapse, G6PD screening must also be undertaken prior to safe use of Primaquine and children are required to adhere to a 14-day treatment regime.[20,21]

Although the relative decrease in symptomatic *P. falciparum* re-infection was greater, this species remained the leading cause of symptomatic episodes within this cohort. Incidence of symptomatic episodes related to *P. falciparum* was 0.13 per child/year, as compared to *P. vivax* symptomatic episodes at 0.04 per child/year. The risks of symptomatic *P. vivax* episodes are considered lower among school-aged children as compared to younger children due to rapidly acquired immunity to *P. vivax* in PNG children.[12,22] Furthermore, the majority of *P. vivax* infections in PNG children are considered to be relapses from long-lasting liver stages and it is generally considered that clinical episodes are more likely to be caused by new mosquito-bite acquired infections.[5,14] Although commonly thought to be more benign than *P. falciparum*, *P. vivax* infection still may be associated with a significant burden of morbidity and associated mortality.[23] At an individual level, multiple relapses of *P. vivax* infection can also result in clinically relevant anaemia.[17]

There were clear differences in the prevalence and proportions of children re-infected according to diagnostic method, reflecting their different limits of detection. Of all 924 *Plasmodium* infections diagnosed by qPCR, 65.3% were not detectable by LM and were therefore sub-microscopic. Additionally, relatively few infections detected by LM were above a species-specific parasite density threshold, confirming that the majority of infections in this age group were low density. Similarly in a 2013 study in East Sepik among children aged 1–5 years, over two thirds of all qPCR detected infections were sub-microscopic.[14] Consistent with a higher proportion of low density or sub-microscopic infections, more infections were asymptomatic.[24] Over the 9-month study duration only 47 symptomatic episodes with concurrent parasitaemia were observed with a maximum of two episodes per child. Incidence of symptomatic malaria episodes

substantially decreased from 1.92 per child/year in 2004 to only 0.17 per child/year. These asymptomatic and low density infections would most likely escape detection and treatment.

The prevalence of *P. vivax* particularly varied by diagnostic method, with 70.9% (537/757) of all qPCR detectable *P. vivax* infections undetected by LM. Similar to Michon et al's findings, relatively fewer qPCR detected *P. vivax* infections were detected by LM, as compared to *P. falciparum.* High density *P. vivax* infections were also rare.[6] Consistently lower parasitaemias by *P. vivax* is in line with recent literature and has been linked to its biological characteristic, such as the tendency for biomass to concentrate in extravascular tissues of the marrow and spleen.[16,23] In relapsing infections typical of *P. vivax,* the host immune system may also be better able to respond adequately to limit density given the prior exposure to the same or similar parasite clones during the primary infection.[25] Older children are considered to have a greater capacity to control *P. vivax* infections at low to moderate densities.[12] However, even low density *P. vivax* infections are potentially infectious and therefore, remain a reservoir of infection.[5,24,25]

There were clear geographical variations in *P. vivax* re-infection risks in this cohort. *P. vivax* re-infection risk was clustered in villages located in the northern coastal region of the study area. Some geographical heterogeneity was evident in 2004. However, cross-sectional studies between 2006 and 2014 indicate increasing geographical heterogeneity in Madang, as well as elsewhere in northern coastal PNG.[4,11] The identification of a higher burden of *P. vivax* re-infection in northern coastal villages of the study area is also consistent with recent analyses of *P. vivax* sero-positivity (Desmond Gul, Burnet Institute, personal communication, 2020 Aug 18). However, driving factors behind the higher burden of infection and the potential contribution of hypnozoites is unknown. Differences in spatial heterogeneity between 2004 and 2013 likely result from the substantial reduction in transmission, as clustering is more likely to occur in low transmission settings.[14,26-29] Spatial clustering may be related to a number of host, vector, and environmental factors including health seeking behaviour related the distances from health centre, as well as mosquito breeding sites and behavioural characteristic of mosquito species.[11,27,29-31] Geographical clusters or hot spots represent important reservoirs of infection and a challenge to control efforts. Detection of hot spots can often be difficult as the increasing proportion of low density infections require sensitive molecular diagnostics, such as qPCR, which are typically not feasible for large-scale routine surveillance or part of routine care in PNG.[32,33] However, once identified, hot spot targeted interventions are considered logistically efficient, particularly in low-transmission or resource-limited settings.[22,28,29]

Age was not found to be associated with risks of *P. falciparum* re-infection or LM detectable re-infections in this cohort. However, the epidemiology of infection with respect to age was likely

impacted by the substantial decrease in transmission. In low transmission settings, the lack of repeated exposure slows the development of immunity, shifting incidence of mild and severe malaria to later in childhood or adulthood.[22,24,29,34,35] Analyses of the 2004 cohort indicated that by nine years of age children had acquired almost complete clinical immunity to *P. vivax* (AHR: 0.67, p<0.05, reference group children ≤9 years) and partial immunity to symptomatic *P. falciparum* (AHR: 0.56, *p*<0.05, reference group children ≤9 years).[6,36] Evidence from 2010 suggests that children still acquired immunity early in life in Madang, yet surveys in Madang indicate that the age of peak *P. falciparum* infection prevalence shifted later by more than 10 years, from 9 years in 2006 to 19.5 years in 2014.[4,37] Although there is evidence indicating a shift in the burden of infection to later adolescence, this school-aged cohort remained less likely to experience a symptomatic episode when compared to younger children. We can see this in the lower proportions of children in our school-aged cohort who were identified with either a symptomatic *P. falciparum* or *P. vivax* episode following treatment (9.4% within 9 months) when compared with a younger cohort (6 months–<5 years of age) in Mugil from a 2011–2013 ACT treatment trial (32.8% within 6 months).[38]

An interaction between age group and sex was observed related to qPCR detectable *P. vivax* re-infection, with male children younger than 9 years of age at increased risk of re-infection. This indication of higher risk of *P. vivax* re-infection among younger school-aged children is relatively consistent with evidence that the peak burden for *P .vivax* is among children 6 years of age.[12] However, this risk was only observed among males which may indicate an underlying gendered behavioural factor. Additionally, male children in the 2013 cohort were observed to have an increased risk of qPCR detectable re-infections (any *Plasmodium* spp.). In 2004 there was no evidence of heterogeneity regarding sex and sex-based differences are not typically considered substantial within this age group. However, increased risk of infection among male school-aged children has been documented elsewhere and school aged boys in Mugil are more likely to engage in outdoor-based chores and have greater freedom to spend time outside of the home, which may increase their exposure risks.[39-41] The identification between sex and *P. vivax* re-infection risk in this cohort compared to 2004, may be related to the smaller-scale of transmission allowing effects of individual variation to be more noticeable.[29]

Another contrast to risk factors identified in 2004 relates to infection status at enrolment. In 2004, enrolment infection status by qPCR was only associated with *P. falciparum* re-infection. This association was not identified in this cohort, yet any *Plasmodium* spp. and *P. vivax* re-infection risk (qPCR and LM) were associated with enrolment infection status (qPCR, same species). A similar association between the presence of same species parasitaemia and

symptomatic episodes has been observed among younger children elsewhere in PNG.[12] The association between enrolment infection status and re-infection risks potentially highlights heterogeneity in transmission among participants, as well as the role of relapsing *P. vivax* infection.[12] Due to the previously high level of transmission, it is likely that the majority of children within the 2004 cohort had hypnozoites. Relapse rates were therefore more likely to be uniform among children with and without infections detected at enrolment. Whilst, in 2013 relapse rates among infection status groups would be expected to vary.

Among this cohort there was high reported bed net usage at enrolment, which has been observed in cross sectional studies within this region.[2,9,11] Large-scale LLIN distribution was the sole vector control strategy in PNG between 2004 and 2014 and has therefore played a crucial role in malaria control.[2,42] However, there was no evidence of an association between reported bed net usage and individual risk of re-infection in this study. This is likely due to the high rates of reported use and the community-level rather than individual-level impact LLINs have on transmission.[11,43] The personal protective effect of LLIN on re-infection among this school-aged cohort is likely highly heterogeneous, particularly in relation to *P. vivax* infection due to the role of relapsing infections.[12,14,42] Bed net usage was also not considered to play a substantial role within the spatial clustering of re-infection risk.

There are some potential limitations to this analysis. Based on the low proportions of children re-infected observed in this study, the median time to re-infection could not be estimated for most outcomes. Yet, this serves to highlight one of the key finding of a substantially lower prevalence of re-infection. Thirteen active clinical follow up dates, of a total of 7,669, were identified as errors and could not be verified by the time of submission. As such, for a small number of episodes the date was estimated based on the plausible range for that follow up event. A violation of the proportional hazards assumption was detected related to age group in the LM detectable *P. falciparum* re-infection Cox regression model. Among older children, LM detectable *P. falciparum* re-infections were more frequent towards the end of the study period. Whilst there may be an underlying behavioural factor increasing exposure, no biologically plausible explanation for variation of re-infection risk over time could be determined for the age group. As such the model was presented unadjusted. Further exploration into potential seasonal behaviours that may contribute to higher density *P. falciparum* re-infection among older school children is warranted.

Conclusion

This study demonstrates the substantial reductions in transmission achieved within school-aged children in northern coastal Madang between 2004 and 2013. Epidemiological patterns of infection were consistent with the experiences of other co-endemic settings achieving low transmission. We identified increasing proportions of infections which were asymptomatic low density or sub-microscopic, as well as greater heterogeneity in re-infection risks. In particular, northern coastal villages were found to have a higher burden of *P. vivax* than other villages in 2013. Although no association was determined between age and re-infection risk, decreased transmission has likely impacted acquisition of immunity for this age group, particularly for *P. falciparum*. The lower level of transmission has also potentially allowed for the identification of increased risk among younger male school-aged children for qPCR detectable *P. vivax* re-infection.

Although both *Plasmodium* spp. prevalence substantially declined, reductions were more pronounced for *P. falciparum* than *P. vivax.* Indeed, by 2013 *P. vivax* had become the primary source of infection for these school-aged children in Mugil. The resilience of *P. vivax* to malaria control activities has been documented across other co-endemic pre-elimination settings globally. The findings of this analysis provide further evidence of the challenges of controlling and eliminating *P. vivax* among school-aged children in PNG, as well as the role of low density, asymptomatic or relapsing infections in sustaining malaria transmission. Malaria control interventions in co-endemic settings, particularly those in pre-elimination stages, should target residual reservoirs of infection. In particular, geographically targeting hot spots and risk groups, as well as prioritising the incorporation of *P. vivax* interventions which aim to efficiently address hypnozoite reservoirs.

References

1. World Health Organization. Malaria eradication: benefits, future scenarios and feasibility. [Internet]. Geneva: WHO; 2020. [cited 2020 May 06]. Available from: https://www.who.int/publications-detail/malaria-eradication-benefits-future-scenarios-feasibility.

2. Hetzel MW, Pulford J, Ura Y, Jamea-Maiasa S, Tandrapah A, Tarongka N, et al. Insecticide-treated nets and malaria prevalence, Papua New Guinea, 2008–2014. Bulletin of the World Health Organization. 2017;95(10):695-705B. DOI: 10.2471/BLT.16.189902

3. Hetzel MW, Morris H, Tarongka N, Barnadas C, Pulford J, Makita L, et al. Prevalence of malaria across Papua New Guinea after initial roll-out of insecticide-treated mosquito nets. Tropical Medicine & International Health. 2015;20(12):1745-55. DOI: 10.1111/tmi.12616

4. Koepfli C, Ome-Kaius M, Jally S, Malau E, Maripal S, Ginny J, et al. Sustained malaria control over an 8-year period in Papua New Guinea: the challenge of low-density asymptomatic *Plasmodium* infections. Journal of Infectious Diseases. 2017;216(11):1434-43. DOI: 10.1093/infdis/jix507

5. Robinson LJ, Wampfler R, Betuela I, Karl S, White MT, Li Wai Suen CS, et al. Strategies for understanding and reducing the *Plasmodium vivax* and *Plasmodium ovale* hypnozoite reservoir in Papua New Guinean children: a randomised placebo-controlled trial and mathematical model. PLoS Medicine. 2015;12(10):e1001891. DOI: 10.1371/journal.pmed.1001891

6. Michon P, Cole-Tobian JL, Dabod E, Schoepflin S, Igu J, Susapu M, et al. The risk of malarial infections and disease in Papua New Guinean children. The American Journal of Tropical Medicine and Hygiene. 2007;76(6):997-1008.

7. Muller I, Genton B, Rare L, Kiniboro B, Kastens W, Zimmerman P, et al. Three different *Plasmodium* species show similar patterns of clinical tolerance of malaria infection. Malaria Journal. 2009;8:158. DOI: 10.1186/1475-2875-8-158

8. Hetzel MW, Gideon G, Mueller I, Siba PM. Papua New Guinea/Global Fund Round 3 malaria control programme evaluation 2008/2009: results from cross-sectional surveys and sentinel sites. [Internet]. Goroka: Papua New Guinea Institute of Medical Research; 2010. [cited 2020 Sep 24]. Available from: https://www.malariasurveys.org/documents/IMR-Evaluation-GFATM-R3-20.03.2010-final2.1.pdf.

9. Hetzel MW, Pulford J, Gouda H, Hodge A, Siba PM, Mueller I. The Papua New Guinea National Malaria Control Program: primary outcome and impact Indicators, 2009–2014. [Internet]. Goroka: Papua New Guinea Institute of Medical Research; 2014. [cited 2020 Sep 29]. Available from: https://www.malariasurveys.org/documents/IMR%20-%20outcome%20and%20impact%202014.pdf.

10. Hetzel MW, Saweri OPM, Kuadima JJ, Smith I, Ura Y, Tandrapah A, et al. Papua New Guinea malaria indicator survey 2016–2017: malaria prevention, infection, and treatment. [Internet]. Goroka: Papua New Guinea Institute of Medical Research; 2018. [cited 2020 Sep 24]. Available from: https://www.malariasurveys.org/documents/PNGIMR%202018%20-%20PNG%20MIS%202016-17%20-%20Final%20Report%2006.04.2018.pdf.

11. Kattenberg JH, Gumal DL, Ome-Kaius M, Kiniboro B, Philip M, Jally S, et al. The epidemiology of *Plasmodium falciparum* and *Plasmodium vivax* in East Sepik Province, Papua New Guinea, pre- and post-implementation of national malaria control efforts. Malaria Journal. 2020;19(1):198. DOI: 10.1186/s12936-020-03265-x

12. Lin E, Kiniboro B, Gray L, Dobbie S, Robinson L, Laumaea A, et al. Differential patterns of infection and disease with P. *falciparum* and P. *vivax* in young Papua New Guinean children. PLoS One. 2010;5(2):e9047. DOI: 10.1371/journal.pone.0009047

13. Senn N, Rarau P, Stanisic DI, Robinson L, Barnadas C, Manong D, et al. Intermittent preventive treatment for malaria in Papua New Guinean infants exposed to *Plasmodium falciparum* and P. *vivax*: a randomized controlled trial. PLoS Medicine. 2012;9(3):e1001195. DOI: 10.1371/journal.pmed.1001195

14. Ome-Kaius M, Kattenberg JH, Zaloumis S, Siba M, Kiniboro B, Jally S, et al. Differential impact of malaria control interventions on P. *falciparum* and P. *vivax* infections in young Papua New Guinean children. BMC Medicine. 2019;17(1):220. DOI: 10.1186/s12916-019-1456-9

15. Hetzel MW, Reimer LJ, Gideon G, Koimbu G, Barnadas C, Makita L, et al. Changes in malaria burden and transmission in sentinel sites after the roll-out of long-lasting insecticidal nets in Papua New Guinea. Parasites & Vectors. 2016;9(1):340. DOI: 10.1186/s13071-016-1635-x

16. Ding XC, Ade MP, Baird JK, Cheng Q, Cunningham J, Dhorda M, et al. Defining the next generation of *Plasmodium vivax* diagnostic tests for control and elimination: target product profiles. PLoS Neglected Tropical Diseases. 2017;11(4):e0005516. DOI: 10.1371/journal.pntd.0005516

17. White MT, Walker P, Karl S, Hetzel MW, Freeman T, Waltmann A, et al. Mathematical modelling of the impact of expanding levels of malaria control interventions on *Plasmodium vivax*. Nature Communications. 2018;9(1):3300. DOI: 10.1038/s41467-018-05860-8

18. *Vivax* Working Group. Targeting *vivax* malaria in the Asia Pacific: The Asia Pacific Malaria Elimination Network *Vivax* Working Group. Malaria Journal. 2015;14:484. DOI: 10.1186/s12936-015-0958-y

19. Jennison C, Arnott A, Tessier N, Tavul L, Koepfli C, Felger I, et al. *Plasmodium vivax* populations are more genetically diverse and less structured than sympatric *Plasmodium falciparum* populations. PLoS Neglected Tropical Diseases. 2015;9(4):e0003634. DOI: 10.1371/journal.pntd.0003634

20. Wells TN, Burrows JN, Baird JK. Targeting the hypnozoite reservoir of *Plasmodium vivax*: the hidden obstacle to malaria elimination. Trends in Parasitology. 2010;26(3):145-51. DOI: 10.1016/j.pt.2009.12.005

21. Kheng S, Muth S, Taylor WR, Tops N, Kosal K, Sothea K, et al. Tolerability and safety of weekly primaquine against relapse of *Plasmodium vivax* in Cambodians with glucose-6-phosphate dehydrogenase deficiency. BMC Medicine. 2015;13:203. DOI: 10.1186/s12916-015-0441-1

22. White NJ, Pukrittayakamee S, Hien TT, Faiz MA, Mokuolu OA, Dondorp AM. Malaria. Lancet. 2014;383(9918):723-35. DOI: 10.1016/S0140-6736(13)60024-0

23. Baird JK. Evidence and implications of mortality associated with acute *Plasmodium vivax* malaria. Clinical Microbiology Reviews. 2013;26(1):36-57. DOI: 10.1128/CMR.00074-12

24. Lindblade KA, Steinhardt L, Samuels A, Kachur SP, Slutsker L. The silent threat: asymptomatic parasitemia and malaria transmission. Expert Review of Anti-infective Therapy. 2013;11(6):623-39. DOI: 10.1586/eri.13.45

25. Joyner CJ, Brito CFA, Saney CL, Joice Cordy R, Smith ML, Lapp SA, et al. Humoral immunity prevents clinical malaria during *Plasmodium* relapses without eliminating gametocytes. PLoS Pathogens. 2019;15(9):e1007974. DOI: 10.1371/journal.ppat.1007974

26. Kreuels B, Kobbe R, Adjei S, Kreuzberg C, von Reden C, Bater K, et al. Spatial variation of malaria incidence in young children from a geographically homogeneous area with high endemicity. Journal of Infectious Diseases. 2008;197(1):85-93. DOI: 10.1086/524066

27. Karl S, White MT, Milne GJ, Gurarie D, Hay SI, Barry AE, et al. Spatial effects on the multiplicity of *Plasmodium falciparum* Infections. PLoS One. 2016;11(10):e0164054. DOI: 10.1371/journal.pone.0164054

28. Bejon P, Williams TN, Liljander A, Noor AM, Wambua J, Ogada E, et al. Stable and unstable malaria hotspots in longitudinal cohort studies in Kenya. PLoS Medicine. 2010;7(7):e1000304. DOI: 10.1371/journal.pmed.1000304

29. Bousema T, Griffin JT, Sauerwein RW, Smith DL, Churcher TS, Takken W, et al. Hitting hotspots: spatial targeting of malaria for control and elimination. PLoS Medicine. 2012;9(1):e1001165. DOI: 10.1371/journal.pmed.1001165

30. Muller I, Smith T, Mellor S, Rare L, Genton B. The effect of distance from home on attendance at a small rural health centre in Papua New Guinea. International Journal of Epidemiology. 1998;27(5):878-84. DOI: 10.1093/ije/27.5.878

31. Rodriguez-Rodriguez D, Maraga S, Jamea-Maiasa S, Tandrapah A, Makita L, Siba PM, et al. Mapping routine malaria incidence at village level for targeted control in Papua New Guinea. Geospatial Health. 2019;14(2). DOI: 10.4081/gh.2019.798

32. Hofmann NE, Gruenberg M, Nate E, Ura A, Rodriguez-Rodriguez D, Salib M, et al. Assessment of ultra-sensitive malaria diagnosis versus standard molecular diagnostics for malaria elimination: an in-depth molecular community cross-sectional study. Lancet Infectious Diseases. 2018;18(10):1108-16. DOI: 10.1016/S1473-3099(18)30411-0

33. Davis WA, Clarke PM, Siba PM, Karunajeewa HA, Davy C, Mueller I, et al. Cost-effectiveness of artemisinin combination therapy for uncomplicated malaria in children: data from Papua New Guinea. Bulletin of the World Health Organization. 2011;89(3):211-20. DOI: 10.2471/BLT.10.084103

34. Boyle MJ, Reiling L, Osier FH, Fowkes FJ. Recent insights into humoral immunity targeting *Plasmodium falciparum* and *Plasmodium vivax* malaria. International Journal for Parasitology. 2017;47(2-3):99-104. DOI: 10.1016/j.ijpara.2016.06.002

35. Stanisic DI, Fowkes FJ, Koinari M, Javati S, Lin E, Kiniboro B, et al. Acquisition of antibodies against *Plasmodium falciparum* merozoites and malaria immunity in young children and the influence of age, force of infection, and magnitude of response. Infection and Immunity. 2015;83(2):646-60. DOI: 10.1128/IAI.02398-14

36. Robinson LJ, D'Ombrain MC, Stanisic DI, Taraika J, Bernard N, Richards JS, et al. Cellular tumor necrosis factor, gamma interferon, and interleukin-6 responses as correlates of immunity and risk of clinical *Plasmodium falciparum* malaria in children from Papua New Guinea. Infection and Immunity. 2009;77(7):3033-43. DOI: 10.1128/IAI.00211-09

37. Koepfli C, Robinson LJ, Rarau P, Salib M, Sambale N, Wampfler R, et al. Blood-stage parasitaemia and age determine *Plasmodium falciparum* and P. *vivax* gametocytaemia in Papua New Guinea. PLoS One. 2015;10(5):e0126747. DOI: 10.1371/journal.pone.0126747

38. Laman M, Benjamin JM, Moore BR, Salib M, Tawat S, Davis WA, et al. Artemether-lumefantrine versus artemisinin-naphthoquine in Papua New Guinean children with uncomplicated malaria: a six months post-treatment follow-up study. Malaria Journal. 2015;14:121. DOI: 10.1186/s12936-015-0624-4

39. Houngbedji CA, N'Dri PB, Hurlimann E, Yapi RB, Silue KD, Soro G, et al. Disparities of *Plasmodium falciparum* infection, malaria-related morbidity and access to malaria prevention and treatment among school-aged children: a national cross-sectional survey in Cote d'Ivoire. Malaria Journal. 2015;14:7. DOI: 10.1186/1475-2875-14-7

40. Kepha S, Nikolay B, Nuwaha F, Mwandawiro CS, Nankabirwa J, Ndibazza J, et al. *Plasmodium falciparum* parasitaemia and clinical malaria among school children living in a high transmission setting in western Kenya. Malaria Journal. 2016;15:157. DOI: 10.1186/s12936-016-1176-y

41. Rodriguez-Rodriguez D, Katusele M, Auwun A, Marem M, Robinson L, Laman M, et al. Human behaviour, livelihood and malaria transmission in two sites of Papua New Guinea. Journal of Infectious Diseases. In press. 2020.

42. Rodriguez-Rodriguez D, Maraga S, Lorry L, Robinson LJ, Siba PM, Mueller I, et al. Repeated mosquito net distributions, improved treatment, and trends in malaria cases in sentinel health facilities in Papua New Guinea. Malaria Journal. 2019;18(1):364. DOI: 10.1186/s12936-019-2993-6

43. Howard SC, Omumbo J, Nevill C, Some ES, Donnelly CA, Snow RW. Evidence for a mass community effect of insecticide-treated bednets on the incidence of malaria on the Kenyan coast. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2000;94(4):357-60. DOI: 10.1016/s0035-9203(00)90103-2

Chapter 6

Lessons from the field and additional teaching activities

Lessons from the field

Background

Teaching and participating in Lessons from the Field (LFF) activities are core requirements of the MAE program. As part of my HIV surveillance project, I developed skills in probabilistic record linkage using the Stata program, *reclink2*. I found this program enabled me to efficiently match routine notifications data with laboratory records. I decided this was a valuable skill and the focus of my LFF.

Lessons learnt

The most challenging aspect was preparing an exercise that was valuable for each participant, regardless of skills and Stata experience (Appendix 1). In preparing the exercise and accompanying dataset, I also had to ensure that there were no unforeseen errors in the dataset or ambiguity in the explanations or instructions to ensure that each participant could complete the exercise. This caused me to reflect much more deeply about my application of record linkage. An additional challenge was scheduling the LFF during a pandemic where participants have unpredictable workloads. Participant feedback indicated that the LFF would be a useful reference for future record matching endeavours and has been shared with first-year MAEs.

Acknowledgements

An important acknowledgement for this LFF is Jonathan Malo. His LFF provided a great example of how to teach Stata through activities and he was responsible for introducing me to *reclink2*.

Additional teaching activities

Background

I was involved in additional experiences during the MAE, these include:

- Coordinating a teaching session titled “Neglected Tropical Diseases” for first-year MAE scholars with Laura Goddard and Hannah Vogt. In this session we used three disease examples from the Asia Pacific region (leptospirosis, melioidosis, lymphatic filariasis) to discuss what defines a Neglected Tropical Diseases and the challenges that can be presented in their surveillance and control.
- Mentoring of the Yangon-based Data Manager to support the analysis of the ECOMORE 2 human leptospirosis case-control study.
- Responding to ad hoc requests from my fellow MAEs with trouble-shooting issues, particularly related to Stata, ethics protocols, and data linkage.

Lessons learnt

Most of my teaching was undertaken remotely. This presented a number of challenges and learnings. Firstly, working with the Yangon-based Data Manager, I learnt the benefit of preparing examples that could be worked through whilst sharing screens. It also highlighted for me how difficult, yet important it is to use clear and simple language to explain epidemiological concepts. Teaching of the first year MAE cohort during Course Block 3 was also completed online. Facilitating and engaging people in this context was much more difficult than anticipated. We used breakout groups as a way to encourage discussion of a case study. Although this may have been successful in person, it was very challenging to encourage engagement and required very conscious facilitation. This experience caused our group to reflect on alternative approaches for teaching online.

Appendix 1: Lesson from the Field

Probabilistic record linkage in *Stata* using *reclink2*

Background

This lesson from the field (LFF) has been developed to assist other MAE scholars to undertake probabilistic record linkage in Stata. The content of this LFF is based on my experience matching routine notifications data with a laboratory record dataset as part of my HIV surveillance project.

Learning objectives

Upon completion of the exercise, you should be able to:

- Describe the differences between deterministic and probabilistic linkage.
- Discuss and recognise some common considerations and issues that arise when undertaking probabilistic record linkage.
- Clean and prepare datasets for linkage using Stata.
- Use *reclink2* to link records from two datasets.

There are two Stata files attached to this LFF; *LFF Exercise (Notifications)*, *LFF Exercise (Study Database)*. Please use these to complete the exercises and submit your responses and annotated .do file to Elenor.kerr@gmail.com by **11th March 2020.**

Introduction to probabilistic record linkage.

There are two common methods to link data from two different data sources; probabilistic and deterministic linkage. Deterministic linkage compares identifier/s across databases and creates a link if there is a complete match. In contrast, probabilistic record linkage (also known as data matching or fuzzy merging) is typically used to merge two datasets when there is no common record identifier (i.e. notification ID or Study ID) or when there is likely to be missing identifier data or a high rate of data entry error.[1]

When two datasets have a common identifier, merging datasets is straightforward and deterministic linkage methods can work well. However, datasets do not always have common identifiers available and error and missing rates for surnames, first names, postcode and date of birth in registries and medical administrative databases can be between 4%–15% and between 0–9%, respectively. Deterministic methods therefore may not capture all matching records.[2]

Probabilistic record linkage allows you to link other partial identifiers, such as names, dates of birth, and addresses and considers that there might be some slight differences in these data (i.e. misspelling, changed addresses etc.). Probabilistic linkage produces a matching score (0–100%) to tell you how probable a match between records is (e.g. a perfect match (100%), some variations (75%)).

For the below example, both datasets below have unique identifiers, but they are not consistent between the datasets and there are several data entry errors. Deterministic linkage in this instance is not appropriate and would have low sensitivity. However, probabilistic matching could be used here to match records using the individual's name and postcode without being thrown off by the data entry errors.

Notification ID	Name	Sex	Postcode
5200232	Miranda Brown	F	3021
6932155	Tom Lui	M	3827
9963220	Kerry Holt	M	3049
3200265	Stacey Grant	F	3190

Lab ID	Full Name	Sex	Postcode
2034	Tom Liu	M	3827
3059	Miranda Brown	F	3021
3695	Grant Stacey	F	3000
4036	Kerry Holt	F	3049

Before deciding to undertake probabilistic linkage and preparing your dataset for matching, there are some important questions you should ask yourself. These include:

- What identifying variables do your datasets have?
- Which of these variables are most specific to an individual? *(i.e. most identifying of that individual)*

- Which of these variables are most likely to be consistent across datasets and over time? What is the likelihood that they are consistently coded? *(e.g. an individual is more likely to move postcodes than to change birth dates)*
- What is the likelihood that you will have missing data in your identifying variables?
- Do you want sensitivity or specificity?
- How important is it for you to have an exact match on all or certain variables?
- Which are the most important variables to be confident about the match for?
- Will there be any subsequent data quality checking?
- Are there confidentiality issues that restrict your access to all identifying variables?

Can you think of any other issues that you might encounter when matching records between two datasets? *Please write any other issues below.*

Using *reclink2* in Stata

reclink2 is a record linkage program that allows us to undertake probabilistic matching in Stata. It can be downloaded and installed in Stata. A benefit of this command is that allows many-to-one matching, which is useful when you have multiple records for an individual in one of your datasets (i.e. multiple tests, repeat episodes etc.). Wasi and Flaaen (2015) have an in-depth article describing linkage techniques in Stata, including *reclink2* (Reference at the end for those interested).[1]

On the next page is an example of how you can use *reclink2* to match individuals between routine notifications of a condition in one database to test result records in a laboratory data system. Brief explanations are provided. The *reclink2* help file is also very useful for understanding how to run and customise this command.

Selecting matching variables

An important first step is to review your dataset to understand which identifying variables are available for matching. In this example we have *name/firstname/surname, sex, date of birth,* and *postcode.* These are great variables to match on. Other useful matching variables could be *address*, *phone number*, or *Medicare number*.

Routine notifications dataset

	dateofbirth	name	sex	postcode	notificati~d	dateofnoti~n
1	17may2000	John Smith	Male	4011	64654687	12aug2016
2	28jan1988	Erin Holmes	Female	4501	58318956	02oct2017
3	09jul1998	Howard Hopkins	Male	4007	45682156	03may2015
4	13apr1981	Julie Tay	Male	4710	75232105	19mar2018
5	23apr1987	William Braden	Male	4187	32113541	12jun2017
6	27oct1976	Lisa Carson	Female	4077	21486745	20oct2010
7	15may1992	William Kelly	Male	4364	22155744	23oct2017
8	08jan1976	Diana Oneil	Female	4885	62000568	24jul2016
9	29oct1976	Wilson Liu	Male	4001	13228463	30jan2014
10	20nov1965	Louis Heath	Male	4750	96513722	12dec2015

Variables

Filter variables here

Name	Label
dateofbirth	Date of Birth
name	Name
sex	Sex
postcode	Postcode
notificationid	Notification ID
dateofnotific...	Date of Notificatio...

Laboratory records dataset

	dob	firstname	surname	sex	postcode	labnumber	testdate
1	30/02/1986	Mark	Wang	M	4297	1509-566	4/20/2018
2	5/17/2000	John	Smith	M	4011	1022-444	7/15/2018
3	7/9/1998	Howard	Hopkins	F	4007	4867-368	3/6/2017
4	5/13/1995	Selina	Donald	F	4058	1422-852	9/29/2018
5	4/23/1987	Brade	William	M	4187	8792-651	2/17/2018
6	6/22/1998	Lisa	Carson	F	4077	5678-896	7/15/2018
7	8/16/2000	Zhou	Lihua	M	4068	9625-584	11/10/2018
8	30/02/1980	Diana	Oneil	F	4885	6542-457	4/23/2017
9	10/29/1976	Wilson	Liu	M	4001	3148-452	8/26/2018
10	11/20/1965	Louis	Heath	M	4750	5656-863	7/30/2018
11	30/02/1980	Diana	Oneil	F	4885	6955-455	1/6/2018
12	30/02/1986	Wang	Mark	M	4297	6335-454	12/12/2018

Variables

Filter variables here

Name	Label
dob	Dob
firstname	First Name
surname	Surname
sex	Sex
postcode	Postcode
labnumber	Lab Number
testdate	Test Date

Cleaning and preparing your dataset

Differences in variables between your two datasets are common. These differences may be in the way in which variable are labelled (e.g. *SEX* vs *GENDER*) or how variables are coded (e.g. *SEX = F* vs *SEX=0*). To match records with *reclink2* your variable names must be identical in both datasets, and to improve the quality of matching, the coding/content of variables should be as consistent as possible.

In our example there are several important differences that should be addressed before we run *reclink2*. We would need to do the following:

- Re-name *dateofbirth* variable to *dob* so the variable names exactly match
- Re-code sex variables consistently (best option is to recode both to binary 0/1)
- Check date variables to ensure they are consistently formatted (recode string to %dm)
- Create a new condensed name variable (*firstname+surname*) in the Laboratory dataset to match *name* in the Notifications dataset

Hint: To create the condensed full name variable you can use *concat()*. Just be sure to include a space between the first and last name using the punctuation option.

```
egen name=concat(firstname surname), punct(" ")
```

Inserts a space between the words

Creates a new variable "name"

Condenses *surname* and *firstname* with *firstname* first

Reclink2 also requires you to remove punctuation (? , . *) from any string variable. To remove these, you can use *sieve*.

```
egen name_clean=sieve(name), keep(a s)
```

Identifies what you want to keep

a – alphabetical characters
s – spaces
n – numerical characters

Creates a new variable *"name_clean"*

Searches through the variable *name* to remove contents

Create a unique identifier

In order to track observations, *reclink2* requires that each observation in both datasets has a unique ID. You can simply create a variable in each dataset using something like the below command.

```
*Unique NOCS observation variable for reclink*
gen notif_obsid=_n // unique observation id number - required for reclink command
```

Set up reclink2

Once your datasets are clean (matching in variable formats and names) and you have unique observation identifiers, you'll need to write the *reclink2* syntax. The command should be run in a Master dataset whilst the other 'using' dataset is closed (see definition below). For our example, the routine notifications dataset will be the Master dataset and the laboratory dataset will be the "using" dataset.

Master dataset: The main dataset you are using and where you will run the command. The program will search each record in this dataset for a match.

"using" dataset: The source dataset, where the program will search for matches from the Master dataset.

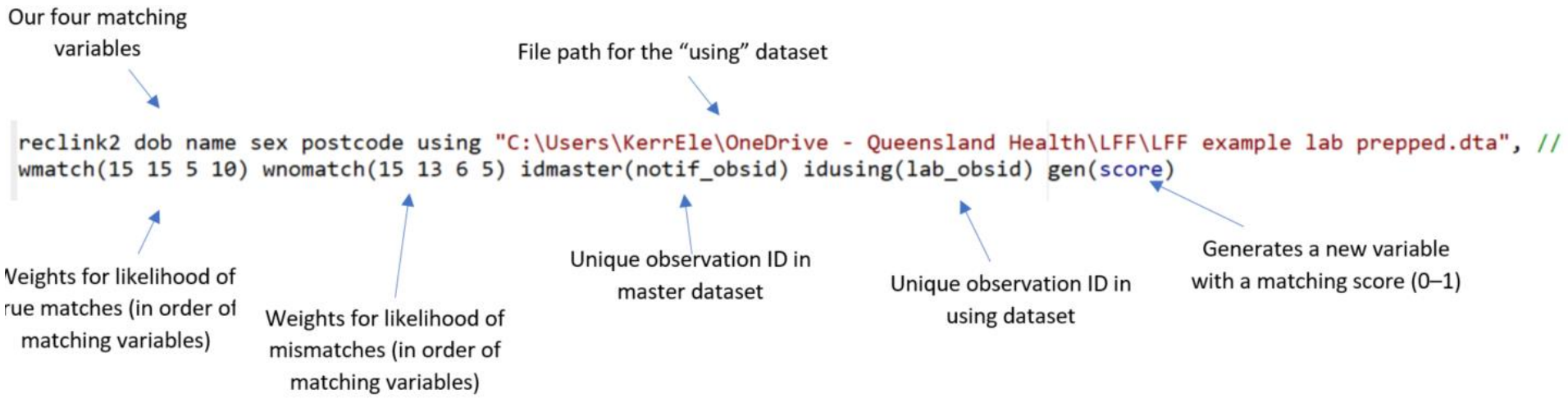
The command syntax (next page) has several required components, as well as optional components that you can add to tailor your matching. Some of recommended optional components are listed in the table below. There are also several more options listed in the *reclink2* help file.

Syntax:

reclink2 varlist ***using*** filename, ***idmaster***(varname) ***idusing***(varname) ***gen***(newvarname) [Options]

Component	Description	Status	Example
idmaster (*varname)*	The variable in your master dataset that uniquely identifies observations.	Required	idm(notif_obsid)
idusing (*varname)*	The variable in your "using" dataset that uniquely identifies observations.	Required	idu(lab_obsid)
gen (*newvarname*)	The new variable created by *reclink2* to store the matching score (0–1) for the linked observations. You can choose what this is called.	Required	gen(score)
wmatch (*match_weight_list)*	Weights given to matches for each variable in *varlist*. Each variable requires a weighting and should be listed in the same order as the first part of the syntax. The default is 1. Weights must be ≥1 and are typically between 1 and 20. The values should reflect the relative likelihood of a variable match truly matching. The higher the value, the higher the likelihood of a true match. The lower the value the more likely that there are lots of duplicates.	Optional	wm(15 10 6)
wnomatch (*nonmatch_weight_list*)	Weights given to mismatches for each variable in *varlist.* These are similar to ***wmatch()*** but instead reflect the relative likelihood that a mismatch on a variable means that the observations truly do not match. A small value indicates that mismatches are expected, even if the observations truly match. I.e. a telephone number variable will have a small value as mismatches may be common.	Optional	wno(10 10 6)
orblock (*varlist*)	Selects one or more variable where only observations that match on at least this/these variable/s in the or-block are examined. I.e. if you include *orblock(date_of_birth, sex),* only observations which match on either date of birth or sex will be included as a match. The default, if not specified, is to *orblock* all variables. If you expect all variables to be unique you can override using *orblock(none)*.	Optional	orblock(dob, sex)
required (*varlist*)	Selects one or more variable that must match exactly for the observation to be considered a match.	Optional	required(dob)
_merge (*newvarname*)	Specifies the name of the variable that will indicate the source of each observation. The default is _merge(_merge)	Optional	_merge(source)
minscore (*#*)	Specifies the lower limit of matching score (0-1) that you are willing to accept. The default is 0.6 (i.e. 60% probability of a true match). A lower value minscore will increase your number of matches but may lead to more false matches.	Optional	mins(0.8)

The syntax for our example would be:



Weights were selected weights based on the below justifications:

	dob	*name*	*sex*	*postcode*
wmatch	*15:* Relatively specific to individuals. A high likelihood of a match indicating a true match.	*15:* Relatively specific to individuals. A high likelihood of a match indicating a true match.	*5:* Likely to be many duplicates.	*10:* May be some duplicates. A match is likely to be moderately indicative of a true match.
wnomatch	*15:* Unlikely to be mismatches and very constant over time.	*13:* Relatively constant over time and a mismatch on full name is likely to indicate a true mismatch. However, there is some potential for misspelling.	*6:* It is unlikely that sex changes over time, but random coding errors commonly occur.	*5:* Mismatches on true matches are likely as people in this population often move locations.

Run command

When you run *reclink2* you will see the below text appear and provide some overall results. With larger datasets it can take some time for the program to run. Our example had a total of 7 matches, 3 of which were perfect (100% match on all variables) and 3 which did not match at all. The remaining 4 cases partially matched and will have a score between 0.8 and 0.99. Unmatched variables will still be in the new dataset but will have a score of ".".

```
. reclink2 dob name sex postcode using "C:\Users\KerrEle\OneDrive - Queensland
> Health\LFF\LFF example lab prepped.dta", wmatch(15 15 5 10) wnomatch(15 13 6
> 5) idmaster(notif_obsid) idusing(lab_obsid) gen(score)

3 perfect matches found


Added: lab_obsid= identifier from C:\Users\KerrEle\OneDrive - Queensland Health
> \LFF\LFF example lab prepped.dta   score = matching score
Observations:  Master N = 10    C:\Users\KerrEle\OneDrive - Queensland Health\L
> FF\LFF example lab prepped.dta N= 12
  Unique Master Cases: matched = 7 (exact = 3), unmatched = 3
```

Sort and check

If you browse the dataset you will see that all variables from the "using" dataset have been merged into the Master dataset. All merged variables begin with "U" and will appear after the Master variable.

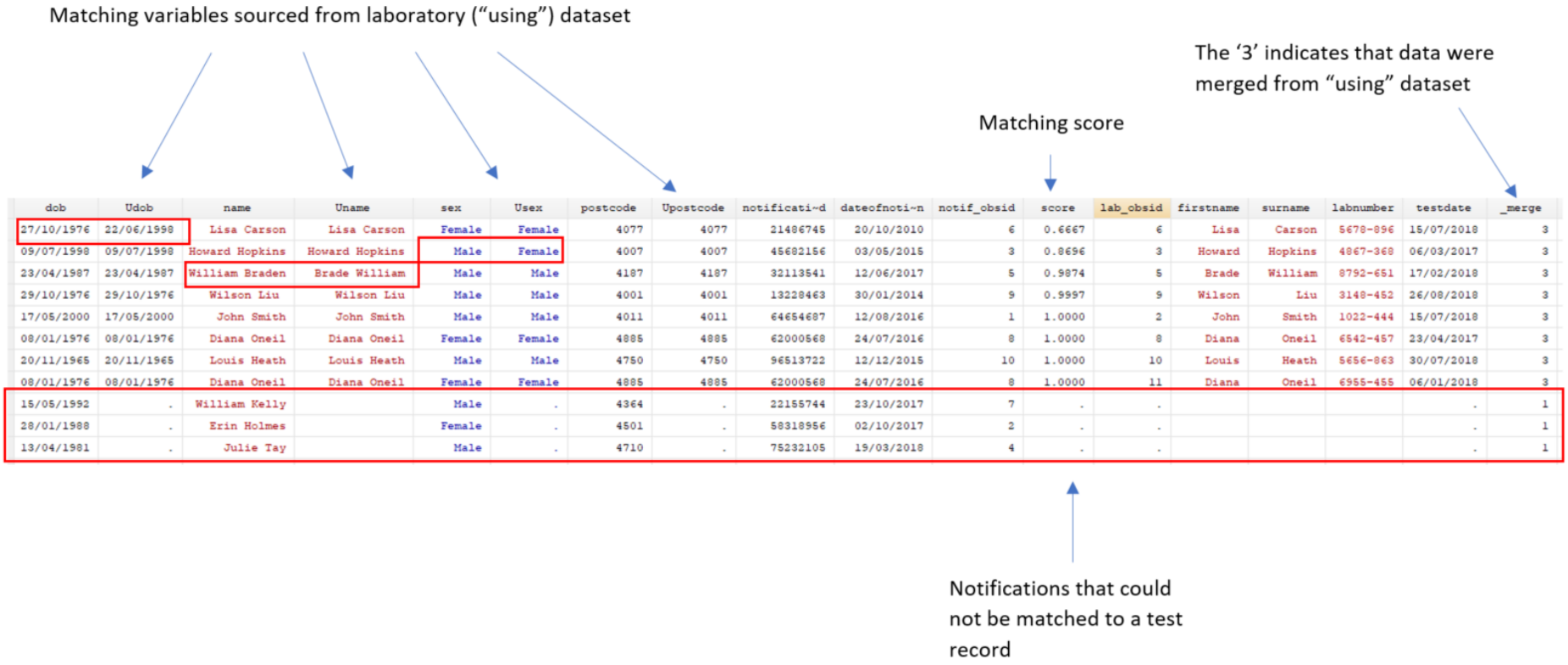
In this example we can visually inspect all matches but with larger datasets you will need to sort by the matching score and inspect to ensure that matching is appropriate. If you are not happy with how it looks, play around with the weights until it is producing matches that meet your requirements.

```
sort score
```
→ Sorts data by score variable

```
drop if score==.
```
→ Drops observations with no matches or a score below 60%

```
drop if score <0.95
```
→ Drops observation with a score below 95%

In the example we can see that there were some coding errors and spelling mistakes in the laboratory dataset. Some issues will be easy to identify (i.e. a mismatched sex), others will be more difficult to identify (i.e. Wilson Liu with a score of 0.9997). You will need to use your judgement to decide which matching errors you can tolerate, which should be investigated further, and which should be excluded.



dob	Udob	name	Uname	sex	Usex	postcode	Upostcode	notificati~d	dateofnoti~n	notif_obsid	score	lab_obsid	firstname	surname	labnumber	testdate	_merge
27/10/1976	22/06/1998	Lisa Carson	Lisa Carson	Female	Female	4077	4077	21486745	20/10/2010	6	0.6667	6	Lisa	Carson	5678-896	15/07/2018	3
09/07/1998	09/07/1998	Howard Hopkins	Howard Hopkins	Male	Female	4007	4007	45682156	03/05/2015	3	0.8696	3	Howard	Hopkins	4867-368	06/03/2017	3
23/04/1987	23/04/1987	William Braden	Brade William	Male	Male	4187	4187	32113541	12/06/2017	5	0.9874	5	Brade	William	8792-651	17/02/2018	3
29/10/1976	29/10/1976	Wilson Liu	Wilson Liu	Male	Male	4001	4001	13228463	30/01/2014	9	0.9997	9	Wilson	Liu	3148-452	26/08/2018	3
17/05/2000	17/05/2000	John Smith	John Smith	Male	Male	4011	4011	64654687	12/08/2016	1	1.0000	2	John	Smith	1022-444	15/07/2018	3
08/01/1976	08/01/1976	Diana Oneil	Diana Oneil	Female	Female	4885	4885	62000568	24/07/2016	8	1.0000	8	Diana	Oneil	6542-457	23/04/2017	3
20/11/1965	20/11/1965	Louis Heath	Louis Heath	Male	Male	4750	4750	96513722	12/12/2015	10	1.0000	10	Louis	Heath	5656-863	30/07/2018	3
08/01/1976	08/01/1976	Diana Oneil	Diana Oneil	Female	Female	4885	4885	62000568	24/07/2016	8	1.0000	11	Diana	Oneil	6955-455	06/01/2018	3
15/05/1992	.	William Kelly		Male	.	4364	.	22155744	23/10/2017	7	.	.				.	1
28/01/1988	.	Erin Holmes		Female	.	4501	.	58318956	02/10/2017	2	.	.				.	1
13/04/1981	.	Julie Tay		Male	.	4710	.	75232105	19/03/2018	4	.	.				.	1

Exercise:

As part of a study on *Ross River Virus*, a survey was undertaken with a group of people notified as confirmed cases to the Department of Health 2016–2018. Research Assistants are maintaining a database with participant survey data with each participant assigned a Study ID. However, they would like to include some information about their notification. Unfortunately, at the beginning of the study they forgot to enter each participant's notification ID into their study database. Rather than manually looking up each participant and enter the data, they decided to undertake probabilistic matching using the demographic information that they have.

The two datasets (study database and notification extract) are attached to this LFF.

Q1: How many observations are in each data set?

Q2: Which variables can be used for matching? How specific is each variable to individuals? Should we use all the variables?

Q3: What changes need to be made to the datasets before matching? (i.e. re-naming variables, new variables etc.)

Q4: If you use the below variables and set the matching weights as per the table, how many exact matches does *reclink2* report? How many matches have scores above 90%?

	dob	***name***	***sex***	***postcode***
wmatch	*15*	*15*	*5*	*10*
wnomatch	*15*	*13*	*6*	*5*

Q5: Are there any matches that you are concerned about? (i.e. incorrectly matched records).

Q6: Are there any individuals in the study database, that were not located in the notifications dataset?

Q7: If you change the weights (wmatch and wnomatch) for *postcode* to 3, how many matches above 90% do you have? In what way did the results change?

References and further reading (only if you are interested!)

1. Wasi N, Flaaen A. Record linkage using Stata: Preprocessing, linking, and reviewing utilities. The Stata Journal. 2015;15(3):672-97.

2. Zhu Y, Matsuyama Y, Ohashi Y, Setoguchi S. When to conduct probabilistic linkage vs. deterministic linkage? A simulation study. Journal of Biomedical Informatics. 2015;56:80-6. doi: https://doi.org/10.1016/j.jbi.2015.05.012.

Chapter 7:

Summary of Cambodia experience and other public health activities and experiences

Background

I had a range of opportunities to undertake additional outbreak and public health activities whilst doing the MAE. Whilst placed at Queensland Health in 2019, I took part in trachoma screening activities in remote northern Queensland, and supported measles outbreak activities in the Metro South region. In Cambodia, I was involved in a Myanmar-based leptospirosis project and provided support for malaria projects, including development of a factsheet and consent for intermittent presumptive treatment of malaria for a forest malaria intervention (Appendix 1). I was also deployed to support the Cambodian Ministry of Health COVID-19 response. Due to the COVID-19 pandemic, I was unable to continue my placement in Cambodia and returned to Melbourne in late March 2020. In July 2020, I was seconded to the Victorian Department of Health and Human Services (DHHS) COVID-19 response.

Activities

Trachoma screening and research in remote northern Queensland

In Australia, the bacterial eye condition trachoma primarily occurs in remote and very remote Aboriginal communities in the Northern Territory, South Australia, and Western Australia, and cases have been found in Queensland. Screening of trachoma is undertaken in at-risk communities and is coordinated in Queensland by the Communicable Diseases Branch (CDB). In October 2019, screening activities were undertaken in Doomadgee, Northern Queensland; public health screening of children aged 1–14 years and sero-surveillance research project to measure historical trachoma infection among people living in the community.

My role

Over the week of screening activities, I worked closely with the public health nurses, ophthalmologist, and research team. During screenings, I provided logistical support by organising and completing sample documentation, as well as providing epidemiological assistance to the research team.

Lessons learned

I was able to gain a better understanding of mass screening activities and the challenges associated both with screening, and undertaking such a public health exercise in a remote Aboriginal community.

Figure 1: Screening and research team, Doomadgee, Queensland

Measles outbreak activities in Southern Brisbane

In early October 2019 an outbreak of measles was detected in Brisbane's Metro South region. The outbreak was primarily related to travellers coming to Queensland with infections associated with epidemics in New Zealand and the Pacific region.

My role

I took part in two activities in support of this measles outbreak. Firstly, in November 2019 I was asked by Jonathan Malo (MAE 2017–2018 cohort) to assist Metro South public health unit (PHU) to identify school children with no documented history of measles containing vaccine (MCV) or history of only 1 dose of MCV who were potentially exposed to a measles case. I completed this task by linking school lists to immunisation records within the school catchment area. Following this activity, I was asked by the CDB Immunisation Branch to conduct a rapid evaluation of a measles outbreak intervention implemented in Logan in November 2019 (Appendix 2).

Lessons learned

I gained experience in measles outbreak response activities and an appreciation for their rapid pace. I also learned a lot about the Australian Immunisation Register. Both projects involved record linkage and allowed me further expand these skills and appreciate its various usages. In instances where there are population lists, record linkage can be a highly effective method to reduce time and effort spent manually identifying individuals.

Determinants of human leptospirosis acquisition in Yangon, Myanmar

The ECOMORE 2 project in Myanmar is a multi-centre hospital-based case-control study to determine the risk factors for human leptospirosis infection in Myanmar's Yangon region. The project is a collaboration between the Institut Pasteur (Cambodia, New Caledonia, and ECOMORE regional team) and the National Health Laboratory in Yangon, Myanmar. This study is based at ten hospitals across the Yangon region and is the first involving laboratory-confirmed human leptospirosis in Myanmar.

My role

This project was intended to be my data analysis project. I completed the following activities:

- Established a comprehensive data analysis plan, including matching, descriptive analysis, and multivariate logistic regression using a theoretical framework for determinants of infection (Appendix 3).
- Undertook a literature review on human leptospirosis risk factors and leptospirosis analysis methods.
- Wrote and piloted Stata code for matching and analysis based on available study data and case report forms.
- Drafted an ethics protocol for submission to the Australian National University Human Research Ethics Committee.

We had planned that I would to spend time in Yangon working closely with the Yangon-based Data Manager to build her epidemiology and analytic skills. However, given that this was no longer possible, I provided remote support and mentorship.

Lessons learned

I gained a real appreciation for leptospirosis as a disease often associated with social inequalities and the complexities of diagnosing and treating the condition in resource-limited settings. This would have been my first solo case-control analysis and preparing the analysis plan and drafting the code was a learning process, especially given that it involved multiple matching criteria and a hierarchical framework for multivariate analysis of determinants of infection. I also experienced the complexities of discussions around publication and authorship on projects with a range of stakeholders.

Support the Cambodian Ministry of Health COVID-19 response

Although Cambodia was among the first countries outside of China to report confirmed cases of COVID-19 (late January 2020), case numbers were low until March 2020. During this period in early 20202, most cases were associated with overseas travel with two large clusters driving case numbers; Cambodian and Malaysian nationals with infections associated with a mass event in Malaysia, and a French tour group. Some mass screening of cruise passengers was undertaken during February. However, testing was limited to symptomatic individuals with overseas exposures or contacts of confirmed cases.

The Ministry of Health's Communicable Diseases Control Department (Cambodian CDC) with support from the US Centers for Disease Control and Prevention (US-CDC) was responsible for coordinating case investigation, contact tracing, and surveillance reporting. The Institut Pasteur Cambodia (IPC) was the nominated laboratory for SARS-CoV-2 testing. In response to increasing case numbers, individuals from partner organisations joined the Cambodian CDC in February/March 2020. Partners included the World Health Organization (WHO), Australian Department of Foreign Affairs and Trade (DFAT), IPC, and Médecins Sans Frontières.

My role

I was deployed twice to the Cambodian CDC in 2020. In February, I assisted in a cruise ship screening activity (Appendix 4) and in March I joined the contact tracing and surveillance team. In this second role, I was responsible for the following:

- Establishing basic outbreak data management systems (line listings and daily surveillance reports), and supporting the development of an online database.
- Maintaining soft and hard copy line listings, including requesting and collating information from the laboratory and contact tracing teams.
- Completed the daily situation report analysis and ad-hoc analyses.

I was also involved in the team operational management including establishing team structures and administrative process and maintaining organisational charts.

Lessons learned

Both deployments were rich learning experiences. The specific learnings related to cruise ship screenings are detailed in Appendix 3. My second deployment was short (10 days) and both professionally and personally chaotic due to my swift repatriation, yet it was also one the most rewarding experiences of the MAE for me. My key learnings include:

- Establishing data management systems with few resources and no prior planning is an incredible challenge, particularly in a low-resource environment. White boards salvaged from around the office and an excel document were perfectly acceptable starting points. However, as case numbers rapidly increased, so to do the limitations and risks of this approach. We also ran out of white boards! Pandemic preparedness in relation to information systems is essential for countries with limited resources.
- Always carry highlighters when on deployment. They are almost always incredibly useful and highly prized.
- Establishing person, place, and time is near impossible when data systems are non-functional and poses real constraints to identifying clusters and following up contacts.
- Although almost everyone wants to do their part, clear delegation and communication of tasks is essential and can mitigate against duplication of efforts, e.g. multiple people working on developing databases in silos.
- The comradery built through shared determination makes it possible to work extraordinarily long hours. However, inevitably you must learn to take breaks in order to avoid falling asleep at your desk.

Figure 2: One of many white boards alongside team members from IPC, Cambodian CDC, and US-CDC

COVID-19 outbreak support to Victorian DHHS

In response to increasing case numbers in Victoria in July 2020, the Victorian DHHS recruited additional surge epidemiology capacity, particularly in outbreak epidemiology support.

My role

For 12 weeks in the second half of 2020, I worked part-time (2–4 days/week) within the Public Health Intelligence team as a field epidemiologist. In this role, I was embedded in the operations teams responsible for case management and contact tracing. I assisted in the coordination of data collection, regularly reviewed outbreak data quality, undertook routine and detailed analyses of outbreaks, and created and reviewed written briefings and outbreak reports.

Lessons learned

This was an excellent counterpoint to my experiences in Cambodia. Being a part of the Victorian response allowed me to see the scale and complexity of organisational structures and data systems that are possible when resources are not so constrained. It also highlighted some universal pandemic experiences. Namely the importance of establishing person, place, and time to link clusters and outbreaks, the challenges of contact tracing and meeting data request demands, as well as the more personal but important issue of burnout and fatigue. The scale of case numbers and new outbreak sites being reported each day in Victoria during July–August 2020 was a valuable lesson on the impacts of scale on data quality, accessibility, and timeliness, as well as the backlogs of missed or low quality data in the wake of high workloads.

Appendix 1: Fact sheet and consent form developed for intermittent presumptive treatment of malaria

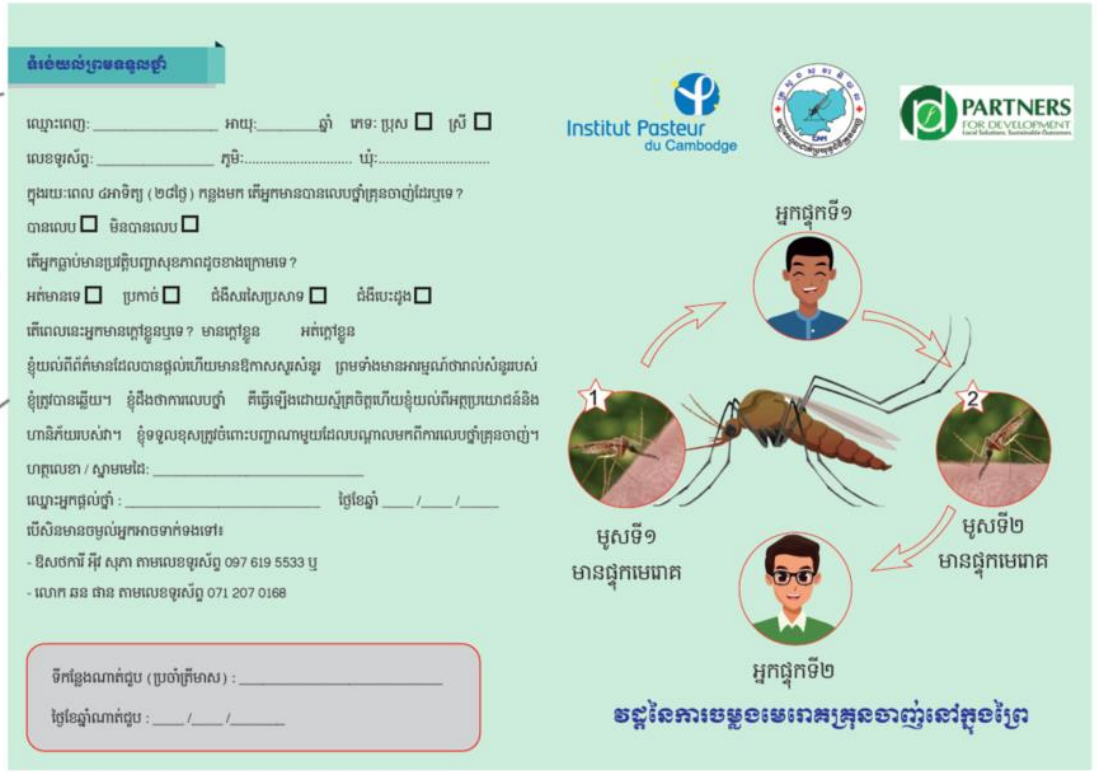
The below leaflet was developed for IPC for use within two donor funded malaria control projects focused on forest malaria workers. The leaflet contains a factsheet about malaria transmission and intermittent presumptive treatment, including details of the treatment program and the benefits and risks of taking treatment. For ease of use by forest malaria workers, a consent form was incorporated. I worked with the project team to design the English version for approval by project donors. Once approved the leaflet was translated into Khmer, with some modifications to content, and was professionally re-designed.

Consent for malaria treatment

Collecting contact and demographic details e.g. name, sex, phone number, village of residence etc.

Medical history and recent medications (e.g. malarial treatment within past 28 days, current fever, history of relevant medical conditions (seizers, heart disorders etc.)

Original text: I understand the information given to me. I had the chance to ask questions and feel that all my questions were answered. I know that I can choose not to have treatment if I like and I understand things that are good about taking treatment, and I know that sometimes there are some problems if you take malaria treatment. If I have any problems with my health after I take the malaria treatment given to me, I will go to see a doctor as soon as I can.



Preventing the spread of malaria

Original text: Malaria is caused by parasites spread to people through bites of infected mosquitos.

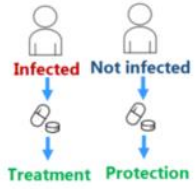
In Cambodia, most malaria is in the forests and people in or near the forest are likely to get malaria.

If infected you can have fever, headache and chills, you might also get very sick. If you are infected and bitten by mosquitos, those mosquitos might bite other people nearby and spread malaria.

How would I be treated?

Original text: We will give you tablets to take over three days. The first tablet is taken on day one with the Forest Malaria Worker, you can take the other tablets home. The second tablet is for day two and the last tablet is for day three.

It is important for you to take three doses. If you share them, you will not be protected. If you know someone who would like treatment, ask them to find a Forest Malaria Worker.



How can we prevent the spread of malaria?

Original text: One way to stop people from getting sick, or getting Malaria is to give everyone in the forest treatment, even if they're not sick. Each month we give people in the forest free treatment (a medicine called artesunate-mefloquine), even if we don't know they are infected.

In the forest your chances of infection are high (one out of three), so monthly treatment will protect you from illness and infection. Treatment is free and because your risk is high we don't need to test. If you are having symptoms, we can help diagnose and provide treatment that suits you.

What are the side effects of malaria treatment?

Original text: The medicine (artesunate-mefloquine) is safe and unlikely to cause any problems after taking it.

For a short time, you may feel dizzy, nauseous, or unease, you may vomit or have trouble sleeping. Few people have these problems, and they are not likely to be serious and will only last a short time.

If you would like more information, please talk to a Forest Malaria Worker.

What are the side effects of treatment?

Original text: Almost anyone in the forest can have free treatment each month. There are two reasons you may not be given treatment: If you have already had malaria treatment (from anyone) in the last 30 days or if you have had seizures, psychiatric or heart disorders in the past. If you are not sure if this applies to you, please talk to a Forest Malaria Worker.

Appendix 2: Logan measles outbreak contact intervention: evaluation report (31/10/2019–30/11/2019)

Summary of intervention

The Australian Immunisation Register (AIR) records of 14,205 individuals aged between 5 and 23 years within the Logan catchment area indicated either no documented history of measles containing vaccine (MCV) or history of only 1 dose of MCV. Due to existing follow up as part of the state-wide '*Immunise to 95'* initiative, children under the age of 5 years were not selected to take part in the intervention and the completeness of AIR records for individuals born prior to the establishment of AIR (over the age of 23 years) was considered to be too poor to provide sufficient evidence of an individual's MCV history.

A master list of individual AIR records for those who met the intervention inclusion criteria was constructed with individuals grouped by priority postcode areas and availability of contact details (Table *1*). Letters, emails and/or SMSs were sent to individuals within priority 1 and 2 postcodes from the 31st October 2019. Contact (letter, emails and/or SMSs) commenced with individuals within priority 3 postcodes from the 4th November 2019.

Table 1: Catchment postcodes by priority level

Priority Level	Postcode
1 (N=3)	4114, 4131, 4132
2 (N=9)	4078, 4110, 4115, 4118, 4124, 4125, 4133, 4205, 4207
3 (N=11)	4117, 4119, 4123, 4127, 4128, 4129, 4130, 4270, 4280, 4285, 4343

Contact was attempted based on the availability of contact details with preference for SMS and/or email. Individuals who were recorded as Return to Sender (RTS) on AIR were contacted if a mobile number and/or email address was available. However, where only a street address was available or the SMS and/or email was rejected, no contact was made. Where SMS and/or emails were rejected, and the individual was not recorded as RTS on AIR and had an address available, a letter was sent. Rejected SMS, emails, and letters returned to the Health Contact Centre (HCC) were recorded. Contact methods were evaluated using the below definitions (Table *2*).

Methods

A master list was established comprising of individuals aged 5 to 23 years located within the catchment postcodes who had either no or only one MCV dose recorded in AIR. Separate databases were then created by the HCC based on priority postcode areas and proposed method of contact. Contact was categorised and analysed using the below contact definitions. The master list and contact databases were linked for the evaluations using a Unique ID generated by the HCC.

Queensland AIR records for individuals aged 5 to 23 years receiving vaccinations at two time points following the beginning of contact were extracted; two-weeks post intervention (15/11/2019), one-month post intervention (30/11/2019). To identify individuals contacted as part of the intervention, AIR records were linked to the master list of individuals and their contact type by full name, date of birth, and sex. Records for vaccinations which were not measles containing were excluded. MCVs included Generic Measles/Mumps/Rubella/MMR, MMR II, Priorix, Priorix-Tetra, and ProQuad.

Individuals identified within AIR as receiving an MCV were analysed by service date (date of vaccination) and the following variables:

- Age group (5–10, 11–18, 19–23 years)
- Sex (male, female, unknown)
- Priority postcode area (1–3)
- Dose number received (1, 2, other)
- Provider Type (aboriginal health service/worker, community health centre, council, general practice, hospital (public & private), state health department, community nurse, flying doctor service, midwife, nurse practitioner, pharmacy, public health unit).
- Contact type (Table *2*)

Table 2: Definition of Contact Types

Contact Type	Definition
SMS and Email	Mobile phone number and email address available and both contacts were sent without record of rejection.
SMS	Mobile phone number was available, and SMS sent. No record of message being rejected from the Telstra instant messaging service
Email	Email address available and email sent. No record of rejection.
Letter	Only address available and letter sent. No record of letter returning to Health Contact Centre (HCC).
Letter after failed SMS and/or Email	SMS and/or email was rejected and address available. A letter was sent and no record of letter returning to HCC.
Incomplete	Personal details insufficient to achieve contact.
Uncontactable	A letter, SMS, and/or email was attempted but unable to be delivered and contact details were insufficient to achieve alternative contact.
No contact after failed SMS and/or Email	SMS and/or email attempted but unable to be delivered, and unable to attempt letter contact due to being recorded as Return to Sender on AIR.
None	Recorded as Return to Sender on AIR and no SMS or email address available.

Analysis of non-intervention areas

To compare the cumulative prevalence of newly administered MCV among individual contacted as part of the intervention to the cumulative prevalence in areas without the intervention, cumulative prevalence was calculated at a state level (excluding the individuals within the master list) and for control postcodes nearby to the catchment area.

Individuals in Queensland aged between 5 and 23 years who are recorded as obtaining an MCV between 31/10/2019–30/11/2019 were analysed. Six control postcodes were selected for proximity to the Logan catchment postcodes and population estimates for individuals aged 5–23 years each postcode was sourced through the Australian Bureau of Statistics (ABS) census of Population and Housing, Community profiles, 2016[1]. The state-wide population for individuals aged 5 to 23 years was estimated using ABS Estimated Resident Population by Single Year Of Age, Queensland (June 2018) and the total number of individuals within the master list was subtracted from the denominator (n=14,205) and any individual identified with a postcode within the Logan catchment but not contacted as part of the intervention was excluded from the numerator.

[1] Postcodes: 4109, 4112, 4113, 4208, 4209, 4310

Contacts sent

As at the 15th of November contact was attempted for 13,483 individuals (email, SMS, and/or letter) across the three priority postcode areas (Figure 1). No contact was attempted for the 653 individuals recorded as RTS on AIR without a mobile number or email address or individuals with incomplete contact details (n=68). One record was identified as a duplicate with a mismatched surname, the individual was contacted using the available information. The below flow diagram depicts individuals within the intervention as at 15 November 2019.

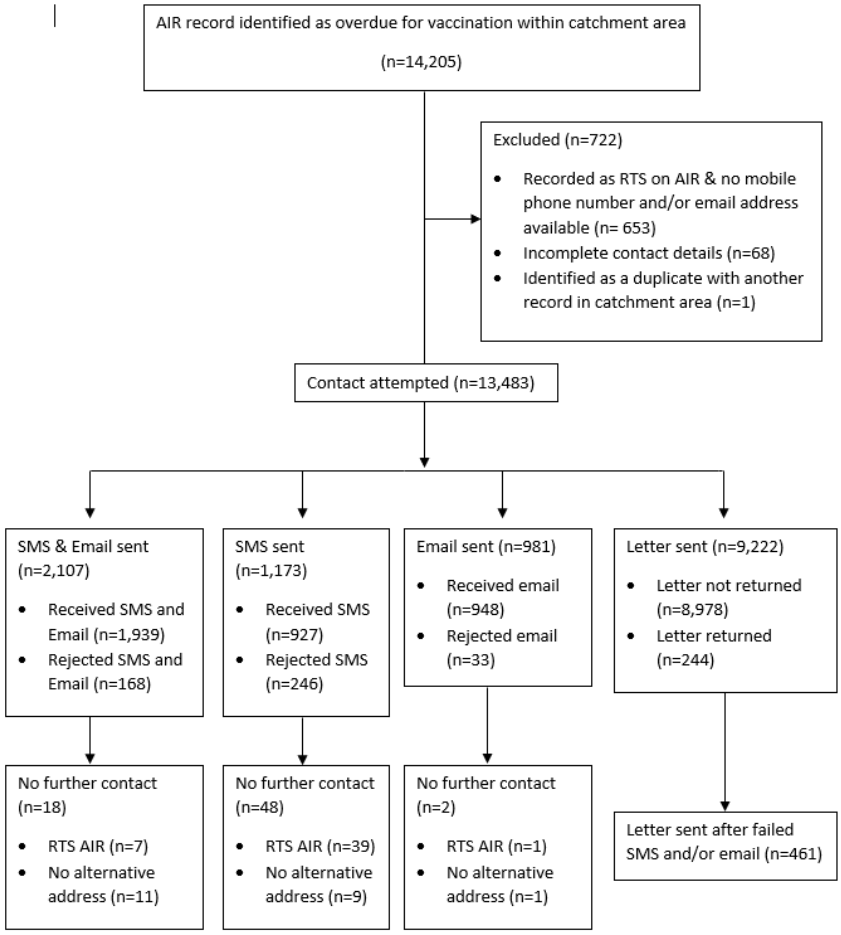


Figure 1: Contact flow diagram for Logan measles intervention group, 31/10/2019–15/11/2019*

* Data are accurate as at the 15/11/2019 and do not reflect any returned/rejected contact or additional contact made after this date.

Vaccination post-contact intervention

Within one month following the commencement of the intervention, 263 individuals contacted as part of the intervention were identified as receiving an MCV. One individual was recorded as receiving both their first and second dose of MCV within the one-month evaluation period. The estimated cumulative prevalence of newly administered MCV within the intervention population was 1.85% (263/14,205). Vaccinated individuals include 142 males (54%) and 121 females (48%) (Table *3*). The age of vaccinated individuals ranged from 6 to 22 years (median 16 years). Vaccinated individuals were predominately within the 11–18-year age group (134/263, 51%) and priority 1–2 postcodes (196/263, 75%). The peak dates of vaccination were Thursday 7th (n=20) and 14th (n=21) November (Figure 2) and 42% of vaccinations occurred in the 3rd week post-intervention (111/263, 14th– 21st November).

Table 3: Summary of individuals within Logan intervention group receiving measles containing vaccine post-intervention, 31/10/2019-30/11/2019

Characteristic	Number Vaccinated (%)		
	31/10–15/11/19 (n=131)	16/11–30/11/19 (n=132)	Total (n=263)
Age group (years)			
5–10	20 (15%)	17 (13%)	37 (14%)
11–18	61 (47%)	73 (55%)	134 (51%)
19–23	50 (38%)	42 (32%)	92 (35%)
Sex			
Female	59 (45%)	62 (47%)	121 (46%)
Male	72 (55%)	70 (53%)	142 (54%)
Priority postcode area			
1	49 (37%)	35 (27%)	84 (32%)
2	50 (38%)	62 (47%)	112 (43%)
3	32 (24%)	35 (27%)	67 (25%)
Dose number received			
1	86 (66%)	85 (64%)	172 (65%)
2	35 (27%)	37 (28%)	71 (27%)
Other	10 (08%)	10 (08%)	20 (08%)
Provider type			
Council	57 (44%)	35 (27%)	92 (35%)
GP	73 (56%)	97 (73%)	170 (65%)
State Health Dept.	01 (01%)	-	01 (00%)
Contact type			
Letter	102 (78%)	110 (83%)	212 (81%)
SMS & email	15 (11%)	09 (07%)	24 (09%)
SMS	07 (05%)	06 (05%)	13 (05%)
Email	05 (04%)	02 (02%)	07 (03%)
Letter after failed SMS &/or email	02 (02%)	04 (03%)	06 (02%)
None	-	01(01%)	01 (01%)

One-hundred and seventy-two individuals (65%) are reported as receiving their first dose and 71 received their second dose (27%). GPs were the largest MCV provider (170/263, 65%), followed by the Council (92/263, 35%). Among those vaccinated by the council, 41% (38/92) of vaccinations occurred over three days (7th, 14th, & 16th November).

Vaccinated individuals were predominately contacted via letter (212/263, 81%) or both email and SMS (24/263, 9%). Within the first seven days of the evaluation period, vaccinated individuals were more likely to be contacted via SMS and/or email (17/263, 65%), yet in the subsequent weeks individuals were more likely to be contacted via letter (Figure 2). Regardless of contact method, individuals contacted by one electronic method only (SMS or email) were less likely to receive an MCV as compared to individuals contacted with both methods (SMS and email). When individuals vaccinated by council clinics are excluded, letters remain the predominant contact method, although at a slightly lower level (127/171, 74%) and the likelihood of vaccination after contact via email and SMS increases slightly (20/171, 12%).

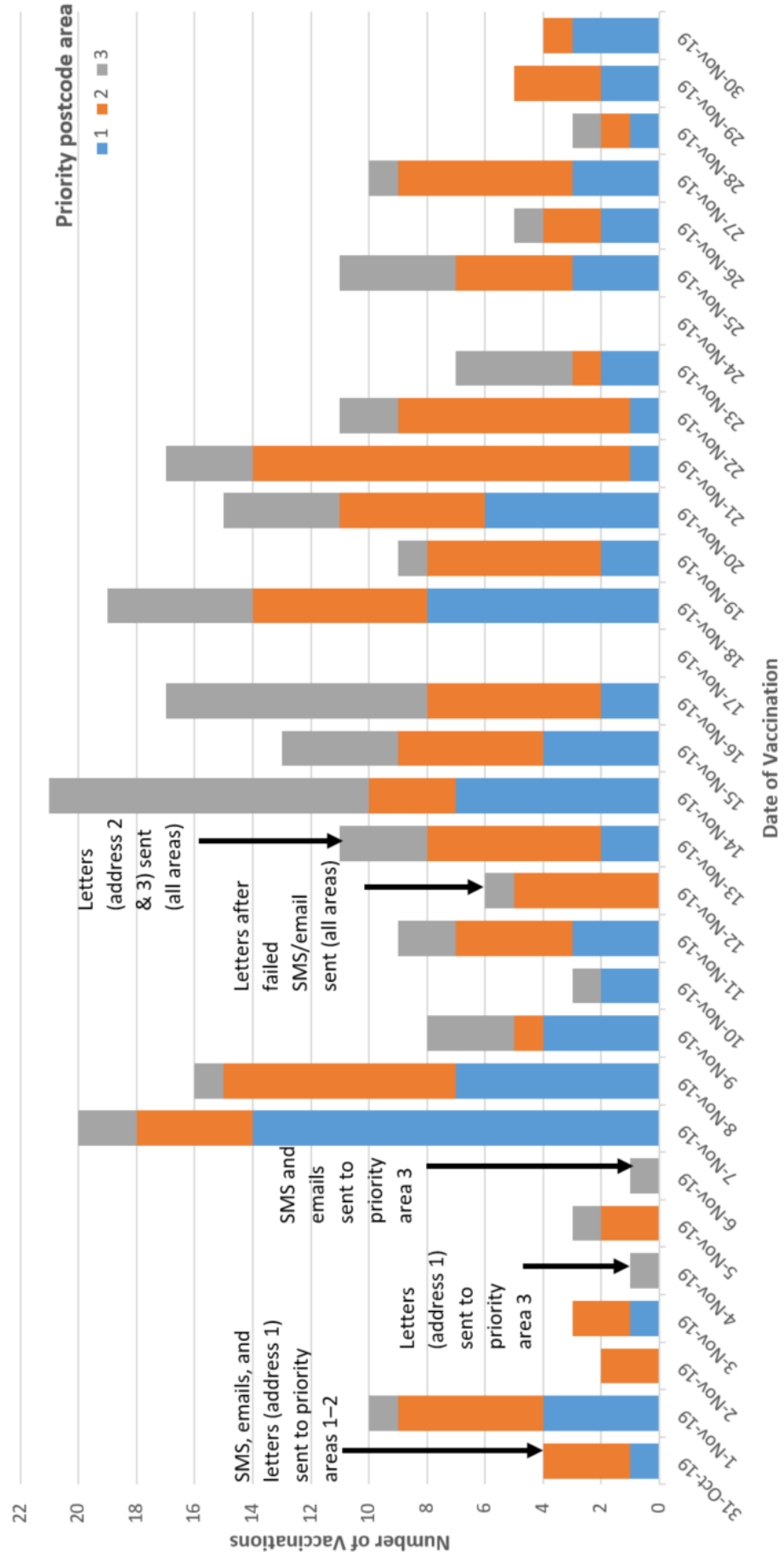


Figure 2: Measles containing vaccinations among Logan intervention group by priority postcode area and date of vaccination, 31/10/2019–30/11/2019

Four individuals received an MCV prior to receiving contact; one individual within priority postcode area 3 was vaccinated before any contacts were sent to this group, two individuals within priority postcode area 3 were contacted via SMS were vaccinated before SMSs were sent, and one individual contacted by letter after a failed email/SMS received an MCV prior to the letter being sent. One individual excluded from contact due to be recorded as RTS on AIR received a second dose of MCV during the evaluation period (Figure 3).

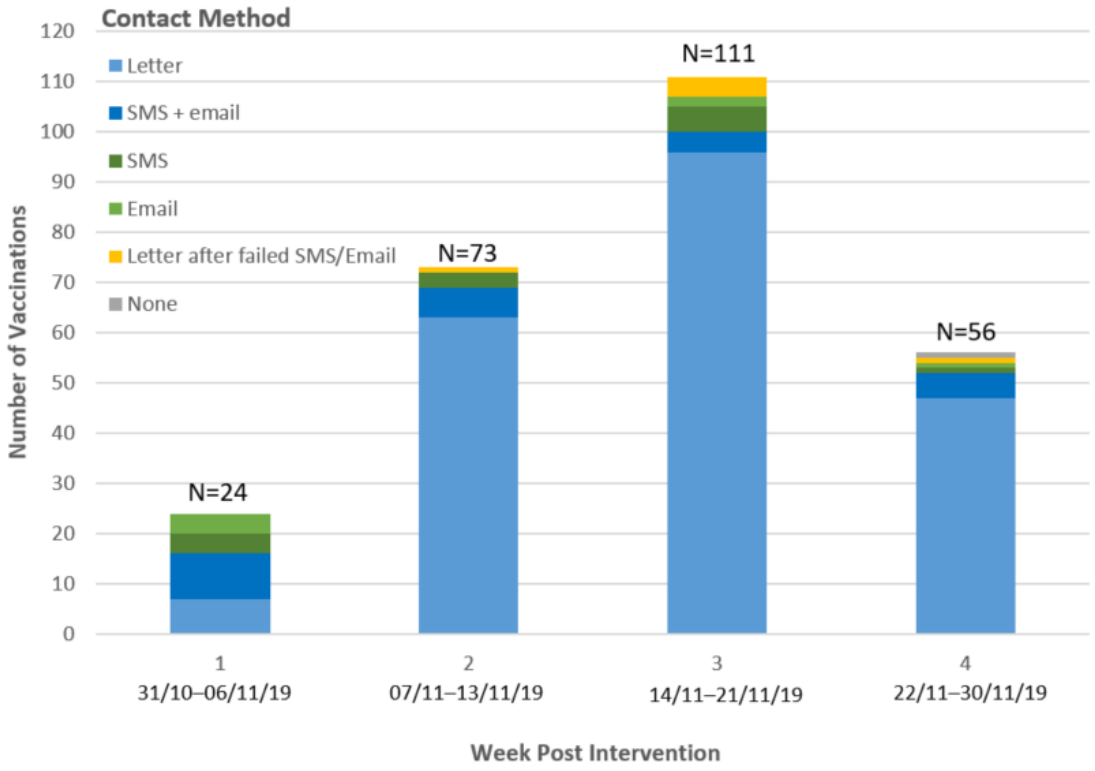


Figure 3: Measles containing vaccinations among Logan intervention group by contact type and week of vaccination, 31/10/2019–30/11/2019

Comparison to non-intervention areas

The estimated cumulative prevalence of newly administered MCV among people aged 5 to 23 years in control postcodes and at a state-level (excluding intervention group) for the 31/10/2019–30/11/15 was 0.12% (50/41,079) and 0.15% (953/622,168) respectively.

As compared to the non-intervention areas, individuals within the intervention group were more likely to be recorded as receiving their first dose in the post-intervention period and be provided the vaccine by the Council (Table *4*). An additional 82 individuals were identified as receiving a measles containing vaccine within the Logan catchment postcodes who were not part of the intervention, they were excluded from analysis. These individuals were recorded as receiving their ≥2nd dose.

Table 4: Comparison of demographic characteristics and vaccine provision for individuals recorded as receiving measles containing vaccines by group, 31/10/2019-30/11/2019

Characteristic	Logan Intervention (N=263)		Control postcodes (N=50)		State (ex. Intervention) (N=953)	
	n	%	n	%	n	%
Age group (years)						
5–10	37	14%	13	26%	180	19%
11–18	134	51%	21	42%	422	44%
19–23	92	35%	16	32%	351	37%
Sex						
Female	121	46%	27	54%	526	55%
Male	142	54%	23	46%	427	45%
Dose number received						
1	172	65%	17	34%	282	30%
2	71	27%	14	28%	327	34%
Other	20	8%	19	38%	344	36%
Provider						
Aboriginal health service	-	-	-	-	01	00%
Community health centre	-	-	-	-	76	08%
Council	92	35%	-	-	06	01%
GP	04	02%	01	02%	10	01%
Medicare GP	166	63%	46	92%	800	84%
Nurse practitioner	-	-	-	-	01	00%
Pharmacy	-	-	-	-	01	00%
Public hospital	-	-	03	06%	42	04%
State health dept.	01	01%	-	-	16	02%

Appendix 3: Presentation of proposed analytical methods for ECOMORE 2 in Myanmar for Cambodian-based study investigators

Case-Control Analysis

Proposed Analytical Methods for ECOMORE 2 in Myanmar

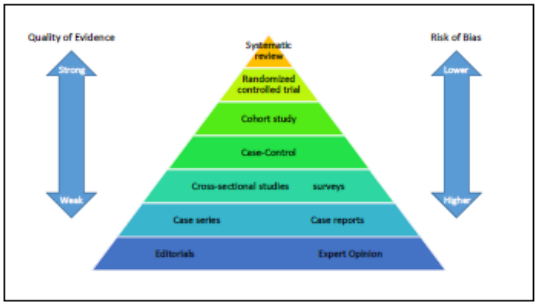
Elenor Kerr
Master of Applied Epidemiology Scholar/ASEAN-Health Security Fellow
Australian National University, Institut Pasteur du Cambodge

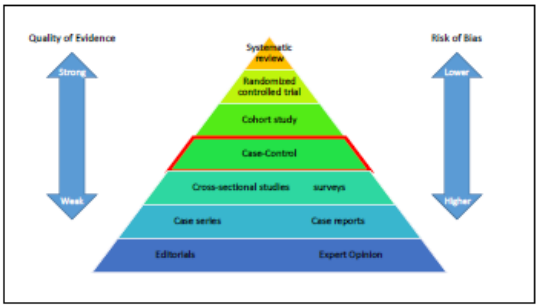
Who am I?

- Master of Applied Epidemiology Scholar/ ASEAN-Australian Health Security Fellow
- Program through Australian National University with field placements at:
  - Queensland Communicable Disease Branch (2019)
  - Institut Pasteur du Cambodge (2020)
- Focus on outbreak investigation, surveillance systems, epidemiological studies

Overview

- Case control – strengths and weaknesses
- Matching of cases and controls
- Descriptive analysis (proportions and differences)
- Conditional Logistic Regression (Odds Ratios)
- Timeline





Study Fundamentals

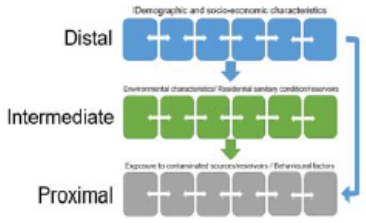
Study Population: All clinically suspected patients recruited from May 2019 to August 2020

Outcome: Laboratory-confirmed leptospirosis

Exposures: Risk factors in the month prior to symptom onset

Risk Factors

- Demographic and socio-economic characteristics — Distal
- Environmental characteristics, residential sanitary conditions, and reservoirs — Intermediate
- Exposure to contaminated sources/reservoirs and behavioral factors — Proximal



Matching

Ratio: 1:2

	Well established risk factors:
Age (± 5 years)	Age
Hospital of recruitment	Geographical location
Week of consultation (± 2)	Climatic period (dry/wet season)

Why match?
- Helps to control for confounding
- Improves precision (roughly equal numbers in each stratum)

Matching

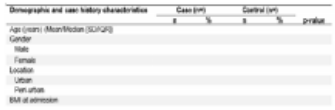
Considerations:

- Analysis must take matching into account → Conditional regression
- Do we have enough controls for each case? → Adjust matching

e.g 80 cases requires at least 160 controls

Descriptive Analysis

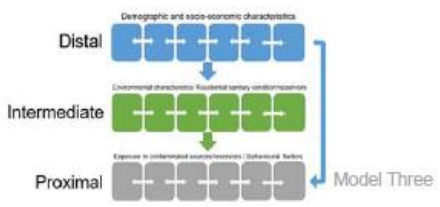
- Calculate %, median/means by covariates
- Compare variation using chi-squared/ t-test/Mann-Whitney test, as appropriate



Logistic Regression

1. Estimate crude Odd Ratio for leptospirosis acquisition (univariate logistic regression)
2. Fit three conditional multivariate logistic regression models using backwards elimination method to estimate adjusted Odds Ratio

Conditional Multivariate Models



Appendix 4: Mission report for Westerdam SARS-CoV-2 screening

Written by: Srean Chhim, ASEAN Health Security Fellow (IPC/ANU), Elenor Kerr, ASEAN-Australia Health Security Fellow (IPC/ANU)

Dates: 16–17/02/2020

Location: Sokha Hotel, Phnom Penh

Purpose: To support the Cambodia Ministry of Health's Communicable Disease Control Department (Cambodia CDC) in screening passengers from the Westerdam cruise ship for COVID-19

Background: On Thursday 13 February an American cruise ship, Westerdam docked in Sihanoukville, Cambodia after being denied entry by several Asian ports (Thailand, Japan, Taiwan, the Philippines, and Guam). The ship departed Hong Kong on the 01 February for a 14-day cruise. Prior to docking, ship doctors reportedly conducted health checks on the 1,455 passengers and 802 crew. Samples from 20 individuals who were thought to be symptomatic were sent to IPC for testing, all of which were negative for COVID-19. From Friday 14 February passengers were allowed to disembark and find flights to depart Cambodia. Those with flights booked were able to disembark, whilst those without bookings were allowed to remain on the ship.

On Sunday 16 February, an 83-year old passenger arrived in Malaysia from Cambodia and tested positive for COVID-19. The individual was identified by thermal temperature scanners at Kuala Lumpur airport. Subsequently, the 255 passengers and 747 crew remaining on the cruise ship were prevented from disembarking and those disembarked passengers awaiting flights in Phnom Penh were prevented from boarding departing flights. Disembarked passengers were required to remain in the Sokha Hotel in Phnom Penh. Some passengers were able to depart from Phnom Penh on Sunday 16 February, yet most airlines required that a certificate of negative test result for COVID-19 be provided before they could travel. Cambodia CDC implemented a COVID-19 screening activity for passengers remaining at the Sokha Hotel. From Sunday 16 to Tuesday 17 February screening was conducted in rooms provided by the hotel. Passengers had temperature readings taken, were administered a questionnaire and nasal and throat swabs were taken by Cambodian National Institute of Public Health (NIPH) staff. Samples were batched at the hotel and transported to Institute Pasteur Cambodia (IPC) laboratory in Phnom Penh. The laboratory capacity was 400 samples per 24-hour period. Results were directly reported to the Director of Cambodia CDC. Certificates of negative test results were issued for those passengers that tested negative.

A majority of passengers were screened on Sunday 16 February which concluded at 6:00 PM when sample capacity was reached. Screening activities resumed the following day from 9:00am to 4:30pm. Between Sunday 16 and Monday 17 February a total of 524 people were screened (including at least one crew member). An additional 10 people were screened on Tuesday 18 February. Results were progressively released and certificates issued. All results were reported by Wednesday 19 February. All samples were negative for COVID-19.

Request: That ASEAN Health Security Fellows based at IPC support screening of remaining passengers at the Sokha Hotel. The request was made by Cambodia CDC via Meghan Counahan (Health Advisor, Australian Department of Foreign Affairs and Trade (DFAT)) and Dr Laurence Barril (Director, IPC).

Personnel: Srean Chhim, ASEAN Health Security Fellow (IPC/ANU) and Elenor Kerr, ASEAN-Australia Health Security Fellow (IPC/ANU)

Activities undertaken:

Sunday 16 February

- Arrived at the Sokha Hotel at around 13:30 and met with Megan Counahan and Michael H. Kinzer (US Centers for Disease Control and Prevention (US- CDC)) for briefing on current situation.
- Assisted Megan and Michael to identify and contact passengers who plan to imminently depart Cambodia using a list of hotel rooms provided by the hotel.
- Introduced to Dr Sidonn Krang (Deputy Director, Cambodia CDC) at the request of Dr Sovann Ly (Director, Cambodia CDC) to provide support to screening activities. Administering screening questionnaires was agreed at the most appropriate support.
- Instructed by NIPH staff on personal protective equipment.
- Conducted screening interviews with Westerdam passengers using Cambodia CDC provided English and Khmer questionnaires. Questionnaires administered included the following topics:
    - Contact information (room number, full name, phone number)
    - Demographic (sex, age (years), nationality)
    - Symptoms in the past 24 hours (fever, cough, shortness of breath, sneezing, runny nose, sore throat, diarrhoea)
    - History of underlying/chronic health conditions (heart disease, diabetes, lung disease, neurologic disease, blood disease, kidney disease, liver disease, cancer, TB, HIV/AIDS, malnutrition, obesity
- Provided communications support when challenges arose with passengers. In particular, when delays or screening closure occurred.
- Departed from the Sokha Hotel at 19:00 when screening was concluded.

Monday 17 February

- Arrived at the Sokha Hotel at 08:00 to meet NIPH staff prior to screening commencing at 09:00.
- Continued to conduct screening interviews with Westerdam passengers and provide direction to passengers going through the screening process.
- Assisted Michael and Dr Sidon to identify rooms which had not yet been screened using lists compiled by Cambodia CDC staff during screening and hotel room lists, and a process for contacting these individuals efficiently.
- Departed from the Sokha Hotel after screening was concluded at 17:00

Lessons learnt:

Due to the operation being established quickly and involving many people from different institutions, there are some gaps which we could learn from for improvement in future exercises. Those gaps include:

- People, including passengers, were freely moving around the hotel and screening area, including busy areas like the hotel swimming pool, which was a potential risk for transmission. There were also reports of passengers leaving the hotel to sightsee in Phnom Penh.

  Recommendation: Passengers should have been advised to remained in hotel rooms until negative results were reported. Passengers should have been provided with clear communication about the risk and potential impact of transmission, particularly the potential health impacts of community spread of COVID-19 within the Cambodian context to deter passengers from leaving the hotel premises.

- There was a lack of consistent communication regarding the restriction of passenger movement inside and outside the hotel, e.g. some passengers were told to remain in their rooms, other passengers were allowed to move freely inside the hotel.

  Recommendation: A simple communication plan and resources, such as a printed flyer should have been developed and shared with passengers, hotel staff, and embassy staff at the hotel.

- There was no clear and efficient way to identify and contact passengers. Although it is required in Cambodia to provide a scanned copy of your passport on checking in to a hotel, lists of passengers and their room numbers were incomplete. This was most likely due to the travel agency making group bookings under the agency name. There were also discrepancies between versions of lists provided. The hotel appeared reluctant to share room lists.

  Recommendation: Establishing a method of communication, potentially with the travel agency or Cruise Ship Company, would have saved a lot of time

- There was a lack of consistent communication around screening activities. Whilst a plan for activities may have been developed, staff were not aware and it was not communicated to passengers. This heightened passenger anxiety and created a burden on screening staff who were constantly fielding difficult questions. Common questions included when screening would be open each day, and how long and how test results would be returned.

  Recommendation: A simple communication plan and involvement of a communications person would have helped address some of these issues. As passengers were freely moving around the lobby and near the screening area, a simple bulletin board with frequently asked questions or a staff member stationed in these area to provide updated information would have assisted.

- Passengers waiting for screening often had to stand in long lines in a hallway with minimal ventilation. Waiting times deterred passengers from being screened early on Sunday. Passengers waiting in lines for long periods were a risk for both potential transmission, as well as for other health issues in this typically elderly population. There was no seating in the screening room for passengers who had difficulties standing.

  Recommendation: Alternative methods of calling passengers for screening should have been explored (e.g. allocating time blocks or calling individual/blocks of rooms for screening). Those with health conditions which made it difficult for them to stand for long periods should also have been prioritized. There could also have been additional seating in the screening area.

- There were language barriers with some passengers could not speak English. Communicating with and completing questionnaires with these passengers was challenging.

  Recommendation: Arrangements with an embassy staff member or translator should have been explored.

- There were notable issues which may affect the validity of screening questionnaire data:
  - Differences between the wording and structure of the English and Khmer questionnaire regarding reported symptoms. The English version was developed by IPC, whilst the Khmer version was developed by the Cambodian CDC. The English questionnaire had additional symptoms (chest tightness, chest pain, conjunctival congestion, nausea, diarrhoea, abdominal pain) and options for various types of cough (productive/dry).
  - People administering the questionnaire were requested not to ask about each symptom individually, rather to ask if the passenger had experienced any symptoms. As passengers may not be aware of all symptoms, there is likely a loss of data capture on these questionnaire items and without a prompt there is also likely reduced recall.

Efficiency was cited as the rationale for asking a consolidated question about symptoms.

- Self-report of symptoms was unlikely to produce accurate results as passengers were anxious and reluctant to declare any symptoms in fear that they would be quarantined.

  Recommendations: One version of the questionnaire should have been selected and then back translated, with both English and Khmer on the form. Patient symptom questions should have been asked individually. As there were concerns about efficiency, alternative time saving measures should have been investigated. Such as asking passengers to write down their name and contact number prior to screening. A short explanation could have been provided making it clear that reporting of symptoms would not impact on whether or not they would be tested or prevented from travel. A communications person could have assisted to develop risk communication plans and materials to provide to passengers.

- There was no plan for providing passengers cleared from onwards travel with infection prevention information/communication materials which may prevent transmission if they later developed symptoms.

  Recommendation: As previously mentioned, a communications team member could have assisted to develop/source materials, such as a leaflet distributed to passengers as they exit the screening rooms and in the hotel lobby.

MAE Outbreak Sub-competencies:

The activities undertaken provided an opportunity to learn-by-doing and contributed towards acquiring the following sub-competencies related to the investigation of an acute public health problem:

Obtain preliminary information: Prior to this request for assistance, in anticipation of further confirmed or suspected COVID-19 cases in Cambodia, we had actively utilized available resources (online, networks, IPC) to keep up to date with the response activities both globally and locally. At the commencement of our deployment, we sought information on the current situation from key stakeholders involved (Megan, Michael, and IPC) to ensure that we understood the nature of the problem and activities underway. In particular, we sourced information on the activity logistics, stakeholders, the internal and external politics involved, and the level of urgency.

Establish the level of investigation/control necessary: From the outset we discussed with stakeholders (Michael and Megan) the priorities of the Cambodian CDC and other stakeholders involved (US-CDC, DFAT). We established that the priority was to identify any positive cases by locating and screening all passengers at the hotel. The other broader priority was facilitating the rapid departure of all passengers from Cambodia.

Participant in an outbreak response team: We participated in the field investigation as a part of the screening team. Due to the hierarchy and dynamics of the stakeholders involved, we had minimal power to shape the investigation. However, we were active members of the screening team; we administered questionnaires to several hundred passengers, assisted key stakeholders with the identification of passengers using hotel room lists, and provided communications assistance.

Our participation provided firsthand experience of the pace and chaos involved in such screening activities. It also provided a better appreciation for the physical, logistical, political, and data collection challenges of mass screening. The activity also gave us the opportunity to identify and assess limitations in process, data collection, and communications and reflect how we may do things differently in the future.