Applied epidemiology of influenza and other respiratory infections in Australia, Bangladesh and Cambodia

M Ximena Tolosa
BAgEng, MAppSc, MIPH, PhD

A thesis submitted for the degree of Master of Philosophy in Applied Epidemiology of the Australian National University

Field Supervisor
A/Prof Sheena Sullivan

Academic Supervisor
Dr Tambri Housen

© Copyright by M Ximena Tolosa. 2019
All rights reserved.
This document makes extensive use of the hyperlinking features of \LaTeX. References to figures, tables, sections, chapters and the literature can be navigated from within the PDF by clicking on the reference. Internet addresses will be displayed in a browser.
Declaration

I 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 proportions of material which have been accepted for the award of any other degree or diploma at ANU or any other educational institution, except where due acknowledgement is made in the thesis. Any contribution made to the research by others, with whom I have worked at the WHO Collaborating Centre for Reference and Research on Influenza, is explicitly acknowledged. 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 projects’ design and conception is acknowledged.

M Ximena Tolosa
20 February 2019
In the spirit of respect,
I acknowledge the traditional owners and
custodians of the land on which I lived and worked,
the Wurundjeri people of the Kulin Nation.

I pay my respects to Aboriginal Elders, past and
emerging and acknowledge the important role
Aboriginal and Torres Strait Islander
peoples of Australia play in
this country.
Acknowledgements

Completing a thesis is never an individual achievement. There are a number of people that deserve credit for enabling the completion of this work. First, I thank my field supervisor Sheena Sullivan for having chosen me to work with her. I appreciate the trust she placed on me, the varied projects she let me lead and for the opportunity to learn how to use \texttt{R} for public health data analysis. I would also like to acknowledge the support of both Sheena and the director of the WHO Collaborating Centre for Reference and Research on Influenza, Kanta Subbarao, in my deployment to Bangladesh to participate in the response to an acute public health emergency. Their support meant so much to me. Other colleagues from the Influenza Centre that I acknowledge are Vivian Leung and Olivia Price for their assistance during crucial parts of data analysis and Heidi Peck, Cleve Rynehart and Sally Soppe for expanding my knowledge on the challenges of egg-grown A/H3N2 influenza viruses for vaccine manufacturing and for the laughs! I also thank Jayde Simpson for her always timely admin support, Patrick Reading for providing feedback in one of my chapters and Meirian Lovelace-Tozer from the University of Melbourne’s The Research Bazaar for her impeccable \LaTeX{} teaching skills.

Massive thanks goes to my academic supervisor Tambri Housen. Tambri has offered me sustained support through the 22-month long MAE program, providing guidance and resources to help me resolve technical challenges and navigate administrative hurdles at ANU. Thanks to Tambri’s encouragement and her own experience as an MAE scholar, I applied for opportunities that –I later learned– were perceived as too hard to get, such as presenting my work at the US CDC Epidemic Intelligence Conference in Atlanta. Tambri also taught several of the MAE courses and I thank her for the time and skill involved in delivering those. The way Tambri encouraged MAE scholars to keep a healthy work-life balance was deeply appreciated. Thank you for encouraging your students with warmth and optimism. Others at ANU I would like to extend my gratitude are Katrina Roper for making the outbreak investigation course super engaging and for sharing with us her fascinating field epidemiology experience; and to Ross Andrews for injecting new perspectives into the MAE program.
For each one of my projects there are a number of people I wish to thank. They are mentioned in the acknowledgement section of each chapter.

Special thanks go to the Training Programs in Epidemiology and Public Health Interventions Network (TEPHINET) for the financial support that enabled me to present my work at the 67th EIS conference in Atlanta. I thank the South Asia Field Epidemiology and Technology Network (SAFETYNET) for supporting me to present at the 9th Southeast Asia and Western Pacific Bi-regional TEPHINET Scientific Conference in Laos. I acknowledge the Center for Excellence in Disaster Management and Humanitarian Assistance for supporting me to attend the Health Emergencies in Large Populations course in Hawaii. The support of my friends Jamie Taratoot and Chad Zimmerman in providing me with the field epi bible and other fantastic Oxford University Press books is gratefully acknowledged.

I thank the Australian Government Research Training Program Scholarship for their generous support.

The MAE 2017 cohort was a key part of my field epidemiology training. They have been a great source of support during this journey but, more importantly, they are now my friends.

Paul Petersen, my partner, has patiently waited for me to achieve the final step in my career transition. Thank you for looking after Bullina and bearing the largest share of our financial responsibilities. I can’t wait to resume our international motorcycle adventures!
Abstract

This thesis documents the work I completed to fulfil the core requirements of the Master of Philosophy in Applied Epidemiology (MAE) while based at the World Health Organization Collaborating Centre for Reference and Research on Influenza in Melbourne.

In May 2017 I travelled to Cambodia to conduct an influenza burden of disease study. I worked in collaboration with Cambodian public health officials and WHO Country Office staff to assist in the estimation of a national hospitalisation rate due to influenza-associated severe acute respiratory infection (SARI). The numerator used in the calculation of rates relied on existing surveillance data. Substantial field work was required to estimate the denominator. Field work entailed conducting two hospital admission surveys in two geographical regions in Cambodia. The results of this study showed that annual rates of influenza-associated hospitalisations were highest in infants and young children (<1 year and 1–4 years). We recommended that public health authorities in Cambodia consider the usefulness of adopting an influenza immunisation policy to reduce the impact of influenza infections in the most vulnerable groups. This work represented the epidemiology study component of the MAE (Chapter 2).

For my data analysis project I used the Australian influenza-like illness (ILI) surveillance system to estimate influenza vaccine effectiveness in Australia for the period of 2012 to 2017 and compare interim and final estimates. Vaccine effectiveness (VE) against influenza viruses was estimated using the case test-negative design and at two time points: mid-season (i.e., interim VE) and at the end of the influenza season (i.e., final VE). VE pairs were also estimated for influenza A and B combined, by subtype/lineage, by age group and by target group of vaccination. We assessed the association between sample size (i.e., the number of ILI specimens tested) and precision of VE pair estimates. This work demonstrated the need for a larger number of specimens to be collected, particularly from children and elderly patients. We recommended that to improve the reliability and usefulness of influenza VE estimates, the national ILI surveillance system be expanded (Chapter 3).
A second data analysis project involved the assessment of a virus isolation external quality assurance program implemented by the Influenza Centre in 2017–2018. This program tested virus isolation and identification performance in 25 National Influenza Centres (NIC) in countries in the WHO regions of the Americas, Africa and Easter Mediterranean. Results of this analysis was used to identify laboratories that required support in meeting their core NIC responsibilities (Chapter 3).

In January 2018, I supported the Bangladesh Ministry of Health and Family Welfare and WHO in responding to a diphtheria outbreak among Rohingya refugees. I worked in Cox’s Bazar for four weeks as a laboratory technical officer and was one of three members of the WHO’s case management team. My main responsibility was to facilitate the urgent establishment of a basic public health laboratory in close proximity to the refugee camps to ensure timely testing of diphtheria specimens as well as rapid confirmation of alert signals emanating from the Early Warning and Alert System. Challenges encountered in establishing laboratory capacity in the context of an acute large scale public health crisis in one of Bangladesh poorest districts were described. Shortcomings in the speed of the response mounted by the international community in supporting Bangladesh to strengthen laboratory diagnostic capacity were highlighted. We recommended the implementation of a global mechanism to support resource-limited countries to include strategies for the rapid establishment of laboratory capacity in their emergency response plans. This work represents my participation in a response to an acute public health problem (Chapter 4).

Despite its relevance for pandemic preparedness, Australia does not conduct surveillance of people working at the animal-human interface for zoonotic respiratory viruses. In my final project I assessed the feasibility of establishing systematic, ongoing epidemiological surveillance of people occupationally exposed to animals for emergent zoonotic influenza viruses. We focused on workers in intensive pig and poultry industries including abattoir workers. Through stakeholder engagement we examined drivers and barriers of conducting this type of surveillance. Furthermore, I developed a framework for conducting surveillance of zoonotic respiratory viruses targeting people working in the intensive pig and poultry production and processing industries in Australia. This work is presented in Chapter 5.
Presentations at conferences


Other presentations

Tolosa MX. WHO End of mission report: Hospital admission review. Siem Reap SARI sentinel site. 23rd May 2017, Ministry of Health, Phnom Penh, Cambodia. The slides are in Appendix 2.H.


Tolosa MX. Influenza sero-surveillance at the animal-human interface: A feasibility study in high-risk groups. 29th November 2018. Presented at a research meeting of the WHO Collaborating Centre for Reference and Research on Influenza, Melbourne.
Publications


## Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>vii</td>
</tr>
<tr>
<td>List of Abbreviations</td>
<td>xxi</td>
</tr>
<tr>
<td>1 Overview of the field placement and MAE course requirements</td>
<td>1</td>
</tr>
<tr>
<td>1.1 The World Health Organization Collaborating Centre for Reference</td>
<td>3</td>
</tr>
<tr>
<td>and Research on Influenza at the Victorian Infectious Diseases</td>
<td></td>
</tr>
<tr>
<td>Laboratory</td>
<td></td>
</tr>
<tr>
<td>1.2 Summary of field activities and public health experience</td>
<td>5</td>
</tr>
<tr>
<td>1.3 Summary of other MAE requirements</td>
<td>6</td>
</tr>
<tr>
<td>References</td>
<td>10</td>
</tr>
<tr>
<td>Appendix 1.A Manuscript submitted to Communicable Diseases Intelligence</td>
<td></td>
</tr>
<tr>
<td>2 Burden of influenza-associated hospitalisations in Cambodia</td>
<td>13</td>
</tr>
<tr>
<td>Prologue</td>
<td>16</td>
</tr>
<tr>
<td>My role</td>
<td>16</td>
</tr>
<tr>
<td>Lessons Learned</td>
<td>17</td>
</tr>
<tr>
<td>Public Health Implications</td>
<td>18</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>19</td>
</tr>
<tr>
<td>Abstract</td>
<td>21</td>
</tr>
<tr>
<td>2.1 Introduction</td>
<td>23</td>
</tr>
<tr>
<td>2.1.1 Influenza surveillance in Cambodia</td>
<td>23</td>
</tr>
<tr>
<td>2.2 Methods</td>
<td>25</td>
</tr>
<tr>
<td>2.2.1 Data sources</td>
<td>25</td>
</tr>
<tr>
<td>2.2.2 Obtaining a numerator: Estimation of influenza-associated</td>
<td></td>
</tr>
<tr>
<td>SARI cases at sentinel sites</td>
<td>26</td>
</tr>
<tr>
<td>2.2.3 Obtaining a denominator: Hospital Admission Surveys</td>
<td>28</td>
</tr>
<tr>
<td>2.2.4 Data analysis</td>
<td>31</td>
</tr>
<tr>
<td>2.2.5 Ethics approval</td>
<td>32</td>
</tr>
</tbody>
</table>
3.2.2 Case definition ........................................... 107
3.2.3 Laboratory methods ..................................... 107
3.2.4 Data analysis ........................................... 107
3.2.5 Ethical approval ......................................... 108
3.3 Results ...................................................... 109
3.3.1 Influenza-like illness consultations and patient characteristics . 109
3.3.2 Overall influenza vaccine effectiveness estimates ................... 112
3.3.3 VE pairs by subtype and lineage ............................ 112
3.3.4 VE pairs by age group ................................... 115
3.3.5 VE pairs by target group of vaccination ....................... 118
3.4 Discussion .................................................. 119
3.5 Conclusions ................................................ 122
References ..................................................... 123
Appendices .................................................... 128
3.A Oral poster presentation at the Annual EIS Conference, April 2018 . 128
3.B Presentation at the National Immunisation Conference, June 2018 . 136
3.C Publication in the WHO Weekly Epidemiological Record, August 2018 141

4 Establishing laboratory capacity in Cox’s Bazar, Bangladesh, in response to a diphtheria outbreak among Rohingya refugees 151
Prologue ......................................................... 154
   My role ....................................................... 154
   Lessons Learned ............................................ 155
   Public Health Implications ................................. 156
   Acknowledgements ........................................ 157
Abstract ....................................................... 159
4.1 Introduction ............................................... 161
   4.1.1 The Rohingya crisis .................................... 161
   4.1.2 Conditions in Rohingya refugee camps and risk of epidemic-prone diseases .................................................. 162
   4.1.3 Diphtheria among Rohingya refugees ..................... 163
4.2 WHO’s response to the diphtheria outbreak and the 10 steps in an outbreak investigation .................................................. 165
   Step 1. Prepare for field work ................................ 166
   Step 2. Confirm the diagnosis ................................ 167
   Step 3. Determine the existence of an outbreak ............... 167
   Step 4. Identify and count cases ............................. 167
   Step 5. Orient the data in terms of time, person and place .... 168
Step 6. Consider whether prevention and control measures can be implemented ........................................... 169
Step 7. Develop and test hypotheses .................................................. 170
Step 8. Plan systematic studies ....................................................... 170
Step 9. Implement and evaluate prevention and control measures .... 171
Step 10. Communicate findings ......................................................... 172
4.3 Challenges in laboratory diagnostic capacity ....................... 172
4.4 Methods ................................................................................. 173
   4.4.1 Establishing laboratory capacity in Cox’s Bazar .............. 173
   4.4.2 Ethics approval ................................................................. 175
4.5 Results .................................................................................. 175
   4.5.1 Timeline of events: From site identification to starting laboratory operations ...................................... 175
   4.5.2 Diagnostic tests recommended for the Cox’s Bazar laboratory .......................................................... 177
   4.5.3 Laboratory testing for diphtheria confirmation and outbreak monitoring ......................................... 178
4.6 Discussion .............................................................................. 179
4.7 Recommendations to improve outbreak diagnostic readiness in developing countries .......................... 182
4.8 Conclusions ........................................................................... 183
References ................................................................................ 185
Appendices ................................................................................ 191
4.B Article published in Global Biosecurity ........................................ 198
4.C Diphtheria Case Report Form .................................................. 202
4.D 30 day follow up of diphtheria patients form .............................. 204
4.E WHO’s Infection prevention and control guide ...................... 205
4.F Site assessment for suitability as a laboratory ......................... 215
4.G Floor plan of the Cox’s Bazar laboratory ................................. 226
4.H Laboratory establishment concept note ................................. 227
4.I Slides presented at TEPHINET’s Bi-regional Conference, Laos, November 2018 .............................................. 238

5 Surveillance of zoonotic influenza viruses at the animal-human interface: is Australia ready? 261
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prologue</td>
<td>264</td>
</tr>
<tr>
<td>My role</td>
<td>264</td>
</tr>
<tr>
<td>Lessons Learned</td>
<td>265</td>
</tr>
<tr>
<td>Public Health Implications</td>
<td>266</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>267</td>
</tr>
<tr>
<td>Abstract</td>
<td>269</td>
</tr>
<tr>
<td>5.1 Introduction</td>
<td>271</td>
</tr>
<tr>
<td>5.1.1 Emergent zoonotic influenza: a public health threat</td>
<td>271</td>
</tr>
<tr>
<td>5.1.2 Zoonotic influenza transmission hostpots also exist in high-income countries</td>
<td>271</td>
</tr>
<tr>
<td>5.1.3 The rationale for adopting influenza surveillance at the pig-human and poultry-human interfaces</td>
<td>272</td>
</tr>
<tr>
<td>5.1.4 Study aims</td>
<td>274</td>
</tr>
<tr>
<td>5.2 Methods</td>
<td>274</td>
</tr>
<tr>
<td>5.2.1 Stakeholder engagement</td>
<td>274</td>
</tr>
<tr>
<td>5.2.2 Surveillance protocol design</td>
<td>276</td>
</tr>
<tr>
<td>5.2.3 Ethical approval</td>
<td>277</td>
</tr>
<tr>
<td>5.3 Results</td>
<td>277</td>
</tr>
<tr>
<td>5.3.1 Stakeholder affiliations</td>
<td>277</td>
</tr>
<tr>
<td>5.3.2 Main findings from stakeholder engagement</td>
<td>278</td>
</tr>
<tr>
<td>5.3.3 Proposed surveillance protocol</td>
<td>290</td>
</tr>
<tr>
<td>5.4 Discussion</td>
<td>291</td>
</tr>
<tr>
<td>5.5 Recommendations</td>
<td>294</td>
</tr>
<tr>
<td>5.6 Conclusions</td>
<td>296</td>
</tr>
<tr>
<td>References</td>
<td>297</td>
</tr>
<tr>
<td>Appendices</td>
<td>306</td>
</tr>
<tr>
<td>5.A Participant information and consent form</td>
<td>306</td>
</tr>
<tr>
<td>5.B Stakeholder questionnaires</td>
<td>309</td>
</tr>
<tr>
<td>5.B.1 Questionnaire for industry, animal health and public health staff</td>
<td>309</td>
</tr>
<tr>
<td>5.B.2 Questionnaire for trade union representatives</td>
<td>330</td>
</tr>
<tr>
<td>5.C Proposed protocol for surveillance of people working in the intensive pig and poultry production and processing industries in Australia</td>
<td>334</td>
</tr>
<tr>
<td>5.C.1 Aim</td>
<td>335</td>
</tr>
<tr>
<td>5.C.2 Description of the proposed surveillance system</td>
<td>335</td>
</tr>
<tr>
<td>5.C.3 Sampling strategy</td>
<td>336</td>
</tr>
<tr>
<td>5.C.4 Data collection and reporting</td>
<td>340</td>
</tr>
<tr>
<td>5.C.5 Surveillance attributes of the proposed system</td>
<td>341</td>
</tr>
<tr>
<td>5.C.6 Important considerations</td>
<td>342</td>
</tr>
</tbody>
</table>
6 Lessons from the field

Prologue

My role and lessons learned

6.1 Lessons from the Field:

Introduction to the open-source R software

6.1.1 Learning objectives

6.1.2 Learning Steps

6.1.3 What is R and RStudio?

6.1.4 Materials needed

6.1.5 Instructions part 1: Software installation

6.1.6 Instructions part 2: Data import and analysis

6.2 Resources for further learning

6.3 A final word about writing code

Acknowledgements

References

6.4 Teaching Experience

6.4.1 Data analysis training workshop in Cambodia

6.4.2 Communication during public health emergencies

Appendices

6.A Lesson from the field: the Rmd output file

6.B Lesson from the field: teleconference slides

6.C Teaching presentation, ANU, March 2018
# List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHC</td>
<td>Angkor Hospital for Children</td>
</tr>
<tr>
<td>ASPREN</td>
<td>Australian Sentinel Practice Research Network</td>
</tr>
<tr>
<td>BDT</td>
<td>Bangladeshi Taka</td>
</tr>
<tr>
<td>CXB</td>
<td>Cox’s Bazar</td>
</tr>
<tr>
<td>DAT</td>
<td>diphtheria antitoxin</td>
</tr>
<tr>
<td>DFC</td>
<td>Direct Financial Cooperation</td>
</tr>
<tr>
<td>DGHS</td>
<td>Director General of Health Services</td>
</tr>
<tr>
<td>DHK</td>
<td>Dhaka</td>
</tr>
<tr>
<td>DipTC</td>
<td>Diphtheria Treatment Centres</td>
</tr>
<tr>
<td>EIS</td>
<td>Epidemic Intelligence Service, US Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>EQA</td>
<td>external quality assurance</td>
</tr>
<tr>
<td>EWARS</td>
<td>Early Warning Alert and Response System</td>
</tr>
<tr>
<td>FETP</td>
<td>Field Epidemiology Training Program</td>
</tr>
<tr>
<td>GISRS</td>
<td>Global Influenza Surveillance and Response System</td>
</tr>
<tr>
<td>GOARN</td>
<td>Global Outbreak Alert and Response Network</td>
</tr>
<tr>
<td>GP</td>
<td>general practitioner</td>
</tr>
<tr>
<td>HAS</td>
<td>hospital admission survey</td>
</tr>
<tr>
<td>HCF</td>
<td>healthcare facilities</td>
</tr>
<tr>
<td>HPAI</td>
<td>highly pathogenic avian influenza</td>
</tr>
<tr>
<td>ICD</td>
<td>International Classification of Diseases</td>
</tr>
<tr>
<td>IEDCR</td>
<td>Institute of Epidemiology Disease Control and Research</td>
</tr>
<tr>
<td>ILI</td>
<td>influenza-like illness</td>
</tr>
<tr>
<td>KCPH</td>
<td>Kampong Cham Provincial Hospital</td>
</tr>
<tr>
<td>LFF</td>
<td>Lesson from the Field</td>
</tr>
<tr>
<td>LPAI</td>
<td>low pathogenic avian influenza</td>
</tr>
<tr>
<td>Acronym</td>
<td>Full Form</td>
</tr>
<tr>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>MAE</td>
<td>Master of Philosophy in Applied Epidemiology</td>
</tr>
<tr>
<td>MHFW</td>
<td>Ministry of Health and Family Welfare</td>
</tr>
<tr>
<td>MOH</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>MSF</td>
<td>Médecins Sans Frontières</td>
</tr>
<tr>
<td>NIC</td>
<td>National Influenza Centre</td>
</tr>
<tr>
<td>NIPH</td>
<td>National Institute for Public Health, Cambodia</td>
</tr>
<tr>
<td>OHS</td>
<td>Occupational Health and Safety</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PPE</td>
<td>personal protective equipment</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RT PCR</td>
<td>reverse transcription polymerase chain reaction</td>
</tr>
<tr>
<td>SAFETYNET</td>
<td>South Asia Field Epidemiology and Technology Network</td>
</tr>
<tr>
<td>SARI</td>
<td>severe acute respiratory infection</td>
</tr>
<tr>
<td>SEARO</td>
<td>World Health Organization South-East Asia Regional Office</td>
</tr>
<tr>
<td>TEPHINET</td>
<td>Training Programs in Epidemiology and Public Health Interventions Network</td>
</tr>
<tr>
<td>TOR</td>
<td>Terms of Reference</td>
</tr>
<tr>
<td>US CDC</td>
<td>United States Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>USD</td>
<td>US dollars</td>
</tr>
<tr>
<td>VE</td>
<td>vaccine effectiveness</td>
</tr>
<tr>
<td>VIDRL</td>
<td>Victorian Infectious Diseases Reference Laboratory</td>
</tr>
<tr>
<td>WASH</td>
<td>water, sanitation and hygiene</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WPRO</td>
<td>World Health Organization Western Pacific Regional Office</td>
</tr>
<tr>
<td>WPSAR</td>
<td>Western Pacific Surveillance and Response Journal</td>
</tr>
</tbody>
</table>
Chapter 1

Overview of the field placement and MAE course requirements
## Contents

1.1 The World Health Organization Collaborating Centre for Reference and Research on Influenza at the Victorian Infectious Diseases Research Laboratory .................. 3

1.2 Summary of field activities and public health experience 5

1.3 Summary of other MAE requirements ...................... 6

References .......................................................... 10

Appendix 1.A Manuscript submitted to Communicable Diseases Intelligence Journal ......................... 11
1.1 The World Health Organization Collaborating Centre for Reference and Research on Influenza at the Victorian Infectious Diseases Research Laboratory

My placement at the World Health Organization (WHO) Collaborating Centre for Reference and Research on Influenza (hereon referred to as the Influenza Centre) commenced on 14th March 2017. The Influenza Centre was designated in 1992 and is located at the Victorian Infectious Diseases Reference Laboratory (VIDRL) in Melbourne. The Influenza Centre is supported by the Australian Government Department of Health through a funding agreement between the Commonwealth and Melbourne Health, and has domestic and international reporting responsibilities. The Centre reports directly to the Department of Health as well as to the WHO.

There are four other WHO Collaborating Centres for Reference and Research on Influenza in humans located in Atlanta, Beijing, London and Tokyo. A sixth Centre deals with influenza viruses in animals (the WHO Collaborating Center for Studies on the Ecology of Influenza in Animals in Memphis, USA). The six Collaborating Centres plus an additional 143 National Influenza Centres (NIC) and other essential regulatory laboratories across the world are part of the WHO Global Influenza Surveillance and Response System (GISRS) (1). The aims of the GISRS network are to provide early warning of changes in influenza viruses circulating in the global population and to inform influenza vaccine composition relative to circulating strains. GISRS is closely linked with the aims of the International Health Regulations (2005) (2) that require countries to notify all human infections with novel influenza viruses. Through its global collaboration and timely sharing of viruses, reagents and information GISRS is a key component of global public health emergency preparedness (3).

The Australian Influenza Centre has four main responsibilities: 1) to conduct ongoing virological and epidemiological surveillance of influenza viruses from Australia and the region, 2) to provide recommendations as what strains are appropriate for inclusion in seasonal vaccines, as well as to provide candidate viruses for vaccine production, 3) to deliver training in laboratory and surveillance capacity in the region, and 4) to conduct research to improve the detection, prevention and treatment of influenza.
The Terms of Reference (TOR) of the Influenza Centre as specified by WHO are:

1. To obtain, isolate and preserve representative viruses from outbreaks and sporadic cases of influenza and characterise their antigenic and other properties including resistance to anti influenza drugs
2. To exchange information and new antigenic variants of influenza viruses with other WHO Collaborating Centres for Reference and Research on Influenza and with Essential Regulatory Laboratories
3. To assist WHO in developing recommendations on viruses to be included in influenza vaccines
4. To provide training and laboratory support to WHO National Influenza Centres and other laboratories, especially those in the developing world in specialised techniques for, diagnosis, isolation and characterisation of influenza virus, according to their needs
5. To collect epidemiological information on the prevalence of influenza, especially in countries and areas in the region;
6. To undertake research to improve the detection, prevention and treatment of influenza
7. To assist WHO and national health authorities in developing and implementing plans for responding to pandemic influenza
8. To comply with the Terms of Reference for WHO Collaborating Centres for Influenza related to work with Pandemic Influenza Preparedness biological materials as specified in Annex 5 of the Pandemic Influenza Preparedness Framework

The work I conducted during my Master of Philosophy in Applied Epidemiology (MAE) contributed to TOR 1 (virus isolation external quality assurance presented in Chapter 3), TOR 3, 5 and 6 (influenza vaccine effectiveness work presented in Chapter 3), TOR 5 (estimation of burden of influenza in Cambodia, Chapter 2), and TOR 6 and 7 (design of a framework for surveillance of zoonotic respiratory viruses at the animal-human interface Chapter 5). Related to TOR 4, I contributed to training health officials in a developing country (Cambodia) on the use of influenza surveillance data to estimate the burden of severe influenza. Further details of these activities are described in the following section.
1.2 Summary of field activities and public health experience

The importance of field epidemiology

Field epidemiology is the application of epidemiological principles and tools to rapidly detect and respond to real-world public health challenges. At a country level, field epidemiology capacity is essential to protect the health of its residents and it is also needed to ensure global health security (2). As I see it, the integrative nature of applied epidemiology means that it can be used to discover public health threats that are not yet immediately obvious and, possibly, before they become widespread. Furthermore, as applied epidemiologists we have the moral obligation to understand the causes of the causes of the health conditions we investigate. That is why applied epidemiology is important to me. The work presented in this thesis represent a contribution to public health in Australia, Bangladesh and Cambodia. This work resulted in evidence-based recommendations aimed at reducing the burden of disease (Chapter 2), improving public health surveillance (Chapter 3 and 5) and responding to an acute public health emergency (Chapter 4).

Summary of field activities

Briefly, my first project was an epidemiology study to estimate the burden of severe influenza in Cambodia. I used a hospital admission survey methodology in which routinely collected severe acute respiratory infection (SARI) data were used to estimate the rate of hospitalisations due to severe influenza. I participated in data collection in the field which consisted of extracting information from hospital medical records and hospital admission logbooks. I designed a qualitative interview guide for clinicians responsible for SARI surveillance, conducted interviews, analysed quantitative and qualitative data, wrote interim and final reports for WHO and a manuscript for publication (see Appendix 2.K) and presented the findings at a virology conference in Melbourne (see Appendix 2.I) and the regional TEPHINET conference in Laos (Appendix 2.L).

For the public health data analysis component of the MAE, I used the Australian influenza-like illness surveillance system to estimate the effectiveness of the influenza vaccine in Australia for 2012 to 2017. A pre-requisite for this project was learning to use the open-source R software. I conducted data cleaning and analysis using R, pre-
pared a report for the Australian Sentinel Practice Research Network (ASPREN) and for the Commonwealth Department of Health and communicated the results at a national and international conferences (see Appendix 3.B). In addition, I conducted data analysis for a virus isolation external quality assurance (EQA) program implemented by the Influenza Centre in 2017-2018 (see Appendix 3.C). This program consisted of an assessment of virus isolation capacity in 25 National Influenza Centres (NIC) in three WHO regions (the Americas, African and Easter Mediterranean regions) (5). The EQA report was published in the WHO’s Weekly Epidemiological Record. Based on EQA performance NICs were selected to receive training to strengthen their ability to isolate and identify influenza viruses. I am grateful for the opportunity to participate in this initiative which is a step forward for global pandemic preparedness.

The requirement to respond to an acute public health event took me to Bangladesh where I participated in the response to a diphtheria outbreak among Rohingya refugees. This outbreak took place in the context of a large-scale complex humanitarian emergency, classified as level 3 by WHO, the highest grading. My role was varied but my main responsibility was to assist the Bangladesh Ministry of Health and Family Welfare to quickly establish a basic public health laboratory capable to serve the needs of the refugee population.

For my ‘surveillance’ project I assessed the feasibility of establishing ongoing surveillance of zoonotic respiratory viruses with pandemic potential in Australia targeting people working at the animal-human interface in the context of intensively produced pigs and poultry. For this work I conducted a review of surveillance systems in operation in other countries, identified stakeholders and administered face to face or phone interviews and an online survey to canvass stakeholders’ views regarding the context, motivation and key challenges of conducting surveillance at the animal-human interface in Australia. A framework for a sentinel disease surveillance system targeting people working at the animal-human interface in Australia was designed.

### 1.3 Summary of other MAE requirements

In addition to completing the core components of the MAE academic course blocks and the projects described above, during the course of my training I met the additional MAE requirements of preparing a manuscript for publication, presenting my work at professional conferences, communicating the results of my work to a non-expert audience and delivering a lesson from the field to my peers.
My work estimating the burden of influenza in Cambodia (epidemiology study, Chapter 2) resulted in two conference presentations and a publication. This work was published in the Centennial Influenza Pandemic special issue of WHO’s Western Pacific Surveillance and Response Journal (see Appendix 2.K). I presented a poster with initial results of the work I conducted in Cambodia at the 9th International Global Virus Network Meeting in Melbourne in September 2017. The title of the poster was ‘Leveraging surveillance data for influenza pandemic preparedness: a Cambodian example’ (see Appendix 2.I). In addition, I presented the national burden of influenza estimates at the 9th Southeast Asia and Western Pacific Bi-Regional TEPHINET Scientific Conference in Laos in November 2018 (see Appendix 2.L).

During my second year, I presented the results of the influenza vaccine effectiveness (VE) work (public health data analysis, Chapter 3) at two scientific meetings. The first meeting was the 67th Annual Epidemic Intelligence Service, US Centers for Disease Control and Prevention (EIS) Conference Field Epidemiology Training Program (FETP) International Night: Improving Global Health Security through Field Epidemiology Training, Surveillance, and Outbreak Response convened in Atlanta in April 2018. I delivered an oral poster presentation at the EIS meeting titled: Influenza Vaccine Effectiveness in Australia, 2012-2017 (see Appendix 3.A). In June 2018, I delivered an oral presentation at the 16th National Immunisation Conference ‘Immunisation for all – Gaps, gains and goals’ held in Adelaide (see Appendix 3.B).

A reflection of my experiences in deploying to Bangladesh as part of WHO’s response to the diphtheria outbreak among Rohingya refugees resulted in two in-house oral presentations, one oral presentation at an international conference and a publication. I delivered a ‘Tales from the Field’ presentation to staff and scholars at the ANU National Centre for Epidemiology and Population Health. A second presentation on my role in establishing laboratory capacity in Bangladesh was delivered to colleagues at the Influenza Centre. Additionally, I delivered a presentation titled: ‘Establishing Basic Public Health Laboratory Capacity in the Context of a Large-Scale Acute Refugee Crisis – Challenges and lessons learned’ at the 9th Southeast Asia and Western Pacific Bi-Regional TEPHINET Scientific Conference in Laos (see Appendix 4.I). In collaboration with other Australians that participated in the Rohingya crisis response, I co-authored a manuscript for the new journal Global Biosecurity describing the operational challenges faced by field epidemiologists in Cox’s Bazar and lessons learned. This publication is included in Chapter 4 under Appendix 4.B.

Delivering a distance ‘Lesson from the Field’ to my peers was also a requirement of the MAE. My lesson included teaching the basic principles of analysing data using the open-source R software. I chose this topic as having at least basic competency
using R is becoming increasingly important for field epidemiologists. For my lesson from the field I prepared an instructional video in which I showed my peers how to code to explore the Titanic dataset. I covered common data management steps and creation of plots. My lesson from the field (Chapter 6) is my legacy to the MAE program. ANU selected it as a teaching tool for future MAE and MPH students.

In addition to the MAE core requirements, I attended weekly laboratory meetings, a staff retreat and assisted colleagues at the Influenza Centre in the preparation of a manuscript titled ‘Report on influenza viruses received and tested by the Melbourne WHO Collaborating Centre for Reference and Research on Influenza in 2017’, which was submitted to the Communicable Disease Intelligence journal (see abstract’s manuscript in Appendix 1.A)

An outline of projects completed in relation to the MAE core competencies is shown in Table 1.1.
Table 1.1: List of projects completed in relation to the MAE core competencies

<table>
<thead>
<tr>
<th>Chapter 1</th>
<th>Chapter 2</th>
<th>Chapter 3</th>
<th>Chapter 4</th>
<th>Chapter 5</th>
<th>Chapter 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction: Overview of the field placement and summary of MAE course requirements</td>
<td>Burden of influenza-associated hospitalisations in Cambodia</td>
<td>Influenza vaccine effectiveness in Australia 2012–2017</td>
<td>Establishing laboratory capacity in Cox’s Bazar, Bangladesh, in response to a diphtheria outbreak among Rohingya refugees</td>
<td>Surveillance of zoonotic respiratory viruses at the animal-human interface: is Australia ready?</td>
<td>Lessons from the field</td>
</tr>
<tr>
<td>Design and conduct an epidemiological study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analyse a public health dataset</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evaluate or establish a public health surveillance system</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✔</td>
</tr>
<tr>
<td>Respond to an acute public health problem</td>
<td></td>
<td></td>
<td></td>
<td>✔</td>
<td></td>
</tr>
<tr>
<td>Conference presentation</td>
<td>National</td>
<td></td>
<td></td>
<td>International</td>
<td>✔</td>
</tr>
<tr>
<td></td>
<td>International</td>
<td></td>
<td></td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Draft a scientific article for publication</td>
<td>Late draft</td>
<td></td>
<td></td>
<td>Published article</td>
<td>✔</td>
</tr>
<tr>
<td></td>
<td>Published article</td>
<td></td>
<td></td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Communicate to a non-expert audience</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✔</td>
</tr>
<tr>
<td>Teaching experience</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>✔</td>
</tr>
</tbody>
</table>

* This publication is in Appendix 3.C. It covered the analysis of an external quality assessment (EQA) of influenza laboratories in 25 countries.
References


Appendix 1.A Publication in the Communicable Diseases Intelligence Journal

My contribution to the manuscript consisted of summarising and tabulating data on viruses received and tested by the Influenza Centre. I performed this analysis using the R software. I reviewed and provided comments on all iterations of the manuscript.

The publication can be accessed on this link doi.org/10.33321/cdi.2019.43.25. The abstract is reproduced below.

Report on influenza viruses received and tested by the Melbourne WHO Collaborating Centre for Reference and Research on Influenza in 2017

Abstract

As part of its role in the World Health Organization’s (WHO) Global Influenza Surveillance and Response System (GISRS), the WHO Collaborating Centre for Reference and Research on Influenza in Melbourne received a record total of 5,866 human influenza positive samples during 2017. Viruses were analysed for their antigenic, genetic and antiviral susceptibility properties and also propagated in qualified cells and hens eggs for potential seasonal influenza vaccine virus candidates. In 2017, influenza A(H3) viruses predominated over influenza A(H1)pdm09 and B viruses, accounting for a total of 54% of all viruses analysed. The majority of A(H1)pdm09, A(H3) and influenza B viruses analysed at the Centre were found to be antigenically similar to the respective WHO recommended vaccine strains for the Southern Hemisphere in 2017. However, phylogenetic analysis of a selection of viruses indicated that the majority of circulating A(H3) viruses had undergone some genetic drift relative to the WHO recommended vaccine strain for 2017. Of 3,733 samples tested for resistance to the neuraminidase inhibitors oseltamivir and zanamivir, only two A(H1)pdm09 viruses and one A(H3) virus showed highly reduced inhibition to oseltamivir, while just one A(H1)pdm09 virus showed highly reduced inhibition to zanamivir.

Keywords: GISRS, influenza, vaccines, surveillance, laboratory, annual report, WHO
Chapter 2

Burden of influenza-associated hospitalisations in Cambodia

Hospital admission review, Thmar Pouk District Hospital, Banteay Meanchey province, Cambodia, May 2017
## Contents

**Prologue** ................................................................. 16  
My role ................................................................. 16  
Lessons Learned ..................................................... 17  
Public Health Implications ........................................... 18  
Acknowledgements ................................................... 19  

**Abstract** .......................................................... 21  

**2.1 Introduction** .................................................. 23  
2.1.1 Influenza surveillance in Cambodia ....................... 23  

**2.2 Methods** .................................................... 25  
2.2.1 Data sources ................................................ 25  
2.2.2 Obtaining a numerator: Estimation of influenza-associated  
SARI cases at sentinel sites ....................................... 26  
2.2.3 Obtaining a denominator: Hospital Admission Surveys ... 28  
2.2.4 Data analysis ............................................... 31  
2.2.5 Ethics approval ............................................. 32  

**2.3 Results** ...................................................... 32  
2.3.1 Influenza-associated SARI cases at sentinel sites ....... 32  
2.3.2 Hospital admission survey results ......................... 37  
2.3.3 Estimated annual influenza-associated SARI incidence rate  
at each sentinel site ............................................. 41  

**2.4 Discussion** .................................................. 42  

**2.5 Recommendations for future influenza burden of disease  
estimations** ......................................................... 45  

**2.6 Conclusions** .................................................. 46  

**References** ....................................................... 47  

**Appendices** ..................................................... 51  
2.A English version of MOH letter to healthcare facilities  
participating in the hospital admission survey* ............. 51  
2.B Hospital admission survey data collection tool ........... 52  
2.C Standard operating procedure for hospital admission sur-  
voy ................................................................. 53
2.D Data collection tool for data validation  
2.E Standard operating procedure for data validation  
2.F Interview guideline for SARI surveillance staff  
2.G Hospital admission survey training materials  
2.H WHO End of mission presentation, Cambodia MOH, Phnom Penh, May 2017  
2.I Poster presented at the International Global Virus Network Meeting, Melbourne, September 2017  
2.J Communication summary for a general, non-specialist, audience  
2.K Article published in the Western Pacific Surveillance and Response Journal  
2.L Poster presented at TEPHINET’s Bi-regional Conference, Laos, November 2018
Chapter 2

Prologue

The Cambodian Ministry of Health requested assistance from World Health Organization Western Pacific Regional Office (WPRO) to conduct an influenza burden of disease study. WPRO contacted my field placement to deliver technical assistance to complete this work and I was selected by my supervisor to travel to Cambodia to support the WHO Country Office in Cambodia to conduct this work.

My role

My epidemiology study consisted of estimating the burden of disease due to severe influenza in two provinces in Cambodia by participating in a hospital admission survey (called hospital admission review in Cambodia). Routinely collected severe acute respiratory infection (SARI) surveillance data for 2016 were used to estimate the rate of hospitalisations due to severe influenza. This work included data collection at two SARI sentinel hospitals (to obtain a numerator) and two hospital admission surveys (to obtain a denominator). I participated in the first hospital admission survey conducted in Siem Reap province. Activities I completed as part of a team included data extraction from medical records using a paper-based tool, supervising data collection teams, data entry into an electronic database and administering interviews to clinicians. I co-delivered a training workshop on hospital admission survey data analysis with staff from the WHO Country Office in Cambodia (See slides in Appendix 2.G).

Tasks I completed independently included analysing data from both surveys, designing a questionnaire for staff responsible for SARI surveillance, preparing interim and final reports for WPRO, presenting an end of mission report to the Cambodian Communicable Disease Control department (See Appendix 2.H), presentation of preliminary findings at a conference in Melbourne (9th International Global Virus Network Meeting, see poster in Appendix 2.I), presentation at the 9th Southeast Asia and Western Pacific Bi-Regional TEPHINET Scientific Conference in Laos (see poster in Appendix 2.L) and preparation of a manuscript published in the Western Pacific Surveillance and Response Journal (WPSAR) Supplement 1 Centennial Influenza Pandemic issue (see Appendix 2.K). Lastly, I authored an article for a general, non-specialist, audience which was published in the Graduate Union of the University of Melbourne website (see Appendix 2.J).
Lessons Learned

This study was a classic example of ‘finding the population denominator’, a critical and often difficult step in calculating an incidence rate, specially in developing countries. Intensive field work was necessary to obtain the denominator due to the structure of the healthcare system in Cambodia. This epidemiological study taught me that there are ways to estimate the burden of disease in countries with numerous small private healthcare providers and fewer larger public providers. As long as there is at least a sentinel surveillance system in place for the disease of interest, even when the proportion of the population that utilises the sentinel site is unknown, a rate can be estimated. I learned that this can be done using a hospital admission survey methodology (1). However, in order to obtain a robust estimate an important characteristic of the surveillance system must be met. The number of sentinel sites included must be proportional to the size of the population. Site location must represent, if possible, both urban and rural areas. Also, the number of sites included in a hospital admission survey is important when the size of the catchment population of each site is small, as was the case in this study. Although I was not able to address these issues I am now more aware of the difficulties involved in estimation of disease burden in developing countries and the need for caution when interpreting the results of a hospital admission survey.

The amount of field work involved in hospital admission surveys meant that to complete the data collection phase within a reasonable amount of time a team of 10+ people were needed. I learned that when working in resource-limited settings one must be flexible as resources will not always match the requirements. I learned the importance of including a training session before data collection starts. This ensures shared knowledge on quality of data collection among enumerators who were selected by the Cambodian Ministry of Health (MOH) and included people with no training in epidemiological methods. Training of data collectors at the end of the survey was an aspect of this work I particularly enjoyed as it contributed to building influenza surveillance capacity in Cambodia, which would also benefit the surveillance of diseases other than influenza.

I also learned how political factors complicate both how field work is conducted and what is included in publications. The manuscript I prepared and submitted to WPSAR (see Appendix 2.K) presents the results of three hospital admission surveys conducted in Cambodia. The first of these was conducted in 2016 by a US CDC Epidemic Intelligence Service officer as a pilot study (2). The pilot used SARI surveillance data for 2015, which was the first year that the surveillance system operated at
that sentinel site. Results from the pilot hospital admission survey revealed critical issues of data availability and quality. The burden of disease due to severe influenza estimated using data from the pilot survey was very low compared with two subsequent surveys conducted in other parts of Cambodia. For example, the rate of influenza-associated SARI cases for the ≤1 year old group was zero. This is inconsistent with the literature (3, 4). Most studies, including lower-middle income tropical climate countries, have found this age group bears the largest burden of influenza (5, 6, 7, 8, 9, 10, 11, 12). For this reason, we decided to use the recommendations from the pilot study to improve subsequent hospital admission surveys. The national influenza burden estimates presented in Chapter two do not include data from the pilot survey as it was deemed of insufficient quality. However, for political reasons these data were included in the manuscript submitted for publication. Hospital admission surveys are expensive and there is an expectation that money and effort invested would be reflected in publications. In addition, although I led the writing and revising of the published manuscript I was listed as a co-first author. We considered it important to list a Cambodian national, Vanra Ieng, as first author in a paper presenting Cambodian data. Vanra made a significant contribution in coordinating all three hospital admission surveys.

Public Health Implications

This was the first time that a burden of disease study used multi-site surveillance data from a recently established sentinel SARI surveillance system to estimate the burden of severe influenza in Cambodia. The logic behind burden of disease studies is that if the scale and impact of a disease are unknown or underappreciated, no measures will be implemented to reduce it. Besides determining the need for public health action, countries that have no baseline data on the burden of influenza –and no influenza vaccination policy– would not be able to evaluate the impact of interventions, such as the introduction of a vaccination program. As a developing and post-conflict country that is under strain to provide adequate primary healthcare to its population and is contending against multiple infectious diseases, Cambodia, must no doubt make difficult decisions when setting their health priorities. We hope the evidence presented here will help the Cambodian MOH make an informed decision regarding the introduction of influenza vaccination.

Through interviews with clinicians at one paediatric SARI sentinel site in Siem Reap we found issues likely impacting on the effectiveness of the SARI surveillance system. An important issue identified was that specimen collection from children for
influenza testing was suboptimal due to lack of parental consent and difficulties swabbing distressed children. This would have resulted in underreporting. To address underreporting of SARI we recommended reinforcing training of surveillance staff in Siem Reap province to ensure children that were not swabbed that fit the SARI case definition were reported.

Graduates of the Cambodian Applied Epidemiology Training Program participated in both influenza burden of disease studies. Through their increased understanding of the challenges present in the collection of surveillance data they are now placed in a better position to train surveillance staff at sentinel sites themselves. In addition, the training provided in data collection and data analysis will hopefully decrease the dependence of the Cambodian MOH on foreign technical assistance for further burden of disease estimations.

Acknowledgements

I thank Vanra Ieng, Yorgos Theocharopoulos and Reiko Tsuyuoka from the WHO Country Office in Cambodia. Vanra did most of the preparatory work for the hospital admission survey, which included engaging senior management of all healthcare facilities to obtain their support and ensure that enumerators were recruited. I also thank Vanra for creating the map in Figure 2.2. I thank Leila Bell from WPRO for contributing to the analysis of survey responses of SARI surveillance staff.

I thank Dr Millya Thyl and the staff at the Angkor Hospital for Children in Siem Reap, Cambodia, for their assistance during the hospital admission survey. I acknowledge the staff from the Provincial Health Department, the National Institute of Public Health Laboratory and the Cambodian Department for Communicable Disease Control for their assistance in data collection: Om SoVantha, Uch Monipheap, Sim Sansam; Vy Phally; Pen Rotha and Tek Bunchhoueng; Sok Sary, Ung Sophanith, Sok Daro, Prak Dara; Bou Sarin, Nhean KhamThol, Sorm Sophanny, Tan Sivorn and Kang Sareth.

Danielle Iuliano and Joshua Mott from the US Centers for Disease Control and Prevention are gratefully acknowledged for their comments on the draft manuscript.

The technical and financial support from World Health Organization (WHO) HQ is acknowledged. This work was supported by WHO Pandemic and Epidemic Diseases grant for Burden of Disease studies HQPED1611421.

Finally, I acknowledge the financial assistance provided by the South Asia Field Epidemiology and Technology Network (SAFETYNET) that enabled me to present
this work at the 9th Southeast Asia and Western Pacific Bi-Regional TEPHINET Scientific Conference in Laos in November 2018.
Abstract

**Background:** The burden of influenza in Cambodia has been understudied. However, it is needed to inform public health policy and set priorities for public health action such as the introduction of an influenza vaccination program. This work presents an estimate of the burden of influenza-associated severe acute respiratory infection (SARI) hospitalisations in two Cambodian provinces for 2016.

**Methods:** We estimated age-specific influenza-associated SARI hospitalisation rates in two sentinel sites: the Angkor Hospital for Children in Siem Reap province and the Kampong Cham Provincial Hospital in Kampong Cham province using data for 2016, the second year of operation of the surveillance system. For each site we used annual influenza-associated SARI surveillance data to estimate the numerator and hospital admission surveys to estimate the population denominator as recommended by WHO. A combined age-stratified influenza-associated SARI hospitalisation rate was estimated using the pooled influenza-associated SARI hospitalisations for both sites as the numerator and the sum of the catchment population of each site as the denominator. Combined influenza-associated SARI case counts for both provinces were estimated by applying hospitalisation rates to the provincial populations which were sourced from the Health Management Information System database managed by the Cambodian Ministry of Health. We administered questionnaires to clinical staff responsible for SARI surveillance at each site to gain insights into the enablers and obstacles for SARI patient recruitment and analysed responses using qualitative content analysis methods.

**Results:** The estimated annual combined rate of influenza-associated SARI hospitalisations per 100,000 population was 397 for children ≤1 year (95% CI: 321–491) and 264 for children 1–4 years (95% CI: 216–323). The second largest burden of influenza-associated SARI hospitalisations were found in the 5–15 and 65+ year age groups (109 and 110 hospitalisations per 100,000 population, respectively). The combined burden among adults of 16–64 years was lowest, between 17 and 45 hospitalisations per 100,000 population. The combined count of influenza-associated SARI hospitalisations in Siem Reap and Kampong Cham provinces were estimated as 1,347 (95% CI: 1,277–1,421), with 49% among children under five years of age. Interviews with surveillance staff indicated that SARI in children was under-estimated at an unknown frequency.

**Conclusion:** We present the first estimates of the burden of severe influenza in Cambodia using surveillance data from two sentinel sites. Our findings indicate that
influenza is an important contributor to hospitalisations in Cambodia, particularly among children under five years of age. These findings can be used by Cambodian health authorities to guide future strategies to reduce the burden of influenza.
Chapter 2

2.1 Introduction

Influenza is a contagious, acute respiratory illness caused by influenza viruses. Although the burden of seasonal influenza is underappreciated by many people outside the field of infectious diseases there is ample evidence that influenza causes significant annual morbidity, mortality and socioeconomic costs globally (13). The latest global annual estimates indicate that seasonal influenza results in 290,000 to 650,000 deaths (14).

Accurate figures of the burden of influenza are difficult to estimate, particularly in developing countries. Robust vital statistics and civil registration, sustainably-funded and well-resourced surveillance systems, hospital discharge databases and the expansion of influenza molecular testing have allowed more developed countries to conduct influenza burden estimation activities. However, due to data quality and availability issues, the burden of seasonal influenza in low and lower middle-income countries, many of which are in tropical areas, is not well documented. Lack of evidence of the burden of influenza as well as resource limitations mean that many countries lack influenza prevention and control policies (15, 16). Limited available data indicate that the burden of influenza in tropical settings is higher than in temperate regions, particularly in children (17). The prolonged circulation of seasonal influenza viruses in tropical areas could explain the higher burden. Recent estimates for the Southeast Asian region which relied on statistical modelling indicated that over 100,000 deaths occur annually in the region as a result of influenza (14).

Cambodia is a lower middle-income country with a tropical climate. The burden of seasonal influenza remains under-studied in Cambodia but the threat of zoonotic avian influenza A(H5N1) –which is perceived as high (18, 19)– has prompted international agencies to work collaboratively to implement and strengthen SARI surveillance systems (20) capable of monitoring influenza circulation and detecting novel influenza viruses associated with severe disease. Effective prevention and control strategies for influenza rely on the routine analysis of surveillance data for the estimation of the burden of disease.

2.1.1 Influenza surveillance in Cambodia

In 2006, the Virology Unit at the Institut Pasteur in Cambodia, the Communicable Disease Control Department of the Ministry of Health (C-CDC/MOH) and the WHO Country Office jointly established a National Influenza Centre (NIC) in Cambodia.
Chapter 2

The aim of the NIC is to monitor and characterise circulating strains of influenza virus associated with mild and severe disease (21).

In 2009, in response to the influenza A(H1N1) pandemic, a surveillance system for influenza-associated severe acute respiratory infection (SARI) was established in Cambodia. The aim of the surveillance system is to characterise the epidemiology of severe respiratory illnesses associated with influenza A and B viruses and other common respiratory pathogens (22). In 2014, the Cambodian SARI surveillance system was expanded from four to eight sentinel surveillance sites. Sentinel sites are located in Phnom Penh (two sites), Kandal, Siem Reap, Takeo, Kampong Cham, Svay Rieng and Kampot provinces. SARI surveillance in Cambodia is conducted throughout the year. National virological and epidemiological SARI surveillance data are reported by the MOH in a monthly bulletin and published online (23). The SARI surveillance system does not capture non-respiratory presentations of influenza, such as myocarditis, nor influenza-associated mortality.

Since 2015 the WHO has been assisting Cambodian health authorities to estimate the influenza burden of disease using a hospital admission survey methodology published by WHO (1). In 2016, a pilot hospital admission survey was conducted in Svay Rieng province and included seven non-sentinel healthcare facilities within the catchment area of the SARI sentinel site, the Svay Rieng Provincial Hospital (2). The main achievement of this pilot survey was to trial the methodology recommended by WHO (1) to estimate the influenza-associated SARI hospitalisation rate for Svay Rieng province. In doing so, the WHO methodology was successfully adapted to the Cambodian context (2). The estimated influenza-associated SARI hospitalisation rate for Svay Rieng province obtained through the pilot hospital admission survey was approximately 7/100,000 all-age population (2). This estimate appeared very low. It remained clear that in order to generate a more reliable burden of influenza estimate further hospital admission surveys were needed. In this chapter, we present the results of a second and third hospital admission surveys.
2.2 Methods

2.2.1 Data sources

SARI sentinel surveillance sites

SARI surveillance in Cambodia includes eight sentinel sites. Sentinel sites were inpatient public healthcare facilities (HCF) where SARI patients were identified and clinical, demographic information and respiratory specimens were collected. We used data from two sentinel sites in this study. Included sites were the Angkor Hospital for Children in Siem Reap province and Kampong Cham Provincial Hospital in Kampong Cham province.

SARI case definition  In Cambodia a SARI case is defined as measured fever (temperature of $\geq 38^\circ C$) or self-reported fever and cough, or sore throat and shortness of breath or difficulty breathing in a hospitalised person with onset of symptoms within 10 days prior to hospitalisation (24). All sentinel sites used the same SARI case definition. Clinical examination of cases was conducted by hospital clinicians.

Specimen collection and frequency of reporting  All hospitalised patients meeting the SARI case definition were eligible to have nasopharyngeal or oropharyngeal swabs collected soon after admission using previously described protocols (21). Specimens from children under five years of age were collected with parental consent. Swabs were transported in viral transport media to the testing laboratory. A standard case report form was completed for each enrolled case. Incident SARI cases were reported weekly by sentinel sites throughout the year. Case report forms accompanied each specimen submitted for testing.

Influenza case definition  An influenza case was defined as laboratory confirmation of influenza infection through detection of influenza A or B ribonucleic acid (RNA) in a person with SARI.

Laboratory methods  Initial testing was conducted at the National Institute for Public Health, Cambodia (NIPH) laboratory in Phnom Penh. Viral RNA was extracted from specimens using the QIAamp Viral RNA Isolation Kit (QIAGEN, CA, USA) and amplified using a multiplex real time reverse transcription polymerase
chain reaction (RT PCR) targeting seasonal influenza A and B viruses as previously described (25). All influenza-positive specimens and 10% of negative specimens were forwarded to the Virology Unit at Institut Pasteur du Cambodge in Phnom Penh for confirmation, using real time RT PCR as previously described (21).

2.2.2 Obtaining a numerator: Estimation of influenza-associated SARI cases at sentinel sites

We enumerated SARI admissions during 2016 at the Angkor Hospital for Children in Siem Reap province and Kampong Cham Provincial Hospital in Kampong Cham province. Patients hospitalised with a diagnosis that matched the SARI case definition were obtained from electronic medical records. Demographic information was also extracted. Only SARI patients that resided within the catchment area were enumerated. We used SARI surveillance laboratory data to enumerate age-specific influenza-associated SARI cases and to calculate age-specific influenza percent positive which represents the proportion of SARI cases that tested positive for influenza from the total number of specimens tested within the same age group. The estimation of the numerator for the influenza-associated SARI hospitalisation rate was informed by the data validation exercise described below.

Data validation

To assess adherence to the SARI case definition by those responsible for surveillance at the sentinel sites we compared the number of SARI cases identified through manual review of paper-based medical records with the number of cases reported by the surveillance system for six selected weeks in 2016. Selection of weeks for data validation was done to include weeks within and outside of the typical influenza virus circulation period. This criteria ensured that weeks with the following characteristics were included:

- Low number of SARI cases and high proportion influenza-positive cases
- Moderate number of SARI cases and moderate proportion of influenza-positive cases
- High number of SARI cases and no influenza-positive cases
- High number of SARI cases and low proportion of influenza-positive cases
- High number of SARI cases and high proportion of influenza-positive cases
Demographic information, dates of illness onset, date of admission and signs and symptoms were recorded from patient records using a paper-based data collection tool designed for this exercise (see Appendix 2.D). The step by step protocol for data validation (referred to as sensitivity analysis in Cambodia) can be found in Appendix 2.E). Both sentinel sites kept electronic medical records. This allowed us to search their databases for SARI cases for comparison with cases reported by the surveillance system for the entire year. We used discrepancies within a six-week period to establish under-reporting (i.e., SARI case counts reported by surveillance/SARI case counts identified in the data validation exercise). We used age-specific SARI case counts identified through electronic medical record search for the entire year and applied the age-specific influenza percent positive to the appropriate age group to obtain numerators for incidence rate calculations.

**SARI surveillance staff survey**

In addition to the data validation activity described above we administered structured, face-to-face questionnaires to all clinical staff responsible for enrolling SARI patients at both sentinel sites (five in Siem Reap: three physicians and two nurses and 19 in Kampong Cham: six physicians and 13 nurses). Questionnaires were designed to gain insight into the enablers, benefits and challenges of SARI surveillance and the mechanisms for case identification, specimen collection and patient management at each site. Interviews were conducted in English with the assistance of a translator at the Siem Reap site and in Khmer at the Kampong Cham site. Questionnaires are in Appendix 2.F.

Qualitative content analysis was applied to interview responses as previously described by Graneheim and Lundman (26). Briefly, interview transcripts were read to get a sense of recurring concepts. Concepts from all relevant data were abstracted into codes and all codes were examined together and sorted into categories. Categories were formulated into overarching themes. The content of the combined responses was analysed independently by two researchers and a coding framework consensus reached upon discussion. The two main themes explored by the questionnaire were perceptions regarding participation in SARI surveillance and technical aspects of SARI surveillance.
2.2.3 Obtaining a denominator: Hospital Admission Surveys

To estimate influenza-associated SARI hospitalisation rates in a context of unknown sentinel site catchment populations we conducted two hospital admission surveys following methods recommended by WHO (1) and piloted at the Svay Rieng sentinel site (27).

Hospital admission surveys – referred to as hospital admission reviews in Cambodia – were conducted in two provinces: Siem Reap and Kampong Cham between May and October 2017. Participating sentinel sites were the Angkor Hospital for Children (AHC), located in Siem Reap province, north-western Cambodia and Kampong Cham Provincial Hospital (KCPH), located in Kampong Cham province, in the central lowlands of Cambodia. AHC is an 85-bed provincial paediatric hospital (admitting children under 16 years of age) located in a township and financed by an international non-governmental organisation (28). KCPH is a 265-bed public general hospital serving a predominantly rural population.

Criteria used for site selection were site acceptance to participate in the hospital admission surveys, sites being in their second year of SARI surveillance and availability of medical records in English. These sentinel sites provided a climatically and demographically representative sample of hospitalisations in Cambodia. Surveillance data were collected in 2016, two years after initiating SARI sentinel surveillance.

The purpose of conducting hospital admission surveys is to estimate the denominator population to use in an incidence rate calculation. The methodology used in conducting hospital admission surveys included the following steps:

1. Estimation of the catchment areas for SARI sentinel surveillance sites
2. Identification of non-sentinel healthcare facilities within the catchment area
3. Enumeration of patients admitted to non-sentinel sites with a proxy SARI diagnosis
4. Calculation of the proportion of respiratory admissions that sought care at the sentinel sites
5. Estimation of denominator population for influenza-associated SARI incidence rates calculation

These elements of the hospital admission surveys are covered in the following subsections. In addition to the steps recommended in the WHO Manual (1), we conducted a data validation exercise and interviewed surveillance staff regarding SARI recruitment practices (see Section 2.2.2).
Step 1. Estimation of the catchment areas for SARI sentinel surveillance sites

Addresses of SARI cases admitted to the sentinel sites were reviewed and the catchment area for each site was defined as the districts where 80% of the SARI cases admitted to the sentinel hospitals resided. We refer to the catchment area of each site as Siem Reap (comprised of 20 districts within five provinces) and Kampong Cham (comprised of 14 districts within two provinces).

Step 2. Identification of non-sentinel healthcare facilities within the catchment area

Non-sentinel HCFs operating in the catchment areas of both sentinel sites that admitted patients overnight were identified by national staff through prior knowledge and internet searches. In preparation for the data collection activities, the MOH Communicable Disease Control Department sent a letter of invitation to all identified HCFs within the catchment areas requesting their collaboration (Appendix 2.A). Once the hospital survey started additional non-sentinel facilities were identified on the ground. These were also invited to participate.

Step 3. Enumeration of patients admitted to non-sentinel sites with a proxy SARI diagnosis

We visited all HCFs and enumerated respiratory admissions consistent with the following diagnoses: acute pulmonary oedema, asthma, asthma-pneumonia, bronchiolitis, bronchitis, broncho-asthma, broncho-pneumonia, flu/cold, laryngitis, lung abscess / empyema, pharyngitis, pneumonia, pneumopathy, pulmonary tuberculosis, respiratory infection, rhino-pharyngitis, severe pneumonia and tonsillitis. These diagnoses, which were collected from HCF admission register books, represent a proxy measure for SARI diagnosis as determined in the pilot hospital admission survey (2). We collected proxy SARI data from non-sentinel HCF from 1st January to 31st December 2016. In addition to admission and discharge diagnoses we collected date, age, gender, district of residence and patient outcome.

The data collection team comprised approximately 12 enumerators and four supervisors. Enumerators worked in pairs with one person identifying proxy SARI cases in admission register books and the other writing this information on a paper-based form. Enumerators visited all non-sentinel HCF and collected data from sites that
granted permission to do so. Teams collected data from 16 HCF in Siem Reap and 14 in Kampong Cham. Non-sentinel HCF kept records in Khmer, French, Vietnamese and English.

Supervisors checked all entries on the first three data collection forms from each team immediately after completion and then spot-checked 10% of forms as data collection progressed (this was equivalent to three entries per sheet). Errors were corrected and training reinforced as needed. Data quality checks involved comparing entries against their source in the logbook as well as reviewing logbooks searching for relevant respiratory admissions/discharges missed by enumerators. All forms were checked for accuracy. Supervisors marked each sheet as ‘checked’ and included their name.

**Data collection tools** Enumerator captured data in a standardised paper-based data collection tool listed in Appendix 2.B. Data were captured in Khmer, French or English as per original entries. When logbook entries were recorded in Vietnamese, assistance from HCF clinicians was sought to translate it to Khmer. Data were subsequently entered from paper forms into an electronic database created in Epi Info 7 (29). All data were entered in the electronic database in English.

**Training of data collectors** All enumerators attended a one day training workshop prior to commencing data collection. This training introduced the data collection protocol (Appendix 2.C) and the paper-based data collection tool (Appendix 2.B). This training taught participants how to identify proxy SARI admissions in HCF register books, how to record information in the data collection tool and the quality control strategy for supervisors to follow. Training was delivered in Khmer and included a practical session in which patient scenarios were presented for participants to appraise whether they represented a respiratory diagnosis that merited inclusion. Participants were provided with the list of relevant diagnoses and districts to include.

**Step 4. Calculation of the proportion of respiratory admissions that sought care at the sentinel sites**

We calculated the age-specific proportion of respiratory hospitalisations at each sentinel site (i.e., SARI cases) relative to all respiratory admissions across all HCFs in the catchment area for 2016 by dividing the number of SARI cases at the sentinel site by the number of respiratory admissions at all HCFs within the catchment area. Admissions from patients that resided outside the catchment area were excluded.
Step 5. Estimation of denominator population for influenza-associated SARI incidence rates calculation

To generate an estimated catchment population for each sentinel site to be used as a population denominator in incidence rate calculations we applied the proportion of respiratory admissions that sought care at the sentinel sites (estimated in Step 4 above) to the age-specific district population (obtained from the Ministry of Health Management Information System).

2.2.4 Data analysis

Site-specific annual hospitalisation rates of influenza-associated SARI were calculated as described in the WHO Manual (1). To address under-reporting of SARI cases, we used SARI case counts identified by manual data extraction from databases at sentinel sites instead of the SARI case count reported by the surveillance system. For each site, we calculated the number of influenza-associated SARI hospitalisations by multiplying the age-specific influenza positive percentages in each age group by the corresponding SARI case count in the same age group. To estimate influenza-associated SARI hospitalisation rates by age group for Siem Reap and Kampong Cham combined we pooled data from both sentinel sites as follows:

\[
\frac{\text{Influenza-associated SARI case counts for Siem Reap + Kampong Cham}}{\text{Estimated catchment populations for Siem Reap + Kampong Cham}} \times 100,000
\]

Estimates of influenza-associated SARI hospitalisation rates for adults were based on data from the Kampong Cham sentinel site only whereas children’s estimates were based on data from both sentinel sites (i.e., one paediatric hospital and one all-ages hospital). The count of SARI hospitalisations in both provinces was estimated by multiplying the combined age-specific rates by the combined provincial population in the corresponding age groups (30).

We calculated 95% confidence intervals (CI) by calculating the error factor, which was the exponentiation of 1.96 divided by the square root of the number of influenza-associated SARI cases. We subsequently calculated the range of the CI by dividing (higher CI) or multiplying (lower CI) the hospitalisation rate by the error factor. Calculations were conducted in Microsoft Excel 2010. The map was created with ArcGIS 10.2 software (Environmental Systems Resource Institute, Redlands, CA, USA).
2.2.5 Ethics approval

Hospital admission surveys consisted of a retrospective review of health data collected by the SARI sentinel surveillance system, which is a public health activity managed by the Cambodia MOH and has standing authorisation from the National Ethics Committee. All parents of ill children who were recruited in SARI surveillance at the AHC sentinel site provided verbal informed consent. Verbal consent was obtained from clinicians prior to the interviews. Ethical aspects of the hospital admission survey conducted in Siem Reap, where I participated in data collection, have been approved by the Australian National University Human Research Ethics Committee (Protocol 2017/337).

2.3 Results

2.3.1 Influenza-associated SARI cases at sentinel sites

The number of SARI admissions and percent positive for influenza by age-group at both sentinel sites is shown in Table 2.1.

Table 2.1: Number of annual severe acute respiratory infection (SARI) cases and influenza positive cases by age group and sentinel site, 1st January - 31st December 2016, Siem Reap and Kampong Cham, Cambodia.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Siem Reap*</th>
<th>Kampong Cham</th>
<th>Combined influenza-associated SARI cases†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SARI cases</td>
<td>Percent positive for influenza (%)†</td>
<td>Influenza-associated SARI cases</td>
</tr>
<tr>
<td>&lt;1</td>
<td>455 (10.4 (15/144))</td>
<td>47</td>
<td>381 (10.0 (2/20))</td>
</tr>
<tr>
<td>1–4</td>
<td>376 (10.9 (19/175))</td>
<td>41</td>
<td>256 (20.8 (10/48))</td>
</tr>
<tr>
<td>5–15</td>
<td>91 (10.0 (1/10))</td>
<td>9</td>
<td>157 (30.0 (3/10))</td>
</tr>
<tr>
<td>16–24</td>
<td>91 (12.0 (3/25))</td>
<td>11</td>
<td>11 (11%)</td>
</tr>
<tr>
<td>25–49</td>
<td>244 (11.4 (8/70))</td>
<td>28</td>
<td>28 (8%)</td>
</tr>
<tr>
<td>50–64</td>
<td>280 (10.0 (3/30))</td>
<td>28</td>
<td>28 (8%)</td>
</tr>
<tr>
<td>≥65</td>
<td>334 (11.1 (5/45))</td>
<td>37</td>
<td>37 (11%)</td>
</tr>
<tr>
<td>Total</td>
<td>922 (10.6 (35/329))</td>
<td>97</td>
<td>1,743 (13.7 (34/248))</td>
</tr>
</tbody>
</table>

* Siem Reap sentinel site was a paediatric hospital and admitted children <16 years of age.
† Percent positive for influenza is the number of positive specimens out of the total number of specimens tested.
‡ Influenza-associated SARI cases were calculated by applying the age-specific influenza percent positive for each age group to the corresponding SARI case count for each age group.
Overall, in 2016 a total of 2,665 SARI admissions were identified at sentinel sites: 922 cases at Siem Reap site and 1,743 cases at Kampong Cham site. The percent positive for influenza among SARI specimens tested on both sites was, on average, 12% (69/557). The number of influenza-associated SARI cases estimated for the Kampong Cham site was more than two fold that of the Siem Reap site. The combined influenza-associated SARI cases estimated from both sentinel sites was 339, 53% of which occurred among children under five years of age (179/339).

The number of SARI cases positive for influenza by subtype and lineage for 2016 as reported by all SARI sentinel surveillance sites is shown in Figure 2.1. Influenza A(H1N1)pdm09 viruses circulated all year around whereas influenza A(H3N2) and B co-circulated predominantly in the second half of the year. Peaks of influenza activity occurred in August and October. Influenza A(H1N1)pdm09, A(H3N2) and B viruses co-circulated in similar proportions (Figure 2.1).

Figure 2.1: Monthly influenza positive SARI cases by subtype and lineage as reported by all SARI surveillance sites, 1st January–31st December 2016, Cambodia.
*Influenza percent positive represents the proportion of specimens collected from SARI cases that tested positive for influenza from the total number of SARI specimens tested. Source: SARI sentinel surveillance system, Cambodia (31).
Data validation at SARI sentinel sites

In Siem Reap, we reviewed 259 records from patients hospitalised during six selected weeks in 2016. These represented all charts for patients admitted during those weeks. In that period, we identified 98 patients who met the SARI case definition. During those same six weeks, the surveillance system identified 55 SARI cases, indicating 44% under-reporting by the surveillance system. In Kampong Cham, 99 records from patients hospitalised during the selected six weeks were reviewed. Of these, 28 patients met the SARI case definition and 19 were not captured by the surveillance system (32% under-reporting).

SARI surveillance staff survey

Content analysis of survey responses from 24 clinical staff (nine physicians and 15 nurses) at the Siem Reap and Kampong Cham SARI sentinel sites is shown in Table 2.2 and Table 2.3. Overall, some respondents reported that surveillance activities represented an acceptable workload and identified difficulties obtaining consent for specimen collection in children and swabbing distressed children as challenges. Further challenges identified were: difficulty applying the SARI case definition due to incomplete or unclear medical histories, parental misunderstanding regarding the purpose of specimen collection as a contributing factor to specimen collection refusal, difficulty in applying the case definition to neonates and fear of reprimand if unable to collect specimens due to lack of parental consent. The main benefits cited from participating in SARI surveillance were the opportunity for professional development through increased understanding of SARI, acquiring technical skills in swabbing patients and gaining confidence educating patient contacts about SARI.

In terms of the technical aspects of SARI surveillance, respondents reported strict adherence to the SARI case definition when determining who to swab and that it was expected that swabbing would assist in differential diagnoses. Flexibility in swabbing practices was mentioned, meaning that swabs were also sometimes collected from patients with suspected influenza or atypical SARI presentation. Clinicians reported that their decision to swab a patient was influenced by their awareness of surveillance reports showing increased community-acquired influenza. Some respondents reported avoiding taking specimens from patients under three months of age and young children.
Table 2.2: Summary of survey responses regarding enablers, benefits and challenges of severe acute respiratory infection (SARI) surveillance among surveillance staff in Siem Reap and Kampong Cham sites, May and July 2017, Cambodia.

<p>| What are the enablers, benefits and challenges of SARI surveillance at your facility? |</p>
<table>
<thead>
<tr>
<th>Enablers</th>
<th>Benefits</th>
<th>Challenges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very acceptable/acceptable workload</td>
<td>Opportunity for professional development</td>
<td>Obtaining parental consent for specimen collection in children</td>
</tr>
<tr>
<td>Sharing surveillance tasks among staff</td>
<td>SARI epidemiology knowledge acquisition</td>
<td>Difficulty swabbing distressed children</td>
</tr>
<tr>
<td>Sharing responsibility in SARI diagnosis</td>
<td>Gain technical skills in swabbing patients</td>
<td>Incomplete/unclear medical history</td>
</tr>
<tr>
<td>Case definition is a useful guide</td>
<td>Increased confidence educating patient contacts about SARI</td>
<td>Delays in obtaining laboratory results, exacerbated by lack of weekend specimen processing</td>
</tr>
<tr>
<td>Flexibleswabbingpractices(requestsand suspicion as drivers)</td>
<td></td>
<td>SARI harder to identify in neonates</td>
</tr>
<tr>
<td>Infants (1 month–2 years) with SARI easier to identify in high season</td>
<td></td>
<td>Lack of knowledge on swabbing technique, stocks of swabbing kits exhausted, lack of access to training</td>
</tr>
<tr>
<td>Usefullwheninternalinfluenzaactivityreportsareavailable</td>
<td></td>
<td>Poor reporting feedback of SARI activity</td>
</tr>
<tr>
<td>Cooperation among staff</td>
<td></td>
<td>Fear of reprimand if unable to obtain consent to swab</td>
</tr>
</tbody>
</table>
Table 2.3: Summary of survey responses regarding technical aspects of severe acute respiratory infection (SARI) surveillance among surveillance staff in Siem Reap and Kampong Cham sites, May and July 2017, Cambodia.

<table>
<thead>
<tr>
<th>Technical aspects of SARI surveillance system</th>
<th>Change in clinical patient management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Most patients with classical SARI symptoms (as per case definition)</td>
<td>No change in antiviral use</td>
</tr>
<tr>
<td>Infants (&lt; 3 months) and young children sometimes avoided due to difficulties swabbing</td>
<td>Reduction in antibiotic use</td>
</tr>
<tr>
<td>Sometimes patients with unusual presentation (SARI suspicion)</td>
<td>SARI became easier to diagnose</td>
</tr>
<tr>
<td>Increases in reported community-acquired influenza prompts to swab</td>
<td></td>
</tr>
<tr>
<td>When seeking differential diagnosis</td>
<td></td>
</tr>
</tbody>
</table>

† Interviewed staff were nine physicians and 15 nurses. Items are listed in no particular order.
An important finding from the staff surveys is that the system does not capture patients that are classified as SARI but for which a swab was not obtained due to parental refusal.

Analysis of responses discussing the impact that participating in SARI surveillance had on clinical patient management showed mixed results (Table 2.3). Some respondents reported no change in use of antivirals while some reported a change although it was not clear if this represented an increase or a decrease in use. A physician stated: ‘Here we don’t use antivirals much. We only use them when we suspect avian flu.’ Some reported that diagnosing SARI became easier and one reported a reduction in use of antibiotics as a result of participation in SARI surveillance.

2.3.2 Hospital admission survey results

Estimation of SARI sites catchment areas

The first step in the hospital admission survey was to estimate the catchment area for each sentinel site. The map in Figure 2.2 shows the location of each sentinel site (red circles) and their catchment areas demarcated by red contour lines. The catchment area for the Angkor Hospital for Children SARI sentinel site comprised 20 districts: 12 in Siem Reap province, two in Oddar Manchey province, one in Preah Vihear province, two in Kampong Thom province and three in Banteay Manchey. The catchment area for the Kampong Cham Provincial Hospital SARI sentinel site comprised 14 districts: 10 in Kampong Cham province and four in Tbong Khmum.
Non-sentinel healthcare facilities within the catchment area

We identified a total of 32 non-sentinel HCFs in the catchment areas of both sentinel sites that admitted patients overnight. In Siem Reap we identified 18 non-sentinel HCFs (10 public and eight private). In Kampong Cham we identified 14 HCFs (nine public and five private). Two private HCF did not consent to participate in the survey, therefore, a total of 30 non-sentinel HCFs were included in the survey.

Patients hospitalised with proxy SARI at non-sentinel sites

A total of 10,750 respiratory admissions with a proxy SARI diagnosis were recorded from 30 non-sentinel HCF located in Siem Reap (n=4,170) and Kampong Cham (n=6,580) catchment areas. International Classification of Diseases (ICD) codes were
not used in any of the HCFs. All diagnoses were recorded in hospital logbooks as free text.

Proportion of respiratory admissions that sought care at the sentinel sites

The first hospital admission survey showed that the proportion of respiratory admissions that sought care at the Siem Reap sentinel site was 18% (922/5,092). In the second hospital admission survey we found that 21% of all respiratory admissions sought care at the Kampong Cham sentinel site (1,743/8,323). Table 2.4 shows the proportion of respiratory admissions that sought care at each SARI sentinel site by age group in 2016.

Table 2.4: Respiratory admissions by age group at severe acute respiratory infection (SARI) sentinel sites and all other healthcare facilities (HCF) in their catchment area, 2016, Cambodia.

<table>
<thead>
<tr>
<th>Age group (Years)</th>
<th>Angkor Hospital for Children</th>
<th>All HCF</th>
<th>Proportion of admissions at AHC* sentinel site</th>
<th>Kampong Cham Provincial Hospital</th>
<th>All HCF</th>
<th>Proportion of admissions at KCPH† sentinel site</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>455</td>
<td>1,300</td>
<td>35.0</td>
<td>381</td>
<td>1,525</td>
<td>25.0</td>
</tr>
<tr>
<td>1–4</td>
<td>376</td>
<td>2,697</td>
<td>13.9</td>
<td>256</td>
<td>1,892</td>
<td>13.5</td>
</tr>
<tr>
<td>5–15</td>
<td>91</td>
<td>1,095</td>
<td>8.3</td>
<td>157</td>
<td>1,978</td>
<td>7.9</td>
</tr>
<tr>
<td>16–24</td>
<td>91</td>
<td>1,095</td>
<td>8.3</td>
<td>91</td>
<td>457</td>
<td>19.9</td>
</tr>
<tr>
<td>25–49</td>
<td>244</td>
<td>891</td>
<td>27.4</td>
<td>244</td>
<td>891</td>
<td>27.4</td>
</tr>
<tr>
<td>50–64</td>
<td>280</td>
<td>741</td>
<td>37.8</td>
<td>280</td>
<td>741</td>
<td>37.8</td>
</tr>
<tr>
<td>≥65</td>
<td>334</td>
<td>839</td>
<td>39.8</td>
<td>334</td>
<td>839</td>
<td>39.8</td>
</tr>
<tr>
<td>Total</td>
<td>922</td>
<td>5,092</td>
<td>18.1</td>
<td>1,743</td>
<td>8,323</td>
<td>20.9</td>
</tr>
</tbody>
</table>

* Angkor Hospital for Children.
† Kampong Cham Provincial Hospital.

Denominator population for influenza-associated SARI incidence rates calculation

We used age-specific population data for each catchment area sourced from the Cambodia Ministry of Health Management Information System (n=510,845 in Siem Reap and n=1,488,678 in Kampong Cham) and the age-specific proportion of admissions at the sentinel site to estimate the catchment population for each sentinel site. For the Angkor Hospital for Children the estimated catchment population was 60,920. For the Kampong Cham Provincial Hospital we estimated a catchment population of 334,812 (Table 2.5).
Table 2.5: Estimated annual influenza-associated severe acute respiratory infection (SARI) hospitalisation rate (and 95% confidence interval) by age group for each sentinel site, Siem Reap and Kampong Cham, 2016, Cambodia.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Siem Reap</th>
<th>Kampong Cham</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Influenza-associated SARI cases</td>
<td>Catchment area population&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt;1</td>
<td>47.4</td>
<td>39,206</td>
</tr>
<tr>
<td>1–4</td>
<td>40.8</td>
<td>142,108</td>
</tr>
<tr>
<td>5–15</td>
<td>9.1</td>
<td>329,531</td>
</tr>
<tr>
<td>16–24</td>
<td>10.9</td>
<td>330,044</td>
</tr>
<tr>
<td>25–49</td>
<td>27.8</td>
<td>458,514</td>
</tr>
<tr>
<td>50–64</td>
<td>37.1</td>
<td>84,554</td>
</tr>
<tr>
<td>Total</td>
<td>97.3</td>
<td>510,845</td>
</tr>
</tbody>
</table>

<sup>*</sup> Catchment area population data was obtained from the Ministry of Health Management Information System.

<sup>†</sup> Angkor Hospital for Children (only admitted children under 16 years).

<sup>§</sup> Site-specific influenza-associated SARI hospitalisation rate were estimated by dividing column A by column D and multiplying by 100,000.

<sup>‡</sup> Kampong Cham Provincial Hospital.

Only one decimal place is displayed but calculations used > decimal places.
2.3.3 Estimated annual influenza-associated SARI incidence rate at each sentinel site

Hospitalisation rates for children estimated for Kampong Cham were higher than for Siem Reap but both had their highest rates in children <1 year (495 and 345 per 100,000 population for Kampong Cham and Siem Reap, respectively. Table 2.5). The second highest hospitalisation rates were seen in children 1–4 years (338 and 206 per 100,000 population for Kampong Cham and Siem Reap, respectively). KCPH, the only site that admitted adult patients showed that hospitalisation rates decreased below 50 for adults 16-64 years and increased to approximately 110 per 100,000 population in adults aged over 65 years.

The combined influenza-associated SARI hospitalisation rates (i.e., Siem Reap and Kampong Cham sites combined) are shown in Table 2.6. Combined rates were highest for children ≤1 year of age and 1–4 years of age (397 [95% CI: 321 - 491] and 264 [95% CI: 216 - 323] per 100,000 population, for ≤1 and 1–4 years respectively).

Table 2.6: Age-specific annual influenza-associated severe acute respiratory infection (SARI) hospitalisation rate (95% confidence interval) for both sentinel sites combined and estimated hospitalisation counts for Siem Reap and Kampong Cham provinces, 2016, Cambodia.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Combined influenza-associated SARI counts</th>
<th>Combined catchment population*</th>
<th>Combined influenza-associated SARI hospitalisation rate per 100,000†</th>
<th>Combined total provincial population‡</th>
<th>Combined influenza-associated SARI counts§</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>85</td>
<td>21,420</td>
<td>396.8 (320.8–490.8)</td>
<td>48,072</td>
<td>191 (166–220)</td>
</tr>
<tr>
<td>1–4</td>
<td>94</td>
<td>35,583</td>
<td>264.2 (215.8–323.4)</td>
<td>176,487</td>
<td>466 (426–511)</td>
</tr>
<tr>
<td>5–15</td>
<td>56</td>
<td>51,455</td>
<td>108.8 (83.8–141.4)</td>
<td>422,948</td>
<td>460 (420–504)</td>
</tr>
<tr>
<td>16–24</td>
<td>11</td>
<td>65,720</td>
<td>16.7 (9.3–30.2)</td>
<td>233,422</td>
<td>39 (29–53)</td>
</tr>
<tr>
<td>25–49</td>
<td>28</td>
<td>125,564</td>
<td>22.3 (15.4–32.3)</td>
<td>324,280</td>
<td>72 (57–91)</td>
</tr>
<tr>
<td>50–64</td>
<td>28</td>
<td>62,329</td>
<td>44.9 (31.0–65.1)</td>
<td>116,656</td>
<td>52 (40–69)</td>
</tr>
<tr>
<td>&gt;65</td>
<td>37</td>
<td>33,660</td>
<td>109.9 (79.6–151.7)</td>
<td>59,801</td>
<td>66 (52–84)</td>
</tr>
<tr>
<td>Total</td>
<td>339</td>
<td>395,731</td>
<td>85.7 (77.0–95.3)</td>
<td>1,381,066</td>
<td>1,347 (1,277–1,421)</td>
</tr>
</tbody>
</table>

* Calculated by adding the estimated catchment population for Siem Reap and Kampong Cham (columns 5 and 10 in Table 2.5)
† The combined influenza-associated SARI hospitalisation rate was estimated by dividing the combined SARI counts by the combined catchment population and multiplying by 100,000.
‡ Provincial population data for Siem Reap and Kampong Cham was obtained from the Ministry of Health Management Information System.
§ Combined influenza-associated SARI counts for Siem Reap and Kampong Cham province were estimated by applying the combined influenza-associated SARI hospitalisation rate to the combined total population of Siem Reap and Kampong Cham provinces and dividing by 100,000.
The total age-adjusted influenza-associated SARI counts (i.e., all-ages) for both provinces combined was 1,347 (95% CI: 1,277–1,421). Almost half of the hospitalisations estimated for Siem Reap and Kampong Cham provinces corresponded to children under five years (657/1,347).

2.4 Discussion

We report the first estimates for severe influenza virus infection in Cambodia using recent influenza surveillance data collected from two SARI surveillance sentinel sites applying methodology described in the WHO Manual for Estimating Disease Burden Associated with Seasonal Influenza (1). The data we used provided a representative sample of hospitalisations in both urban and rural areas in two provinces in Cambodia. In agreement with what was observed in previous reports from Cambodia (21) and other countries in the region and globally (11, 9), influenza activity was detected throughout the year with sustained peaks between March and December.

Our findings indicate that influenza is an important contributor to hospitalisations in Cambodia, particularly among children under five years of age. In both sentinel sites we observed that infants (≤1 year) had the highest hospitalisation rates due to severe influenza (345 and 495 hospitalisations per 100,000 population in Siem Reap and Kampong Cham, respectively) followed by children aged 1–4 years (206 and 338 cases per 100,000 population in Siem Reap and Kampong Cham, respectively).

The combined influenza-associated respiratory hospitalisations we estimated for Cambodian children are close to those for developing countries published in a global meta-analysis using data from hospital-based passive surveillance (4). In that study, authors reported annual hospitalisation rates of 300 and 200/100,000 population for children ≤1 year and ≤5 years, respectively. Similarly high rates of hospitalisation due to influenza-associated SARI in children were found in the Philippines (368 per 100,000 children ≤2 years) (7), Kenya (290–470 per 100,000 children ≤5 years) (6) and Zambia (188 per 100,000 children ≤5 years) (12). These studies were multi-centered, used a similar methodology to that recommended by WHO and used multiple years of surveillance data.

Several studies published recently from countries in Central and South America, Africa and Asia (9, 32, 10, 33) reported two to four fold lower estimates of influenza-associated respiratory hospitalisations in children compared to ours. For example, influenza-associated SARI hospitalisation rates in children ≤5 years in Chile were 72 per 100,000 children, 156 in Laos and 158 in Rwanda compared to the 314 per 100,000
children \leq 5 \text{ years estimated in our study. Our influenza-associated hospitalisation rate estimates for children are also 2.6 times higher than those from a population-based study among children} \leq 5 \text{ years in rural India that estimated a rate of 118/100,000 population for the 2009-2011 seasons (34).}

There are several reasons why it is difficult to compare the burden of severe influenza between countries. Estimates from different years show differences in the predominant strains of influenza virus circulating. Different populations are likely to differ in health status, in health seeking behaviour and in access to healthcare. Differences in hospital admission threshold would result in differences in influenza burden estimates. Varying SARI case definitions, SARI case recruitment challenges, proficiency in diagnosing SARI, proportion of patients tested and heterogeneity of study designs would compound the challenge of inter-country comparisons of influenza burden. However, comparing our estimates with those of Indonesia, a tropical lower-middle income country that used a similar methodology based on data from sentinel sites servicing an all-age population, shows that the burden of influenza in Cambodian children was almost three times higher than in Indonesian children (hospitalisation rate in Indonesian children 0–4 years in 2015-2016 was 114/100,000 (11)).

Hospitalisation rates estimated for all-age groups showed a similar pattern of high rates in infants and young children, lower rates in working-age adults and higher rates in people over 65 years of age. The same pattern of influenza burden across age groups estimated using similar methods has been reported from tropical countries with similar population age structures. For example, Indonesia reported hospitalisation rates of 114, 36, 1–2, 0 and 38 per 100,000 population in 0–4, 5–14, 15–59 and \geq 60 age groups, respectively (11). Zambia also reported a similar influenza-associated hospitalisation rate pattern (484, 109, 6, 19–26 and 57 per 100,000 population in \leq 1, 1–4, 5–24, 25–64 and \geq 65 age groups, respectively (12)). Similarly, Rwanda reported influenza-associated hospitalisation rates in children \leq 1 that were 24 times higher than in working age adults and rates in older adults that were three times as high as those of the working age population (10). The combined burden of influenza hospitalisations across all age-groups estimated for Cambodia (86/100,000 population) is similar to that reported for the Philippines (100/100,000 population (7)) but higher than the rates found in most published studies from tropical low-income countries such as Indonesia (19/100,000 population (11)), Rwanda (35/100,000 population (10)), Laos (37/100,000 population (33)) and Zambia (44/100,000 population (12)). All-age influenza hospitalisation burden is likely to vary both within and between countries for the reasons described above.
One important strength of our study is the staff survey that enabled the exploration of operational challenges in SARI surveillance at the two sentinel sites. To our knowledge, previous burden of disease studies have not included staff surveys. These add value as they aid interpretation of the data validation exercise conducted to understand the extent of underreporting.

This work uncovered evidence of under-reporting of SARI cases from both sentinel sites studied. Approximately one third to almost half of all SARI cases admitted to the sentinel sites in Siem Reap and Kampong Cham during 2016 had not been reported by the surveillance system nor laboratory tested. This highlights the need for strengthening SARI surveillance in these sites, which were on their second year of surveillance operations. The importance of strengthening influenza surveillance capability in Cambodia goes beyond its function as data source for burden of disease estimation and rests on its central role in the early detection and response to emerging threats (35). Due to its relevance to global health security building surveillance capacity in Cambodia is a vital component of global pandemic preparedness.

Limitations

Several limitations were identified in our work. Our estimates –as in most studies that used a similar methodology– represent only a subset of the overall disease burden associated with influenza. This is because we were unable to account for: SARI cases that did not seek healthcare; hospitalisations due to influenza in respiratory patients that did not meet the SARI case definition; nor patients admitted for conditions triggered by influenza infection (i.e., acute myocardial events). In addition, the role of cultural and geographical factors in influencing health seeking behaviour and therefore burden estimation was not measured.

Through staff surveys at two sentinel surveillance sites we found that bias in recruitment of SARI cases among children occurred at an unknown frequency. The SARI surveillance system in Cambodia does not have a way to record cases who meet the SARI definition but refuse specimen collection. Clinicians reported that the frequency of specimen collection refusal was higher for children. This would have resulted in underreporting of SARI cases in children and therefore underestimation of hospitalisation rates for children. In addition, it is also likely that the case definition used in Cambodia – which is less sensitive than the WHO recommended case definition (2) – contributed to an underestimation of influenza-associated hospitalisations in children.
Population data were based on projections derived from the 2013 census and at the district-level it did not capture population by sex. Due to this lack of data we were unable to estimate the burden of severe influenza by sex.

We were not able to estimate mortality due to influenza-associated SARI due to lack of available data. In-hospital deaths in Cambodia are either not well captured or are rare due to cultural practices where families might prefer to tend to the terminally ill at home. The estimation of mortality due to influenza in Cambodia needs further attention.

Finally, we estimated the burden of influenza using surveillance data from only two SARI sentinel sites. Our estimates are therefore not representative of the entire country. Despite these limitations this work provides evidence that the burden of severe influenza in Cambodia, particularly in children and older adults, is substantial and warrants the introduction of influenza control policies.

2.5 Recommendations for future influenza burden of disease estimations

We estimated the burden of influenza based on only two of the eight SARI sentinel sites that function in Cambodia. The sites included in our study serve approximately 3% of the Cambodian population. We recommend future burden studies use data from all sentinel sites collected in multiple calendar years (at least three years) to ensure more reliable quantification of the burden of severe influenza that could be used for policy decision making. If including all sites is not feasible it would be valuable to at least include a site in the most densely populated province of Phnom Penh as they are likely to represent a more complex and diverse demographic.

We recommend strengthening SARI surveillance in Cambodia to ensure that all sites operate to the same standard and are able to generate data of sufficient quality to allow reliable estimation of the burden of severe influenza in the population. Specifically, we recommend the SARI surveillance system captures information for SARI cases that are not swabbed. To address the problem of parent refusal for specimen collection, education campaigns targeting parents are recommended. In addition, to estimate gender-specific influenza-associated hospitalisation rates we recommend that efforts are made to disaggregate population data by gender at the district level. Finally, to stimulate the translation of evidence of the burden of influenza into public health
action it would be helpful for Cambodia to conduct further operational research to understand the economic burden of seasonal influenza.

2.6 Conclusions

We produced up-to-date estimates of the burden of severe influenza in Cambodia contributing to narrowing the gap in understanding the burden of influenza disease in a lower-middle income tropical country. Our work indicates that the impact of severe seasonal influenza was highest in the youngest and oldest age groups. These findings reinforce the need for Cambodia to take action to prevent and control the threat of influenza. The evidence presented here can be used by the Ministry of Health in Cambodia to support the decision to introduce influenza vaccination to reduce the impact of influenza-associated hospitalisations in the most vulnerable population groups, children and older adults. Furthermore, this work underscores the value of investing in routine influenza surveillance in lower-middle income countries as key drivers of population health and pandemic preparedness.
References


27 Centers for Disease Control and Prevention, Atlanta, GA. Using a Hospital Admission Survey to Determine Rates of Influenza-Associated Severe Acute Respiratory


Appendices

2.A English version of MOH letter to healthcare facilities participating in the hospital admission survey*

24 April, 2017

To healthcare facility manager,

Subject: Request of collaboration on the review of inpatients’ logbook and patient charts at SARI sentinel site, provincial hospitals and private healthcare facilities to understand the influenza disease burden in Cambodia.

As mentioned in the above subject, I would like to inform you that the Communicable Disease Control department in collaboration with WHO and US CDC is aiming to better understand and estimate the influenza disease burden in Cambodia and therefore conduct a hospital admission review (HAR), a patient records review method.

The Ministry of Health has launched in 2015 the process to estimate the burden of severe influenza piloting a hospital admission review and sensitivity analysis of the severe acute respiratory illness (SARI) surveillance system. To understand the burden of influenza in Cambodia we need to expand this HAR activity. HARs will allow us to estimate the population seeking treatment for severe acute respiratory illness at public and private hospitals. Further HARs are needed to determine the national burden of severe influenza.

The next HAR will be conducted between 8–23 May 2017 and visits are anticipated to be carried out to selected hospitals for this purpose between those dates. A detailed schedule will be sent to you in the coming weeks. We are hereby seeking your collaboration to conduct this activity.

Sincerely,

Communicable Disease Control Department, Cambodia

*Note regarding terminology: In Cambodia the methodology used is referred to as ‘hospital admission review’ instead of the ‘hospital admission survey’ used in this thesis and the published literature.
## 2.B Hospital admission survey data collection tool

<table>
<thead>
<tr>
<th>No.</th>
<th>Relevant District</th>
<th>Relevance Relevant Diagnosis</th>
<th>Admission Diagnosis</th>
<th>Onset date (DDMMYY)</th>
<th>Admission Date (DDMMYY)</th>
<th>Discharge Date (DDMMYY)</th>
<th>Discharge Diagnosis</th>
<th>Outcome</th>
<th>Referral from/to</th>
</tr>
</thead>
</table>
2.C Standard operating procedure for hospital admission survey

These are the instructions for filling the data collection form shown in Appendix 2.B. During the training workshop prior to data collection, we introduced this protocol to enumerators and supervisors.

1. Ensure each data collection form sheet is given a unique number starting with one and continuing sequentially. The supervisor should label each sheet before data entry begins.

2. Hospital district: Record the district of the hospital

3. Facility name: Record the name of the hospital

4. Ward: Record the ward of the logbook or logbook entry

5. Logbook number: All logbooks for the facility should be numbered sequentially starting with 1 by the supervisor before data entry begins. Record the logbook number

6. Logbook start date: Find the date of the first entry in the logbook and record this date even if the date is before your data collection period

7. Logbook end date: Find the date of the last entry in the logbook and record this date even if the date is after your data collection period

8. Data collector name: write your name

9. Data collector phone: write your phone number

10. Data collection date: record today’s date (date in which you are collecting data)

11. Number: Pre filled (28 rows per sheet, 14 on each side)

12. Relevant district of residence: Review each logbook entry and look first to find ones within the catchment area by reviewing patient addresses. If patient is from a district within the catchment area, check this box

13. Relevant diagnosis: Then review each patient from relevant districts for a selected respiratory diagnosis either on admission or discharge. If patient has a relevant respiratory diagnosis, check this box

14. Order: record the entry number written in the logbook. If a star, record a star
15. Patient ID: record the patient ID as it appears in the logbook

16. Age in years: record the age of the patient in years. If less than one year, skip this column and proceed to next column to write the age of the patient in months

17. Age in months: If less than one year, write the age of the patient in months

18. Age in days: If less than one month, write the age of the patient in days

19. Sex: Write M for males and F for females

20. District: record the patient’s district of residence

21. Admission diagnosis: Write all listed admission diagnoses

22. Onset date: Some logbooks will record the date of onset. Record this date in DDMMYY format. If missing, leave this cell blank

23. Admission date: write the date of admission in DDMMYY format

24. Discharge date: write the date of discharge in DDMMYY format

25. Discharge diagnosis: Write all listed discharge diagnoses

26. Outcome: If listed, write the outcome of the patient: still admitted (moved to another ward), discharged (recovered or discharged against medical advice), died or transferred (if available record location of transfer)

27. Referral to/from: If referred to or from another ward, record this information here

28. Repeat with next entry and subsequent entries until all cases for the selected time period have been recorded
### Data collection tool for data validation

<table>
<thead>
<tr>
<th>No</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>District</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Admission (DBMMYY)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Admission Diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (Y)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (M)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (D)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date Onset (DDMMYY)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start of Tympany (Y/N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start of Cough (Y/N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start of Fever (Y/N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temp in °C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever Reported (Y/N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever Measured (Y/N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difficulty Breathing (Y/N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sore Throat (Y/N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diifficulty Coughing (Y/N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Data collection tool for data validation activity**

**Hospital Name:**

**Data Collection Date:**

**Ward:**

**Chart Week:**

**Data Collector:**

---

**Chapter 2**

2.D Data collection tool for data validation
2.E Standard operating procedure for data validation

1. Ensure you know your target dates (starting and ending dates for the epidemiological weeks selected)

2. Select medical records with mention of fever (reported or recorded) for review

3. For each medical record selected, review and record the following information in the appropriate field of the data collection tool

   (a) Hospital Name: write the name of the hospital
   (b) Data collection date: write today’s date in DDMMYY format
   (c) Ward: write the ward of the chart (i.e., paediatrics, medicine)
   (d) Chart week: write the epi week of the chart.
   (e) Data collector: write your name
   (f) Number: Write a number in the field starting with 1 in the first data line. Use consecutive numbers for each entry
   (g) Date of admission: this is the date that the patient was admitted to the hospital. It should be within the specified weeks. Record the date in DDMMYY format
   (h) District of residence: Write the district where the patient resides
   (i) Sex: Record F (female) or M (male)
   (j) Admission diagnosis: this is the diagnosis reported at admission. Write all admission diagnoses listed
   (k) Age in years: write the age of patient in years (only if the patient is older than 12 months). If the age is less than one, leave blank
   (l) Age in months: write the age of patient in months (only if the patient is younger than 12 months). If the patient is over one year old, leave blank
   (m) Age in days: write the age of patient in days (only if the patient is less than 1 month)
   (n) Date of symptom onset: record the date when the patient’s symptoms began (DDMMYY)
   (o) Fever reported: review the chart for any mention of fever or fever symptoms (chills, sweats, skin hot to touch, etc.) and record Y for yes, N for no
   (p) Measured fever: review the chart for measured fever and record Y for yes, N for no, or U for unknown
(q) Temperature in degree Celsius: If a measured fever is recorded, write the temperature reading in Celsius

(r) Cough: review the chart for any mention of cough (either reported or observed by the clinician) and record Y for yes, N for no, or U for unknown

(s) Sore throat: review the chart for any mention of reported sore throat and record Y for yes, N for no, or U for unknown

(t) Difficulty breathing: review the chart for any mention of shortness of breath, tachypnoea (fast breathing), increased work of breathing, etc. and record Y for yes, N for no, or U for unknown

(u) Respiratory rate: review the chart for measured respiratory rate and record the fastest rate (RR)

(v) Discharge diagnosis: this is the diagnosis reported by the physician at discharge. Write all discharge diagnoses listed

4. Record data for all medical records within the specified time period (six epidemiological weeks).

5. Supervisors need to check all entries in the first form for each data collector, make corrections and reinforce training as necessary. For later forms, supervisors should check at least 50% of entries

6. After the data collection is completed, transfer each entry into an Excel spreadsheet and add three extra columns: sheet number, days sick and SARI (Y/N)

(a) Write the corresponding sheet number for each entry

(b) Create a formula to calculate the number of days sick: date of admission minus date of onset of symptoms

(c) Use the countif function to determine whether entries constitute a SARI case. Record Y for yes and N for no.

   Remember that to be recorded as Y, it must meet the SARI case definition:

   • Symptoms started within 10 days of hospital admission
   • Measured temperature \( \geq 38^\circ C \) or history of fever is present in chart
   • Has either a cough or a sore throat
   • Has some evidence of difficulty breathing
2.F Interview guideline for SARI surveillance staff

1. How do you rate the amount of work that you have to undertake for SARI surveillance?
   - □ very acceptable
   - □ acceptable
   - □ unacceptable
   - □ highly unacceptable

2. What factors make participating in SARI surveillance difficult?

3. What factors make participating in SARI surveillance easy?

4. Has participating in influenza surveillance changed:
   (a) Your use of antivirals?
      - □ Yes
      - □ No
      i. If yes, how and since when?
   (b) Your use of antibiotics?
      - □ Yes
      - □ No
      i. If yes, how and since when?
   (c) Your clinical practice to assist you in the diagnosis of influenza?
      - □ Yes
      - □ No
      i. If yes, how and since when?

5. From the list below, what makes you decide to swab a patient? (Choose all that apply)
   - □ A patient who has a typical presentation of influenza
6. Would you sometimes swab a patient who does not exactly fit the SARI case definition? If so, what things influence your decision to swab a person?

7. During the low influenza season do you think it is easier to know that a patient has influenza (without testing) in any of the following age groups?
   - Under 1 year of age
   - 1 to 2 years
   - 2 to 5 years
   - 5 to 14 years

8. During the high influenza season do you think it is easier to know that a patient has influenza (without testing) in any of the following age groups?
   - Under 1 year of age
   - 1 to 2 years
   - 2 to 5 years
   - 5 to 14 years

9. From the list below, are there any age groups that you would avoid taking swabs from? (Please choose ONE or MORE)
   - Under 1 year of age
   - 1 to 2 years
   - 2 to 5 years
   - 5 to 14 years
   - There are no age groups that I avoid swabbing

10. Considering your answer to question 9, what are some of the reasons that you would avoid swabbing certain age groups?
11. Have you received any reports summarising Cambodian SARI surveillance data?
   □ Yes
   □ No
   (a) If yes, what did you find interesting about these reports?

12. Have you searched for reports summarising Cambodian SARI surveillance data?
   □ Yes
   □ No
   (a) If yes, what kind of data did you search for?

13. How many years have you worked at AHC?

14. What is your employment status?
   □ Part-time
   □ Full time
2.G Hospital admission survey training materials

The following slides were used to deliver a training workshop in Phnom Penh in May 2017. The aim of the workshop was to present an overview of the activities conducted and how to use hospital admission survey data to estimate influenza burden of disease. This workshop included interactive sessions using Excel and provided opportunity for discussion and reflection on challenges and lessons learned.
Estimating Influenza Disease Burden
Data Analysis Workshop
Monday – Tuesday
22.5.17 to 23.5.17
Cambodia CDC

Mr Vanra Ieng, WHO Cambodia
Dr Ximena Tolosa, WHO Collaborating Centre for Reference and Research on Influenza, Australia

Hospital Admission Review Data Analysis Training
Part 1: Monday, 22 May 2017
Cambodia CDC

Overall training objectives

- Understand the importance of quality SARI surveillance data in estimating the burden of influenza disease
- Identify challenges in the process of influenza burden estimation in Cambodia
- Adapt the WHO influenza burden estimation tool to the context of Cambodia

Learning Objectives Part 1 (22/05/2017)

- Handle and interpret surveillance data
- Decide which case definitions to use in disease burden estimation
- Assess the completeness, representativeness and accuracy of surveillance data through sensitivity analysis
- Assess bias
- Define the numerator using data based on a proportion of SARI cases

Many countries in WPRO region and around the world aim to estimate the influenza burden using their own SARI surveillance

Cambodia is using the HAR method for second time in another sentinel site in order to develop a protocol and tools
Burden of Influenza Studies

- Globally, influenza is known to cause a large amount of illness and deaths.
- First study in Cambodia, launched in August 2015.
- MOH commits to continue the burden of influenza studies in 2017 using SARI data from Siem Reap sentinel surveillance site, (children only).
- Knowing the burden of influenza in Cambodia would help decision makers to better manage prevention and treatment programs for influenza-related illness:
  - Risk communication during the yearly influenza season
  - Introducing influenza vaccine
  - Enable comparisons of annual seasonal influenza data with a baseline data

Burden of Influenza Studies I

- Kampong Cham, August 2015
- Concerns with catchment population

Burden of Influenza Studies II

- Svay Rieng, May 2016
- Concerns with hospital and population data quality

Study in Siem Reap, Angkor Hospital for Children sentinel surveillance site

- Characteristics of the 2016 influenza season, preliminary results
  - Increased influenza activity was between June and December based on national ILI surveillance.
  - Increased in number SARI reported cases occurred between July and November peaking in weeks 10, 46 and 48.
  - Sensitivity analysis in AHC revealed under-reporting of SARI cases

Burden of Influenza Studies III

- Siem Reap, May 2017
- Concerns with under-reporting of SARI cases

Study in Siem Reap, Angkor Hospital for Children sentinel surveillance site (cont.)

- Challenges
  - To estimate the true number of cases in Siem Reap sentinel site
  - To determine the catchment population of sentinel surveillance site (denominator)
  - Hospital Admission Review (HAR) is chosen as the most appropriate method to address this problem
REFINING THE HAR TOOL

SARI Surveillance

- Counts cases of SARI at eight sentinel surveillance sites around the country
- Can be used to estimate burden of severe influenza disease in Cambodia
- Severe influenza is defined as cases requiring hospitalization and/or resulting in death
- Provides a numerator for the burden estimate

Reporting System

Summary data at sentinel site

- Number of new SARI cases admitted
- Number of SARI cases that were sampled
- Number of sampled SARI cases that were positive for influenza
- Population of catchment area

Define SARI cases

Fever or history of fever (≥38.0°C)

AND

Cough or sore throat

AND

Shortness of breath or difficulty breathing

AND

Hospitalization

Great within 10 days prior to admission

Discussion:
Is Cambodia case definition different from WHO case definition?
How would that change who gets enrolled in SARI?

Fill in table with 2016 SARI surveillance data from Siem Reap sentinel surveillance site

<table>
<thead>
<tr>
<th>Positive SARI cases / SARI cases sampled (%)</th>
<th>Month</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤6 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;6 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Econtained</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥5 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NBR: National Respiratory Disease and Influenza Bulletin

64
Population age groups used in Cambodia
- Birth to 28d
- 1m to <1y
- 1y to <5y
- 5y to <15y
- 15y to <25y
- 25y to <50y
- 50y to <65y
- ≥65y

WHO age groups for global comparisons
- <6 months
- 6m to <1y
- 1y to <2y
- 2y to <5y
- 5y to <15y
- 15y to <50y
- 50y to <65y
- ≥65y

What other age groups might also work and why?
- Birth to <1y
- 1 to <5y
- 5 to <16y
- 16 to <50y
- 50 to <65y
- ≥65y

Are SARI cases reported through the surveillance the ‘true number’ of cases in the sentinel site?

- YES – why?
- NO – why?

SENSITIVITY OF SARI SURVEILLANCE

Sensitivity estimation
Sensitivity = Number of cases reported by surveillance in a defined time period / Number of cases meeting the case definition identified at hospital during the same time period
Sensitivity
• How many SARI cases in hospital?

All SARI Cases

100%

Sensitivity
• How many SARI cases in hospital are reported by SARI surveillance staff?

SARI cases not reported

25%

SARI Cases Detected by Surveillance Staff

75%

What are the reasons SARI cases are not reported?
• 1
• 2
• 3
• 4

How can this bias BOD estimates derived from the SARI surveillance?

How to conduct a sensitivity analysis
• Review SARI surveillance and reported cases
• Pick weeks to review with a variety of SARI and influenza activity in the site
• Count cases reported to CDC for those weeks
• Review ALL charts in the hospital for the selected weeks and count number of patients who meet SARI case definition

Review SARI Surveillance

Selected weeks to review
• Six weeks were selected (10, 12, 20, 35, 44, 45)
Count cases reported at central level for those weeks

<table>
<thead>
<tr>
<th>Age group</th>
<th>Week 10</th>
<th>Week 12</th>
<th>Week 20</th>
<th>Week 33</th>
<th>Week 44</th>
<th>Week 45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth to &lt;1y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1y to &lt;5y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 to &lt;16y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chart Review Methods
(Discussion)

- Charts not logbooks → Why?
- What are we looking for?
- What counts as fever?
- What counts as cough or sore throat?
- What counts as difficulty breathing?

Chart Review Methods
(Discussion cont.)

- Fever: history or measured - admission (?)
- Cough when written in chart - children <1month (?)
- Sore throat when written or diagnosis of pharyngitis – reported by parent (?)
- Difficulty breathing
  - Dyspnea
  - Tachypnea

Sensitivity Analysis Form

Exercise: Step 1. Count cases identified through chart review

Let’s try to pull this information together using excel
Exercise: Step 2. Compare surveillance data with identified cases through chart review

| Identified through surveillance/All cases identified through chart review with evidence of difficulty breathing (%) |
|---|---|---|---|---|---|
| Week | 10 | 12 | 20 | 33 | 44 | 45 |
| Birth to <1y | | | | | | |
| 1y to <5y | | | | | | |
| 5 to <16y | | | | | | |
| Total | | | | | | |

Exercise: Step 3. What if we used the WHO case definition?

| Identified through surveillance/All cases identified through chart review with no evidence of difficulty breathing (%) |
|---|---|---|---|---|---|
| Week | 10 | 12 | 20 | 33 | 44 | 45 |
| Birth to <1y | | | | | | |
| 1y to <5y | | | | | | |
| 5 to <16y | | | | | | |
| Total | | | | | | |

Identified differences with surveillance data:

- Is reported data accurate?
- Is the system sensitive?
- What dataset to trust?
- What are the next steps?

Respiratory Admission Diagnoses

Apply the weekly positivity rate to weekly reported SARI cases from the sentinel site, to estimate the ‘true number’ of SARI positive cases = numerator.

We used respiratory diagnoses instead of SARI cases in sentinel site.

- Data availability
- Time and resources constraints
Exercise: respiratory diagnoses as proxy for SARI

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Pediatrics</th>
</tr>
</thead>
<tbody>
<tr>
<td>SARI (a)</td>
<td></td>
</tr>
<tr>
<td>Not SARI (b)</td>
<td></td>
</tr>
<tr>
<td>% SARI (a/(a+b))</td>
<td></td>
</tr>
</tbody>
</table>

- Pneumonia
- Severe Pneumonia
- Bronchopneumonia
- Bronchial asthma
- Bronchitis
- Bronchiolitis
- Respiratory illness
- Pneumopathies
- Pulmonary TB
- Asthma
- Pharyngitis
- Rhino-pharyngitis
- Tonsillitis
- Laryngitis
- Lung abscess

Exercises
- Surveillance data extraction
- Sensitivity analysis using two case definitions
- Respiratory admissions compared to SARI cases

Siem Reap, May 2017
- Hospital Admission Review (to be continued)

Hospital Admission Review Data Analysis Training

Part 2: Tuesday, 23 May 2017
Cambodia CDC

Learning Objectives Part 2 (22/05/2017)
- Learn the importance of denominators in influenza burden of disease (BOD) estimation
- Learn how to define a catchment area
- Learn how to use HAR to find a denominator
  - Learn how to clean HAR data using Epi-info
- Learn how to calculate influenza BOD using the adjusted numerator and denominator obtained from the HAR
- Reflect on lessons learned for improved surveillance and reliable influenza BOD estimation

HOSPITAL ADMISSION REVIEW IN SIEM REAP
**Hospital Admission Review**

**Day 1**: Define numerator using SA at AHC

**Today**: Define denominator -> HAR to obtain information on catchment area for the selected SARI surveillance site: Angkor Hospital for Children in Siem Reap

To obtain a denominator for BOD estimation we need to:

1. Define a catchment area for the sentinel site
2. Locate all admitting HCF within the catchment area
3. Count respiratory admissions at sentinel site (AHC)*, public hospitals and private clinics
4. Divide respiratory admissions at sentinel site (AHC)* / respiratory admissions at all facilities within catchment area

\[
\text{SARI admissions @ AHC} \quad \text{respiratory admissions @ all health facilities}
\]

\[\Rightarrow\] proportion of SARI cases admitted at sentinel site

*Note: cases included are those that reside within catchment area (relevant districts)

---

**Location of sentinel site:**
Siem Reap province

**Catchment Area**

Where at least 80% of children SARI cases @ AHC live

---

**What is the catchment area for Angkor Hospital for Children (AHC) in Siem Reap?**

Cases at AHC could be residents from:
- Siem Reap
- Banteay Meanchey
- Oddar Meanchey
- Kampong Thom
- Preah Vihear

---

**Selected Boundary**

20 districts in 5 provinces

- Location of referral hospital
- Location of sentinel hospital

We are interested in all HCF that could admit children in this area
Public Hospitals in AHC Catchment Area (10)

Siem Reap Province:
• Angkor Chum Referral Hospital
• Kralanh Referral Hospital
• Pouk Referral Hospital
• Sotr Nikum Referral Hospital
• Kantha Bopha Children's Hospital (Jayavarman VII)

Kampong Thom Province:
• Stong Referral Hospital

Banteay Manchey Province:
• Mongkol Borey Provincial Hospital
• Phnom Srok Referral Hospital
• Preah Netr Preah Referral Hospital

Oddar Manchey Province:
• Anlong Veng Referral Hospital

Private health facilities in AHC Catchment Area

Private clinics in Siem Reap Province (10):
• Angkor Sante Polyclinic ✓
• Neak Tep Clinic ✓
• 777 Clinic ✓
• Sokha Pheap Clinic ✓
• Komapich Clinic ✓
• Chea Leng Clinique (Puok District) ✓
• Mekong SR International Polyclinic ✗
• Reaksmeay Angkor International Hospital ✗
• Sing Rithireth Clinic ✗
• Taprum Clinic ✗

Private clinics in Banteay Manchey Province:
• 77 Clinic ✓
• Sokha Pheap Clinic ✓
• Komapich Clinic ✓

Generating and managing the HAR database

• All respiratory admissions at 17 facilities (10 public + 6 private + 1 sentinel site) were counted and recorded to paper forms
• Data from paper forms was entered twice into Epi Info by different data collectors
• Data cleaning to ensure quality and completeness of data
• 1st and 2nd entry databases consolidated into one. First deal with discrepancies between collectors (example in next slide)
• Data aggregated into groups for analysis (combining ages into groups for example)

Data cleaning example – Consolidating 1st and 2nd entry

Data quality/completeness issues found

Summary of data issues
• Anlong Veng Referral Hospital uses its own outcome codes ≠ to HAR codes => discrepancies between entries
• When several relevant diagnosis where listed only one was entered in Epi Info => discrepancies between entries
• Stoung Referral Hospital, Angkor Chum Referral Hospital and Phnom Srok Referral Hospital don’t record DoO => need for training
• Phnom Srok Referral Hospital and Stoung Referral Hospital don’t record outcomes
Data cleaning
Data completeness issues found

- Signs listed instead of a diagnosis
- Questionable patient path:
  Admission dx: foot abscess, discharge dx: laryngitis
  Admission dx: bronchiolitis, discharge dx: infected wound

⇒ Need to reinforce training
- Often location of patient transfer not listed

Count all respiratory admissions at all health facilities

<table>
<thead>
<tr>
<th>Health care facility name</th>
<th>AHC</th>
<th>All sites</th>
<th>AHC/All sites (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Divide respiratory admissions at sentinel site by respiratory admissions at all hospitals

<table>
<thead>
<tr>
<th>AHC</th>
<th>All sites</th>
<th>AHC/All sites (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth to &lt; 1 y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 y to &lt; 5 y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 y to &lt; 16 y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CATCHMENT POPULATION DATA

<table>
<thead>
<tr>
<th>Age group</th>
<th>Birth to &lt; 1 y</th>
<th>1 y to &lt; 5 y</th>
<th>5 y to &lt; 16 y</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth to &lt; 1 y</td>
<td>26,281</td>
<td>2,995</td>
<td>1,811</td>
<td>3,632</td>
</tr>
<tr>
<td>1 y to &lt; 5 y</td>
<td>208,481</td>
<td>31,086</td>
<td>21,145</td>
<td>20,322</td>
</tr>
<tr>
<td>5 y to &lt; 16 y</td>
<td>208,481</td>
<td>31,086</td>
<td>21,145</td>
<td>46,808</td>
</tr>
<tr>
<td>Total</td>
<td>208,481</td>
<td>31,086</td>
<td>21,145</td>
<td>46,808</td>
</tr>
</tbody>
</table>

*Note: from catchment area

Obtain Population Data for Catchment Area

AHC Catchment Population

Class to complete

<table>
<thead>
<tr>
<th>Age group</th>
<th>Birth to &lt; 1 y</th>
<th>1 y to &lt; 5 y</th>
<th>5 y to &lt; 16 y</th>
<th>Total Catchment Area Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth to &lt; 1 y</td>
<td>208,481</td>
<td>31,086</td>
<td>21,145</td>
<td>46,808</td>
</tr>
<tr>
<td>1 y to &lt; 5 y</td>
<td>208,481</td>
<td>31,086</td>
<td>21,145</td>
<td>20,322</td>
</tr>
<tr>
<td>5 y to &lt; 16 y</td>
<td>208,481</td>
<td>31,086</td>
<td>21,145</td>
<td>46,808</td>
</tr>
<tr>
<td>Total</td>
<td>208,481</td>
<td>31,086</td>
<td>21,145</td>
<td>46,808</td>
</tr>
</tbody>
</table>
AHC Catchment Population (CA) calculation

- Estimated denominator for each age group = Proportion of respiratory cases for same age group from CA that were admitted to AHC (1 in slide 18) X Total population for that age group in CA (2 in slide 21)

**AHC SARI INCIDENCE RATE**

**SARI Incidence Rate Formula**

Calculate the annual incidence rate of influenza-associated SARI using the formula:

\[
\text{Incidence rate per 100,000} = \left( \frac{\text{Annual SARI cases} \times \text{SA adjustment}}{\text{Estimated catchment population of AHC}} \right) \times 100,000
\]

**AHC CATCHMENT AREA INFLUENZA-ASSOCIATED SARI INCIDENCE RATE**
### AHC Catchment Area

**Incidence Rate of Influenza-Associated SARI**

<table>
<thead>
<tr>
<th>Age group</th>
<th>Birth to &lt; 1 y</th>
<th>1 to &lt; 5 y</th>
<th>5 to &lt; 16 y</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza-associated SARI cases (a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SARI cases with adjustment (a X 1.5 SA adjustment)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estimated catchment population of AHC (b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence rate of SARI for AHC catchment population (per 100,000)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[117x220]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**SARI INCIDENCE RATE FOR AHC CATCHMENT AREA**

Calculating Incidence Rate for the Catchment Area

Estimate the number of new cases in the district for the year by using the formula in Equation B.

\[
\text{Annual estimated number of new cases of influenza-associated SARI} = \text{Incidence rate (IR) of influenza-associated SARI for sentinel catchment area} \times \text{District Population at midyear}
\]

<table>
<thead>
<tr>
<th>Age group</th>
<th>SARI Incidence Rate (IR)</th>
<th>CA Population</th>
<th>IR x Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth to &lt; 1 y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 to &lt; 5 y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 to &lt; 16 y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Influenza-associated SARI Incidence Rate for AHC Catchment Area

<table>
<thead>
<tr>
<th>Age group</th>
<th>Influenza-associated SARI Incidence Rate (IR)</th>
<th>Provincial Population</th>
<th>IR x Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth to &lt; 1 y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 to &lt; 5 y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 to &lt; 16 y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
How do we use this information?

- To improve surveillance
- To provide adjustment for our numerator calculation
- Numerator = Adjusted No SARI cases \times \% Influenza Positivity

Lessons learned & Recommendations

- Reinforce training surveillance staff at all HCF
  - Enrolment of cases
  - Registration of enrolled cases
  - Consistency between private / public health facilities
- Refine the HAR tool
  - Simplify data collection by reducing n of relevant diagnosis
    (more efficient/effective use of resources)
- Obtain necessary support for full Applied Epidemiology Trainees involvement

References

End of Mission Report
Hospital Admission Review
Siem Reap Sentinel Site
7 - 23 May, 2017

23 May 2017, Cambodian Communicable Disease Control Department, Ministry of Health
Phnom Penh, Cambodia

Ximena Tolosa, PhD, MIPH
MPhil in Applied Epidemiology Scholar at
The Australian National University

Objectives of the HAR

- Conduct sensitivity analysis at Siem Reap province SARI surveillance sentinel site, Angkor Hospital for Children (AHC) to obtain an appropriate population numerator for BOD estimation
- Conduct a Hospital Admission Review to determine healthcare seeking behavior and therefore an appropriate population denominator for BOD estimation

What was achieved

- Sensitivity analysis at Angkor Hospital for Children (AHC) 9-10 May 2017
- Six weeks were selected for sensitivity analysis (10, 12, 20, 35, 44, 45)
- Criteria for choosing weeks: variety of influenza activity and positivity rate as per graph below
- Interviews conducted with SARI surveillance staff at AHC

<table>
<thead>
<tr>
<th>Week</th>
<th>No. potential SARI cases identified in sensitivity analysis</th>
<th>No. SARI cases reported to CDC</th>
<th>Proportion reported</th>
<th>Proportion not reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>13</td>
<td>5</td>
<td>38.5%</td>
<td>61.54%</td>
</tr>
<tr>
<td>12</td>
<td>11</td>
<td>7</td>
<td>63.6%</td>
<td>36.36%</td>
</tr>
<tr>
<td>20</td>
<td>7</td>
<td>3</td>
<td>42.9%</td>
<td>57.14%</td>
</tr>
<tr>
<td>35</td>
<td>9</td>
<td>10</td>
<td>111.1%</td>
<td>-11.11%</td>
</tr>
<tr>
<td>44</td>
<td>28</td>
<td>24</td>
<td>85.7%</td>
<td>14.29%</td>
</tr>
<tr>
<td>45</td>
<td>30</td>
<td>6</td>
<td>20.0%</td>
<td>80.00%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>98</td>
<td>55</td>
<td>56.1%</td>
<td>43.9%</td>
</tr>
</tbody>
</table>

What was achieved – Field work preparation

- Prior to field work:
  - Support from Angkor Hospital for Children was obtained
  - Data from 2016 was selected for HAR
  - A catchment area (CA) was defined
- HAR team members
  - MOH CDC (5)
  - AET graduates (6)
  - Provincial Health Department (4)
  - NIPH (1)
  - US CDC (1)
  - WHO (4)
- Most hospitals prepared for our visit (relevant logbooks selected)

What was achieved – Field work

- Sensitivity analysis at Angkor Hospital for Children (9-10 May 2017)
- Full patient record review
- More cases detected by chart review compared to SARI surveillance system => underreporting
Catchment area and location of referral hospitals visited

- Banteay Meanchey
- Oddar Meanchey
- Preah Vihear
- Kampong Thom
- Siem Reap

All districts in SR (12), 3 districts in BM, 2 districts in OM, 1 district in PV and 2 districts in KT

What was achieved – Field work

Training was provided before starting data collection (10/5/17 at Siem Reap Provincial Hospital)

Explained the SARI case definition and importance of capturing cases within catchment area

What was achieved – Field work

Public hospitals visited in 4 provinces (10):

- Siem Reap Province:
  - Angkor Chum Referral Hospital
  - Kralanh Referral Hospital
  - Pouk Referral Hospital
  - Sotrnikum Referral Hospital
  - Kantha Bopha Children’s Hospital (Jayavarman VII)*

- Banteay Meanchey Province:
  - Mongkol Borey Provincial Hospital
  - Phnom Srok Referral Hospital
  - Peakh Netr Peakh Referral Hospital

- Kampong Thom Province:
  - Stong Referral Hospital

- Oddar Meanchey Province:
  - Anlong Veng Referral Hospital

Kampong Thom Province:
- Stong Referral Hospital

Oddar Meanchey Province:
- Anlong Veng Referral Hospital

*KTB provided data as pdf, yet to be entered into our database

What was achieved – Field work

Private clinics visited in Siem Reap Province (10):

- Angkor Sante Polyclinic ✓
- Neak Tep Clinic ✓
- 777 Clinic ✓
- Sokha Pheap Clinic ✓
- Komapich Clinic ✓
- Chea Leng Clinic (Puok District) ✓
- Reaksmey Angkor International Hospital ✓
- Sing Rithireth Clinic ✓
- Mekong SR International Polyclinic ✓
- Taprum Clinic ✓

✓ Data obtained (6)
✗ Unable to obtain data (2)
✗ No patients admitted (2)

What was achieved – Field work

Private clinics visited in Siem Reap Province (10):

- Angkor Sante Polyclinic ✓
- Neak Tep Clinic ✓
- 777 Clinic ✓
- Sokha Pheap Clinic ✓
- Komapich Clinic ✓
- Chea Leng Clinic (Puok District) ✓
- Reaksmey Angkor International Hospital ✓
- Sing Rithireth Clinic ✓
- Mekong SR International Polyclinic ✓
- Taprum Clinic ✓

✓ Data obtained (6)
✗ Unable to obtain data (2)
✗ No patients admitted (2)

What was achieved – Field work

HCF visited – Puok Referral Hospital

Referral Hospitals Visited

Axong Veng Referral Hospital

What was achieved – Field work

Private clinics visited in Siem Reap Province (10):

- Angkor Sante Polyclinic ✓
- Neak Tep Clinic ✓
- 777 Clinic ✓
- Sokha Pheap Clinic ✓
- Komapich Clinic ✓
- Chea Leng Clinic (Puok District) ✓
- Reaksmey Angkor International Hospital ✓
- Sing Rithireth Clinic ✓
- Mekong SR International Polyclinic ✓
- Taprum Clinic ✓

✓ Data obtained (6)
✗ Unable to obtain data (2)
✗ No patients admitted (2)
Private Health Care Facilities Visited

Some private clinics visited did not admit patients.

Counting cases and managing data

- Using logbooks, all respiratory admissions at 21 facilities (10 public + 10 private + 1 NGO) were counted and recorded to paper forms.
- Data from paper forms was entered twice into Epi Info by different data collectors.
- Data cleaning to check errors & completeness of data
  - Common errors noted for training data collectors.
- Data aggregated into age groups for analysis.
- Proportion of respiratory cases from CA that were admitted to AHC vs other HCF was calculated (by age group).
- Calculations for denominator in progress.
  - Proportion of flu-associated cases @ AHC x Catchment area population.

HAR data management – next steps

- Kantha Bopha Hospital for Children data needs to be digitised & entered into database.
- Once we enter KTB data and find population data for all relevant districts (challenging) we will calculate SARI-associated incidence rates by age group.

Data quality challenges found

- Some hospitals don’t record date of onset or outcomes in logbooks.
- Many admission and discharge diagnoses consisted of signs & symptoms only (cases missed?).
- Often location of patient transfer not listed.
- Important implications for the SARI surveillance system.
- Need to reinforce training of surveillance staff.

Next steps

- Estimation of influenza BOD for Siem Reap to be finalised.
- National influenza BOD estimation.
- Planning for a further HAR in Kampong Cham (to start 19 June 2017).
The Melbourne WHO Collaborating Centre for Reference and Research on Influenza is supported by the Australian Government Department of Health.

Acknowledgements

- Cambodian Communicable Disease Control Department
- Applied Epidemiology Training Program graduates
- Provincial Health Department
- National Institute of Public Health Laboratory
- Angkor Hospital for Children
- US CDC
- WHO Cambodia country office

The Melbourne WHO Collaborating Centre for Reference and Research on Influenza is supported by the Australian Government Department of Health.
2.1 Poster presented at the International Global Virus Network Meeting, Melbourne, September 2017

LEVERAGING SURVEILLANCE DATA FOR INFLUENZA PANDEMIC PREPAREDNESS: A CAMBODIAN EXAMPLE

TOLOSA MX1,2, Ieng V3, Tek B4, Theocharopoulos G5, Kheng B6, Seng H4, Ly S4, Leung VK2, Sullivan SG2,6,7

1National Centre for Epidemiology and Population Health, The Australian National University; 2WHO Collaborating Centre for Reference and Research on Influenza; 3WHO Cambodia office; 4Cambodian Ministry of Health; 5US CDC office in Cambodia; 6Department of Epidemiology, University of California, Los Angeles, USA; 7School of Population and Global Health, University of Melbourne

Background: The importance of public health surveillance data

Regular and timely analysis of public health surveillance data allows interpretation of the impact of seasonal epidemics and pandemics. Moreover, surveillance data can be used to provide an estimate of the burden of a disease in the population which is needed to design targeted policies for public health action.

Objective
• To estimate the burden of influenza illness in children in one province in Cambodia using a recently established system for routine surveillance of seasonal influenza

Methods used to calculate incidence rate of influenza-associated hospitalisations

• ESTIMATING INCIDENCE RATE NUMERATOR: Using 2016 surveillance data, we enumerated laboratory-confirmed influenza-associated severe acute respiratory illness (SARI) cases admitted at the Angkor Hospital for Children, a paediatric sentinel site in Siem Reap province
  → Conducted a sensitivity analysis to adjust numerator for under-reporting of SARI cases at the sentinel site

• ESTIMATING INCIDENCE RATE DENOMINATOR: To estimate the size of the population that sought care at the Angkor Hospital for Children (sentinel site catchment population) we conducted a hospital admission survey consisting of the following steps:
  1. DEFINE the catchment area for the SARI sentinel surveillance site (area where 80% SARI cases live)
  2. IDENTIFY all healthcare facilities that admit respiratory patients under 16 years of age within catchment area of the sentinel site
  3. ENUMERATE respiratory admissions at non-sentinel healthcare facilities using a validated list of diagnoses (pneumonia, bronchitis, pharyngitis, etc.) as a proxy for SARI
  4. CALCULATE proportion of respiratory cases that seek care at the sentinel site
  5. OBTAIN district-level population data for the catchment area
  6. ESTIMATE the sentinel site catchment population

• Estimated the number of hospitalisations due to influenza-associated SARI of children under 16 years of age in the entire province of Siem Reap by multiplying the influenza-associated SARI incidence rate by the provincial population

Results: Influenza-associated SARI hospitalisation rate in Cambodian children

Field work at a glance SARI sentinel surveillance sites in Cambodia

Influenza-associated SARI hospitalisation rate was highest among children under 1 year of age

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Influenza-associated SARI cases @ sentinel site</th>
<th>Sentinel Site Catchment Population</th>
<th>Influenza-associated SARI incidence rate (A/RE/7/2000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1 y</td>
<td>47</td>
<td>13,722</td>
<td>345</td>
</tr>
<tr>
<td>1-4 y</td>
<td>41</td>
<td>18,912</td>
<td>206</td>
</tr>
<tr>
<td>5-16 y</td>
<td>9</td>
<td>27,836</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>60,920</td>
<td>160</td>
</tr>
</tbody>
</table>

By extrapolation, we estimated 354 influenza-associated SARI hospitalisations in children under 16 years of age in Siem Reap province in 2016

Conclusions: Children under 1 year of age had highest burden of influenza

• First estimates of influenza-associated SARI hospitalisation rates in Cambodian children for the catchment area of the Angkor Hospital for Children
• Children under 1 year of age had highest burden of influenza due to severe respiratory illness (345/100,000 population)
• Recommendations included addressing data quality issues at sentinel site and non-sentinel healthcare facilities
• Estimation of the burden of influenza in Siem Reap province is an important step in estimating the national burden influenza in Cambodia

The Melbourne WHO Collaborating Centre for Reference and Research on Influenza is supported by the Australian Government Department of Health.

The presenting author can be contacted at ximena.tolosa@anu.edu.au
2.J Communication summary for a general, non-specialist, audience

This article was published by the Graduate Union of the University of Melbourne in July 2017. Click here to access it online.

Understanding the impact of influenza virus infections in Cambodia

by Graduate House Admin | Jul 26, 2017 | Good health and well-being, Our Members, Reduced Inequalities, Sustainable Cities and Communities, Sustainable Development Goals | 0 comments

Photo: Dr Ximena Tolosa (front row, first from the right) with Cambodian public health authorities, infectious disease control personnel and colleagues from the World Health Organization in Phnom Phen, Cambodia, May 2017
Dr Ximena Tolosa is a microbiologist with qualifications in international public health. In February 2017 she joined the Australian Field Epidemiology Training Program (FETP) run by the National Centre for Epidemiology and Population Health at the Australian National University. The FETP is a two-year program that prepares Australian public health professionals to address a wide range of public health problems, from tackling the emergence of zoonotic diseases to setting up public health surveillance systems and responding to outbreaks of disease. A key goal for the field epidemiologist is to improve population health by preventing and responding to health threats. Of course, disseminating lessons learned is critical to epidemiology as it ensures that for each health problem addressed there is a shared understanding regarding best practice. FETPs currently exist in 61 countries and form a network called Training Programs in Epidemiology and Public Health Interventions Network (TEPHINET). While placed in state health departments and research institutes, FETP scholars provide a public health service to their countries and regions while strengthening international public health capacity.

“I am one of 16 field epidemiology scholars in the 2017 Australian cohort. Professional backgrounds in the 2017 cohort range from medicine, nursing, microbiology, anthropology, Indigenous health, environmental health and public health,” says Dr Tolosa.

“My field placement is at the World Health Organization (WHO) Collaborating Centre for Reference and Research on Influenza located at the Peter Doherty Institute for Infection and Immunity. My first project involved a one month trip to Cambodia to better understand the impact of influenza virus infections in Cambodian children. This is part of a larger study that aims to estimate the burden of influenza in the entire population. Currently, the burden of influenza in Cambodia and other developing countries in the region is not well understood. If the scale of a health problem is unknown it is unlikely that action will be taken to prevent it, leaving subpopulations vulnerable to severe disease and at further disadvantage. In practical terms, understanding the burden of influenza infections in Cambodia is important so public health officials can design evidence-based public health policies, and conduct prevention and control activities to limit the spread of disease. Armed with knowledge of seasonal influenza activity and a more robust public health system, Cambodia will also be better prepared to protect its population against the threat of pandemic influenza.

In May 2017 I travelled to Cambodia to work with a team from the Ministry of Health and the WHO Cambodia country office to estimate the influenza burden of disease. For this we visited several healthcare facilities in the north west of Cambodia and reviewed hospital admission registries for 2016 to identify cases of severe acute respiratory infection. Importantly, the methodology we used
accounted for specific challenges that developing countries face when trying to calculate the incidence rates of diseases.

Besides assisting public health officials in Cambodia in conducting an epidemiological study I co-delivered two training courses to enhance the skills of local epidemiologists in data management and data analysis. It is energising to participate in building public health capacity in our region. More important than assisting a country to perform a specific task is ensuring that technical knowledge is transmitted to relevant individuals so that they can carry on working independently. This is how I understand international solidarity. As the recent Ebola epidemic has taught us, a swift and collaborative response is needed to halt disease transmission and further spread, but what is needed to prevent another large-scale humanitarian emergency is strengthening public health systems in low-income countries. When dealing with the threat of infectious diseases in a highly interconnected world, this approach becomes critical.

I would like to acknowledge the support of my supervisors Dr Sheena Sullivan and Dr Tambri Housen for facilitating my participation in this project. This work was supported by an Australian Government Research Training Program (RTP) Scholarship.”

If you are interested in a career as a disease detective you can learn more about the Australian FETP program.
2.K  Article published in the Western Pacific Surveillance and Response Journal

National burden of influenza-associated hospitalizations in Cambodia, 2015 and 2016

Vannar Ieng,* M Ximena Tolosa,* Bunchhoeng Tek, Borann Sar, Kheng Sim, Heng Seng, Miliya Thy, Chan Dara, Mey Moniborin, Rebekah J. Stewart, Leila C. Bell, Georgios Theocharopoulos, Sovath Chin, Dorapheak Chau, Danielle Iuliano, Ann Moen, Reiko Tsuyuoka, Erica L. Dueger, Sheena G. Sullivan, and Sovann Ly

Correspondence to M Ximena Tolosa (email: ximena.tolosa@anu.edu.au)

Introduction: The burden of influenza in Cambodia is not well known, but it would be useful for understanding the impact of seasonal epidemics and pandemics and to design appropriate policies for influenza prevention and control. The severe acute respiratory infection (SARI) surveillance system in Cambodia was used to estimate the national burden of SARI hospitalizations in Cambodia.

Methods: We estimated age-specific influenza-associated SARI hospitalization rates in three sentinel sites in Svay Rieng, Siem Reap and Kampong Cham provinces. We used influenza-associated SARI surveillance data for one year to estimate the numerator and hospital admission surveys to estimate the population denominator for each site. A national influenza-associated SARI hospitalization rate was calculated using the pooled influenza-associated SARI hospitalization rates for all sites as a numerator and the pooled catchment population of all sites as denominator. National influenza-associated SARI case counts were estimated by applying hospitalization rates to the national population.

Results: The national annual rates of influenza-associated hospitalizations per 100 000 population was highest for the two youngest age groups at 323 for <1 year and 196 for 1–4 years. We estimated 7547 influenza-associated hospitalizations for Cambodia with almost half of these represented by children younger than 5 years.

Discussion: We present national estimates of influenza-associated SARI hospitalization rates for Cambodia based on sentinel surveillance data from three sites. The results of this study indicate that the highest burden of severe influenza infection is borne by the younger age groups. These findings can be used to guide future strategies to reduce influenza morbidity.

Influenza is a contagious, acute respiratory infection caused by influenza viruses. Globally, seasonal influenza causes significant morbidity, mortality and socioeconomic costs. Accurate figures of the burden of influenza are difficult to estimate. Robust vital statistics and civil registration, well-functioning surveillance systems, hospital discharge databases and the expansion of influenza molecular testing have allowed more countries to complete influenza burden estimations. However, due to data quality and availability issues, the burden of seasonal influenza in low-income, lower middle-income and tropical climate countries is not well documented. Consequently, many countries lack influenza prevention and control policies. Limited available data indicate that influenza burden in tropical settings, defined as areas with humid or...
METHODS

Data sources

SARI sentinel surveillance sites

SARI surveillance in Cambodia includes eight sentinel surveillance sites. For this study, sentinel sites were public health care inpatient facilities (HCFs) where SARI patients were identified and clinical, demographic information and respiratory specimens were collected. A SARI case was defined as measured fever (temperature ≥38 °C) or history of fever, and cough or sore throat, and shortness of breath or difficulty breathing in a hospitalized person with onset of symptoms within 10 days before hospitalization. All data were recorded in a secure online database. Sentinel sites were located in Phnom Penh (two sites), Kandal, Siem Reap, Takeo, Kampong Cham, Svay Rieng and Kampot provinces (Fig. 1). New SARI cases were reported weekly by sentinel sites throughout the year. National virological and epidemiological surveillance data were reported in a monthly respiratory bulletin and published online.

To estimate SARI rates, we used data from the three sentinel sites where Hospital Admission Surveys (HAS) had been conducted (Fig. 1). Two sites were rural and one was urban. Only three of the eight sites were included in the HAS due to resource limitations. Criteria used for site selection were site acceptance to participate in HAS and either the perceived quality of their data or availability of medical records in English. Additional details on sentinel sites, case definitions and laboratory methods are available in Appendix I and II.

Hospital admission surveys

Hospital admission surveys were conducted in three locations to estimate the catchment population of each sentinel site using methods recommended by WHO and piloted at the Svay Rieng sentinel site. First, the addresses of the SARI cases admitted to the sentinel site were reviewed, and the catchment area for each site was defined as the districts from which 80% of the SARI cases admitted to the sentinel hospitals came (Fig. 1).
Influenza-associated hospitalizations in Cambodia

Ieng et al

We refer to the catchment area of each site as Svay Rieng, Siem Reap and Kampong Cham.

Second, we listed the non-sentinel health facilities in the catchment areas of the sentinel sites that admitted patients overnight. We visited these health facilities to enumerate respiratory admissions consistent with the following diagnoses: acute pulmonary oedema, asthma, asthma-pneumonia, bronchiolitis, bronchitis, broncho-asthma, broncho-pneumonia, flu/cold, laryngitis, lung abscess/empyema, pharyngitis, pneumonia, pneumopathy, pulmonary tuberculosis, respiratory infection, rhino-pharyngitis, severe pneumonia and tonsillitis. These diagnoses, which were collected from hospital log books, represent a proxy measure for SARI diagnosis. We collected information from 38 privately operated non-sentinel HCFs from 1 January–31 December 2015 (Svay Rieng site) and 1 January–31 December 2016 (Siem Reap and Kampong Cham sites). The data collection team (approximately 12 enumerators and four supervisors) used paper-based forms to collect data from eight non-sentinel HCFs in Svay Rieng, 16 in Siem Reap and 14 in Kampong Cham. Non-sentinel HCFs kept records in Khmer, French, Vietnamese and English. Enumerators captured data recorded in Khmer or English. HAS data were entered in data collection forms and subsequently entered into Epi Info 7 in English.

We calculated the age-specific proportion of SARI cases that sought care at each sentinel site out of all respiratory admissions across all HCFs in the catchment area. Admissions from patients that resided outside the catchment area were excluded from both the numerator and the denominator. We assume the proportion of catchment population of the sentinel site to the total population density by province, 2008.

HAS: Hospital Admission Surveys; SARI: severe acute respiratory infection

* Red circles represent the SARI sentinel surveillance sites that participated in the HAS, and black circles indicate all other SARI sentinel surveillance sites. Red contour lines surrounding HAS sites represent catchment areas for the three sentinel sites that participated in the HAS. Map created with ArcGIS 10.2 software by Environmental Systems Resource Institute (Redlands, CA, USA).
RESULTS

Counting SARI cases at sentinel sites: findings from SARI surveillance

Overall, 2868 SARI cases were enrolled: 203 cases at Svay Rieng site, 922 cases at Siem Reap site and 1743 cases at Kampong Cham site. The majority of influenza-associated SARI cases in all sites combined were children under 5 years of age (51%) followed by the two older age groups (50–64 years and ≥65 years) representing 21% of SARI admissions (Table 1).

Validation of SARI data at three sentinel sites

In Siem Reap, 259 records from patients hospitalized during six weeks in 2016 were reviewed and 98 met the SARI case definition. The surveillance system identified 55 of these cases, indicating that 56% of SARI cases were identified and enrolled in surveillance. In Kampong Cham, we reviewed 99 records from patients hospitalized during six weeks in 2016. Of these, 28 patients met the SARI case definition and only 19 of these were captured by the surveillance system (32% underreporting). In Svay Rieng, we did not find underreporting. Instead we found overreporting by the surveillance system (i.e. 50 SARI cases were reported by the surveillance system compared to 41 identified by medical records review).15

Some respondents of the staff surveys reported that surveillance activities represented an acceptable workload. Challenges identified in the survey included difficulties in obtaining consent for specimen collection in children, swabbing distressed children, difficulties in applying the SARI case definition due to incomplete or unclear medical histories, parental misunderstanding regarding the purpose of specimen collection, difficulties in applying the case definition to neonates and fear of reprimand if unable to collect specimens due to lack of parental consent. Through staff surveys we found that SARI surveillance underestimated SARI in infants and children as those without swabs were not counted as SARI.

Influenza viruses circulated year-round with peaks in July and August. Multiple influenza virus types and subtypes were detected in 2015 and 2016; the predominant viruses were influenza A(H3N2) in 2015 and both A(H1N1)pdm09 and B in 2016 (Fig. 2).
Table 1. Number of annual severe acute respiratory infection (SARI) cases and influenza-positive cases by age group and sentinel site, 1 January–31 December 2015 (Svay Rieng) and 1 January–31 December 2016 (Siem Reap and Kampong Cham, Cambodia)

| Age group (years) | SARI cases | Svay Rieng | Per cent positive for influenza | Influenza-associated SARI cases | Siem Reap* | SARI cases | Per cent positive for influenza | Influenza-associated SARI cases† | Kampong Cham | SARI cases | Per cent positive for influenza | Influenza-associated SARI cases† | Total influenza-associated SARI cases
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>8</td>
<td>0% (0/8)</td>
<td>0</td>
<td>455</td>
<td>10.4% (15/144)</td>
<td>47</td>
<td>381</td>
<td>10.0% (2/20)</td>
<td>38</td>
<td>85</td>
<td>24%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–4</td>
<td>18</td>
<td>11.1% (2/18)</td>
<td>2</td>
<td>376</td>
<td>10.9% (19/175)</td>
<td>41</td>
<td>256</td>
<td>20.8% (10/48)</td>
<td>53</td>
<td>96</td>
<td>27%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5–15</td>
<td>6</td>
<td>33.3% (2/6)</td>
<td>2</td>
<td>91</td>
<td>10.0% (1/10)</td>
<td>9</td>
<td>157</td>
<td>30.0% (3/10)</td>
<td>47</td>
<td>58</td>
<td>16%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16–24</td>
<td>4</td>
<td>25.0% (1/4)</td>
<td>1</td>
<td></td>
<td>NA</td>
<td></td>
<td>91</td>
<td>12.0% (3/25)</td>
<td>11</td>
<td>12</td>
<td>3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25–49</td>
<td>40</td>
<td>7.5% (3/40)</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>244</td>
<td>11.4% (8/70)</td>
<td>28</td>
<td>31</td>
<td>9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50–64</td>
<td>61</td>
<td>6.6% (4/61)</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>280</td>
<td>10.0% (3/30)</td>
<td>28</td>
<td>32</td>
<td>9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥65</td>
<td>66</td>
<td>7.6% (5/66)</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td>334</td>
<td>11.1% (5/45)</td>
<td>37</td>
<td>42</td>
<td>12%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>203</td>
<td>8.4% (17/203)</td>
<td>17</td>
<td>922</td>
<td>10.6% (35/339)</td>
<td>97</td>
<td>1743</td>
<td>13.7% (34/248)</td>
<td>242</td>
<td>356</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Siem Reap sentinel site was a pediatric hospital and admitted children <16 years of age.
† Per cent positive for influenza is the proportion of SARI cases that tested positive for influenza.
‡ Influenza-associated SARI cases were calculated by applying the age-specific influenza per cent positive for each month to the corresponding SARI case count for each month.

Fig. 2. Number of influenza-positive SARI cases by month and subtype/linage reported by all (eight) SARI surveillance sites, 1 January 2015–31 December 2016, Cambodia

* Influenza per cent positive is the proportion of SARI cases that tested positive for influenza.
Source: SARI sentinel surveillance system, Cambodia.19,20
Estimated annual influenza-associated SARI hospitalization rate

The site-specific influenza-associated SARI hospitalizations rate varied widely. In 2015, the all-age influenza-associated hospitalized rate in Svay Rieng was 7/100 000 population (Table 2). In 2016, the all-age rates in Kampong Cham were 72/100 000 population and much higher in the pediatric population (160). The combined influenza-associated SARI hospitalization rate was highest for children <1 year (323/100 000 population) and 1–4 years (196) followed by those aged ≥65 years (91). Influenza-associated SARI hospitalization rates varied by site – with the largest differences seen in the <1 years age group – from 0 for Svay Rieng to 495 per 100 000 in Kampong Cham. Hospitalization rates for Kampong Cham were higher compared with other sites for all age groups. Estimated age-adjusted influenza-associated SARI hospitalizations in Cambodia in 2016 were 7547 with most hospitalizations among children <16 years of age (5328/7547).

DISCUSSION

We present the first national burden estimate of severe influenza in Cambodia using hospital-based influenza surveillance data representing a climatically and demographically representative sample of hospitalizations in Cambodia in both rural and urban areas. Our findings indicate that influenza is an important contributor to hospitalizations in Cambodia particularly among children <5 years of age. In two sites, we observed that infants (<1 year) had the highest influenza-associated SARI hospitalization rates (345 and 495 hospitalizations per 100 000 population) followed by children aged 1–4 years (206 and 338 cases per 100 000 population). Our combined estimates of influenza-associated SARI hospitalizations in children are consistent with findings from African countries21,22 but higher than those reported for Indonesia and India (82–114 and 118/100 000 children 0–4 years, respectively).23,24

When age-specific influenza-associated SARI hospitalization rates could be estimated across all age groups, we observed higher rates in infants and young children, lower rates in working-age adults and higher rates among those >65 years of age. The same patterns of influenza burden have been reported in tropical climate countries. For example, the Lao People’s Democratic Republic reported hospitalization rates of 156, 44, 9 and 42 per 100 000 population in 0–4, 5–14, 15–64 and 65 years age groups, respectively.25 In both Zambia and Rwanda influenza-associated hospitalization rates in infants were highest compared to all other age groups (484 and 295/100 000 children <1 year, respectively), and rates were lowest for the 5–24 years age group (6 and 11/100,000 5–24 years, respectively).21,22 Compared to the hospitalization rates we estimated for older Cambodian adults, those reported for Zambia and Rwanda were lower (57 and 34/100 000 population >65 years).21,22

The combined burden of influenza hospitalizations across all age-groups estimated for Cambodia (56/100 000 population) is similar to that reported for Zambia (44)21 but higher than Rwanda (35)22 and Indonesia (19).23 Influenza hospitalization burden likely varies both within and between countries. This may be explained by virological, geographical, sociological (health care-seeking behaviour), underlying health status of the population and burden estimation approaches.

Consistent with previous reports from Cambodia, countries in the region and globally,2,21,22 influenza activity was detected throughout the year with peaks between March and December. In 2015 the predominant strain was influenza A(H3N2), whereas in 2016 A(H1N1)pdm09 and B co-circulated. Influenza A(H3N2) typically causes more severe disease in children and older adults compared with other seasonal influenza strains.2 Therefore, differences in the predominant strain may not entirely explain the lower rates observed in 2015 in Svay Rieng.

Several limitations were identified in this study. The burden of influenza for Svay Rieng was estimated using data from 2015, the first year of operation of surveillance, whereas the other sites used 2016 data, the second year of surveillance. Using data from well-established systems collected in the same calendar year would improve comparability among sites and years. This is particularly important given that the predominant influenza circulating strains usually differ between years, which is associated with specific disease severity and therefore differing impacts on hospitalization rates. Additionally, multiple years of surveillance data are needed to reliably quantify the burden of influenza.

Furthermore, we estimated the burden of influenza based on three of the eight sentinel sites. The associated
### Table 2. Estimated annual influenza-associated severe acute respiratory infection (SARI) hospitalization rate (and 95% confidence interval) by age group for each sentinel site and nationally, 2015 (Svay Rieng) and 2016 (Siem Reap and Kampong Cham, Cambodia)

<table>
<thead>
<tr>
<th>Age group (Years)</th>
<th>Site-specific influenza-associated SARI hospitalization rate (HR) per 100 000 population*</th>
<th>Combined influenza-associated SARI hospitalization rate per 100 000 population†</th>
<th>Cambodian population</th>
<th>National influenza-associated SARI case count‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>0.44 (245.8–459.2)</td>
<td>245.9 (360.3–679.9)</td>
<td>323.0 (261.3–399.3)</td>
<td>348.518</td>
</tr>
<tr>
<td>1–4</td>
<td>1.48 (3.7–59.3)</td>
<td>206.1 (151.6–280.0)</td>
<td>338.2 (258.6–442.3)</td>
<td>1.235.655</td>
</tr>
<tr>
<td>5–15</td>
<td>4.7 (1.2–18.7)</td>
<td>33.2 (17.4–63.6)</td>
<td>195.7 (147.1–260.4)</td>
<td>2.880.177</td>
</tr>
<tr>
<td>16–24</td>
<td>1.9 (0.3–13.4)</td>
<td>NA</td>
<td>16.6 (9.2–30.1)</td>
<td>3.334.307</td>
</tr>
<tr>
<td>25–49</td>
<td>3.6 (1.2–11.2)</td>
<td>22.2 (15.3–32.2)</td>
<td>14.8 (10.4–21.1)</td>
<td>5.066.335</td>
</tr>
<tr>
<td>50–64</td>
<td>12.9 (4.8–34.4)</td>
<td>44.9 (31.0–65.1)</td>
<td>35.4 (25.1–49.7)</td>
<td>1.544.946</td>
</tr>
<tr>
<td>≥65</td>
<td>36.2 (15.0–86.9)</td>
<td>110.3 (79.9–152.1)</td>
<td>90.9 (67.4–122.4)</td>
<td>677.422</td>
</tr>
<tr>
<td>Total</td>
<td>7.0 (4.4–11.3)</td>
<td>159.7 (130.1–194.9)</td>
<td>72.4 (63.8–82.1)</td>
<td>561 (50.6–62.2)</td>
</tr>
</tbody>
</table>

* Site-specific HRs were estimated using methodology described in the WHO Manual for Estimating Disease Burden Associated with Seasonal Influenza.‡ The national influenza-associated SARI case count was estimated by applying the combined influenza-associated SARI hospitalization rate (A) to the Cambodian population (B) and dividing by 100 000.§ The influenza-associated SARI hospitalization rate for Svay Rieng was calculated using 2015 surveillance data (whereas data for Siem Reap and Kampong Cham used 2016 data). The Svay Rieng HR slightly differs from previously published rates due to a different population data source used for their calculation.† The combined influenza-associated SARI hospitalization rate was estimated by adding influenza-associated SARI cases from all three HAS sites (i.e. adding column A for each site in Table 2 in Appendix IV), dividing by the sum of the three catchment populations (i.e. adding column D for each site in Table 2 in Appendix IV) and multiplying by 100 000.‡ The national influenza-associated SARI case count was estimated by applying the combined influenza-associated SARI hospitalization rate (A) to the Cambodian population (B) and dividing by 100 000.§ The influenza-associated SARI hospitalization rate for Svay Rieng was calculated using 2015 surveillance data (whereas data for Siem Reap and Kampong Cham used 2016 data). The Svay Rieng HR slightly differs from previously published rates due to a different population data source used for their calculation.† The sentinel site in Siem Reap was a pediatric hospital that admitted children under 16 years of age.** The total national influenza-associated SARI case count was calculated as the sum of all values in the column. Only two decimal places are displayed, but calculations used >10 decimal places.
hospitalized with severe respiratory illness attributed to influenza in Cambodia (10.9% of all SARI hospitalizations, all-ages average) is comparable to that reported for Thailand (10.4%) and Indonesia (14%).\textsuperscript{23,28}

One important strength of the study is the data validation conducted to understand the extent of underreporting and the potential surveillance operational challenges.

The results of this study can be used by the Ministry of Health in Cambodia to consider the introduction of influenza vaccination to reduce the impact of influenza-associated hospitalizations in the most vulnerable population groups: children and elderly people. Furthermore, this work underscores the value of investing in routine influenza surveillance in low–middle-income countries as key drivers of population health and pandemic preparedness.

Conflict of interest

None.

Funding information

This work was financially supported by the World Health Organization Pandemic and Epidemic Diseases grant for Burden of Disease studies HQPED1611421. The Melbourne WHO Collaborating Centre for Reference and Research on Influenza is supported by the Australian Government Department of Health. The corresponding author was supported by an Australian Government Research Training Program Scholarship.

Acknowledgements

We would like to thank the following institutions and organizations for their contribution in supporting the sentinel surveillance system for severe acute respiratory infection in Cambodia: the World Health Organization (WHO) Country Office in Cambodia; Centers for Disease Control and Prevention, Atlanta, USA; Centers for Disease Control and Prevention, Country Office in Cambodia; National Institute of Public Health, Phnom Penh, Cambodia; Communicable Disease Control Department, Ministry of Health, Phnom Penh, Cambodia; and WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia.

References


Influenza-associated hospitalizations in Cambodia


2.1 Poster presented at TEPHINET’s Bi-regional Conference, Laos, November 2018

The burden of influenza infections in Cambodia

Severe illness is known to cause significant annual mortality, mostly in elderly and socioeconomic groups globally [5]. However, the national burden of influenza in Cambodia has not yet been quantified or likely under-estimated. Cambodia does not have an influenza immunization program.

Surveillance of severe acute respiratory infections in Cambodia

In 2006 a National Influenza Centre was designated at the Institut Pasteur du Cambodge in Phnom Penh to monitor circulating strains of influenza virus associated with mild and severe disease.

Surveillance of severe acute respiratory infections (SARI) started in 2009 with four sites. Its aim was to characterize the epidemiology of severe respiratory illnesses associated with influenza A and B viruses and other common respiratory pathogens [2]. By 2014 there were a total of eight SARI sentinel sites (shown as black and/or circles in the map below).

Figure 1: Location of the eight severe acute respiratory infection (SARI) sentinel surveillance sites, Cambodia, 2017. This hospital-based and laboratory-based surveillance is conducted throughout the year.

Field work summary measures

Resources needed to conduct the three hospital admission surveys, area covered and data collected in Siem Reap, Siem Reap and Kampeng Cheang can be summarized as follows:

- Examiners: 1.5
  - Days of data collection: 24
  - Province covered: 0
  - District covered: 0
  - Area covered: 82,117 km² or 20% of Cambodia’s area
  - Healthcare facilities visited: 0
  - Total respiratory admissions examined: 14,000
  - SARI surveillance staff interviewed: 24

Results: Influenza-associated SARI hospitalizations were highest in children

We provide the first estimates of the national burden of severe influenza in Cambodia using sentinel surveillance data from three sites. Our results indicate that the impact of severe seasonal influenza was highest in the young and oldest age groups. For these findings to translate into public health action we recommend that Cambodian public health authorities:

- Consider this evidence to support the decision to introduce influenza vaccination to reduce the impact of severe influenza in the most vulnerable population groups: children and older adults.
- Continue to invest in routine influenza surveillance as key drivers of population health and pandemic preparedness.

Acknowledgments

We thank the Ministry of Health in Cambodia for the support and guidance during the course of this field work. We also thank all the field supervisors and enumerators for their assistance and comments. The results presented here were supported in part by a fellowship from the Australian Government’s Research Training Program Scholarship.

References


Conclusion and recommendations

We present the first estimates of the national burden of severe influenza in Cambodia using sentinel surveillance data from three sites. Our results indicate that the impact of severe seasonal influenza was highest in the young and oldest age groups. For these findings to translate into public health action we recommend that Cambodian public health authorities:

- Consider this evidence to support the decision to introduce influenza vaccination to reduce the impact of severe influenza in the most vulnerable population groups: children and older adults.
- Continue to invest in routine influenza surveillance as key drivers of population health and pandemic preparedness.

Acknowledgments

We thank the Ministry of Health in Cambodia for the support and guidance during the course of this field work. We also thank all the field supervisors and enumerators for their assistance and comments. The results presented here were supported in part by a fellowship from the Australian Government’s Research Training Program Scholarship.

References


Conclusion and recommendations

We present the first estimates of the national burden of severe influenza in Cambodia using sentinel surveillance data from three sites. Our results indicate that the impact of severe seasonal influenza was highest in the young and oldest age groups. For these findings to translate into public health action we recommend that Cambodian public health authorities:

- Consider this evidence to support the decision to introduce influenza vaccination to reduce the impact of severe influenza in the most vulnerable population groups: children and older adults.
- Continue to invest in routine influenza surveillance as key drivers of population health and pandemic preparedness.

Acknowledgments

We thank the Ministry of Health in Cambodia for the support and guidance during the course of this field work. We also thank all the field supervisors and enumerators for their assistance and comments. The results presented here were supported in part by a fellowship from the Australian Government’s Research Training Program Scholarship.

References

Chapter 3

Influenza vaccine effectiveness in Australia, 2012–2017

Selection of candidate viruses for the influenza vaccine, WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia
Chapter 3

Contents

Prologue .................................................. 97
   My role ................................................. 97
   Lessons Learned ..................................... 97
   Public Health Implications ......................... 99
   Acknowledgements .................................... 99
Abstract ................................................. 100
3.1 Introduction ......................................... 102
3.2 Methods ............................................. 106
   3.2.1 Data sources: Sentinel surveillance of influenza-like illness . 106
   3.2.2 Case definition ................................. 107
   3.2.3 Laboratory methods .............................. 107
   3.2.4 Data analysis ................................... 107
   3.2.5 Ethical approval ................................ 108
3.3 Results ................................................ 109
   3.3.1 Influenza-like illness consultations and patient characteristics109
   3.3.2 Overall influenza vaccine effectiveness estimates ............ 112
   3.3.3 VE pairs by subtype and lineage .................... 112
   3.3.4 VE pairs by age group ......................... 115
   3.3.5 VE pairs by target group of vaccination ............ 118
3.4 Discussion ............................................ 119
3.5 Conclusions .......................................... 122
References ............................................... 123
Appendices .............................................. 128
3.A Oral poster presentation at the Annual EIS Conference, April 2018 . 128
3.B Presentation at the National Immunisation Conference, June 2018 136
3.C Publication in the WHO Weekly Epidemiological Record, August 2018 141
Chapter 3

Prologue

My data analysis project involved using data from the Australian Sentinel Practices Research Network (ASPREN), a national influenza-like illness (ILI) surveillance system, to estimate the effectiveness of the influenza vaccine in Australia for the 2012 to 2017 influenza seasons. The WHO Collaborating Centre for Reference and Research on Influenza assists ASPREN in the estimation of vaccine effectiveness (VE) each year. Among the reporting commitments that the Influenza Centre had with ASPREN in 2017 was the requirement to compare VE estimated in 2017 to previous years for all influenza types as well as disaggregated by subtype or lineage, age group and target group for vaccination.

My role

I was responsible for preparing a data analysis plan, obtaining ethics clearance from ANU, cleaning influenza-like illness surveillance data and conducting the required analysis. A pre-requisite for this project was learning to use the open-source statistical computing language R. I conducted data cleaning and analysis using R, prepared a report for ASPREN and communicated the results at a national public health conference and an international applied epidemiology conference (see Appendix 3.A and Appendix 3.B). A second data analysis project, presented in Appendix 3.C, involved the analysis of the outcomes of a virus isolation external quality assurance (EQA) program implemented by the Influenza Centre in 2017–2018. This program consisted of an assessment of virus isolation capacity in 25 National Influenza Centres (NIC) in countries in the WHO regions of the Americas, Africa and Eastern Mediterranean.

Lessons Learned

In conducting this study I learned about multiple factors affecting influenza immunity conferred by the vaccine and therefore VE. After working on this project and seeing evidence of the modest performance of the influenza vaccine against subtypes such as A/H3 I am convinced of the pressing need to develop an influenza vaccine with improved performance across influenza types and age groups.

Of importance to my future work as a field epidemiologist I learned how to use R for data manipulation, graphing and analysis. It took me several months to get a decent
command of this tool and I am grateful for this learning opportunity. I also learned that data cleaning can require more effort and time than the actual data analysis.

This was the first time I worked with surveillance data. I now have a much better understanding of the importance of routinely collected surveillance data. In the case of influenza, if sufficient numbers of patients (with known influenza vaccination status) are tested for influenza when they present to a sentinel GP, a more robust VE estimate disaggregated by subtype/lineage, age group and target group for vaccination—of most importance to public health—can be calculated. Unfortunately for three of the years I examined vaccination coverage and the number of influenza-positive patients was too low to allow for the estimation of VE disaggregated by what matters most: at-risk groups and viral subtype/lineage. This taught me two things. We need to advocate for more financial resources for influenza surveillance so that more GPs can be recruited throughout Australia which in turn would result in more patients being recruited. Additionally, a more targeted specimen collection approach (i.e., aiming to sample more children, pregnant women, older adults, people with co-morbidities and Indigenous Australians) is important to close this gap and make better use of our national ILI surveillance system.

In my second data analysis project I was able to gain extensive practice in descriptive data analysis and I learned how to use R programming to generate automatic reports. This function is useful for reporting of public health surveillance data. The R script can be written once and reports can be generated at regular intervals with new data as is done at the Influenza Centre monthly to fulfil certain reporting requirements.

The second project taught me that a small number of laboratories in developing countries—although designated National Influenza Centres (NIC)—do not have the capacity to isolate and/or identify influenza viruses in a timely manner or at all despite virus isolation being one of their Terms of Reference. This deficit appears to be due to challenges in obtaining reagents, workforce development and shortages issues and lack of financial resources to deal with equipment malfunction. Of concern is that some of the laboratories struggling to meet its obligations as NIC are in areas where arguably the risk of zoonotic influenza with pandemic potential are greatest. In my experience working in developing countries I observed that laboratories that are designated NIC are those that have a relatively high profile in the country. Systemic issues that challenge their capacity to diagnose influenza would also impact on the diagnosis of other diseases of public health concern.
Public Health Implications

The work that my field supervisor has been conducting for the past six years and to a smaller degree the results of this project pointed to the need of a larger sample size than was available until 2017 through ASPREN in order to reliably estimate influenza VE by subtype/lineage, age and target groups for vaccination reliably. This work, the introduction of new enhanced influenza vaccines for older Australians and, likely, the severity of the 2017 influenza season resulted in more funds being available in 2018 to increase the number of specimens collected for testing as part of the national ILI surveillance system. This is an important public health gain.

The results of virus isolation EQA were published in the WHO’s Weekly Epidemiological Record. Based on EQA performance NICs were selected to receive training to strengthen their ability to isolate and identify influenza viruses. A small number of countries were identified to receive further training in diagnostic techniques for virus isolation and identification from WHO. These techniques are also used for the identification of other viral pathogens. Therefore, this work will contribute to closing the gap in diagnostic capacity between high- and low-income countries and improve global pandemic preparedness.

Acknowledgements

I thank Nigel Stocks, ASPREN director, and Monique Chilver, ASPREN manager, for facilitating access to their national influenza-like illness database. I thank ASPREN GPs for recruiting patients and laboratory staff of the SA Pathology, PathWest and the WHO Collaborating Centre for Reference and Research on Influenza for testing hundreds of specimens each year. Without the combined efforts of clinicians and laboratory staff we would know little about how the seasonal influenza vaccine performs each season. The dedication of practitioners to record vaccination status and patients’ demographic characteristics in a high-workload environment does not go unnoticed. Their work results in the evidence needed for decision making and allows epidemiologists to estimate the protection conferred by the influenza vaccine to vulnerable population groups.

I acknowledge the financial assistance provided by the Training Programs in Epidemiology and Public Health Interventions Network (TEPHINET) that enabled me to present this work at the 67th Annual Epidemic Intelligence Service Conference in Atlanta in April 2018.
Chapter 3

Abstract

**Background:** Understanding the protection conferred by vaccination against community-acquired influenza assists experts in planning strategies to protect vulnerable populations during severe influenza seasons. This report summarises influenza vaccine effectiveness (VE) estimates for Australia for the period 2012–2017.

**Methods:** Data from the Australian Sentinel Practices Research Network (ASPREN) were used to estimate the interim and final VE against medically-attended influenza A and B. We used the case test-negative design in which cases were individuals of known influenza vaccination status that present to a sentinel general practitioner during the influenza season, met the national influenza-like illness (ILI) case definition and tested positive for influenza A and/or B virus by real time reverse transcription polymerase chain reaction (PCR). Non-cases were participants with a negative PCR test result. VE was estimated as \((1-\text{OR}) \times 100\%\) by logistic regression, where OR is the odds of being a vaccinated case divided by the odds of being a vaccinated non-case. Estimates were adjusted for age and time (date of consultation). VE was estimated during and at the end of the influenza season to assess the extent to which interim VE reliably predicts final VE. Statistical analyses were conducted in R version 3.4.1.

**Results:** Overall, interim and final VE point estimate pairs were closest for 2015 [56% (95% CI 38–69; n=1,255) and 56% (95% CI 38–68; n=1,386)] and 2017 [36% (95% CI 16–51; n=1,534) and 35% (95% CI 19–48; n=2,357) for interim and final estimates, respectively]. Concordance between VE pairs was the lowest for 2016 [60% (95% CI 37–75; n=678) and 46% (95% CI 22–63; n=1,013) for interim and final estimates, respectively]. Concordance between interim and final VE estimates were highest when interim VE was estimated after the peak of the season. A larger number of participants available for final estimation also resulted in more precise estimates.

Final VE estimates by subtype, showed higher VE for A(H1N1)pdm compared to A(H3N2) in all years. The lowest VE estimate for A(H3N2) was observed in the 2017 season (i.e., nil final VE for all ages combined). Precision of VE estimate pairs by subtype was very low for seasons with too few positive swabs available (i.e., A(H1N1)pdm in 2012 and 2015, and A(H3N2) in 2013). VE estimates by age group varied greatly by year. In years with larger sample sizes (2015 and 2017), VE pairs in adults were more concordant. VE estimation by target group for vaccination was moderate to low and the precision of the estimate was low. VE estimates specific to influenza A(H3N2) by age group had very poor precision in all years. For this
analysis, large fluctuations were observed in elderly adults between interim and final estimates for all years.

**Conclusion:** Overall VE estimates for the last six influenza seasons in Australia were moderate. As the total sample size increased and the consistency of data collection improved, an increase in the precision of the VE estimates was observed. The reliability of VE estimates by subtype/lineage and by age and target group for vaccination was questionable due to insufficient sample size. Our analysis lends support to the expansion of national ILI surveillance to increase the number of patients that are laboratory-tested for influenza. Increased testing results in a more robust influenza VE estimate and, in particular, more reliable VE estimates by virus subtype and subpopulations of interest. We recommend that the ASPREN sampling scheme for ILI patients be further refined to include 40% sampling of working-age adults, 100% of people over 65 years of age and 100% of children (i.e., under 18 years of age). Emphasis in collecting more swabs from children and the older patients would enable more reliable VE estimates in these subpopulations.
3.1 Introduction

The importance of estimating influenza vaccine effectiveness

Knowledge of vaccine performance is an important tool for public health decision making (1). Vaccine effectiveness (VE) provides evidence of a vaccine’s capacity to protect a person against infection. Evidence of moderate to high VE against influenza virus infection is used to promote immunisation while vaccines with low VE indicate the need to adopt alternative control measures to prevent infection as well as the need for further vaccine research to obtain an improved vaccine.

Increasingly, VE is being used by public health authorities in Australia and globally to evaluate the performance of publicly-funded influenza immunisation programs (2, 3, 4). Further, VE estimates are also useful to the WHO influenza vaccine committee at the time of deciding the composition of the vaccine to be used in the following season (5).

Reasons why influenza vaccine effectiveness must be estimated annually

The predominant types and subtypes of influenza viruses that circulate in the community change each year. In addition, there are antigenic changes that occur within virus types and subtypes due to continuous antigenic drift (6). The influenza vaccines currently available are unable to confer protection against drifted influenza virus strains. For this reason the composition of the influenza vaccine is often changed to accommodate changes in viral antigenicity (7).

Annual influenza immunisation with a vaccine containing seasonal viral strains closely matched to those predicted to be circulating is required to keep pace with the changing antigenicity of the virus. As influenza immunisation remains the most important tool to prevent influenza infection and mortality it is important to estimate influenza vaccine effectiveness each year. This can be done during the season (to obtain an interim estimate in real time) and at the end of the season (final estimate) (8).

Factors that affect influenza vaccine effectiveness

The effectiveness of the influenza vaccine depends on host, agent and vaccine factors. Age and immunocompetence of the vaccine recipient, the influenza strains in circulation in the community and the degree of similarity between the vaccine and circulating viral strains influence VE (7, 9). Age is expected to affect VE given
that levels of exposure to influenza vary across the lifespan. Immunocompetence is associated with both health status (i.e., presence of underlying health conditions) and age. VE in individuals at both extremes of age is expected to be lower than in adults due to the relative immaturity of the immune system in children (10) and immunosenescence in older adults (11).

The strains included in the seasonal influenza vaccines used in Australia and available on the national immunisation programme in the 2012–2017 period are shown in Table 3.1. Only trivalent vaccines were available in Australia between 2012 and 2014. Trivalent vaccines contain representative viruses for both influenza A subtypes (H1N1pdm and H3N2) and one influenza B lineage (Victoria or Yamagata). Quadrivalent influenza vaccine, which became available on the national immunisation programme in 2016 (9), contain representative viruses for both influenza A subtypes and both influenza B lineages. Although data of vaccine manufacturer could be used to assess VE by vaccine brand these are not currently recorded by ASPREN. The influenza strains circulating in corresponding years identified from ASPREN specimens is shown in Figure 3.1, Section 3.3.1.

Table 3.1: Composition of the seasonal influenza vaccine available through the national immunisation programme, Australia, 2012–2017

<table>
<thead>
<tr>
<th>Year</th>
<th>H1</th>
<th>H3N2</th>
<th>B</th>
<th>B lineage</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>A/California/7/2009</td>
<td>A/Perth/16/2009</td>
<td>B/Brisbane/60/2008</td>
<td>Victoria</td>
</tr>
<tr>
<td>2013</td>
<td>A/California/7/2010</td>
<td>A/Victoria/361/2012</td>
<td>B/Wisconsin/1/2011</td>
<td>Victoria</td>
</tr>
<tr>
<td>2014</td>
<td>A/California/7/2012</td>
<td>A/Texas/50/2012</td>
<td>B/Massachusetts/2/2012</td>
<td>Victoria</td>
</tr>
<tr>
<td>2015</td>
<td>A/California/7/2012</td>
<td>A/Switzerland/9715293/2013</td>
<td>B/Phuket/3073/2013</td>
<td>Yamagata</td>
</tr>
<tr>
<td>2016</td>
<td>A/California/7/2012</td>
<td>A/Hong Kong/4801/2014</td>
<td>B/Brisbane/60/2008</td>
<td>Victoria</td>
</tr>
<tr>
<td>2017</td>
<td>A/Michigan/45/2015</td>
<td>A/Hong Kong/4801/2014</td>
<td>B/Phuket/3073/2013</td>
<td>Yamagata</td>
</tr>
</tbody>
</table>

The main factor that affects the precision of VE estimates is the number of people captured by the surveillance system which influences the sample size used for VE calculation (12). This depends on the rate at which people with ILI present to a sentinel GP and the specimen collection behaviour of doctors and nurses in sentinel practices. Even when the sample size is large, precision can be negatively impacted by the relative proportion of vaccinated and influenza-infected patients (6). Both very low and very high vaccination coverage and proportions of infected patients may result in decreased precision.
In an effort to produce robust overall VE estimates and to enable VE by subtype and lineage for specific age groups, Australian epidemiologists combine data from three GP-based and laboratory-based ILI surveillance systems: national, Victorian and Western Australian (9). This method increases sample size and produces VE estimates with narrower confidence intervals and greater statistical power to detect an effect compared to estimates from the national surveillance system only. For example, summary VE for 2012 using Australian Sentinel Practice Research Network (ASPREN) data and pooled surveillance data was 23% (95% CI -4–43) and 38% (95% CI 24–49), respectively (9).

**Influenza vaccine effectiveness in subpopulations**

Policy makers are interested in the summary measure of VE, that is a single point estimate for all influenza virus subtypes and lineages for the whole population. However, knowledge of vaccine performance against specific influenza subtypes and lineages in subpopulations at risk of the more serious complications of influenza is particularly important since these groups are associated with poorer disease outcomes (13). Subpopulations for which the Australian Government funds influenza vaccine include: 1) people aged six months and over with certain medical risk factors such as chronic illnesses and immunocompromising conditions; 2) Aboriginal and Torres Strait Islander children aged six months to less than five years; 3) Aboriginal and Torres Strait Islander adults aged 15 years and over; 4) older adults (65 years and over); and 5) pregnant women (15).

When the sample size of these subpopulations allows it, and surveillance data is captured comprehensively, it is useful to estimate VE disaggregated by age group (i.e., children, adults and older adults), and patients targeted by public influenza immunisation programs. The estimation of summary VE by logistic regression allows adjustment for confounding factors. These are variables that are associated with both the outcome and the exposure such as age group, comorbidities, Indigenous status and pregnancy.

**Influenza vaccine effectiveness in Australia and globally**

The latest multi-year estimates (2012–2014) published for Australia show moderate influenza VE of 38 to 60% (9). Summary VE, estimated using the three ILI surveillance

---

†A list of medical conditions that increase the risk of influenza can be found in the Australian Immunisation Handbook (14)
systems operating in Australia, was lowest for 2012 compared to the other two years. This was likely due to A(H3N2) being the dominant strain and the poor match between the strains in the vaccine and those circulating in the community that year. It is hypothesised that the influenza vaccine is typically less effective against influenza A(H3N2) viruses due to the genetic changes that occur during the vaccine production process which involves growing the virus in hen’s eggs (16, 17).

Sullivan et al. (9) reported higher VE estimates against influenza B and A(H1N1)pdm compared to A(H3N2) for the 2012–2014 Australian seasons (between 54 and 56% for influenza B, 54–59% for A(H1N1)pdm and 38–60% for A(H3N2). Evidence of improved protection against influenza B and influenza A(H1N1)pdm viruses compared to A(H3N2) viruses was also reported in a systematic review and meta-analysis that included 56 publications mainly from the Northern Hemisphere (18). That review found minimal differences across age groups for influenza B and A(H1N1)pdm. However, for A(H3N2) VE was highest in paediatric age groups and lowest in older adults.

**Timing of influenza vaccine effectiveness estimation**

Monitoring VE as the influenza season unfolds would be particularly important in the event of a pandemic or a more severe season as it would provide an early warning to public health authorities of the need to implement additional control measures for vulnerable population groups. Interim VE also contributes to the decision to update the influenza vaccine composition made each year by the WHO influenza vaccine committee twice. Despite its importance, interim VE estimates are based on incomplete data and could potentially differ from final VE estimates. Comparing interim and final VE allows the assessment of the extent to which interim estimates reliably predict end of season estimates.

**Study aims**

The aim of this study is to estimate influenza VE using the Australia-wide influenza-like illness (ILI) surveillance system for the period 2012–2017. Specifically we aimed to:

• Compare VE pairs by subtype and lineage (influenza A(H1N1)pdm, influenza A(H3N2) and influenza B), by age group and by target group for vaccination.

### 3.2 Methods

#### 3.2.1 Data sources: Sentinel surveillance of influenza-like illness

Data from ASPREN were used to estimate the interim and final VE against influenza A and B. ASPREN is an Australia-wide network of approximately 200 general practitioners and nurse practitioners, who report syndromic presentations of influenza-like illness and other medical conditions each week throughout the year (19). ASPREN was established in 1991 and has been expanding in its functions since then. Virological surveillance of influenza commenced in 2010. As the largest national sentinel practice surveillance system ASPREN is funded by the Australian Government Department of Health to monitor influenza activity in the community. ASPREN provides timely information on which to base decisions on control measures to lessen the burden of influenza in the community (20). Its stated aim, although difficult yet to achieve, is also to act as an early indicator of pandemic influenza (21).

The target for ASPREN participation is of one general practitioner (GP) per 200,000 population in urban settings and one GP per 50,000 population in rural and remote settings or one GP per Division of General Practice (20, 1). General practitioners from all eight states and territories in Australia participate in ASPREN. During 2016 and in the first quarter of 2017, there were a total of 203 general practitioners regularly contributing data (22, 23). Fifty-eight per cent of ASPREN practitioners are located in metropolitan areas, 32% in rural and 10% in remote areas of Australia (21).

For their participation ASPREN practitioners receive monetary compensation for specimens collected and continuing professional development points from the Royal Australian College of General Practitioners (24).

#### Specimen and data collection

ASPREN practitioners collect respiratory specimens of a proportion of patients meeting the ILI case definition for laboratory testing (20). ASPREN practitioners collect de-identified demographic information, influenza vaccination history and nasal
swab specimens of a proportion of influenza-like illness (ILI) patients for influenza A and influenza B testing (23). Patients were selected for testing at the discretion of the sentinel GP. Vaccination status is collected through patient self report or via patient medical records (25). Systematic and consistent collection of vaccination status data commenced in 2012 therefore enabling influenza VE estimation. In 2014 ASPREN GPs began collecting data on medical conditions that could increase the risk of severe influenza.

3.2.2  Case definition

ASPREN GPs use the national ILI case definition to identify patients for surveillance. ILI is defined as history of fever with cough and fatigue (26, 21)

3.2.3  Laboratory methods

In 2012–2017, nasopharyngeal specimens were collected from selected ILI patients using pre-prepared kits consisting of flocked swabs (Copan Diagnostics). Specimens were shipped at room temperature via Australia Post’s Express post sachets to SA Pathology in 3 ml universal transport medium. Influenza was detected using an in house real time reverse transcription polymerase chain reaction (PCR) assay (1). With the exception of 2012, the multiplex real time PCR test was capable of detecting influenza A (un-subtyped), influenza A(H1N1)pdm09, influenza A(H3N2), influenza A(H5N1) and influenza B. In 2012, the PCR panel did not include influenza A(H3N2) (25).

3.2.4  Data analysis

VE was estimated for the 2012–2017 period using the case test-negative design in which cases were individuals of known influenza vaccination status that present to a sentinel GP during the influenza season, met the national influenza-like illness (ILI) case definition and tested positive for influenza A and/or B virus by real time PCR (6, 27). Non-cases were participants with a negative PCR test result for influenza. VE was calculated by logistic regression where influenza test result was the outcome variable and vaccination status, the exposure. VE was defined as (1-OR)*100%, where OR is the odds of being a vaccinated case divided by the odds of being a vaccinated non-case. Using this method, vaccine effectiveness measures the reduction in the occurrence of medically-attended influenza infection amongst those that have been
vaccinated compared to those unvaccinated. Statistical analyses and graphics were conducted in R version 3.4.1 (28). We excluded patients with presentation outside of the influenza season, duplicate laboratory results and with missing data for influenza status, vaccination status, age and consultation date.

VE estimates were adjusted for known confounders, selected a priori, including age group and week of presentation to the sentinel practice in relation to the peak of influenza circulation.

VE was estimated for an interim and final period of influenza epidemic activity. The influenza season was defined as commencing when a positive case had been reported for two consecutive weeks at least two weeks after the annual vaccination campaign (which is usually conducted in week 16) and ended in the post-peak week when no positive cases had been reported for at least three consecutive weeks. The peak week of influenza activity is defined as the week with the highest number of cases during that influenza season. The interim period was defined as the period between the beginning of the season until week 36, which coincides with the WHO vaccine composition meeting. The final period included the entire season. We compared interim and final VE estimates for the study period by calculating their difference in percentage points. We assessed VE pairs as concordant when the difference between the interim and final estimates was no more than 10%. Likewise, a difference between estimates of more than 10 percentage points was considered meaningful.

We report summary VE estimates (i.e., for all age groups against all influenza), estimates by subtype and lineage, by age group: children (<18 years); adults (18-64) and older adults (65+ years) against all influenza and by age group against influenza A(H3N2). Lastly, we report VE for people in target groups for vaccination. All estimates were reported with 95% confidence intervals.

### 3.2.5 Ethical approval

This study used data routinely collected for ILI and virological surveillance purposes and carried negligible risk as defined by the National Statement on Ethical Conduct in Human Research 2007. Participation in ASPREN was voluntary. Respiratory specimens from ILI patients were collected with informed written consent. This study has been approved by the Australian National University Human Research Ethics Committee (Protocol 2017/894).
3.3 Results

3.3.1 Influenza-like illness consultations and patient characteristics

The number of ILI consultations by influenza test result for 2012–2017 is shown in Figure 3.1. ILI consultations peaked before week 36 in all seasons, although in 2017 consultations peaked slightly later. 2012 and 2017 showed higher peaks of influenza positive ILI patients compared to other years. The number of respiratory specimens collected for influenza testing each year and the number of specimens used for the estimation of interim and final VE in each year is presented in Table 3.2. The 2016 season had the smallest sample size closely followed by 2013. Sample size for interim VE estimation ranged from 678 and 1534. For final VE estimation the sample size ranged from 990 and 2357.

![Figure 3.1](image_url)

Figure 3.1: Number of influenza-like illness (ILI) consultations per week by laboratory test result status captured by the Australian Sentinel Practices Research Network (ASPREN), 2012–2017. The shaded rectangle represents the period included for final VE estimation. The period included for interim VE estimation is represented by the shaded rectangle up to the broken line.
Table 3.2: Total number of respiratory specimens collected for influenza testing each year and total number of specimens used for the estimation of interim and final vaccine effectiveness against influenza A and B, Australia, 2012–2017

<table>
<thead>
<tr>
<th>Year</th>
<th>Total number of swabs taken</th>
<th>Total number of swabs used for interim VE estimation</th>
<th>Total number of swabs used for final VE estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>1775</td>
<td>1306</td>
<td>1417</td>
</tr>
<tr>
<td>2013</td>
<td>1338</td>
<td>728</td>
<td>990</td>
</tr>
<tr>
<td>2014</td>
<td>1925</td>
<td>1273</td>
<td>1469</td>
</tr>
<tr>
<td>2015</td>
<td>1626</td>
<td>1255</td>
<td>1386</td>
</tr>
<tr>
<td>2016</td>
<td>1136</td>
<td>678</td>
<td>1013</td>
</tr>
<tr>
<td>2017</td>
<td>2417</td>
<td>1534</td>
<td>2357</td>
</tr>
</tbody>
</table>

Note: Laboratory test results used to estimate influenza VE correspond to dates of collection within the influenza season. The difference between total number swabs taken and the total number swabs used for final VE estimation represent the number of swabs collected outside of the influenza season. These specimens are used to ensure continuous virological surveillance of circulating influenza viruses.

Participant demographic and clinical characteristics by interim and final periods are summarised in Table 3.3. The number of female and male participants was similar between interim and final periods and most years had more female participants (55–57%). Adults were the largest group in all years in both the interim and final periods. Older adults represented the smallest group (between 10 to 15% in most years). The proportion of children participants ranged from 18 to 29% in most years but was particularly low in 2014 at only 1%. Vaccination coverage among ASPREN participants ranged from 24 to 40% and was very similar for both timepoints each year. Both the 2012 and 2017 seasons had higher proportions of influenza positive tests (41 and 42%, respectively for the final period). Influenza A(H3N2) was the dominant strain in 2012 and 2016 but also represented half of viruses detected in ILI patients in 2014 and 2017. Influenza B viruses dominated the 2015 and 2013 seasons. Data on Indigenous status began being collected in 2014 and was very low for most years (1.8–2.9%). The highest proportion of Indigenous ILI patients was recorded for 2016 (3.7 and 6.0% for the interim and final periods, respectively). The proportion of patients in the target group for vaccination increased from approximately 10% in 2012 and 2013 to 31–49% in the following years.
Table 3.3: Characteristics of patients with ILI as captured by the ASPREN surveillance system, Australia, 2012–2017

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>2012 n (%)</th>
<th>2013 n (%)</th>
<th>2014 n (%)</th>
<th>2015 n (%)</th>
<th>2016 n (%)</th>
<th>2017 n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>643 (49.2)</td>
<td>695 (49.0)</td>
<td>418 (57.4)</td>
<td>563 (56.9)</td>
<td>700 (55.0)</td>
<td>809 (55.1)</td>
</tr>
<tr>
<td>Male</td>
<td>641 (49.1)</td>
<td>700 (49.4)</td>
<td>309 (42.4)</td>
<td>425 (42.9)</td>
<td>573 (45.0)</td>
<td>660 (44.9)</td>
</tr>
<tr>
<td><strong>Age group (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children (&lt;18)</td>
<td>332 (25.4)</td>
<td>359 (25.3)</td>
<td>168 (23.1)</td>
<td>221 (22.3)</td>
<td>12 (0.9)</td>
<td>13 (0.9)</td>
</tr>
<tr>
<td>Adults (18-64)</td>
<td>841 (64.4)</td>
<td>915 (64.6)</td>
<td>478 (65.7)</td>
<td>666 (67.3)</td>
<td>735 (57.7)</td>
<td>841 (57.2)</td>
</tr>
<tr>
<td>Older adults (65+)</td>
<td>133 (10.2)</td>
<td>143 (10.1)</td>
<td>82 (11.3)</td>
<td>103 (10.4)</td>
<td>526 (41.3)</td>
<td>615 (41.9)</td>
</tr>
<tr>
<td><strong>Vaccination status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccinated</td>
<td>311 (23.8)</td>
<td>337 (23.8)</td>
<td>225 (30.9)</td>
<td>301 (30.4)</td>
<td>380 (29.9)</td>
<td>444 (30.2)</td>
</tr>
<tr>
<td>Unvaccinated</td>
<td>995 (76.2)</td>
<td>1080 (76.2)</td>
<td>503 (69.1)</td>
<td>689 (69.6)</td>
<td>893 (70.1)</td>
<td>1025 (69.8)</td>
</tr>
<tr>
<td><strong>Influenza status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>548 (42.0)</td>
<td>577 (40.7)</td>
<td>153 (21.0)</td>
<td>400 (31.4)</td>
<td>442 (30.1)</td>
<td>417 (33.2)</td>
</tr>
<tr>
<td>Negative</td>
<td>758 (58.0)</td>
<td>840 (59.3)</td>
<td>759 (79.0)</td>
<td>873 (68.6)</td>
<td>1027 (69.9)</td>
<td>938 (66.8)</td>
</tr>
<tr>
<td><strong>Influenza type and subtype/lineage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A(H1N1)pdm</td>
<td>7 (1.3)</td>
<td>7 (1.2)</td>
<td>90 (45.8)</td>
<td>99 (42.9)</td>
<td>150 (35.5)</td>
<td>157 (35.5)</td>
</tr>
<tr>
<td>A(H3N2)</td>
<td>459 (83.8)</td>
<td>467 (80.9)</td>
<td>10 (6.5)</td>
<td>17 (7.4)</td>
<td>206 (51.5)</td>
<td>226 (51.1)</td>
</tr>
<tr>
<td>A(Hx) not subtyped</td>
<td>0 (0.0)</td>
<td>2 (1.3)</td>
<td>5 (2.2)</td>
<td>8 (2.0)</td>
<td>9 (2.0)</td>
<td>20 (4.8)</td>
</tr>
<tr>
<td>B</td>
<td>82 (15.0)</td>
<td>103 (17.9)</td>
<td>71 (46.4)</td>
<td>110 (47.6)</td>
<td>36 (9.0)</td>
<td>50 (11.3)</td>
</tr>
<tr>
<td><strong>Indigenous status (i.e., Aboriginal and Torres Strait Islander people)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indigenous</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>10 (1.8)</td>
<td>12 (1.8)</td>
<td>14 (2.0)</td>
</tr>
<tr>
<td>Non-Indigenous</td>
<td>NA</td>
<td>NA</td>
<td>552 (98.2)</td>
<td>668 (98.2)</td>
<td>684 (98.0)</td>
<td>783 (98.0)</td>
</tr>
<tr>
<td><strong>In target vaccination group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>133 (10.2)</td>
<td>143 (10.1)</td>
<td>78 (10.7)</td>
<td>98 (9.9)</td>
<td>618 (48.5)</td>
<td>725 (49.4)</td>
</tr>
<tr>
<td>No</td>
<td>1173 (89.8)</td>
<td>1274 (89.9)</td>
<td>1274 (89.9)</td>
<td>892 (90.1)</td>
<td>655 (51.5)</td>
<td>744 (50.6)</td>
</tr>
</tbody>
</table>

* People in the target group for vaccination exclude patients over 65 years.
3.3.2 Overall influenza vaccine effectiveness estimates

Overall VE pair estimates against any influenza infection (A or B) for all age groups by year are presented in Figure 3.2. Overall VE was lowest in 2012 and 2017 compared to other years (final VE: 20 and 35%, respectively). VE for seasons 2013 to 2016 was moderate and ranged from 45 to 56%. Concordance between interim and final VE estimate was highest for years with the largest sample sizes (i.e., 2012, 2014, 2015 and 2017). In these years, interim VE was estimated after the influenza peak.

<table>
<thead>
<tr>
<th>Year</th>
<th>Positive V</th>
<th>UV</th>
<th>Negative V</th>
<th>UV</th>
<th>VE [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>Interim 107</td>
<td>441</td>
<td>204</td>
<td>554</td>
<td>18 [-12.4, 40]</td>
</tr>
<tr>
<td></td>
<td>Final 113</td>
<td>464</td>
<td>224</td>
<td>616</td>
<td>20 [-8.4, 41]</td>
</tr>
<tr>
<td>2013</td>
<td>Interim 26</td>
<td>127</td>
<td>199</td>
<td>376</td>
<td>55 [24.7, 73]</td>
</tr>
<tr>
<td></td>
<td>Final 42</td>
<td>189</td>
<td>259</td>
<td>500</td>
<td>52 [27.6, 68]</td>
</tr>
<tr>
<td>2014</td>
<td>Interim 83</td>
<td>317</td>
<td>297</td>
<td>576</td>
<td>50 [31.6, 63]</td>
</tr>
<tr>
<td></td>
<td>Final 98</td>
<td>344</td>
<td>346</td>
<td>601</td>
<td>45 [27.5, 63]</td>
</tr>
<tr>
<td>2015</td>
<td>Interim 81</td>
<td>336</td>
<td>303</td>
<td>535</td>
<td>56 [38.6, 69]</td>
</tr>
<tr>
<td></td>
<td>Final 94</td>
<td>366</td>
<td>344</td>
<td>583</td>
<td>56 [38.6, 69]</td>
</tr>
<tr>
<td>2016</td>
<td>Interim 46</td>
<td>164</td>
<td>182</td>
<td>286</td>
<td>60 [37.7, 75]</td>
</tr>
<tr>
<td></td>
<td>Final 79</td>
<td>226</td>
<td>264</td>
<td>444</td>
<td>46 [22.6, 63]</td>
</tr>
<tr>
<td>2017</td>
<td>Interim 177</td>
<td>642</td>
<td>293</td>
<td>522</td>
<td>36 [16.5, 51]</td>
</tr>
<tr>
<td></td>
<td>Final 251</td>
<td>710</td>
<td>515</td>
<td>881</td>
<td>35 [19.4, 48]</td>
</tr>
</tbody>
</table>

Figure 3.2: Overall influenza VE pair estimates—including 95% confidence intervals—against influenza A and/or B, Australia, 2012–2017. Notes: V=vaccinated, UV=unvaccinated, 95% CI=95% confidence interval.

3.3.3 VE pairs by subtype and lineage

VE estimates for influenza A(H1N1)pdm, A(H3N2) and B are shown in Figure 3.3, Figure 3.4 and Figure 3.5, respectively. Final VE estimates were higher for A(H1N1)pdm compared to A(H3N2) in all years and concordance between interim and final estimate pairs was within 10 percentage points for both subtypes and for almost all years. VE
for A(H1N1)pdm could not be estimated for 2012 due to the absence of vaccinated cases. Estimates against influenza A(H1N1)pdm for 2015 and 2017 had very wide confidence intervals (i.e., ranging from 89 to 146 percentage points). In 2012 and 2017, VE estimates for A(H3N2) (both interim and final) were the lowest compared to other years with final VE for A(H3N2) in 2017 estimated as zero. The highest VE estimates for A(H3N2) were observed in 2015. The least concordant VE pair for A(H3N2) was seen in 2013, a year with very low vaccinated cases (≤5).

<table>
<thead>
<tr>
<th>Year</th>
<th>Positive</th>
<th>Negative</th>
<th>VE [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V</td>
<td>UV</td>
<td>V</td>
</tr>
<tr>
<td>2012</td>
<td>Interim</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>2013</td>
<td>Interim</td>
<td>9</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>16</td>
<td>83</td>
</tr>
<tr>
<td>2014</td>
<td>Interim</td>
<td>24</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>29</td>
<td>128</td>
</tr>
<tr>
<td>2015</td>
<td>Interim</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>2016</td>
<td>Interim</td>
<td>4</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>4</td>
<td>35</td>
</tr>
<tr>
<td>2017</td>
<td>Interim</td>
<td>6</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>9</td>
<td>59</td>
</tr>
</tbody>
</table>

Figure 3.3: VE pair estimates –including 95% confidence intervals– against influenza A(H1N1)pdm, Australia, 2012–2017. Estimates for 2012 could not be estimated due to the lack of vaccinated cases. Notes: V = vaccinated, UV = unvaccinated, 95% CI = 95% confidence interval.
VE estimates for influenza B showed good agreement between interim and final estimate pairs for most years. For 2015 and 2017 VE estimates for influenza B showed the highest precision and highest concordance between estimate pairs. In addition, VE for influenza B was highest for the years where the quadrivalent vaccine was widely used (2016–2017) compared to other years. In 2016, interim VE for influenza B predicted final VE poorly (i.e., 53 vs 10%, respectively). This was a year with very few vaccinated cases (n=3) and the smallest sample size (n=486 and n=747 for the interim and final periods, respectively). Although VE pair concordance for influenza B was within 10 percentage points for 2012–2014, precision was poor and most of the confidence intervals included zero.

<table>
<thead>
<tr>
<th>Year</th>
<th>Positive</th>
<th>Negative</th>
<th>VE [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V</td>
<td>UV</td>
<td>V</td>
</tr>
<tr>
<td>2012</td>
<td>Interim</td>
<td>97 362</td>
<td>204 554</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>101 366</td>
<td>224 616</td>
</tr>
<tr>
<td>2013</td>
<td>Interim</td>
<td>2 8</td>
<td>199 376</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>5 12</td>
<td>259 500</td>
</tr>
<tr>
<td>2014</td>
<td>Interim</td>
<td>50 156</td>
<td>297 576</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>56 179</td>
<td>346 681</td>
</tr>
<tr>
<td>2015</td>
<td>Interim</td>
<td>27 75</td>
<td>303 535</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>31 85</td>
<td>344 583</td>
</tr>
<tr>
<td>2016</td>
<td>Interim</td>
<td>36 104</td>
<td>182 286</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>56 150</td>
<td>264 444</td>
</tr>
<tr>
<td>2017</td>
<td>Interim</td>
<td>119 259</td>
<td>293 522</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>164 313</td>
<td>515 881</td>
</tr>
</tbody>
</table>

Figure 3.4: VE pair estimates -including 95% confidence intervals- against influenza A(H3N2), Australia, 2012–2017. Notes: V=vaccinated, UV=unvaccinated, 95% CI=95% confidence interval.
3.3.4 VE pairs by age group

VE estimate pairs against any influenza infection (A or B) by age group are shown in Figure 3.6. VE pairs by age group varied by year. Precision of VE estimates by age group was lower for children and elderly adults in most years. Out of all 15 VE pairs estimated, only 5 showed good concordance (i.e., estimates within 10 percentage points). These were three VE pairs for adults (2013, 2015 and 2016) and two for children (2014 and 2015). Of note, although in 2014 the interim and final VE for children were very similar, among children there were zero vaccinated cases and zero vaccinated non-cases. VE pair concordance for elderly adults was particularly low on all six years with the difference between interim and final equal to or higher than 20 percentage points.

<table>
<thead>
<tr>
<th>Year</th>
<th>Positive</th>
<th>Negative</th>
<th>VE [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V</td>
<td>UV</td>
<td>V</td>
</tr>
<tr>
<td>2012</td>
<td>Interim</td>
<td>10</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>12</td>
<td>91</td>
</tr>
<tr>
<td>2013</td>
<td>Interim</td>
<td>14</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>19</td>
<td>91</td>
</tr>
<tr>
<td>2014</td>
<td>Interim</td>
<td>7</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>11</td>
<td>39</td>
</tr>
<tr>
<td>2015</td>
<td>Interim</td>
<td>44</td>
<td>236</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>53</td>
<td>254</td>
</tr>
<tr>
<td>2016</td>
<td>Interim</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>13</td>
<td>26</td>
</tr>
<tr>
<td>2017</td>
<td>Interim</td>
<td>37</td>
<td>209</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>60</td>
<td>298</td>
</tr>
</tbody>
</table>

Figure 3.5: VE pair estimates –including 95% confidence intervals– against influenza B, Australia, 2012–2017. Notes: V=vaccinated, UV=unvaccinated, 95% CI=95% interval.
### Figure 3.6: Influenza VE pair estimates –including 95% confidence intervals– against influenza A and/or B by age group, Australia, 2012–2017.

Notes: V=vaccinated, UV=unvaccinated, 95% CI=95% confidence interval.

<table>
<thead>
<tr>
<th>Year</th>
<th>Age</th>
<th>Positive V</th>
<th>Positive UV</th>
<th>Negative V</th>
<th>Negative UV</th>
<th>VE [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2012</td>
<td></td>
<td>2012</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Children</td>
<td>Interim</td>
<td>9</td>
<td>19</td>
<td>30 [-21, 60]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Final</td>
<td>9</td>
<td>177</td>
<td>11 [-139, 67]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adults</td>
<td>Interim</td>
<td>69</td>
<td>255</td>
<td>40 [16, 88]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Final</td>
<td>75</td>
<td>267</td>
<td>9 [-26, 35]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Elderly</td>
<td>Interim</td>
<td>29</td>
<td>17</td>
<td>-13 [-156, 50]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Final</td>
<td>20</td>
<td>20</td>
<td>65 [20, 85]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2013</td>
<td></td>
<td>2013</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Children</td>
<td>Interim</td>
<td>2</td>
<td>47</td>
<td>80 [51, 90]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Final</td>
<td>3</td>
<td>66</td>
<td>67 [-33, 83]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adults</td>
<td>Interim</td>
<td>20</td>
<td>78</td>
<td>50 [13, 72]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Final</td>
<td>31</td>
<td>119</td>
<td>59 [-32, 75]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Elderly</td>
<td>Interim</td>
<td>4</td>
<td>2</td>
<td>68 [-64, 94]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Final</td>
<td>8</td>
<td>4</td>
<td>9 [-158, 72]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2014</td>
<td></td>
<td>2014</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Children</td>
<td>Interim</td>
<td>0</td>
<td>2</td>
<td>54 [14, 76]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Final</td>
<td>0</td>
<td>2</td>
<td>53 [-17, 73]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adults</td>
<td>Interim</td>
<td>30</td>
<td>220</td>
<td>52 [30, 66]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Final</td>
<td>31</td>
<td>237</td>
<td>39 [-16, 57]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Elderly</td>
<td>Interim</td>
<td>53</td>
<td>95</td>
<td>44 [-31, 76]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Final</td>
<td>67</td>
<td>105</td>
<td>63 [-14, 84]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2015</td>
<td></td>
<td>2015</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Children</td>
<td>Interim</td>
<td>7</td>
<td>155</td>
<td>52 [14, 73]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Final</td>
<td>7</td>
<td>165</td>
<td>62 [-33, 79]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adults</td>
<td>Interim</td>
<td>47</td>
<td>172</td>
<td>57 [37, 71]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Final</td>
<td>54</td>
<td>189</td>
<td>50 [40, 70]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Elderly</td>
<td>Interim</td>
<td>27</td>
<td>9</td>
<td>85 [59, 94]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Final</td>
<td>33</td>
<td>11</td>
<td>62 [7, 65]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2016</td>
<td></td>
<td>2016</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Children</td>
<td>Interim</td>
<td>2</td>
<td>44</td>
<td>70 [38, 86]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Final</td>
<td>3</td>
<td>77</td>
<td>32 [-25, 63]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adults</td>
<td>Interim</td>
<td>28</td>
<td>111</td>
<td>49 [15, 69]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Final</td>
<td>46</td>
<td>138</td>
<td>40 [21, 64]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Elderly</td>
<td>Interim</td>
<td>15</td>
<td>9</td>
<td>52 [-78, 87]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Final</td>
<td>30</td>
<td>11</td>
<td>25 [-117, 74]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2017</td>
<td></td>
<td>2017</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Children</td>
<td>Interim</td>
<td>11</td>
<td>214</td>
<td>24 [-19, 52]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Final</td>
<td>14</td>
<td>260</td>
<td>52 [27, 68]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adults</td>
<td>Interim</td>
<td>91</td>
<td>309</td>
<td>48 [30, 61]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Final</td>
<td>129</td>
<td>426</td>
<td>37 [20, 50]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Elderly</td>
<td>Interim</td>
<td>75</td>
<td>19</td>
<td>32 [-44, 66]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Final</td>
<td>108</td>
<td>23</td>
<td>-18 [-115, 35]</td>
</tr>
</tbody>
</table>

Vaccine effectiveness, overall by age group.
VE pairs against influenza A(H3N2) by age group

VE pair estimates specific for influenza A(H3N2) by age group are shown in Figure 3.7. Age-specific VE estimation for A(H3N2) was characterised by non-significance, very low precision and poor interim-final pair concordance for most years studied.

Figure 3.7: Influenza VE pair estimates –including 95% confidence intervals– for influenza A(H3N2) by age group, Australia, 2012–2017. Notes: V=vaccinated, UV=unvaccinated, 95% CI=95% confidence interval.
An exception was VE for adults in 2014 which were significant and showed reasonable precision and good concordance. Although shown in the figure below VE estimates for the 2013 season had zero cases among children and older adults, making the estimates meaningless. There were less than five cases among children, adults and older adults in different categories in three of the years studied. Sparse data resulted extremely large confidence intervals containing infinite negative values and inflated or negative estimates.

Concordance among age-specific interim and final VE estimates for influenza A(H3N2) was lowest for elderly adults for all years (i.e., the difference between interim and final VE was more than 25 percentage points).

### 3.3.5 VE pairs by target group of vaccination

Data from 2015 to 2017 were used to estimate VE pairs for people in a target group for vaccination as defined by the national immunisation program (14). Prior to 2015, data availability on target group status were insufficient to allow VE estimation. Figure 3.8 shows VE estimates for people in target groups for vaccination excluding those over 65 years of age. Concordance among VE pairs was reasonably high for this group but precision was poor. 2017 had the lowest VE for people in a target group for vaccination.

<table>
<thead>
<tr>
<th>Year</th>
<th>Positive</th>
<th>Negative</th>
<th>VE [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V</td>
<td>UV</td>
<td>V</td>
</tr>
<tr>
<td>2015</td>
<td>Interim</td>
<td>15</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>20</td>
<td>47</td>
</tr>
<tr>
<td>2016</td>
<td>Interim</td>
<td>11</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>21</td>
<td>64</td>
</tr>
<tr>
<td>2017</td>
<td>Interim</td>
<td>51</td>
<td>118</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>77</td>
<td>153</td>
</tr>
</tbody>
</table>

Figure 3.8: Influenza VE pair estimates –including 95% confidence intervals– for people in a target group for vaccination, Australia, 2012–2017. Notes: V=vaccinated, UV=unvaccinated, 95% CI=95% confidence interval. Patients over 65 years of age were excluded from this group.


3.4 Discussion

We estimated influenza VE during the season and at the end of the season for 2012 to 2017 using nationally-collected outpatient ILI surveillance data. Our study showed that the final adjusted all-age VE against all influenza was moderate for 2013 to 2016 seasons (45–56%) and lower for the 2012 and 2017 seasons (20 and 35%, respectively). A similarly low and non-significant VE (23%) was also reported for Australia in 2012 when the same surveillance system was used (1). Our overall final VE of 52% for 2013 is in agreement with that previously reported for Victoria (55%), which was generated using outpatient data from a different surveillance system (29). In contrast with that study, our overall VE estimate for 2013 was significant, reflecting the larger sample size we used. Our overall final VE estimate for 2014 is similar to that reported in the literature using pooled surveillance data from Victoria, Western Australia and ASPREN (45 vs. 44 %). We did not find published Australian VE data for 2016 for comparison. Lastly, our low overall interim VE estimate for 2017 (36%) is consistent with that reported by Sullivan et. al (30) (33%) which used pooled surveillance data and a slightly longer interim period (i.e., two weeks).

In terms of the ability of the interim VE to predict final VE we found that larger sample sizes produced interim overall VE that reliably predicted final VE against all influenza and against specific subtypes/lineages. Other studies that found good overall VE pair concordance attributed it to interim VE being estimated after the peak of influenza activity, as was also the case in our study (5, 8). For seasons with smaller sample sizes, the variation within VE pairs was more pronounced.

Poor concordance in VE pairs was observed in age-specific all-influenza estimates in this study, particularly for children and older adults. VE pairs for older adults were generally non-significant. Large VE pair discrepancies were found for H3-specific VE where the enormously wide confidence intervals rendered most estimates meaningless for children and older adults in all years. Imprecise subtype- and age-specific VE estimates for these age groups have also been reported previously. Using all three ILI surveillance systems in Australia in an effort to overcome sample size limitations Sullivan et. al found that when overall age-specific VE was less precise, subtype-specific VE could not be estimated for children and/or elderly people. This was partly due to very low influenza immunisation coverage in children and the low number of vaccinated cases in older adults (9).

When analysis was restricted to people in the target groups for vaccination, which excluded those over 65 years, we found very good estimate concordance for two out of
the three years studied. Variable VE pair concordance for people in a target group for vaccination has been reported by others. Using the same study design and outpatientILI surveillance data, a Spanish study found that for some years there was little variation between interim and final VE estimates for target groups whereas for other years the final estimate was much lower than the interim one (44 and 22%, for interim and final VE respectively for the 2013/14 season) (31). Similarly, Sullivan et. al (5) found a decline in final VE for people in target groups for vaccination of 13% points compared to interim VE for the 2011 Victorian season. Differences in interim and final VE for subgroups are influenced by the same factors that influence the precision of summary estimates: the length of the season, the timing of the peak of influenza activity, sample size and number of vaccinated cases.

In addition, we noted that final estimates were generally lower than interim estimates. Several studies from Australia and elsewhere shared these findings (32, 31). Differences between interim and final VE could occur mainly due to agent and vaccine factors. Host factors would not be expected to change sufficiently within a few months in a given population to have a substantial effect on VE pair concordance. Differences in the predominant influenza subtype circulating mid season versus late season could also result in VE pair discordance. This change in predominant strains could cause a mismatch between circulating and vaccine strains. VE would be higher for the seasonal period in which vaccine match is highest. Furthermore, the vaccine immunogenicity is known to decrease with time post-vaccination (33). As vaccination campaigns span April to May, by the time final VE is estimated (in December) a larger cohort of people would be expected to have waned immunity compared to the time when interim VE is estimated.

When VE was estimated by influenza subtype/lineage, age group and target group for vaccination issues of sparse data (i.e., where there are few or no observations at key combinations of the outcome, exposure, and covariates (34)) precluded reliable VE estimation. The most severe example of this problem was observed in the age-specific VE estimate for influenza A(H3N2) where the nil or very low number of both vaccinated and unvaccinated cases—particularly among children—produced inflated estimates, impossibly wide confident intervals of several thousand percentage points and also infinite values. Caution is needed when interpreting estimates generated with fewer than five observations per cell in the two by two table.

All-ages final H3-specific VE for 2017 was centered around the null. Final VE estimates for 2017 using all available Australian outpatient surveillance data have not yet been published but our interim VE for H3 (8%) closely matched the previously published interim estimate of 10% (30). Although not statistically significant, these
results indicate a lack of vaccine protection against this strain. The 2017 season was characterised by increased length, predominance of influenza A(H3N2) viruses, an increase in ILI consultations compared to previous years and a poor phylogenetic match between the H3 subtypes in circulation and those included in the vaccine likely due to egg-induced changes introduced during the vaccine manufacturing process (35, 17, 30, 36).

In addition to season severity, the null VE against all influenza in older adults seen in 2017 could be due to the effect of repeated vaccination, as vaccination coverage in older Australian adults is relatively high. Further research is needed to better understand the effect of repeated vaccination on influenza VE in all age groups so that sound influenza immunisation policies can be designed (37).

Our results indicate that a larger sample size (i.e., increased numbers of specimens tested for influenza) improves the quality of overall VE estimates, however, the sampling scheme currently in place for ASPREN practitioners does not generate a sufficient sample size to enable reliable and accurate subtype/lineage-, age- and sub-population-specific VE estimates during the season. A hypothetical comparison of the impact of swabbing rates on the precision of VE estimates using data from the Victorian Sentinel Practice Influenza Network demonstrated that as the annual sample size declines, confidence intervals become excessively wide, estimates tended to include the null and the study power to detect an effect was reduced (38). For some subtypes/lineages it was not possible to generate an estimate at all with a reduced sample size. Sparse data can lead to bias (39) and statistical ways to reduce it have been suggested (34). However, it is prudent to revise swabbing recommendations for ASPREN practitioners and advocate for the inclusion of as many children and older outpatients as possible to prevent sparse data in the first place.

**Strengths and limitations**

The test-negative design has similar limitations as case control studies. Poor patient recall or incomplete medical records can result in misclassification of vaccination status which is likely a source of non-differential error (6). Importantly, results from test-negative studies may not be generalisable to the entire population because cases and controls are selected only from people that seek medical care (40).

No single surveillance system is able to adequately capture the dynamics between influenza disease severity, predominant circulating strain and population susceptibility. As such, this study was impacted by ASPREN limitations, many of which have previously been described. These are: 1) lack of geographic representativeness (i.e.,
most GPs are located in urban areas compared to rural and remote areas) (41, 42); 2) incomplete data collection by practitioners – particularly vaccination status, date of vaccination, Indigenous status and comorbidities; 3) predominance of adult patients that present to sentinel practices; and 4) bias in practitioners’ swabbing behaviour against children and older ILI patients (38). The combination of poor representativeness of the surveillance system and incomplete collection of Indigenous status data meant that influenza VE in Aboriginal and Torres Strait Islander people could not be estimated. Specifically, the low influenza vaccine coverage and the fairly low intensity of surveillance activity by ASPREN (i.e., relatively small weekly numbers of specimens collected from ILI patients for influenza testing) resulted in low numbers of vaccinated cases and sometimes a lack of power to detect even a modest effect of vaccination in young children and elderly adults. Sample size limitations were also a barrier to meaningfully estimating VE for specific influenza type and subtypes.

Finally, no genetic and antigenic data was used in this study for assessment of the match between vaccine strains and those isolated from ASPREN patients to aid VE interpretation. Despite these limitations, ASPREN data could be used to summarise protection conferred by the vaccine against the most common types of influenza virus – A(H1N1)pdm, A(H3N2) and B- which circulate in fluctuating proportions at different times of the season and in different parts of Australia while varying levels of vaccine strain match are at play.

3.5 Conclusions

Concordance between interim and final overall influenza VE estimates was higher in years where the number of specimens collected and tested was higher. Precise VE estimation for subtypes and lineages and by age group was not possible in most years due to the small number of specimens collected. In contrast to VE for adults, the number of specimens collected from children and elderly adults was too low to produce a meaningful estimate.

We recommend that the ASPREN sampling scheme for ILI patients be further refined to include 40% of working-age adults, 100% of people over 65 years of age and 100% of children (i.e., under 18 years of age). Increasing the number of respiratory specimens collected from the youngest and oldest age categories of ILI patients would improve the usefulness of ASPREN data to reliably estimate influenza VE during the season, by subtype/lineage for vulnerable groups: children, older adults and people in target groups for vaccination.
References


Chapter 3


18 Belongia EA, Simpson MD, King JP, Sundaram ME, Kelley NS, Osterholm MT, et al. Variable Influenza Vaccine Effectiveness by Subtype: A Systematic Re-


33 Immune History and Influenza Vaccine Effectiveness. Vaccines. 2018;6(2). doi:10.3390/vaccines6020028.


Appendices

3.A Oral poster presentation at the Annual EIS Conference, April 2018

I delivered an interactive oral poster presentation at the 67th Annual Epidemic Intelligence Service Conference TEPHINET International Night in Atlanta, United States, on 17 April 2018. The conference theme was ‘Improving Global Health Security through Field Epidemiology Training, Surveillance, and Outbreak Response’. An image of the digital poster and each of its frames are shown below.
1. What is Vaccine Effectiveness...

- VE is a measure of vaccine performance in reducing disease in a population, under real life circumstances.¹

- An important public health tool. Low VE estimated mid-season indicates that alternative prevention and control measures are needed.

- Assists experts in deciding the

2. Surveillance of Influenza-like...

Figure 1. Australian General Practices that Participate in ILI Surveillance in Australia.

- A selection of patients seeking care with influenza-like illness are tested for influenza

- Vaccination status is recorded.

- The odds of vaccination among the test-positives is compared with the negatives by adjusted logistic regression.

3. How is Vaccine Effectiveness...

We estimated VE using the test-negative design² during and at the end of the season. The test-negative design, represented in Figure 2, is a variant of the case–control study.

Figure 2. Estimating Vaccine Effectiveness from the Test-negative Design

4. Results

Figure 3. Number of Influenza-like illness (ILI) Consultations per Week by Laboratory Test Result Status for the Australian Sentinel Practices Research Network (ASPREN), 2012-2017

Notes:
The shaded area represents the period included for final VE estimation. The broken line represents the end of the interim period.

Figure 4. Overall influenza VE estimates against influenza A and/or B, Australia, 2012-2017

5. Public Health Significance of the...

- Our findings indicate that to improve the reliability and usefulness of influenza VE estimates for public health action, the national ILI surveillance system needs to be expanded.

- We advocate for a larger number of specimens to be collected, particularly from children and elderly patients that present to sentinel general practice with ILI.

- Expanding routine ILI surveillance in Australia will allow more precise VE estimates overall, be cost-effective, and...

Acknowledgements

We thank all practices, and general and nurse practitioners who participated in the sentinel surveillance for their time and data contribution; staff of the Data Management and Analysis Centre at the University of Adelaide for design and programming of the ASPREN database.

We also acknowledge the laboratory staff of the SA Pathology, PathWest, the Victorian Infectious Diseases Reference Laboratory, and the WHO Collaborating Centre for Reference and Research on Influenza.
1. WHAT IS VACCINE EFFECTIVENESS (VE)?

- VE is a measure of vaccine performance in reducing disease in a population, under real life circumstances.¹

- An important public health tool. Low VE estimated mid-season indicates that alternative prevention and control measures are needed.

- Assists experts in deciding the composition of the annual influenza vaccine.

2. SURVEILLANCE OF INFLUENZA-LIKE ILLNESS IN AUSTRALIA

Figure 1. Australian General Practices that Participate in ILI Surveillance in Australia.

ASPREN is the Australian Sentinel Practices Research Network. It conducts routine influenza-like illness (ILI) surveillance.

ILI surveillance data emanating from ASPREN is used to estimate vaccine effectiveness in Australia.

Over 200 GPs, Australia-wide, report to ASPREN. Their locations are shown in the map below (Figure 1).

ASPREN is a key part of pandemic preparedness for Australia.

For more information on surveillance of influenza-like illness in Australia you can watch this video:

[VIDEO] https://www.youtube.com/embed/CoZuDMfhVUw?feature=oembed&fs=1&modestbranding=1&rel=0&showinfo=0
3. HOW IS VACCINE EFFECTIVENESS ESTIMATED?

We estimated VE using the test-negative design\(^2\) during and at the end of the season. The test-negative design, represented in Figure 2, is a variant of the case–control study.

**Figure 2. Estimating Vaccine Effectiveness from the Test-negative Design**

- A selection of patients seeking care with influenza-like illness are tested for influenza
- Vaccination status is recorded
- The odds of vaccination among the test-positives is compared with the negatives by adjusted logistic regression,
- VE is calculated as \((1-OR)\times 100\%\)
4. RESULTS

**Figure 3.** Number of Influenza-like illness (ILI) Consultations per Week by Laboratory Test Result Status for the Australian Sentinel Practices Research Network (ASPREN), 2012-2017

Notes:
The shaded area represents the period included for final VE estimation. The broken line represents the end of the interim period.

**Figure 4.** Overall influenza VE estimates against influenza A and/or B, Australia, 2012-2017

Notes:
V = vaccinated, UV = unvaccinated

Overall VE against influenza A and B (estimated both during and at the end of the season) was approximately 50% in 2013-2016.

Precision of the VE estimates increased with a larger sample size.
In 2017, estimates for A/H3N2 by age group had very poor precision and VE pair concordance was low, especially for children and the elderly. Similar patterns were observed for previous seasons.

Children and elderly are under-represented in national ILI surveillance.

Most ILI patients that had a specimen collected were adults (~60%). Approximately 20% of patients were children and elderly.
5. PUBLIC HEALTH SIGNIFICANCE OF THE STUDY

- Our findings indicate that to improve the reliability and usefulness of influenza VE estimates for public health action, the national ILI surveillance system needs to be expanded.

- We advocate for a larger number of specimens to be collected, particularly from children and elderly patients that present to sentinel general practice with ILI.

- Expanding routine ILI surveillance in Australia will allow more precise VE estimates overall, by subtype/lineage and, importantly, for vulnerable groups such as children, the elderly, people with underlying medical conditions and Indigenous Australians.

Influenza immunisation remains the most important intervention in preventing or attenuating influenza infection and mortality. A national ILI surveillance system capable of producing robust VE estimates in near real time is one of the tools we have to monitor vaccine performance and consider the need for a better vaccine.

ACKNOWLEDGEMENTS

We thank all practices, and general and nurse practitioners who participated in the sentinel surveillance for their time and data contribution; staff of the Data Management and Analysis Centre at the University of Adelaide for design and programming of the ASPREN database.

We also acknowledge the laboratory staff of the SA Pathology, PathWest, the Victorian Infectious Diseases Reference Laboratory, and the WHO Collaborating Centre for Reference and Research on Influenza.

The Australian Sentinel Practices Research Network and the Melbourne WHO Collaborating Centre for Reference and Research on Influenza are supported by the Australian Department of Health. The presenting author was supported by an Australian Government Research Training Program Scholarship.

This study has been approved by the Australian National University Human Research Ethics Committee (Protocol N 2017/894).
AUTHOR INFO

Ximena is an applied epidemiology trainee completing the Australian Field Epidemiology Training Program at the WHO Collaborating Centre for Reference and Research on Influenza. She holds postgraduate qualifications in international public health and microbiology. Her work experience spans the prevention and control of infectious diseases of public health and veterinary importance, in both developed and resource-constrained countries. As a field epidemiologist Ximena will work to reverse Indigenous disadvantage, and respond to humanitarian emergencies.

For more information about the work of the WHO Collaborating Centre for Reference and Research on Influenza in Melbourne, Australia, you can watch this video.

[VIDEO] https://www.youtube.com/embed/Yug2QsvbCDU?feature=oembed&fs=1&modestbranding=1&rel=0&showinfo=0

REFERENCES


3.B Presentation at the National Immunisation Conference, June 2018

I delivered an oral presentation at the 16th Public Health Association of Australia National Immunisation Conference ‘Immunisation for all: Gaps, gains and goals’ on 6 June 2018 in Adelaide, South Australia. The slides are shown below.

Reliability of interim estimates of influenza vaccine effectiveness, 2012-2017
Tolosa MX, Chilver M, Stocks NP, Leung VK, Sullivan SG
16th National Immunisation Conference
6 June 2018

Background

Robust influenza VE for subtypes is needed during the influenza season to guide public health action to reduce influenza morbidity and mortality.

Objective


Methods

ASPREN ILI surveillance data
Test-negative design
Interim VE (week 18 – 36)
Final VE (week 18 – season end)

Methods

Robust influenza VE for subtypes is needed during the influenza season to guide public health action to reduce influenza morbidity and mortality.

ASPREN ILI surveillance data

Test-negative design

Interim VE (week 18 – 36)

Final VE (week 18 – season end)

Methods

Sentinel patients

Skewed patients

Test-positive

Test-negative

Patient data:
- vaccination history
- age
- comorbidities
- sex, etc.

VE = (1 - ORadj) x 100%

## Methods

### Interim and final influenza VE estimates, 2012-2017

#### Overall

<table>
<thead>
<tr>
<th>Year</th>
<th>( n )</th>
<th>( f )</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>1,417</td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>990</td>
<td></td>
</tr>
<tr>
<td>2014</td>
<td>1,469</td>
<td></td>
</tr>
<tr>
<td>2015</td>
<td>1,386</td>
<td></td>
</tr>
<tr>
<td>2016</td>
<td>1,013</td>
<td></td>
</tr>
<tr>
<td>2017</td>
<td>2,357</td>
<td></td>
</tr>
</tbody>
</table>

#### By subtype & age group

**Children**

- Interim (\( n = 368 \))
  - VE range: \( -12 \) to \( -41 \)
- Final (\( n = 509 \))
  - VE range: \( -11 \) to \( -22 \)

**Adults**

- Interim (\( n = 922 \))
  - VE range: \( -27 \) to \( -59 \)
- Final (\( n = 1,362 \))
  - VE range: \( -14 \) to \( -28 \)

**Elderly**

- Interim (\( n = 208 \))
  - VE range: \( -21 \) to \( -50 \)
- Final (\( n = 329 \))
  - VE range: \( -37 \) to \( -75 \)

---

**VE for influenza A/H3N2 by age group, Australia, 2017**

---

![Graph showing vaccine effectiveness by week in 2017](image.png)

---

**Children**

- Interim (\( n = 368 \))
  - VE range: \( -12 \) to \( -41 \)
- Final (\( n = 509 \))
  - VE range: \( -11 \) to \( -22 \)

**Adults**

- Interim (\( n = 922 \))
  - VE range: \( -27 \) to \( -59 \)
- Final (\( n = 1,362 \))
  - VE range: \( -14 \) to \( -28 \)

**Elderly**

- Interim (\( n = 208 \))
  - VE range: \( -21 \) to \( -50 \)
- Final (\( n = 329 \))
  - VE range: \( -37 \) to \( -75 \)
Conclusion

- Insufficient sample size -> VE by subtypes and population groups of interest
  - Low precision
  - Unreliable interim VE
- Highlights the need to increase representation of children and people in target groups for vaccination in ILI surveillance

We recommend:
Expansion of routine ILI surveillance in Australia to allow reliable and precise influenza VE estimation by subtype for children, older adults and others in target groups for vaccination such as people with chronic illnesses and Indigenous Australians
Acknowledgements

ASPREN practitioners

Laboratories
SA Pathology
PathWest
VIDRL
WHO CCRRI

Sheena Sullivan,
WHOCCRRI
Tambri Housen,
ANU

The Melbourne WHO Collaborating Centre for Reference and Research on Influenza is supported by the Australian Government Department of Health.
3.C Publication in the WHO Weekly Epidemiological Record, August 2018

This project consisted of assessing the ability of National Influenza Centers in 25 countries to isolate and correctly identify influenza viruses. The assessment was done within the framework of an external quality assurance program conducted by the WHO Collaborating Centre for Reference and Research on Influenza. Using the statistical software R, I cleaned, compiled, tabulated and graphed data provided by each laboratory in a standard Excel sheet. I identified issues of data completeness and clarity and updated data accordingly once we received clarifications from laboratories. I revised and provided feedback on all versions of the manuscript.
to the April sewage isolate was identified in stool samples from 2 AFP cases in Middle Shabelle province and 1 AFP case in Hiran province (which consisted of coinfection with cVDPV2). A bOPV response vaccination campaign is planned for the southern and central provinces of Somalia. AFP surveillance has been intensified in all 3 countries through active case finding at health facilities and other reporting sites. Further field investigations are ongoing.

Affiliations

* Global Immunization Division, Centers for Disease Control and Prevention, Atlanta (GA), USA; † Division of Viral Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta (GA), USA; ‡ Polio Eradication Department, World Health Organization, Geneva, Switzerland; § World Health Organization Regional Office for the Eastern Mediterranean, Amman, Jordan; ¶ World Health Organization, Horn of Africa Coordination Office, Nairobi, Kenya; ¶ World Health Organization, Kenya Country Office, Nairobi, Kenya; ¶ World Health Organization Liaison Office for Somalia, Nairobi, Kenya (Corresponding author: Victor Eboh, nqy1@cdc.gov).


M. Ximena Tolosa,* Vivian K. Leung,† Ivona Buettner,‡ Angela Todd,* Yi-Mo Deng,* Robert Shaw,§ Kanta Subbarao,† Ian G. Barr,¶ Belinda Herring,* Amal Barakat,¶ Rakhee Palekar,* Wenqing Zhang,* Christian Fuster,* Magdi D. Samaan and Patrick C. Reading*

Introduction

Global influenza virus surveillance has been conducted through the WHO Global Influenza Surveillance and Response System (GISRS) for more than 65 years. 1 The laboratory network associated with GISRS comprises more than 140 National Influenza Centres (NICs) in >110 WHO Member States, 6 WHO collaborating centres (CCs), 4 WHO essential regulatory laboratories and 13 WHO H5 reference laboratories. NICs perform preliminary analyses on virus specimens collected in the country before shipping representative clinical specimens and influenza virus isolates to WHO CCs for advanced antigenic and genetic analyses. The results of these analyses form the basis for WHO recommendations on the composition of influenza vaccines twice a year. The ability to isolate and propagate influenza virus from clinical specimens is essential for ongoing surveillance of circulating virus strains and sharing of viruses. Virus isolates generated from clinical specimens provide the necessary antigenic and genetic information to

PVDCv3 apparent to l’isolat prélevé dans les eaux usées en avril a été identifié dans des échantillons de selles provenant de 2 cas de PFA dans la province du Moyen Shabelle et d’un cas de PFA dans la province d’Hiran (un cas de coinfection par le PVDCv2). Une campagne de vaccination de riposte par le VPOb est prévue dans les provinces du sud et du centre de la Somalie. Des efforts d’intensification de la surveillance de la PFA ont été faits dans les 3 pays moyennant une recherche active des cas dans les centres de santé et autres sites de notification. Des recherches plus approfondies se poursuivent sur le terrain.

Affiliations des auteurs

* Global Immunization Division, Centers for Disease Control and Prevention, Atlanta (GA), États-Unis d’Amérique; † Division of Viral Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta (GA), États-Unis d’Amérique; ‡ Département Eradication de la poliomyélite, Organisation mondiale de la Santé, Genève, Suisse; ¶ Bureau régional de la Méditerranée orientale de l’Organisation mondiale de la Santé, Amman, Jordanie; © Bureau de coordination pour la Corne de l’Afrique de l’Organisation mondiale de la Santé, Nairobi, Kenya; * Bureau de liaison de l’Organisation mondiale de la Santé pour la Somalie, Nairobi, Kenya (auteur correspondant: Victor Eboh, nqy1@cdc.gov).

Isolation et identification de virus grippaux humains en culture cellulaire: analyse sommaire du programme d’évaluation externe de la qualité de l’OMS pour les centres nationaux de la grippe dans les régions OMS des Amériques, de l’Afrique et de la Méditerranée orientale, 2017

M. Ximena Tolosa,* Vivian K. Leung,† Ivona Buettner,‡ Angela Todd,* Yi-Mo Deng,* Robert Shaw,§ Kanta Subbarao,† Ian G. Barr,¶ Belinda Herring,* Amal Barakat,¶ Rakhee Palekar,* Wenqing Zhang,* Christian Fuster,* Magdi D. Samaan and Patrick C. Reading*

Introduction

Depuis plus de 65 ans, le Système mondial OMS de surveillance de la grippe et de riposte (GISRS) assure la surveillance des virus grippaux au niveau mondial. 1 Le réseau de laboratoires associé au GISRS comprend plus de 140 centres nationaux de la grippe (CNG) dans plus de 110 États Membres de l’OMS, 6 Centres collaborateurs de l’OMS, 4 laboratoires essentiels de réglementation et 13 laboratoires H5 de référence de l’OMS. Les CNG pratiquent des analyses préliminaires sur des échantillons de virus recueillis dans le pays avant l’expédition d’échantillons cliniques représentatifs et d’isolements de virus grippaux aux centres collaborateurs (CC) de l’OMS pour des analyses antigéniques et génétiques poussées. Les résultats de ces analyses servent de base à la formulation des recommandations de l’OMS concernant la composition des vaccins antigrippaux deux fois par an. La capacité à isoler et à propager le virus grippal à partir d’échantillons cliniques est déterminante pour la surveillance continue des souches virales circulantes et l’échange de virus. Les isolements de virus obtenus à partir des échantillons cliniques fournissent les informations antigéniques et

informs selection of vaccine strains and are used for other assays, such as phenotypic assays for assessing susceptibility to antiviral drugs.

NIICs and diagnostic laboratories perform proficiency tests to confirm the quality and reliability of their testing procedures. In 2007, after a number of outbreaks of A(H5N1) in Asia, WHO initiated a global external quality assessment (EQA) programme for influenza A virus subtype detection by polymerase chain reaction (PCR). The EQA programme has been extended to include seasonal influenza A, influenza B and other non-seasonal influenza A viruses reported in human infections such as influenza A(H5N1) and A(H7N9) as well as analysis of the susceptibility of influenza viruses to current antiviral drugs. Over the years, a growing number of NICs worldwide have participated in this programme. Of the 151 laboratories that reported results for the 2016 panel (panel 15), 87.4% returned correct results for all EQA samples.2

Influenza virus isolation in cell culture is a key function of NICs and is in their WHO terms of reference. In 2016, an initiative was begun to assess the performance of NICs in the WHO Western Pacific and South-East Asian regions in isolating and identifying a panel of influenza viruses in cell culture (panel 13). In 2017, this programme was extended to assess isolation and identification of influenza viruses with cell culture techniques by NICs in the WHO regions of the Americas (AMR), Africa (AFR) and the Eastern Mediterranean (EMR) in a second panel of viruses (panel 2). This programme, under the coordination of WHO's Global Influenza Programme, was implemented by the WHO Collaborating Centre for Reference and Research on Influenza Melbourne, Australia (Melbourne WHO CC), with support from the associated WHO regional offices. This report summarizes the results for panel 2, which was dispatched to participating laboratories starting in 2017.

Study design

Preparation and composition of panel

EQA panel 2 comprised 16 samples containing isolates of influenza A or B viruses and negative control samples labelled VIP-2017-01–VIP-2017-16. All were originally isolated in MDCK or MDCK-SIAT-1 cells at the WHO CC between 2015 and 2016 and stored at −80 °C. The virus isolates used to generate the EQA panel were A/H1N1pdm09 (A/H3N2 subtype), A/Sydney/31/2016 (H1N1pdm09 subtype), B/Victoria/726/2015 (B/Victoria lineage (B/Vic)) and B/New Caledonia/47/2015 (B/Yamagata lineage (B/Yam)). Isolates were diluted as appropriate in serum-free Dulbecco's modified Eagle's medium (Gibco), separated into 0.5-ml aliquots and frozen at −80 °C. The composition of the panel is shown in Table 1.

EQA sample VIP-2017-01 (containing a B/Yam virus isolate) was included in the panel to test the ability of laboratories to perform haemagglutination (HA) assays with their choice of red blood cells. Participating laboratories were instructed not to perform virus isolation on this sample. VIP-2017-01 was tested in 3 independent genetic pathways necessary to identify the selection of the vaccine strains and are used in other endpoints, such as those tests phenotypic assays to evaluate the sensitivity to antiviral drugs.

The CNG and laboratories of diagnostic origin prent part to the tests of aptitude to confirm the quality and the reliability of their procedures of analysis. In 2007, a suite to the survenue of one certain number of flammé de grippe A(H5N1) in Asia, the OMS has launched a programme mondial d'évaluation externe de la qualité (EQA) portant on the detection of the virus gripaux appartenant au sous-type A par amplification génique (PCR). Ce programme d'EQA a été étendu pour couvrir également la grippe A saisonnière, la grippe B et d'autres virus gripaux non saisonniers signalés comme responsables d'infections humaines, tels que les virus A(H5N1) et A(H7N9), ainsi que l'analyse de la sensibilité des virus gripaux aux médicaments antiviraux actuels.2 With several years, in plus de plus in CNG in the monde ont été part au programme. Sur les 151 laboratoires ayant rapporté des résultats pour la série d'échantillons de 2016 (série 15), 87,4% ont soumis des résultats corrects pour l'ensemble des échantillons d'EQA.2

L'isolement des virus de la grippe en culture cellulaire est une fonction essentielle des CNG et figure dans leur mandat selon l'OMS. En 2016, une initiative a été lancée pour évaluer la capacité de ces centres, dans les Régions OMS du Pacifique occidental et de l’Asie du Sud-Est, à isoler et à identifier les virus gripaux en culture cellulaire dans une série de 16 échantillons (série 1).3 En 2017, ce programme a été largi pour intégrer l'évaluation de l'isolement et de l'identification des virus gripaux par des techniques de culture cellulaire par les CNG dans les Régions OMS des Amériques (AMR), de l'Afrique (AFR) et de la Méditerranée orientale (EMR) sur une deuxième série de virus (série 2). Ce programme, sous la coordination du Programme mondial de l'OMS de lutte contre la grippe, a été mis en œuvre par le centre collaborateur de référence et de recherche pour la grippe (CC) de Melbourne (Australie), avec l'appui des bureaux régionaux de l'OMS associés. Le présent rapport résume les résultats pour la série 2, qui a été distribuée aux laboratoires participants à partir de 2017.

Conception de l'étude

Préparation et composition de la série

La série 2 de l'EQA comprenait 16 échantillons, dont des échantillons de virus gripaux A ou B et des échantillons témoins négatifs, étiquetés VIP-2017-01–VIP-2017-16. Tous avaient initialement été isolés dans des cellules MDCK ou MDCK-SIAT-1 dans le CC de l'OMS à Melbourne entre 2015 et 2016, puis conservés à +4°C. Les isolats de virus utilisés pour générer la série d'EQA appartenaient aux lignées A/H1N1pdm09 (sous-type A/H3N2), A/Sydney/31/2016 (sous-type H1N1pdm09), B/Victoria/726/2015 (B/Victoria lineage (B/Vic)) et B/New Caledonia/47/2015 (B/Yamagata lineage (B/Yam)). Les isolats ont été dilués dans un milieu d'Eagle modifié par Dulbecco exempt de sérum (Gibco), la solution obtenue étant ensuite fractionnée en aliquotes de 0,5 ml et congelée à −80 °C. La composition de la série est présentée dans le Tableau 1.

L'échantillon d'EQA VIP-2017-01 (contenant un isolement de virus B/Yam) a été inclus dans la série pour tester la capacité des laboratoires à pratiquer les épreuves d'hémagglutination (HA) avec des érythrocytes de leur choix. Les laboratoires participants ont reçu l'instruction de ne pas réaliser d'isolement viral sur cet échantillon. Le VIP 2017-01 a été soumis à 3 épreuves d’HA indé-
Table 1 EQA panel 2 samples provided to National Influenza Centres for influenza virus isolation and identification and corresponding results

<table>
<thead>
<tr>
<th>Sample identity – Identité de l’échantillon</th>
<th>Identity – identité(a)</th>
<th>Virus load – Charge virale(b)</th>
<th>Correct isolation result – Résultat d’isolement correct(c)</th>
<th>Correct Identification result – Résultat d’identification correct(d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>VIP-2017-02</td>
<td>A/H pdm</td>
<td>++</td>
<td>14</td>
<td>56</td>
</tr>
<tr>
<td>VIP-2017-03</td>
<td>B/Vic</td>
<td>++</td>
<td>15</td>
<td>60</td>
</tr>
<tr>
<td>VIP-2017-04</td>
<td>A/H3</td>
<td>++</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td>VIP-2017-05</td>
<td>B/Vic</td>
<td>++</td>
<td>14</td>
<td>64</td>
</tr>
<tr>
<td>VIP-2017-06</td>
<td>Negative – Négatif</td>
<td>–</td>
<td>22</td>
<td>88</td>
</tr>
<tr>
<td>VIP-2017-07</td>
<td>B/Yam</td>
<td>++++</td>
<td>21</td>
<td>84</td>
</tr>
<tr>
<td>VIP-2017-08</td>
<td>A/H pdm</td>
<td>++</td>
<td>17</td>
<td>68</td>
</tr>
<tr>
<td>VIP-2017-09</td>
<td>B/Vic</td>
<td>++++</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>VIP-2017-10</td>
<td>Negative – Négatif</td>
<td>–</td>
<td>23</td>
<td>92</td>
</tr>
<tr>
<td>VIP-2017-11</td>
<td>Negative – Négatif</td>
<td>–</td>
<td>22</td>
<td>88</td>
</tr>
<tr>
<td>VIP-2017-12</td>
<td>A/H pdm</td>
<td>++++</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>VIP-2017-13</td>
<td>A/H3</td>
<td>++++</td>
<td>18</td>
<td>72</td>
</tr>
<tr>
<td>VIP-2017-14</td>
<td>A/H3</td>
<td>++</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td>VIP-2017-15</td>
<td>B/Yam</td>
<td>++</td>
<td>13</td>
<td>52</td>
</tr>
<tr>
<td>VIP-2017-16</td>
<td>A/H3</td>
<td>++++</td>
<td>21</td>
<td>84</td>
</tr>
</tbody>
</table>

CPE: cytopathic effect; EQA: external quality assessment; HA: haemagglutination. – ECP: effet cytopathologique; EQA: évaluation externe de la qualité; HA: hémagglutination

* The identity of each sample was confirmed by real-time RT-PCR with CDC primers and probes. – L’identité de chaque échantillon a été confirmée par RT-PCR en temps réel au moyen d’amorces et de sondes des CDC.

* The amount of virus in each EQA sample provided to laboratories is shown. ++++, ++, – = real-time RT-PCR values of 20–25, 25–30 and >35, respectively, for type A/B assays. Negative = no A/B virus added to EQA sample. – Indication de la quantité de virus contenu dans chaque échantillon d’EQA fourni aux laboratoires. ++++, ++, – = valeurs de Ct de 20–25, 25–30 et >35, respectivement, lors de la RT-PCR en temps réel pour les types A/B. Négatif = aucun virus A/B ajouté à l’échantillon d’EQA.

* Isolation was assessed on the basis of HA results from the 21/25 laboratories that performed this test and on the basis of CPE results from 3/25 laboratories. The 1 laboratory that performed neither CPE nor HA was assigned a value of 0 correct. – L’isolement des virus a été évalué à partir des résultats des épreuves d’HA effectuées par 21/25 laboratoires et des résultats des tests d’ECP pour 25/25 laboratoires. Le seul laboratoire qui n’avait ni évalué l’ECP, ni réalisé d’épreuve d’HA, s’est attribué une valeur de 0 résultat correct.

HA assays at the Melbourne WHO CC with turkey, guinea-pig or human type O erythrocytes (all HA titre = mean of 32 haemagglutinating units (HAU/25 µL)). HA assays were performed with erythrocyte suspensions prepared at 1% vol/vol in phosphate-buffered saline by standard procedures.

EQA samples VIP-2017-02–VIP-2017-16 were included in the panel to test the ability of the laboratories to isolate and identify viruses. The samples were thawed and tested at the Melbourne WHO CC with the real-time RT-PCR influenza A/B typing and subtyping kits FR-198, FR-929 and FR-1209 from Influenza Reagent Resource, Influenza Division, WHO Collaborating Centre for Surveillance, Epidemiology and Control of Influenza, Centers for Disease Control and Prevention, Atlanta, GA, USA. Each sample contained only one virus type/subtype or type/lineage, and no evidence was found of mixed samples (data not shown). Samples from the EQA panel were inoculated onto MDCK cells, and virus was isolated and identified at the Melbourne WHO CC on independent 2 occasions, with consistent results.

Participants were instructed how to store and test the panel of viruses. A questionnaire on the laboratory methods used for cell culture and influenza virus isolation and identification was included for completion by each participant.

Distribution of panel and response of participants

NICs were invited to participate before the panels were dispatched. The panel was dispatched between November 2017 and June 2018 to 25 NICs, namely 12 NICs in pendants au CC de l’OMS à Melbourne avec des érythrocytes de dinde, de cochin d’Inde ou humains de groupe O (pour tous les titres d’HA = moyenne de 32 unités hémagglutinantes (HAU/25 µL)). Les épreuves d’HA ont été pratiquées avec des suspensions d’érythrocytes à 1% en volume dans une solution saline tamponnée au phosphate, selon des procédures standard.


Les participants ont reçu des instructions pour conserver et tester la série de virus. Un questionnaire sur les méthodes de laboratoire appliquées pour la culture cellulaire, l’isolement et l’identification des virus grippe A/B était joint aux envois d’échantillons pour être rempli par chacun des participants.

Distribution de la série et réponse des participants

Les CNG ont été invités à participer avant que les séries ne soient envoyées. Cette série a été distribuée entre novembre 2017 et juin 2018 à 25 CNG, dont 12 en la Région africaine.
AFR, 4 NICs in AMR and 9 NICs in EMR. The panels were shipped on dry ice, and temperature monitors were included in each shipment to ensure that the panels remained frozen throughout transport. Three receiving laboratories reported that the panel did not arrive frozen on dry ice but that they had nonetheless tested the panel. Participating laboratories were asked to report results within 6 weeks of the date of sample reception. Of the 25 participating laboratories, 12 (48%) reported results within 6 weeks, 6 reported results within 6–8 weeks, and 7 reported results after 8 weeks. The average turnaround time from the receipt of the EQA panel to submission of result was 52 days, the shortest and longest times being 19 days and 131 days, respectively. All the results received were analysed, irrespective of time taken for submission.

Methods for assessing virus growth and for determining type, subtype and/or lineage after isolation

Participating laboratories isolated viruses on either MDCK (20/25), MDCK-SIAT-1 (3/25), Vero (1/25) or RhMK (1/25) cells. Cells were prepared in tissue culture flasks (14/25), multi-well plates (3/25) or vials/tubes (8/25). All the laboratories reported that the cells were ≥70% confluent at the time of inoculation. Negative and positive controls for virus isolation were used by 20/25 and 13/25 NICs, respectively. Cells were inoculated with 0.5 mL of EQA sample, as stated in the instructions, by 17/25 laboratories, while 8/25 laboratories used smaller volumes. The protocols used for virus isolation were the WHO Global Influenza Surveillance Network Manual for the laboratory diagnosis and virological surveillance of influenza (12/25), in-house standard operating procedures (2/25) and protocols obtained from the WHO CCRRI (8/25) or other sources (3/25).

Exogenous trypsin was added to virus isolation media at a final concentration of <1 µg/mL (3/25), 1–5 µg/mL (17/25) or >5 µg/mL (3/25).

The first tests used to determine virus growth after inoculation of EQA samples were assessment of cytopathic effect (CPE) and/or HA assays. Overall, 22/25 participating laboratories assessed CPE and 21/25 performed HA assays after inoculation of cells with VIP-2017-02–VIP-2017-16, and 19/25 used both CPE and HA. Only one laboratory assessed neither CPE nor HA but used RT-PCR for the first assessment of virus growth. The methods used to identify the type, subtype and/or lineage of influenza virus present in virus isolates included immunofluorescence assay (IF), haemagglutination inhibition (HI) assay and/or RT-PCR. Overall, 18/25 laboratories performed RT-PCR, 14/25 performed HI, and 2/25 performed IF assays; 12/25 laboratories used more than one assay to confirm virus identity, with combinations of HI and PCR (9/25), HI and IF (1/25), PCR and IF (1/25) and HI, PCR and IF (1/25). The ability of laboratories to identify virus isolates differed, most (23/25) being able to determine influenza type (A or B), 23/25 reporting the influenza A subtype (A/H3 or A/H1pdm) and 20/25 reporting the influenza B lineage (B/Yam or B/Vic). The 2 laboratories that did not report type or subtype used HI assays to identify isolates but reported that they had not obtained sufficient HA titres for subsequent assays.

4 dans la Région des Amériques et 9 dans la Région de la Méditerranée orientale. Les échantillons ont été expédiés sur de la glace sèche et des moniteurs de température ont été inclus dans chaque envoi pour s’assurer que les séries restent à l’état congelé tout au long du transport. Trois laboratoires destinataires ont signalé que la série n’était pas arrivée congelée sur de la glace sèche, mais ils l’ont néanmoins analysée. Il a été demandé aux laboratoires participants de fournir les résultats dans les 6 semaines suivant la réception des échantillons. Sur les 25 laboratoires participants, 12 (48%) ont rapporté les résultats dans le délai de 6 semaines, 6 l’ont fait entre 6 et 8 semaines après la réception et 7 après 8 semaines. Le temps de traitement moyen des échantillons depuis la réception de la série d’EQA jusqu’à la soumission des résultats était de 52 jours, avec un minimum de 19 jours et un maximum de 131 jours. Tous les résultats reçus ont été analysés, indépendamment du temps écoulé avant la soumission.

Méthodes pour évaluer la croissance virale et pour déterminer le type, le sous-type et/ou la lignée après l’isolement du virus


Performance of laboratories

Analysis of VIP-2017-01 with the HA assay

HA titres of sample VIP-2017-01 were provided by 22/25 participating laboratories, and 15/22 reported titres that were within 4-fold of the minimum/maximum HA titres (compared with 32 HAU/25 μL, as determined at the WHO CCRRRI). Overall, 7/25 laboratories reported HA titres of ≤4. Participating laboratories used guinea-pig (9/22), turkey (8/22), human O (4/22) and/or chicken (3/22) erythrocytes; 2 laboratories reported results with both guinea-pig and chicken erythrocytes. All laboratories used 0.5–1.0% suspensions of erythrocytes for HA assays.

Isolation and identification of influenza viruses from samples in the EQA panel

The ability of participating laboratories to obtain the correct result for virus growth, indicative of virus isolation and virus identification, in each sample in the panel are presented in Figure 1A and B, respectively. Virus isolation and identification from samples that did not contain virus, no laboratories identified virus and virus identification was performed by 21/25 laboratories that performed this test and for 3/25 from CPE results. The laboratory that did not perform either CPE or HA was assigned a value of 0 correct analyses. Laboratories were successful (72–84% of the 25 participating laboratories) in isolating virus from the 5 EQA samples that contained large amounts of virus, indicated by a lower cycle threshold (Ct) of 20–25 by real-time RT-PCR (Table 1, Figure 1A). Fewer laboratories (52–68%) were able to isolate virus from the 7 EQA samples that contained smaller amounts of virus (Ct 5–30 for influenza A or influenza B virus) (Table 1). The smallest proportions of laboratories reported influenza virus isolation from EQA samples VIP-2017-15 (52% correct), VIP-2017-02 (56% correct) and VIP-2017-05 (56% correct). Generally, participating laboratories did not report virus isolation from the 3 samples in the EQA panel that did not contain virus (88–92% correct), although 2/25 laboratories reported a positive HA result for VIP-2017-10 (Table 1, Figure 1A).

The results for virus identification showed similar trends to those for virus isolation (Figure 1B). Two laboratories were unable to isolate virus from any samples and therefore correctly identified only negative samples. Of the remaining laboratories, 3/25 performed typing and subtyping assays but did not assess type B lineage; therefore, the results of A/H3, A/H1pdm or type B type were considered correct. For the majority (20/25) of laboratories that assessed isolates for type, subtype and lineage, the results of A/H3, A/H1pdm, B/Yam and B/Vic were considered correct. High percentages (80–86%) of participating laboratories correctly identified the 5 EQA samples containing large amounts of virus (Ct = 20–25 for type A/B, Table 1) but fewer (56–80%) for EQA samples that contained less virus (Ct = 25–30 for type A/B, Table 1). With regard to the negative EQA samples, which did not contain virus, no laboratories identified virus in VIP-2017-06, while 1/25 laboratories identified influenza virus in both VIP-2017-10 (type B) and VIP-2017-11 (type B).

Performances des laboratoires

Analyse du VIP-2017-01 par une épreuve d’HA

Sur les 25 laboratoires participants, 22 ont communiqué des titres d’HA pour l’échantillon VIP-2017-01, et 15 de ces 22 laboratoires ont donné des titres moins de 4 fois supérieurs aux titres d’HA minimum/maximum (par rapport à la valeur de 32 HAU/25 μL établie par le CCRRRI de l’OMS). En tout, 7 laboratoires sur 25 ont fourni des titres d’HA ≤4. Les laboratoires participants ont utilisé des érythrocytes de cochon d’Inde (9/22), de dinde (8/22), humains de groupe O (4/22) et/ou de poulet (3/22); 2 laboratoires ont soumis des échantillons obtenus à la fois avec des érythrocytes de cochon d’Inde et de poulet. Tous les laboratoires ont utilisé des suspensions d’érythrocytes à 0.5-1.0% pour les épreuves d’HA.

Isolement et identification des virus grippaux dans les échantillons de la série d’EQA

La capacité des laboratoires participants à obtenir des résultats corrects pour la croissance virale, qui est un indicateur de l’isolement et de l’identification des virus, dans chaque échantillon de la série est présentée dans les Figures 1A et B, respectivement. L’isolement des virus a été évalué à partir des résultats des épreuves d’HA effectuées par 21/25 laboratoires et des résultats des tests d’ECP pour 3/25 laboratoires. Le laboratoire qui n’avait ni évalué l’ECP, ni réalisé d’épreuve d’HA s’est vu attribuer une valeur de 0 analyse correcte. La grande majorité des 25 laboratoires participants (72–84%) a réussi à isoler le virus dans les 5 échantillons d’EQA qui contenaient une grande quantité de virus, dénotée par une faible valeur de cycle seuil (Ct), de 20-25, lors de la RT-PCR en temps réel (Tableau 1, Figure 1A). Un nombre moins important de laboratoires (52–68%) a soumis des échantillons d’EQA qui contenaient une quantité plus faible de virus (valeur de Ct de 25-30 pour les virus grippaux A ou B) (Tableau 1). La proportion de laboratoires ayant isolé le virus gripsilla était particulièrement faible pour les échantillons d’EQA VIP-2017-15 (52% de résultats corrects), VIP-2017-02 (56% de résultats corrects) et VIP-2017-05 (56% de résultats corrects). De manière générale, les laboratoires participants n’ont pas signalé d’isolement viral dans les 3 échantillons de la série d’EQA qui ne contenaient pas de virus (88–92% de résultats corrects), mais 2/25 laboratoires ont donné un résultat positif à l’épreuve d’HA pour VIP-2017-06 et VIP-2017-11, et 1/25 un résultat positif d’HA pour VIP-2017-10 (Tableau 1, Figure 1A).

Pour l’identification des virus, les résultats suivraient une tendance comparable à celle de l’isolement des virus (Figure 1B). Deux laboratoires n’ont pas été en mesure d’isoler le virus dans un quelconque échantillon et ont donc correctement identifié uniquement des échantillons négatifs. Parmi les autres laboratoires, 3/25 ont effectué des tests de typage et de sous-typage, mais n’ont pas évalué la lignée de type B; leurs résultats pour A/H3, A/H1pdm et les virus de type B ont donc été considérés comme corrects. Pour la majorité (20/25) des laboratoires ayant analysé les isolèments de virus en vue d’en établir le type, le sous-type et la lignée, les résultats pour A/H3, A/H1pdm, B/Yam et B/Vic ont été considérés comme corrects. La proportion de laboratoires participants ayant correctement identifié les virus était élevée (80-88%) pour les 5 échantillons d’EQA qui contenaient une grande quantité de virus (Ct=20-25 pour les types A/B, Tableau 1), mais plus faible (56-80%) pour les échantillons d’EQA qui contenaient moins de virus (Ct=25-30 pour les types A/B, Tableau 1). S’agissant des échantillons négatifs d’EQA, qui ne contenaient pas de virus, aucun laboratoire n’a identifié de virus dans l’échantillon VIP-2017-06, tandis que 1/25 laboratoire a identifié un virus gripilla aussi bien dans l’échantillon VIP-2017-10 (type B) que dans l’échantillon VIP-2017-11 (type B).
The 15 EQA samples tested for virus isolation were grouped according to the amount of influenza virus into "high" (5 panel samples, Ct = 20–25 for type A or B by real-time RT-PCR), "low" (7 panel samples, Ct = 26–30 for type A or B by real-time RT-PCR) or "negative" (3 panel samples, Ct >35 for type A or B by real-time RT-PCR). The percentages of the 25 participating laboratories that obtained the correct result for (A) virus isolation and (B) virus identification are shown. Note that isolation was assessed on the basis of HA results from 21/25 laboratories that performed this test and on the basis of CPE results from 3/25 laboratories. The 1 laboratory that performed neither CPE nor HA was assigned a value of 0 correct.

A. Correct results for virus isolation by amount of virus in EQA sample –

B. Correct results for virus identification by amount of virus in EQA sample

The 15 EQA samples tested for virus isolation were grouped according to the amount of influenza virus into "high" (5 panel samples, Ct = 20–25 for type A or B by real-time RT-PCR), "low" (7 panel samples, Ct = 26–30 for type A or B by real-time RT-PCR) or "negative" (3 panel samples, Ct >35 for type A or B by real-time RT-PCR). The percentages of the 25 participating laboratories that obtained the correct result for (A) virus isolation and (B) virus identification are shown. Note that isolation was assessed on the basis of HA results from 21/25 laboratories that performed this test and on the basis of CPE results from 3/25 laboratories. The 1 laboratory that performed neither CPE nor HA was assigned a value of 0 correct.
Capacity of participating laboratories to isolate and identify viruses

We assessed the overall performance of the participating laboratories in correctly isolating and identifying influenza viruses from the EQA panel samples (Figure 2). For virus isolation, 8/25 laboratories (7/12 in AFR, 0/5 in AMR and 1/8 in EMR) obtained correct results for all 15 EQA samples, and 18 laboratories obtained correct results for ≥10/15 samples (9/12 in AFR, 4/5 in AMR and 5/8 in EMR); 7/25 laboratories reported correct isolation results for <10/15 EQA samples (3/12 in AFR, 1/5 in AMR and 3/8 in EMR).

With regard to virus identification, 12/25 laboratories also obtained correct results for 15/15 EQA samples (6/12 in AFR, 2/5 in AMR and 4/8 in EMR), and 20/25 laboratories obtained correct results for ≥10 EQA samples (10/12 in AFR, 4/5 in AMR and 6/8 in EMR). Overall, 5 laboratories reported correct identification results for <10/15 EQA panel samples (2/12 in AFR, 1/5 in AMR and 2/8 in EMR).

Discussion

Panel 2 is the first EQA of influenza virus isolation and identification in cell culture distributed to NIC laboratories in WHO AFR, AMR and EMR. Most of the participating laboratories could detect influenza virus growth and could identify virus amplified from EQA samples in cell culture distributed to NIC laboratories in WHO AFR, AMR and EMR. Most of the participating laboratories in correctly isolating and identifying influenza viruses from the EQA panel samples (Figure 2). For virus isolation, 8/25 laboratories (7/12 in AFR, 0/5 in AMR and 1/8 in EMR) obtained correct results for all 15 EQA samples, and 18 laboratories obtained correct results for ≥10/15 samples (9/12 in AFR, 4/5 in AMR and 5/8 in EMR); 7/25 laboratories reported correct isolation results for <10/15 EQA samples (3/12 in AFR, 1/5 in AMR and 3/8 in EMR).

With regard to virus identification, 12/25 laboratories also obtained correct results for 15/15 EQA samples (6/12 in AFR, 2/5 in AMR and 4/8 in EMR), and 20/25 laboratories obtained correct results for ≥10 EQA samples (10/12 in AFR, 4/5 in AMR and 6/8 in EMR). Overall, 5 laboratories reported correct identification results for <10/15 EQA panel samples (2/12 in AFR, 1/5 in AMR and 2/8 in EMR).

Discussion

Panel 2 is the first EQA of influenza virus isolation and identification in cell culture distributed to NIC laboratories in WHO AFR, AMR and EMR. Most of the participating laboratories could detect influenza virus growth and could identify virus amplified from EQA samples in cell culture distributed to NIC laboratories in WHO AFR, AMR and EMR. Most of the participating laboratories in correctly isolating and identifying influenza viruses from the EQA panel samples (Figure 2). For virus isolation, 8/25 laboratories (7/12 in AFR, 0/5 in AMR and 1/8 in EMR) obtained correct results for all 15 EQA samples, and 18 laboratories obtained correct results for ≥10/15 samples (9/12 in AFR, 4/5 in AMR and 5/8 in EMR); 7/25 laboratories reported correct isolation results for <10/15 EQA samples (3/12 in AFR, 1/5 in AMR and 3/8 in EMR).

With regard to virus identification, 12/25 laboratories also obtained correct results for 15/15 EQA samples (6/12 in AFR, 2/5 in AMR and 4/8 in EMR), and 20/25 laboratories obtained correct results for ≥10 EQA samples (10/12 in AFR, 4/5 in AMR and 6/8 in EMR). Overall, 5 laboratories reported correct identification results for <10/15 EQA panel samples (2/12 in AFR, 1/5 in AMR and 2/8 in EMR).

Discussion

Panel 2 is the first EQA of influenza virus isolation and identification in cell culture distributed to NIC laboratories in WHO AFR, AMR and EMR. Most of the participating laboratories could detect influenza virus growth and could identify virus amplified from EQA samples in cell culture distributed to NIC laboratories in WHO AFR, AMR and EMR. Most of the participating laboratories in correctly isolating and identifying influenza viruses from the EQA panel samples (Figure 2). For virus isolation, 8/25 laboratories (7/12 in AFR, 0/5 in AMR and 1/8 in EMR) obtained correct results for all 15 EQA samples, and 18 laboratories obtained correct results for ≥10/15 samples (9/12 in AFR, 4/5 in AMR and 5/8 in EMR); 7/25 laboratories reported correct isolation results for <10/15 EQA samples (3/12 in AFR, 1/5 in AMR and 3/8 in EMR).

With regard to virus identification, 12/25 laboratories also obtained correct results for 15/15 EQA samples (6/12 in AFR, 2/5 in AMR and 4/8 in EMR), and 20/25 laboratories obtained correct results for ≥10 EQA samples (10/12 in AFR, 4/5 in AMR and 6/8 in EMR). Overall, 5 laboratories reported correct identification results for <10/15 EQA panel samples (2/12 in AFR, 1/5 in AMR and 2/8 in EMR).

Discussion

Panel 2 is the first EQA of influenza virus isolation and identification in cell culture distributed to NIC laboratories in WHO AFR, AMR and EMR. Most of the participating laboratories could detect influenza virus growth and could identify virus amplified from EQA samples in cell culture distributed to NIC laboratories in WHO AFR, AMR and EMR. Most of the participating laboratories in correctly isolating and identifying influenza viruses from the EQA panel samples (Figure 2). For virus isolation, 8/25 laboratories (7/12 in AFR, 0/5 in AMR and 1/8 in EMR) obtained correct results for all 15 EQA samples, and 18 laboratories obtained correct results for ≥10/15 samples (9/12 in AFR, 4/5 in AMR and 5/8 in EMR); 7/25 laboratories reported correct isolation results for <10/15 EQA samples (3/12 in AFR, 1/5 in AMR and 3/8 in EMR).

With regard to virus identification, 12/25 laboratories also obtained correct results for 15/15 EQA samples (6/12 in AFR, 2/5 in AMR and 4/8 in EMR), and 20/25 laboratories obtained correct results for ≥10 EQA samples (10/12 in AFR, 4/5 in AMR and 6/8 in EMR). Overall, 5 laboratories reported correct identification results for <10/15 EQA panel samples (2/12 in AFR, 1/5 in AMR and 2/8 in EMR).

Discussion

Panel 2 is the first EQA of influenza virus isolation and identification in cell culture distributed to NIC laboratories in WHO AFR, AMR and EMR. Most of the participating laboratories could detect influenza virus growth and could identify virus amplified from EQA samples in cell culture distributed to NIC laboratories in WHO AFR, AMR and EMR. Most of the participating laboratories in correctly isolating and identifying influenza viruses from the EQA panel samples (Figure 2). For virus isolation, 8/25 laboratories (7/12 in AFR, 0/5 in AMR and 1/8 in EMR) obtained correct results for all 15 EQA samples, and 18 laboratories obtained correct results for ≥10/15 samples (9/12 in AFR, 4/5 in AMR and 5/8 in EMR); 7/25 laboratories reported correct isolation results for <10/15 EQA samples (3/12 in AFR, 1/5 in AMR and 3/8 in EMR).

With regard to virus identification, 12/25 laboratories also obtained correct results for 15/15 EQA samples (6/12 in AFR, 2/5 in AMR and 4/8 in EMR), and 20/25 laboratories obtained correct results for ≥10 EQA samples (10/12 in AFR, 4/5 in AMR and 6/8 in EMR). Overall, 5 laboratories reported correct identification results for <10/15 EQA panel samples (2/12 in AFR, 1/5 in AMR and 2/8 in EMR).
samples; however, a number of laboratories obtained <715 correct results for isolation and identification. In addition, many laboratories had difficulty in isolating and identifying viruses from EQA samples that contained low titres, indicating differences in the range of sensitivity of influenza virus isolation among laboratories.

The majority of the participating laboratories (19/25) used both CPE and HA to confirm virus growth. RT-PCR (18/25) and HI (14/25) assays were the most commonly used tests for identifying viruses; 9/25 laboratories performed both assays. IF was used in combination with RT-PCR or HI in 3/25 laboratories. While many laboratories were less successful in correctly isolating and identifying viruses in EQA samples that contained smaller amounts (Figure 1), the EQA identified certain issues for particular laboratories. For example, a number of laboratories identified EQA samples correctly by RT-PCR, but recorded high Ct values, in the range of 30–40 instead of <25, as would be expected for virus isolates, indicating suboptimal virus isolation and/or lack of sensitivity of RT-PCR detection. Another laboratory obtained low HA titres (indicative of low levels of virus) but low Ct values (indicative of high levels of virus) for EQA samples after isolation, suggesting issues in HA test performance. Although one laboratory obtained accurate HA test results for VIP-2017-01, they did not succeed in detecting any virus isolates with the HA test, indicating a major problem in their virus isolation technique. Individual follow-up will be required to correct these issues and to improve the accuracy of virus isolation and identification in these laboratories.

The EQA samples provided to participating laboratories in panel 2 were not original clinical specimens, which is the material most NTCs would receive for routine virus isolation. Instead, the panel consisted of virus isolates propagated in MDCK cells at the WHO CCRRI that were diluted to contain amounts of virus similar to those present in clinical specimens (as determined by Ct values obtained with RT-PCR assays for influenza A or B viruses). Re-isolation of previously isolated influenza viruses is likely to be more successful than isolation from true respiratory clinical samples. It should be noted that, in recent years, H3N2 viruses have proven particularly difficult to isolate from clinical specimens and identifying viruses in EQA samples that contained low titres, indicating differences in the range of sensitivity of influenza virus isolation among laboratories.

It was found that, in recent years, H3N2 viruses have proven particularly difficult to isolate from clinical material, both in eggs and in MDCK cell cultures. Changes in the agglutination of different erythrocytes and reduced growth capacity have created challenges to laboratories for isolating and characterizing H3N2 viruses (in particular, clade 3C.2a viruses) antigenically in the HI assay. 5, 6, 7 Use of MDCK cells engineered to express a high density of human-type sialic acid recep-

d’identifier les virus amplifiés dans les échantillons d’EQA; cependant plusieurs laboratoires ont obtenu <715 résultats corrects pour l’isolement et l’identification. En outre, de nombreux laboratoires ont eu des difficultés à isoler et à identifier les virus dans les échantillons d’EQA qui contenaient un faible titre de virus, ce qui indique que la sensibilité de la procédure d’isolement des virus grippaux varie entre les laboratoires.

La majorité des laboratoires participants (19/25) se sont appuyés à la fois sur l’évaluation de l’ECP et des tests d’HA pour confirmer la croissance virale. Les épreuves de RT-PCR (18/25) et d’HI (14/25) étaient les plus fréquemment employées pour identifier les virus; 9/25 laboratoires ont effectué les deux types de test. L’immunofluorescence a été utilisée en combinaison avec la RT-PCR ou l’HI dans 3/25 laboratoires. Les laboratoires ont été nombreux à avoir des difficultés à isoler et à identifier correctement les virus des échantillons d’EQA contenant une quantité plus faible de virus (Figure 1), mais l’EQA a identifié des problèmes spécifiques à certains laboratoires. Par exemple, plusieurs laboratoires ont correctement identifié les virus des échantillons d’EQA par RT-PCR, mais ont enregistré des valeurs de Ct élevées, de l’ordre de 30-40, au lieu de la valeur <25 normalement escomptée pour les isolements, ce qui témoigne d’un isolement viral sous-optimale et/ou d’une insensibilité insuffisante de la détection par RT-PCR. Un autre laboratoire a obtenu des titres faibles d’HA (signe d’une quantité faible de virus), mais aussi des valeurs faibles de Ct (signe d’une quantité élevée de virus) pour les échantillons d’EQA après l’isolement, ce qui est révélateur de problèmes dans la réalisation des tests d’HA. Dans un laboratoire, les résultats obtenus au test d’HA étaient exacts pour l’échantillon VIP-2017-01, mais aucun isolement de virus n’a pu être détecté au moyen du test d’HA, signe d’un problème majeur dans la méthode d’isolement utilisée. Un suivi individuel sera nécessaire pour remédier à ces problèmes et améliorer l’isolement et l’identification des virus dans ces laboratoires.

Dans cette série 2, les échantillons d’EQA fournis aux laboratoires participants n’étaient pas des échantillons cliniques originaux, comme ceux que la plupart des CNG reçoivent ordinairement à des fins d’isolement viral, mais plutôt des isolements de virus qui avaient été propagés sur cellules MDCK au CCRRI de l’OMS, puis dilués de sorte à contenir une quantité de virus analogue à celle des échantillons cliniques (déterminée à partir des valeurs de Ct obtenues par RT-PCR pour les virus grippaux A ou B). Le réisolement de virus grippaux précédemment isolés est plus aisément réalisable que l’isolement des virus à partir d’échantillons cliniques respiratoires réels. Il convient de noter que depuis ces dernières années, les virus H3N2 se sont avérés particulièrement difficiles à isoler à partir d’échantillons cliniques, que ce soit sur œufs ou sur cellules MDCK. 4 En raison des changements observés dans l’agglutination de différents érythrocytes et de la capacité de croissance réduite de ces virus, il est devenu difficile pour les laboratoires d’identifier et de caractériser antigéniquement les virus H3N2 (en particulier ceux appartenant au clade 3C.2a) par des épreuves d’HI. 5, 6, 7 Des cellules MDCK qui ont été modifiées pour exprimer une forte densité de récepteurs sialiques de
tors (MDCK-SIAT1) has been adopted in many laboratories to improve isolation of H3N2 (and other influenza viruses), including 3/25 laboratories participating in this EQA. Future EQA panels should include samples that accurately reflect the challenges associated with isolation and characterization of the most recent H3N2 viruses.

Participation in EQA programmes is relevant to core NIC functions and is important to establish the reliability of laboratory diagnostic procedures for accurate, reliable, timely test results. EQA permits laboratories to identify problems in their practices and to find corrective actions, and it is required for quality management systems and laboratory accreditation. Given the diverse protocols and procedures used for influenza virus isolation and identification in the laboratories that participated in this EQA, individual feedback will be given to those laboratories that reported difficulty in isolating and/or identifying influenza viruses in this EQA. For example, considerable differences were seen in (i) the source of the standard operating procedure used to perform virus isolation, (ii) the tissue culture vessels used to perform virus isolation (e.g., flasks, tubes and multi-well plates were all used) and (iii) the laboratory tests used to detect virus growth and to identify the virus present in isolates derived from EQA samples. In addition to individual recommendations, standardization of reagents and protocols is a simple approach for improving evaluation (and perhaps the performance) of NICs in future EQA panels for assessing influenza virus isolation and identification in cell culture.

**Author affiliations**

* WHO Collaborating Centre for Reference and Research on Influenza, Victorian Infectious Diseases Reference Laboratory at the Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia; * National Centre for Epidemiology and Population Health, The Australian National University, Canberra, Australia; * Infectious Hazard Management, WHO Health Emergencies, WHO Regional Office for Africa, Brazzaville, Congo; * Infectious Hazard Management Unit, WHO Health Emergency Department, WHO Regional Office for the Eastern Mediterranean, Cairo, Egypt; * WHO Regional Office for the Americas, Washington DC, USA; * Global Influenza Programme, Influenza Preparedness and Response, Infectious Hazard Management, WHO Health Emergencies Cluster, World Health Organization, Geneva, Switzerland (Corresponding author: Patrick C. Reading, Patrick.Reading@influenzacentre.org).

**Acknowledgements**

We thank Jayde Simpson and Katie Milne at the Melbourne WHO CC for excellent administrative assistance in this project. The Melbourne WHO CC is supported by the Australian Government Department of Health.

**Affiliations des auteurs**

* Centre collaborateur OMS de référence et de recherche pour la grippe, Victorian Infectious Diseases Reference Laboratory at the Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australie; * National Centre for Epidemiology and Population Health, Australian National University, Canberra, Australie; * Gestion des risques infectieux, Programme OMS de gestion des situations d’urgence sanitaire, Bureau régional OMS de l’Afrique, Brazzaville, Congo; * Unité Gestion des risques infectieux, Département OMS de gestion des situations d’urgence sanitaire, Bureau régional OMS de la Méditerranée orientale, Le Caire, Égypte; * Bureau régional OMS des Amériques, Washington DC, États-Unis d’Amérique; * Programme mondial de lutte contre la grippe, Préparation et riposte à la grippe, Gestion des risques infectieux, Groupe OMS de gestion des situations d’urgence sanitaire, Organisation mondiale de la Santé, Genève, Suisse (auteur correspondant: Patrick C. Reading, Patrick.Reading@influenzacentre.org).

**Remerciements**

Nous tenons à remercier Jayde Simpson et Katie Milne, du CC de l’OMS à Melbourne, pour l’excellent soutien administratif apporté à ce projet. Le CC de l’OMS à Melbourne est soutenu par le Département de la Santé du Gouvernement australien.
Chapter 4

Establishing laboratory capacity in Cox’s Bazar, Bangladesh, in response to a diphtheria outbreak among Rohingya refugees

From top to bottom and left to right: Newly established laboratory at Cox’s Bazar Medical College; Médecins Sans Frontières Rubber Garden diphtheria treatment centre; Kutupalong refugee camp; and diphtheria case in investigation in host community, Bangladesh, 2018
Chapter 4

Contents

Prologue .............................................................. 154
My role ................................................................. 154
Lessons Learned ...................................................... 155
Public Health Implications ........................................... 156
Acknowledgements .................................................. 157

Abstract ............................................................ 159

4.1 Introduction ..................................................... 161
4.1.1 The Rohingya crisis ........................................... 161
4.1.2 Conditions in Rohingya refugee camps and risk of epidemic-prone diseases ................................. 162
4.1.3 Diphtheria among Rohingya refugees ..................... 163

4.2 WHO’s response to the diphtheria outbreak and the 10 steps in an outbreak investigation ......................... 165
Step 1. Prepare for field work ...................................... 166
Step 2. Confirm the diagnosis ...................................... 167
Step 3. Determine the existence of an outbreak ................. 167
Step 4. Identify and count cases ................................... 167
Step 5. Orient the data in terms of time, person and place .... 168
Step 6. Consider whether prevention and control measures can be implemented ........................................ 169
Step 7. Develop and test hypotheses .............................. 170
Step 8. Plan systematic studies .................................... 170
Step 9. Implement and evaluate prevention and control measures ......................................................... 171
Step 10. Communicate findings ..................................... 172

4.3 Challenges in laboratory diagnostic capacity .................. 172

4.4 Methods .......................................................... 173
4.4.1 Establishing laboratory capacity in Cox’s Bazar ......... 173
4.4.2 Ethics approval ................................................ 175

4.5 Results .......................................................... 175
4.5.1 Timeline of events: From site identification to starting laboratory operations ................................. 175
4.5.2 Diagnostic tests recommended for the Cox’s Bazar laboratory

4.5.3 Laboratory testing for diphtheria confirmation and outbreak monitoring

4.6 Discussion

4.7 Recommendations to improve outbreak diagnostic readiness in developing countries

4.8 Conclusions

References

Appendices


4.B Article published in Global Biosecurity

4.C Diphtheria Case Report Form

4.D 30 day follow up of diphtheria patients form

4.E WHO’s Infection prevention and control guide

4.F Site assessment for suitability as a laboratory

4.G Floor plan of the Cox’s Bazar laboratory

4.H Laboratory establishment concept note

4.I Slides presented at TEPHINET’s Bi-regional Conference, Laos, November 2018

4.J Presentation at ANU National Centre for Epidemiology and Population Health, March 2018
Chapter 4

Prologue

An outbreak of diphtheria among the Rohingya refugee population that fled from Myanmar into Bangladesh was declared by World Health Organization (WHO) in November 2017. This outbreak was just one aspect of a large and complex acute humanitarian emergency. The government of Bangladesh requested technical assistance from WHO. In response, the Global Outbreak Alert and Response Network (GOARN) issued a global request for assistance from partner institutions. In late December 2017 my academic supervisor indicated that ANU would support my participation in the outbreak response. Without hesitation I submitted my offer of support to GOARN. After assessment by the Bangladesh Ministry of Health and Family Welfare (MHFW) and by the WHO Country Office in Bangladesh, my offer was accepted.

My role

I arrived in Bangladesh on the 10th of January 2018. My deployment was four weeks long. I worked within the WHO case management team in the newly established WHO field office in Cox’s Bazar. The case management team consisted of three people: the team manager, an infection prevention and control expert and myself, the laboratory technical officer. My primary responsibility was to assist the Bangladesh MHFW to establish a public health laboratory close to the refugee camps. I worked independently but also collaborated closely with the chief medical officer at the Institute of Epidemiology, Disease Control and Research (IEDCR), an agency within MHFW responsible for outbreak response. My main responsibilities were to 1) compile a list of resources, including equipment, reagents, consumables and human resources needed to establish a new laboratory; 2) prepare a budget for the new laboratory to operate for a period of 12 months; and 3) negotiate with my MHFW counterpart the list of diagnostic tests to be offered in the new laboratory.

Other activities I supported included conducting field investigations of diphtheria case-patients from the host community and planning and conducting a survey of healthcare facilities in the camps. The main purpose of this survey was to ascertain the number of functional facilities, services offered and conditions under which they operated, in particular, if they had access to safe water and sanitation and to update the WHO database on the number of facilities offering the basic package of essential health services, map gaps in healthcare coverage and plan referral pathways.
In addition, I reported data on the bed occupancy rate in all diphtheria treatment centres (DipTC) and diphtheria antitoxin (DAT) use by health partners operating DipTC on a daily basis to the epidemiology team. Data on DAT use were not captured by the Early Warning Alert and Response System (EWARS) early in the outbreak. Monitoring of DAT use was important as it was a proxy measure for a ‘true’ diphtheria case in the absence of laboratory confirmation.

Communication materials I developed included an end of mission report to WHO GOARN, an oral presentation at the 9th Southeast Asia and Western Pacific Bi-Regional TEPHINET Scientific Conference in Laos (see slides in Appendix 4.I), an oral presentation at ANU (see slides in Appendix 4.J), and drafting part of a manuscript published in the Global Biosecurity journal (this publication is presented in Appendix 4.B).

Lessons Learned

This was my first experience responding to a complex, large-scale humanitarian emergency and is unlikely to be my last. What I learned ranged from gaining a better appreciation of the way humanitarian emergencies are wrapped in political complexities to the need to close the gap in laboratory outbreak preparedness and the soft skills that are critical for effective emergency work which often takes place in a chaotic and fast-evolving environment characterised by resource and logistical limitations.

It is unreasonable to expect that milestones that are known to require careful long-term planning in a developed country would happen any faster in a developing country grappling with long-term systemic poverty and a sudden, massive humanitarian emergency in one of their most neglected districts. Nevertheless, I found myself in a situation were, with counterparts from the Bangladesh MHFW and the WHO Bangladesh Country Office, I was tasked with mounting a basic public health laboratory in one of the most impoverished district of Bangladesh within weeks. To add to this challenge I had to quickly learn to navigate WHO’s bureaucracy and business processes – such as budgeting and procurement – to be effective in my work.

The laboratory opened 10 weeks after I completed the necessary preparatory work. During those 10 weeks diphtheria testing had been minimal. This taught me the importance of investing in the deployment of a mobile laboratory until local diagnostic capacity has been built and is able to provide consistent and quality diagnostic services during an emergency.
I learned that field epidemiologists and laboratory experts must press the point that it is as necessary to include laboratory capacity in emergency response plans as it is to consider the provision of shelter, food, water and healthcare. The laboratory is a critical element in outbreak response. It allows monitoring of an outbreak and guides appropriate clinical case management. Yet, there are no global financial mechanisms in place that allow the rapid establishment of a comprehensive laboratory package as part of the response to emergencies.

Diphtheria is a vaccine-preventable disease that caused significant morbidity and mortality globally almost 100 years ago. It is due to violent conflict and weakened public health systems that outbreaks of diphtheria are occurring today. The still ongoing diphtheria outbreak among Rohingya refugees and the neighbouring host community in Bangladesh and the recent outbreak in Yemen are examples of this.

Through my involvement in this outbreak response I was able to appreciate how Myanmar’s colonial history has impacted on the wellbeing of an entire minority population. To prevent further humanitarian crises we must remain alert to health inequities globally and work towards achieving political solutions.

**Public Health Implications**

My main contribution to the Rohingya emergency response was to lay the groundwork necessary to establish a laboratory for diphtheria diagnosis. The laboratory opened in April 2018 and resulted in an improvement in the testing capability, specifically the number of specimens that could be tested per week and their turnaround time. Testing capacity increased from five specimens per week in January to 40 specimens per day in August 2018. Specimen turnaround time increased from several weeks in January to 48 hours in August 2018. As a result of having a dedicated facility to test specimens from refugee camps a larger proportion of cases could be tested and confirmed for diphtheria. As a comparison, only 5% (223/4,021) of cases had been tested by the first week of January 2018 whereas 90% (86/100) of cases were being tested by the last week of August. Testing more specimens allowed better monitoring of the outbreak and therefore assessment of the need for public health action.

The new laboratory recruited and trained five technical staff in molecular and serology pathogen detection techniques. This represents an expansion to Bangladesh’s public health laboratory workforce. I hope that the Ministry of Health and Family Welfare will take ownership of the Cox’s Bazar laboratory and continue managing
its operations beyond the 12 month budget provided by WHO so that the host and refugee populations can access better healthcare.

Acknowledgements

I wish to acknowledge my field supervisor, Sheena Sullivan, and the director of the WHO Collaborating Centre for Reference and Research on Influenza, Professor Kanta Subbarao, for supporting my deployment to Bangladesh. A special thanks also goes to Dr Tambri Housen, my academic supervisor, for selecting me from the 2017 MAE cohort to participate in the early stages of this humanitarian emergency response.

Personally, responding to the Rohingya refugee crisis was the most meaningful work I was involved in during my Master of Philosophy in Applied Epidemiology (MAE). This was an experience I had been preparing for since 2014 when I completed the WHO course in communicable disease control in humanitarian emergencies and disasters. I am deeply grateful to GOARN for trusting me to provide assistance and for organising every detail of my trip. In particular, I would like to thank Dr Tony Stewart, Dr Lucky Sangal (my supervisors in WHO Cox’s Bazar field office) and the WHO Bangladesh Country Office. I thank Caroline Voute (technical operations manager at the WHO Cox’s Bazar field office) for her leadership and encouragement when the obstacles to achieving my key objectives seemed to be exceedingly prolific. I acknowledge Ali Khan, epidemiology team leader at the WHO Cox’s Bazar field office, for including me in aspects of field epidemiology investigations, for organising team-bonding dinners and assisting when technology failed me. The WHO case management Team, Angie Jackson, Anne Lickliter, Noore Alam and Masrura Kabir were very supportive. It was a pleasure working with them.

It was an honour to work alongside epidemiologists, emergency medicine and infectious diseases specialists, medical scientists and public health professionals from the Bangladesh MHFW, GOARN, WHO World Health Organization South-East Asia Regional Office (SEARO) and international humanitarian health partners from the UN International Organization for Migration, Samaritan’s Purse and Médecins Sans Frontières (MSF). Witnessing the work done by MSF in the camps was inspiring. Not that I needed any more evidence of the impactful and unique work they do. I hope to —in the near future— count myself among their cadre. It was an honour to work with Bangladeshi public health experts and fellow FETPs. Special thanks go to Md. Mazhar for keeping the team’s spirits up and looking after us in the face of long days, little rest and punishing road trips to the camps. I hope to work with you all again.
I acknowledge the generous financial assistance provided by the South Asia Field Epidemiology and Technology Network (South Asia Field Epidemiology and Technology Network (SAFETYNET)) that enabled me to present this work at the 9th Southeast Asia and Western Pacific Bi-Regional Training Programs in Epidemiology and Public Health Interventions Network (TEPHINET) Scientific Conference in Laos in November 2018.
Abstract

**Background:** Over 650,000 Rohingya fled Myanmar in August 2017 following violent conflict and sought refuge in neighbouring Cox’s Bazar, Bangladesh, triggering the beginning of a protracted humanitarian and public health crisis. An under-immunised population living in over-crowded makeshift shelters, unsanitary conditions and with very limited access to healthcare are at high-risk of infectious disease outbreaks. In December 2017 an outbreak of diphtheria was declared resulting in a major international response. Here we describe the steps taken by members of the WHO case management team to support the Bangladeshi Ministry of Health and Family Welfare (MHFW) to establish a basic public health laboratory in proximity to the refugee camps to address the lack of local laboratory diagnostic capacity in Cox’s Bazar. The aim of the new laboratory was to test specimens from suspected diphtheria patients and to assist in the investigation of alerts emanating from the early warning, alert and response system, a surveillance system covering most healthcare facilities and all diphtheria treatment centres in the refugee camps.

**Methods:** To establish a laboratory we took the following steps: 1) identify a suitable site; 2) assess the selected facility for basic infrastructure; 3) refurbish the space to allow molecular diagnostics workflow; 4) select laboratory tests (i.e., diphtheria and relevant outbreak-prone diseases); 5) anticipate material and human resources needed to operate the laboratory for 12 months; 6) prepare a budget, draft a memorandum of understanding to be signed by MHFW and WHO, procure laboratory equipment, reagents and consumables; and 7) recruit and train personnel.

**Results:** An agreement between the Institute of Epidemiology, Disease Control and Research (MHFW) and WHO was reached on 17 February 2018, four weeks after planning activities had commenced. A budget of approximately 450,000 USD was allocated to operate the laboratory for 12 months. The laboratory opened on 20 of April 2018 at the Cox’ Bazar Medical College. Between 1 May and 30 November 2018 the new laboratory tested a total of 614 specimens for diphtheria, 54 of which were positive. Test expansion occurred in late October 2018 to include diagnosis of dengue, chikungunya and Zika. Challenges encountered were lack of a functional public health laboratory facility that could be enhanced or expanded, time needed to source equipment and difficulties recruiting skilled staff willing to relocate to Cox’s Bazar. Despite these delays, the establishment of a laboratory dedicated to serving the refugee population and the host population living in proximity to the camps resulted in a larger proportion of diphtheria suspect cases being tested and a faster
turnaround time. This allowed better monitoring of the changing epidemiology of diphtheria.

**Conclusion:** Lessons learned while establishing a basic laboratory in the context of a large-scale acute refugee crisis and complex political environment add evidence on the need for a global mechanism to support resource-limited countries to include strategies for the rapid establishment of laboratory capacity in their emergency response plans. With these experiences we hope to contribute to identifying best strategies to address the current gap in diagnostic emergency preparedness in countries with fragile health systems to ensure adequate patient management and effective disease control in resource-limited settings.
4.1 Introduction

4.1.1 The Rohingya crisis

The Rohingya population are a Muslim ethnic minority living in Rakhine State, Myanmar. They have been systematically deprived of their rights since the 1970’s and have been stateless since 1982 (1). The oppression that the Rohingya population face include restricted access to healthcare, to state education, to employment and restrictions to freedom of movement and reproductive rights (2). As a result the Rohingya in Myanmar, and children in particular, have been suffering poor health outcomes, mainly low birth weight, malnutrition and diarrhoeal illnesses (3, 1).

In Myanmar the Rohingya have faced several violent military crackdowns. The latest, on the 25th of August 2017, resulted in over 600,000 Rohingya fleeing their homelands in Myanmar seeking a safe haven in neighbouring Bangladesh (4). This was the second largest exodus of an ethnic minority since the Rwanda genocide of 1994. Within a period of three weeks Rohingya refugees settled in temporary makeshift settlements mainly in Cox’s Bazar district joining approximately 300,000 others who had fled in earlier waves of displacement (5). The location of the camps is shown in Figure 4.1. The refugee population took shelter in 6,000 acres of land made available by the Bangladeshi government (6).

Figure 4.1: Location of Rohingya refugee camps, Cox’s Bazar district, Bangladesh, October 2017.
NB: The size of the orange circles represent the size of the camp’s population.
Source: Al Jazeera Media Network based on data from the International Organization for Migration (7).
By October 2017, over 500,000 people were sheltering along the Kutupalong-Balukhali expansion site. Approximately 250,000 people had settled in other camps and informal settlements and 46,000 Rohingya were living within host communities. The government of Bangladesh and a coordinating body of international organisations called the Inter Sector Coordination Group were jointly responsible for the management of camps.

The sudden influx of hundreds of thousands of forcibly displaced people arriving in a very short period of time to a small area lacking basic infrastructure created a major humanitarian crisis classified as an emergency grade 3 by WHO. This is the highest severity level, requiring a significant response from WHO as outlined in the Emergency Response Framework (8). Further complicating this picture, incoming refugees started occupying the land newly allocated by the Bangladeshi government for temporary settlements before the sites were prepared. Therefore humanitarian actors were unable to adhere to the universal minimum standards in humanitarian response laid out in the Sphere Handbook (9, 1).

With a steady number of refugee arrivals, by January 2018 the capacity of the Bangladesh MHFW and numerous international health actors to mount an effective response continued being compromised. Cox’s Bazar host communities living near the refugee camps, among the poorest in Bangladesh, were also impacted by the refugee crisis. Land available for rice farming shrunk to give way to shelters, deforestation increased significantly driven by the refugees’ need for firewood and the inadequate placement of latrines resulted in environmental pollution. In addition, the host communities felt the impact of this crisis in terms of the increasing costs of living. Competition for healthcare services and employment which was limited before the crisis began was high (10).

4.1.2 Conditions in Rohingya refugee camps and risk of epidemic-prone diseases

Rohingya refugees live in temporary shelters made of bamboo sticks and plastic sheeting. Ventilation in shelters is poor. During the initial two months of the crisis people living in spontaneous settlements had no access to water, sanitation and hygiene (11). In the early stages of the crisis access to healthcare in the newly formed camps, which sprawled along hilly terrain and rice paddies, was very limited and road access into the camps non existent (12). The Rohingya were, and still are, completely dependent on humanitarian assistance for all their basic needs: shelter, food and non-
food items, water, sanitation and healthcare. The total population of the latest wave of refugee arrivals, the pre-existing Rohingya refugee population and the surrounding host community in need of humanitarian assistance totalled 1.2 million (13).

Squalid conditions in the densely populated and poorly constructed settlements were favourable to the rapid spread of infectious diseases (14). The underimmunised status of the Rohingya population compounded with their living conditions placed them at heightened vulnerability to multiple diseases of epidemic potential (11, 15). Infectious diseases common in emergencies in developing country settings where the prevalence of malnutrition is high include measles, diarrheal diseases, acute respiratory infections and, in endemic areas, malaria and other vector-borne diseases (i.e., dengue, Zika, chikungunya, Japanese encephalitis and scrub typhus) (16). In this context the epidemic risks identified as early as October 2017 were cholera, bloody diarrhoea, typhoid, shigellosis and hepatitis A and E (12, 17, 18). The cyclone-prone and low-lying characteristics of the area in which people have settled coupled with insufficient sewage infrastructure meant that the risk of outbreaks of water-borne diseases was assessed as substantial (14).

4.1.3 Diphtheria among Rohingya refugees

The epidemiology of diphtheria

Diphtheria is a vaccine-preventable infectious disease caused by three toxigenic species of Corynebacterium: C. diphtheriae, C. ulcerans and C. pseudotuberculosis. Diphtheria can cause either invasive or noninvasive disease within the respiratory tract or skin lesions (19).

All three Corynebacterium species have been described as emerging zoonotic pathogens in humans (20). C. diphtheriae is considered primarily a human pathogen and is only sporadically found in animals whereas C. ulcerans and C. pseudotuberculosis have been implicated in zoonotic infections, notably the consumption of raw cow and goat’s milk but also through transmission involving other animals species (21, 22).

Respiratory diphtheria is characterised by the local growth of the bacterium in the pharynx and the formation of an adherent pseudo-membrane. The incubation period is typically between two and five days (range 1-10 days) (23). Symptoms include low grade fever, sore throat and swollen lymph nodes. Systemic dissemination of the toxin causes lesions in peripheral nerves and distant organs such as the heart and kidneys potentially resulting in death (24). Diphtheria complications include loss of motor
function and difficulty in swallowing. *C. diphtheriae* is transmitted by direct contact or by breathing in the aerosolised droplets from coughs or sneezes of infected persons (25). Treatment involves administering diphtheria antitoxin (DAT) to neutralise the effects of the toxin, and antibiotics.

Diphtheria, which has a case fatality rate of 5–10% (26), used to be one of the leading causes of childhood death. Globally, morbidity and mortality from diphtheria have dropped considerably since the introduction of a vaccine in the 1930s (23). There are claims that, as a result of systematic childhood vaccination campaigns of adequate coverage, diphtheria has been nearly eradicated from Bangladesh (17). As a result, most physicians from both developed and developing countries have never seen a case of diphtheria (20) and, in some countries, such as Bangladesh, diphtheria diagnostic capacity has been neglected. Past diphtheria epidemics, notably the outbreak in the former Soviet Union between 1991 and 1996, were associated with inadequate vaccine coverage in young children, waning vaccine-induced immunity in adults, poverty and mass population movements (27).

### The first eight weeks of the diphtheria outbreak in Cox’s Bazar

The first suspected case of diphtheria was reported by a Médecins Sans Frontières (MSF) health clinic in the Balukali camp, Cox’s Bazar, on 10th of November 2017 (28). In the following month 440 cases were reported from nearby camps. On 9 December 2017 alone 168 diphtheria cases were reported. WHO declared an outbreak on 13 December 2017 (28). By the end of December 2017 34 deaths had been recorded. Early Warning Alert and Response System (EWARS), a mobile surveillance platform developed by WHO for outbreak detection in emergency settings, was implemented in December 2017 (29). By the first week of January 2018 all DipTC operating in the refugee camps started submitted diphtheria case data to EWARS. Combined diphtheria cases reported by week 1 of 2018 (the time I arrived in Cox’s Bazar) were mostly children under 14 years of age (75%) and female (54%). The majority of diphtheria deaths occurred in children under 9 years (approximately 80%) (30). Figure 4.2 shows the epidemic curve of diphtheria cases in Cox’s Bazar by 11 January 2018.
Diphtheria reporting and case definitions

Case definitions used in the daily reporting of diphtheria cases through EWARS are listed below (31).

**Confirmed**: case-patients reported as positive for toxigenic *C. diphtheriae* by real time polymerase chain reaction (PCR).

**Probable**: case-patients with an upper respiratory tract illness with laryngitis or nasopharyngitis or tonsillitis AND sore throat or difficulty swallowing and an adherent membrane/pseudo-membrane OR gross cervical lymphadenopathy.

**Suspected**: any case with a clinical suspicion of diphtheria. Includes case-patients that are unclassified due to missing values.

### 4.2 WHO’s response to the diphtheria outbreak and the 10 steps in an outbreak investigation

This chapter is concerned with addressing challenges in laboratory confirmation of diphtheria diagnosis. However, to provide context to the overall outbreak response
I briefly describe response activities in relation to the 10 steps for conducting an outbreak investigation as set out in the United States Centers for Disease Control and Prevention (US CDC) Field Epidemiology Manual (32). These activities were coordinated by WHO and were implemented by national and international health actors.

**Step 1. Prepare for field work**

On 20 December 2017 the Government of Bangladesh issued a formal request for technical assistance to WHO for experts to work in collaboration with national health authorities and national and international partners to support the response to the diphtheria outbreak among Rohingya refugees. Experts in diphtheria case management, EWARS, data management, infection prevention and control and laboratory were requested. Additionally, a request for five specialist Emergency Medical Teams with expertise in infectious diseases was also made to provide care to the large expected diphtheria patients caseload. The Bangladesh MHFW also requested assistance from the US CDC to conduct initial laboratory testing of specimens for diphtheria confirmation (33).

Following the incident management system WHO set up a field office in Cox’s Bazar township (approximately four hours drive to the refugee camps), designated an incident manager and formed teams to cover the following incident management critical functions: 1) partner coordination; 2) information and planning; 3) health operations and technical expertise; 4) operations support and logistics; and 5) finance and administration (8). WHO also assisted MHFW to set up an emergency operations centre at the Civil Surgeon’s Office in Cox’s Bazar.

GOARN deployees arrived in Bangladesh during the first week of January 2018 and commenced work within the epidemiology team (which was responsible for surveillance and contact tracing) and the case management team, which sat under the health operations team. The case management team was composed of a case management expert, an infection prevention and control advisor and a laboratory technical officer (the author of this chapter), medical coordinators of the main humanitarian health agencies and representatives of the Bangladesh MHFW.
Step 2. Confirm the diagnosis

In late December 2017 the first throat swabs from clinically suspected diphtheria case-patients were tested by multiplex real-time PCR at the Institute of Epidemiology Disease Control and Research (IEDCR) central laboratory in Dhaka with the assistance of the US CDC. This assistance included technology transfer, the provision of DNA extraction kits, PCR reagents (specifically primers and probes) and training of technical staff. The \textit{Corynebacterium} species triplex real-time PCR assay used was able to identify the presence of the diphtheria toxin gene (\textit{tox}) and differentiate \textit{C. diphtheriae} from the two other \textit{tox}-bearing \textit{Corynebacterium} species, mainly \textit{C. ulcerans} and \textit{C. pseudotuberculosis}. This assay was a modified version of Mothershed \textit{et al.} (34) assay which has a reported 100% sensitivity and specificity. Tests were conducted on an AB7500 FAST Dx instrument.

Initial testing for diphtheria was done at IEDCR’s central laboratory in Dhaka due to the lack of public health diagnostic capacity in Cox’s Bazar. Section 4.3 describes the logistical and operational challenges involved in obtaining timely results of diphtheria testing during the initial phase of the outbreak. Supporting the Bangladesh MHFW to address this challenge was my main responsibility.

Step 3. Determine the existence of an outbreak

A diphtheria outbreak was declared by WHO on 13 December 2017 (28) as covered in Section 4.1.3. By mid December, a total of 804 suspected cases had been reported among the newly arrived Rohingya refugees by MSF clinics located in the camps.

At this time WHO mobilised $ 3 million US dollars (USD) from its Contingency Fund for Emergencies and later launched an appeal to obtain funds to support the delivery of essential health services to the Rohingya refugee population in Bangladesh (28, 18).

Step 4. Identify and count cases

EWARS was established by the WHO field epidemiology team in the first week of January 2018 (35). Staff from 151 reporting healthcare facilities located in the camps received equipment and training in the use of EWARS (35). Reporting facilities included the six newly established Diphtheria Treatment Centres (DipTC) which were operated by MSF, the International Organization for Migration, the UK’s Emergency
Medical Team and Samaritan’s Purse. DipTCs had a combined capacity of almost 500 beds.

As the only public health surveillance system EWARS’s aim was to capture information on diphtheria (and other diseases) among refugees and the host community.

In addition to monitoring the diphtheria outbreak, EWARS facilitated the early detection of the following diseases and disease syndromes: acute watery diarrhoea, bloody diarrhea, acute respiratory infection, measles/rubella, acute flaccid paralysis, suspected meningitis, acute jaundice syndrome, suspected hemorrhagic fever, tetanus, malaria, unexplained fever and severe malnutrition (35).

An epidemic curve of diphtheria cases reported by date of presentation from the 8 November 2017 to 11 January 2018 and case definitions are presented in Section 4.1.3. Most diphtheria cases were found among Rohingya refugees, however, nine cases from the host community were also reported through EWARS in December 2017 (36).

**Step 5. Orient the data in terms of time, person and place**

Data collected in diphtheria case report forms included age, sex, ethnicity (i.e., Rohingya/host community), location of reporting facility, clinical details (symptoms and signs), treatment details, specimen collection for laboratory testing, admission and discharge dates and follow up details. The diphtheria case report form is presented in Appendix 4.C. I was tasked with creating a 30 day follow up form for diphtheria patients to be used by healthcare workers. The aim of this form was to collect data that would enable monitoring of diphtheria complications. This form is presented in Appendix 4.D.

The EWARS system reported data from all DipTC and automatically transformed data into epidemiologic curves, spot maps and tables. Refugee population data, sourced from the International Organization for Migration (37), was used to calculate diphtheria attack rates. Diphtheria bulletins were issued daily, weekly and monthly by EWARS. Diphtheria epidemiological highlights from EWARS were shared weekly through the WHO SEARO website (38). Daily diphtheria reporting started on 16 of January 2018. Figure 4.3 shows an example of how case demographic data was presented.
Figure 4.3: Section of an EWARS Weekly Diphtheria Bulletin from January 2018 showing a breakdown of cases by sex, age, nationality, immunisation status and signs and symptoms. (31)

Step 6. Consider whether prevention and control measures can be implemented

Implementation of prevention and control measures started in mid December 2017 and consisted of mass immunisation campaigns, supporting partners in terms of patient treatment and contact tracing. In addition WHO supported MHFW to establish diphtheria diagnostic capacity in close proximity to the refugee camps. The Bangladesh MHFW with assistance from WHO, United Nations Children’s Fund (UNICEF) and Gavi the Vaccine Alliance (formerly known as Global Alliance for Vaccines and Immunizations) conducted a three-round diphtheria immunisation campaign targeting children under 15 years living in camps and surrounding areas as well as healthcare workers in Rohingya camps (39). Tetanus-diphtheria vaccines were used for children aged seven to 15 years and pentavalent vaccines (diphtheria, pertussis, tetanus, *Haemophilus influenzae* type b and hepatitis B) and pneumococcal conjugate vaccines (PCV) for children aged six weeks to six years (40). Diphtheria immunisation rounds were conducted at four week intervals. Vaccine cold chain was maintained.
WHO supported health partners treating diphtheria patients by providing materials to build and furnish temporary DipTC, sourcing and distributing supplies of antibiotics and diphtheria antitoxin vials. In addition, WHO conducted training of clinicians working in DipTC, in case management according to their clinical care guidelines. Staff working in DipTC, and other healthcare facilities also received training in infection prevention and control. The aim of this training was to protect healthcare workers from infection while caring for patients and during specimen collection and to prevent infection between patients and family members. The infection prevention and control guideline, to which I contributed (see Appendix B. Specimen collection guidelines for diphtheria treatment centres) is presented in Appendix 4.E.

Contact tracing was coordinated by WHO and conducted by health partners. Close contacts of diphtheria cases were given post-exposure prophylaxis in the form of penicillin or erythromycin for seven days and were immunised for diphtheria. By clearing the bacteria antibiotics prevent further transmission to susceptible individuals and limit carriage that can persist after clinical recovery.

Due to the specific context in Cox’s Bazar the response to the diphtheria outbreak included supporting the MHFW to establish laboratory capacity close to the camps. This is the focus of this chapter and is covered in section Section 4.4.1 onward.

**Step 7. Develop and test hypotheses**

Hypotheses related to the risk factors that resulted in the emergence of diphtheria among the refugee population and later spread from person to person in the newly established camps have been postulated by MSF (17) and WHO (40). These were covered in Section 4.1.2. Briefly, the lack of access to immunisations and healthcare, poor nutritional status, substandard living conditions and hardship experienced by the Rohingya population would have increased the risk of contracting diphtheria.

No studies were conducted, to the author’s knowledge, to test hypotheses that sought to explain the exposures that caused the disease. This was because all partners involved in the health response, including MHFW and WHO were struggling to address the overwhelming acute health needs of the affected population.

**Step 8. Plan systematic studies**

No studies were conducted to determine the source of diphtheria infection and risk exposures. All three *Corynebacterium* species capable of producing toxin are known
to infect animals such as buffaloes, cows, sheep and goats (25). There is evidence that the Rohingya migrated to Bangladesh with these livestock species (41), therefore, investigation of this zoonotic source of exposure using a One Health approach is warranted.

Laboratory studies to genetically characterise the causative agent were not conducted due to lack of resources, both human and material. The importance and public health implications of high level laboratory capacity for diphtheria diagnosis have been discussed in the literature (42). Likewise, information of the antimicrobial sensitivity profile of *C. diphtheria* isolates could not be obtained due to the lack of diphtheria culture capacity. This is aspect of the outbreak investigation, which has serious implications for clinical management, remains unresolved.

Two months into the diphtheria outbreak clinicians working in DipTC raised the need to conduct descriptive epidemiological analysis of diphtheria patients that presented with serious complications 30 days after diagnosis. This is a delicate issue given that there were no tertiary level facilities in the camps or elsewhere in Cox’s Bazar that could provide the healthcare needed for a patient suffering post-diphtheria complications such as cardiac dysfunction, polyneuropathy, localised neuropathy (which can range from facial to total paralysis) and renal failure.

Diphtheria is treated with diphtheria antitoxin (DAT), a hyperimmune antiserum produced in horses developed more than a century ago that carries the risk of severe allergic reactions (43, 44). The need for research into new and improved therapeutics for diphtheria has become pressing. We need an antitoxin that is effective, safe and can be administered quickly and with less pain.

**Step 9. Implement and evaluate prevention and control measures**

Mass diphtheria immunisation campaigns concluded on 25 March 2018. Reported coverage of the three rounds was high (88% average) (40). Case numbers decreased from 720 in epidemic week 50 of 2017 to 98 in week 15 of 2018 (36, 31). The \( R_0 \) for diphtheria, which was estimated specifically for this epidemic as 7 (45), indicates that the mass vaccination coverage achieved was sufficient to halt the epidemic (a 86% coverage was estimated to be necessary to interrupt diphtheria transmission). Likely the reduction in the size of the susceptible population through immunisation, natural infection and deaths combined with other countermeasures resulted in reduced transmission.
Step 10. Communicate findings

EWARS reports were issued daily since 16 January 2018 and were disseminated through the WHO website. Updates regarding the evolution of the outbreak were presented at health sector coordination meetings by the epidemiology team lead. The case management team held daily meetings with the medical coordinators of all diphtheria treatment centres. Operational challenges and planning for anticipated healthcare needs were discussed at these meetings. An important function of the case management meetings early in the outbreak was to monitor daily DAT use and DipTC to help forecasting demands and ensure sufficient DAT stockpiling.

GOARN deployees completed end of mission reports that were available to subsequent deployees to help orient them in their new roles. Aspects of the early response were documented by at least two GOARN deployees, including myself, in the form of a publication and a conference presentation to share lessons learned (35, 46). Australian experts that served in the GOARN Rohingya crisis response co-authored a manuscript describing their experiences, challenges and lessons learned. This manuscript titled ‘Field epidemiology in action: an Australian perspective of epidemic response to the Rohingya health emergencies in Cox’s Bazar, Bangladesh’ is presented in Appendix 4.B.

4.3 Challenges in laboratory diagnostic capacity

By the end of January 2018, two months after the outbreak had begun, only 7% of diphtheria cases (both probable and clinically suspected) had been laboratory tested (377/5,172). To address this problem WHO attempted to strengthen existing local laboratory capacity. The only pathology laboratory serving the Cox’s Bazar population was located in Sadar District Hospital. This laboratory was reviewed by WHO personnel and found to lack sufficient space and technical staff to allow the test expansion needed to respond to the needs of the affected population. The diagnostic capacity available at the district hospital was limited to haematology, blood and urine biochemistry, rapid diagnostic testing of selected sexually transmitted infections (HIV, syphilis and hepatitis B), stool microscopy and malaria microscopy. This laboratory operated without a qualified pathologist or microbiologist. Due to the lack of a public health laboratory facility in Cox’s Bazar, specimens had to be transferred to the IEDCR laboratory in Dhaka.
Although the US CDC had transferred diphtheria diagnostics technology to the IEDCR laboratory to conduct diphtheria real time PCR testing, as the only laboratory servicing a population of 163 million, IEDCR did not have sufficient human resources to absorb the extra testing required to monitor the diphtheria outbreak. Financial resource limitations were also a challenge as reagents for molecular diagnostics, such as DNA extraction kits, are expensive. The US CDC had donated diagnostic materials to commence diphtheria testing, however, the IEDCR laboratory did not have a financial mechanism in place to continue testing diphtheria specimens once donated reagents were depleted. Testing demands also included the need to confirm signals emanating from the EWARS surveillance system.

Specimen turnaround time was also severely compromised due to long specimen transport times. Delays were due to congested roads between the refugee camps and the Cox’s Bazar airport, limited daily available flights between Cox’s Bazar and Dhaka and insufficient human resources to transport specimens with adequate frequency from DipTC to the WHO field office.

Logistical and operational challenges impacted on reporting times, with diphtheria test results taking weeks to reach clinicians in diphtheria treatment centres.

In this context it became critical to set up a laboratory in proximity to the refugee camps. In partnership with MHFW and IEDCR we planned the establishment of a basic public health laboratory in Cox’s Bazar.

### 4.4 Methods

The following section describes how we addressed the gap in local laboratory capacity. Due to the lack of an adequate public health laboratory facility in Cox’s Bazar that could be enhanced or expanded we started by scouting for a suitable site.

#### 4.4.1 Establishing laboratory capacity in Cox’s Bazar

Steps taken to establish the new laboratory were:

1. **Identify** a suitable site. Knowledge of potentially suitable buildings was provided by staff from IEDCR to guide this step. Road access, proximity to the camps and availability of basic infrastructure informed site selection.

2. **Assess** building infrastructure. A standard tool developed by WHO and previously used during the response to the Ebola virus outbreak in West Africa was
used to assess the selected facility. The building was assessed for its capacity to safely sustain laboratory operations. Power supply, space, hand washing and capacity to be secured were assessed. Site assessment was conducted by one scientist from IEDCR and two from the WHO Cox’s Bazar field office including myself. The site assessment form I completed is presented in Appendix 4.F.

3. **Refurbish** the selected room to allow appropriate workflow for molecular testing. We designed a space that would allow separation of work to avoid cross-contamination. The laboratory was sub-divided to include a nucleic acid extraction room, a clean room for master mix preparation and a PCR amplification room. Carpentry work was procured to install partitions, benches, blinds and additional power points. A floor plan of the laboratory, equivalent to a biosafety level 2 facility, that I co-designed with the case management team lead indicating partitions and power points to be installed is shown in Appendix 4.G.

4. **Select** laboratory tests. In addition to diphtheria we aimed to include other tests relevant to the context. Expert knowledge (WHO Country Office epidemiologist and humanitarian health agencies with experience serving the Rohingya population in Myanmar), signals emanating from EWARS and the literature were used to identify diseases endemic to Cox’s Bazar. Vaccine-preventable diseases known to cause outbreaks in underimmunised populations during humanitarian emergencies were considered. The lists of tests I proposed for the new laboratory is shown in Table 4.2 under Appendix 4.H.

5. **Prepare** a budget, procure materials and draft a MHFW–WHO memorandum of understanding outlining the purpose of the new laboratory and the roles and responsibilities of each party. Procurement of laboratory materials was done in collaboration with WHO Cox’s Bazar field office logisticians. The budget I prepared is shown in Table 4.5. The concept note I drafted, which was the precursor of a memorandum of understanding signed in February 2018, is presented in Appendix 4.H.

6. **Recruit** and train personnel. IEDCR was responsible for recruiting and training laboratory personnel for the new facility. Recruitment commenced two weeks after the direct financial cooperation agreement between IEDCR and WHO was approved.
4.4.2 Ethics approval

This work was carried out in the context of a large-scale public health emergency. Outbreak response activities were conducted in line with the International Health Regulations. Ethics approval to initiate immediate response efforts is not required in this context.

4.5 Results

The laboratory, established at the Cox’ Bazar Medical College, opened on 20th April 2018 after a memorandum of understanding (MOU) was signed, equipment was procured and training of newly recruited technical staff was completed. The purpose of the laboratory was to test specimens for diphtheria as a matter of urgency and, in time, for other pathogens of concern as required to assist the investigation of EWARS alerts. WHO supported the establishment of the laboratory with a budget of 450,000 USD. The budget, reduced by approximately 110,000 USD compared to that proposed, included the necessary refurbishment, salaries, equipment, reagents, consumables and running costs to operate the laboratory for a period of 12 months. The budget did not include maintenance service contracts for diagnostic equipment (PCR and enzyme-linked immunosorbent assay (ELISA) instruments).

Challenges that delayed the opening of the laboratory were time needed to source equipment and difficulties recruiting skilled technical personnel willing to relocate to Cox’s Bazar. The non-overlapping short-term nature of WHO technical staff overseeing the establishment of the laboratory impacted on the timeliness of test expansion when no qualified personnel were available to install and calibrate new equipment. Conflicting opening hours between the the Cox’s Bazar Medical College and the working hours required for efficient laboratory work had an impact on specimen turnaround time as the laboratory could only operate until 14.00 on weekdays (i.e., the closing time for the college).

4.5.1 Timeline of events: From site identification to starting laboratory operations

The timeline of events from the date of site identification to laboratory inauguration date is presented below.
Timeline for the establishment of a laboratory in Cox’s Bazar, Bangladesh, 2018

15 January  • Site identified
17 January  • Building infrastructure assessed
20 January  • Refurbishment and partitioning installed
25 January  • Laboratory tests selected
27 January  • HR and material needs identified
30 January  • Budget completed
17 February  • MHFW–WHO memorandum of understanding signed
19 February  • Equipment procurement commenced
  05 March  • Direct Financial Cooperation (DFC) approved*
  19 March  • Staff recruitment commenced
  31 March  • Staff recruitment completed
  04 April  • PCR instrument and consumables arrived
  05 April  • Staff training commenced (two weeks)
  20 April  • Laboratory commenced operations
  21 April  • Diphtheria testing commenced
  30 June  • Serology equipment arrived
20 August  • Serology equipment installed and calibrated
22 October  • Serology testing began for dengue, chikungunya and Zika

*DFC arrangements are payments made by WHO to cover the cost of items and activities that would otherwise be borne by the Government of Bangladesh, in order to meet their diphtheria outbreak response commitments.

A potentially suitable site was identified on the 17\textsuperscript{th} of January 2018. This site was the newly built Cox’s Bazar Medical College which had a laboratory on the second floor that was partly furnished but had not been used since construction was completed. The laboratory consisted of a large lockable room with two functional hand basins and power supply. A site assessment was conducted on the 18\textsuperscript{th} of January 2018 and the space was deemed suitable for molecular biology work with the installation of partitions and addition of power points, laboratory benches, chairs and blinds (see Appendix 4.F for the results of the site assessment). Refurbishment was completed within two days.
4.5.2 Diagnostic tests recommended for the Cox’s Bazar laboratory

The list of diagnostic tests we recommended for the new laboratory is shown below. Most proposed tests were serological (i.e., ELISA) except for diphtheria which was proposed as a molecular test (i.e., real time PCR). The proposal included commencing diphtheria testing as a matter of urgency and expanding testing to other pathogens as soon as practically possible. Not all proposed tests were accepted by MHFW. Measles, mumps, rubella, influenza and *H. influenzae* were not accepted at the time of signing the memorandum of understanding. Influenza testing, however, commenced in November 2018 with assistance of the US CDC. See Table 4.2 in Appendix 4.H for further details regarding proposed diagnostic tests required to confirm outbreaks of selected infectious diseases.

Molecular and serological diagnostic tests recommended

- Diphtheria
- Dengue
- Chikungunya
- Zika
- Japanese encephalitis
- Scrub typhus
- Measles*
- Mumps*
- Rubella*
- Influenza*
- *H. influenzae*
- *Streptococcus pneumoniae*
- Rotavirus
- Hepatitis A, B, C and E*
- Leptospirosis

Tests marked with a star were not accepted by MHFW at the time of signing the memorandum of understanding in February 2018.
4.5.3 Laboratory testing for diphtheria confirmation and outbreak monitoring

Between 1 May and 30 November 2018 the new laboratory had tested a total of 614 specimens for diphtheria, 54 of which were positive. After diphtheria diagnostic testing started at the Cox’s Bazar laboratory, the proportion of cases tested increased from an average of 12% (836/6,751) from 8 November 2017 to 30 April 2018 to 55% (104/189) during May 2018. Between June and November 2018 the majority of reported cases were being tested. The increase in the proportion of cases tested coincided with a sharp decrease in the number of cases reported. For example, an average of 1,350 cases were being reported monthly between December 2017 and April 2018 compared to 141 reported cases between May and November 2018. Figure 4.4 shows the timing of the opening of the laboratory in Cox’s Bazar in relation to the evolution of the diphtheria epidemic.

Figure 4.4: Epidemic curve of diphtheria cases (laboratory confirmed, probable, suspected and discarded) among Rohingya refugees reported by date of presentation, 8 November 2017–30 November 2018, Cox’s Bazar, Bangladesh. Laboratory confirmed cases are shown in red (n=285), laboratory negative cases, in grey (n=1,165) and cases not tested, in orange (i.e., probable, n=3,647) and yellow (i.e., suspected, n=3,166).

Specimen turnaround time, defined as the time between specimen collection and reporting of results, decreased from several weeks during the first two months of the
outbreak to 48 hours soon after the new laboratory opened. The main contributors to the turnaround time were shipping time from the camps to the laboratory and the time of specimen arrival at the laboratory. Specimens arriving after 14.00 on weekdays would be tested the following day with results reported the same day.

Increased diphtheria diagnostic capacity allowed health authorities to monitor the changing epidemiology of diphtheria. At the peak of the outbreak (December 2017) 72.5% of cases (confirmed, probable and suspected) were children under 14 years and 24% were adults between 15 and 44 years. However, as the outbreak progressed the number of cases decreased but the proportion of cases aged 15-44 slowly increased from 24% in January to 30% in November 2018 (i.e., approximately one percent point per month). Cases among adults stabilised and represent approximately one third of all reported cases.

4.6 Discussion

This work describes how WHO supported Bangladeshi health authorities to establish laboratory diagnostic capacity in Cox’s Bazar in response to a large diphtheria outbreak among newly arrived Rohingya refugees in the context of an acute, complex humanitarian emergency. In the face of limited local laboratory capacity that could be enhanced or expanded to include diphtheria testing and due to the logistical and operational challenges inherent to the shipment of specimens to the central laboratory in Dhaka we opted to establish a new laboratory close to the refugee camps. Equipment was procured and human resources developed to operate the new laboratory in the Cox’s Bazar Medical College, located approximately four hours by road from the refugee camps. Introducing diagnostic capacity in the impoverished district of Cox’s Bazar was a step forward to address the urban-rural imbalance in diagnostic services usually seen in developing countries.

Planning a new laboratory, choosing an appropriate site, anticipating resource needs, selecting tests, choosing suitable equipment and recruiting and training staff require time. We achieved these tasks in slightly over three months. The groundwork necessary to arrive at an agreement with MHFW took four weeks while procuring human resources and materials was completed in approximately two months. We could argue that three months to establish local diagnostic capacity in response to a large outbreak of an infectious disease is not adequate particularly in the absence of a reliable alternative to test specimens in the meantime. By the time the laboratory opened –five months after the first diphtheria case had been reported –the outbreak
had shrunk considerably, as a result of the reduction in the size of the susceptible population as described in Section 4.2.

Establishing a basic public health laboratory is a substantial undertaking. Had a functioning public health laboratory been available in Cox’s Bazar we would have been able to transfer diphtheria testing technology and train laboratory staff within weeks. Robust diphtheria diagnostic capacity earlier in the outbreak would have provided much needed support to clinicians who had largely no experience treating diphtheria patients. This would have empowered DipTCs to conduct contact tracing in order to interrupt transmission chains. This was not the case. Treating physicians had to rely on their clinical acumen to diagnose diphtheria during the first crucial months of the outbreak. One of the main factors that delayed the establishment of the laboratory were difficulties in recruiting qualified staff willing to relocate to Cox’s Bazar. Possible reasons for this was that the influx of international health agency staff to Cox’s Bazar had contributed to an increase in the cost of living. Due to this challenge local staff were recruited and trained. Procuring equipment internationally were also a limiting factor. It took six weeks for PCR equipment to arrive.

The delay observed in expanding the diagnostic capacity of the new laboratory from diphtheria to other pathogens was in part due to the short-term, non-overlapping nature of the technical assistance provided by international staff managed by WHO. The ELISA instrument arrived in June 2018 but it was not until August that skilled international staff were able to install and calibrate it.

We encountered additional challenges when planning the establishment of the laboratory. Selection of tests to be offered at the Cox’s Bazar laboratory had political and health equity implications as the government was not able to authorise tests for the refugee population if these tests were not available to the host population of Cox’s Bazar or elsewhere in Bangladesh. In addition testing for pathogens not considered in the Bangladesh immunisation schedule, such as mumps, were not supported. The reasoning being that diseases not considered a public health priority are not monitored. The government therefore did not consider it appropriate to apply a different testing policy to the refugee population.

The slow shift seen in diphtheria incidence from children ≤ 14 years to adults (15-44 years) indicates that immunisation policies might need revision if this trend continues. Immunisation campaigns targeting children under 15 years conducted at the beginning of the outbreak with diphtheria-tetanus vaccine were shown to be effective in protecting this age group. However, as shown in the previous largest diphtheria outbreak in the former Soviet states, without diphtheria immunisation boosters the adult
population remains at risk and it is this susceptible group that can allow diphtheria transmission to persist (27).

Similar laboratory capacity challenges were reported during the West Africa Ebola virus epidemic. For example, there was no laboratory in Liberia able to test for Ebola when the outbreak started and it was challenging to find laboratory staff trained in molecular diagnostics (47). To address these issues laboratories equipped with GeneXpert instruments were established close to Ebola treatment centres. The use of the GeneXpert (Cepheid, Sunnyvale, CA, USA), an easy to use automated cartridge-based system for nucleic acid extraction and amplification, allowed the rapid testing of specimens by laboratory staff with minimal training (48). Future outbreak responses in under-resourced locations should consider similar strategies. Investing in technologies that can be deployed quickly, require minimal training, produce rapid and accurate results and can be repurposed to test other pathogens would also result in a more sustainable laboratory. The superiority of this technology over real time PCR in emergency contexts has also been reported by Raftery et al. 2018 (49). However, commercial diphtheria GeneXpert tests have not yet been developed. Development of this technology to cover more outbreak-prone diseases will improve diagnostic readiness and response capacity globally.

Strengths and limitations

Important limitations that remained after the new laboratory commenced functioning that hindered outbreak response were the lack of diphtheria culture capacity at the central laboratory and unavailability of diphtheria isolates for antimicrobial susceptibility testing and for genotyping. The full implementation of all diagnostic tests recommended has not yet occurred. Only six months after opening the laboratory did ELISA testing for arboviruses begin. The timing for this expansion was inadequate as it coincided with the end of the monsoon season. In terms of laboratory quality, the lack of service contracts for maintenance and repair of critical diagnostic equipment will likely threaten the continuity and sustainability of laboratory operations.

Despite these limitations there were also important achievements. The district of Cox’s Bazar, which had been historically neglected, is now better able to respond to future infectious diseases outbreaks. This is significant due to the anticipated long-term nature of the humanitarian emergency taking place within its borders. The establishment of the new laboratory also resulted in an expansion of Bangladesh laboratory workforce.
4.7 Recommendations to improve outbreak diagnostic readiness in developing countries

The diphtheria outbreak among newly arrived Rohingya refugees in Cox’s Bazar revealed long-standing deficiencies in laboratory services coverage in Bangladesh. This outbreak also exposed weaknesses in the capacity of the international community to respond to the critical diagnostic needs of the affected population. To address these issues we propose a number of recommendations. Our recommendations are in line with the latest report of the Joint External Evaluation conducted in Bangladesh which assessed progress made to improve its public health security and meet its obligations under the International Health Regulations. The Joint External Evaluation also pointed to the need to strengthen the national laboratory network to reduce the reliance on central laboratories as well as to improve its laboratory quality management systems (50).

We recommend the following actions:

- **Strengthening of laboratory networks and upgrade central laboratories within developing countries.** Laboratory strengthening needs to be prioritised not done as a short-term response to an outbreak. A sharper focus must be placed in laboratory capacity building to ensure that a network of basic public health laboratories is in place in all districts/provinces to confirm outbreaks and that central/reference laboratories have the capacity to conduct advanced genetic and microbiological analysis such as antimicrobial susceptibility testing and whole genome sequencing. It is important that at least one laboratory in the country maintains the capacity to diagnose vaccine-preventable diseases considered rare. An in-depth set of recommendations has recently been proposed to enhance laboratory capacity in developing countries. These address financial mechanisms, workforce, infrastructure, education and training and quality assurance issues (51, 52).

- **Advocate for a financial and operational global strategy for quick implementation of a comprehensive laboratory package to provide diagnostic capacity during emergencies.** This is particularly important for large-scale emergencies in remote and low-resource settings. Efforts are also needed to ensure that capacity established in response to a public health emergency continues being utilised after the crisis ends. This means that field laboratories become part of the country’s laboratory network and are supported to attain minimum laboratory quality management standards and obtain accred-
itation. Important challenges such as the lack of service contracts for equipment maintenance and repair must be addressed to ensure diagnostic continuity and trust in laboratory services.

- **Evaluate diagnostic readiness in proximity to conflict zones.** To help select laboratories to strengthen the international community needs to evaluate laboratory capacity in areas proximate to conflict. The health status of prosecuted minorities must be monitored and advocacy efforts directed to prevent political conflict escalating to a humanitarian crisis.

- **Consider deploying mobile laboratories to provide diagnostic capacity until local laboratory capacity is established.** An example of a deployable laboratory is that designed by the European Mobile Lab Consortium which can provide real time PCR and ELISA diagnostics in remote and austere conditions (53).

### 4.8 Conclusions

The availability of laboratory services during an outbreak is important for clinicians, epidemiologists and public health authorities. Diagnostic results inform patient management and treatment and guide public health interventions such as the need to immunise certain population groups or to implement other infection prevention and control measures. To better manage the response to the diphtheria outbreak among Rohingya refugees in the context of lack of a local diagnostic capacity WHO supported the Bangladesh MHFW to establish a laboratory in Cox’s Bazar. Despite these efforts, the new laboratory was ready to commence operations five months after the first diphtheria case was reported. Beyond confirmation of a larger proportion of cases, the ability of the new laboratory to provide useful information for public health action was limited. By the time the laboratory opened the diphtheria outbreak had been largely controled as a result of successful immunisation campaigns targeting the most vulnerable population: children under 14 years as well as other countermeasures.

Through our involvement in the laboratory response to a large-scale outbreak we learned an important lesson. That even 11 years after the International Health Regulations came into effect authorities are yet to recognise the centrality of laboratory diagnostic capacity to outbreak response and to a well-functioning and equitable health system.
Chapter 4

The main recommendation that stems from this work is the need to strengthen field and central laboratories in developing countries not only as a response to an outbreak but in anticipation to outbreaks. A strong in-country public health laboratory network would be better able to respond to future outbreaks. The availability of basic public health laboratory capacity throughout a developing country would also provide more equitable services to its population whether they live in rural or urban areas. Epidemiologists and laboratory experts need to become stronger advocates for laboratory strengthening in developing countries.
References


Chapter 4


Chapter 4


Appendices


Summary

Background: An outbreak of respiratory diphtheria among Rohingya refugees was declared in Cox’s Bazar, Bangladesh, on 13 December 2017. The outbreak occurred during the acute phase of a large-scale complex humanitarian emergency involving around 700,000 underimmunised refugees sheltering in overcrowded makeshift shelter with poor access to sanitation.

Methods: Multiple measures were implemented simultaneously to control the outbreak. Six diphtheria treatment centres were established in the camps. Patients were treated with antibiotics and, when appropriate, with diphtheria antitoxin. A facility-based disease surveillance system was implemented and case definitions were established. Active case finding, health promotion and contact tracing commenced soon after the outbreak started. activities within the affected population. Three mass immunisation campaigns targeting children under 15 years were conducted at monthly intervals starting in December 2017. A basic public health laboratory was established close to the camps.

Results: A total of 7,098 diphtheria cases were identified between 10 November 2017 and 30 November 2018 (285 confirmed, 3,647 probable and 3,166 suspected). The majority of cases were children under 14 years (67%). Most cases were female (57%). All cases were refugees sheltering in seven camps except 72 who were host nationals living in the proximity to the camps. The attack rate was estimated as 101 cases per 10,000 population. The case fatality rate was 0.5% (43 deaths). Presence of a pseudomembrane (35%) and gross cervical lymphadenopathy (33%) were the most common signs reported. Common symptoms included sore throat (95%), fever (88%) and difficulty swallowing (41%). Medical complications defined as respiratory distress, shock, neuropathy and kidney failure were reported in 1% of cases (n=83). At the time of writing (November 2018) the outbreak had not yet been declared over.

Conclusion: This outbreak highlighted the need to recognise diphtheria as an outbreak prone-disease in humanitarian emergencies. To prevent further outbreaks in emergency settings it is essential to immunise displaced populations on arrival.
Introduction

Respiratory diphtheria is a vaccine-preventable infection caused by the diphtheria toxin-producing bacterium Corynebacterium diphtheriae. Diphtheria is transmitted through droplets and close physical contact (1). The incubation period is typically between 2 and 5 days (range 1–10). Diphtheria causes fever, sore throat, and malaise. Symptoms may include the formation of a pseudo-membrane on the pharynx—which can obstruct the airway—or enlarged lymph nodes. The diphtheria toxin can cause life-threatening complications in 10-20 % of cases (2) including myocarditis, polyneuropathy and other systemic toxic effects. Diphtheria was a global public health threat until the 1980s when diphtheria vaccines became widely available. However, diphtheria remains a significant problem where vaccination coverage is suboptimal which is associated with fragile states, forced migration and a breakdown in routine public health services due to war or severe economic crisis (3).

In August 2017, over 650,000 people from the Rohingya ethnic minority fled their homelands due to a violent crackdown by the Myanmar military. The Rohingya sought refuge in neighbouring Cox’s Bazar, Bangladesh, triggering a major humanitarian and public health crisis. Within a few weeks, the Rohingya settled in makeshift shelters in Kutupalong and Balukali joining other refugees that were already present. The camps quickly became overcrowded and unsanitary. The Rohingya were under-immunised and suffered malnutrition prior to their mass displacement (4). These factors placed them at high risk of outbreak-prone diseases.

A suspected case of respiratory diphtheria was reported by an MSF health clinic in the Balukali camp on 10 November 2017 (5). Over 3,000 cases were notified the following month. On a single day (9 December 2017) 168 diphtheria cases were reported. WHO declared an outbreak on 13 December 2017 A major international response coordinated by WHO followed (5).

This of this report presents a summary of the epidemiology of the diphtheria outbreak and the public health measures implemented. Lessons learned to prevent future outbreaks of diphtheria in emergency contexts are provided.

Methods

Determining the beginning and end of the outbreak

The outbreak of diphtheria began on 10 November 2017 with the detection of a single clinical case. The outbreak was declared by WHO on 13 December 2017 At the time of writing (November 2018) the outbreak had not been declared over. To determine the end date of the outbreak, 20 days—or two incubation periods—must pass since the end of symptom onset of the last reported case. The role of the laboratory in declaring the outbreak over will be crucial.
Establishment of an incident command system

An incident management team (IMT) was set up by WHO staffed by GOARN deployees. IMT functions included the coordination and support to health actors for case management, infection prevention and control, laboratory, surveillance, data management and contact tracing. An emergency operations centre managed by the Bangladesh Ministry of Health and Family Welfare (MHFW) was activated. Major partners involved in the response followed emergency disaster management principles which allowed for streamlined communication and collaboration.

Case finding, data collection and epidemiological analysis

Early Warning, Alert and Response System (EWARS), a facility-based surveillance system specially designed for emergency settings was deployed in mid December 2017. All diphtheria treatment centres (DipTC) were reporting sites. Cases were reported on a diphtheria case report form via EWARS.

Case definitions for confirmed, probable and suspect cases are listed in Table 4.1. Descriptive epidemiology was completed for the cases on a daily basis. Diptheria attack rates were calculated using refugee population data sourced from the International Organization for Migration (6).

Table 4.1: Confirmed, probable and suspect case definitions for the diphtheria outbreak in Cox’s Bazar, Bangladesh.

<table>
<thead>
<tr>
<th>Case classification</th>
<th>Definition</th>
</tr>
</thead>
</table>
| **Confirmed**       | A laboratory confirmation of infection meeting the following criteria:  
- Upper respiratory tract illness with laryngitis or nasophayngitis or tonsillitis;  
- Sore throat or difficulty swallowing and an adherent membrane or gross cervical lymphadenopathy  
- Detection of toxigenic *C. diphtheriae* by real time PCR. |
| **Probable**        | Clinical illness meeting the following criteria:  
- Upper respiratory tract illness with laryngitis or nasophayngitis or tonsillitis;  
- Sore throat or difficulty swallowing and an adherent membrane or gross cervical lymphadenopathy. |
| **Suspect**         | Clinical suspicion of diphtheria. Includes cases that were unclassified due to missing data. |

Laboratory investigations

Throat/pseudomembrane specimens were collected using swabs containing Amies transport media. When possible, specimens were collected before the initiation of antibiotic therapy. During the first six months of the outbreak, specimens were transported from the camps to the IEDCR central laboratory in Dhaka, almost 400 km away. Specimens were tested.
in the new laboratory in Cox’s Bazar from April 2018 onward. Testing was by multiplex real-time PCR. The *Corynebacterium* species triplex real-time PCR assay was used to detect the diphtheria toxin gene (*tox*) and differentiate *C. diphtheriae* from the two other *tox*-bearing *Corynebacterium* species, *C. ulcerans* and *C. pseudotuberculosis*. The assay has a reported 100% sensitivity and specificity (7). Tests were conducted on an AB7500 FAST Dx instrument.

**Public health measures implemented**

Implementation of prevention and control measures started soon after the outbreak was declared and consisted of:

**Case management:** Cases were triaged and admitted to DipTC. There were six DipTC throughout the camps with a combined capacity of almost 500 beds. DipTC were managed by Médecins Sans Frontières (MSF), the International Organization for Migration (IOM), the UK’s Emergency Medical Team and Samaritan’s Purse. Cases were treated with antibiotics (penicillin or erythromycin) for two weeks. By clearing the bacteria antibiotics prevent further transmission to susceptible individuals and limit carriage that can persist after clinical recovery. Diphtheria antitoxin was given to cases with clinical warning signs (presence of pseudomembrane of the pharynx). During convalescence patients were immunised as needed.

**Infection prevention and Control:** Clinicians practised standard, droplet and contact precautions while caring for patients and collecting specimens. Triage and patient cohorting was practised at DipTC (i.e., keeping suspect and confirmed cases separate to reduce the likelihood of diphtheria transmission in DipTC). Relatives caring for patients were given prophylactic treatment and were immunised as needed.

**Surveillance:** A new surveillance system designed for emergency settings, EWARS, was set up by WHO within two weeks. WHO provided equipment and training to DipTC staff in the use of EWARS to report diphtheria cases in real time, depending on internet access. Demographic, clinical, treatment and outcome details were reported. Data were analysed daily and shared with the Ministry of Health and health partners involved in the response. Descriptive epidemiological data informed immunisation campaign design.

**Contact tracing and active case finding:** Contact tracing was conducted by Ministry of Health, MSF, IOM and other NGOs with the support of WHO. Close contacts of diphtheria cases were given chemoprophylaxis (penicillin or erythromycin) for seven days and were referred for diphtheria immunisation.

**Mass immunisation campaigns:** The Bangladesh Ministry of Health with assistance from WHO, United Nations Children’s Fund (UNICEF) and Gavi the Vaccine Alliance conducted a three-round diphtheria immunisation campaigns targeting children under 15 years living in camps and surrounding areas as well as healthcare workers in Rohingya camps (8). Tetanus-diphtheria vaccines were used for children aged seven to 15 years and pentavalent vaccines (diphtheria, pertussis, tetanus, *Haemophilus influenzae* type b and hepatitis B) and
pneumococcal conjugate vaccines for children aged six weeks to six years. Diphtheria immunisation rounds were conducted at four week intervals. Vaccine cold chain was maintained and coverage was reported at 80% (9).

Laboratory capacity strengthening: WHO worked with the Ministry of Health to establish diphtheria diagnostic capacity in close proximity to the refugee camps.

Risk communication: Frequent, timely and consistent communication to stakeholders such as religious and community leaders as well as healthcare workers staffing the 150 healthcare facilities in the camps. Communications with the affected population were conducted in the Rohingya language to inform about the outbreak and encourage implementation of measures to minimise further spread of disease.

Results and discussion
This was the first widespread outbreak of diphtheria in an emergency setting. A total of 7,098 diphtheria cases were identified between 10 November 2017 and 30 November 2018 (285 confirmed, 3,647 probable and 3,166 suspected).

Figure 4.5: Epidemic curve of diphtheria cases (laboratory confirmed, probable, suspected, discarded and deaths) among Rohingya refugees reported by month of presentation, 1 November 2017–30 November 2018, Cox’s Bazar, Bangladesh. Laboratory confirmed cases are shown in red (n=285), probable cases, in orange (n=3,647), suspected cases in yellow (n=3,166), laboratory negative cases in grey (n=1,165) and deaths in black (n=43). Syringe icons represent the start of each immunisation campaign. The blue arrow points at the date the new laboratory opened in Cox’s Bazar.

The majority of cases were children under 14 years (67%). Most reported cases were female (57%). Only 285 of the 7,098 cases were laboratory confirmed (4%). Genotyping, culture
and antimicrobial susceptibility testing were not available. An epidemic curve of diphtheria cases reported by month of presentation from 1 November 2017 to 30 November 2018 is shown in Figure 4.5.

All cases were refugees except 72 who were host nationals living in the proximity to the camps. The attack rate was estimated as 101 cases per 10,000 population. Presence of a pseudomembrane (35%) and gross cervical lymphadenopathy (33%) were the most common signs reported. Common symptoms included sore throat (95%), fever (88%) and difficulty swallowing (41%). Medical complications defined as respiratory distress, shock, neuropathy and kidney failure were reported in 1% of cases (n=83). Forty three deaths were associated with this outbreak (case fatality rate was 0.5%). At the time of writing (November 2018) the outbreak had not yet been declared over.

There were several challenges associated with this outbreak. First, time between the detection of the first case to outbreak confirmation was five weeks. As a result there was a delay in implementing control measures. The delay in confirming the outbreak was due to the lack of diphtheria diagnostic capacity in Bangladesh. Due to the presence of a large population of susceptible individuals the diphtheria outbreak was large and protracted. In an attempt to address this issue, in early January 2018 the IMT began preparations to establish a basic public health laboratory in Cox’s Bazar. It took almost four months to establish the laboratory due to difficulties sourcing equipment and staff. Second, exhaustive contact tracing was no possible as finding patients’ shelter in the chaos of the mega camps was challenging. Between 1 January and 30 November 2018, 73% of contacts had been traced. This likely contributed to the long tail of the outbreak.

Conclusions
This outbreak in an underimmunised population affected by a humanitarian crisis demonstrated how quickly one diphtheria case can turn into a widespread outbreak and emphasise the importance of preparing in advance and taking immediate action. Simultaneous implementation of multiple public health interventions was a key component in controlling the outbreak.

Lessons learned and recommendations
To prevent future protracted outbreaks of diphtheria among displaced and underimmunised populations of the magnitude seen Cox’s Bazar, diphtheria immunisation should be administered to displaced persons at the point of registration in camps, or soon after arrival, in a culturally sensitive manner. Furthermore, this outbreak serves as a reminder that maintaining diagnostic capacity for diseases perceived as no longer a public health threat is crucial for effective outbreak response. In a humanitarian emergency context, we recommend that mobile laboratories be deployed until reliable local laboratory capacity is established.
References


Field epidemiology in action: an Australian perspective of epidemic response to the Rohingya health emergencies in Cox’s Bazar, Bangladesh

Noore Alam, Bernadette Kenny, Julia Maguire, Samuel McEwen, Meru Sheel, M. Ximena Tolosa

1Queensland Health, Brisbane, QLD, Australia
2Griffith University, Nathan, QLD, Australia
3National Centre for Epidemiology and Population Health, Australian National University, Acton, ACT, Australia
4Communicable Disease Control Branch, Department for Health and Wellbeing, Government of South Australia, Adelaide, SA, Australia
5National Centre for Immunisation Research and Surveillance, Westmead, NSW, Australia
6The University of Sydney, The Children’s Hospital at Westmead Clinical School, Westmead, NSW, Australia
7WHO Collaborating Centre for Reference and Research on Influenza, Victoria, Australia
8Deployed through the Global Outbreak Alert and Response Network, Geneva, Switzerland

Approximately one million Rohingya persons who fled waves of violence in Myanmar at different times, the latest being 25 August 2017, now live in two coastal districts in Bangladesh; Cox’s Bazar and Bandarban (1). In makeshift shelters made from bamboo and tarpaulin in camps sprawling through rough terrains, the Rohingya live in conditions of overcrowding, poor sanitation, high malnutrition and, on arrival, extremely low vaccination coverage (1-3). The United Nations described the situation as the fastest growing refugee crisis and a major humanitarian emergency (4).

In November 2017, an outbreak of diphtheria that was first identified in Kutupalong mega campsite quickly spread and became a major public health threat – an emergency within an emergency. The World Health Organization’s (WHO) Global Outbreak Alert and Response Network (GOARN) responded to the emergency with the deployment of a team of trained epidemiologists, vaccine specialists and technical experts (5). This report describes the experience of some of the Australian experts who served in the GOARN mission in Cox’s Bazar, Bangladesh in 2018.

This mass displacement event provided an ideal setting for large-scale outbreaks of communicable diseases. The refugee camps in Cox’s Bazar endure monsoonal rains as one of the wettest parts of the world, prone to landslides and flooding (Image 1). These conditions contribute to the proliferation of diseases spread via person to person, vector-borne, airborne, waterborne and zoonotic transmission. Complex emergencies such as this also pose the risk of environmental health threats, gender-based violence, decreased mental health and a rise in non-communicable diseases (6).

Image 1. Rohingya refugee camps in Cox’s Bazar established on sandy hills prone to landslide and flooding (left), and immediately after the first rain in April 2018 (right). Photos: Meru Sheel (left), Julia Maguire (right). Reproduced with permission of the photographers.
Table 1. Epidemic prone syndromes/diseases reported through the Early Warning, Alert and Response System, 1 January 2018 – 11 November 2018

<table>
<thead>
<tr>
<th>Disease/Syndrome</th>
<th>Total Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute respiratory infection</td>
<td>481,294</td>
</tr>
<tr>
<td>Unexplained fever</td>
<td>361,781</td>
</tr>
<tr>
<td>Acute watery diarrhoea</td>
<td>202,384</td>
</tr>
<tr>
<td>Other diarrhoea</td>
<td>96,473</td>
</tr>
<tr>
<td>Bloody diarrhoea</td>
<td>38,905</td>
</tr>
<tr>
<td>Suspected malaria</td>
<td>68,102</td>
</tr>
<tr>
<td>Acute jaundice syndrome</td>
<td>2,920</td>
</tr>
<tr>
<td>Suspected measles/rubella</td>
<td>1,541</td>
</tr>
</tbody>
</table>


The identification of the first case of diphtheria was rapidly followed by one of the world’s largest and protracted diphtheria outbreaks. As of mid-August 2018, the number of diphtheria cases exceeded 8,000, including 44 deaths. This outbreak marked the first major resurgence of diphtheria in the post-universal vaccine era in Bangladesh since the 1980s. By comparison, the world largest known diphtheria epidemic was recorded in the 1990s throughout the former Soviet Republics with over 140,000 cases and 4,000 deaths and the protracted outbreak in Indonesia (2011–2017) with over 3,000 cases and 110 deaths (7, 8). This diphtheria outbreak in Bangladesh was unique in that it occurred among a largely unvaccinated refugee population living in overcrowded camps, and spilled over to the local host Bangladeshi community, which generally had a high (>90%) immunisation rate (9).

There were also other infectious disease outbreaks and health conditions that occurred concurrently with diphtheria. Table 1 provides a summary of key epidemic-prone diseases monitored using the Early Warning, Alert and Response System (EWARS), a web-based mobile application used for disease notification, outbreak detection and response in emergency situations (10).

The Australian Response MAE (ARM) network deployed three epidemiologists and three Master of Philosophy in Applied Epidemiology (MAE, Australia’s FETP – Field Epidemiology Training Program) scholars to the response between January and April 2018 to support activities of the WHO’s emergency operations in Cox’s Bazar (2, 12). The overall objectives of the GOARN deployees were to provide technical support for capacity building and training for improved prevention and control of outbreaks, while working in collaboration with other health organisations as well as the Bangladesh Ministry of Health and Family Welfare (MoHPW).

The WHO supported MoHPW in conducting activities to contain the diphtheria outbreak and prevent additional outbreaks. These activities included the provision of technical and logistical support for capacity building and training, strengthening disease surveillance, supplying essential medical supplies and laboratory materials, and adapting preparedness and response activities on disaster management to the local context. The GOARN team supported the WHO’s mass immunisation campaigns for diphtheria, tetanus, measles, rubella, and cholera, and launched the Extended Program on Immunization through outreach clinics within the camps.

The main contributions of the Australian GOARN team included: 1) supporting the establishment and monitoring of the EWARS for diphtheria and diseases listed in Table 1; GOARN deployees conducted in-field risk assessments and supported health partners in the use of EWARS; 2) coordinating contact tracing within the camps and the host community; 3) coordinating the establishment of local laboratory capacity for diphtheria and selected epidemic-prone diseases; and 4) providing infection prevention and control assessment, training and support with a focus on anticipated potential health needs (13).

Image 2. Field hospital in Rohingya refugee camp; external (left) and internal (right). Photos: Noore Alam. Reproduced with permission of the photographer.
Providing large-scale health support in crisis conditions predictably involved challenges due to lack of resources, poor infrastructure and limited capacity for patient follow-up in the refugee camps. Timely identification of patients and their contacts was a challenge due to poorly or unmarked dwellings leaving high likelihood of missing. The WHO-GOARN team established a strong network of partner agencies that acted as focal points for treatment, contact tracing and referrals for diphtheria and other outbreaks, and facilitated daily sharing of information between agencies.

The local host community has historically relied on basic health services that are considered standard compared to urban Bangladesh (14, 15). The host community had limited availability of public health laboratory services prior to this crisis, however this situation has improved with the establishment of the new laboratory in Cox’s Bazar by a collaboration between the WHO case management team and the Institute of Epidemiology, Disease Control and Research (IEDCR). There was a rapid expansion and strengthening of primary health services and, as of April 2018, there were more than 200 health facilities across the Rohingya camps (Image 2). There are sensitive and complex political aspects of humanitarian assistance that must be navigated by all organisations involved.

As expected, language was an obstacle in effective health service delivery. Chittagonian, spoken in the Chittagong region and by local staff, is the primary dialect of the Cox’s Bazar population, while other regions of Bangladesh primarily speak Bengali (Bangla), and the Rohingya speak the Rohingya language. The WHO office in Cox’s Bazar operates in English, which promotes a chain of communication from English to Bengali or Chittagonian to Rohingya. The use of translators and local personnel aided communication, especially since Chittagonian is not dissimilar to Rohingya. Language barriers coupled with cultural differences made it difficult to communicate with affected populations and highlighted the role of building capacity by identifying and training bilingual local staff.

Our participation in this emergency response demonstrated a strong partnership between several players: volunteers, front-line healthcare workers, epidemiologists, laboratory specialists, immunisation experts, operations and logistics managers and high-level policy makers. The experience for the Australian FETP trainees was a first glimpse of field epidemiology in a complex humanitarian emergency and acutely demonstrated the barriers and challenges for success in communicable disease prevention in a crisis. The impact and efficiency of the response in Cox’s Bazar will improve over time with increased training of local staff, collaborative efforts of health organisations and the establishment of a greater number of health facilities, including vital services such as laboratories. While some may suggest that emergency response is like “blowing out the fire”, a strong response based on underlying principles of capacity development and preparedness can lead to long term gains in outbreak response capacity. This is particularly critical in a crisis that is expected to continue for many years.

Acknowledgements
All authors would like to thank the Global Outbreak Alert and Response Network and the Australian MAE Network for facilitating their deployments. BK, JEM and MXT were supported by Australian Government Research Training Program (RTP) Scholarships.

Competing interests
The authors have no competing interests to declare.

References


How to cite this article: Alam N, Kenny B, Maguire JE, McEwen S, Sheel M, Tolosa MX. Field epidemiology in action: an Australian perspective of epidemic response to the Rohingya health emergencies in Cox’s Bazar, Bangladesh. Global Biosecurity, 2019; 1(1).
4.C Diphtheria Case Report Form

ID: □□□□□□ □□□□

DIPHTHERIA CASE REPORTING FORM

I. CASE IDENTIFICATION/ DEMOGRAPHIC DETAILS

<table>
<thead>
<tr>
<th>Camp</th>
<th>Block</th>
<th>Mahji name</th>
<th>Phone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient name</td>
<td>Fathers name</td>
<td>House ID</td>
<td>Ethnicity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>National</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Non-national</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Phone</th>
<th>Male</th>
<th>Female</th>
<th>Pregnancy</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Date of examination: (dd/mm/yy)</th>
<th>School name, if applicable:</th>
</tr>
</thead>
</table>

If date of birth unavailable please indicate age in month or years:

Age: (Years)                         (Months)  

II. VITALS:

<table>
<thead>
<tr>
<th>Heart rate:</th>
<th>Respiratory Rate:</th>
<th>Temp:</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP:</td>
<td>O₂ saturation:</td>
<td>AVPU:</td>
</tr>
</tbody>
</table>

III. BACKGROUND INFORMATION:

<table>
<thead>
<tr>
<th>Diphtheria containing vaccine doses (DPT/Pentavalent/Td)</th>
<th>Contact with known case of Diphtheria/similar illness within 10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of total doses</td>
<td>□ Yes □ No □ Unknown</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date of last vaccination (dd/mm/yy)</th>
<th>Number of persons living in the household?</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Number of accompanying care givers treated with antibiotics?</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Other people with similar illness in the family?</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Attended health care facility in last 10 days:</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>□ Yes □ No</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>If Yes, date:</th>
<th>Location:</th>
</tr>
</thead>
</table>

IV. CLINICAL DETAILS

<table>
<thead>
<tr>
<th>Date onset of fever and sore throat:</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Signs</th>
<th>Complications</th>
<th>Comorbid conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Fever</td>
<td>□ Pseudomembrane</td>
<td>□ Respiratory distress</td>
<td></td>
</tr>
<tr>
<td>□ Sore throat</td>
<td>□ Gross cervical lymphadenopathy</td>
<td>□ Shock</td>
<td></td>
</tr>
<tr>
<td>□ Difficulty swallowing</td>
<td>□ Chest indrawing</td>
<td>□ Irregular heart rate</td>
<td></td>
</tr>
<tr>
<td>□ Difficulty breathing</td>
<td>□ Fast breathing rate</td>
<td>□ Peripheral neuritis/neuropathy</td>
<td></td>
</tr>
<tr>
<td>□ Nasal regurgitation</td>
<td>□ Stridor</td>
<td>□ Kidney failure</td>
<td></td>
</tr>
<tr>
<td>□ Bloody nasal discharge</td>
<td>□ Central cyanosis</td>
<td>□ Cutaneous necrotic lesions</td>
<td></td>
</tr>
<tr>
<td>□ Ear discharge</td>
<td>□ Fast heart rate</td>
<td>□ Other, specify</td>
<td></td>
</tr>
<tr>
<td>□ Drooling of saliva</td>
<td>□ Decreased capillary refill (&gt;3 s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>□ Change in voice</td>
<td>□ Weakness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>□ Swollen neck</td>
<td>□ Lethargy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>□ Skin ulcers</td>
<td>□ Restlessness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>□ Other, specify</td>
<td>□ Other, specify</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date of admission: (dd/mm/yy)</th>
<th>Admitted as: bed colour</th>
</tr>
</thead>
</table>

31 December 2017
**V. TREATMENT INFORMATION:**

<table>
<thead>
<tr>
<th>Administered antibiotic therapy?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV/IM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start date</td>
<td></td>
<td></td>
</tr>
<tr>
<td>End date</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Azithromycin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steroids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other, specify</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excluded from DAT according to criteria:</td>
<td>No</td>
<td>Yes,</td>
</tr>
<tr>
<td>If No, is DAT treatment given:</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Sensitivity test:</td>
<td>not done</td>
<td>If done, test results is:</td>
</tr>
<tr>
<td>Pre-treatment:</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Manufacturer:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date of DAT administration (dd/mm/yy)</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Dose received (units)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Side effects of DAT?</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>DAT infusion continued?</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*Initiate SAE form for all patients with reactions*

**VI. SPECIMEN COLLECTION**

<table>
<thead>
<tr>
<th>Data collection done?</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of collection: (dd/mm/yy):</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Type of sample:</td>
<td>throat swab</td>
<td>nasal swab</td>
</tr>
<tr>
<td>Use of transport media?</td>
<td>Amies</td>
<td>Amies with charcoal</td>
</tr>
<tr>
<td>Transported in cold chain (2-8 C)?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Lab results for C. Diphtheria</td>
<td>Positive</td>
<td>Undetermined</td>
</tr>
<tr>
<td>Date of results:</td>
<td>/</td>
<td>/</td>
</tr>
</tbody>
</table>

**VII. DISCHARGE DETAILS**

| Date of Discharge | / | / | |
| Outcome at discharge |
| Discharged clinically well |
| Death: date ______ / ________ /_____ Cause: ________________ | *(Fill mortality line list)* |
| Referred to __________ date: ______ / ________ /____ |
| Left against medical advice |
| Recovery with clinical sequela: | palatal palsy | neurologic deficit | renal failure | arrhythmia/heart failure |
| Other, specify ________ |

**VIII. 30 DAY FOLLOW-UP**

| Date: | / | / |
| Full recovery |
| Death: date ______ / ________ /_____ Cause: ________________ |
| Recovery with clinical sequela: | palatal palsy | neurologic deficit | renal failure | arrhythmia/heart failure |
| Other, specify ________ |
### 30 Day Follow up of Diphtheria Patient Interview Form

<table>
<thead>
<tr>
<th>Case ID:</th>
<th>DipTC Name:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient Name:</th>
<th>Age</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Father’s Name:</th>
<th>Mazee’s Name:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Camp:</th>
<th>Block/Zone:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Other locating information:**
_________________________________________________________________
_________________________________________________________________

**Name of volunteer conducting interview:** ____________________________________________________

**Date of interview:** ____________________________

**Interviewer mobile N:**

### QUESTIONNAIRE

**Section 1:**
1. Did the patient complete antibiotic treatment?  
   - Yes  
   - No
2. Were contacts in the household traced and given antibiotic prophylaxis?  
   - Yes  
   - No
3. Was anyone else in the household diagnosed with diphtheria?  
   - Yes  
   - No
4. Is the patient alive?  
   - Yes (go to Section 2)  
   - No (go to Section 3)

**Section 2:**
1. Has the patient experienced any of the following? *(check all that apply)*
   - Difficulty walking / moving
   - Tingling or numbness of the arms or legs
   - Swelling of the feet
   - Change in voice
   - Difficulty swallowing liquids
   - Puffiness of the face
   - Breathlessness when running or playing or doing any activity
   - Facial droop: ask the patient to smile and check if smile is symmetric / normal
   - Significant weight loss since diagnosis with diphtheria
   - Physically unable to perform daily activities since diagnosis with diphtheria
   - Other (specify) ________________________________________________
   - None of the above (patient is feeling completely normal)

2. Has the patient gone back to the health centre?  
   - Yes  
   - No

   **If yes, where:** ____________________________
   **Date:** _____________________

   **Has anyone in your household become sick with diphtheria?**  
   - Yes  
   - No

   **If yes, how many days after the patient became ill?** ________________

**Section 3:**
1. What date did the patient die? *(dd/mm/yy)* ________________
2. What was the cause / reason for death? ________________________________________________
3. Did the death occur in a health care facility?  
   - Yes  
   - No

   **If yes, which facility?** __________________________________________

4. Could we return to interview you again about this patient?  
   - Yes  
   - No

   **If yes, please write the contact’s name:** ____________________________________________

   **Phone number (if applicable):** ____________________________

---

*Please return this form to your DipTC*
4.E WHO’s Infection prevention and control guide

Infection Prevention and Control Practical Guide for the Care of Patients with Suspected or Confirmed Diphtheria in Health Care Setting in Cox’s Bazar, Bangladesh

(Version 29th January 2018)

1. Background

Diphtheria is a disease caused by toxin-producing strains of Corynebacterium diphtheriae. Humans are the only reservoir of diphtheria. Transmission occurs from person to person through droplets from the nose and throat and through physical touch with cutaneous diphtheria. The organism produces a toxin, which causes tissue damage and leads to respiratory or cutaneous diphtheria. Incubation period is usually 2-5 days (range 1-10 days). Untreated patients are infectious for approximately 2-3 weeks. Antibiotics usually render patients non-infectious within 48 hours. The disease is preventable by toxoid vaccination.

2. Goals of Infection Prevention and Control (IPC) Practices

- To protect oneself and other health care workers (HCWs) while providing care
- To prevent cross infection between patients and family/community members

3. Summary

- Apply standard precautions for all patients in addition to droplet precautions for patients with suspected or confirmed diphtheria.
- Add contact precautions when touching patients with cutaneous diphtheria.

4. Triage

- At each health facility and diphtheria treatment centre (DipTC), a triage area is required to screen all patients for signs and symptoms of diphtheria.
- Staff must wear a surgical mask when within 1m of the patient
- If a patient is suspected of having diphtheria place a surgical mask on the patient if condition allows. Adjust the mask ties or elastics for paediatric patients so that the mask fits securely.
- The patient must be referred to a DipTC/isolation ward for ongoing management as outlined below.
- If the patient is NOT suspected to have diphtheria they are either discharged or referred to the designated Primary Health Centre (PHC) or elsewhere as per established referral process.

5. Patient Admission to the Diphtheria Treatment Centre/Isolation Ward

a. Place the patient in a designated isolation area or DipTC only with patients suspected to have diphtheria (cohorting). The optimum spacing between patients or beds is ≥1m.

b. Limit patient movement. Patients should be moved for essential purposes only. During transfer the patient must wear a surgical mask.

c. Wear PPE (surgical mask, gown, gloves, eye protection/goggles) when examining the patient and when obtaining a throat swab/pathology samples (Refer to appendices A and B).

d. Educate patients on the importance of hand hygiene and provide advice on cough etiquette i.e. to cover their mouth and nose with a tissue when coughing or sneezing and to place used tissues in a bin.

e. One caretaker/family member per patient is recommended and they must be educated on the correct use of personal protective equipment (PPE) and hand hygiene.
6. Apply standard precautions for all patients at all times\textsuperscript{4,5} 

- Hand hygiene as per the WHO 5 Moments for Hand Hygiene (Appendix B)
- Respiratory hygiene (cough etiquette)
- Use of PPE based on risk assessment (Appendix C)
- Cleaning and disinfection of the environment and equipment
- Waste management
- Safe handling of linen and laundry
- Prevention of sharps injuries/spillages

7. Transmission-based precautions

<table>
<thead>
<tr>
<th>Droplet Precautions</th>
<th>Contact Precautions</th>
</tr>
</thead>
</table>
| • Place the patient in a designated area*.
  • Wear gloves before touching the patient.
  • Wear a gown before your body touches the patient, environmental surfaces or items. Gowns should be changed between patients. | • Place the patient in a designated area*.
  • Wear a surgical mask when working within 1 metre of the patient.
  • Wear a face shield or goggles (eye protection) if you anticipate exposure to splashes.
  • Place a surgical mask on the patient if condition allows. |

* A designated area means a diphtheria isolation ward or an area separated from other patients.

Duration of precautions: from admission until discharge if laboratory testing is not readily available

8. Personal protective equipment\textsuperscript{6}

<table>
<thead>
<tr>
<th>Surgical mask</th>
<th>Gloves</th>
<th>Gown</th>
<th>Eye protection</th>
<th>Goggles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scenario</td>
<td>Hand hygiene</td>
<td>Mask</td>
<td>Gloves</td>
<td>Gown</td>
</tr>
<tr>
<td>Receiving patient in reception area e.g guard of the DipTC</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Working in the isolation area with multiple patients, keeping ≥1m away from patients</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Examining the patient’s throat when within 1 m of the patient e.g nurse or doctor</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Touching patient with cutaneous diphtheria</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>
10. Discharge planning

- Diphtheria is usually not contagious after completing 48 hours of effective antibiotic therapy.
- Discharge may be considered at this time if the patient is clinically well.

11. Care of the deceased

a. Apply contact precautions to avoid touching of discharge/body fluids/cutaneous lesions.

b. Ensure that the body fully wrapped in a clean cloth before being removed from the isolation area.

c. Encourage the family to limit the number of people directly touching the deceased person and follow hand hygiene during funeral and burial after touching the deceased or any associated items.

d. Take cultural and religious aspects into consideration.

12. Environmental management

a. Water, sanitation and hygiene requirements

   - Water requirement: 40–60 litres/inpatient/day.
   - At least four latrines, clearly separated for staff and patients, and for male and female, and at least one per 20 inpatients.
   - Functioning hand hygiene stations within 5m of latrines.
   - Functioning hand hygiene stations (alcohol-based handrub and for exposure to blood/body fluids safe, running water, soap and disposable towels) at all points of patient care.

b. Management of hospital environment

   - Those performing cleaning should always wear appropriate PPE.
   - Always perform WHO 5 Moments for Hand Hygiene as appropriate, i.e. after touching patient surroundings which includes patient care equipment, and after blood/body fluid exposure risk, even if gloves have been worn.
   - Prior to any disinfection procedures, cleaning with detergent and water is necessary to remove organic matter. General cleaning of the environment should continue routinely with detergent and water.
   - Any areas or items visibly contaminated with blood/body fluids should be cleaned immediately with detergent and water and then disinfected with 0.5% sodium hypochlorite.
   - Patient isolation areas should be cleaned with detergent at least daily.
   - All toilet areas should be cleaned at least daily as per routine hospital cleaning.
   - Shared patient care equipment (e.g. stethoscope, oxygen saturation probe, blood pressure apparatus, AMBU bag and mask) should be cleaned after use between patients.

c. Waste management

   - Appoint a person responsible for management of waste collection, handling, storage and disposal.
   - All personnel handling the waste should use standard precautions, and perform hand hygiene as per the 5 Moments for Hand Hygiene and after completing any tasks when finally disposing of waste. Heavy-duty tasks require more resistant PPE.
   - Segregate clinical (infectious) waste from non-clinical waste in dedicated containers.
   - Transport and store waste in specified areas with restricted access.
• Mark the storage areas of clinical waste with a biohazard symbol.
• Liquid waste: dispose patient’s faeces and urine in the latrine, and clean the latrine after disposal.

**d. Linen and laundry**
• Collect linen and laundry in the bag, and wash them in a designated laundry area.
• Precautions should be taken when transporting used linen.

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Standard procedure</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linen</td>
<td>Soak linen in clean water with bleaching powder 0.5% for 30 minutes. Wash again with detergent and water and then rinse.</td>
<td>Ideally dry in the sun.</td>
</tr>
<tr>
<td>Mops</td>
<td>Soak linen in clean water with bleaching powder 0.5% for 30 minutes. Wash again with detergent and water and then rinse.</td>
<td>Mops should be changed routinely. Store dry.</td>
</tr>
</tbody>
</table>

**e. Prevention of sharps injuries/spillages**

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Standard procedure</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Needles and syringes and other sharp objects involved in patient care</td>
<td>Discard in puncture proof container with international biohazard symbol</td>
<td>When the container is two thirds full, seal it and send it for disposal. Report any sharps injuries immediately</td>
</tr>
<tr>
<td>Spillages of blood/body fluids</td>
<td>Use 0.5% sodium hypochlorite to soak up the spillage, carefully pouring on to the affected area and using disposable towels. After disposal of the towels, disinfect the area with fresh 0.5% solution and dry.</td>
<td>Should be used in well-ventilated areas. Rinse with clean water after use of sodium hypochlorite as it may be corrosive.</td>
</tr>
</tbody>
</table>

**13. Staff health**
In an outbreak, HCWs are at greater risk of diphtheria than the general population. Special attention should be paid to vaccinating HCWs who may have occupational exposure to *Corynebacterium diphtheriae*.

**a. Vaccination**
Health care workers who provide direct patient care should be vaccinated against diphtheria.

• If vaccinated in childhood:
  Td or Tdap booster vaccine once.
  Td: Tetanus and diphtheria vaccine, adult/adolescent formulation
  Tdap: Tetanus, diphtheria and acellular pertussis vaccine, adult/adolescent formulation
If not vaccinated, an accelerated schedule as follows should be administered:

<table>
<thead>
<tr>
<th>Dose</th>
<th>Vaccine</th>
<th>Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary 1</td>
<td>Td or Tdap</td>
<td>---</td>
</tr>
<tr>
<td>Primary 2</td>
<td>Td</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Primary 3</td>
<td>Td</td>
<td>4 weeks</td>
</tr>
</tbody>
</table>

b. Daily health check
On a daily basis, appoint a person who monitors health status of each staff member who enters isolation areas. Those HCWs who are exposed to or infected with diphtheria should be excluded from duty. See Appendix D.

14. References
1) Operational protocol for clinical management of diphtheria – Bangladesh, Cox’s Bazar (version 10 Dec 2017)
10) http://www.who.int/gpsc/5may/Your_5_Moments_For_Hand_Hygiene_Poster.pdf
Appendix A. Specimen collection guidelines for diphtheria treatment centres

Testing strategy

During the first week of January 2018, on an average 50-80 diphtheria cases were being reported from seven diphtheria treatment centres (DipTC). The capacity of the laboratory in Dhaka to test specimens for diphtheria was 10-15 specimens per day. Due to the limited testing capacity, a strategy was developed for case selection for diphtheria testing.

Priority for specimen collection should be given to the following cases:

1. Suspected case with typical signs and symptoms with pseudomembrane.
2. Suspected case in host community
3. Suspected case in new geography
4. Atypical presentation with no pseudomembrane but with bull neck or other danger signs
5. Extremes of age: infants and older adults

Important sampling considerations for Diphtheria Treatment Centres

- Timing: aim to collect specimens before initiation of antibiotic therapy.
- Specimen type: throat swab or pieces of pseudomembrane can be collected.
- Swab type: throat swab can be collected using polyester, rayon or nylon swabs containing transport media such as Amies. If available collect specimens in duplicate using duo swabs.
- Storage and transport conditions: Shipment to WHO office Cox Bazar at room temperature on the same day collected if possible. If unable to ship specimen quickly store for no more than 4 days and ship at 2-8 ˚C
- Shipment days: The IEDCR laboratory is closed on Fridays and Saturdays. Specimens should be collected from Saturday to Wednesday and shipped to the WHO office in Cox’s Bazar (CXB) the same day. Specimens should reach the WHO office in CXB before 17.00. WHO will ship specimens to the IEDCR laboratory in Dhaka.

Materials required for collection of specimens for diphtheria testing

- Wooden disposable tongue depressors
- Sterile Duo throat swab –with plain Amies transport media
- Zip lock bag and indelible ink pen
- Latex gloves
Instructions for collection of specimen for diphtheria testing

1. Fill the laboratory request form (upper section)
2. Label the empty swab with the unique patient identification code, patient’s name and date of collection
3. Put on a surgical mask, eye protection (face shield or goggles), a disposable gown and a pair of gloves.
4. Swab the inflamed area of tonsils and posterior pharynx, the junction of membrane and mucosal lining is the best site for specimen collection. If membrane is visible then rub the swab beneath the membrane with care. Do not try to dislodge the membrane as it may lead to bleeding.
5. A piece of membrane can also be collected on the swab
6. Immediately place the throat swab specimen in Amies transport media. Push the swab to the bottom of the media, then cut the shaft of the swab to fit into the tube and cap it securely.
7. Remove the gown and the gloves, roll them inside out and dispose safely. Perform hand hygiene.
8. Remove eye protection/face shield from behind and place in allocated container for reprocessing. Remove the mask from behind and dispose safely.
10. Place the throat swab in a tightly sealed zip lock bag and the laboratory request form in the bag’s outside pocket.
11. Organise shipment to the WHO office. If shipping swabs on same day they were collected, this can be done at room temperature. Otherwise swabs should be shipped with ice packs. It is the responsibility of the DipTC to ship specimens to the WHO CXB office.
Appendix B. Five moments for hand hygiene

Your 5 Moments for Hand Hygiene

1. BEFORE TOUCHING A PATIENT
   WHEN? Clean your hands before touching a patient when approaching his/her.
   WHY? To protect the patient against harmful germs carried on your hands.

2. BEFORE CLEAN/ASEPTIC PROCEDURE
   WHEN? Clean your hands immediately before performing a clean/aseptic procedure.
   WHY? To protect the patient against harmful germs, including the patient’s own, from entering his/her body.

3. AFTER BODY FLUID EXPOSURE RISK
   WHEN? Clean your hands immediately after an exposure risk to body fluids (and after glove removal).
   WHY? To protect yourself and the health-care environment from harmful patient germs.

4. AFTER TOUCHING A PATIENT
   WHEN? Clean your hands after touching a patient and his/her immediate surroundings, when leaving the patient’s side.
   WHY? To protect yourself and the health-care environment from harmful patient germs.

5. AFTER TOUCHING PATIENT SURROUNDINGS
   WHEN? Clean your hands after touching any object or furniture in the patient’s immediate surroundings, when leaving – even if the patient has not been touched.
   WHY? To protect yourself and the health-care environment from harmful patient germs.

World Health Organization
Patient Safety
A WHO Alliance for Safer Health Care
SAVE LIVES
Clean Your Hands

All reasonable precautions have been taken by the World Health Organization to verify the information contained in this document. However, the published material is used distributed without warranty of any kind, either expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall the World Health Organization be liable for any damages arising from its use.

May 2009

212
Appendix C. How to don and doff personal protective equipment

How to put on PPE (when all PPE items are needed)

Step 1
- Identify hazards & manage risk. Gather the necessary PPE.
- Plan where to put on & take off PPE.
- Do you have a buddy? Mirror?
- Do you know how you will deal with waste?

Step 2
- Put on a gown.

Step 3a
- Put on a face shield.

OR

Step 3b
- Put on a medical mask and eye protection (e.g., eye, face, or goggles).

Note: If performing an aerosol-generating procedure (e.g., aspiration of respiratory tract, intubation, resuscitation, bronchoscopy, autopsy), a particulate respirator (e.g., US NIOSH-certified N95, EU FFP2, or equivalent respirator) should be used in combination with a face shield or an eye protection. Do user seal check it using a particulate respirator.

Step 4
- Put on gloves over cuffs.

How to take off PPE

Step 1
- Avoid contamination of self, others & the environment.
- Remove the most heavily contaminated items first.
- Remove gloves & gown
- Peel off gown & gloves and roll inside out.
- Dispose gloves and gown safely.

Step 2
- Perform hand hygiene.

Step 3a
- If wearing a face shield:
  - Remove face shield from behind.
  - Dispose of face shield safely.

Step 3b
- If wearing eye protection and mask:
  - Remove goggles from behind.
  - Remove mask from behind and dispose of safely.

Step 4
- Perform hand hygiene.
Appendix D. Sample of daily monitoring form for healthcare workers

Name: 
Contact number: 
Job title: 
Work location: 
First day on duty: 
Last day on duty: 

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Temperature</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
</tr>
<tr>
<td></td>
<td>☐Yes</td>
<td>☐Yes</td>
<td>☐Yes</td>
<td>☐Yes</td>
<td>☐Yes</td>
</tr>
<tr>
<td>Sore throat</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
</tr>
<tr>
<td>Other diphtheria-like symptoms (If yes, specify)</td>
<td>(     )</td>
<td>(     )</td>
<td>(     )</td>
<td>(     )</td>
<td>(     )</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Temperature</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
</tr>
<tr>
<td></td>
<td>☐Yes</td>
<td>☐Yes</td>
<td>☐Yes</td>
<td>☐Yes</td>
<td>☐Yes</td>
</tr>
<tr>
<td>Sore throat</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
</tr>
<tr>
<td>Other diphtheria-like symptoms (If yes, specify)</td>
<td>(     )</td>
<td>(     )</td>
<td>(     )</td>
<td>(     )</td>
<td>(     )</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Day 11</th>
<th>Day 12</th>
<th>Day 13</th>
<th>Day 14</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Temperature</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
</tr>
<tr>
<td></td>
<td>☐Yes</td>
<td>☐Yes</td>
<td>☐Yes</td>
<td>☐Yes</td>
<td>☐Yes</td>
</tr>
<tr>
<td>Sore throat</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
</tr>
<tr>
<td>Other diphtheria-like symptoms (If yes, specify)</td>
<td>(     )</td>
<td>(     )</td>
<td>(     )</td>
<td>(     )</td>
<td>(     )</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Day 16</th>
<th>Day 17</th>
<th>Day 18</th>
<th>Day 19</th>
<th>Day 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Temperature</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
</tr>
<tr>
<td></td>
<td>☐Yes</td>
<td>☐Yes</td>
<td>☐Yes</td>
<td>☐Yes</td>
<td>☐Yes</td>
</tr>
<tr>
<td>Sore throat</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
<td>☐No</td>
</tr>
<tr>
<td>Other diphtheria-like symptoms (If yes, specify)</td>
<td>(     )</td>
<td>(     )</td>
<td>(     )</td>
<td>(     )</td>
<td>(     )</td>
</tr>
</tbody>
</table>
### Site visit assessment, Cox Bazar Medical College, January 2018

#### PART I: LABORATORY PROFILE

<table>
<thead>
<tr>
<th>Date of Assessment/Audit</th>
<th>17-JAN-2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name(s) and Affiliation(s) of Assessor(s)</td>
<td>Dr Tahmina Shirin, Dr Lucky Sangal, Dr Ximena Tolosa</td>
</tr>
<tr>
<td>Facility contact name(s)</td>
<td>Dr Sonia Afroz</td>
</tr>
<tr>
<td>Facility/laboratory Name</td>
<td>Cox’s Bazar Medical College</td>
</tr>
<tr>
<td>Region</td>
<td></td>
</tr>
<tr>
<td>District</td>
<td>Cox Bazar</td>
</tr>
<tr>
<td>Town</td>
<td>Cox Bazar</td>
</tr>
<tr>
<td>Postal Address</td>
<td></td>
</tr>
<tr>
<td>Laboratory Telephone</td>
<td>NA</td>
</tr>
<tr>
<td>Fax</td>
<td>NA</td>
</tr>
<tr>
<td>Email</td>
<td>NA</td>
</tr>
<tr>
<td>Head of Laboratory</td>
<td>Dr Sonia Afroz</td>
</tr>
<tr>
<td>Telephone (Head of Lab)</td>
<td>0167 302 4935</td>
</tr>
<tr>
<td>Personal</td>
<td></td>
</tr>
<tr>
<td>Work</td>
<td></td>
</tr>
<tr>
<td>Regional or District coordinator</td>
<td></td>
</tr>
<tr>
<td>Contact details</td>
<td></td>
</tr>
<tr>
<td>Partners involved with Lab</td>
<td>IEDCR</td>
</tr>
<tr>
<td>Main aims</td>
<td>Begin operations asap for molecular and serology testing of FDMN specimens</td>
</tr>
<tr>
<td>Main access to site (road, air...)</td>
<td>Road, in close proximity to Cox’s Bazar township and 20 min to domestic airport terminal</td>
</tr>
<tr>
<td>Nearest supply warehouse</td>
<td>Cox’s Bazar district medical store</td>
</tr>
<tr>
<td>Laboratory Level (check those that apply)</td>
<td>National, Regional / Provincial</td>
</tr>
<tr>
<td>Laboratory Affiliation (check those that apply)</td>
<td>Public, Academic</td>
</tr>
</tbody>
</table>
## Laboratory Staffing Summary – this section must be completed prior to starting operations

<table>
<thead>
<tr>
<th>Profession</th>
<th>Number of Full Time Equivalents</th>
<th>Adequate for facility operations?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory Scientist (degree or higher)</td>
<td>NA</td>
<td>Yes</td>
</tr>
<tr>
<td>Laboratory Technicians (Certificate)</td>
<td>NA</td>
<td>Yes</td>
</tr>
<tr>
<td>Other – Laboratory assistants</td>
<td>NA</td>
<td>Yes</td>
</tr>
<tr>
<td>Cleaner/ lab attendant/Data Clerk *IPC training for all cleaners</td>
<td>NA</td>
<td>Yes</td>
</tr>
<tr>
<td>Driver for sample transportation</td>
<td>NA</td>
<td>Yes</td>
</tr>
<tr>
<td>Quality manager - Name:</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Safety officer - Name:</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Stock manager / store person - Name:</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Data manager - Name:</td>
<td></td>
<td>Yes</td>
</tr>
</tbody>
</table>

Complete prior to starting operations

- Patient
- Quality manager - Name:
- Safety officer - Name:
- Stock manager / store person - Name:
- Data manager - Name:

What are there arrangements to process samples out of hours / out of working days?

- Can laboratory staff be identified as potential candidates for training based on language, computer and laboratory skills? Provide names/details

The following sections must be revisited prior to starting operations, paying particular attention to answers marked 'NA', 'No' and 'unknown'
### Part 2: Laboratory Physical Infrastructure

<table>
<thead>
<tr>
<th>Checklist Question</th>
<th>YES/NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Does the laboratory have a lockable door and secure windows?</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Comment</strong></td>
<td></td>
</tr>
<tr>
<td>The laboratory is secure. Doors are lockable, it is located on the 5th floor and the windows are fitted with security screens.</td>
<td></td>
</tr>
<tr>
<td>2. Does the Laboratory have adequate separation to conduct molecular work?</td>
<td>No</td>
</tr>
<tr>
<td>3. Is there enough bench space to install serology equipment?</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Comment</strong></td>
<td></td>
</tr>
<tr>
<td>The space consists of one large room only. Partitions need to be added to create the following 3 spaces:</td>
<td></td>
</tr>
<tr>
<td>- ‘Dirty area’ DNA/RNA extraction and serology lab</td>
<td></td>
</tr>
<tr>
<td>- Clean room for PCR master mix preparation</td>
<td></td>
</tr>
<tr>
<td>- PCR amplification room</td>
<td></td>
</tr>
<tr>
<td>Prior to partitioning installation, the central workbenches need to be removed (see photo below)</td>
<td></td>
</tr>
</tbody>
</table>

![Laboratory Image](image-url)
There is adequate space for installation of BSC, and other necessary equipment.

| 4. | Does the laboratory have stable power supply? – count the plug sockets available, which type? | No |
| Comment | 24 hours power supply but not stable. are approx. 6 power plugs. More need to be installed |

| 5. | Does the laboratory have backup generators or solar power? Are they started manually? Is there sufficient fuel? How long does it take to start the alternate power supply? | Unknown |
| Comment | Need more information regarding back-up generator, no evidence seen of this. There is no solar power |

| 6. | Does the laboratory have sufficient storage space for all consumables in temperature controlled environment (2-28°C)? | No |
| Comment | There is one cupboard only for storage of consumables. This will remain in the main lab area for storage of consumables needed for sample preparation and serology work. Cupboards need to be |
7. Does the laboratory have adequate waste disposal for infections materials? Describe below. Where possible follow waste disposal from lab to incinerator. | No |

**Comment**
Biohazard waste bins and biohazard bags are needed
Information regarding incinerators, and a plan to train operators is needed

8. Does the laboratory have internet? Which company? | No |

**Comment**
Internet connectivity must be arranged for reporting results to lab users, including WCO

9. Does the laboratory have an LIS (lab information system) system in place? | No |

**Comment**
The following systems need to be in place prior to starting operations:
A system for specimen registration at point of entry to the lab.
A system for registration of test results.
A system for dissemination of laboratory results to all stakeholders.

### Part 3: Laboratory Environment

1. Does the laboratory have a means of controlling temperature between 2-30°C e.g. air circulation, thermometers, regular charting. What is the average room temperature? | Yes |

**Comment**
There are three functional air conditioning units.
When the lab starts operating, thermometers need to used and, ambient temperature needs to be recorded daily

2. Review laboratory space, workflow compared to requirements and then identify appropriate location for clean room, location for template addition, PCR room (for real time PCR instruments, computers, and printer) | NA |

**Comment:**
Partitioning will be of 2.4m height and not join with the ceiling; therefore fans must not be operated to avoid air flow between the different laboratory spaces. **I suggest that fans (or fan switches) be removed/disabled.** Real time PCR instruments, computers and printer will be located in the PCR room partition. Blinds/curtains must be installed in all windows to protect equipment from the sun.
3. Does the laboratory have enough hand wash facilities with soap available? | NA
---|---
**Comment**
Must be in stock before operations commence

4. Does the laboratory have other large, electronic and or computer based equipment? | No
---|---
**Comment**

5. Does the laboratory have enough regular supply of PPE (gloves, masks, goggles)? | Yes
---|---
**Comment**
The budget we prepared should cover supply of PPE for 12 months

6. Does the laboratory have refrigerator for storage? Are temperatures monitored and charted? What is the average temperature? | No
---|---
**Comment**
Fridge will be purchased by WHO, average ambient temperature not currently monitored.

### Part 4: Laboratory Human Resources

1. Does the Laboratory have sufficient personnel to implement real time PCR? | No
---|---
**Comment**
Personnel recruitment to begin as soon as agreement is reached between IEDCR and WCO

2. Do laboratory staff have adequate computer skills for real time PCR and reporting needs? | NA
---|---
**Comment – score 1-7, (1 being no skills 7 being hacker level)**
WHO lab officer to train new recruits and oversee first weeks of work
Senior IEDCR staff will conduct regular supervisory visits as per concept note we prepared in late January

3. Is there a hospital IT department or someone responsible for computer maintenance, virus scanner updates etc.? | No
---|---
**Comment**
4. Do laboratory staff need additional training in order to perform testing? Specify type of training | Yes

Comment
Recruits will be trained by WHO lab officer and supervised by senior IEDCR staff, SOP’s must be prepared for all tests/procedures

<table>
<thead>
<tr>
<th>Part 5: Supply chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Describe the current supply chain for reagents</td>
</tr>
<tr>
<td>Comment: Must be implemented before operations commence. Supply mechanisms must be documented in SOPs</td>
</tr>
<tr>
<td>2. Is the supply chain adequate, have there been any stock outs or expirations in last 6 months?</td>
</tr>
<tr>
<td>Comment</td>
</tr>
<tr>
<td>3. How long is the average lead time from ordering to receiving stock?</td>
</tr>
<tr>
<td>Comment</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Part 6: Clinic/hospital readiness</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Have hospital clinicians been sensitized to new lab in CXB and list of tests available?</td>
</tr>
<tr>
<td>Comment</td>
</tr>
<tr>
<td>2. Is there an established sample transportation system from sites to this laboratory (Describe the current system, its adequacy, efficiency and coverage)</td>
</tr>
<tr>
<td>Comment To be devised, documented and communicated to relevant stakeholders</td>
</tr>
<tr>
<td>3. Is a sample referral system in place for any additional testing at referral laboratories (transportation of samples and results, schedules, triple packaging materials)</td>
</tr>
<tr>
<td>Comment To be devised and documented</td>
</tr>
</tbody>
</table>
4. Are there any additional training needs for clinical staff from this facility or referring facilities? | Yes

**Comment**
Clinical staff in DipTCs and other HCF in camps need to be informed of testing algorithm, requesting, and result reporting mechanism. These also need to be documented.

5. Are patients initiated at this site and how long does it take to initiate treatment? | NA

<table>
<thead>
<tr>
<th>Part 7: Current Diagnostics methods</th>
</tr>
</thead>
</table>
| 1. Does the facility currently have another diagnostic method in use? If yes please specify methods | NO

**Comment**
If the answer is YES above, indicate average monthly tests done including positivity rates

2. How are results currently returned to clinician? | NA

**Comment**
To be decided upon, documented and communicated to relevant stakeholders

<table>
<thead>
<tr>
<th>Part 8: Work flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Where will samples be collected and by who?</td>
</tr>
</tbody>
</table>

**Comment:**
To be decided upon, documented and communicated to relevant stakeholders

| 2. Is there a dedicated sample collection area and isolation area within the facility? | NA

**Comment:**
<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3.</td>
<td>Do laboratory have training in biosafety requirements?</td>
</tr>
<tr>
<td><strong>Comment</strong></td>
<td>To be arranged by WHO lab officer and IEDCR senior supervisory staff</td>
</tr>
<tr>
<td>4.</td>
<td>If the collection team is separate from laboratory team, how will the laboratory supply additional materials required, such as swabs and buffer?</td>
</tr>
<tr>
<td><strong>Comment</strong>:</td>
<td>To be decided upon, documented and communicated to relevant stakeholders</td>
</tr>
<tr>
<td>5.</td>
<td>Where collection teams bring samples to the laboratory, where will infection control of the sample occur? Are there additional materials required to follow current country guides?</td>
</tr>
<tr>
<td><strong>Comment</strong>:</td>
<td>To be decided upon, documented and incorporated into training of new recruits</td>
</tr>
<tr>
<td>6.</td>
<td>Who currently receives laboratory samples? How are samples currently registered in the laboratory and what is the protocol to send samples to correct laboratory work stations?</td>
</tr>
<tr>
<td><strong>Comment</strong>:</td>
<td>To be decided upon, documented and incorporated into training of new recruits</td>
</tr>
<tr>
<td>Where would real-time PCR preparation area and equipment be located?</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Comment</strong>:</td>
<td>Open area lab for extractions, clean room for master mix prep and PCR room for amplification (where the PCR equipment will be located)</td>
</tr>
<tr>
<td>7.</td>
<td>Is bleach available for decontamination and are there suitable waste containers including biohazard bags, available in the working area?</td>
</tr>
<tr>
<td><strong>Comment</strong>:</td>
<td>Chlorine has been included in the materials list we prepared</td>
</tr>
<tr>
<td>Are there spill kits, eye wash and PEP available?</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Comment</strong>:</td>
<td>Ensure availability of spill kits, eye wash stations, training in needle stick injuries (reporting, and PEP treatment)</td>
</tr>
<tr>
<td>How are results recorded, who is responsible to verify and record results? How are results currently released to clinical staff?</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Comment</strong>:</td>
<td>Very important to establish a protocol for this prior to commencing operations. This must be documented in an SOP and incorporated into training of new recruits. Reporting of results to WHO Cox’s Bazar office must be specifically included in this SOP</td>
</tr>
</tbody>
</table>
**Part 9: Management Involvement**  
(This maybe clinic, hospital, Ministry of Health etc.)

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Is Management aware of the implementation of all tests conducted at this laboratory?</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Comment</strong></td>
<td></td>
</tr>
<tr>
<td>The Principal of the Cox’s Bazar Medical College, Dr Subash Chandra Saha, was very supportive of our initiative to open a basic public health lab at this campus. <strong>Dr Subash might not be aware of which tests will be conducted in this lab and will need to be briefed prior to commencing operations.</strong></td>
<td></td>
</tr>
<tr>
<td>2. During the assessment, did the assessors meet with management to discuss the implementation of a basic public health lab and the expectations from Laboratory and Management (This could take place before or after the assessments), what are the main concerns about implementation?</td>
<td>YES</td>
</tr>
<tr>
<td><strong>Comment:</strong></td>
<td></td>
</tr>
<tr>
<td>Main concerns are unstable power supply could potentially damage expensive equipment and threaten lab operations. Supply mechanisms can be a potential challenge. In particular DNA extraction kits and diphtheria PCR reagents. This will need strengthening.</td>
<td></td>
</tr>
<tr>
<td>3. What are management expectations for real time PCR testing?</td>
<td></td>
</tr>
<tr>
<td><strong>Comment</strong></td>
<td></td>
</tr>
<tr>
<td>It is expected that real time PCR equipment will be used for diphtheria testing, however, it could be also used to tests for other pathogens of concern in both human and environmental pathogens (see lab concept note)</td>
<td></td>
</tr>
</tbody>
</table>
### Overall rating (Tick appropriate box and give reasons in the general comments section)

<table>
<thead>
<tr>
<th>Not ready</th>
<th>X</th>
<th>Ready to work</th>
<th>Ready with minor changes</th>
<th>Major changes required</th>
</tr>
</thead>
</table>

**General comments**

1. Partitions and lab benches to be installed. Lab chairs to be purchased
2. Ensure there is a backup generator and that there is an inverter + batteries and voltage stabilisers for key equipment ( -80C freezers, PCR instrument, etc.) to protect equipment in the event of power cuts. Electrical sockets to be added to each partition room
3. Curtains needed to protect equipment from direct sunlight
4. Fans should be disabled to avoid airflow on testing area and airflow moving between partitions.
5. Cupboards to be installed to store consumables
6. Internet connected must be established to ensure timely reporting of test results

**Comments regarding technical operations**

- Training in biosafety and IPC must be provided to new recruits (safe working procedures, use of PPE, appropriate waste management, etc.)
- SOPs must be available and must cover all lab procedures and processes (including sample collection, transportation, registration, testing, result recording and result reporting)
- A timeline for the expansion from diphtheria testing to other tests must be in place within the first month of operations
4.G Floor plan of the Cox’s Bazar laboratory

The light blue lines in the figure below indicate the partitions needed to create separate spaces for the clean room and the PCR amplification room. Purple lines on the left indicate the location of windows. PP indicates power points to be installed. The DNA/RNA extraction area is to be shared with serology testing.
4.H Laboratory establishment concept note

IEDCR Decentralised diphtheria laboratory capacity within Cox’s Bazar Medical College, Bangladesh.
January 2018

Context and justification

Since 25th August 2017, an estimated 688,000 Rohingya have crossed the border into Cox’s Bazar, joining approximately 300,000 others who had fled in earlier waves of displacement. Currently, over 548,000 arrivals are in Kutupalong-Balukhali expansion site, 242,000 are located in other camps and settlements, and 79,000 arrivals in host communities—impacting an already congested health response. Risks remain high for a variety of diseases of epidemic potential due to increasingly crowded living conditions, inadequate water, sanitation and hygiene (WASH) facilities and generally low vaccination coverage.

As of 8th November 2017 an outbreak of diphtheria has been detected among the displaced Rohingya communities. Measured against a burden of 7,097 reported diphtheria cases globally in 2016 (WHO) – as of 4 February 2018, a total of 5,193 clinically suspected cases have been reported in Cox’s Bazar, Bangladesh. Laboratory specimen information has only reported for 303 cases, 103 (34%) of which tested positive by PCR. At present, IEDCR Dhaka-level laboratory capacity is limited to 10-15 specimens a day, with delays in reporting up to 2 weeks—limitations that make timely patient care and accurate response to epidemic dynamics extremely difficult.

Due to the sensitivity of suspect case definition, it is estimated (IEDCR/CDC) that up to 70% of patients admitted to Diphtheria Treatment Centres (DipTC) may be negative. In parallel, a total of 37 diphtheria attributed deaths have been recorded to date. Early, accurate diagnosis is imperative since delay in specific therapy may result in death. The microbiologic diagnosis of the disease, the identification and management of contacts and carriers, and the appropriate clinical management of case-patients must be swift and of sufficient scale to match current needs.

Recommendations and actions

- There is urgent need to increase timeliness and specimen testing volume for diphtheria PCR, in order to adequately respond to the current outbreak.
- Discussions with IEDCR have resulted in the proposal of establishing decentralised laboratory capacity at Cox’s Bazar’s level in proximity to the ongoing outbreak. This
service would operate in support of Bangladesh Director General of Health Services (DGHS) capable of testing up to 150 diphtheria specimens per day using real time PCR technology.

- Six (6) IEDCR laboratory staff (microbiologist level to medical technologist) and the concurrent use of two real time PCR instruments are recommended as sufficient to test all patients admitted to the six existing DipTCs and provide results within 24 hours.

- Discussions at Cox’s Bazar level have revealed suitable space for this laboratory facility exists within the Cox’s Bazar Medical College. WHO has undertaken to renovate the space provided by the CXB Medical College to meet minimum standards and norms for laboratory activities, as well as to provide necessary equipment, materials, and reagents to run this facility for a period of 12 months.

- In the interest of time, IEDCR and WHO have agreed that the most rapid way forward is to transfer selected existing resources from IEDCR laboratories in Dhaka to Cox’s Bazar. WHO will procure replacement materials and ship them to the IEDCR facilities in Dhaka.

- Decentralised laboratory presence with investment in two real time PCR instruments should expand sample testing capacity from current limitation of 15 samples per day to 150 samples per day.

- In the interest of timely detection of outbreaks within the ongoing humanitarian crisis; discussions between IEDCR and WHO have concluded that the laboratory includes the capacity to diagnose other epidemic-prone diseases. Serological and molecular testing capacity for Cox’s Bazar laboratory includes selected pathogens listed in Table 4.2. IEDCR and WHO have agreed on this list which was compiled based on the epidemiological profile for the Cox’s Bazar area and vector, food and water borne diseases expected in the extreme living conditions experienced in the camps.

- Given the very uncertain and likely long-term presence of the Rohingya population in Cox’s Bazar, the initiative is proposed for an initial duration of one (1) year. This project will be implemented in at least two phases, each with it’s specific budget and evaluation of outcomes.
Table 4.2: Proposed list of diagnostic tests for the new Cox’s Bazar Medical College Laboratory

<table>
<thead>
<tr>
<th>Agent</th>
<th>Serology</th>
<th>Molecular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphtheria</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Dengue (type 1, 2, 3, and 4)*</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>Chikungunya†</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>Hepatitis (A, B, C, and E)‡</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>Pertussis</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Influenza (A and B)</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>*Streptococcus pneumoniae</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>*Haemophilus influenzae</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Leptospirosis</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Rubella</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Japanese encephalitis</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Rotavirus</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Scrub typhus</td>
<td>×</td>
<td></td>
</tr>
</tbody>
</table>

* Dengue serology for dengue diagnosis (untyped). Dengue molecular testing will be used for serotyping.

† Chikungunya serology for human specimens. Chikungunya molecular testing for human specimens as well as to detect the pathogen in mosquito specimens.

‡ Hepatitis serology will be used for diagnosis of each of the four hepatitis types in humans and hepatitis molecular testing will be used to detect the pathogen in human specimens as well as in water/other environmental specimens. This will be needed for outbreak investigations.

**Primary objective**

Establishing a decentralised IEDCR laboratory with proximity to DipTC and other communicable disease treatment centres to ensure timely detection of outbreaks by supporting the investigation of alerts received via the Early Warning, Alert and Response System EWARS currently covering the Rohingya population in Cox’s Bazar.

**Secondary objectives**

1. Establish real time PCR testing capacity able to handle a volume of up to 150 specimens a day for diphtheria in the first phase of implementation and additional key pathogens within four weeks of opening the laboratory.
2. Test relevant specimens collected by rapid response teams when investigating alerts received via EWARS.
3. Expand activities to provide both serological and molecular testing for selected outbreak-prone diseases Table 4.2.
Chapter 4

Staff roles and responsibilities

The table below lists the type and number of technical staff required to operate the Cox’s Bazar (CXB) laboratory and to support the Dhaka (DHK) laboratory and their responsibilities.

Table 4.3: Laboratory staffing requirements

<table>
<thead>
<tr>
<th>Staff role</th>
<th>#</th>
<th>Location</th>
<th>Responsibilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbiologist</td>
<td>1</td>
<td>CXB</td>
<td>Officer in charge of CXB laboratory (focal point for test reporting)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>More experienced staff in charge of fortnightly supervisory visits to CXB for a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>period of 3 months followed by monthly supervisory visits until end of year.</td>
</tr>
<tr>
<td>Microbiologist</td>
<td>1</td>
<td>DHK</td>
<td>One expert in serology and one expert in molecular testing able to trouble shoot</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>and maintain equipment</td>
</tr>
<tr>
<td>Senior Medical Technologist</td>
<td>2</td>
<td>CXB</td>
<td>Perform all laboratory testing of specimens referred from DipTC and other facilities</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>within FDMN camps</td>
</tr>
<tr>
<td>Medical Technologist</td>
<td>3</td>
<td>CXB</td>
<td>To perform culture of diphtheria specimens.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Note: Due to a lack of biosecurity, IEDCR does not recommend culturing diphtheria</td>
</tr>
<tr>
<td>Medical Technologist</td>
<td>1</td>
<td>DHK</td>
<td>in the CXB laboratory and advises that culture work be conducted at their Dhaka</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>laboratory.</td>
</tr>
</tbody>
</table>

Notes: CXB=Cox’s Bazar, DHK=Dhaka, DipTC=diphtheria treatment centre, FDMN=forcibly displaced Myanmar nationals

Focal points for implementation and follow up

- Prof Meergadi Flora (IEDCR Director)
- Prof Tahmina Shirin (IEDCR Chief Medical Officer)
- Dr Ximena Tolosa (WHO GOARN Case Management–Laboratory technical officer, until 5 February 2018)
- Mr Francis Yesurajan (WHO laboratory consultant commencing in late February 2018)

Planned activities, proposed timeline for implementation

The table below shows the steps in the laboratory establishment process, person responsible and target date for completion.
<table>
<thead>
<tr>
<th>Activity</th>
<th>Responsible</th>
<th>Target date</th>
<th>Status</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification and refurbishment of laboratory facilities at</td>
<td>Prof Tahmina Shirin (IEDCR Chief Scientific Officer), Dr Lucky Sangal (WHO),</td>
<td>25 Jan 2018</td>
<td>Completed</td>
<td>In collaboration with IEDCR, WHO has refurbished a space at the CXB Medical College to ensure biosecurity standards of a molecular laboratory are met. List of materials has been shared with Dhamari Naidoo (WHO HQ) who checked and assessed it as complete and realistic in terms of cost.</td>
</tr>
<tr>
<td>Cox’s Bazar.</td>
<td>Dr Ximena Tolosa (WHO GOARN)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Identification of materials (equipment, reagents and consumables),</td>
<td>Ximena Tolosa (WHO GOARN), Purvi Paliwal (WHO), Miquel Serra (WHO)</td>
<td>25 Jan 2018</td>
<td>Completed</td>
<td>List of materials has been shared with Dhamari Naidoo (WHO HQ) who checked and assessed it as complete and realistic in terms of cost.</td>
</tr>
<tr>
<td>human resources needed for 12-month operations.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IEDCR and WHO to sign letter of agreement</td>
<td>Prof Meergadi Flora (IEDCR Director)</td>
<td>7 February 2018</td>
<td>In progress</td>
<td></td>
</tr>
<tr>
<td>Support IEDCR to recruit CXB based personnel</td>
<td>Dr Art Pesigan</td>
<td>7–15 Feb 2018</td>
<td>Not started</td>
<td></td>
</tr>
<tr>
<td>Ship laboratory materials from Dhaka to Cox’s Bazar</td>
<td>Prof Meergadi Flora (IEDCR Director), Prof Tahmina Shirin (IEDCR), Mr Francis Yesurajan</td>
<td>7–15 Feb 2018</td>
<td>Not Started</td>
<td>Materials to move together with personnel so they can be set up and calibrated soon after arrival. Mr Francis Yesurajan to be actively involved in this step.</td>
</tr>
<tr>
<td>Training of medical technologists on real time PCR testing</td>
<td>Prof Tahmina Shirin (IEDCR), Prof Meergadi Flora (IEDCR Director)</td>
<td>18–21 Feb 2018</td>
<td>Not started</td>
<td></td>
</tr>
<tr>
<td>Commence diphtheria testing</td>
<td>Mr Francis Yesurajan (WHO), Prof Tahmina Shirin (IEDCR)</td>
<td>22 Feb 2018</td>
<td>Not started</td>
<td>Diagnostic test expansion will occur when all reagents and consumables procured by WHO arrive at the facility. Miquel Serra (WHO) will be able to provide ETA of materials. Mr Francis Yesurajan will train technicians in ELISA testing.</td>
</tr>
<tr>
<td>Expanded laboratory diagnostic capacity to cover other selected</td>
<td>Mr Francis Yesurajan (WHO), Prof Tahmina Shirin (IEDCR)</td>
<td>22 Mar 2018</td>
<td>Not started</td>
<td></td>
</tr>
<tr>
<td>pathogens (as per verbal agreement between WHO and IEDCR; see Table 1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Annex 1. Estimated budget summary

Table 4.5 below shows the estimated budget in USD required to establish and operate a basic public health laboratory in Cox’s Bazar for 12 months testing selected pathogens relevant to the current humanitarian context (February 2018 to February 2019). The total budget required was 559,391 USD. A detailed breakdown of the budget for laboratory materials, namely equipment, reagents and consumables is available in Annex 2 under Table 4.6, Table 4.7 and Table 4.8, respectively.

Budget rationale

- HR costs are based on the full-time presence of six (6) staff at CXB laboratory: one (1) laboratory supervisor, two (2) senior laboratory technicians, and three (3) junior laboratory technicians.
- *Per diem* calculations are based on WHO direct financial cooperation agreement with MHFW (Annex 3). We applied the local resource person grade 9 and above *per diem* at divisional HQ (Bangladeshi Taka (BDT) 3000 = USD 36).
- IEDCR supervisory visit costs will include expenses related to travel (DHK to CXB flights) and accommodation for one (1) IEDCR microbiologist. It is calculated as 15 visits (2 days each visit) in one year (February 2018-February 2019). This is equivalent to 30 days of *per diem*.
- Costs related to laboratory resources include all equipment, reagents, and consumables as per list in Annex 2.
- Equipment, reagents, and consumables’ quantities have been estimated based on a capacity of up to 150 PCR tests per day.
- A lump sum for structural rehabilitation and refurbishment is included for the needs related to establishing a decentralised laboratory in CXB Medical College structure including construction, IT materials, IPC materials, etc.
- Shipping costs are both international (including importation duties) as well as national (transport of materials from Dhaka to Cox’s Bazar).
- Two (2) IEDCR staff will travel to CXB to assist in initial laboratory setup and equipment installation and calibration.
- Running costs are estimated monthly for 12 months for internet, water, electricity, paper, printer cartridges, etc.
Table 4.5: Estimated budget summary (USD) to establish and operate a microbiology laboratory in Cox’s Bazar.

<table>
<thead>
<tr>
<th>Budget (Feb 2018 - Feb 2019)</th>
<th>Unit cost</th>
<th>Unit(s)</th>
<th>Total Cost</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human Resources</strong></td>
<td></td>
<td></td>
<td><strong>63,25,300</strong></td>
<td></td>
</tr>
<tr>
<td>Microbiologist</td>
<td>25,930.00</td>
<td>1</td>
<td>25,930.00</td>
<td>CXB based, full time</td>
</tr>
<tr>
<td>Microbiologist</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>Based in DHK, travels to CXB for supervision - costs covered in per diem and flights</td>
</tr>
<tr>
<td>Senior medical technologist</td>
<td>8,103.00</td>
<td>2</td>
<td>16,206.00</td>
<td>CXB based, full time</td>
</tr>
<tr>
<td>Medical technologist CXB</td>
<td>6,303.00</td>
<td>3</td>
<td>18,909.00</td>
<td>CXB based, full time</td>
</tr>
<tr>
<td><strong>Per diem during equipment</strong></td>
<td>36.00</td>
<td>30</td>
<td>1,080.00</td>
<td>Per diem for 15 supervisory visits (2 days, one night) for one microbiologist. Per diem as per WHO-MHWF guidelines (see Table 4.9)</td>
</tr>
<tr>
<td>Travel costs (IEDCR personnel to CXB)</td>
<td>96.00</td>
<td>23</td>
<td>2,208.00</td>
<td>Travel in/out for technicians recruited in DHK (6 flights) + supervisory visits (15 flights) + 2 technicians for initial setup (2 flights)</td>
</tr>
<tr>
<td><strong>Laboratory Materials</strong></td>
<td></td>
<td></td>
<td><strong>388,387.81</strong></td>
<td></td>
</tr>
<tr>
<td>Equipment (serology and molecular)</td>
<td>108,151.00</td>
<td>1</td>
<td>108,112.29</td>
<td>See list of laboratory materials in Annex 2</td>
</tr>
<tr>
<td>Reagents</td>
<td>195,708.00</td>
<td>1</td>
<td>218,208.00</td>
<td></td>
</tr>
<tr>
<td>Consumables</td>
<td>29,751.00</td>
<td>1</td>
<td>62,067.51</td>
<td></td>
</tr>
<tr>
<td><strong>Other Costs</strong></td>
<td></td>
<td></td>
<td><strong>107,751.00</strong></td>
<td></td>
</tr>
<tr>
<td>Refurbishment of CXB medical college to accommodate laboratory space (lumpsum)</td>
<td>5,000.00</td>
<td>1</td>
<td>5,000.00</td>
<td>Construction and building rehabilitation, IT equipment, furniture</td>
</tr>
<tr>
<td>Internet, electricity, water, stationary supplies</td>
<td>200.00</td>
<td>12</td>
<td>2,400.00</td>
<td>$200 monthly</td>
</tr>
<tr>
<td>International freight</td>
<td>70,058.10</td>
<td>1</td>
<td>70,058.10</td>
<td>International order</td>
</tr>
<tr>
<td>National freight</td>
<td>268.00</td>
<td>1</td>
<td>268.00</td>
<td>DHK to CXB (road transport)</td>
</tr>
<tr>
<td>Insurance and customs (~30% of item cost)</td>
<td>30,024.90</td>
<td>1</td>
<td>30,024.90</td>
<td>Importation into Bangladesh</td>
</tr>
<tr>
<td><strong>ESTIMATED TOTAL (USD)</strong></td>
<td></td>
<td></td>
<td><strong>559,391.18</strong></td>
<td></td>
</tr>
</tbody>
</table>
Annex 2: List of required laboratory equipment, reagents and consumables

Materials required for the CXB laboratory and their cost are listed in the three tables below. The rate used to convert BDT to BDT was 83.3. To molecular reagents and consumables we assumed 150 tests per day multiplied by 240 working days (20 working days per month times 12 months) plus a margin of approximately an extra 27 days of testing, giving a total of 40,000 tests in 12 months.

Table 4.6: Resources required for the CXB laboratory and their cost: Equipment

<table>
<thead>
<tr>
<th>Equipment type</th>
<th>Quantity</th>
<th>Unit cost USD</th>
<th>Total cost USD</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biosafety cabinet II</td>
<td>3</td>
<td>4,800</td>
<td>14,400</td>
<td>Including UV light</td>
</tr>
<tr>
<td>Fridge/freezer</td>
<td>2</td>
<td>1,000</td>
<td>2,000</td>
<td></td>
</tr>
<tr>
<td>Centrifuge specs 1</td>
<td>1</td>
<td>1,801</td>
<td>1,801</td>
<td>Available in Bangladesh to be purchased by local tender.</td>
</tr>
<tr>
<td>Mini-centrifuge</td>
<td>3</td>
<td>155</td>
<td>465</td>
<td></td>
</tr>
<tr>
<td>Refrigerated centrifuge</td>
<td>2</td>
<td>6,900</td>
<td>13,800</td>
<td></td>
</tr>
<tr>
<td>Distilled water system</td>
<td>2</td>
<td>2,000</td>
<td>4,000</td>
<td></td>
</tr>
<tr>
<td>ELISA Reader</td>
<td>1</td>
<td>7,000</td>
<td>7,000</td>
<td></td>
</tr>
<tr>
<td>ELISA Washer</td>
<td>1</td>
<td>2,800</td>
<td>2,800</td>
<td></td>
</tr>
<tr>
<td>Incubator</td>
<td>1</td>
<td>3,601</td>
<td>3,601</td>
<td></td>
</tr>
<tr>
<td>Heat block</td>
<td>2</td>
<td>200</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>PCR thermocycler* + PC + associated software</td>
<td>2</td>
<td>25,000</td>
<td>50,000</td>
<td>Capacity to detect 5 targets per reaction, 96 reactions per run</td>
</tr>
<tr>
<td>Uninterruptible power source</td>
<td>2</td>
<td>500</td>
<td>1,000</td>
<td></td>
</tr>
<tr>
<td>Voltage stabiliser</td>
<td>1</td>
<td>144</td>
<td>288</td>
<td></td>
</tr>
<tr>
<td>Racks</td>
<td>5</td>
<td>125</td>
<td>625</td>
<td></td>
</tr>
<tr>
<td>PCR cooler/cold packs</td>
<td>3</td>
<td>100</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>Multichannel pipette</td>
<td>350000</td>
<td>2</td>
<td>420</td>
<td>840</td>
</tr>
<tr>
<td>Pipette spec 1</td>
<td>4</td>
<td>300</td>
<td>1,200</td>
<td>5-100 uL–no filter</td>
</tr>
<tr>
<td>Pipette spec 2</td>
<td>3</td>
<td>300</td>
<td>900</td>
<td>100-1000 uL–no filter</td>
</tr>
<tr>
<td>Pipette spec 3</td>
<td>2</td>
<td>300</td>
<td>600</td>
<td>200 uL–no filter</td>
</tr>
<tr>
<td>Pipette spec 4</td>
<td>1</td>
<td>300</td>
<td>300</td>
<td>5-100 uL–with filter</td>
</tr>
<tr>
<td>Pipette spec 5</td>
<td>2</td>
<td>300</td>
<td>600</td>
<td>100-1000 uL–with filter</td>
</tr>
<tr>
<td>Pipette spec 6</td>
<td>2</td>
<td>300</td>
<td>600</td>
<td>200 uL–with filter</td>
</tr>
<tr>
<td>Vortex</td>
<td>2</td>
<td>250</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td><strong>108,112</strong></td>
<td></td>
</tr>
</tbody>
</table>

*The cost of the PCR instrument was based on the cost of QuantStudio 5 Real-Time PCR System instrument (Thermo Fischer Scientific).

Purchase of biosafety cabinets, ELISA and PCR equipment must include maintenance and repair service contracts.
Table 4.7: Resources required for the CXB laboratory and their cost: Reagents

<table>
<thead>
<tr>
<th>Reagents</th>
<th>N kits</th>
<th>Price p kit USD</th>
<th>Total USD</th>
<th>Comment</th>
<th>Total N tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA kit Dengue NS1</td>
<td>50</td>
<td>195</td>
<td>9,750</td>
<td>Only order enough for initial 6 months, re-order later</td>
<td>4,500</td>
</tr>
<tr>
<td>ELISA kit Dengue IgM</td>
<td>50</td>
<td>210</td>
<td>10,500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA kit Chikungunya IgM</td>
<td>50</td>
<td>200</td>
<td>10,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA kit Chikungunya ICT</td>
<td>50</td>
<td>200</td>
<td>10,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA kit Rubella</td>
<td>50</td>
<td>170</td>
<td>8,500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA kit Hep A</td>
<td>50</td>
<td>200</td>
<td>10,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA kit Hep B</td>
<td>50</td>
<td>200</td>
<td>10,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA kit Hep C</td>
<td>50</td>
<td>200</td>
<td>10,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA kit Hep E</td>
<td>50</td>
<td>200</td>
<td>10,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA kit Japanese encephalitis</td>
<td>50</td>
<td>195</td>
<td>9,750</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA kit Pertussis</td>
<td>50</td>
<td>200</td>
<td>10,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA kit Rotavirus</td>
<td>50</td>
<td>145</td>
<td>7,250</td>
<td>30 tests per kit</td>
<td>1,500</td>
</tr>
<tr>
<td>ELISA kit Scrub typhus</td>
<td>25</td>
<td>436</td>
<td>10,900</td>
<td>96 tests per kit</td>
<td>2,250</td>
</tr>
<tr>
<td>RNA extraction kits, i.e., Qiagen kits or MagMax kits</td>
<td>25</td>
<td>1,342</td>
<td>33,550</td>
<td>250 columns per kit, manual</td>
<td>6,250</td>
</tr>
<tr>
<td>DNA extraction kit , i.e., Qiagen kits or MagMax kits</td>
<td>25</td>
<td>700</td>
<td>17,500</td>
<td>250 columns per kit</td>
<td></td>
</tr>
<tr>
<td>Primers/probes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>250</td>
</tr>
<tr>
<td>Oligonucleotides</td>
<td></td>
<td></td>
<td></td>
<td>For diphtheria, influenza, S pneumoniae, H influenzae, leptospirosis, hepatitis, dengue and chikungunya</td>
<td></td>
</tr>
<tr>
<td>Mastermix QuantiFast for qPCR</td>
<td>10</td>
<td>2,500</td>
<td>25,000</td>
<td>40,000 reactions (4,000 rxn/k)</td>
<td>40,000</td>
</tr>
<tr>
<td>Mastermix SuperScript III</td>
<td>8</td>
<td>1,876</td>
<td>15,008</td>
<td>500 reactions per kit</td>
<td>4,000</td>
</tr>
<tr>
<td>Platinum one step qRT-PCR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td><strong>218,208</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Ensure long shelf life of reagents prior to purchasing!
Table 4.8: Resources required for the CXB laboratory and their cost: Consumables

<table>
<thead>
<tr>
<th>Consumables</th>
<th>BDT price</th>
<th>USD price</th>
<th>Quantity (packs)</th>
<th>Total cost USD</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pipette tips spec 1</td>
<td>25</td>
<td>0.2</td>
<td>500</td>
<td>5-100 uL- no filter</td>
<td></td>
</tr>
<tr>
<td>Pipette tips spec 2</td>
<td>25</td>
<td>0.2</td>
<td>500</td>
<td>100-1000 uL- no filter</td>
<td></td>
</tr>
<tr>
<td>Pipette tips spec 3</td>
<td>18</td>
<td>0.13</td>
<td>360</td>
<td>200 uL- no filter</td>
<td></td>
</tr>
<tr>
<td>Pipette tips spec 4</td>
<td>35</td>
<td>0.26</td>
<td>350</td>
<td>5-1000 uL- with filter</td>
<td></td>
</tr>
<tr>
<td>Pipette tips spec 5</td>
<td>35</td>
<td>0.26</td>
<td>350</td>
<td>100-1000 uL- with filter</td>
<td></td>
</tr>
<tr>
<td>Pipette tips spec 6</td>
<td>28</td>
<td>0.18</td>
<td>280</td>
<td>200 uL- with filter</td>
<td></td>
</tr>
<tr>
<td>Pipette Asl</td>
<td>448</td>
<td>3.56</td>
<td>896</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disposable pipette, 10 mL</td>
<td>40</td>
<td>3.2</td>
<td>400</td>
<td>Serological, 200 units/pack</td>
<td></td>
</tr>
<tr>
<td>PCR tubes, 0.1 mL</td>
<td>95</td>
<td>0.76</td>
<td>30,400</td>
<td>40,000 needed, 125 tubes/pack</td>
<td></td>
</tr>
<tr>
<td>Microcentrifuge tubes</td>
<td>5000</td>
<td>39.6</td>
<td>500</td>
<td>1.5 mL</td>
<td></td>
</tr>
<tr>
<td>Tube rack</td>
<td>300</td>
<td>2.39</td>
<td>72</td>
<td>For 15 mL and 50 mL Falcon tubes</td>
<td></td>
</tr>
<tr>
<td>Reagent reservoir (boats)</td>
<td>250</td>
<td>19.5</td>
<td>180</td>
<td>V bottom</td>
<td></td>
</tr>
<tr>
<td>Storage box</td>
<td>400</td>
<td>3.12</td>
<td>960</td>
<td>for freezer storage of PCR reagents</td>
<td></td>
</tr>
<tr>
<td>Glass beaker</td>
<td>700</td>
<td>5.6</td>
<td>42</td>
<td>1 L</td>
<td></td>
</tr>
<tr>
<td>Conical flask</td>
<td>700</td>
<td>5.6</td>
<td>42</td>
<td>1 L</td>
<td></td>
</tr>
<tr>
<td>Measuring cylinder spec 1</td>
<td>200</td>
<td>1.55</td>
<td>12</td>
<td>100 mL</td>
<td></td>
</tr>
<tr>
<td>Measuring cylinder spec 2</td>
<td>300</td>
<td>2.38</td>
<td>18</td>
<td>500 mL</td>
<td></td>
</tr>
<tr>
<td>Measuring cylinder spec 3</td>
<td>700</td>
<td>5.6</td>
<td>42</td>
<td>1 L</td>
<td></td>
</tr>
<tr>
<td>Flask spec 1</td>
<td>1200</td>
<td>9.48</td>
<td>54</td>
<td>5 L, round bottom</td>
<td></td>
</tr>
<tr>
<td>Flask spec 2</td>
<td>900</td>
<td>7.12</td>
<td>54</td>
<td>2 L, conical bottom</td>
<td></td>
</tr>
<tr>
<td>Screw cap glass bottle spec 1</td>
<td>1200</td>
<td>9.48</td>
<td>144</td>
<td>1 L</td>
<td></td>
</tr>
<tr>
<td>Screw cap glass bottle spec 2</td>
<td>900</td>
<td>7.12</td>
<td>108</td>
<td>500 mL</td>
<td></td>
</tr>
<tr>
<td>Screw cap glass bottle spec 3</td>
<td>800</td>
<td>6.24</td>
<td>96</td>
<td>200 mL</td>
<td></td>
</tr>
<tr>
<td>Swabs, Amies</td>
<td>500</td>
<td>3.9</td>
<td>300</td>
<td>50 boxes (50 units/box)</td>
<td></td>
</tr>
<tr>
<td>Gloves, S</td>
<td>800</td>
<td>6.24</td>
<td>960</td>
<td>100 boxes (100 pairs/box)</td>
<td></td>
</tr>
<tr>
<td>Gloves, M</td>
<td>800</td>
<td>6.24</td>
<td>960</td>
<td>100 boxes (100 pairs/box)</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>5000</td>
<td>39.6</td>
<td>600</td>
<td>10 bottles (Abs, 99.9% 2.5 L)</td>
<td></td>
</tr>
<tr>
<td>Vacutainer tubes</td>
<td>1000</td>
<td>7.84</td>
<td>300</td>
<td>25 boxes (50 boxes)</td>
<td></td>
</tr>
<tr>
<td>Hexisol</td>
<td>150</td>
<td>1.19</td>
<td>540</td>
<td>bottles (250 mL/bottle)</td>
<td></td>
</tr>
<tr>
<td>Savlon</td>
<td>300</td>
<td>2.38</td>
<td>360</td>
<td>bottles (500 mL/bottle)</td>
<td></td>
</tr>
<tr>
<td>Hypochlorite solution</td>
<td>500</td>
<td>3.9</td>
<td>600</td>
<td>bottles (5 L/bottle)</td>
<td></td>
</tr>
<tr>
<td>Coton</td>
<td>100</td>
<td>0.79</td>
<td>240</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biohazard bag spec 1</td>
<td>100</td>
<td>7.84</td>
<td>6,092</td>
<td>autoclavable, 12 x 24 in</td>
<td></td>
</tr>
<tr>
<td>Biohazard bag spec 2</td>
<td>120</td>
<td>9.48</td>
<td>7,203</td>
<td>autoclavable, 24 x 36 in</td>
<td></td>
</tr>
<tr>
<td>Biohazard bag stand</td>
<td>3000</td>
<td>23.7</td>
<td>1,080</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biohazard mat</td>
<td>3000</td>
<td>23.7</td>
<td>1,080</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First aid kits</td>
<td>50</td>
<td>0.39</td>
<td>250</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First-aid bands</td>
<td>200</td>
<td>1.59</td>
<td>360</td>
<td>100/box</td>
<td></td>
</tr>
<tr>
<td>Tourniquet spec 1</td>
<td>200</td>
<td>1.59</td>
<td>60</td>
<td>adults</td>
<td></td>
</tr>
<tr>
<td>Tourniquet spec 2</td>
<td>200</td>
<td>1.59</td>
<td>60</td>
<td>children</td>
<td></td>
</tr>
<tr>
<td>Syringes spec 1</td>
<td>500</td>
<td>3.9</td>
<td>180</td>
<td>3 mL, disposable, sterile (Box/100)</td>
<td></td>
</tr>
<tr>
<td>Syringes spec 2</td>
<td>500</td>
<td>3.9</td>
<td>180</td>
<td>5 mL</td>
<td></td>
</tr>
<tr>
<td>Syringes spec 3</td>
<td>800</td>
<td>6.24</td>
<td>96</td>
<td>10 mL</td>
<td></td>
</tr>
<tr>
<td>Syringes spec 4</td>
<td>1000</td>
<td>7.84</td>
<td>12</td>
<td>50 mL</td>
<td></td>
</tr>
<tr>
<td>Transfer pipette</td>
<td>800</td>
<td>6.24</td>
<td>288</td>
<td>5.8 mL, disposable (100/pack)</td>
<td></td>
</tr>
<tr>
<td>Autoclave tape</td>
<td>200</td>
<td>1.59</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squeeze bottle</td>
<td>500</td>
<td>3.9</td>
<td>60</td>
<td>250 mL, for 70% alcohol</td>
<td></td>
</tr>
<tr>
<td>Falcon Tube spec 1</td>
<td>2000</td>
<td>15.84</td>
<td>480</td>
<td>15 mL</td>
<td></td>
</tr>
<tr>
<td>Falcon Tube spec 2</td>
<td>2500</td>
<td>19.8</td>
<td>300</td>
<td>50 mL</td>
<td></td>
</tr>
<tr>
<td>Laboratory coats</td>
<td>1500</td>
<td>11.88</td>
<td>450</td>
<td>Various sizes</td>
<td></td>
</tr>
<tr>
<td>Laundry costs</td>
<td>10,400</td>
<td>82.56</td>
<td>1,248</td>
<td>For laboratory coats, 12 month estimate</td>
<td></td>
</tr>
</tbody>
</table>

TOTAL: 62,068
Annex 3: Per Diem Rates

The following rates –in BDT– which were agreed upon between WHO and the Ministry of Health and Family Welfare were used in the budget calculations presented in Table 4.5.

Table 4.9: *Per Diem* Rates (BDT) for supervisory staff during visits to CXB.

<table>
<thead>
<tr>
<th>Status of Officials</th>
<th><em>Per diem</em> rate at Div HQ including Narayanganj, Gazipur town &amp; Savar municipal area</th>
<th><em>Per diem</em> rate at other district towns</th>
<th><em>Per diem</em> rate at Upazila/Union level</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Local Resource Person</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 9 &amp; above</td>
<td>3000</td>
<td>2100</td>
<td>1680</td>
</tr>
<tr>
<td>Grade 10</td>
<td>1800</td>
<td>1200</td>
<td>1000</td>
</tr>
<tr>
<td><strong>Non-Local Resource Person</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 9 &amp; above</td>
<td>3000</td>
<td>2100</td>
<td>1680</td>
</tr>
<tr>
<td>Grade 10</td>
<td>2000</td>
<td>1400</td>
<td>1150</td>
</tr>
</tbody>
</table>
Establishing Public Health Laboratory Capacity in the Context of a Large-Scale Acute Refugee Crisis

Rohingya crisis response, 2018, Cox’s Bazar, Bangladesh

Outline

• Context to the crisis: the host country and the refugee population
• The problem: lack of laboratory capacity
• How we addressed the problem
• Successes, challenges and lessons learned
Host country context

Bangladesh

- 8th most populated country in the world
- Lower middle-income country, 2014
- Among top 10 causes of premature mortality two are ID (LRI, DD)
- Vulnerable to cyclones & flooding

Source: Health Data http://www.healthdata.org/bangladesh
Who are the Rohingya?

- Muslim ethnic minority living in Myanmar
- Prosecuted and stateless
- Systematically deprived of rights
- Discrimination & barriers to health care


Rohingya Crisis – Level 3 PH emergency

- Vulnerable to epidemic-prone diseases due to poor living, inadequate WASH, lack of access to immunisations/healthcare

Refugee camps

Cox's Bazar, Bangladesh

Refugee Camps in Cox's Bazar
The problem

Diphtheria outbreak in Rohingya refugee camps

- Diphtheria detected in November 2017
- Expanded from mega camp to smaller camps 4,000+ cases by Wk1, 2018
- No local public health laboratory
- Specimens shipped to central lab in Dhaka-working at capacity
- Congested roads, limited personnel & flights → Delays
- Difficult to monitor outbreak
Congested roads – CXB to camps 4h

EWARS – Early Warning Alert & Response System

Syndromes under surveillance in camps
- Acute watery diarrhoea
- Acute jaundice syndrome
- Acute flaccid paralysis
- Measles/rubella
- Mumps
- Acute respiratory infection
- Suspected meningitis
- Suspected haemorrhagic fever
- Tetanus
- Malaria
- Unexplained fever
- Severe malnutrition

Rapid laboratory confirmation required to support clearance of EWARS alerts - unavailable
Addressing the problem

Establishing a laboratory near Rohingya refugee camps, Cox’s Bazar, Bangladesh

- Locate site close to camps
- Design a space suitable for molecular biology work → Partitioning
- Test selection
- Anticipate equipment, reagents, consumables needs
- Prepare budget & procure materials
- Recruit personnel
- FAST!
Establishing a laboratory in Cox’s Bazar

Addressing the problem

**Proposed tests**

- Diphtheria
- Dengue
- Chikungunya
- Zika
- Japanese encephalitis
- Rotavirus
- Hepatitis A, B, C & E
- Leptospirosis
- Scrub typhus
- Measles
- Mumps
- Rubella
- Influenza
- H influenzae
Addressing the problem

WHO warehouse – swabbing kits, PPE

Successes

Establishing a laboratory near Rohingya refugee camps, Cox’s Bazar, Bangladesh
Laboratory establishment in Cox’s Bazar

**LOCATION**
- Suitable space identified & refurbished
- Materials identified

**17 Feb**
- MOU SIGNED
  - MoHFW & WHO Country Office
  - Budget & Material
- 15-25 Jan

**LAB OPENED**
- Diphtheria PCR testing commenced
- Staff recruitment & training completed

**21 April**
- TEST EXPANSION
  - Flu RT-PCR
  - Zik-Den-Chik RT-PCR

**Nov-Dec**

**Diphtheria epi curve, Cox’s Bazar, 2018**

8 Nov 2017 – August 9 2018

- Laboratory opened

**Source:** https://twitter.com/PeteSalama WHO SEARO 19 Aug 2018
Challenges

Establishing a laboratory near Rohingya refugee camps, Cox’s Bazar, Bangladesh

- Recruitment of skilled personnel
- Technical assistance interrupted
- Equipment installation & calibration → test expansion
- Health equity → consistency tests available host community & refugee population
- Conflicting working hours Medical College & lab → TAT
- Service contracts critical equipment
Emergency response perspective

• Address lack of outbreak diagnostic preparedness
• Advocate financial global strategy for comprehensive laboratory package quickly implemented in emergencies
• Deployment of mobile lab to cover initial gap?
• Strengthening lab networks in developing countries

FETP perspective

• Understand political context
• Cultural competency critical—colleagues from all corners of the world
• Navigate unfamiliar bureaucracy
• Keep a diary
• Opportunity to put into practice what you learn during your FETP
Want to know more?

The Rohingya
Inside Myanmar’s Genocide
Azem Ibrahim

Ibrahim’s searing book documents the airliftgenocide of the Muslim Rohingya and exposes the culpability of the Buddhist clergy in fuelling the religious cleansing of Myanmar.

REVISED AND UPDATED EDITION
Foreword by Nobel Peace Prize winner Muhammad Yunus

WHO Rohingya Weekly Situation Report:

EWARS
http://ewars-project.org/
http://www.who.int/emergencies/kits/ewars/en/

Acknowledgements
4.J Presentation at ANU National Centre for Epidemiology and Population Health, March 2018

The slides below were used for a lunchtime seminar presentation at the National Centre for Epidemiology and Population Health, The Australian National University, Canberra on 8th March 2018.

Diphtheria outbreak among Rohingya refugees in Cox’s Bazar, Bangladesh
My experience deploying with GOARN January-February 2018

Ximena Tolosa
MSc scholar, WHO Collaborating Centre for Reference & Research on Influenza, Melbourne

Preparation

Pre-deployment steps – GOARN 1
- WHO medical certificate of fitness for work*
- Immunisations mandatory / highly recommended for Bangladesh
- UNDSS online courses - security in the field
- Other forms: designation of beneficiary, designation of representative, declaration of interest form
- GOARN reimburses vax costs

<table>
<thead>
<tr>
<th>Vaccines</th>
<th>Advice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphtheria</td>
<td>MANDATORY</td>
</tr>
<tr>
<td>Tetanus</td>
<td>MANDATORY</td>
</tr>
<tr>
<td>Polio</td>
<td>MANDATORY</td>
</tr>
<tr>
<td>Hep A &amp; B</td>
<td>MANDATORY</td>
</tr>
<tr>
<td>MMR</td>
<td>MANDATORY</td>
</tr>
<tr>
<td>Typhoid</td>
<td>Strongly recommended</td>
</tr>
<tr>
<td>Rabies</td>
<td>Strongly recommended</td>
</tr>
<tr>
<td>Japanese encephalitis</td>
<td>Recommended if &gt;4 wk</td>
</tr>
<tr>
<td>Malaria prophylaxis</td>
<td>Strongly recommended</td>
</tr>
</tbody>
</table>

Pre-deployment steps – GOARN 2
- Obtained WHO emergency contract US$ 1
- Set up profile in UNDSS TRIP system
- Submit a Security Clearance Request in the UNDSS TRIP system, make sure it is approved before departure

Packing

- FOOD! Tins, beans, tuna, noodles, crackers, cereal bars
- Coffee & coffee cup cold
- Quick dry clothes
- Hat
- Hiking shoes, crocs
- OTR hand rub, insect repellent
- Vit C and Vit B stress formula
- Decent laptop (Stats, R)
- Hybrid backpack
- Adapters - 3 kinds, ext cord, multi tool, spork, luggage lock
- Laundry powder
- Earplugs
Pre-deployment steps - ANU

- Apply for travel approval
- Register with DFAT Smartraveller
- Bangladesh DFAT level 3 (high threat of terrorism)
- High risk travel - more signatures are required: 6 days
- Risk assessment plan

Arriving in Bangladesh

- BNE-SIN-DAC (16h)
- Obtained visa on arrival (WHO provided me with a letter, 4 wk)
- If arriving at night hotel driver transfer
- During working hours, WHO driver takes you to WHO office in Dhaka
- per diem - You pay for all your expenses
- Cost of hotels: US 45 – 75 pn
- Meals USD 4 -7

Visa USD 50 (reimbursed by GOARN)

Day 1 – Dhaka to CXB

- WHO country office – all morning
- Security briefing
- Political situation briefing from WR
- Obtained WHO ID cards
- Flight to Cox’s Bazar (CXB)
- Issued with a phone with lots of data 😊
- Arrived at WHO CXB office 19.00
- Case management meeting!

Working in CXB - Security

- 24/7 radio room for all UN staff
- Emergency contact details provided on arrival
- Booked into UNDSS approved hotel
- UNDSS security briefing
- Travel to camps in WHO vehicles only (MOSS compliant with VHF radio and other emergency equipment)
- Field trips restricted to daylight hours
- No walking around @ night, no rickshaws @ night

Hotel Sea Palace

[Image of Hotel Sea Palace]

- www.hotelseapalacebd.com
- 45 USD pn
- SAFE (UNDSS approved)
- 3 star, 24th restaurant, better wifi than @ WHO office
- Good b'fast
- 1 AUD = 64 BDT

Country context
**Country profile**

- Densely pop
- ↑ Poverty
- Improvements in H & edu
- Independence in 1971
- 15 y military rule (1965)
- Political volatility
- Religious tolerance with rising Islamist extremism
- Vulnerable to cyclones & flooding

**Rohingya Crisis**

- Government sponsored violence
- Since 25 August 2017, 600th + Rohingya have crossed over from Myanmar into CXB, joining other waves of displacement!
- 548,000 in Kutupalong-Balukhali expansion site
- 185,000 in other camps
- 110,000 in host communities
- Risk of epidemic-prone diseases due to poor living conditions, inadequate WASH, low vaccination coverage

**My roles**

- Setting up a PH laboratory in CXB

**Tests proposed for new lab in CXB**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Serology</th>
<th>Molecular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphtheria</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Dengue (1, 2, 3, and 4)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Chikungunya</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Hepatitis (A, B, C, and E)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Pertussis</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Influenza (A and B)</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>MR</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Leptospirosis</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Japanese encephalitis</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Rotavirus</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Scrub typhus</td>
<td>x</td>
<td></td>
</tr>
</tbody>
</table>

*Denotes serology for dengue diagnosis (untyped), Dengue molecular testing for genotyping.
**Denotes serology for diagnosis and hepatitis molecular testing to detect the pathogen in watertable/environmental specimens.
***Authors needed for confirmatory investigations.

**Survey of HCF in camps – services, WASH, IPC**
mWater app

The mWater Explorer app is used for data collection on mobile smart phones and tablets.

The data is uploaded to an online (cloud) database. The data may be reviewed in the app
or mobile devices with internet connection.

This information is also stored in the online database. Both the data and the online store
This is available for review and analysis.

New GOARN partner. Training local volunteers NGO. Team of 12. 9 days in the field
Survey of HCF in camps

Dip case investigation in host community

N patients treated with DAT – proxy for confirmed cases

Cases within host community

EWARS – Early Warning Alert & Response System

Syndromes under surveillance in camps:
- Acute diarrhoeal disease
- Acute respiratory infection
- Malaria
- Severe malnutrition
- Tetanus
- Mumps
- Measles
- Rubella
- Suspected meningitis
- Suspected haemorrhagic fever
- Severe acute respiratory infection

Chapter 4
WHO warehouse – RDT kit, swabs stocks

Reaching out to partners – MSF DipTC

Comms officer!

Filming at SP DipTC
Case management team leader describing diphtheria symptoms for WHO website

Life in Cox’s Bazar

Morning walk to the office

Congested roads – CXB to camps 4h
Refugee camps

Rohingya refugee camps in CXB

Rohingya refugee camps in CXB

Rohingya refugee camps in CXB
Outputs

• Lab proposal acceptable for MOH & WHO
• List of resources required (HR, consumables, reagents)
• Budget
• Report on HCF in camps, co-author
• Daily reporting to EWARS: DAT use, bed occupancy
• Contributed to IPC guideline for DipTC

Challenges

• Maintaining data security
  Data sharing agreement - one single WHO email address to share line lists. Line lists only to be used for the purposes of case investigation and to identify duplicates across lists from multiple facilities. They are not to be shared with a third party, nor via any non-WHO email address.
• Tension between patient case management and epi research
  Unreasonable demands on clinicians in DipTC due to research projects
• Antiquated treatment (late 1800s).
  Controversy reactivity to DAT (sensitivity test increases delivery time, painful for children)
• Rapid staff turn over. Frustrating for BMOH

End of Mission Report

• Background
• Introduction
• Objectives
• Activities and findings
• Conclusions
• Recommendations
• Annexes (key persons met, tools used, references, photos, travel claim)

Lessons Learned

• Keep a diary, daily entries. Who you meet, when, for what
• Remain calm as you deal with the monstrous bureaucracy
• Cultural sensitivity is critical – BMOH and colleagues from all corners of the world
• Opportunity to put into practice what you learn during your MAE

Skills GOARN is looking for – CXB response

Want to know more?

WHO Rohingya Weekly Situation Report:
EWARS
http://ewars-project.org/
http://www.who.int/emergencies/kits/ewars/en/
Acknowledgements

Tambri Housen, ANU
Sheena Sullivan, WHOFLU
Kanta Subbarao, WHOFLU
Tony Stewart, GOARN
GOARN colleagues
ARM network

ximena.tolosa@anu.edu.au
Chapter 5

Surveillance of zoonotic influenza viruses at the animal-human interface: is Australia ready?

The One Health approach to public health recognises the interconnection between people, animals, plants and their shared environment.
## Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prologue</td>
<td>264</td>
</tr>
<tr>
<td>My role</td>
<td>264</td>
</tr>
<tr>
<td>Lessons Learned</td>
<td>265</td>
</tr>
<tr>
<td>Public Health Implications</td>
<td>266</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>267</td>
</tr>
<tr>
<td>Abstract</td>
<td>269</td>
</tr>
<tr>
<td>5.1 Introduction</td>
<td>271</td>
</tr>
<tr>
<td>5.1.1 Emergent zoonotic influenza: a public health threat</td>
<td>271</td>
</tr>
<tr>
<td>5.1.2 Zoonotic influenza transmission hostpots also exist in high-income countries</td>
<td>271</td>
</tr>
<tr>
<td>5.1.3 The rationale for adopting influenza surveillance at the pig-human and poultry-human interfaces</td>
<td>272</td>
</tr>
<tr>
<td>5.1.4 Study aims</td>
<td>274</td>
</tr>
<tr>
<td>5.2 Methods</td>
<td>274</td>
</tr>
<tr>
<td>5.2.1 Stakeholder engagement</td>
<td>274</td>
</tr>
<tr>
<td>5.2.2 Surveillance protocol design</td>
<td>276</td>
</tr>
<tr>
<td>5.2.3 Ethical approval</td>
<td>277</td>
</tr>
<tr>
<td>5.3 Results</td>
<td>277</td>
</tr>
<tr>
<td>5.3.1 Stakeholder affiliations</td>
<td>277</td>
</tr>
<tr>
<td>5.3.2 Main findings from stakeholder engagement</td>
<td>278</td>
</tr>
<tr>
<td>5.3.3 Proposed surveillance protocol</td>
<td>290</td>
</tr>
<tr>
<td>5.4 Discussion</td>
<td>291</td>
</tr>
<tr>
<td>5.5 Recommendations</td>
<td>294</td>
</tr>
<tr>
<td>5.6 Conclusions</td>
<td>296</td>
</tr>
<tr>
<td>References</td>
<td>297</td>
</tr>
<tr>
<td>Appendices</td>
<td>306</td>
</tr>
<tr>
<td>5.A Participant information and consent form</td>
<td>306</td>
</tr>
<tr>
<td>5.B Stakeholder questionnaires</td>
<td>309</td>
</tr>
<tr>
<td>5.B.1 Questionnaire for industry, animal health and public health staff</td>
<td>309</td>
</tr>
<tr>
<td>5.B.2 Questionnaire for trade union representatives</td>
<td>330</td>
</tr>
</tbody>
</table>
5.C Proposed protocol for surveillance of people working in the intensive pig and poultry production and processing industries in Australia

5.C.1 Aim

5.C.2 Description of the proposed surveillance system

5.C.3 Sampling strategy

5.C.4 Data collection and reporting

5.C.5 Surveillance attributes of the proposed system

5.C.6 Important considerations

5.D Oral presentation at the Influenza Centre, November 2018
Chapter 5

Prologue

This project was borne out of the long-term interest by scientists at the WHO Collaborating Centre for Reference and Research on Influenza in elucidating what respiratory pathogens circulate at the animal-human interface in Australia. Knowledge on the types of influenza viruses that infect people who work in specific intensive animal industries, which is currently lacking, is necessary from an occupational health equity point of view and important for pandemic preparedness.

My role

With input from a team of researchers and following the suggestion by the Director of the Influenza Centre, I drafted an internal grant proposal to the Australian Partnership for Preparedness Research on Infectious Disease Emergencies (APPRISE). The title of the proposal was ‘Influenza sero-surveillance at the animal-human interface: a feasibility study in high-risk groups’. We were successful in obtaining a AU$25,000 grant. Our work had two major objectives: 1) to develop protocols for surveillance and for outbreak investigation of respiratory viruses in people working in specific animal industries; and 2) to assess the feasibility of conducting a sero-epidemiological pilot study of influenza and coronavirus infection in at-risk populations. I worked on part of objective one (i.e., design a framework for a surveillance protocol). Development of acceptable protocols requires the input of all stakeholders. Given the political sensitivities involved in conducting surveillance in an intensive animal industry context, active participation of stakeholders in protocol development is crucial.

As project coordinator, specific activities I was responsible for included administrative (i.e., schedule and chair monthly teleconferences with our interstate and international APPRISE collaborators† and circulate agendas and minutes), research (i.e., identify stakeholders within the intensive pig and poultry industries and search for relevant surveillance protocols, nationally and internationally) and epidemiological tasks. The latter consisted of stakeholder engagement (to obtain stakeholder views regarding the implementation of surveillance), interview guideline and survey design, preparation of participant information and consent form, conducting face to face and phone interviews, build an online survey using Qualtrics, analysis of responses, preparation of a progress report for the funding body, drafting a final report to respondents and designing a surveillance framework informed by existing protocols and stakeholder input.

†A list of collaborators and their affiliations is presented in the acknowledgements
Lessons Learned

This project gave me the opportunity to design questionnaires for four types of stakeholders: animal industries, animal health, public health and trade unions. I learned the importance of carefully balancing the amount of information sought with keeping the interview to a reasonable length. Close to 10 hours of voice recordings and over 50 pages of survey responses also gave me further experience in qualitative data management and analysis.

Early on during this project it became apparent that systematic surveillance of people at high-risk of contracting zoonotic respiratory infections does not occur in high-income country contexts in a systematic manner. Through our professional networks (mainly the US CDC Influenza Division) we were able to obtain surveillance protocols from known geographic hotspots, i.e., countries in South East Asia and Africa that had previously experienced outbreaks of zoonotic diseases with high mortality rates such as highly pathogenic avian influenza. The settings in which disease transmission between animals and humans occur in low-income countries is very different to the Australian context of large-scale intensive animal production. Through this project I learned that the potential risk for emerging zoonotic diseases also exists in high-income countries like Australia. Intensive pig and poultry production and processing establishments have been identified as locations where emerging zoonotic pathogen spillovers can occur. It is therefore important to monitor pathogen circulation at the animal-human interface in this context so that we can move from a reactionary to a preventive approach to zoonotic outbreaks.

The reason for the lack of surveillance of workers in intensive pig and poultry industries in Australia became clearer once I engaged or attempted to engage industry stakeholders. Despite repeated attempts to engage the peak body for the Australian pig industry we were unable to obtain their participation. It is possible that lack of trust and fear of the repercussions this work could have on the industry influenced their decision not to participate. Engagement with the poultry industry was comparatively much easier, although they did express scepticism regarding the feasibility of worker surveillance as well as concern about the potential negative financial consequences to producers that positive findings may have.

Not surprisingly, representatives from trade unions with coverage in Australia’s largest pig and poultry abattoirs displayed interest and in-principle support in a surveillance system targeting workers. They believed that understanding the types of exposures associated with large pig/poultry meat processing plants are necessary to improve workers’ occupational health and safety. Unfortunately, we were unable to reach
farm workers. This sector of the workforce is characterised by immigrant workers on short-term contracts who are largely non-unionised and therefore lack representation.

Through this work I learned that trans-disciplinary effort and trust among stakeholders are key to overcoming barriers to adopting a One Health approach to public health surveillance at the animal-human interface. Indeed, the foundation of One Health is built upon mutual trust among participating disciplines and sectors and mutual trust should be reflected on communication, collaboration and coordination.

I learned that an active participation of animal industries in the design of surveillance protocols is crucial to designing protocols acceptable to their respective industries. This will happen if industry believes there is value in conducting such surveillance. A possible solution to the current climate of resistance to surveillance at the animal-human interface was proposed by several stakeholders: the formation of a dedicated One Health government agency. A more pessimistic view that emerged from stakeholder engagement suggested that surveillance of people working with animals will only commence with full industry support as a consequence to a serious zoonotic outbreak occurring in an Australian intensive pig/poultry production or processing establishment.

**Public Health Implications**

One Health surveillance consists of a trans-disciplinary approach to preventing disease outbreaks among people or animals and for maintaining ecosystem integrity to reduce the risk of infectious disease transmission at the animal-human-ecosystems interface. This project, although not dealing with integrated One Health surveillance but rather part of it, represents an initial step to reignite the debate on the need for One Health surveillance in Australia. This debate has a long history of industry resistance and progress in adopting a One Health strategy in Australia has been remarkably slow. As one epidemiologist in our team put it "just having stakeholders from animal industries, animal health and public health in the same room will be an achievement in itself".

We envisage that the future stakeholder workshop we planned, as part of this study, will foster collaboration among stakeholders and result in an agreement to conduct a pilot study that will help understand the risks present at selected animal-human interfaces in Australia. We anticipate that such a study would also help justify the need for One Health surveillance implementation in Australia targeting intensive pig and poultry production and processing establishments.
The work presented in this chapter highlighted the need for a sero-epidemiological pilot study. This is important because it is possible that undocumented transmission of respiratory zoonotic pathogens is already occurring at the animal-human interface in Australia. If people don’t show symptoms when infected, or if those infected display non-specific symptoms that are mistaken for other diseases it is unlikely that a diagnosis will be obtained. This is how the emergence of a new zoonotic pathogen could be missed. Further compounding the problem is the fact that not all state public health laboratories in Australia have the capacity to subtype influenza viruses; early diagnosis of a new strain of influenza of animal origin is therefore unlikely. In this case if an influenza type A virus result is obtained from a person infected with a novel animal strain it would be reported as seasonal influenza. Therefore, the protocol to detect spillover events at the pig/poultry-human interfaces presented in this chapter will be an important resource going forward in efforts to improve the surveillance of zoonotic influenza (and other respiratory viruses) in Australia.

Better understanding of zoonotic respiratory viruses circulation at the animal-human interface would result in the protection of workers’ health, many of which are vulnerable due to their temporary immigrant status. Similarly, if evidence of undocumented zoonotic respiratory disease transmission is found, this would lead to improved animal health through the enhancement of on-farm biosecurity measures, thus reducing disease transmission from humans to pigs/poultry and wild birds to pigs/poultry. Lastly, implementation of surveillance at the animal-human-environment interface would better prepare Australia for a future pandemic.

It is encouraging that 17 out of the 20 participants –representing all relevant sectors– indicated an interest in attending a future workshop to discuss the feasibility of adopting surveillance at the animal-human interface in Australia.

**Acknowledgements**

I acknowledge the productive discussions had with members of the APPRISE team:

- Prof David Smith, Clinical Virologist, PathWest Laboratory Medicine, WA Department of Health, Western Australia
- A/Prof Paul Effler, Medical coordinator, Communicable Disease Control Directorate, WA Department of Health, Western Australia
- Prof Soren Alexandersen, Director, Geelong Centre for Emerging Infectious Diseases, Victoria
Chapter 5

- Dr Frank Wong, Molecular Microbiologist, Australian Animal Health Laboratory, CSIRO, Victoria
- Dr James Watson, Veterinary Investigation Leader, Australian Animal Health Laboratory, CSIRO, Victoria
- Prof Marion Koopmans, Head of Viroscience, Erasmus Medical Centre, The Netherlands
- A/Prof Sheena Sullivan, Senior Epidemiologist, WHO Collaborating Centre for Reference and Research on Influenza, Victoria
- Prof Kanta Subbarao, Director, WHO Collaborating Centre for Reference and Research on Influenza, Victoria

I appreciate the feedback the team gave me on the stakeholders’ interview guide and thank them for introducing our work to relevant high-level government stakeholders. We thank colleagues at the US CDC in Atlanta and elsewhere for sharing information on surveillance for zoonosis.

Before embarking on this work I sought guidance from Simon Firestone, an MAE graduate with a veterinary background who conducted a One Health-like project as part of his MAE. I thank Simon for expanding my list of relevant stakeholders and for taking the time to set the scene for me as to the challenges of implementing surveillance of respiratory pathogens in humans in the context of pig and poultry industries.

Special thanks go to my ex colleague Dr Pat Blackall (Biosecurity Queensland) who personally introduced myself and my work to his colleagues from the pig and poultry industries. I also thank my friend and ex colleague Aggie Dawainavesi (Mataika House) for productive discussions on how to develop a surveillance system and to Noore Alam (Queensland Health) for his feedback on this chapter. Lastly, I acknowledge all interview participants for making time within their very busy schedules.
Abstract

**Background:** People engaged in activities involving high contact with poultry, wild birds or pigs are considered at risk of contracting novel zoonotic viral infections such as influenza. Surveillance of people occupationally exposed to emerging zoonotic respiratory viruses has been proposed for the early detection and control of novel influenza viruses with pandemic potential. This study aimed to assess the feasibility of establishing systematic, ongoing epidemiological surveillance of people occupationally exposed to intensively-produced pigs and poultry in Australia for emergent zoonotic influenza viruses.

**Methods:** We identified stakeholders within the Australian pig and poultry industries, relevant trade unions, animal health and public health experts within government and academia. Through interviews and surveys we examined drivers and barriers of conducting surveillance of people occupationally exposed to intensively-produced pigs and poultry for zoonotic influenza viruses. We searched the peer-reviewed and grey literature for surveillance protocols applied to the intensive production and processing context in high-income countries. Informed by the stakeholder engagement and literature findings we drafted a framework for the surveillance of emerging zoonotic influenza viruses in humans.

**Results:** Twenty out of the 34 (59%) invited stakeholders participated in our study. Most participants were engaged in animal health (55%) but all stakeholder types were represented in our sample. Stakeholders expressed concern about the potential financial consequences to the industry if public health surveillance were to be implemented within their industries. Engagement with a sector of the workforce revealed limited awareness of the potential risk of zoonotic respiratory viruses among pig and poultry abattoir workers. In addition, it was highlighted that when abattoir workers seek medical care GPs fail to elicit animal contact information. We were unable to reach people that work in large pig and poultry production establishments. This is a population comprised of an unknown proportion of immigrants working under seasonal contracts and other short-term arrangements. Several animal health and public health experts agreed that although the burden of zoonotic respiratory viruses at the animal-human interface is unknown, they represent a threat. Potential gaps in early detection of a spillover event were identified by stakeholders from different sectors. As an aid to a future consultation process we drafted a framework for conducting surveillance of people at risk of contracting zoonotic influenza infections.
Conclusion: The benefits of implementing surveillance of emerging zoonotic influenza viruses in people occupationally exposed to intensively-produced pigs and poultry were readily accepted by public health stakeholders and viewed with optimism by unions representing abattoir workers. In contrast, industry and animal health stakeholders regarded surveillance with caution. Prior to the implementation of a surveillance system targeting people working at the pig/poultry-human interface, the risk of zoonotic influenza must be assessed. A trans-disciplinary and multi-sectoral consultation process is necessary to work through challenges such as competing priorities, divergent agendas, silo working cultures and limited resources before an acceptable surveillance protocol can be agreed upon.
5.1 Introduction

5.1.1 Emergent zoonotic influenza: a public health threat

Animals used for food and wildlife carry viruses that are known or potential threats to human health (1, 2). Wild aquatic birds are the ancestral hosts of influenza A viruses that infect humans (3). Viral reassortment can occur in pigs, poultry and humans. However, pigs are thought to be more efficient at this process but the exact mechanism is not clear (4). The way viral reassortment works is that when two different influenza viruses (i.e., an animal and a human influenza A virus) simultaneously infect a host swapping of gene segments can occur and through this process, novel viruses can be generated (5).

Novel viruses generated in pigs have the potential to cause severe disease in humans and, if capable of sustained human-to-human transmission, could also cause an epidemic or pandemic. Indeed, transmission of avian influenza viruses to humans via pigs gave rise to the last A(H1N1) influenza pandemic in 2009 (6, 7). The influenza pandemics of 1957 and 1968 were the result of reassortment of avian and human influenza viruses (8, 9). The current understanding of the complex ecology of influenza in pigs suggests that the generation of novel viruses occurs through the following sequence of events: 1) a cycle of human-to-pig or bird-to-pig transmission transmission; 2) evolution of the virus in pigs; and 3) viral ‘re-entry’ into the human population (5, 10).

5.1.2 Zoonotic influenza transmission hostpots also exist in high-income countries

Transmission of novel influenza viruses between pigs and humans and between poultry and humans has been documented in both low- and high-income countries (11, 12, 13, 14, 15, 16, 17). Among high-income countries there is evidence of pig to human transmission in the United States (18, 19), Canada (20), Hong Kong (21), the Netherlands (22), the United Kingdom (23), Italy (24), other European countries (25, 26) and most recently, Australia (personal communication). Due to the absence of influenza surveillance in pigs, evidence of the bi-directional nature of influenza transmission between pigs and humans in Australia did not emerge until 2009, when influenza A(H1N1) pandemic was identified in a pig herd in New South Wales (27). Since then transmission of pig and human influenza A viruses within commercial pig
herds has been documented in Victoria, Western Australia and Queensland (27, 28). Reports of transmission of influenza viruses between humans and pigs may be expected to continue to increase with the establishment of systematic ongoing surveillance and improved diagnostic tests able to detect novel strains (29).

In the last 10 years Australia has had four outbreaks of highly pathogenic avian influenza (HPAI) in intensively produced poultry (30). Although the pathway for infection had not been identified, it has been hypothesised that wild water birds infected commercial poultry with low pathogenic avian influenza (LPAI) –directly or indirectly through consumption of contaminated drinking water– and the virus later evolved into an HPAI strain (30). The possibility of introducing HPAI from wild birds directly to commercial poultry also exists as HPAI subtypes, including H5, have been detected through sporadic surveys of Australian wild water birds (31). These spillover events demonstrate that biosecurity measures associated with the intensive pig and poultry production and processing industries in a high-income country like Australia are not perfect.

5.1.3 The rationale for adopting influenza surveillance at the pig-human and poultry-human interfaces

The ability of reassortant influenza viruses to infect humans, spread among humans and potentially cause a pandemic provides a strong rationale for ongoing surveillance at the animal-human interface. Surveillance is fundamental to disease control efforts and facilitates ongoing risk assessment. The importance of establishing surveillance in pigs, poultry and people in contact with them utilising a One Health approach is emphasised by influenza epidemiologists and animal scientists globally (25, 18, 27, 29, 32, 28, 17). Increasingly, it is being recognised that adopting influenza surveillance at the animal-human interface is as important in large-scale intensive pig and poultry operations in industrialised countries as it is in developing countries where farming systems might differ in scale and ability to implement biosecurity measures (33). This is because biosecurity failures can occur anywhere.

In the last 15 years sufficient evidence has been accumulated globally regarding the risk that working at the intensive pig/poultry-human interface represents (34, 35, 36, 37, 38, 4). In Australia outbreaks of both high- and low-pathogenic avian influenza in commercial poultry and wild birds have been documented (39, 40) and evidence of influenza infection has been found in intensively produced pigs as well as in wild pigs (41, 28). To our knowledge there is only one published report that documents
transmission of influenza viruses from poultry to humans in Australia (42). This detection occurred in abattoir workers after processing birds from a commercial flock in New South Wales that had experienced an outbreak of low pathogenic avian influenza A(H10N7). Partial sequences of the viruses detected in both birds and humans were identical.

Despite evidence of the risk involved in working at the animal-human interface Australia does not conduct targeted systematic surveillance of people working in the intensive pig and poultry industries. Instead we rely on the investigation of respiratory disease outbreaks. These are detected if there is a drop in production due to reduced food intake or an increase in weekly morbidity and mortality rates among animals. Recommendations to adopt more stringent on-farm biosecurity measures usually follow (43). In addition, the Commonwealth Department of Health recommends seasonal influenza immunisation for people associated with the commercial pig and poultry industries only during an outbreak of avian or swine influenza (44). Complicating the early detection of emerging zoonotic viruses is the fact that these viruses are sometimes responsible for mild disease and negligible mortality in animals (45, 46) and are often unrecognised, not diagnosed or not reported until they spread into humans. The same could be true for humans infected with novel zoonotic viruses.

It is possible that the current reactive, fixed-term approach to prevent and contain potential zoonotic infections in pigs and poultry is based on the seemingly low frequency of spillover events in Australia and in the assumption that an emergent zoonotic influenza strain would be quickly detected should it arise in a person working at the animal-human interface. We argue that this approach is inadequate, is not in line with the International Health Regulations nor does it meet the requirements for pandemic preparedness (47, 48).

Australia had, as of September 2018, an estimated 1.4 million pigs and 164 million chickens raised for food (49) and approximately 24 million wild pigs (50). Large herd and flock size, high animal stocking densities and animal confinement, which are the hallmark of intensive pig and poultry production, are associated with influenza infection in animals (51, 33). We argue that the conditions for the emergence of a novel zoonotic influenza virus of public health concern are present in Australia and therefore surveillance at the animal-human interface is warranted. Sentinel populations for the detection of emergent influenza viruses of public health concern could include people working in high-risk occupations such as workers on pig and poultry production and processing establishments, pig/poultry transporters, and veterinarians.
5.1.4 Study aims

This study aimed to assess the feasibility of establishing systematic, ongoing epidemiological surveillance of people occupationally exposed to intensively-produced pigs and poultry in Australia for emergent zoonotic influenza viruses.

**Study objectives:** In the context of an absent influenza-like illness surveillance system that targets people occupationally exposed to intensively produced pigs and poultry the objectives of this project were to:

- Engage Australian stakeholders in the intensive pig and poultry industries, unions that represent workers in these industries, animal health and public health/one health experts within government and academia in order to seek their views on drivers and obstacles for surveillance of people working in the intensive pig and poultry industries.
- Draft the framework for a surveillance protocol for zoonotic influenza viruses in people working at the animal-human interface in intensive pig and poultry industries in Australia.

5.2 Methods

5.2.1 Stakeholder engagement

**Participant recruitment**

We used purposive followed by snowball sampling techniques (52) targeting stakeholders in the pig and poultry peak industries bodies, pig industry veterinarians, unions representing workers in pig and poultry abattoirs, animal health and public health experts within government (including surveillance officers and staff engaged in One Health activities) and academia. Stakeholders were identified by tapping into previous and current professional networks of research team members, such as the Australian Animal Health Laboratory, Biosecurity Queensland and Queensland Health. Official websites were also used to find the contact details of the Chief Veterinary Officer for the Commonwealth and for the states of Queensland, Victoria and Western Australia. We recruited a stakeholder at a One Health workshop held during the National Antimicrobial Resistance and Stewardship Forum in Melbourne in November 2018. The
initial list of stakeholders was expanded by asking participants to provide contact details of other relevant stakeholders.

Prior to commencing the stakeholder engagement we obtained support from Animal Health Australia (AHA) for conducting this work. AHA is a not-for-profit public company that links Commonwealth, state and territory governments, livestock industries and other stakeholders to protect animal health and improve biosecurity (53).

We identified 34 stakeholders and made initial contact by email to invite them to participate in our study. The initial email contained the participant information and consent form (see Appendix 5.A), a brief introduction to the study, the composition of the research team and the funding body.

**Data collection**

To increase response rates stakeholders were given the choice to participate in a phone interview, a face to face interview or an online survey. The self-administered online surveys were designed in Qualtrics and distributed via email. Face to face interviews took place in Brisbane and Melbourne during September 2018. The survey was active between September and October 2018. One participant requested an extension. Data collection was completed by mid November 2018.

Questionnaires and surveys were designed for each stakeholder type and consisted of three parts: 1) the threat of zoonotic influenza within the industry; 2) overall support for a new surveillance system targeting people working within the industry including views regarding drivers and constraints for adopting this surveillance; and 3) suggestions to overcome obstacles and interest in participating in future consultations. Union officials were also asked questions regarding awareness of zoonosis, the size and location of the workforce and access to healthcare for workers. Participants were given four weeks to complete the online survey. Two email reminders were sent one week apart to increase the response rate.

Questions asked to pig and poultry industry representatives, animal health and public health stakeholders are shown in Appendix 5.B.1 and questions asked to trade union representatives are presented in Appendix 5.B.2. Notes were taken during face to face interviews. Audio from interviews was recorded with consent. The average length of interviews was 72 minutes (range: 35–90 minutes).
Data analysis

We analysed written survey responses and interview notes using content and thematic analysis methods (54) to identify stakeholder perceptions as to whether zoonotic influenza is a threat, whether they support surveillance in at-risk humans and obtain their views regarding drivers and obstacles for adoption of this surveillance. The main themes identified corresponded with the questionnaire parts described above. This was followed by identifying codes from the data by an inductive process in which broad generalisations are made from specific observations.

Notes from interviews and survey responses were read thoroughly multiple times to gain familiarity with the data and a deeper understanding of recurring concepts. Sections of text were manually coded (55). Initial codes were generated by scanning text for concepts that shared the same central meaning. Codes that were similar or comparable were grouped into categories. Sections of text were assigned into categories and labelled using a unique coloured pen. Categorised text from all responses was sorted into themes and tabulated (54, 56).

We ensured that the analysis was grounded in the data by regularly referencing back to the raw data. Responses were re-examined and quotes extracted to illustrate the main themes.

5.2.2 Surveillance protocol design

We contacted our colleagues at the US CDC Influenza Division and requested surveillance protocols for zoonotic influenza in people occupationally exposed to pigs and poultry in any country. These included protocols from the US as well as other countries in which the US CDC has projects. In August 2018 we conducted a literature search for published protocols using PubMed Central and Google Scholar. Our search strategy for protocols for pig workers included combinations of the following terms: (i) ‘influenza’ OR ‘flu’, (ii) ‘surveillance’ OR ‘protocol’, (iii) ‘pig’ OR ‘swine’. The search for protocols for poultry workers had the same first two terms and the third was replaced by ‘poultry’ OR ‘avian’. Searches were limited to articles in English and to the period 2000–2018. Abstracts of potentially relevant papers were reviewed for eligibility and the full text of eligible articles was reviewed. Articles describing a surveillance system or a methodology to monitor the health of people occupationally exposed to pigs and poultry were considered. The reference lists of selected articles were reviewed to identify additional publications.
The US CDC Updated Guidelines for Evaluating Public Health Surveillance Systems (57) were used when considering selected attributes required for the system. The attributes considered here were those during the planning phase of the system were:

- **Acceptability:** a reflection of the willingness of stakeholders to implement the system and of the end users to accept and use the data generated through the system.
- **Sensitivity:** the ability for the system to capture all spillover events.
- **Timeliness:** the ability for the system to quickly report spillover events of potential public health significance in order to facilitate a swift response.
- **Usefulness:** the ability of the system to detect spillover events, including the ability to monitor changes in the number of these events over place and time.

The detailed information needed to evaluate the proposed surveillance system against the five other attributes was not available as it was beyond the scope of this chapter to craft a detailed protocol without appropriate input from all stakeholders. These surveillance system attributes—namely simplicity, flexibility, data quality, representativeness and stability—will need to be considered prior to piloting the system and with appropriate stakeholder consultation. Sensitive issues of responsibilities, financing and potential industry impact must be reconciled before moving to the next stage of protocol design.

### 5.2.3 Ethical approval

Participants provided informed consent to take the online survey or be interviewed. Interviews were recorded with participants’ permission. Participant affiliation remained confidential if requested. Ethical aspects of this study were approved by the Australian National University Human Research Ethics Committee under protocol number 2017/909. This research followed the National Statement on Ethical Conduct in Human Research (58).

### 5.3 Results

#### 5.3.1 Stakeholder affiliations

A total of 20 stakeholders agreed to participate in this study out of the 34 invited. Stakeholders had the following affiliations:
Chapter 5

- AgriFutures Australia (previously known as Rural Industries Research and Development Corporation), Chicken Meat Program
- Australian Chicken Meat Federation Inc.
- Australian Egg Corp Limited
- Australasian Meat Industry Employees’ Union (Queensland Branch Secretary and national Occupational Health and Safety official)
- Chief Veterinary Officers: Commonwealth, Queensland, Victoria and Western Australia
- Commonwealth Department of Agriculture and Water Resources (animal health)*
- Commonwealth Scientific and Industrial Research Organisation (animal health)*
- Biosecurity Queensland (animal health surveillance, animal health diagnostics)
- Queensland Health (public health surveillance)
- Academia (animal health, public health diagnostics)*

*Participants within these categories had experience in the practice of One Health.

We were unable to recruit representatives from Australian Pork Limited, the peak body for the pig industry, despite repeated attempts and two instances (by phone and face to face) in which details of this qualitative work were described and their questions answered. We were unable to obtain a justification as to the reason for the decision to decline participation. However, we were able to recruit one private pig industry veterinarian with 45 years of combined experience within government, industry and private practice. We were also unable to reach workers on farms due the non-unionised nature of the sector (i.e., the lack of a representative to contact).

The majority of participants (n=15) chose to complete the online survey. All industry participants –except for one who opted for a phone interview– chose face to face interviews (n=3) as did all trade union participants (n=2).

5.3.2 Main findings from stakeholder engagement

The main findings from the stakeholder engagement, presented in the following sections, cover the perceived need and support for surveillance of people working at the animal-human interface, drivers, obstacles and suggestions for overcoming obstacles. Participants affiliated with the pig and poultry industries and animal health experts are sometimes broadly referred to as animal sector respondents and workforce representatives and public health experts, as human health sector respondents.
1. Perceptions regarding the threat of zoonotic influenza in Australia

The main themes identified in how participants perceived the threat of zoonotic influenza are covered below. These included lack of baseline data, gaps in our current capacity to detect spillover events and the need for research in this field to better understand the threat of zoonotic influenza to humans working at the animal-human interface.

The scale of the threat of zoonotic influenza arising from pigs is largely unknown: Animal health experts working within and outside of the pig industry expressed difficulty in ascertaining how much of a threat zoonotic influenza represents for the pig industry due to the lack of systematic testing in pigs, constraints in data sharing between private industry laboratories and the Department of Agriculture and the limited number of viruses isolated from Australian pigs available for characterisation. Most animal health respondents were clear that the lack of evidence of influenza viruses circulating at the pig/poultry-human interface was due to the significant business risk it represents to the industries. The overall view of respondents with One Health expertise is illustrated by the following statement:

*The risk of swine influenza, zoonotic or pandemic, events occurring in Australia is unknown, as there is no active surveillance to determine which influenza A viruses are circulating endemically in piggeries, or the extent of the genetic diversity, and there is currently limited characterisation of influenza-like illness in people.* (Respondent with One Health expertise).

Despite the lack of surveillance of influenza viruses in pigs and the belief that Australian pigs were free from swine influenza until 2010, several respondents agreed that 'low path' influenza in pigs is likely to be endemic.

A spillover event of zoonotic influenza can occur in Australia, in both the commercial pig and the poultry setting: Stakeholders with a One Health background provided the most comprehensive answers regarding the threat that zoonotic influenza poses to animal health, human health and the intensive animal industries. One respondent pointed out that seasonal influenza viruses can be readily transmitted from humans to pigs therefore contributing to new genetic diversity within pigs and the potential for a zoonotic influenza strain to emerge after reassortment.

In the intensive poultry setting, respondents agreed that there is a risk that LPAI, which likely circulates undocumented in Australian flocks, could be a source of in-
fection to poultry workers. This is significant to public health given the known association between circulation of some LPAI subtypes in commercial poultry and the emergence of novel strains with zoonotic potential (i.e., China H9N2 outbreak). Additionally, the risk that LPAI could mutate to HPAI within poultry, which represents a direct threat to workers and animals, was identified by participants.

Zoonotic influenza is a recognised threat to Australia’s poultry industry: Some respondents were acutely aware of the risk that avian influenza viruses represent for the poultry industry and indicated that the risk is perceived to be higher in the egg industry compared to the chicken meat industry due to the longer life span of laying hens. In terms of transmission of LPAI to workers in the industry, respondents stated that the risk is managed by enhancing biosecurity measures immediately after an LPAI outbreak occurs in birds. Additionally, some respondents perceived the risk of transmission of seasonal influenza from humans to poultry to be low.

Several gaps exist in the early detection of spillover events: Participants revealed several ways in which a zoonotic influenza spillover event could be missed. Participants discussed ways in which workers, general practitioner (GP)s, laboratories and the national influenza-like illness (ILI) surveillance system contribute to these gaps.

It was discussed that workers in pig and poultry abattoirs have very limited knowledge of zoonotic influenza or the potential link between their occupation and respiratory illnesses in general. Further, respondents from multiple sectors indicated that awareness of this link is also low among GPs given that they fail to elicit information on animal exposure and occupation when workers seek care for an ILI. Lack of awareness of zoonoses among GPs has been a challenge for Australian meat workers occupational health and safety officials for many decades, in particular due to Q fever. In response, the meat workers union distributed a ‘zoonosis card’ (see Figure 5.1) to its members and instructed them to show it to their GPs whenever they sought healthcare.
Figure 5.1: Zoonosis card given to meat workers by the Australasian Meat Industry Employees’ Union to present to clinicians to encourage them to consider zoonoses in their diagnoses when investigating an acute febrile illness.

The threat of zoonotic influenza to human health in regions where intensive animal industries are a major employer was stated as moderate by some respondents. However, respondents acknowledged that there is no system currently in place for the early detection of spillover events at the pig/poultry-human interface. Additionally, gaps in the current ILI surveillance system were a recurrent theme. For example, not all state laboratories are able to subtype influenza from surveillance specimens, animal contact information of ILI patients is not routinely collected and influenza-positive patients are not followed up to seek animal exposure information.

**Research to elucidate the burden of disease of zoonotic influenza is needed:** A recurrent theme among participants from multiple sectors was that research to address the knowledge gap regarding the risk of occupational exposure to pigs/poultry should be conducted.

2. **Current level of support for surveillance of people occupationally exposed to pigs and poultry**

There was a marked contrast in the opinions of respondents regarding their level of support for a surveillance system targeting people working with intensively produced pigs and poultry. Respondents from the animal sector either failed to see any benefit in adopting such surveillance or outright opposed it. Referring to the poultry setting, respondents considered that surveillance of workers is not warranted due to the (perceived) low risk of transmission of avian influenza to occupationally-exposed people. Respondents expressed that a serious outbreak of zoonotic influenza in
occupationally-exposed people would provide convincing evidence of the need for such surveillance.

In contrast, respondents with an interest in human health expressed support for this initiative providing that sufficient industrial protections and confidentiality of test results were guaranteed. Further, public health experts, although aware of the potential negative consequences for the intensive animal industries, reasoned that the surveillance of at-risk workers would allow the early detection of spillover events.

3. Drivers for surveillance of people occupationally exposed to pigs and poultry

The themes we found regarding drivers for surveillance of workers are listed in Table 5.1. Stakeholders from the animal sectors struggled to identify drivers for surveillance of workers (the reasons for this are covered in the following section) but mentioned that two factors could make surveillance more palatable: 1) if it resulted in an economic gain; 2) if the Government pays for the cost of implementing and managing the surveillance of workers; and 3) if pig and poultry producers are fairly compensated for the consequences of positive findings. Respondents added that surveillance data could be used to assess the quality of biosecurity practices on farms and therefore could be useful as a tool to identify enterprises that need to improve their biosecurity. Respondents stated that evidence of influenza circulation at the pig-human interface would help clarify the epidemiology of influenza in pigs in Australia.

Again, animal health experts with an understanding of One Health clearly articulated the need for this surveillance as a driver at least at a research level. A representative viewpoint was:

*The burden of respiratory disease is not known for people that work with pigs in Australia. There is very little information regarding syndromic surveillance, detection of active infection with viruses such as influenza or corona viruses, or seroprevalence to indicate exposure to zoonotic infections. This is an important research question that needs to be answered.*

(Respondent with One Health expertise).

Stakeholders from the human health sector agreed that surveillance of workers has the potential to identify zoonotic influenza as a previously unrecognised work hazard. This could result in the addition of zoonotic influenza to the list of occupational diseases and therefore improved work practices being implemented at abattoirs with
Table 5.1: Drivers for adopting influenza surveillance of people occupationally exposed to intensively produced pigs and poultry: Findings from stakeholder engagement, Australia, November 2018.

<table>
<thead>
<tr>
<th>Drivers for adopting surveillance of people working with animals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No drivers exist</strong></td>
</tr>
<tr>
<td>Surveillance poses more risk to industry than benefit.</td>
</tr>
<tr>
<td><strong>Potential economic gain</strong></td>
</tr>
<tr>
<td>Perhaps proof of freedom from spill over events could allow access to new markets.</td>
</tr>
<tr>
<td><strong>Receive financial assistance</strong></td>
</tr>
<tr>
<td>Government to provide a reasonable cost-sharing agreement to respond to positive findings.</td>
</tr>
<tr>
<td>Government to pay for the cost of conducting surveillance and for the consequences of such surveillance.</td>
</tr>
<tr>
<td><strong>Tool for biosecurity assessment</strong></td>
</tr>
<tr>
<td>Surveillance data could be used to assess the quality of biosecurity practices.</td>
</tr>
<tr>
<td>Assessment could result in potential improvements in biosecurity.</td>
</tr>
<tr>
<td><strong>Elucidate influenza transmission dynamics</strong></td>
</tr>
<tr>
<td>Knowing what viruses are circulating between pigs and people would be of benefit.</td>
</tr>
<tr>
<td><strong>Clarify the epidemiology of influenza in pigs</strong></td>
</tr>
<tr>
<td>Long-term accumulation of evidence of flu at the pig-human interface would clarify the epidemiology of flu in pigs in Australia.</td>
</tr>
<tr>
<td><strong>Improve occupational health and safety (OHS)</strong></td>
</tr>
<tr>
<td>Improved OHS as consequence of better knowledge of a previously unrecognised occupational disease.</td>
</tr>
<tr>
<td>Improved engineering controls to reduce exposure (i.e., better abattoir design).</td>
</tr>
<tr>
<td><strong>Enhance cross-sectoral collaboration</strong></td>
</tr>
<tr>
<td>In QLD the collaboration between DoAg, DoH and WHSQ has been formalised and is very good as a consequence of joint responses to several serious zoonotic disease outbreaks.</td>
</tr>
</tbody>
</table>

DoH = Department of Health; DoAg = Department of Agriculture; WHSQ = Workplace Health and Safety Queensland.

the obvious improvement in Occupational Health and Safety (OHS). Unions noted that OHS regulators are often engaged in investigations related to injuries and might not have sufficient experience in zoonoses.

Stakeholders working in public health expressed a lack of sufficient knowledge of the industry to suggest what factors could be interpreted as drivers but hypothesised that educating producers and pig/poultry workers on zoonotic influenza could be helpful. They also highlighted that better collaboration between sectors would facilitate surveillance and referred to the very good collaboration between the relevant actors.
from the Department of Agriculture, the Workplace Health and Safety agency and the Department of Health in Queensland due to previous joint responses to zoonotic events. This driver was also identified by stakeholders working in animal health at the state government level.

4. Obstacles for surveillance of people occupationally exposed to pigs and poultry

The main themes identified in obstacles for surveillance are summarised in Table 5.2. Common responses regarding a major obstacle for adopting surveillance of people working in the pig and poultry industries in Australia voiced by all stakeholders was the concern of substantial economic losses for the industry sector as a result of positive findings.

Table 5.2: Obstacles for adopting influenza surveillance of people occupationally exposed to intensively produced pigs and poultry: Findings from stakeholder engagement, Australia, November 2018.

<table>
<thead>
<tr>
<th>Obstacles for adopting surveillance of people working with animals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Major negative consequences to industry</strong></td>
</tr>
<tr>
<td>Loss of trade as a consequence of positive findings is a major concern.</td>
</tr>
<tr>
<td>Intense negative media attention during previous outbreaks in piggeries resulting in reduced product consumption and a drop in price.</td>
</tr>
<tr>
<td><strong>Fear of regulatory over-response</strong></td>
</tr>
<tr>
<td>Fear that regulatory response might not be proportional to the impact caused by the disease (i.e., ‘the response could be worse than the disease’)</td>
</tr>
<tr>
<td><strong>Too costly</strong></td>
</tr>
<tr>
<td>This surveillance would be very resource intensive.</td>
</tr>
<tr>
<td>Who will bear the costs?</td>
</tr>
<tr>
<td>Deep concern that ongoing surveillance could jeopardise the cost sharing agreement set out in the Emergency Animal Disease Response Agreement (chicken industry has more at stake compared to egg industry as they split cost of responses 70-30).</td>
</tr>
<tr>
<td>Producers might shift resources from biosecurity to surveillance to cover the costs. This will be detrimental to animal health.</td>
</tr>
</tbody>
</table>
Table 5.2 continued from previous page

**Distrust among main actors**
Limited trust between industry and public health authorities is a primary obstacle.
Limited trust between regulatory vets and industry vets/producer.
Industry has limited experience working with public health authorities.
Uncertainty re communications with people working in the industry if positive findings.
Mismatch between who benefits from proposed surveillance and who risks serious business losses.

**Risk to business**
Surveillance poses more risk to industry than benefit.
Difficult to justify surveillance if novel zoonotic flu is detected in worker and traced to pigs but herd morbidity and mortality is not affected (i.e., the producer might not find this result relevant).
Producers do not want to find a virus of concern in workers that could trigger serious responses if they are not experiencing production (and economic) losses.

**Risk to workers**
Worker suspicion due to data confidentiality concerns and potential loss of employment.
Positive findings can have a negative impact on industry and therefore on jobs.
Fear of being stigmatised (i.e., being blamed as a spreader in the workplace).

**Specific worker vulnerabilities**
People on temporary work visas or employed through labour hire companies might not report ILI for fear of dismissal.

**Difficult to implement**
Lack of consistent regulatory framework.
Politics and practicalities of adopting surveillance in workers perceived as barriers.
SafeWork Australia deals primarily with workplace injuries and might not have adequate experience with zoonoses.

**Perception that influenza is not a serious disease**
Attitude among industry, animal health and also influenza researchers that influenza in pigs and people is not an important health issue.
The statement below illustrates the idea of surveillance as a risk:

*It’s the “what if” - the what if we do find a zoonotic respiratory virus.*  
*International level: trade risks, access to certain markets [that] are reliant on our freedom from exotic avian influenza strains. Impact on domestic market: detection of some viruses may also have an impact on domestic consumption. This then drives how the industry may view such activities and dictate how they engage... Sadly conservative approaches. Action and interest may only occur if people start getting sick, then it gets a public health approach which often will trump animal industry.* (Animal health respondent).

Another respondent expanded the idea that surveillance represents a major risk to animal industries as follows:

*[Obstacles] for [surveillance in the] poultry [setting are the same as for piggeries] —except perhaps more so because the trend towards free-range has increased the exposure of commercial birds to endemic LPAI in wild birds— and so the likelihood of finding something of concern [in the poultry setting] may be relatively high.*

*Surveillance potentially poses more business risk than benefit. Altruistic contribution to science, even better human health, is a poor motivator compared to profit at the farm level. ...the issue is that the benefit is to one group (human population, medicine, health agencies) —but the risk applies to the participating piggery [and poultry] businesses. Expecting individuals to take potentially significant risk for the benefit of others is a hard sell. It’s all fine until you find something. Resolve that disconnect/misalignment and you solve the problem.* (Animal health respondent).

Both industries agreed that the lack of benefit, fear of a regulatory over-response and lack of trust were major obstacles. In addition, respondents emphasised that positive findings in poultry workers, if epidemiologically linked to poultry, could jeopardise the current Emergency Animal Disease Response Agreement (in which Animal Health Australia and the affected industry agree on sharing the cost of a disease response) (59). They also expressed that workers ‘would make too much out of positive findings’.

Human sector respondents raised the potential for workers’ test results being covertly used as grounds for dismissal by management. The issue of lack of confidentiality of test results was highlighted as potentially more acute for migrant workers, who might object to providing specimens due to their more vulnerable employments status.
This potential obstacle was echoed by respondents from a different sector. Lastly, respondents discussed that any industry shake ups due to the consequences of this surveillance could have a negative impact on job availability and sustainability and therefore be interpreted as undesirable.

Respondents with expertise in surveillance stated that surveillance of people working with pigs and poultry would be very resource intensive and difficult to implement due to the complex politics of intersectoral work and practical barriers to reaching workers in the animal production and processing industries. The perception that influenza is not considered a serious disease by relevant stakeholders was also raised as an obstacle.

5. Prerequisites for the implementation of surveillance of people occupationally exposed to pigs and poultry

Information that can help shape a protocol for the surveillance of people that work with pigs and poultry was offered by all stakeholder categories and is presented in Table 5.3.

The need to first estimate the burden of disease of zoonotic influenza in workers was proposed by several respondents. Respondents also discussed the need of research that clarifies the epidemiology of zoonotic influenza at the pig-human interface and evaluates the effectiveness of interventions to reduce the risk of zoonotic influenza, such as worker immunisation, bird proofing and dam water chlorination. Research findings should be communicated in producer and veterinary forums.

Most respondents were clear about the importance that all stakeholders work together in the design of a new surveillance system to ensure its acceptance. Stakeholders from several sectors proposed that a One Health agency or an intersectoral committee within a newly established Centre for Communicable Disease Control could guide the drafting of a national framework for surveillance of workers as well as administer the funding and operationalise the surveillance. It was proposed that sector-specific discussion groups should be convened first, followed by meetings with all stakeholders. Obtaining support from leadership within each sector and building cross-sectoral relationships were identified as key factors to achieve effective collaborations to prevent and detect emergent zoonotic influenza viruses at the pig/poultry-human interface.

To facilitate the adoption of surveillance of workers by the industry, some experts indicated that the public health sector must acknowledge that they are the main beneficiary of the surveillance. In terms of funding, several stakeholders agreed...
that the Commonwealth government should be responsible to cover the costs of surveillance. Some respondents added that industry should also contribute financially to the surveillance.

Table 5.3: Overcoming obstacles to influenza surveillance of people occupationally exposed to intensively produced pigs and poultry: Findings from stakeholder engagement, Australia, November 2018.

<table>
<thead>
<tr>
<th>Proposed ways to overcome obstacles to surveillance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Research</strong></td>
</tr>
<tr>
<td>Close knowledge gap in diversity of zoonotic influenza viruses and mechanisms of influenza transmission at the animal-human interface within intensive pig and poultry industries in Australia.</td>
</tr>
<tr>
<td>Establish the burden of disease (BoD) of zoonotic influenza among industry workers. Share research findings in industry, producer, worker and veterinary forums.</td>
</tr>
<tr>
<td>If BoD indicates a need for ongoing surveillance workers will more readily accept it.</td>
</tr>
<tr>
<td><strong>Agreements</strong></td>
</tr>
<tr>
<td>Reach a national agreement among all stakeholders on the details of a response to positive findings (i.e., outbreak response protocol, cost-sharing agreement, content of messages to workers and the public).</td>
</tr>
<tr>
<td>Once agreement is reached assurances are needed regarding compliance by government.</td>
</tr>
<tr>
<td><strong>Governance</strong></td>
</tr>
<tr>
<td>Convene a national consultative group/intersectoral committee to advice government and industry in drafting a national surveillance framework (including funding and operational mechanisms of the proposed surveillance).</td>
</tr>
<tr>
<td>Establish a national CDC –Centre for Disease Control– with a One Health focus to lead this work.</td>
</tr>
<tr>
<td><strong>Funding</strong></td>
</tr>
<tr>
<td>Ensure the public health sector acknowledges that they are the main beneficiary of this surveillance.</td>
</tr>
<tr>
<td>Commonwealth government to bear the main cost of operating this surveillance (both DoH and DoAg) with an industry contribution to be negotiated.</td>
</tr>
<tr>
<td>Establish an industry fund to pay incentives to surveillance participants and offset cost to business in case of positive findings.</td>
</tr>
<tr>
<td>Personnel taking swabs from animals (if needed) need to get paid (finances cannot only go to laboratories).</td>
</tr>
<tr>
<td><strong>Cost-benefit</strong></td>
</tr>
<tr>
<td>Resolve the imbalance between who will largely bear the costs of a positive finding (industry) and who would largely benefit from having surveillance data (public health).</td>
</tr>
</tbody>
</table>
Table 5.3 continued from previous page

**Ethics**
Support to surveillance by industry more likely if it is done on a voluntary basis. Companies cannot force employees or contract farmers to participate. Ensure findings are communicated to industry and that workers that test positive suffer no repercussions.

**Specific strategies**

1. **Participatory approach**
   If a new system is adopted all stakeholders must be involved in its design, and operation, from the very beginning (i.e., not a single agency). Open the framework for consultation to reach consensus prior to implementation. In addition to listed stakeholders WorkSafe Australia needs to be part of future consultations (they would be involved in a response to positive findings). Obtain leadership support from key industry and government organisations.

2. **Zoonotic flu champions within government**
   Ensure DoAg has ‘zoonotic flu champions’ and enable them to visit the 15 industry pig vet practices serving the pig industry and travel to relevant conferences.

3. **Remove influenza from list of notifiable pig diseases**
   To encourage industry acceptance only strains of influenza A viruses in pigs not previously seen in Australia should be notifiable –not all flu A in pigs (i.e., stakeholders need to recognise that flu in pigs is a ‘fact of life’). This can be done via the Animal Health Committee.

4. **Recruit regional GPs in a pilot surveillance system**
   Number of GPs in regional communities with large pig/poultry abattoirs is very small, enrolling them as sentinel GPs in a pilot surveillance program should be considered. Target locations: Kingaroy, QLD (pig abattoir); Mareeba, QLD (poultry abattoir); Hanwood, NSW (largest poultry abattoir in Australia, and possibly the Southern Hemisphere); Corowa, VIC (pig abattoir).

5. **Enhance ILI national sentinel surveillance**
   In addition to targeted surveillance consider enhancing existing ILI surveillance by following up positive flu cases to obtain information on occupational exposure to pigs/poultry.

6. **Timing of implementation**
   Commence efforts in surveillance implementation during the next influenza pandemic so industry would see the need for surveillance.

Notes: DoH=Department of Health; DoAg=Department of Agriculture; GP=general practitioner; ILI=influenza-like illness.
Some respondents expressed that to decrease the resistance of the pig industry to any surveillance the following approach could be taken:

*Deregulate the response. Treat the disease [influenza infection in pigs] like salmonella. Monitor it but recognise that it is a fact of life. That people are as likely responsible for outbreaks on an individual farm as are pigs or birds...* (Industry respondent)

A more pessimistic view was held by one respondent who stated:

*A strategy to facilitate cross-sectoral collaboration could be to* conduct stakeholder round tables and find what their common goal is. But need to consider and factor industry conservative approach to not rocking the boat too much. Personally– wait for the next big influenza outbreak globally and use that as the platform to stage such a meeting. But industry needs to feel like there is a demand or drive for such surveillance otherwise they’d feel it would carry a risk. (Animal sector respondent).

Respondents identified ethical issues that need to be addressed such as the need to seek consent not just from individuals but also from production and processing establishments to participate in surveillance.

A number of specific strategies to fast track the adoption of surveillance were proposed. This ranged from ensuring there is a ‘zoonotic influenza champion’ embedded in the Department of Agriculture to running a pilot sentinel surveillance program in a small regional town where pig/poultry farms or abattoirs are a major employer (see Table 5.3). GPs could be recruited in the selected towns to participate in the pilot surveillance program. Another alternative discussed by respondents was to enhance the current ILI surveillance system by following up positive cases to obtain information on occupational exposure to pigs/poultry.

To address GPs failure to recognise the link between animal contact and illness, one respondent recommended that the zoonoses component of occupational medicine training curriculum for medical students be enhanced (in Victoria, according to a respondent, it consists of three hours during the entire medical training program).

### 5.3.3 Proposed surveillance protocol

The proposed protocol for surveillance of people occupationally exposed to intensively-produced pigs and poultry in Australia is shown in Appendix 5.C. Peer-reviewed publications, grey literature and stakeholders’ suggestions informed this protocol.
Protocols supplied by the US CDC were all from low-income countries and were not amenable for adaptation to the Australian context of large-scale intensive animal production. Only two publications were found that dealt with surveillance of pig and poultry workers. One from Taiwan (60) and one from Canada (61). These protocols were part of comprehensive surveillance targeting both animals and humans which we recognise is more useful than just the surveillance of people working at the animal–human interface. A One Health protocol would be more appropriate but was beyond the scope of this chapter.

A description of how the system would meet acceptability, sensitivity, timeliness and usefulness attributes is presented in Table 5.4.

5.4 Discussion

Prevention and early detection of zoonotic influenza viruses at the animal-human interface within the intensive pig and poultry production industries in Australia: Current gaps

Our stakeholder engagement revealed several factors that frustrate Australia’s capacity to adequately prevent and detect zoonotic influenza viruses at the animal-human interface within the intensive pig and poultry production and processing setting.

Gaps can occur at the following levels:

- farm/abattoir (lack of compliance with biosecurity guidelines such as facilitating free, on site influenza vaccination of workers, eliminating contact between wild birds and pigs/poultry, providing personal protective equipment (PPE) to farm workers and encouraging its use)
- worker (failure to recognise the link between ILI and occupational exposure to pig/poultry; disincentive to miss work if experiencing ILI due to fear of loosing job or, for foreign workers, being deported)
- attending GP (failure to elicit occupational exposure to pig/poultry, failure to collect specimens for influenza testing)
- laboratory (subtyping of influenza viruses is not conducted in all laboratories, not all untyped specimens are referred for further characterisation).

These gaps represents opportunities in which a zoonotic influenza virus spillover event could be missed in Australia. There was strong agreement among experts from...
multiple sectors regarding the existence of gaps in detection of zoonotic influenza viruses which enhanced the strength of the evidence.

Our findings stand in contrast to those of the recent report ‘Australia’s Joint External Evaluation of International Health Regulations Implementation’ (62) which gave the highest possible rank to compliance to the ‘Zoonotic diseases’ target (i.e., adopted measured behaviours, policies and/or practices that minimise the transmission of zoonotic diseases from animals into human populations). It is possible that the evaluation team did not specifically assess compliance with the International Health Regulations within the intensive animal industries due to time constraints.

**Surveillance in humans working with pigs and poultry: A partial solution**

These potential gaps in the early detection of zoonotic influenza viruses could be addressed by adopting an ILI surveillance system targeting people working with intensively produced pigs and poultry. Our stakeholder engagement provided insights into how different sectors view such surveillance. We have used stakeholders input on a range of issues to design a protocol for surveillance. At least half of the respondents acknowledged that zoonotic influenza might represent a threat to animal health, to the workforce in intensive pig and poultry settings and to public health. However, only the human health sector supports a proactive approach to influenza zoonotic detection in workers.

Australia is not the only high-income country that does not conduct surveillance of pig/poultry workers as part of a pandemic preparedness strategy. Canada, which has similar challenges to those we found for Australia, appears to rely on its multiple influenza surveillance systems to be able to detect a spillover event at the pig-human interface (63). A qualitative study published in 2014 found that Canadian experts from all relevant sectors agreed that to enhance influenza surveillance in humans a question could be added to laboratory requisition forms, completed for outpatients and also for hospitalised patients, about contact with animals (all animals not just pigs). They reasoned that this would result in faster detection of variant influenza strains, which would need to be isolated and genetically characterised using whole genome sequencing (63). It is not clear if this proposal was adopted.

Similarly, the United States also relies on its comprehensive national influenza system in humans for the early detection of a spillover event from intensively produced pigs and poultry into humans (Personal communication with scientist from US CDC Influenza Division). We argue that even a strong and well-functioning surveillance system might not be able to detect infections that spillover from intensively produced
animals to immigrant workers whom, for multiple reasons, might not seek healthcare when ill. This is particularly relevant in Australia given that seasonal workers are sought by animal industries to work in piggeries and poultry enterprises for up to nine months (64). Moreover, union membership among immigrant seasonal workers is rare, increasing their vulnerability to infection (i.e., PPE not provided) and exploitation such as the denial of paid sick leave (65).

It would be unwise not to adopt a surveillance system targeting these workers until the intensive animal industries are able to trust animal health regulators and public health officials and become more transparent in how they conduct their business (i.e., share the results of private laboratory tests with the Department of Agriculture); and until temporary immigrant workers are able to access the same rights to health as their Australian counterparts (i.e., access to PPE, healthcare and paid sick leave). However, we also recognise that enhancing human-based surveillance of zoonotic influenza would only detect a spillover event after it occurs. Indeed, at the time of writing the WHO Collaborating Centre for Reference and Research on Influenza detected the first human infection with a swine-origin A(H3N2) variant influenza virus (Personal communication with laboratory scientists are the Influenza Centre). This reassorting event, which demonstrates the first pig to human influenza infection in Australia, was detected more than three months after the specimen had been collected.

A proactive approach for the early detection of zoonotic influenza would involve the simultaneous surveillance of animals, humans and their shared environment. This would inform the risk of disease emergence before human exposure occurs and enable mitigation strategies to prevent disease transmission to humans (66). However, work is needed before Australian animal industries embrace the One Health approach to zoonotic influenza prevention (67). As a starting point we propose a surveillance system targeting pig and poultry workers within production and processing settings. In the longer term, addressing the current vacuum in One Health approaches to public health in Australia requires dedicated formal governance. This has been proposed almost 15 years ago for the specific purpose of surveillance of zoonosis (68).

We developed a framework for surveillance of people working within the intensive pig and poultry production and processing industries using suggestions from stakeholders, the published literature and the US CDC guidelines for the evaluation of public health surveillance systems (57). It is important that during the consultation and planning stages key surveillance system attributes are considered. At this point acceptability, or the willingness of persons and organisations to participate in the surveillance system, appears contested. In particular, who should be responsible for implementing,
managing and financing the system—which we envisage will be resource-intensive initially, the logistics of data and specimen collection and the type of response that will be enacted following positive findings need to be agreed upon to ensure system acceptance.

Limitations

A limitation of this work was our inability to obtain the views of the Australian pig industry. Although we did interview animal health experts with industry experience, their views might not represent those of the pig industry. Given the historically controversial nature of our work and the known challenges in engaging industry stakeholders, having the perspective of animal health sector representatives with decades of industry experience provided us with important insights.

We were also unable to reach people working in intensive pig and poultry farms due to their lack of representation. Future work will need to engage with this essential stakeholder. We limited our stakeholder engagement to people from some states that had previously experienced outbreaks of zoonotic influenza. Most of the animal health and public health respondents resided in Queensland and their views and experiences are unlikely to represent those of similar stakeholders in other Australian jurisdictions. To progress the adoption of national surveillance in workers future consultative work will need to include stakeholders from all jurisdictions.

The stakeholder questionnaires were not piloted due to time constrains and this was a limitation. Lastly, designing a surveillance protocol without direct input and feedback from stakeholders was also a limitation. However, we hope that this protocol provides a starting point for future consultations.

Despite these limitations our work was the first of its kind to include the views of the meat processing workforce that is regularly exposed to intensively produced pig and poultry in Australia.

5.5 Recommendations

The transmission of zoonotic influenza viruses from intensively produced pigs and poultry in Australia remains an understudied field. Although spillover events from animals to humans within this interface appear to be infrequent and these events have not resulted in human to human transmission, we recommend that the need to
adopt a surveillance system targeting the population working within this neglected interface be appropriately assessed.

The difficulties associated with this proposal mean that to progress it the concerns and views of all stakeholders must be considered from the very beginning. As a starting point for future consultations we have designed a surveillance protocol (see Appendix 5.C).

The following recommendations aim to address issues highlighted by the stakeholders consulted. They are related to the prevention and detection of zoonotic influenza.

1. Establish a One Health agency to lead the development of comprehensive surveillance at relevant understudied animal-human-environment interfaces in Australia.

2. Use the memorandum of understanding between Agriculture, Health and Workplace Health and Safety agencies in place in Queensland (69) and New South Wales (70) as a blue print for a similar nation-wide mechanism of joint response, coordination and communication in the event of zoonotic disease spillover events of public health significance.

3. Include relevant stakeholders from all jurisdictions in future stakeholder consultations.

4. Enhance the training of Workplace Health and Safety officials in zoonoses control.

5. Address the specific vulnerabilities of seasonal workers by involving the Department of Jobs and Small Businesses (which manages the Australian government’s Seasonal Workers Programme). Seasonal workers, like their Australian counterparts, should have access to paid sick leave if absent from work due to an ILI. Seasonal workers should have access to Medicare and workers’ compensation if they contract a zoonotic disease. In addition, if a research study or surveillance system is adopted seasonal workers need to participate.

6. We recommend that Australian Technical Advisory Group on Immunisation (ATAGI) adds pig workers and poultry workers to the free influenza immunisation program to minimise opportunities for viral reassortment (in addition to the current recommendation for workers to receive the vaccine during an outbreak of influenza in pigs or poultry). Potential challenges in vaccine uptake among workers would need to be considered (for example holding immunisation clinics on farms/abattoirs).
5.6 Conclusions

The benefits of implementing surveillance of emerging zoonotic influenza viruses in people occupationally exposed to intensively-produced pigs and poultry in Australia were readily accepted by public health stakeholders and viewed with optimism by unions representing abattoir workers but regarded with caution by industry and animal health stakeholders. A recurrent concern among the pig and poultry industry and animal health stakeholders was that surveillance of workers risks significant economic and financial losses to the industry.

Prior to the implementation of a surveillance system targeting this high-risk population – a section of which is vulnerable due to the non-unionised nature of farm work and their migrant status – a number of challenges must be addressed. These are the perceived imbalance between which sector will benefit and which sector will be disadvantaged, competing priorities, divergent agendas, lack of trust between some sectors and limited resources. Importantly, the risk of emerging zoonotic influenza at the animal-human interface must be assessed. Following this, a trans-disciplinary and multi-sectoral committee must work through the design of an acceptable and useful surveillance protocol. This will better prepare Australia to quickly detect a spillover event of public health concern and clarify the epidemiology of influenza at the animal-human interface. We hope this work will represent the first step in the development of a One Health surveillance system that integrates animal, human and environmental health.
References


57 German RR, Lee LM, Horan JM, Milstein RL, Pertowski CA, Waller MN, et al. Updated Guidelines for Evaluating Public Health Surveillance Systems: Recom-


65 Maritime Union of Australia. From Paddock to Port: Organising Migrant Workers. Interview with National Union of Workers National President Cat Cinnani Radio
Chapter 5


Appendices

5.A Participant information and consent form

PARTICIPANT INFORMATION STATEMENT AND CONSENT FORM
Stakeholders in Pig & Poultry Industry, Trade Unions, Animal Health, and Public Health
Zoonotic respiratory disease surveillance at the animal-human interface - A feasibility study

1. What is the research study about?
   You are invited to take part in this research study. The study aims to canvass stakeholders’ views regarding enablers and barriers to surveillance of emergent zoonotic respiratory viruses in people that work with animals in intensive pig and poultry industries in Australia. Regular monitoring of people occupationally exposed to animals is considered important because it could enable the early detection of emergent zoonotic respiratory viruses of public health concern. In addition, having an agreed-upon framework in place would provide clear guidelines on how each stakeholder would need to respond and communicate in the event of an outbreak of zoonotic respiratory disease of pandemic potential occurring at the animal-human interface.

   You have been invited because your views are important to help us shape the future of surveillance for the early detection of emergent zoonotic respiratory viruses at the animal-human interface in Australia. Contact details for the animal production industries were obtained from Animal Health Australia and from the student investigator professional networks.

   The ethical aspects of this research have been approved by the ANU Human Research Ethics Committee (Protocol 2017/909).

2. Who is conducting this research?
   The study is being carried out by the following researchers:
   - Prof David Smith, Clinical Virologist, PathWest Laboratory Medicine, WA (Lead investigator)
   - Dr Ximena Tolosa, Applied Epidemiology Scholar, National Centre for Epidemiology and Population Health, The Australian National University (Student investigator)
   - A/Prof Paul Effler, Medical coordinator, Communicable Disease Control Directorate, WA Department of Health
   - Prof Soren Alexandersen, Director, Geelong Centre for Emerging Infectious Diseases, a collaboration of Deakin University, Barwon Health/University Hospital Geelong and Australian Animal Health Laboratory (AAHL), CSIRO
   - Dr James Watson, Veterinary Investigation Leader, AAHL, CSIRO
   - Dr Frank Wong, Agent Characterisation Research Team Leader, AAHL, CSIRO
   - Prof Kanta Subbarao, Director, WHO Collaborating Centre for Reference and Research on Influenza (WHO CCRI), The Peter Doherty Institute for Infection and Immunity
   - Prof Soren Alexandersen, Director, Geelong Centre for Emerging Infectious Diseases, a collaboration of Deakin University, Barwon Health/University Hospital Geelong and Australian Animal Health Laboratory (AAHL), CSIRO
   - Dr James Watson, Veterinary Investigation Leader, AAHL, CSIRO
   - Dr Frank Wong, Agent Characterisation Research Team Leader, AAHL, CSIRO
   - Prof Kanta Subbarao, Director, WHO Collaborating Centre for Reference and Research on Influenza (WHO CCRI), The Peter Doherty Institute for Infection and Immunity
   - A/Prof Sheena Sullivan, Senior Epidemiologist, WHO Collaborating Centre for Reference and Research on Influenza, The Peter Doherty Institute for Infection and Immunity

   Research Funder: This research is funded by the Australian Partnership for Preparedness Research on Infectious Disease Emergencies (APPRISE).

3. Inclusion/Exclusion Criteria
   If you are considering participation in this research project, we need to ensure that it is ok for you to take part. The research study is looking to recruit people who meet one of the following criteria:
   - Is affiliated with/spokesperson for the following animal production industry bodies
     - Australian Pork Ltd
     - Australian Chicken Meat Federation Inc.
     - Australian Eggs Limited
     - AgriFutures Australia
   - Is an official for a trade union with coverage in intensive pig/poultry production/processing industries
   - Is affiliated with a federal/state government agency or is an academic working in
     - Animal health
     - Public health
     - One health
4. **Do I have to take part in this research study?**
   Participation in this research is voluntary. If you decide to take part and change your mind later, you are free to withdraw from the project at any stage. If you consent to participate, you may withdraw at any time. You can do so by writing to the research team and telling them you no longer want to participate. If you decide to leave the research study, the researchers will not collect additional information from you. Your decision not to participate will not affect your relationship with APPRISE.

5. **What does participation in this research require, and are there any risks involved?**
   If you decide to take part in the research study, we will ask you to complete an online questionnaire or face-to-face/phone interview. We will ask you questions about your views on the context, motivations, and challenges of conducting surveillance among people working at the animal-human interface in your industry. We will also seek your opinion on the most appropriate way to reduce the threat of emergent zoonotic respiratory infections at the animal-human interface. The questionnaire/interview will take approximately 45 min to complete. If you provide permission to do so, audio of your interview will be recorded.

   We do not anticipate this questionnaire will cause any harm. You are free to withdraw from the research at any time. If you withdraw from the research we will destroy any information you provided that has already been collected.

6. **What are the possible benefits to participation?**
   We hope the information we collect in this study will lead to improved dialogue, collaboration and trust between key stakeholders, including livestock industries, trade unions, animal health and public health. We envisage that improved inter-sectoral collaboration will lead to the future development of protocols for monitoring, early detection and response to emerging zoonotic respiratory diseases at the animal-human interface. Agreed-upon plans for a coordinated, multi-sectoral response to a novel zoonotic disease outbreak of public health concern should mitigate unpredictable impacts on trade and therefore employment.

7. **What will happen to information about me?**
   We will store information about you in an identifiable format, unless you request it be anonymised. Your responses will contribute to a final report that will be shared with APPRISE, the funding body. A report based on the findings of this study will also form the basis of a MPhil thesis chapter that the student investigator will submit to ANU. If you chose to participate as a non-identified respondent, all information included in the report will appear in a way that will not identify you.

   We will store transcribed interview responses and audio files on a secure server at WHO CCRRI in Melbourne for a period of seven years after the date of report preparation in line with the ANU Code of Research Conduct. After this date electronic files will be destroyed unless further approval for data retention is obtained. If retained and approved for further use, data will continue to exist in the format you specified (identifiable/anonymised) for analysis and storage as per National Statement on the Ethical Conduct of Human Research.

8. **How and when will I find out what the results of the research study are?**
   If you would like to receive a copy of the final report, you can let the research team know by providing your email or postal address to the interviewer or in the online survey form. We will only use these details to send you the results of the research. We anticipate that the final report will be completed by December 2018.

9. **What should I do if I have further questions about my involvement in the research study?**
   If you want any further information concerning this project or if you have any problems related to your involvement in the project, you can contact the following member/s of the research team:
Research Team Contact Details

<table>
<thead>
<tr>
<th>Name</th>
<th>Prof David Smith</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position</td>
<td>Clinical Virologist</td>
</tr>
<tr>
<td></td>
<td>PathWest Laboratory Medicine</td>
</tr>
<tr>
<td></td>
<td>Western Australian Department of Health</td>
</tr>
<tr>
<td>Telephone</td>
<td>08 6383 4438</td>
</tr>
<tr>
<td>Email</td>
<td><a href="mailto:david.smith@health.wa.gov.au">david.smith@health.wa.gov.au</a></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name</th>
<th>Dr Ximena Tolosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position</td>
<td>Applied Epidemiology Scholar</td>
</tr>
<tr>
<td></td>
<td>National Centre for Epidemiology and Population Health</td>
</tr>
<tr>
<td></td>
<td>The Australian National University</td>
</tr>
<tr>
<td></td>
<td>Field placement: WHO Collaborating Centre for Reference and Research on Influenza</td>
</tr>
<tr>
<td></td>
<td>The Peter Doherty Institute for Infection and Immunity</td>
</tr>
<tr>
<td>Telephone</td>
<td>03 9342 9324</td>
</tr>
<tr>
<td>Email</td>
<td><a href="mailto:ximena.tolosa@anu.edu.au">ximena.tolosa@anu.edu.au</a></td>
</tr>
</tbody>
</table>

10. What if I have a complaint or any concerns about the research study?
If you have any complaints about any aspect of the project including, the way it is being conducted, then you may contact:

Complaints Contact

<table>
<thead>
<tr>
<th>Position</th>
<th>Human Research Ethics Coordinator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telephone</td>
<td>02 6125 3427</td>
</tr>
<tr>
<td>Email</td>
<td><a href="mailto:Human.Ethics.Officer@anu.edu.au">Human.Ethics.Officer@anu.edu.au</a></td>
</tr>
<tr>
<td>HC Reference Number</td>
<td>Protocol 2017/909</td>
</tr>
</tbody>
</table>

Consent Form

Declaration by the participant

☐ I agree to participate in this research project;
☐ I agree to be recorded for this purpose.
☐ I do NOT agree to be recorded

☐ I would like to receive a copy of the study results via email, I have provided my details below and ask that they be used for this purpose only;

Email Address: ______________________________

Record details of participant consent here

Name of Participant
(please print)
Name of Interviewer
Date of interview
5.B  Stakeholder questionnaires

Questionnaires were designed for each type of stakeholder. Questions asked to pig and poultry industry representatives, animal health and public health stakeholders are shown in Appendix 5.B.1 and questions asked to trade union representatives, in Appendix 5.B.2.

5.B.1  Questionnaire for industry, animal health and public health staff

Consent

PIC.
Welcome to the survey:

'Zoonotic respiratory disease surveillance at the animal-human interface:
A feasibility study'

We are interested in your views on the early detection of emergent zoonotic respiratory viruses of public health concern at the animal-human interface in Australia. This survey aims to canvass stakeholders’ views regarding enablers and barriers to surveillance of emergent zoonotic respiratory viruses in people that work with animals in intensive pig and poultry industries in Australia.

Regular monitoring of people occupationally exposed to animals is considered important because it could enable the early detection of emergent zoonotic respiratory viruses of public health concern. In addition, having an agreed-upon framework in place would provide clear guidelines on how each stakeholder would need to respond and communicate in the event of an outbreak of zoonotic respiratory disease of pandemic potential occurring at the animal-human interface.

Please refer to the participant information statement emailed to you for details on the research team, funding body (APPRISE), invited stakeholders, data use and storage, and contact details of investigators. If you would like to contact the study investigator to discuss this research, please email ximena.tolosa@anu.edu.au.

The survey should take you around 45 minutes to complete. Your participation is voluntary.

If you would like a copy of the final report, you will have the option to provide your email address in the final question of this survey.

We thank you very much for your participation.

☐ I consent, begin survey
☐ I do not consent, I do not wish to participate

STH: Please indicate your affiliation
Animal.Context

A.CTI. The burden swine influenza virus (SIV) in pigs in your state

Is swine influenza virus (SIV) regarded as a problem to the pig industry in your state?

If known, comment on what is the estimated prevalence of SIV in pigs in your state.

Would the response differ if an emergent unknown respiratory virus is detected as opposed to zoonotic flu?
A.CT2.
Once influenza virus in intensively produced pigs/poultry is notified what are the immediate actions taken by the state department of agriculture?

Please comment on the response to flu notification in pigs.

Please comment on the response to flu notification in poultry.

Please comment on what the consequences of these actions are for the pig producer.

Please comment on what the consequences of these actions are for the poultry producer.

A.CT3.
Under what circumstances are tests for influenza virus conducted among intensively produced pigs/poultry in your state?

Please comment on pigs vs. poultry

Where are specimens tested (private lab/state lab)?
A.CT4. Does the state department of agriculture monitor swine influenza virus (SIV) in pigs and their evolution?

- Yes
- Unsure
- No

In your opinion, would free flu testing for animals encourage industry to submit more specimens to state labs?

A.CT5. Is unconfirmed influenza-like-illness (ILI) in intensively produced pigs/poultry notified to the state department of agriculture?

Please comment on ILI in pigs.

Please comment on ILI in poultry.
A.C76. In your state, under the current practices, how would a novel zoonotic influenza virus become detected?

Please comment on detection of zoonotic influenza virus in pigs.

Please comment on detection of zoonotic influenza virus in poultry.

Please comment on the timeliness of this detection.

Animal.Views

A.VI.

Would surveillance of flu in pigs/poultry for evolution of viruses be beneficial?

Please comment on benefits for pig industry.

Please comment on benefits for poultry industry.
A.V2.
In your opinion what are the biggest obstacles to conducting surveillance of zoonotic respiratory viruses in animals and people that work with animals in intensive pig and poultry industries in Australia?

Please comment on obstacles for intensive pig industry.

Please comment on obstacles for intensive poultry industry.

A.V3.
How could surveillance of zoonotic respiratory viruses in animals and people that work with animals in intensively produced pigs/poultry be conducted without adversely impacting industry?

Please comment in relation to the pig industry.
A.V4.
Who should be responsible for coordinating surveillance efforts for emergent zoonotic respiratory viruses at the animal-human interface?

Please comment in relation to the poultry industry.

A.V5.
In the United States, the USDA National Surveillance Plan for Swine Influenza Monitoring is comprised of laboratory surveillance of pigs exhibiting influenza-like illness either on farms or in places of commingling such as auctions or fairs AND surveillance of pigs that are epidemiologically linked to a human with influenza A infection including humans with swine influenza virus (SIV) or pdmH1N1. Pig producers may choose to remain anonymous.
Would a similar monitoring program work in Australia? Please elaborate.

Animal.Working Together

_A.WT1._
Can you provide any general comments regarding how pig and poultry industries, unions, animal health and human public health professionals could work together to prevent a novel zoonotic respiratory virus with pandemic potential (such as flu) from emerging at the animal-human interface in your state?

Please comment on pig-human interface.

Please comment on poultry-human interface.

_A.WT2._
Do you have any general comments regarding how pig and poultry industries, unions, animal health and human public health professionals could work together to ensure the early detection of a novel zoonotic respiratory virus with pandemic potential (such as flu) emerging at the animal-human interface in your state?
A.WT3. Is there any reporting from human public health labs or from public health authorities to the state department of agriculture of confirmed or suspected zoonotic flu cases among pig/poultry workers including pig/poultry vets?

- If yes, please comment on the timeliness of this reporting.

A.WT4. In the event of a swine influenza (SIV) outbreak in pigs would the investigating vet ask about influenza-like illness (ILI) among any persons in contact with the pigs?

Please comment on outbreak being investigated by a government vet.

Please comment on outbreak being investigated by an industry vet.

A.WT5. Is there anything the state could do, or is currently doing, to reduce the risk of interspecies transmission (animal<>human) of zoonotic influenza virus or other emergent zoonotic respiratory virus of pandemic potential?

Please comment on infection going from human to pig.
If it was demonstrated that immunisation of pig/poultry workers against the seasonal flu could help prevent a new zoonotic influenza virus emerging from an Australian herd/flock would the state department of agriculture support a campaign to promote seasonal flu vaccination among pig/poultry workers including vets?

Please comment on what you think would be the challenges to establishing seasonal flu vaccination among pig workers.

Please comment on what you think would be the challenges to establishing seasonal flu vaccination among poultry workers.
A.WT7.
In your opinion, what needs to be done in advance organisationally and politically to achieve effective cross-sectoral* collaboration so that a framework to prevent, detect and mitigate emergent zoonotic respiratory viruses at the animal-human interface can be devised?

*industry, unions, animal health and human public health community.

PH.Context

PH.CT1. In your state how much of a threat is zoonotic influenza to human health?

Please comment on threat of swine flu.

Please comment on threat of avian flu.

Is the burden of zoonotic respiratory disease known for people that work with pigs in Australia?

Is the burden of zoonotic respiratory disease known (i.e., unknown) for people that work with poultry in Australia?

PH.CT2. In the event of a case of human infection with a zoonotic influenza virus in a person occupationally exposed to animals, what would the public health response be?
Please comment on response in the event of human infection with *swine flu*.

<table>
<thead>
<tr>
<th>Please comment on response in the event of human infection with <em>avian flu</em>.</th>
</tr>
</thead>
</table>

Please comment if the pigs/poultry in contact with the infected person would be sampled and tested for influenza viruses.

<table>
<thead>
<tr>
<th>Please comment on what <strong>legal or regulatory tools</strong> are available to public health officials to test pigs/poultry.</th>
</tr>
</thead>
</table>

Please comment on the circumstances under which legal/regulatory tools could be used.

<table>
<thead>
<tr>
<th>Would the response differ if an <em>emergent novel respiratory virus</em> is detected in an animal worker as opposed to zoonotic flu?</th>
</tr>
</thead>
</table>

**PH.CT3.** In your state, under the current practices, how would a human infection with a novel zoonotic respiratory virus become detected?

Please comment on detection of *zoonotic influenza virus* in humans.

<table>
<thead>
<tr>
<th>Please comment on detection of <em>emergent unknown respiratory virus</em> in humans.</th>
</tr>
</thead>
</table>
**Prevention of interspecies transmission of zoonotic respiratory viruses.**

- Are there specific programs to encourage pig/poultry workers and their families to be vaccinated with seasonal flu vaccine?

- Are there any (other) programs aimed at prevention of interspecies transmission of respiratory viruses in your state?

- Are temporary foreign workers in intensive animal industry (incl. abattoir workers) eligible for these programs?

**Surveillance for emergent zoonotic respiratory viruses.**

- Is the question about occupational exposure/animal contact part of routine influenza-like illness (ILI) surveillance?

- In your opinion, how good is the ability of your state public health laboratory to detect a novel zoonotic influenza A or an emergent zoonotic respiratory virus in a human?

**Inter-agency communications:**
If the state department of agriculture were to identify an unusual influenza virus strain in intensively produced pigs/poultry would human public health officials be alerted? If possible, please use examples of such events when answering these questions.

Please comment on pigs vs poultry.

If yes, how would these unusual findings be communicated?

What is your opinion regarding the timeliness of initial animal to human health agency communications?

What is your opinion regarding the openness of these communications?

Does the Chief Medical Officer have regular contact with the Chief Veterinary Officer of your state regarding zoonotic diseases?

Do human health labs and animal labs
Would surveillance of flu in pigs/poultry be beneficial to surveillance of influenza virus in humans?

Please comment on benefits of flu surveillance in pigs.

Please comment on benefits of flu surveillance in poultry.

If beneficial, then how could flu surveillance in pigs be conducted to maximize the benefit?

If beneficial, then how could flu surveillance in poultry be conducted to maximize the benefit?

In your opinion what are the biggest obstacles to conducting surveillance of zoonotic respiratory viruses in animals and people that work with animals in intensive pig and poultry industries in Australia?
PH.V3.
How could surveillance of zoonotic respiratory viruses in animals and people that work with animals in intensively produced pigs/poultry be conducted without adversely impacting industry?

Please comment on obstacles for intensive pig industry.

Please comment on obstacles for intensive poultry industry.

PH.V4.
Who should be responsible for coordinating surveillance efforts for emergent zoonotic respiratory viruses at the animal-human interface?

Please comment in relation to the pig industry.

Please comment in relation to the poultry industry.

Please comment on who should conduct this surveillance.

Please comment who should be responsible for the cost.
What are your views regarding the need for a surveillance system targeting people that work with animals in the intensive pig and poultry industries for emergent zoonotic respiratory viruses?

Please comment in relation to the pig industry.

Please comment in relation to the poultry industry.

In your opinion, what would be the most beneficial form of surveillance for emergent zoonotic respiratory viruses. Please rank the options below by moving each block to position 1, 2 or 3.

To strengthen current human influenza surveillance systems in Australia to ensure they include high-risk groups such as people that work with animals in the intensive pig and poultry industries AND ensure that lab capacity to timely detect novel influenza A subtypes exists.

To create, in collaboration with stakeholders (animal industries, unions, animal health and public health officials), a new surveillance system targeting people that work with animals in the intensive pig and poultry industries.

Another option. Please elaborate
PH. Working Together

PH.WT1.
Can you provide any general comments regarding how pig and poultry industries, unions, animal health and human public health professionals could work together to prevent a novel zoonotic respiratory virus with pandemic potential (such as flu) from emerging at the animal-human interface in your state?

Please comment on pig-human interface.

Please comment on poultry-human interface.

PH.WT2.
Do you have any general comments regarding how pig and poultry industries, unions, animal health and human public health professionals could work together to ensure the early detection of a novel zoonotic respiratory virus with pandemic potential (such as flu) emerging at the animal-human interface in your state?

PH.WT3. Is there any reporting from human public health labs or from public health authorities to the state department of agriculture of confirmed or suspected zoonotic flu cases among...
pig/poultry workers, including pig/poultry vets? If yes, please comment on the timeliness of this reporting.

\[PH.WT4.\]

Does the state department of agriculture report to public health authorities when there is an outbreak of swine influenza virus in pigs?

\[PH.WT5.\]

Is there anything the state could do, or is currently doing, to reduce the risk of inter-species transmission (animal<>human) of zoonotic influenza virus or other emergent zoonotic respiratory viruses of pandemic potential?

Please comment on infection going from pig to human.

Please comment on infection going from human to pig.

Please comment on infection going from poultry to human.

Please comment on infection going from human to poultry.
In your opinion, what needs to be done in advance organisationally and politically to achieve effective cross-sectoral* collaboration so that a framework to prevent, detect and mitigate emergent zoonotic respiratory viruses at the animal-human interface can be devised?

*industry, unions, animal health and human public health community.

Future Workshop

WS1.
Would you be interested in participating in a workshop to discuss the context, drivers, obstacles, and usefulness of conducting surveillance of people working at the animal-human interface with other relevant stakeholders (intensive pig/poultry industry, trade unions, government and academics)?

○ Yes
○ Maybe
○ No

WS2.
What conditions would need to be met to facilitate your participation in such workshop (i.e., finances for travel, etc.)?

WS3.
Can you indicate your preference regarding the location and timing of such workshop, for example, before/after an industry meeting or conference?
Please provide names of suitable meeting/conferences and dates.

Final question

F1. Is there anything else that you would like to add that has not been covered by these questions? Are there any other persons that you suggest that I contact?

F2. If you would you like a copy of the final report please add your email address below.
5.B.2 Questionnaire for trade union representatives

You are invited to take part in this research study. The study aims to canvass stakeholders’ views regarding enablers and barriers to surveillance of emergent zoonotic respiratory viruses in people that work with animals in intensive pig and poultry industries in Australia. Regular monitoring of people that work with animals is considered important because it could enable the early detection of emergent zoonotic respiratory viruses of public health concern. In addition, having an agreed-upon framework in place on how each stakeholder would need to respond and communicate in the event of an outbreak of zoonotic respiratory disease of pandemic potential at the animal-human interface would provide clear guidelines to stakeholders while reducing negative impact of such event.

You have been invited because your views are important to help us shape the future of surveillance for the early detection of emergent zoonotic respiratory diseases at the animal-human interface in Australia.

The ethical aspects of this research have been approved by the ANU Human Research Ethics Committee (Protocol 2017/909).

Goals

The goals of this questionnaire are:

- To understand union coverage of people working in intensive pig and poultry industries in Australia, both on farms and abattoirs
- To understand the current state of surveillance for zoonotic respiratory diseases in people that work in pig and poultry farm and abattoirs
- To seek your opinion regarding surveillance of abattoir workers for zoonotic respiratory diseases, factors that would enable it and critical obstacles
- To understand the level of healthcare available to abattoir workers and abattoirs

It is hoped that the information provided will form the basis for knowledge, trust, dialogue, collaboration and future planning among stakeholders to ensure that Australia has protocols in place to prevent, detect and respond to an outbreak of a zoonotic respiratory virus of public health concern at the animal-human interface.

Coverage and Strength of Union Membership

1. Does your union have coverage in:
(a) Large pig farms?
□ Yes
□ No
(b) If not, which unions have coverage on large pig farms?

(c) Large poultry farms?
□ Yes
□ No
(d) If not, which unions have coverage on large poultry farms?

(e) In abattoirs that slaughter pigs?
□ Yes
□ No
(f) If not, which unions cover workers on large pig abattoirs?

(g) In abattoirs that slaughter poultry?
□ Yes
□ No
(h) If not, which unions cover workers on large poultry abattoirs?

2. What are your views regarding the strength of union membership among people that work in large pig and poultry farms in Australia?
3. Are you aware of where are the abattoirs that process the largest numbers of pigs per day?

**Zoonotic disease surveillance in abattoirs**

1. Are you aware of any system or program that monitors the health of people that work in abattoirs that slaughter pigs and poultry for zoonotic respiratory diseases or other zoonotic diseases?
2. Are you aware of any measures in place at abattoirs to reduce the threat of zoonotic respiratory diseases to workers?
3. What (further) measures could be taken to reduce the threat of zoonotic respiratory viruses and protect the health of workers?

4. What can industry and government agencies do to help protect the health of workers from the threat of zoonotic respiratory infections?

5. In your experience what has been the response from public health authorities to cases of zoonotic disease in an abattoir worker?

6. If there were public health responses, what has been the impact on workers and on the abattoir management?

7. What public health response would be the most appropriate in the event of a pig/poultry abattoir worker becoming infected with a known strain of pig/avian flu?

8. What public health response would be the most appropriate in the event of workers becoming infected with a new strain of influenza or an unknown zoonotic respiratory disease of public health concern?

9. Given that there is no information regarding the burden of disease in people that work with pigs/poultry in Australia, would the union support an initial study to quantify this burden among abattoir workers?

10. In your opinion, would the union support ongoing surveillance of abattoir/farm workers for zoonotic respiratory disease? (surveillance could involve testing of ‘healthy’ people (collecting nasopharyngeal swabs, and/or blood specimens) at regular intervals and or testing people showing influenza-like illness (ILI), and/or testing of people in direct contact with infected pigs/poultry (i.e., pigs/poultry diagnosed with respiratory illness)

11. What conditions would need to exist for the union to support ongoing health surveillance of abattoir/farm workers?

12. In your opinion, what would be the challenges (or ethical concerns) in conducting health surveillance of workers?

13. Are there any other considerations that have not been covered by these questions?

14. Are there other unions or persons that you think I should talk to?

**Workers’ access to healthcare**

1. In your experience, do most abattoir/farm workers have ready access to healthcare services if they should become ill?
2. If your organisation represents workers with foreign backgrounds and/or those on special visas that may not have Medicare, would these workers typically have ready access to healthcare services if they became ill?

3. In your opinion, would workers be able to report an influenza-like illness that they thought might be related to their work to management?

4. Do you think there would be negative repercussions if a worker reported a potentially work-related respiratory illness to management? i.e. time-off work without pay or other unfavourable consequences?

5. In your opinion, if annual influenza vaccination for workers was recommended as a measure to mitigate the emergence of a zoonotic influenza strain of public health concern, do you foresee any problems in accepting or implementing the recommendation on the part of the workforce and/or management? [this question maybe more relevant for farm workers].

Workshop questions

1. Would you be interested in participating in a workshop to discuss the context, drivers, constraints, and usefulness of conducting surveillance of people working at the animal-human interface with other relevant stakeholders (intensive pig and poultry industries, government and academics from the animal and public health fields)?

2. What conditions would need to be met to facilitate your participation in such workshop (i.e., finances for travel, etc.)?

3. Can you indicate your preference regarding the timing and location of such workshop?
5.C Proposed protocol for surveillance of people working in the intensive pig and poultry production and processing industries in Australia

We propose an active workplace-based and laboratory-based sentinel surveillance system managed by a One Health trans-disciplinary agency with a coordinator in each jurisdiction. The design of this system aims to capture events in which novel influenza strains are transmitted from pigs or poultry into humans as well as influenza reassortant strains. This surveillance would fill current gaps in the national sentinel ILI surveillance system which has not been designed to capture influenza at the animal-human interface, i.e., occupational animal exposure is not recorded nor are positive influenza patients followed up to seek animal exposure information.

Surveillance of emerging zoonotic influenza at the animal-human interface will help establish, and in some cases strengthen, partnerships between animal industries, labour, occupational health and safety, animal health and public health stakeholders. These partnerships are expected to contribute to timely and effective outbreak investigation, the implementation of robust prevention and control measures and crafting clear messages to address public concern. Additionally, this system would have the ability to provide candidate viruses for pandemic vaccine production.

Definition of intensive pig and poultry industries

We define intensive piggeries as enterprises with more than 1,000 pigs which maintain close herds, use artificial insemination and market directly to abattoirs. This are the characteristics of the few enterprises that hold the majority of Australia’s pig population. Notably, there are two enterprises, in New South Wales and South Australia, that hold more than 10,000 pigs (71).

We define intensive poultry production enterprises as those with more than 20,000 birds per shed. A report on the structure of the Australian poultry industry published in 2009 indicated that establishments with multiple sheds housing 50,000 birds each were becoming more common (72).
5.C.1 Aim

The aim of this protocol is to achieve the early detection of zoonotic influenza spillover events associated with occupational exposure to intensively produced pigs and poultry. The proposed protocol has the following objectives:

- To monitor people occupationally exposed to intensively produced pigs and poultry for potential zoonotic influenza spillover events in Australia.
- To assist in developing an understanding of the epidemiology of zoonotic influenza viruses at the animal-human interface in Australia in order to identify risk factors and prevent transmission of a potential influenza pandemic strain to humans.
- To describe the antigenic and genetic characteristics of influenza viruses circulating at the animal-human interface in Australia.

5.C.2 Description of the proposed surveillance system

The flow chart in Figure 5.2 shows the main actors, steps in data collection and the data flow for the proposed surveillance system. We propose obtaining respiratory and conjunctival specimens as well as demographic data through the following mechanism. Participating pig and poultry production and processing enterprises in every jurisdiction (see Appendix 5.C.3 for selection criteria) will be reached by a mobile team at frequency to be established. In each jurisdiction, a team consisting of a minimum of two people will collect specimens from workers with and without symptoms of conjunctivitis or respiratory infection and complete a report form. Mobile teams will be part of a newly formed trans-disciplinary One Health agency with representation at jurisdictional and national level. Specimens are shipped to the selected laboratory. Laboratories would report test results to the state One Health surveillance coordinator. The latter analyses virological and demographic data and forwards it to the Commonwealth One Health surveillance coordinator who is responsible for data consolidation, analysis, interpretation and reporting to stakeholders and decision makers. Individual test results are communicated to workers –observing confidentiality principles– by a member of the mobile surveillance team.

We propose an alternative method of specimen collection to mobile teams. This would involve recruiting workplace medical clinics –when they exist– and delegating to them the responsibility of specimen collection and shipment to laboratories. A further variation to this protocol involves recruiting GPs in areas where the intensive
industries are present, usually regional towns (see Table 5.3 for examples of towns where very few GPs serve a small population where very large pig and poultry abattoirs are located).

![Flowchart for the proposed surveillance system of people occupationally exposed to intensively produced pigs and poultry in Australia.](image)

**Figure 5.2**: Flowchart for the proposed surveillance system of people occupationally exposed to intensively produced pigs and poultry in Australia. Notes: *Refers to workers in production or processing facilities. **Stakeholders include pig and poultry peak industry bodies, labour force, occupational health and safety regulators, industry veterinarians, government veterinarians and public health officers.

### 5.C.3 Sampling strategy

**Where to sample:** Select sentinel intensive pig and poultry (chicken meat and egg) production and processing enterprises ('farm' or abattoir) according to the criteria below.

**Production establishments**

- has the largest number of animals in the jurisdiction
- has very high animal stocking densities
Chapter 5

- piggery (of any size) is located near intensive poultry production establishment and vice versa
- establishment (of any size) is located near wetlands
- establishment (of any size) has free ranging animals

Processing establishments

- slaughters the highest volume of pigs/poultry in the jurisdiction per week

To be established: the number of intensive production establishments to be sampled and how many of the above criteria need to be met must be established with expert trans-disciplinary stakeholder input. Consensus is also needed regarding the definition of high stocking density in the pig and poultry production contexts. We propose piloting the system in one poultry and one pig production and processing site per jurisdiction and expand to more sites once the system is functioning well (73).

How many people to sample: Select at least 10 people per workplace (refer to the section ‘Who to sample’ below for details on how to select workers for testing). If the workforce in contact with animals is less than 10 people –a common characteristic of intensive animal industries (72, 71) – attempt to obtain specimens from each of them.

To be established: Through our stakeholder engagement we learned that the number of people employed in intensive production and processing of pigs and poultry is generally small (i.e, 10 people or less perform animal husbandry activities on ‘farms’ and a similarly small number of people work –in shifts– in the slaughtering and evisceration of animals in Australia’s largest abattoirs, which can process up to three million chickens per week and several thousand pigs per day). However, these numbers need to be corroborated and the number of people to sample on animal production establishments and abattoirs must be established with input from experts.

Who to sample: Collect specimens from people that work in close contact with live animals for at least one hour per day (61). In abattoirs include people that are involved in slaughter, evisceration, de-feathering and other procedures that generate bio-aerosols that could contain influenza viruses (74). Include veterinarians and animal transporters.

A mix of workers displaying ILI and/or conjunctivitis as well as workers who are not displaying any symptoms of respiratory or eye infection should be sampled. Consent for specimen collection should be sought.
Important considerations: It is important to include foreign workers on seasonal contracts and people contracted through labour hire companies. The US CDC warns that these workers are difficult to reach for several reasons due to strategies used by management to block their participation in surveillance programs but reminds epidemiologists that these workers may be at the highest risk of occupational illness (75). In fact, our stakeholder engagement (i.e., poultry industry and unions) and other sources (76, 65) indicated that workers engaged by labour hire companies are not guaranteed access to PPE nor influenza immunisation as neither the workplace management nor the labour hire company assume responsibility for its provision. In contrast, workers employed by large companies directly have access to PPE and, depending on the policy of each establishment, to free influenza vaccines.

**Case definition:** Person occupationally exposed to intensively produced pigs and poultry experiencing conjunctivitis (with or without rhinorrhea or sore throat) OR influenza-like illness (defined as fever with cough and fatigue) (42, 35).

To be established: The inclusion of symptoms such as sore throat, runny or stuffy nose, muscle or body aches and headache needs to be considered.

**When to sample:** Collect specimens from selected workers once during the influenza season as a minimum. The timing of the visit could be either randomly selected or could –if the necessary warning is received by the mobile team– coincide with a recent ILI event in animals (60, 61).

To be established: The frequency at which selected workplaces are to be sampled must be established.

**Sampling logistics:** Due to the likely large distances between intensive animal production/processing establishments and public health units we propose that a mobile workforce visits the selected establishments. A similar type of occupational surveillance is in place in the United States, managed by the US CDC National Institute for Occupational Safety and Health, to monitor the health of mine workers and study the causes and effects of respiratory diseases related to coal mine dust exposure (77). For on site specimen collection, management cooperation from production and processing facilities would be needed to accommodate specimen collection with production schedules.

As an alternative to mobile teams we propose that establishments that have their own medical clinics (our stakeholder engagement revealed that very large pig and
poultry abattoirs have nurses or physicians on site) take responsibility for specimen collection and shipping. Another option is to train Health and Safety (workplace) Representatives (HSR) in each selected establishment to identify workers for sampling, collect specimens and ship them to the appropriate laboratory. Negotiation with management to obtain approval for the site clinic or HSR worker to conduct those activities would be needed.

A less costly alternative to the above could be to recruit GPs in areas where large pig and poultry production and/or processing establishments are located for participation in the proposed surveillance system.

**Who should collect specimens:** We propose that the mobile workforce, part of a newly formed trans-disciplinary One Health government agency, would be responsible for specimen collection and transportation. Other alternatives include utilising workplace clinic staff (if available), HSR workers or community GPs.

**What type of specimen to collect:** Members of the mobile team collect conjunctival (if worker is displaying conjunctivitis) and nasopharyngeal swabs of selected workers and transport or ship them to the closest laboratory capable of conducting molecular testing for influenza A viruses. Specimen collection, transportation and storage conditions and initial screening tests should be done as per the World Health Organization recommendations (73).

**What data to collect:** Demographic information should be collected in a case report form from each worker that consents to participate. As a minimum the following data should be collected from each worker: unique identifier, sex, date of birth, job type (i.e., piggery/poultry shed/abattoir), employment type (i.e., directly employed by the company, employed through labour hire or foreign seasonal worker), workplace location, history of conjunctivitis and/or ILI in previous 10 days, date of specimen collection and presence of chronic conditions. If the option of enhancing the current ILI surveillance system is chosen, extra fields that need to be collected are contact with animals through work or otherwise, employment details and recent ILI symptoms.

**What laboratory tests to perform:** Initial screening for influenza A viruses by real time reverse transcription polymerase chain reaction (RT PCR) assay is performed at the designated laboratory. Positive specimens are referred to the WHO
Collaborating Centre for Reference and Research on Influenza (WHOCC) and/or the Australian Animal Health Laboratory (AAHL) for virus characterisation. Virus isolation, genetic (i.e., whole genome sequencing) and antigenic characterisation of virus isolates to identify novel zoonotic influenza strains to be performed at WHOCC/AAHL (60, 61).

5.C.4 Data collection and reporting
A case report form must be completed at the same time a specimen is collected from a worker. The frequency of data analysis would depend on how often the mobile teams collect data and specimens from workplaces (if data collection is done once during the influenza season reporting could be done at the end of the season). Laboratories should report test results to the state One Health surveillance coordinator who estimates:

- the proportion of specimens that test positive for zoonotic influenza per jurisdiction: overall, by industry, workplace type (animal production/processing facility), age and sex.
- the clinical characteristics of laboratory-confirmed zoonotic influenza cases.
- the antigenic and genetic characteristics of zoonotic influenza viruses circulating in people occupationally exposed to pigs/poultry.

These reports are sent to the Commonwealth One Health surveillance coordinator for the calculation of the proportion of influenza positive tests nationally overall and disaggregated as above. Individual results should be reported to each worker by the state One Health surveillance coordinator as soon as available. Counselling of workers when communicating results could be done by a member of the mobile team. The content of messages to workers needs to be carefully considered.

What would trigger a response
The following scenarios would merit an outbreak investigation.

- A detection of a zoonotic influenza strain associated with illness in a worker.
- An increase in the number of workers at one sentinel site that test positive for zoonotic influenza above a certain level (with or without associated illness). This threshold will be determined once sufficient information had been collected by the system.

How an outbreak investigation should be carried out is beyond the scope of this chapter. A draft protocol for outbreak response to a spillover event of zoonotic influenza is being developed by our APPRISE partners.
Detection of novel influenza strains in humans should be immediately reported to the World Health Organization (WHO) through official channels (i.e., the Commonwealth Department of Health) in line with the International Health Regulations (2005) (47).

5.C.5 Surveillance attributes of the proposed system

The attributes that the proposed surveillance system would seek to achieve are listed in Table 5.4. Attributes assessed were based on the US Centres for Disease Control Updated Guidelines for Evaluating Public Health Surveillance Systems (2001) (57).

Table 5.4: Attributes of the proposed surveillance system and description of how the system would seek to address each attribute

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acceptability</strong></td>
<td>The proposed system is not currently acceptable to animal production industry stakeholders. This stakeholder would consider implementing the system and participating in surveillance if there is an assurance that their commercial interests would be protected if a spillover event is detected. A surveillance system that benefits all stakeholders and is financed in an way that is deemed fair would meet the acceptability attribute.</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>For the system to be able to detect all spillover events quickly, the selected focal point in each workplace must notify the One Health mobile team as soon as a worker displays ILI. The team would then visit the facility and obtain specimens and complete the report form. A pre-requisite for this attribute to be met, is a relationship of trust and clear agreements between all stakeholders.</td>
</tr>
<tr>
<td><strong>Timeliness</strong></td>
<td>The system would meet the timeless requirement by:</td>
</tr>
<tr>
<td></td>
<td>- Focal points are willing and able to recognise an ILI in a worker and quickly contact the One Health team.</td>
</tr>
<tr>
<td></td>
<td>- One Health team deploy to the facility as soon as they receive a call, collect and transport specimens to the lab quickly.</td>
</tr>
<tr>
<td></td>
<td>- The lab tests the specimens as soon as they receive them.</td>
</tr>
<tr>
<td></td>
<td>- Positive results are acted upon without delay (the specific actions involved in this step need careful consideration).</td>
</tr>
</tbody>
</table>
Table 5.4 continued from previous page

Usefulness
The proposed system is useful because it would avert a potentially serious epidemic. Specifically, the system’s usefulness would be demonstrated by:
- Detecting zoonotic influenza spillover events.
- Improve our understanding of the epidemiology of zoonotic influenza which is not currently under surveillance anywhere in Australia.
- Monitoring the genetic and antigenic characteristics of zoonotic influenza.
- Providing first-time estimates of the magnitude of morbidity and mortality associated with zoonotic influenza.
- Stimulating epidemiologic and scientific research leading to prevention strategies
- Raising awareness among clinicians and workers in relevant industries of zoonotic influenza.
- Improving on farm biosecurity to reduce transmission of zoonotic influenza from humans to animals.

5.C.6 Important considerations

Aspects of surveillance that need to be embedded in the system and worked through during a future cross-sectoral consultation process include the following:

- Confidentiality: To ensure workers are not disadvantaged in the event of a positive test result. Experience from the United States indicates that workplace management sometimes resists protecting workers’ confidentiality (75).

- Communication: In case of a positive test result the content of communications to the affected worker needs to be agreed upon and expectations of compensation and action to reduce further exposure need to be considered. It is essential that in the event of a detection of a spillover event of potential public health significance the Department of Health, the Department of Agriculture, the OHS agency and other relevant stakeholders craft a clear and consistent message to the public regarding the safety of meat and egg products for human consumption and release it simultaneously across the country.
5.D Oral presentation at the Influenza Centre, November 2018

I presented the results of the stakeholder engagement to my colleagues at the Influenza Centre on 29 November 2018. The slides are shown below.

Influenza sero-surveillance at the animal-human interface: a feasibility study in high-risk groups

Ximena Tolosa, MAE scholar
29 November 2018
WHO Collaborating Centre for Reference and Research on Influenza

Outline
- Justification
- My responsibilities
- Results of s/holder engagement
- Are other countries conducting surveillance in occupationally exposed to animals at A-H interface?
- Drafting a protocol for Australian intensive pig/poultry industry context
- Next steps
Influenza sero-surveillance at the animal-human interface

**Phase 1**
- engage stakeholders to obtain views on drivers & obstacles to conducting surveillance of workers
- assessment of existing protocols for investigation of respiratory disease outbreaks at the animal-human interface and development of a draft protocol for Au

**Phase 2**
- a workshop with stakeholders to assess the draft protocol and determine the feasibility of ongoing, systematic surveillance of workers
- conduct pilot sera collection in VIC, WA & QLD

---

**Research team**

- **Prof David Smith**, WA PathWest
- **A/Prof Paul Effler**, WA DoH
- **Dr Frank Wong**, AAHL
- **Prof Marion Koopmans**, Erasmus Medical Centre, The Netherlands
- **Prof Soren Alexandersen**, Geelong Centre for Emerging Infectious Diseases
- **Ximena Tolosa**, A/Prof Sheena Sullivan, Prof Kanta Subbarao, WHO CCRRI
What is public health surveillance?

Public Health Surveillance is...

‘The ongoing systematic collection, analysis and interpretation of health-related data essential to the planning, implementation, and evaluation of public health practice, closely integrated with the timely dissemination of those data to those who need to know. The final link in the surveillance chain is the application of these data to prevention and control’

Centres for Disease Control and Prevention
Principles and Practice of Public Health Surveillance, Lee et al. 2010

Importance of surveillance at animal-human interface

- Zoonotic infections in in food animals: evidence from developing countries overwhelmingly but also from Au, Europe, US
- Wong et al. 2018 debunked myth Australian pigs are flu-free
  - IV isolated from pigs in 2 Au states
  - Flu viruses circulating undetected in pigs for decades
Importance of surveillance at AH interface

Evidence of antibodies to flu A in feral pigs living near wetlands in SA, 2014 (3/23=13%)
H type not ascertained

Proposed Surveillance for Influenza A in Feral Pigs

Antonia E. Dalziel,1 Heidi A. Peck,2 Aoron C. Hure,2 Julie Cooke,2 and Phillip Casey3

1Barham Laboratories, School of Biological Sciences, University of Adelaide, Adelaide, SA, 5005, Australia
2EcoHealth Association/WHO Collaborating Centre for Reference and Research on Influenza, UEWH, at the Peter Doherty Institute, Melbourne, VIC, 3010, Australia
3Diagnostic and Surveillance Response Laboratory, Australian Animal Health Laboratory, CSIRO (Commonwealth Scientific and Industrial Research Organisation), Geelong, VIC, 3216, Australia

Outbreaks of HPAI in commercial poultry

<table>
<thead>
<tr>
<th>Type</th>
<th>State</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>H7N7</td>
<td>VIC</td>
<td>1976</td>
</tr>
<tr>
<td>H7N7</td>
<td>VIC</td>
<td>1985</td>
</tr>
<tr>
<td>H7N3</td>
<td>VIC</td>
<td>1992</td>
</tr>
<tr>
<td>H7N3</td>
<td>QLD</td>
<td>1994</td>
</tr>
<tr>
<td>H7N4</td>
<td>NSW</td>
<td>1997</td>
</tr>
<tr>
<td>H7N7</td>
<td>NSW</td>
<td>2012*</td>
</tr>
<tr>
<td>H7N2</td>
<td>NSW</td>
<td>2013*</td>
</tr>
</tbody>
</table>

*Detections of LPAI in commercial poultry

<table>
<thead>
<tr>
<th>Type</th>
<th>State</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>H4N8</td>
<td>VIC</td>
<td>1994</td>
</tr>
<tr>
<td>H5</td>
<td>TAS</td>
<td>2006</td>
</tr>
<tr>
<td>H6N4</td>
<td>QLD</td>
<td>2006</td>
</tr>
<tr>
<td>H6N4</td>
<td>NSW</td>
<td>2006</td>
</tr>
<tr>
<td>H10N7</td>
<td>NSW</td>
<td>2010</td>
</tr>
<tr>
<td>H5N3</td>
<td>VIC</td>
<td>2012*</td>
</tr>
<tr>
<td>H9N2</td>
<td>NSW</td>
<td>2012</td>
</tr>
<tr>
<td>H4N6</td>
<td>NSW</td>
<td>2012</td>
</tr>
<tr>
<td>H10N7</td>
<td>QLD</td>
<td>2012</td>
</tr>
<tr>
<td>H5N3</td>
<td>WA</td>
<td>2013*</td>
</tr>
</tbody>
</table>

*Free range
Suspected contact w/ water birds

LPAI also isolated or detected (several subtypes)

Documented transmission to poultry abattoir workers

Importance of surveillance at AH interface

Evidence of influenza A in Australian
• Domestic pigs
• Feral pigs – small study
• Poultry

Experts pointing need of surveillance in animals and people working with animals for decades

Pop size  
- Domestic pigs: 1.4 M
- Feral pigs: 164 M
- Poultry: 24 M

Source: ABS, Sep 2018; BQ, Apr 2018

Importance of surveillance at the AH interface


- The animal health-human health link getting research attention since 1970s (Linton 1977)
- HK pig abattoir study – Ma et al. 2011
- Need for surveillance of people at the interface
- Global efforts in pandemic influenza preparedness
Why is this not already in place anywhere in the world?

- Commercial sensitivities – intensive animal industries
- Catastrophic financial impact of outbreak responses
- Flu in pig & poultry in Australia is notifiable ► animal emergency disease (AUSVETPLAN) ► destroy-dispose-decontaminate-wait to restock
- Complexities of trans-disciplinary, multi-sector collaboration – limited resources, competing priorities, trust issues, coordination & leadership challenges

Our study: Stakeholder engagement - Methods

Questionnaire design
- Face to face
- Phone
- Online survey

Participant selection

Participants’ information sheet & verbal informed consent was obtained (ANU Ethics approval)
Stakeholder engagement - Questions

- Threat?
- Surveillance
  - Drivers
  - Obstacles
  - Way forward
- Future workshop

Stakeholders invited: 34

Content analysis

What research is about
- Research team
- Funding source
- Why/How they were selected
- Ethics requirements:
  - Confidentiality
  - Consent
Stakeholder engagement - Results

Participants: 20/34 59% response rate

<table>
<thead>
<tr>
<th>Category</th>
<th>Count (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal health</td>
<td>11 (55%)</td>
</tr>
<tr>
<td>Industry</td>
<td>4 (20%)</td>
</tr>
<tr>
<td>Public Health</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>Unions</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>Poultry industry</td>
<td>n=3</td>
</tr>
<tr>
<td>Pig industry</td>
<td>n=1</td>
</tr>
<tr>
<td>Unions in poultry/pig industry</td>
<td>n=3</td>
</tr>
<tr>
<td>Animal health – government</td>
<td>(inc. state CVO, surveillance) n=11</td>
</tr>
<tr>
<td>Public health – government</td>
<td>(inc. surveillance) n=3</td>
</tr>
</tbody>
</table>

Poultry industry Stakeholder engagement – Results

- Support for investigations in response to outbreaks
- No support for ongoing surv. > Worry about consequences of positive findings to industry
- Agreement on communication to workers if there is a finding.
- Major obstacle: Lack of trust in public health authorities
- Cost: shift of resources from biosecurity to surveillance?
- Cost: Would surveillance jeopardise cost sharing arrangements in Emergency Animal Disease Response Agreement (EADRA)
Stakeholder engagement – Results

Pig industry vet

“In 2010 the consequences were extreme. The TV people brought in helicopters, the agency people came in like storm troopers, the notifying veterinarian was treated like shit by the regulators, the press had a field day and consumers stopped buying pork. Even though there was no health risk to consumers pork consumption slumped and with it the price. It was all unnecessary. The pig industry is still scarred.”

Impact of SIV detection in pigs – action by DoAg

“If a novel flu infection in pigs does not cause high MM it’s quite likely it would be missed. In any case it takes ages for AAHL to report a result. In fact in my case we got the influenza A dx within a couple of weeks but the full typing of the virus (H3N2 or whatever it was – can’t remember) took ages for it to be reported.”

Union

Pig & poultry abattoirs

Note: workers on farms largely non-unionised temporary immigrants

Unaware of risk of zoonotic respiratory viruses

Detection zoonotic respiratory viruses

- Worker not aware of potential link between illness & occupation
- GP does not ask about animal contact history/occupation (Zoonosis card)
- People on 417 visas might not seek healthcare (fear of loosing job). No access to Medicare (Taiwan, Korea) – 30-90% workforce in poultry abattoirs (temp visas)

Supportive of a study/surveillance among pig/poultry abattoir workers to determine BoD provided positive findings do not result on job losses. Who will have access to data?
Public health

Stakeholder engagement – Results

- Threat pig to human transmission: real – perhaps not immediate
- BoD of zoonotic resp viruses in people that work in intensive pig/poultry industries in Australia is unknown
- Detection
  - Not all PH labs subtype flu
  - If flu subtyping unsuccessful > refer specimen if severe disease
  - Most flu cases not followed up for occupational exposure info

Animal health

Stakeholder engagement – Results

- Au CVO - High level support to this study
- AHA - Will host our workshop in 2019

State CVOs – PVOs (Surveillance)
- Prevalence of SIV in pigs in Au not known
- Suspect ‘low path’ SIV in pigs common
- No surveillance in pigs due to business risk (response worse than disease. AUSVETPLAN)
- Industry private vets & labs test ILI
- Poor awareness of zoonotic risk among workers
- GPs not testing pig/poultry workers
- Workplaces that do not provide flu vax

Producers don’t want to risk finding a virus of concern that would trigger serious responses if not experiencing economic losses
Stakeholder engagement – Results

Future workshop

<table>
<thead>
<tr>
<th></th>
<th>Poultry</th>
<th>Pig vet</th>
<th>Union</th>
<th>Animal H</th>
<th>Public H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attendance</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Financial</td>
<td>[X]</td>
<td>✔</td>
<td>[X]</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>assistance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=3</td>
<td>n=1</td>
<td>n=2</td>
<td>n=6</td>
<td>n=5</td>
<td></td>
</tr>
</tbody>
</table>

What are other countries doing about surveillance at the animal-human interface?

Canada

- Nurses ILI surveillance
- Visited farms 2 x wk
- NP swabs & acute & conv sera from ILI
- Swabs qPCR tested
- PCR +ve cultured
- MN for ab to H4, H5, H7, H9
- +ve flu findings trigger testing in pigs – vice versa
- Routine vet visits
- Ill pigs purchased-euthanised-lung tissue/swabs/blood
What are other countries doing about surveillance at the animal-human interface?

Serological evidence of transmission of SwiV/reassortants to swine workers will be defined as antibodies to one or more of the swine strains or reassortants with a titre > 1/40 [34]. This is valid only for classical swine H1N1 virus in human sera collected before April 2009, as, due to shared epitopes, there may be cross reactivity on serological tests between endemic human and swine strains, including the new pandemic influenza strain. Evidence for transmission of other swine strains/reassortants will require culture from the swine workers. Serological evidence of transmission of H1N1V/reassortants to pigs is strongest if there is an eightfold or higher increase in titre between acute and convalescent serum samples to contemporary human H3N2 and H1N1 viruses. Further evidence of transmission from human to pig is the isolation of these human strains from pigs, the strongest evidence obtained from comparison of the full length sequences showing that the same strains are isolated from both humans and animals.

What are other countries doing about surveillance at the animal-human interface?

Ethics
We do not do virus subtyping or sequencing or serological analyses in real time. All samples are shipped to laboratories under code and all testing done in an anonymized fashion to preclude linkage of results to a specific farm and is done at periodic intervals such that evidence of infection would be historical (i.e., sufficient time would have passed since specimen collection that any infection events would be over, precluding the need for any public health action). This is required to abate concerns that study herds would be quarantined or depopulated or the market value of the swine from the colony or the Alberta swine industry, generally, be adversely impacted by test results. This study was approved by the Conjoint Health Research Ethics Board of the University of Calgary (Ethics ID 18970), McMaster University H1S/FHS Research Eth-
What are other countries doing about surveillance at the animal-human interface?

Hong Kong

Surveillance of influenza viruses in swine in Hong Kong abattoir: methods and feasibility

Edward S. K. Ma, Po L. Ho, Chung Yan Choung, Tsosmay M. Tao, Andy Chan, Dhanasekaran Vijaykrishna, Loo L.M, Poon, Yi Guan, Joseph Srijal Malik Poins

*Department of Microbiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China. "Program in Emerging Infectious Diseases, Duke-NUS Graduate School, Singapore.

Kwanzikind: influenza, methods, surveillance, epidemiology.

Introduction

The concept that swine are a mixing-pot for the re-assortment of influenza viruses and for the emergence of pandemic influenza viruses has been re-enforced by the emergence of the recent pandemic. The pandemic H1N1 addition to endemic swine virus lineages, swine influenza viruses such as H1N2 and highly pathogenic avian influenza (HPAI) H5N1 have been occasionally identified in pigs in parts of Asia.17,18 It has been shown that H5N1/10 poultry outbreaks with H5N1 to generate viable progeny in vitro.19 It is therefore essential to monitor the

- In place > decade
- Central abattoir
- 4,000 pigs/day
- 250 swabs/month
- VI, HI, sequencing
- Small subset qPCR
- 1.4% rate VI - acceptable
- FEASIBLE
- Anonymous
- Not real time
- No consequences to supplier if +ve detections

What next?

- Drafting a protocol – aspects from Canadian & HK studies
- Consultation in 2019
- Refine protocols
- Plan a sero-survey
- Hopefully gather evidence to support surveillance at the animal-human interface
- Expand to comprehensive animal, environment, human surveillance ► One Health
Other projects completed!

Acknowledgements

- APPRISE team
- Respondents
- My supervisors
Chapter 6

Lessons from the field
## Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prologue</td>
<td>359</td>
</tr>
<tr>
<td>My role and lessons learned</td>
<td>359</td>
</tr>
<tr>
<td><strong>6.1 Lessons from the Field:</strong></td>
<td></td>
</tr>
<tr>
<td>Introduction to the open-source R software</td>
<td>360</td>
</tr>
<tr>
<td>6.1.1 Learning objectives</td>
<td>360</td>
</tr>
<tr>
<td>6.1.2 Learning Steps</td>
<td>360</td>
</tr>
<tr>
<td>6.1.3 What is R and RStudio?</td>
<td>360</td>
</tr>
<tr>
<td>6.1.4 Materials needed</td>
<td>361</td>
</tr>
<tr>
<td>6.1.5 Instructions part 1: Software installation</td>
<td>361</td>
</tr>
<tr>
<td>6.1.6 Instructions part 2: Data import and analysis</td>
<td>363</td>
</tr>
<tr>
<td><strong>6.2 Resources for further learning</strong></td>
<td>366</td>
</tr>
<tr>
<td><strong>6.3 A final word about writing code</strong></td>
<td>366</td>
</tr>
<tr>
<td><strong>Acknowledgements</strong></td>
<td>367</td>
</tr>
<tr>
<td><strong>References</strong></td>
<td>368</td>
</tr>
<tr>
<td><strong>6.4 Teaching Experience</strong></td>
<td>369</td>
</tr>
<tr>
<td>6.4.1 Data analysis training workshop in Cambodia</td>
<td>369</td>
</tr>
<tr>
<td>6.4.2 Communication during public health emergencies</td>
<td>369</td>
</tr>
<tr>
<td><strong>Appendices</strong></td>
<td>371</td>
</tr>
<tr>
<td>6.A Lesson from the field: the Rmd output file</td>
<td>371</td>
</tr>
<tr>
<td>6.B Lesson from the field: teleconference slides</td>
<td>383</td>
</tr>
<tr>
<td>6.C Teaching presentation, ANU, March 2018</td>
<td>386</td>
</tr>
</tbody>
</table>
Chapter 6

Prologue

My role and lessons learned

In the Lesson from the Field (LFF) I delivered to my peers I used the R software to analyse data. I believe that using open source software is an asset for epidemiologists. For those of us that engage in capacity building in developing countries it has an obvious benefit: no need to pay for expensive statistical software licences when resources are limited. In addition, I chose to introduce R to my peers because this software is able to perform user-defined sophisticated data analysis.

In this session I used the Titanic dataset (i.e., survival of passengers on the Titanic) to illustrate how to use code for data cleaning and graphic creation. To gently introduce R, which was completely unfamiliar to my peers, I created a 50 minute instructional video. I also selected the Titanic dataset as we had used it with Stata for our course POPH 8313 Analysis of Public Health Data. Familiarity with the data and the types of analysis proposed were meant to allow my peers to gauge differences between both software packages.

The complete lesson from the field is shown in Appendix 6.A. It shows the instructions given to students, the code as it should be entered by the students, the Rmd output executed after each chunk of code and my questions. This session included a teleconference in which I presented an overview of R and addressed issues that my peers faced when completing the exercise including demonstrating how to approach ‘debugging’ or error handling. The slides used at the teleconference are presented in Appendix 6.B. Feedback from my peers, which I collected through an online survey, was positive with all reporting that they are likely to seek further training so that they can use R for public health data analysis in the future. They also commented on the usefulness of learning how to address error messages.

Preparing this activity taught me how to use Bloom’s Taxonomy to inform the design of my lesson plan. Of course, teaching is the best way to learn. This activity consolidated my own understanding of R and its capabilities. Through this LFF I also learned how to use screen recording software needed to make the instructional video. As a result I have a healthier digital toolbox in my kit. It is rewarding that the ANU National Centre for Epidemiology and Population Health selected my LFF as a teaching tool for future MAE and MPH students.

I acknowledge my field supervisor for encouraging me to learn R and Associate Professor Beth Beckmann for her session on foundational principles of teaching and learning.
6.1 Lessons from the Field:  
Introduction to the open-source R software

6.1.1 Learning objectives

In this session, you will learn the basic principles of analysing data using R. You will learn how to code to explore the Titanic dataset using a collection of packages called the tidyverse. Packages are sets of tools available in R to manage data. Packages we will use (dplyr and ggplot2) perform functions such as filter, sort and graphic creation.

At the end of this lesson you will:

- Learn why R is the preferred software for modern epidemiologists
- Practice writing code in R for simple data manipulation and analysis
- Create publication-quality graphs using R
- Gain confidence in your ability to learn how to use data analysis software famous for being non-user friendly and difficult to learn

6.1.2 Learning Steps

We will use R to explore the Titanic dataset and analyse characteristics and the fate of the passengers of the Titanic. You will be provided with an instructional video so you can write code with me in ‘real time’. There will be a series of tasks that you will complete, such as creating tables and graphs and you will have to answer questions as we progress in our analysis. The aim of this lesson is to become a beginner level user of R.

6.1.3 What is R and RStudio?

R is a free software environment for statistical computing and creation of publication-quality graphics from CRAN, the Comprehensive R Archive Network. R uses a command line interface. Because this software was created by computer scientists over four decades, the language it uses (i.e., meaning ascribed to a command) is not always intuitive. The R Help tab is where you go to find out the meaning of a command. Increasingly, R is becoming the preferred data analysis software for
epidemiologists. R is favoured not only because it is free but also because it permits tailored and sophisticated statistical analysis (1). Software developers in collaboration with epidemiologists, statisticians and others regularly contribute R tools. A relevant example for us is OutbreakTools, an R package for storing, handling and visualising outbreak data (2). RStudio is a more user friendly interface for R. An analogy that helps explain why we need both R and RStudio is that R is like a car’s engine and RStudio is like the car’s dashboard. For an overview of RStudio you can watch this 1.5 min video.

6.1.4 Materials needed

1. Preferably you need a PC with Windows installed. I have not used R in a Mac or Linux (some commands are different in Mac and Linux), therefore, I am unable to provide guidance. Of course, you can always google any issues you may have when using R on a Mac or Linux.

2. Software: R and RStudio (see instructions below).

3. Video tutorial (3).

4. LFF_XT.Rmd file template (equivalent to a do file in Stata. We will write code in this file).

5. TitanicPassengers.csv (data file)

6.1.5 Instructions part 1: Software installation

Please note that text in blue is the text you need to enter in the console and text in bold is the output or the text you will see once you execute a command. If you have trouble installing R and RStudio on a PC I can provide support.

Program installation

- Install R from here. Note: If you have R already installed but it is not the latest version you will need to update it to the latest version. The easiest way to ‘update’ is to remove the program and re-install it.
- Install RStudio from here.
- Follow the instructions on the following section to test your set-up
Testing RStudio

1. Launch RStudio

2. Place your cursor in the panel labelled ‘Console’, which is where you interact with the live R process (see Figure 6.1).

3. Create a simple object with code by typing this:
   \[
   x \leftarrow 2 \times 4
   \]
   The above line can be read as follows: 2 times 4 is assigned to x. An equal sign can also be used instead of a left arrow.
   Click enter or return. Then inspect the x object by typing:
   \[
   x
   \]
   Click enter or return.

You should see the value 8 appear in the line immediately below, as per screenshot in Figure 6.1

If you can see \[1\] 8 as per the screenshot above, you are good to go.

Proceed to installing the following three packages: knitr (needed to compile a document containing the code you will type, the output and all comments), ggplot2 (this is the package needed for creating graphs) and plyr (we will use this package to do some
common data manipulation operations such as filtering, sorting, converting variable type, etc.)

For this, type the following into the console, one line at a time pressing enter after typing each line:

```r
install.packages("knitr", dependencies = TRUE)
install.packages("ggplot2", dependencies = TRUE)
install.packages("plyr", dependencies = TRUE)
```

You will see some writing in red. Don’t worry. R is doing its thing. After you run the last line you will see something like what’s on the screen shot in Figure 6.2.

![Figure 6.2: Screen shot of package installation](image)

Finally, you need to install a collection of packages called the tidyverse. Type the line of code that appears in blue below and press enter (you can see that pressing enter is equivalent to executing/running a line of code):

```r
install.packages("tidyverse")
```

When you are ready to start the data analysis exercise move on to the next section.

### 6.1.6 Instructions part 2: Data import and analysis

1. Locate the following files (which were emailed to your ANU email address).
2. Watch the instructional video.

3. Open the Rmd file. The video shows that when I double click on the Rmd file it automatically opens in RStudio. However, if you have EndNote installed in your PC, the Rmd file will open in EndNote if you double click it. To avoid this, right click on LFF_XT.Rmd, select open with, then select RStudio.

4. Make sure you type your code on the Rmd file and below the green comments. See Figure 6.3 for guidance.

![Image of RStudio interface showing code input locations](Image)

Figure 6.3: Where to enter commands (R syntax)

The first arrow shows the name of the file where you should be working (i.e., the Rmd file). The other arrows point at where you should type the first three lines of code. Enter each line of code under its corresponding comment in green.
If you cannot see the syntax clearly in the video you need to select the highest quality setting. On the YouTube video click on Settings > select Quality > select (720pHD). See screenshots in Figure 6.4 for instructions.

Figure 6.4: Viewing the video on highest quality setting
5. Record your answers to the three questions in the places indicated in the Rmd file.

6. Once you have completed all tasks save the file as follows: Go to knitr, select ‘Knit to HTML’. RStudio will compile the output document. Once the html document opens, click on Open in browser. In the browser, right click anywhere in the file, select print and save as pdf. Name the pdf Intro to R plus your initials and email it to me. This step appears on the video tutorial.
Note: there is a way to save the Rmd file directly to pdf but it involves the installation of a \LaTeX compiler which is beyond the scope of this lesson.

Note on this lesson: The data manipulations covered in this lesson can also be performed using base R functionalities. That is, without the need to install extra packages. I have chosen to introduce R to you using the tidyverse because the main feature of the tidyverse of ‘one verb - one function’ makes it easy for beginners to remember how to use functions. However, it is important that you also learn R base.

6.2 Resources for further learning

There are many free resources available online if you wish to continue using R. I highly recommend the free course ‘Introduction to R’ from DataCamp (4). It has videos and an interactive interface where you can type your code and get instant feedback.

6.3 A final word about writing code

When writing code for data cleaning and analysis, you need to aim for:

- code that is nicely formatted
- well-annotated
- governed by simple rules
- understood by all the team members

As with any statistical analysis, your code must yield reproducible results and be easy to follow by another person or if you get back to it months later, which is fairly common. You don’t want to have to go through code like the one in the image below. This is what R experts say about code syntax: ‘Code should be clean and robust and
handle errors in grace and style. That is the mark of a great software craftsman.’ Apparently, ‘Some people are born with it and some have to painfully acquire it through practice, persistence and perseverance.’ If you feel inspired and would like to know what constitutes code that is ‘clean and beautiful’ read this article (5).

Your aim is to write simple code that will be understood across person, time and place. However, avoiding code as pictured above is an art. Image source: Utilika.com

**Acknowledgements**

This tutorial drew on material created by Bowne-Anderson from DataCamp (6).
References


6.4 Teaching Experience

In addition to the LFF delivered to my peers I had three other teaching experiences. Two are described below. The third consisted of assisting in two LaTeX workshops delivered by the University of Melbourne’s Research Platform Services to a multidisciplinary group of postgraduate students.

6.4.1 Data analysis training workshop in Cambodia

In May 2017 I co-delivered a training workshop for staff from the Cambodian Ministry of Health involved in the hospital admission survey in Siem Reap. The aim of this two-day workshop was to present an overview of the activities completed, interpret the results of the data validation exercise and present a step by step guide on how to analyse hospital admission survey data. This workshop included practical exercises in Excel to calculate the burden of influenza for the SARI sentinel site catchment population. Slides used for this training workshop are in Appendix 2.G.

6.4.2 Communication during public health emergencies

The 2017 MAE cohort delivered a teaching lesson to the 2018 intake of MAE scholars during their first course block as per academic requirements. We split ourselves into four groups and delivered lessons that integrated epidemiology, with general scientific skills and MAE specific skills. The teaching session was two hours long and was delivered on the last day of a three week course block. I was part of a team composed by Aurysia Hii, Patiyan Andersson and Sophie Phelan. We delivered a lecture on communicating as a field epidemiologist during public health emergencies. Other teams covered ethical considerations when recruiting participants for a study, using logic models for project planning and evaluation and tips to help MAE scholars stay on top of their writing during all stages of their program. These were issues we identified as important to enable a new MAE scholar to hit the ground running.

The learning objectives for the session I co-delivered were:

1. Understand your role as a field epidemiologist investigating an outbreak within the context of a complex, fast evolving, humanitarian emergency.

2. Learn how to communicate to different audiences as a part of your role as field epidemiologist.
3. Practice delivering relevant information to specific audiences in a limited amount of time.

Our teaching session was scenario-based and required students to form 3-4 person groups and work through a series of questions (presentation slides are shown in Appendix 6.3). Exercise questions were based on real-life experiences and observations of team members that had worked in international responses that ranged from serious outbreaks within hospitals to a large-scale humanitarian emergency with multiple health actors. We aimed to share with students practical information that might not be covered in formal MAE lectures.

We presented a post-tsunami scenario in a Pacific Island Country in which the scholar was deployed to work on the response to a cholera outbreak. After appraising the scenario, each group presented their responses to the class. This was followed by a short presentation by a designated team member describing how they approached the situation on deployment and their lessons learned.

We asked students to identify key messages they would need to present at a health interagency daily meeting. We also prompted students to think about how to approach a problem with one health centre that had stopped reporting through the early warning and response system. To conclude, we asked students to devise a handover strategy for the epidemiologist that was going to replace them.

We send an online evaluation survey to all first-year student. Results of the evaluation showed that over 90% of participants, on average, rated the content, format and delivery style for our session either useful or highly useful. Some participants reported that all teaching sessions fostered interaction among students and presenters. In terms of aspects that needed improvement most respondents highlighted that considering the time constrains we were able to deliver an engaging session in an easy to digest format. One issue raised was the need for presenters and facilitators to ensure that shy students are encouraged to participate and that the dominant types are made aware of the importance of this.
Welcome to Ximena’s lesson from the field.

We will start by installing the tidyverse collection of packages. Please note that anything that appears after the hashtag is a comment, therefore not executable. Also, the following syntax removes R messages and warnings (r message = FALSE, warning=FALSE)

```
# Install the tidyverse
# install.packages("tidyverse")
```

Note that for the file to compile, after installing the tidyverse, you must comment out that line (i.e., remove the #). Installing the tidyverse package does not mean that the package is ready to use, we also need to import it.

```
# Import tidyverse
library(tidyverse)
```

```
# Import data
passengers <- read_csv("TitanicPassengers.csv")
```

```
# Check out the first observations of the dataframe
passengers
```

```
## A tibble: 891 x 12
##   PassengerId Survived Pclass Name  Sex Age SibSp Parch Ticket Fare
##      <int>   <int> <int> <chr> <chr> <dbl> <int> <int> <chr> <dbl>
## 1       1       0     3 Brau~ male  22   1   0 A/5 2~   7.25
## 2       2       1     1 Cumi~ fema~ 38   1   0 PC 17~  71.3
## 3       3       1     3 Heik~ fema~ 26   0   0 STON/~  7.92
## 4       4       1     1 Futr~ fema~ 35   1   0 34009~  8.05
## 5       5       1     3 Alle~ male  35   0   0 330877~  8.46
## 6       6       0     3 Mora~ male NA   0   0 350486~  8.42
## 7       7       1     4 McCa~ male 54   0   0 349909~  21.1
## 8       8       0     3 Pals~ male  2   3   1 347742~ 11.1
## 9       9       1     3 John~ fema~ 27   0   0 347742~ 11.1
## 10      10      1     3 Hans~ fema~ 14   1   0 327736~  30.1
## # ... with 881 more rows, and 2 more variables: Cabin <chr>,
## #   Embarked <chr>
```

Using the function ‘summary’ let’s have an initial look at the data.

```
# Use the function summary to get an overview of data
summary(passengers)
```

```
## PassengerId Survived Pclass Name  Sex Age SibSp Parch Ticket Fare
## Min. :1.0   Min. :0.0000 Min. :1.0000 Length:891
## 1st Qu.:223.5 1st Qu.:0.0000 1st Qu.:2.0000 Class :character
## Median :646.0 Median :0.0000 Median :3.0000 Mode :character
## Mean :646.0 Mean :0.3838 Mean :2.309
## 3rd Qu.:668.5 3rd Qu.:1.0000 3rd Qu.:3.0000
## Max. :891.0 Max. :1.0000 Max. :3.000
```
Using the pipe operator, let’s check the data again.

```r
# Summarise data using a pipe operator
passengers %>%
  summary()
```

```
## PassengerId Survived Pclass Name
## Min. : 1.0 Min. :0.0000 Min. :1.000 Length:891
## 1st Qu.:223.5 1st Qu.:0.0000 1st Qu.:2.000 Class :character
## Median :446.0 Median :0.0000 Median :3.000 Mode :character
## Mean :446.0 Mean :0.3838 Mean :2.309
## 3rd Qu.:668.5 3rd Qu.:1.0000 3rd Qu.:3.000
## Max. :891.0 Max. :1.0000 Max. :3.000
##
## ## Sex Age SibSp Parch
## Length:891 Min. : 0.42 Min. :0.000 Min. :0.0000
## Class :character 1st Qu.:20.12 1st Qu.:0.000 1st Qu.:0.0000
## Mode :character Median :28.00 Median :0.000 Median :0.0000
## Mean :29.70 Mean :0.523 Mean :0.3816
## 3rd Qu.:38.00 3rd Qu.:1.000 3rd Qu.:0.0000
## Max. :80.00 Max. :8.000 Max. :6.0000
##
## ## Ticket Fare Cabin Embarked
## Length:891 Min. : 0.00 Length:891 Length:891
## Class :character 1st Qu.: 7.91 Class :character Class :character
## Mode :character Median :14.45 Mode :character Mode :character
## Mean :32.20
## 3rd Qu.: 31.00
## Max. :512.33
##```
You will now see why the pipe operator is so useful.

```r
# Summarise data excluding missing values (NA's) by concatenating/linking pipes
passengers %>%
  drop_na() %>%
  summary()
```

<table>
<thead>
<tr>
<th>#</th>
<th>PassengerId</th>
<th>Survived</th>
<th>Pclass</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min.</td>
<td>2.0</td>
<td>0.0000</td>
<td>1.000</td>
<td>Length:183</td>
</tr>
<tr>
<td>1st Qu.:</td>
<td>263.5</td>
<td>0.0000</td>
<td>1.000</td>
<td>Class :character</td>
</tr>
<tr>
<td>Median :</td>
<td>457.0</td>
<td>1.0000</td>
<td>1.000</td>
<td>Mode :character</td>
</tr>
<tr>
<td>Mean :</td>
<td>455.4</td>
<td>0.6721</td>
<td>1.191</td>
<td></td>
</tr>
<tr>
<td>3rd Qu.:</td>
<td>676.0</td>
<td>1.0000</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Max. :</td>
<td>890.0</td>
<td>1.0000</td>
<td>3.000</td>
<td></td>
</tr>
<tr>
<td>#</td>
<td>Sex</td>
<td>Age</td>
<td>SibSp</td>
<td>Parch</td>
</tr>
<tr>
<td>---</td>
<td>-----</td>
<td>-----</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Min.</td>
<td>0.92</td>
<td>0.0000</td>
<td>0.0000</td>
<td></td>
</tr>
<tr>
<td>1st Qu.:</td>
<td>24.00</td>
<td>0.0000</td>
<td>0.0000</td>
<td></td>
</tr>
<tr>
<td>Median :</td>
<td>36.00</td>
<td>0.0000</td>
<td>0.0000</td>
<td></td>
</tr>
<tr>
<td>Mean :</td>
<td>35.67</td>
<td>0.4645</td>
<td>0.4754</td>
<td></td>
</tr>
<tr>
<td>3rd Qu.:</td>
<td>47.50</td>
<td>1.0000</td>
<td>1.0000</td>
<td></td>
</tr>
<tr>
<td>Max. :</td>
<td>80.00</td>
<td>3.0000</td>
<td>4.0000</td>
<td></td>
</tr>
<tr>
<td>#</td>
<td>Ticket</td>
<td>Fare</td>
<td>Cabin</td>
<td>Embarked</td>
</tr>
<tr>
<td>---</td>
<td>-------</td>
<td>------</td>
<td>-------</td>
<td>----------</td>
</tr>
<tr>
<td>Min.</td>
<td>0.00</td>
<td>Length:183</td>
<td>Length:183</td>
<td></td>
</tr>
<tr>
<td>1st Qu.:</td>
<td>29.70</td>
<td>Class :character</td>
<td>Class :character</td>
<td></td>
</tr>
<tr>
<td>Median :</td>
<td>57.00</td>
<td>Mode :character</td>
<td>Mode :character</td>
<td></td>
</tr>
<tr>
<td>Mean :</td>
<td>78.68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd Qu.:</td>
<td>90.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max. :</td>
<td>512.33</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

It is easier to keep track of all manipulations you want to apply to the data if you use the pipe operator, which is essentially equivalent to ‘and’. For example, in one chunk of code and using syntax that is easy on the eye (i.e., not too hard to follow) you can exclude missing values, convert a numeric variable to factor and then summarise.

**QUESTION 1:** Look at how dropping missing values impacted on mean age and mean fare. What does that tell you? How would you want to interrogate the data to explore the fate of those with missing age? What hypothesis would you want to test? Note: You don’t need to use code to answer the question. You need to explain in your own words what else you might want to know about those with missing values for age.

Type your answer here:

Next we will use the package dplyr to manipulate and convert data
Manipulating data

To interrogate parts of the data we will explore functions such as ‘filter’, ‘arrange’ and ‘mutate’

```r
# Select female passengers only
passengers %>%
  filter (Sex == "female")
```

```r
## A tibble: 314 x 12
## # A tibble: 314 x 12
##  ##  PassengerId Survived Pclass Name  Sex Age SibSp Parch Ticket Fare
## 1     <int>  <int> <int> <chr> <chr> <dbl> <int> <int> <chr> <dbl>
## 2   2      1     1  Cumi~ fema~  38   1    0 PC 17~  71.3
## 3   3      1     3 Heik~ fema~  26   0    0 STON/~  7.92
## 4   4      1     1 Futr~ fema~  35   1    0 113803  53.1
## 5   5      1     3 John~ fema~  27   0    0 347742  11.1
## 6   6      1     1  Nass~ fema~  14   1    0  237736  30.1
## 7   7      1     3  Sand~ fema~  4   1    1  PP 95~  16.7
## 8   8      1     1  Bonn~ fema~ 58   0    0  113783  26.6
## 9   9      1     2  Vann~ fema~ 31   1    0  345763  18.1
## 10 10      1     3  Bonn~ fema~ 62   0    0  345763  18.1
## # ... with 304 more rows, and 2 more variables: Cabin <chr>,
## # Embarked <chr>
```

Now, let’s arrange observations by increasing Fare

```r
# Arrange by increasing Fare
passengers %>%
  arrange (Fare)
```

```r
## A tibble: 891 x 12
## # A tibble: 891 x 12
##  ##  PassengerId Survived Pclass Name  Sex Age SibSp Parch Ticket Fare
## 1    <int>  <int> <int> <chr> <chr> <dbl> <int> <int> <chr> <dbl>
## 2    180   0     3  Leon~ male  36   0    0 LINE 0
## 3    284   0     1 Harri~ male  40   0    0  112059  0
## 4    272   1     3  Torn~ male  25   0    0 LINE 0
## 5    278   0     2 "Par~ male NA   0    0  239853  0
## 6    303   0     3 John~ male  19   0    0 LINE 0
## 7    414   0     2 Cunn~ male NA   0    0  239853  0
## 8    467   0     2 "Camp~ male NA   0    0  239853  0
## 9    482   0     2 "Fro~ male NA   0    0  239853  0
## 10   598   0     3 John~ male  49   0    0 LINE 0
## # ... with 881 more rows, and 2 more variables: Cabin <chr>,
## # Embarked <chr>
```

**QUESTION 2:** Look at pages 2 to 6 in the above output. How many men that paid at the bottom bracket of fare survived? How many women? Does this make you think of a particular way to examine survival by sex? Note: You don’t need to use code to answer the question, just mention another way in which you could further explore the association between fare and survival

Type your answer here:

Next, we will create a new variable “FamSize” as the sum of “Parch” which means parents & children, and “SibSp” which means siblings & spouses
# Create a new variable “FamSize” as the sum of “Parch” and “SibSp”

```r
passengers %>%
mutate(FamSize = Parch + SibSp)
```

### # A tibble: 891 x 13
###    PassengerId Survived Pclass Name Sex Age SibSp Parch Ticket Fare
###      <int>   <int> <int> <chr> <chr> <dbl> <int> <int> <chr> <dbl>
### 1       1       0     3     Brau~  male     22    1     0    A/5 2~     7.25
### 2       2       1     1     Cum~  fema~     38    1     0     PC 17~     71.3
### 3       3       1     3     Heik~  fema~     26    0     0     STON~     7.92
### 4       4       1     1     Futr~  fema~     35    1     0     113803    53.1
### 5       5       0     3     Alle~  male     35    0     0     373450     8.05
### 6       6       0     3     Mora~  male     NA    0     0     330877     8.46
### 7       7       0     1     McCa~  male     NA    0     0     17463    51.9
### 8       8       0     3     Pals~  male     NA    2     3     139909    21.1
### 9       9       1     3     John~  fema~     27    0     2     347742    11.1
### 10      10      1     2     Nass~  fema~     14    1     0     237736    30.1
### ... with 881 more rows, and 3 more variables: Cabin <chr>,
###     Embarked <chr>, FamSize <int>

Arrange “FamSize” in decreasing order

```r
passengers %>%
mutate(FamSize = Parch + SibSp) %>%
arrange(desc(FamSize))
```

### # A tibble: 891 x 13
###    PassengerId Survived Pclass Name Sex Age SibSp Parch Ticket Fare
###      <int>   <int> <int> <chr> <chr> <dbl> <int> <int> <chr> <dbl>
### 1       1       0     3     Sage~  male     NA    8     2     CA 21~    69.6
### 2       2       0     3     Sage~  male     NA    8     2     CA 21~    69.6
### 3       3       1     3     Sage~  male     NA    8     2     CA 21~    69.6
### 4       4       0     3     Sage~  female  NA    8     2     CA 21~    69.6
### 5       5       1     3     Sage~  female  NA    8     2     CA 21~    69.6
### 6       6       1     3     Sage~  male     NA    8     2     CA 21~    69.6
### 7       7       0     3     Sage~  female  NA    8     2     CA 21~    69.6
### 8       8       0     3     Good~  male   11    5     2     CA 21~    46.9
### 9       9       1     3     Good~  female  16    5     2     CA 21~    46.9
### 10      10      0     3     Good~  male   11    5     2     CA 21~    46.9
### ... with 881 more rows, and 3 more variables: Cabin <chr>,
###     Embarked <chr>, FamSize <int>
The function `mutate` can be used to create a new variable and to change an existing variable.

Convert `Survived` from 1/0 to “Yes” and “No” and save as a new dataframe:

```r
# Convert Survived variable to "Yes" and "No" and save as a new dataframe
passengers_new <- passengers %>%
  mutate(Survived = ifelse(Survived == 0, "No", "Yes"))
```

```
## # A tibble: 891 x 12
## # Row names: A/5 2~ 7.25
##   PassengerId Survived   Pclass Name Sex Age SibSp Parch Ticket Fare
##    <int>     <chr> <int> <chr> <chr> <dbl> <int> <int> <chr> <dbl>
##  1       1    No       3    Brau~ male  22    1    0    A/5  2~   7.25
##  2       2   Yes       1   Cumi~ fema~ 38    1    0    PC 17~ 71.3
##  3       3   Yes       3   Heik~ fema~ 26    0    0    STON/~ 7.92
##  4       4   Yes       1   Futr~ fema~ 35    1    0  113803 53.1
##  5       5    No       3   Alle~ male  35    0    0  373450  8.05
##  6       6    No       3   Mora~ male  NA    0    0   330877  8.46
##  7       7    No       1  McCa~ male  54    0    0  17463   51.9
##  8       8    No       3   Pals~ male  2    3    1  349909 21.1
##  9       9   Yes       3   John~ fema~ 27    0    2  347742 11.1
## 10      10   Yes       2   Nass~ fema~ 14    1    0  237736 30.1
## # ... with 881 more rows, and 2 more variables: Cabin <chr>, Embarked <chr>
```

Plotting data

We’ll create a bar plot of age by fare using `ggplot2`. We need to specify three things:

- data
- what to plot on the x and y axis - R calls this aesthetics
- plot type (e.g. barplot, scatterplot, etc) - R calls this layers

```r
# Create a scatter plot of Age by Fare
ggplot(passengers, aes(x = Age, y = Fare)) + geom_point()
```
Let's now create a scatter plot of fare by age using colour to distinguish sex.

```r
# Create a scatter plot of Age by Fare coloured by Sex
ggplot(passengers, aes(x = Age, y = Fare, colour = Sex)) + geom_point()
```
This is how you read the above code: We take the data and we map Age to the x axis and Fare to the y axis to create a scatter plot.

We’ll create two scatter plots of age by fare, side by side, one for survived and not survived

```r
# Create two scatter plots of Age by Fare coloured by Sex faceted by Survived
ggplot(passengers_new, aes(x = Age, y = Fare, colour = Sex)) +
  geom_point() +
  facet_grid(~Survived)
```

![Scatter plot of Age vs Fare, colored by Sex and faceted by Survived](image)
Note that you can use both British and American spelling, the *tidyverse* works with both.

**QUESTION 3:** what does the above figure tell you? Do you think this is a good way to explore the association between fare, sex and survival? How else would you want to graph survival if you want to zero in on those that paid between 6 and 15 monetary units? Note: Six being the lowest fare (not counting those that did not pay anything) and 15 being the median fare. You don’t need to use code to answer the question, just explain another way to dig in further into the data.

Type your answer here:

Now, we’ll create a bar chart of Survived count by sex

```r
# Create a bar chart of Survived by Sex
ggplot(passengers_new, aes (x = Sex, fill = Survived)) + geom_bar()
```
Recap

- We’ve imported and inspected data
- We’ve learnt about the pipe operator %>%
- We used dplyr to filter and arrange data and mutate to create a new var
- We’ve learned how to plot data using the graphics package ggplot2

Grouping data

Let’s now summarise data across one dimension, such as “Sex”

Let’s find out what was the mean fare paid

```r
# Calculate mean Fare
passengers %>%
  summarise (meanFare = mean (Fare))
```

## # A tibble: 1 x 1
## #  meanFare
##   <dbl>
## 1  32.2

Note: summarise() takes a dataset with n observations, calculates the requested summary, and returns a dataset with 1 observation.
How much did men pay for their fares on average?

```r
# Calculate mean Fare for men
passengers %>%
  filter (Sex == 'male') %>%
  summarise (meanFare = mean (Fare))
```

## # A tibble: 1 x 1
##    meanFare
##       <dbl>
## 1       25.5

What was the mean fare paid by women?

```r
# Calculate mean Fare for women
passengers %>%
  filter (Sex == 'female') %>%
  summarise (meanFare = mean (Fare))
```

## # A tibble: 1 x 1
##    meanFare
##       <dbl>
## 1       44.5

What was the mean fare paid by women & how many women survived?

```r
# Calculate mean Fare for women and count female survivors
passengers %>%
  filter (Sex == 'female') %>%
  summarise (meanFare = mean (Fare),
             numSurv = sum (Survived))
```

## # A tibble: 1 x 2
##    meanFare numSurv
##       <dbl>   <int>
## 1       44.5   233

What was the mean fare paid & the survival count for both sexes?

```r
# Calculate mean Fare and count survivors by sex
passengers %>%
  group_by (Sex) %>%
  summarise (meanFare = mean(Fare),
             numSurv = sum (Survived))
```

## # A tibble: 2 x 3
##   Sex    meanFare numSurv
##   <chr>     <dbl>   <int>
## 1 female    44.5   233
## 2 male     25.5    109
What was the mean fare paid & proportion survivors by sex?

```r
# Calculate mean Fare and proportion of survivors by sex
passengers %>%
group_by(Sex) %>%
summarise(meanFare = mean(Fare),
propSurv = sum(Survived) / n())
```

```
## # A tibble: 2 x 3
## Sex meanFare propSurv
## <chr> <dbl> <dbl>
## 1 female 44.5 0.742
## 2 male 25.5 0.189
```

Saving the output file

Go to the `knitr` tab and select ‘Knit to HTML’. RStudio will compile the output document. Once the html document opens, click on Open in browser. In the browser, right click anywhere in the file, select print and save as pdf. Name the pdf Intro to R plus your initials and email it to me. Note that you can output directly to pdf (by selecting ‘Knit to PDF’) but you will need a \{\LaTeX\} compiler to do this. Installation of a compiler is beyond the scope of this lesson.

Make sure you write your responses to the three questions in this file and then save it.

You can ‘knit’ your file several times, if you need to, until you are happy with the contents of the file.

I hope you feel inspired to start your journey with R. There are lots of free resources available online. I highly recommend the free course ’Introduction to R’ from DataCamp https://www.datacamp.com/courses/free-introduction-to-r
6.B Lesson from the field: teleconference slides

Overview

- Free open source stats/graphics software
- Based on software created by IT scientists four decades ago
- Preferred software -> tailored and more sophisticated stat analysis compared to Stata
- Tools/packages developed for epi — OutbreakTools (Jombart et al. 2014)

Your questions

1. What is tidyverse?
2. Are there multiple operating systems to choose from?
3. Can you explain the pipe operator?
4. Does R allow you to use multiple datasets at once?

What is tidyverse?

- Collection of R packages designed for data science (all in one, avoid installation of dozens of packages)
- Has it’s own ‘language’ that is more intuitive than R base (written in the 70’s by IT specialists)

Are there multiple packages to choose from?

- Yes. The list of available packages is huge.
  For example, there are packages specific for creating forest plots, for loading, creating and saving tables, etc.
The pipe operator

- Becomes useful when you need to do many manipulations
- Easier to follow when you are a beginner
- Alternative is a much longer line of code

Does R allow you to use multiple datasets at once?

- Yes!
- It can read xlsx, stata, access files and compile to further manipulate

Rmd files

An R Markdown (.Rmd) file is used to
- Save and execute code
- Build a report of your analysis (embeds results of your code)

Structure
- Chunks of code to run
- Text to display
- Metadata to guide doc building process

Supports many types of high quality, reproducible outputs (reports, thesis, books, presentations, websites, apps, etc).

Doc output > .html, .pdf, .doc...
https://vimeo.com/178485416

Dealing with errors

Google it!
Example
Error in contrib.url(repos, "source") : trying to use cran without setting a mirror calls: <anonymous> ... withVisible -> eval -> eval -> install.packages -> contrib.url Execution halted
Stackoverflow

Dealing with errors

Answer
You don't need install.packages() line every time.
Normally you should install packages in console or a separate interactive session or delete that line after installation of that library (here it's ggplot).
Interpreting Stackoverflow answer when dealing with error

For the Rmd file to ‘knit’ and create the html output
the `install.packages` command must be commented out in the .Rmd file
OR
The `install.packages` is run in the console not the Rmd file

Resources

• R for Data Science (Garrett Grolemund & Hadley Wickham, 2017) e-Book
  http://r4ds.had.co.nz/index.html
• Cheat sheets. You can find them for most R packages
  Rmarkdown cheat sheet:
• Debugging: Google errors. This is how you learn. Stackoverflow will have answers
Learning objectives

1. **Understand** your role as a Field Epidemiologist investigating an outbreak within the context of a complex, fast evolving, humanitarian emergency.

2. **Communicate** to different audiences as a part of your role as Field Epidemiologist.

3. **Work** and **deliver** relevant information under time pressure.
Scenario

You are the Field Epi on deployment to a post disaster zone. There has been a cholera outbreak following a tsunami in a coastal urban centre in a Western Pacific country.

Today, you have three stakeholders that you need to communicate to:

1. Interagency members at daily Situation Report meeting
2. Data collectors at local health care clinic
3. Your incoming replacement Field Epi (another GOARN volunteer)

Lead Field Epi presenting at a daily interagency Situation Report meeting

1. Recent data (24 hours)
2. Any problems? E.i, increase in cases in clinic 4 (they have asked for help), no reporting from clinic 2.
3. In-country situation (is there still an influx of people?)

Method: short talk, use PowerPoint/whiteboard to assist if available
Field epi discussing data collection with local clinic staff (who have stopped reporting)

1. Ask what is going on, and discuss what additional support would be helpful
2. Explain the significance of the outbreak in terms of how it is affecting their local area (local stats so far), and how important their data is in contributing to the bigger picture of solving the outbreak.
3. Develop a feedback loop so they can see how their data is contributing to the big picture (ie brief 1 page epi report disseminated among health care clinics)

Method: Arrange a field visit to discuss in person. Be aware of cultural faux pas!

Handover to the next Field Epi

1. Contact details of MoH, WASH, etc people you were dealing with
2. Explain what stage the work is at
3. Hard and soft copies of all documents

Method: meet in person, connect over email prior if possible, leave soft/hardcopy backups if you can.