Epidemic Modelling Studies of Hendra virus and Coronavirus in Australian Bats

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Submitted in fulfilment of the requirements of the degree of Doctor of Philosophy

June 2017
ABSTRACT

Bats (order Chiroptera) are known as natural reservoir hosts of many emerging zoonotic diseases. The increasing trend in outbreaks of bat-borne emerging zoonotic diseases in recent years poses serious risks to public health. Coronaviruses in bat populations have demonstrated their potential to bring about deadly pandemics, such as SARS (severe acute respiratory syndrome) and MERS (Middle East respiratory syndrome). Hendra virus in Pteropus spp. (fruit bats or flying foxes) is a lethal zoonotic virus that has repeatedly emerged to infect horses, leading to fatal human infections in eastern Australia. However, more research has been needed on mechanisms how bats maintain zoonotic pathogens in their populations and on factors that stimulate the reservoir hosts to excrete the pathogens. This knowledge would help understand the spillover mechanism and manage the diseases effectively in their natural reservoir hosts before the diseases spillover. This thesis explores the transmission dynamics of bat-borne viruses (coronavirus and Hendra virus) in their natural reservoir hosts of bats, by employing mathematical epidemic models to simulate the dynamics.

Chapter 1 commences with the story of the emergence of Hendra virus. From the story, particular questions are extracted. I review the knowledge previously available to answer those questions and explain how approaches for mathematical modelling of infectious diseases can be used to study these topics. Relevant information on bat biology and ecology is suggested. Management strategies for bat zoonotic diseases are also previewed. Finally, the aims and structure of the thesis are outlined.

Chapter 2 analyses the effect of persistent infection on coronavirus maintenance in a population of Australian bats (Myotis macropus). By using a previously performed
capture-mark-recapture (CMR) study, more intensive mathematical methods were employed. The multi-model selection processes supported the notion that it is appropriate to divide coronavirus infectious bats into two groups of persistently infectious and transiently infectious bats, based on the infectious period. The epidemic models predicted that the grouping of bats increases the probability of coronavirus maintenance in the bat population.

Chapter 3 explores the effects of maternally-derived immunity in seasonally breeding wildlife on epidemic patterns by using a system of Hendra virus infection in black flying foxes (*Pteropus alecto*). Deterministic models were used to simulate epidemics, which were characterised by a variety of timings of viral introduction and a range of pre-existing herd immunities. Waning maternally-derived immunity dispersed the timing of supply of susceptible individuals from births and losses of maternally-derived immunity and thereby diluted the effect of seasonal breeding on epidemics. The dispersion of timing increased the probability of viral persistence and contributed to shifting the timing of epidemic peaks further away from the peak of a birth pulse.

Chapter 4 numerically examines whether a metapopulation of flying foxes (*Pteropus* spp.) can support the maintenance of Hendra virus. The implications of metapopulation structure of flying foxes on Hendra virus dynamics needs more investigations. A single population of flying foxes in the context of a metapopulation structure was stochastically simulated to repeat the cycle of viral extinction and recolonisation in the population. The simulation results predicted that viral recolonisation should occur more frequently than extinction in a colony in a metapopulation, supporting the hypothesis
that the metapopulation structure of flying foxes can maintain long-term persistence of Hendra virus.

Chapter 5 examines the effects of culling and dispersal of flying foxes on the spillover risk of Hendra virus. Metapopulation models were simulated stochastically using various culling and dispersal scenarios. The models used the most favourable possible assumptions about Hendra virus epidemiology for the application of these management strategies. Nevertheless, many scenarios were predicted to be counter-productive in reducing the spillover risk of Hendra virus. Even though the scenarios expected positive effects on decreasing the spillover risk, the degree of benefits was not realistic if the cost was considered. I, therefore, concluded that culling or dispersal were not effective strategies to manage Hendra virus spillover.

Chapter 6 describes the findings provided in each chapter. Then, I discuss the findings, focusing on the viral dynamics in reservoir populations of emerging infectious diseases. Based on the dynamics, I suggest the disease management strategies. I discuss how to do proper modelling research using insufficient data on wildlife diseases. Finally, this chapter provides suggestions for further research.
STATEMENT OF ORIGINALITY

This work has not previously been submitted for a degree or diploma in any university.
To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

(Signed) __ (Date) 15 June 2017_________

Jaewoon JEONG
ACKNOWLEDGEMENT

First and foremost, I am deeply appreciative of my principal supervisor, Hamish McCallum. This work could not have been possible without his guidance and encouragement. His comments will remain in my mind while I am building my career as a researcher. I would also like to thank my supervisor, Alison Peel. She took great care of my research and has always provided attentive advice all the time. I am very grateful to my supervisor, Raina Plowright. She has offered me thoughtful comments on my thesis. In particular, her PhD work and papers have been the great stepping stones for my research. If my work can be a stepping stone for another researcher as hers was for me, it would be the greatest honour for me. I would also like to thank James McBroom. His assistance for Bayesian methods was essential for the Chapter 2. Moreover, while I have been tutoring his statistics course, I was able to learn the precious value of teaching at the university level.

I am grateful to Craig Smith. His sharing of coronavirus data enabled the study of Chapter 2. I also thank Olivier Restif. His interest in chapter 3 has been a great driving force for me to proceed the topic. I thank Peggy Eby. Her work on the data on Movebank was essential for me to conduct Chapter 5.

I have been so pleased and proud of being a member of the excellent research team, “Griffith Wildlife Disease Ecology Group.” I would like to thank all members, Doug, Konstans, Laura, John, and Nick. I am also thankful for Environmental Futures Research Institute for supporting my research.
While I have been working as a PhD student here, I have been greatly thankful for that I have all I need for doing my research. I have enjoyed the benefits. In return, I did my best to fulfil my responsibility. As I generate this thesis as an output of my PhD study, I hope that the input invested for me was worthwhile.

Finally, and most importantly, I want to thank my family. My mother! Her love remains on me, and it will do forever. My father, who has always supported and trusted me, is another author of this thesis. I hope my sister’s family had a fantastic break in Australia. I believe that my sister enjoyed the benefit of having a brother who lives overseas. My best friend, Yong-ki, has contributed to this thesis by kindly responding to my nagging text messages.
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STATEMENT OF CONTRIBUTION TO CO-AUTHORED PUBLISHED PAPER

Chapter 2 in this thesis includes a co-authored paper. The bibliographic details/status of the co-authored paper, including all authors, are:


My contribution to the paper involved: formulation of the study, most of the computer programming and analysis of results, and leading of writing and editing of the manuscript.

(Signed) __________ (Date) 14 June 2017

Jaewoon JEONG

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(Countersigned) _______ (Date) ______ 14 June 20017

Supervisor: Hamish McCallum

Note that Chapters 3-5 have also been prepared a form suitable for publication, and hence there is some repetition in the introductions to each of these chapters. They will xvi
require further input from the supervisory team before publication. In each case I was responsible for design of the study, computer programming and analysis, and writing the chapters.
CHAPTER 1

1. General Introduction

Bats (order Chiroptera) are increasingly recognised as natural reservoir hosts of emerging infectious diseases that can be transmitted to and can cause fatal illnesses in other mammalian species including humans (Dobson 2005, Calisher et al. 2006, O'Shea et al. 2014). The significance of bat-borne viruses for human public health emphasises the need to research bat virus dynamics. This thesis uses compartmental mathematical models to explore the transmission dynamics of one such virus, Hendra virus, with an emphasis on understanding the processes that enable its maintenance in flying fox (genus Pteropus) populations. In addition, I analyse a unique mark-recapture dataset on a coronavirus to further examine maintenance of viral infections in bat populations.

1.1. HENDRA VIRUS OUTBREAKS

In 1994, thoroughbred horses in a training complex in Hendra, Queensland, Australia showed acute respiratory syndrome. During the outbreak, 18 horses became ill and 14 horses subsequently died (Selvey et al. 1995). A trainer and a stablehand who were closely involved with infected horses were infected, and the trainer died (Selvey et al. 1995). In the same year, there was also an outbreak of the same virus in Mackay, Queensland, which is approximately 1000 km north of Hendra, Queensland. In the outbreak, two horses and one person died (Field et al. 2001). At that time, this virus was completely novel. The virus was initially named as equine morbillivirus (Murray et al.
CHAPTER 1.

1995), but later re-named as Hendra virus after the suburb where the disease first broke out (Young et al. 1996). After the initial outbreak of Hendra virus, serosurveys of wildlife were conducted to identify the natural reservoir hosts of the virus, but no evidence was found in 168 individuals of more than 16 species such as rodents, marsupials, birds, amphibians and insects (Field et al. 2001). Flying foxes were identified as potential reservoirs for a number of reasons: their distributions include outbreak locations, they often migrate long distances, and they can easily be in contact with horses (Young et al. 1996). Finally, in 1996, anti-Hendra virus antibodies were identified in four species of flying foxes: black flying foxes (Pteropus alecto), grey-headed flying fox (P. poliocephalus), spectacled flying fox (P. conspicillatus), and little red flying fox (P. scapulatus) (Young et al. 1996). As a result, it was demonstrated that flying foxes are the natural reservoir hosts of Hendra virus.

Black and spectacled flying foxes are more likely to be associated with Hendra virus spillover than grey-headed and little red flying foxes, because of the correlation between spatial distribution of each species and the locations of Hendra virus outbreaks (Smith et al. 2014) and the higher Hendra virus positivity rates in the black and spectacled flying foxes than in the other two species (Field et al. 2015). Hendra virus has claimed more than 70 horses in east Australia, with a 75% case fatality rate (Field et al. 2011), and the virus has claimed 4 out of 7 human cases as of July 2016 (World Health Organization 2017). While the number of cases is not substantially high, the case fatality rate is markedly high in horses and humans (Breed et al. 2006). The high fatality in humans emphasised the significance of the management of Hendra virus for public health. Hendra spillover events have occurred mostly in the east coast of Queensland and northeast New South Wales, and the frequency of occurrences peaked in 2011 (Plowright et al. 2015).
1.2. QUESTIONS FROM HENDRA VIRUS OUTBREAKS

From the Hendra virus outbreaks, I suggest three issues that are important and significant to improve our understanding of Hendra virus transmission dynamics in flying fox populations and thereby to contribute to the reduction of the spillover risk. These three questions are based on current knowledge and refer to areas that require more attention.

The first is why spillover events of Hendra virus are seasonally clustered in the subtropics. In south-east Queensland and north-east New South Wales, all spillover events have occurred in the cooler months, in contrast to tropical North Queensland where a seasonal pattern is not as apparent (Plowright et al. 2015). Exploration of factors stimulating spillover during the spillover season and inhibiting spillover during the non-spillover season is expected to improve our understanding of the underlying mechanism of spillover. A variety of factors encompassing environment, horses, and flying foxes might be involved in the seasonal clustering of spillover events. For example, virus survival outside hosts in the environment might be affected by temperature or humidity (Martin et al. 2015, Martin et al. 2017). Horses and flying foxes may have seasonally different behavioural characteristics and thereby may generate seasonally different contact frequencies (Smith et al. 2014). This thesis concentrates on seasonal breeding of flying foxes. Seasonal breeding and subsequent waning maternally-derived immunity make the population size and structure fluctuate on a seasonal basis. Because transmission dynamics is strongly affected by the population structure, it is necessary to investigate how the seasonality of breeding activities affects the seasonal pattern of spillover events (Altizer et al. 2006). Moreover,
temporal prediction of the high spillover risk would allow management resources to be concentrated on much-needed situations.

The second question concerns the role of metapopulation structure of flying foxes for the maintenance of Hendra virus. A metapopulation is a network of habitat patches, in which individuals form discrete local populations connected by migration (Hanski and Gilpin 1997). As flying foxes are the natural reservoir hosts of Hendra virus (Halpin et al. 2000), mechanisms must exist to enable indefinite maintenance of the virus in flying fox populations, although flying foxes have unfavourable conditions to maintain Hendra virus in their populations. An adverse condition is a short infectious period (7 (95% CI: 4, 10) days (Plowright et al. 2011)) that limits the likelihood of Hendra virus transmission in the bat populations (Halpin et al. 2011). In addition, lifelong immunity has been presumed for flying foxes against Hendra virus infection, based on evidence that long-lived antibodies were detected in captive flying foxes (Field 2005). These factors made it difficult to envision how Hendra virus is maintained in bat populations. Not only is the short infectious period a disadvantage for maintenance of infection to be due to fewer number of infectious individuals, but also lifelong immunity inhibits maintenance because there is no supply of susceptible individuals from a recovered pool (Vynnycky and White 2010). Although no conclusive results have been obtained, studies have suggested several possible maintenance mechanisms: endemic infection (Breed et al. 2011); metapopulation structure (Plowright et al. 2011); and recrudescent infection (Wang et al. 2013). In particular, metapopulation structure has been suggested to play a vital role in the transmission dynamics of bat populations (Hayman et al. 2013) and it would be necessary to investigate the effects of metapopulation dynamics on Hendra virus maintenance in flying fox populations. The establishment of a maintenance mechanism for a virus in its reservoir populations is a fundamental step to
explore ongoing transmission to target hosts. Furthermore, the understanding of these mechanisms may help to identify potential interventions to reduce transmission to target hosts such as horses and humans.

Similarly, the mechanisms by which coronaviruses are maintained in bat populations are not clear. The significance of bat coronavirus as an emerging infectious disease has been well demonstrated by pandemic SARS (severe acute respiratory syndrome) and MERS (Middle East respiratory syndrome) (Hilgenfeld and Peiris 2013). It would be valuable to monitor coronaviruses that have potential to transmit in other species. CMR (capture-mark-recapture) data of Australian bats (*Myotis macropus*) with coronavirus infection state became available (Smith 2015). The data are rare and valuable in that it is difficult to obtain longitudinal demographic data including virus infection state. Thus, the CMR data can be used to confirm whether bats can be grouped into persistently or transiently infectious bats and whether the grouping improves the persistence of coronavirus in a bat population.

The third question is how the Hendra virus spillover risk would be affected by the population management strategies of flying foxes. Identification of the reservoir hosts of Hendra virus brings up the issue of managing the reservoir hosts to reduce the frequency of spillover events. Considering that flying foxes are abundant free ranging wildlife, individual management of flying foxes is not a plausible option. Instead, population management may be available. However, it is important to acknowledge that controlling wildlife populations is not an easy task, and is one that can have major flow-on effects on ecosystem functioning. Therefore, it is important to emphasise that detailed assessment of control strategies must precede their execution. Otherwise, the
controls may result in unacceptable and irreversible damage to the natural environment and may aggravate the situations in which wildlife diseases emerge.

1.3. EMERGING INFECTIOUS DISEASES

The significance of infectious diseases is well demonstrated by the statistic that more than 20% of annual deaths worldwide in humans are estimated to be directly related to infectious diseases (Morens et al. 2004, 2008). The striking feature of infectious diseases over the past several decades has been the substantial increase in the number of outbreaks of emerging infectious diseases (Jones et al. 2008). Emerging infectious diseases are defined as “infectious diseases that have newly appeared in a population or have existed previously but are rapidly increasing in incidence or geographic range” (Morse 2001). The majority of emerging infectious diseases originate from wildlife (Jones et al. 2008). This emphasises the importance of studying pathogens in wildlife reservoir hosts that have a potential to cause fatal infectious diseases in domestic species or humans.

Transmission of a pathogen between different host species is hindered by species barriers (Woolhouse et al. 2005). However, some pathogens jump the species barriers and transmit to new host species (Parrish et al. 2008). Emerging infectious diseases are often those that can infect multiple host species, seldom cause clinical symptoms in their original host species, but tend to cause serious illnesses in the new host species, bringing about critical public health concerns (Woolhouse et al. 2001). The multiple species infected by emerging pathogens can be classified into ‘reservoir’ and ‘target’ host species. A reservoir is a host species that can indefinitely maintain a pathogen and occasionally transmit the pathogen to other species, and a target is another host species
that is occasionally infected and does not maintain the pathogen (Haydon et al. 2002). The definition of reservoir depends on two features: ‘indefinite maintenance’ and ‘source of infection for target’ (Haydon et al. 2002). The correct identification of reservoir, based on the two features, is a foundational step to understanding emerging infectious diseases (Viana et al. 2014).

Regarding the first feature, the maintenance mechanism of pathogens in reservoir hosts is an important issue to study. Understanding the transmission dynamics in reservoir populations has been crucial in progressing our understanding of emerging infectious diseases (Haydon et al. 2002, Calisher et al. 2006). When there has been an outbreak of emerging infectious diseases originating from wildlife, studies have ensued to reveal the maintenance mechanism of the pathogen in the wildlife reservoir hosts. For example, Hayman (2015) proposed that filoviruses were maintained within African fruit bat populations because of biannual birth pulses that allowed the population structure to support viral persistence, and George et al. (2011) suggested that rabies virus was maintained in big brown bats (Eptesicus fuscus) because of long incubation periods that allowed the virus to persist during hibernation. It is important to recognise that there is no universal rule for virus maintenance mechanism in bat-borne infectious diseases. The maintenance mechanism of Hendra virus also needs to be investigated in the context of the virus epidemiology and the reservoir host ecology.

The second feature of reservoir hosts, ‘source of infection for target’, is related to spillover, that is, the invasion of infectious agents from a reservoir host into a new host (Keesing et al. 2010). Even if a deadly virus is retained by wildlife hosts, they are not reservoir hosts unless transmission to another species occurs. Multiple barriers must be
overcome for the spillover to occur, and the barriers are related to reservoir hosts, target hosts, and environment (Plowright et al. 2017). Therefore, it is necessary to identify the conditions to which interventions can exert to reduce spillover events. The identification can contribute to the discovery of how we can suppress spillover.

1.4. MATHEMATICAL MODELLING OF INFECTIOUS DISEASES

Research into emerging infectious diseases can be categorised into three types: laboratory, field, and modelling studies. This thesis utilises the modelling, which has the potential to investigate the complex systems of emerging infectious diseases (Heesterbeek et al. 2015). The factors related to emerging infectious diseases are often diverse and layered, with the systems involved ranging from reservoir hosts to target hosts and from molecular to global scales (Plowright et al. 2008b). Mathematical modelling is an appropriate tool to explore this apparently intractable complexity by simplifying the system to reveal its features. Besides, another beauty of modelling studies comes from the fact that models do not use actual subjects but use virtual subjects. Studying wildlife diseases by directly contacting wildlife has limits due to the unmanageable nature of wildlife. However, models using virtual subjects can be free from this disadvantage. Particularly when the research topics are related to a large number of populations that interact each other to transmit pathogens, the interaction can be studied by models but not by experiments. The models allow us to explore the effects of one or a few factors on the results of interest while eliminating the effects of other factors on the results. This feature of modelling can simplify the system to make the elements of interest stand out, and it can elucidate the mechanisms of the system.
Models of infectious diseases can be used for either prediction or understanding (Keeling and Rohani 2008). When the primary aim of a study is to predict, we need to design models that are as accurate as possible by using exact parameter values and model structure. When the main objective is understanding, we need to design transparent and straightforward models to clarify how diseases behave. This thesis aims to advance the understanding of two emerging infectious diseases of bats, a coronavirus and Hendra virus, the dynamics of which remain obscure. The level of accuracy with which it is possible to estimate parameter values and specify model structure is not great. Therefore, the overall emphasis of the models in this thesis is on understanding how the emerging infectious diseases behave, by developing transparent and simple models, rather than to predict the impact of the diseases.

In this thesis, information from empirical studies, inferences from conceptual studies, and findings from previous modelling studies are all integrated to build models. I use epidemic compartmental models, in which hosts are categorised based on their infection state, rather than being individually modelled. For example, individuals in the population are classified into ‘susceptible’, ‘infected’, or ‘immune’ compartment, and each individual in a compartment it is assumed to have the same average characteristics (Heesterbeek et al. 2015). The transition of individuals between compartments is calculated by using ordinary differential equations, in which individuals move between compartments depending on the rate of change between compartments (Hethcote 2000). This modelling framework is relevant to exploring the effects of population dynamics on transmission dynamics rather than of investigating within-host virus ecology. After choosing the modelling framework, it needs to identify key parameters (Russell et al. 2017). This thesis concentrates on the properties of the social behaviour of the bat population. These properties substantially affect the transmission dynamics by
determining the contact patterns among individuals (Craft and Caillaud 2011). For example, wildlife hosts often have seasonally different behaviour patterns or spatially uneven distribution, both of which need to be considered to simulate the transmission appropriately.

1.5. BATS AND BAT INFECTIOUS DISEASES

Bats are the second most common mammalian order, after rodents (Ng and Baker 2013). Bats are distributed virtually worldwide and have a diverse range of ecological attributes (Calisher et al. 2006). The fact that bats host more zoonotic viruses per species than even rodents (Luis et al. 2013) exemplifies that bats are a major source of emerging zoonoses for humans. Bats are the known or suspected natural reservoirs for filovirus, SARS coronavirus, Hendra virus, Nipah virus, and rabies viruses (Wood et al. 2012). The increasing trend of outbreaks of bat-borne zoonotic diseases means that understanding of the ecology and epidemiology of bat viruses is more urgent and essential than ever (Ng and Baker 2013).

Many studies have explored why bats are particularly involved in emerging infectious diseases of humans, and several reasons have been suggested (Dobson 2005, Calisher et al. 2006, Luis et al. 2013, O'Shea et al. 2014, Brierley et al. 2016). The reasons can be categorised into bats’ role in harbouring many viruses and their role in transmitting these viruses to other species. Regarding the role of harbouring, first, it has been proposed that certain viruses do not cause clinical symptoms in bats, while the viruses are highly pathogenic for humans and other vertebrates (Calisher et al. 2006). Bats have experienced long term co-evolution with their viruses and as a result many viruses do not cause substantial pathogenicity in bats (Halpin et al. 2007, Halpin et al. 2011).
During the long processes of co-evolution with pathogens, bats have developed an unique immune mechanisms associated with the control of viral replication (Bean et al. 2013). Identification of a large number of unique bat characteristics related to immunity and the antiviral response showed that bats are able to control viral replication very early in the immune response through innate antiviral mechanisms (Ng and Baker 2013, Zhang et al. 2013). Second, the flying ability of bats may contribute to developing unique physiological characteristics. Higher body temperature during flight may mimic a fever response caused by viral infections and help them control viral infections (O'Shea et al. 2014). Third, reduced body temperature and metabolic rate during hibernation can delay the clearance of viruses from bats (George et al. 2011, Wang et al. 2011). Fourth, bats have a high contact rate within their large populations, many living in very high-density colonies. The high contact rate contributes to increasing the probability of persistence of pathogens with relatively low transmissibility that may fail to persist in other species of hosts. (Kuzmin et al. 2011, Holmes 2013). Regarding the role of transmission to other species, first, bats are volant mammals, making it more difficult for people to create barriers between bats and livestock. Bats are more likely to share zoonotic agents with other mammalian species (including domestic animals and humans) than are other volant species (such as birds). Second, some bats, particularly flying foxes, are nomadic (Roberts et al. 2012b). This high mobility allows bats to have more opportunities to exchange novel variants of viruses with other bat species or even other mammals (Calisher et al. 2006). Regarding both roles, bats have relatively long lifespans compared to other similar sized mammals, providing more favourable conditions to develop persistent infections and more opportunities to transmit viruses to other hosts (Calisher et al. 2006).
CHAPTER 1.
Understanding of bat biology is necessary to understand the diseases bats harbour. An important aspect of bat biology that may affect viral dynamics is that bats often breed seasonally. Because seasonal breeding results in fluctuating population structure, varying contact rate, or unstable immune system, it has been suggested as a crucial factor affecting bat virus diseases (Groseth et al. 2007, George et al. 2011, Hayman et al. 2012). However, the effects of fluctuating population on transmission are not straightforward. An annual pattern of population dynamics may generate annual or biannual patterns of prevalence (Altizer et al. 2006). Moreover, if immune female hosts give their newborns passive immunity, the viral dynamics in the host populations would become more complicated. Therefore, to understand seasonality of infectious diseases in bats, the seasonal factors need to be understood comprehensively (Grassly and Fraser 2006).

Bats often live in periodically interacting and spatially discrete subpopulations forming metapopulations (Calisher et al. 2006). Migration of bats among colonies in a metapopulation has been found to be a major driver of bat disease dynamics (Hayman et al. 2013). Particularly, the metapopulation structure of bats has been reported as a contributor in maintaining pathogens, where otherwise extinctions may occur (Hayman et al. 2013). Metapopulations are an important concept in flying fox ecology due to their high mobility. Flying foxes move between feeding sites and roosting sites daily, and they may migrate long distances to seek seasonally and interannually varying food resources. To describe aggregations of bats, “colony”, “roost”, and “camp” have been used interchangeably, which has led to some confusion in the literature. In this thesis, I principally use “colony” to indicate the aggregation of bats, and “roost” is used only to describe the sites where bats rest and sleep during day. The usage of “camp” has been restricted. Also, I assume that a bat metapopulation consists of connected colonies.
In metapopulation structures, pathogen persistence could be greatly facilitated by an ensemble of various forms of epidemics across subpopulations (Earn et al. 1998). The ensemble means that local extinction does not extend to global extinction. This phenomenon can be described by using the ecological terminology of a ‘rescue effect’, which means that areas of local extinction of individuals can be recolonized by migration of new individuals into the areas (Brown and Kodricbrown 1977). Likewise, the rescue effect concept can be used in the perspective of epidemiology, meaning that a subpopulation in which hosts become free of pathogens can be re-infected by the pathogens with the migration of infectious hosts from other subpopulations. The rescue effect is a fundamental concept of viral maintenance and epidemic spread in metapopulation structures. The impact of the rescue effect needs to be explored in bat metapopulation structures to improve our understanding of bat virus dynamics. In particular, flying foxes are highly mobile and travel long distances, which may facilitate the rescue effect, playing a major role in the transmission dynamics (Plowright et al. 2011).

1.6. MANAGEMENT STRATEGIES FOR INFECTIOUS DISEASES OF BATS

Models can be used to provide formal guidelines for the management of wildlife diseases (McCallum 2016). Simulation of various scenarios of management strategies can be used to compare the relative efficacy of the scenarios, and the simulation can help choose optimal strategies (Russell et al. 2017). There are logistical, ethical and ecological limitations in experimental studies dealing with real wildlife. In contrast, modelling studies that do not result in actual manipulation of wildlife can benefit from
simulating virtually without the concerns of cost and conservation (Beeton and McCallum 2011).

Wildlife species that harbour zoonotic agents have often been the subject of management actions (Mathews 2009). Wildlife disease management strategies can be categorised as focusing on either prevention, control, eradication, or doing nothing (Wobeser 2002). First, prevention strategies aim to block the introduction of pathogens to unaffected populations. Second, control strategies aim to reduce the impact of infectious diseases to an acceptable level. Third, eradication strategies aim to remove infections completely. Last, doing nothing is not attempting any active management. Deciding which among these management strategies is the optimal approach should depend on individual situations, such as the reasons for management, available techniques, logistical cost, and a likelihood of success (Wobeser 2002). Among those strategies, this thesis focuses on “control” through population management of bats because reducing the spillover of virus from bats is a reasonable goal. Blocking spatially migratory bats is an unrealistic goal and eliminating spillover is unlikely to be achievable.

Reduction of Hendra virus spillover through direct management of flying fox populations has been considered (Martin and McIlwee 2002, Roberts et al. 2011). Such management has also been motivated by other reasons than Hendra virus, such as protection of fruit industry or mitigation of conflicts between humans and flying foxes related to perceptions of foul odour, noise, and droppings (Kung et al. 2015). Implementation of management actions without taking Hendra virus into account may transform the population structure in a manner that may stimulate the spillover of
Hendra virus. Therefore a compelling need to assess the effect of the management strategies on the spillover risk.

Although culling of flying foxes in Australia has been proposed by some groups to reduce Hendra virus spillover risk, previous examples of culling in other bat infectious diseases have showed unpromising results. A simulation study that investigated culling of bats for management of White-Nose Syndrome determined that culling was not an appropriate measure to reduce the prevalence because the contact rate among bats is too high, contacts take place at multiple locations and bats keep moving periodically (Hallam and McCracken 2011). Culling of vampire bats in Peru has been used in attempts to control human rabies outbreaks, but modelling studies found out that culling the vampire bats is not a successful method to control the transmission of rabies virus from bats to humans, because localised culling was not effective in managing the highly mobile species (Streicker et al. 2012a, Blackwood et al. 2013). Also, although juvenile and sub-adult bats were potentially more important for transmission, the culling action removed preferentially adult bats (Streicker et al. 2012b). An attempt to eradicate a population of Egyptian fruit bats (*Rousettus aegyptiacus*) to control Marburg virus ended up significantly increasing prevalence of the virus after the cull (Amman et al. 2014). These unsuccessful implementations of culling bats emphasise the importance of well-designed modelling studies that can predict the benefits and costs of culling flying foxes before the culling actions. In particular, improved prediction of the results of bat culling by using modelling studies is expected to ameliorate the social conflicts between proponents and opponents of bat culling (Franklin 2016). Also, the culling of wildlife should be carefully considered in that it causes changes in complex ecosystems.
Another strategy proposed to manage flying fox populations is dispersal, which aims at moving bats from a region where they are more likely to cause conflicts with people to an area where they are less liable to cause conflicts (Tidemann et al. 1999). Dispersal is arguably more humane and ethical than culling and might be a more ethically and ecologically acceptable strategy to culling (Phillips et al. 2007). Unlike other bat species, for which dispersal has rarely been considered, colony dispersal has been more often reviewed and implemented than culling for flying foxes in Australia. This is because the growing number of flying foxes in urban areas is decreasing amenity for people and therefore many people want to disperse flying foxes away from their houses, parks etc (Tait et al. 2014). However, unfortunately, trials of relocation of flying foxes into new colonies have often produced poor results, for several reasons (Roberts and Eby 2013). First, it has been difficult to make bats move to proposed regions. Conflicts can be aggravated if bats move to unexpected regions. Second, conflicts between neighbourhoods are likely to occur, because few neighbourhoods want bats – perceived to be noisy, smelly, and carrying zoonotic disease – close to their homes. It is hard to choose acceptable regions to move bats. Last, even ‘successful’ dispersal is often only effective temporarily, because bats have a high attachment to their original habitats and they keep trying to come back to their original habitat (Roberts et al. 2011). Continued efforts are likely required to prevent the dispersed flying fox from re-establishing their colonies (Phillips et al. 2007).

1.7. AIMS AND STRUCTURE OF THIS THESIS

The overarching objective of this thesis is to provide new information on the epidemiology and ecology of emerging infectious diseases. For that purpose, this thesis uses bat viral diseases in Australia. Mathematical modelling was employed to simulate
the transmission of the viruses in the bat populations. The modelling results are relevant to the maintenance mechanism and management strategies of other emerging infectious diseases.

While it mostly focuses on Hendra virus, this thesis also considers a related example of coronaviruses in Australian bats. The chapters describe the persistence mechanisms of coronavirus and Hendra virus and the factors that trigger epidemics in their reservoir host populations. Persistence and epidemics are important to understand spillover mechanisms and to prepare appropriate management strategies to prevent spillover.

The thesis consists of six chapters: four chapters that address the four objectives listed above, this general introduction, and a general discussion. Chapters 2 to 5 are structured as manuscripts for publication. As these are intended to be stand-alone publications, there is a certain amount of repetition in the introductions to these chapters. More specifically, the ensuing chapters are summarised as follows:

Chapter 2 examines the persistent infection of coronavirus in Australian bats (Myotis macropus). It quantitatively analyses the capture-mark-recapture data of M. macropus with coronavirus infection status. It examines whether the grouping of infectious bats into persistently infectious bats and transiently infectious bats based on their infectious period is statistically valid. Then, it simulates the transmission of coronavirus in varying scenarios to determine whether the persistent infection improves the probability of the virus maintenance in a population of M. macropus.
Chapter 3 explores the effects of seasonal births and waning maternally-derived immunity on epidemic patterns. This chapter is motivated by the seasonal clustering of the Hendra virus spillover events during austral winter in the southern subtropics in Australia. It develops deterministic models to simulate invasion and persistence of Hendra virus in a population of black flying foxes (*P. alecto*). In particular, it examines how the timing of viral introduction into the population affects the timing of epidemic peaks.

Chapter 4 explores how a metapopulation consisting of subpopulations smaller than critical community size can support viral maintenance. It simulates stochastically the circulation of three stages that occur in the process of Hendra virus extinction and recolonization in a colony. It focuses on a single colony of flying foxes, but in the context of a metapopulation. By using Levins’ (1969) metapopulation model, this chapter compares the recolonization rate and the extinction rate to evaluate the plausibility of metapopulation structure as a mechanism for viral maintenance.

Chapter 5 examines how a variety of metapopulation structures differently affect epidemic occurrence. This chapter is inspired by the interest to assess the effectiveness of culling and dispersal of flying foxes on managing the spillover risk of Hendra virus. It stochastically simulates how triggering of an epidemic is affected by metapopulation-related factors. It investigates how likely it is that the introduction of infectious hosts into an infection-free metapopulation can trigger an epidemic in various metapopulation structures that may be formed by culling and dispersal scenarios.
Chapter 6 integrates the findings from previous chapters in the perspective of emerging infectious diseases. It discusses desirable management strategies of flying fox populations. It also explains how to create productive models with insufficient data of wildlife diseases. It describes significant knowledge gaps to be filled and suggests further studies by which this thesis should be followed.
CHAPTER 2

2. Persistent infections support Maintenance of a Coronavirus in a Population of Australian Bats (Myotis macropus)

2.1. ABSTRACT

Understanding viral transmission dynamics within populations of reservoir hosts can facilitate greater knowledge of the spillover of emerging infectious diseases. While bat-borne viruses are of concern to public health, investigations into their dynamics have been limited by a lack of longitudinal data from individual bats. Here, we examine capture-mark-recapture (CMR) data from a species of Australian bat (Myotis macropus) infected with a putative novel Alphacoronavirus within a Bayesian framework. Then, we developed epidemic models to estimate the effect of persistently infectious individuals (which shed viruses for extensive periods) on the probability of viral maintenance within the study population. We found that the CMR data analysis supported grouping of infectious bats into persistently and transiently infectious bats. Maintenance of coronavirus within the study population was more likely in an epidemic model that included both persistently and transiently infectious bats, compared to the epidemic model with non-grouping of bats. These findings, using rare CMR data from longitudinal samples of individual bats, increase our understanding of transmission dynamics of bat viral infectious diseases.
2.2. INTRODUCTION

Coronaviruses have been increasingly recognized as a human public health issue following the emergence of high-impact zoonotic diseases from bats—the mammalian order that hosts the largest diversity of coronaviruses (Drexler et al. 2014). Examples include the coronaviruses that caused severe acute respiratory syndrome (SARS), which claimed 916 lives out of 8,422 cases from November 2002 to August 2003 (World Health Organization 2003), and Middle East respiratory syndrome (MERS), which has claimed 608 lives out of 1,449 cases as of 31 August 2016 (ProMED-mail. 2016).

Severe acute respiratory syndrome-like coronaviruses (SL-CoV) are maintained in bats (Li et al. 2005), and Middle East respiratory syndrome coronavirus (MERS-CoV) is assumed to have originated in bats (Memish et al. 2013). The findings that genetically diverse SL-CoV strains share high similarity with severe acute respiratory syndrome coronavirus (SARS-CoV) and that bats harbour diverse coronaviruses which can be classified to the same coronavirus species as MERS-CoV suggest that recurrent coronavirus epidemics and pandemics in humans are likely (Hu et al. 2015). Genetic analyses of coronaviruses in bats suggested that the diversity of coronaviruses in bats may provide further opportunities for spillover into other species (Osborne et al. 2011). Mitigation of spillover of the viruses is based on understanding of the maintenance mechanism of multi-host viruses in reservoir hosts.

Bats may have idiosyncratic immune responses, allowing them to be infected (with no signs of disease) with viruses that are highly pathogenic in other species (Baker et al. 2013). This feature of bats may contribute to their ability to host zoonoses, including SARS, MERS, Ebola, Marburg, Nipah, and Hendra viruses. A number of hypotheses have been proposed to explain this: some authors suggest that bats’ ability to fly induces fever, helping to control viruses (O'Shea et al. 2014); others suggest that immune
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system adaptation allows tolerance of intracellular pathogens (Brook and Dobson 2015) and that co-evolution during a long history between specific viruses and bat hosts results in no pathogenicity to bats whereas recent host shifts from bats to other mammals result in high fatality rates (Calisher et al. 2006, Cui et al. 2007).

Two conditions must be met for bats to be reservoir hosts of emerging infectious diseases: first, the ability to maintain pathogens in their populations, and second, the ability to transmit those pathogens to another species. This chapter concentrates on the first of these conditions. Features that may contribute to viral maintenance in bat populations include: 1) a metapopulation structure that avoids viral extinction across the total population via sub-populations, which allow reinfection through movement (Drexler et al. 2011, Plowright et al. 2011); 2) reduced metabolism during hibernation that facilitates viral survival (George et al. 2011); and 3) persistent infections with chronic shedding or intermittent recrudescence (Sohayati et al. 2011, Peel et al. 2012). This study focuses on the feature of persistent infection. Persistent infections of SARS-CoV \textit{in vitro} studies support the plausibility of persistent infections of coronaviruses in bats (Chan et al. 2004, Mizutani et al. 2005). In persistent infections, a virus is not cleared from the host but remains associated with specific cells for a long period. Regardless of the mechanisms operating within hosts, persistent infection contributes to viral maintenance within populations and has been considered as a mechanism of viral maintenance in reservoir hosts of other emerging infectious diseases (Plowright et al. 2016).

This study is based on data obtained in a previously-reported capture-mark-recapture (CMR) study (Smith 2015) of a maternal roost of \textit{Myotis macropus}. This microbat
Persistent infection of coronavirus 

(Microchiroptera), also known as large-footed myotis, is widely distributed in Australia (Campbell 2009). *M. macropus* inhabits areas close to waterways in small groups (Smith 2015), foraging on aquatic invertebrate and small fish (Campbell 2009). The bats in the study region form maternity roosts biannually in October and January, and deliver single young (Smith 2015). Gestation and lactation periods are 12 and eight weeks, respectively (Smith 2015).

Smith undertook a CMR study of *M. macropus* in the lifting holes of a bridge in south-east Queensland, Australia (Appendix 2-1) (Smith 2015). Full details of the methodology are described in the original study (Smith 2015). Briefly, the CMR data of 52 Australian bats (*M. macropus*) were collected during 9 capturing occasions over 12 weeks from 13 January to 31 March 2009, which overlapped with the January breeding season and subsequent lactation period for this species (Smith 2015). Coronavirus RNA was detected in faecal pellets or anal swabs using reverse transcription polymerase chain reaction (RT-PCR) targeting a conserved region of a coronavirus gene (Smith 2015). Of the 52 unique individuals, 42 were recaptured at least once, and of these, 7, 16, and 19 bats respectively showed multiple detections, a single detection, and no detection of coronavirus RNA (Table 2-1). The data from bats with multiple detections of coronavirus RNA were suggestive of persistent infections (Smith 2015). The CMR dataset is unusual and particularly valuable in that the data contain individual tracking records with infection states. Longitudinal sampling of individual bats is necessary to explore persistent infection and to test whether persistent infection is a possible viral maintenance mechanism in a bat population. For many bats, large population size and migratory behaviour have impeded obtaining individual data. Most field studies have therefore been cross-sectional (Dominguez et al. 2007, Gloza-Rausch et al. 2008,
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Drexler et al. 2011) rather than following individuals through time, limiting development of understanding the maintenance mechanism of bat borne viruses.

Here, we apply quantitative analyses in a Bayesian framework to the CMR data from Smith (Smith 2015) to derive parameter estimates for epidemic models to investigate the effects of persistent infection on viral maintenance in a bat population.

2.3. METHODS

2.3.1. Capture-Mark-Recapture (CMR) data analyses

We analyzed the CMR data using multistate models to estimate survival rates ($\phi$), recapture rates ($p$), and transition rates ($\psi$) (Figure 2-1). The multistate models included two states, infectious and non-infectious, with transitions in both directions (Kéry and Schaub 2011). Each time step was a week. In addition, to investigate persistent infection in the bat population, we explored multistate models in which bats were divided into two groups, based on the frequency of coronavirus RNA detection. Recaptured bats with a single detection were referred to as transiently-infectious bats and recaptured bats with multiple detections were referred to as persistently-infectious bats. Bats that were only captured once were excluded from analysis (Table 2-1). Five bats were once captured with a single detection of coronavirus RNA, and those five bats were excluded from the two groups.

To analyze the CMR data, we chose a Bayesian method over a frequentist approach because the Bayesian method is more appropriate in dealing with a small amount of data, relying less on large sample asymptotic approximations (Dunson 2001). The
Bayesian analyses were conducted in R (R Core Team 2016) and in OpenBUGS using the R package “R2OpenBUGS” (Sturtz et al. 2005). OpenBUGS was used to run three independent chains of an MCMC (Markov chain Monte Carlo) sampler for 10,000 iterations each, after discarding the initial 1,000 samples as a ‘burn in’. The mean of each of the parameters was calculated, as were the 2.5th and 97.5th percentiles of the parameter distributions (95% Bayesian credible intervals (CrI)). The relevant R code is provided as appendices (Appendix 2-2 and 2-3).

Table 2-1. The Capture-Mark-Recapture (CMR) data composition of coronavirus in 52 Myotis macropus. 7 ‘persistently-infectious bats’ were recaptured bats with multiple detections of coronavirus RNA, and 16 ‘transiently-infectious bats’ were recaptured bats with a single detection of coronavirus RNA. 23 ‘infectious bats’ were persistently or transiently infectious bats. ‘Infectious bats’ did not include 5 bats, which were not recaptured, with a single detection of coronavirus RNA.

<table>
<thead>
<tr>
<th>Detection</th>
<th>Recapture</th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 7 recaptured bats with multiple detections of coronavirus RNA were identified with total 18 detections of coronavirus RNA.

2 Persistently infectious bats

3 Transiently infectious bats
CHAPTER 2.

Figure 2-1. Capture-Mark-Recapture data analyses across eight recapturing occasions. (a) Survival and recapture rates. Black and white circles represent survival and recapture rates, respectively. (b) Transition rates between un-infectious and infectious states. Black and white circles represent transition from un-infectious to infectious state and from infectious to un-infectious state, respectively. Error bars indicate 95% credible interval.

2.3.2. Multistate model selection

We used CMR multistate model selection to determine whether ‘grouping’ of persistently and transiently-infectious bats and the inclusion of infectious and non-infectious states were supported by the CMR data. The grouping and the multiple states were applied to survival, recapture, and transition rates of the CMR data. Comparisons between the candidate multistate models were assessed with DIC (deviance information criterion) (Spiegelhalter et al. 2002), and the most parsimonious model was selected for subsequent simulated epidemic models.

2.3.3. Parameterization

Survival rates (φ) were used to calculate mortality rates (µ). Transition rates (ψ) from the infectious to non-infectious state (ψ_{ui}) were used to calculate infectious period.
Persistent infection of coronavirus which is a reciprocal of recovery rate (\(\gamma\)) (Table 2-2). Failure to detect coronavirus RNA in faeces or anal swabs may not necessarily imply recovery from the infection, and could conceivably represent intermittent viral excretion or false negative laboratory results. However, recovery from SARS coronavirus infection in humans has been shown to occur when virus is no longer detected in faecal samples (Bermingham et al. 2004). In the absence of specific information about recovery from coronavirus infection in these bats, we assumed that failure to detect coronavirus in faecal samples or anal swabs similarly corresponded to recovery.

The transmission rate (\(\beta\)) could not be directly calculated from the CMR data analyses, and was instead calculated from basic reproduction number (\(R_0\)) equations. From Drexler et al.’s study (Drexler et al. 2011) and from observation of the CMR data (Smith 2015), Smith (Smith 2015) hypothesized that the initial epidemic peak was caused by the formation of a maternity roost of *M. macropus* from weeks 1 to 6, and that a second epidemic peak, after parturition, was caused by newborn pups who lost their passive immunity from weeks 6 to 12. Thus, with an assumption that a new epidemic began from week 7, we used an equation of \(R_0=1+\Lambda D\) (Vynnycky and White 2010), where \(\Lambda\) represents the growth rate in an epidemic and \(D\) represents the average duration of the infectious period. We calculated \(\Lambda\) as 0.3328 per week by using the CMR data from week 7 to week 12 (Appendix 2-4). We estimated mean \(D\) as 1.7737 weeks, from the infectious period of recaptured bats (1/\(\gamma\)) (Table 2-2). Thus, we estimated \(R_0\) to be 1.5903. We estimated \(\beta\) using \(R_0=\beta ND\), where \(N\) is the total population size (Vynnycky and White 2010). By assuming that \(N\) was 86 (the mean estimated size of the study population (Smith 2015)) we could calculate \(\beta\), 0.0104. A rate of waning immunity (\(\omega\)) was unable to be estimated from this CMR data and has not been estimated in other studies. In the absence of data to suggest otherwise, we
assumed the rate of waning immunity was comparable to that in human SARS-CoV infections (Bermingham et al. 2004). Uncertainty in parameter values was included by sampling mortality and recovery rates from PERT distributions using the R package “mc2d” (Vose 2008).

2.3.4. Epidemic model framework

We built a deterministic density-dependent susceptible-infectious-recovered-susceptible (SIRS) model, using ordinary differential equations (ODE). We assumed that density dependence was appropriate for a coronavirus because SARS-CoV is transmitted in bats via a fecal-oral route, which is more suggestive of density-dependent transmission than frequency-dependent transmission (Wang et al. 2006, Peel et al. 2013). Further, the number of bats in the roost was relatively small and this would allow homogenously mixed contacts among bats (Smith 2015). We used a SIRS model for coronavirus infection in bats, following previous authors, who have used SIRS models for coronavirus infection in *Miniopterus* spp. (Smith 2015). Additionally, high SARS-CoV sero-prevalence in bats supports the existence of a recovered class (Li et al. 2005). In the CMR data, with the course of time, positive detections of coronavirus RNA occasionally encompassed negative detections and negative detections occasionally encompassed positive detections, implying that infectiousness and non-infectiousness were not permanent (Smith 2015).
Table 2-2. Parameters of coronavirus infection in *Myotis macropus*. Each model time step is one week. “All” represents infections without grouping of persistent and transient infections. Subscripts *p*, *t*, *u*, and *i* represent persistent infection, transient infection, non-infectious state, and infectious state, respectively. CrI represents credible interval.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Infection type</th>
<th>Symbol</th>
<th>Estimate or range</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transmission rate</td>
<td></td>
<td>$\beta$</td>
<td>0.0104</td>
<td>Calculated from equations for basic reproductive rate (Vynnycky and White 2010)</td>
</tr>
<tr>
<td>Recovery rate</td>
<td>All</td>
<td>$\gamma$</td>
<td>0.5638 (95% CrI: 0.3236, 0.8031)</td>
<td>Transition rates from infectious state to non-infectious state ($\psi_{ui}$) (Smith 2015)</td>
</tr>
<tr>
<td></td>
<td>Persistent</td>
<td>$\gamma_p$</td>
<td>0.3354 (95% CrI: 0.1210, 0.6518)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Transient</td>
<td>$\gamma_t$</td>
<td>0.8582 (95% CrI: 0.4985, 0.9943)</td>
<td></td>
</tr>
<tr>
<td>Rate of waning immunity</td>
<td></td>
<td>$\omega$</td>
<td>0.0833</td>
<td>(Bermingham et al. 2004)</td>
</tr>
<tr>
<td>Mortality rate in non-infectious state</td>
<td>All</td>
<td>$\mu_u$</td>
<td>0.0152 (95% CrI: 0.0732, 0)</td>
<td>1-survival rate (Smith 2015)</td>
</tr>
<tr>
<td></td>
<td>Persistent</td>
<td>$\mu_{up}$</td>
<td>0.0617 (95% CrI: 0.2817, 0.0017)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Transient</td>
<td>$\mu_{ut}$</td>
<td>0.0166 (95% CrI: 0.0815, 0)</td>
<td></td>
</tr>
<tr>
<td>Mortality rate in infectious state</td>
<td>All</td>
<td>$\mu_i$</td>
<td>0.0269 (95% CrI: 0.1134, 0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Persistent</td>
<td>$\mu_{ip}$</td>
<td>0.0252 (95% CrI: 0.1289, 0)</td>
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</tr>
<tr>
<td></td>
<td>Transient</td>
<td>$\mu_{it}$</td>
<td>0.0684 (95% CrI: 0.266, 0.0033)</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER 2.
To test effects of grouping of bats into persistently infectious and transiently infectious bats on the probability of viral persistence in the bat population, we designed two alternative models: a “one-group” model and a “two-group” model (Figure 2-2). The one-group model did not differentiate between persistently infectious and transiently infectious states, instead assumed that all recaptured bats (n=23) with multiple or single detection of coronavirus RNA in the CMR data had the same recovery and mortality rates, regardless of the number of coronavirus RNA detections. On the other hand, the two-group model split bats into persistently infectious and transiently infectious groups. We assumed that recaptured bats with multiple detection of coronavirus RNA were “persistently-infectious” (n=7) and recaptured bats with single detection of coronavirus RNA in the CMR data were “transiently-infectious” (n=16). Given the multistate model selection preferred the inclusion of the multistate effect (Table 2-3), we estimated different mortality rates for infectious bats (I class) and non-infectious bats (S and R classes).

In the one-group model, the mean prevalence (P=0.2786) of coronavirus in the CMR data was used to set the initial number of infectious bats in the model with the population size (N=86) and the remaining bats were considered susceptible (S=(1-P)N, I=PN, R=0). In the two-group model, the initial number of infectious bats was set based on the proportion of persistently infectious bats in infectious bats out of recaptured bats in the CMR data (f=7/23) (S=(1-P)N, Ip=fPN, It=(1-f)PN, and R=0).
2.3.5. Scenarios

Six scenarios were set up based on different infectious periods. Scenario 1 was developed to describe the dynamics of coronavirus infection in *M. macropus* without grouping bats based on infectious period (the ‘one-group’ model). Scenarios 2-6 were ‘two-group’ models. Scenario 2 was developed to describe the dynamics when bats were grouped into persistently-infectious and transiently-infectious bats, and scenarios 3-6 were modifications of scenario 2, with extended periods of persistent infection.

While we used the infectious periods calculated from the CMR data analyses in scenario 1 and 2, we assumed extended infectious period of persistently-infectious bats to 5, 7, 9, and 11 weeks in scenario 3 to 6, respectively, following a previous study in which *M.*
macropus could be identified with a putative novel Alphacoronavirus infection for up to 11 weeks (Smith 2015).

We simulated the model with 10,000 iterations, sampling from the range of parameter values calculated from the CMR data and estimated the probability of viral persistence in the population of *M. macropus*. The R package “deSolve” (Soetaert et al. 2010) was used to build the epidemic model. Time steps were weekly (following the time interval of the CMR data (Smith 2015)) (See Appendix 2-5). We assumed that the virus persisted in the population when at least one infectious bat remained at week 12.

2.4. RESULTS

2.4.1. CMR data analyses

The multistate models enabled estimation of survival and recapture rates, of *M. macropus*, and transition rates between infectious and non-infectious states (Table 2-2). The survival rates were relatively constant during the period, whereas the recapture rates, generally decreased in the first half and increased in the second half of capturing occasions (Figure 2-1 A). As a result, the transition rates should be considered with caution when recapture rate was low (for example, only one bat was captured at the fifth recapturing occasion) (Figure 2-1 B). Paucity of the CMR data resulted in wide error bars.
Persistent infection of coronavirus

Table 2-3. Multistate model selection with survival, recapture and transition probabilities of Macropus myotis. Transition probabilities are probabilities that bats transit between infectious state and non-infectious state. Group means grouping of infectious bats into persistently-infectious bats and transiently-infectious bats. Multistate means the multistate effect of infectious and non-infectious states. Model 1 was found to be the most parsimonious model that best fits the capture-mark-recapture (CMR) data.

<table>
<thead>
<tr>
<th>Model number</th>
<th>Survival Probability</th>
<th>Recapture Probability</th>
<th>Transition Probability</th>
<th>DIC(^4)</th>
<th>ΔDIC(^5)</th>
<th>Parameter number</th>
<th>Deviance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group, Multistate</td>
<td>Group</td>
<td>Group, Multistate</td>
<td>273.9</td>
<td>0</td>
<td>10</td>
<td>258.2</td>
</tr>
<tr>
<td>2</td>
<td>Group</td>
<td>Group</td>
<td>Group, Multistate</td>
<td>274.1</td>
<td>0.2</td>
<td>8</td>
<td>259.3</td>
</tr>
<tr>
<td>3</td>
<td>Multistate</td>
<td>Multistate</td>
<td>Group, Multistate</td>
<td>279.5</td>
<td>5.6</td>
<td>5</td>
<td>267.3</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td>Multistate</td>
<td>280.1</td>
<td>6.2</td>
<td>4</td>
<td>268.9</td>
</tr>
<tr>
<td>5</td>
<td>Group, Multistate</td>
<td>Group, Multistate</td>
<td>Group, Multistate</td>
<td>305.5</td>
<td>31.6</td>
<td>12</td>
<td>251.1</td>
</tr>
<tr>
<td>6</td>
<td>Group</td>
<td>Group, Multistate</td>
<td>Group, Multistate</td>
<td>319</td>
<td>45.1</td>
<td>10</td>
<td>252.7</td>
</tr>
<tr>
<td>7</td>
<td>Multistate</td>
<td>Multistate</td>
<td>Multistate</td>
<td>342.1</td>
<td>68.2</td>
<td>6</td>
<td>258.4</td>
</tr>
<tr>
<td>8</td>
<td>Multistate</td>
<td>Multistate</td>
<td></td>
<td>352.7</td>
<td>78.8</td>
<td>5</td>
<td>259.8</td>
</tr>
</tbody>
</table>

2.4.2. Multistate model selection

The most parsimonious model supported grouping of bats into two groups based on the detection frequency of coronavirus RNA (single and multiple detections) for all three rates of survival, recapture, and transition. There was little support for multistate effects on the survival rate (ΔDIC=0.2, comparing models 1 and 2; Table 2-3). This was in accordance with previous findings that coronavirus infection in bats showed no signs of illness (Baker et al. 2013, Hu et al. 2015). The results of model selection processes also

\(^4\text{DIC (Deviance Information Criterion; a Bayesian analogy of the AIC (Akaike's Information Criterion)) is a measure of the relative quality of statistical models, and the model with the smallest DIC is estimated to be the model that would best fit the data.}\)

\(^5\text{ΔDIC is the change in DIC from the top-ranked model to each model.}\)
indicated that the multistate effect should be excluded for recapture rate, and should be intrinsically considered for transition rate.

2.4.3. Epidemic model simulations

The SIRS epidemic models were simulated to generate the probabilities of coronavirus persistence in a bat population in the six scenarios. The simulated probability of viral persistence in scenario 2 (two-group model, 0.5210) was somewhat higher than the probability in scenario 1 (one-group model, 0.4094) (Table 2-4). This result showed that viruses were more likely to be maintained in the bat population when bats could either be persistently-infectious or transiently-infectious than when bats were not split into these groups. As the period of persistent infection was lengthened to 5 weeks in scenario 3, the probability of persistence within the population reached to almost 1. In scenario 4 to 6, in which the periods of persistent infection were lengthened to 7, 9, and 11 weeks, respectively, the virus persisted in the population in every simulation. Although Smith (Smith 2015) found that *M. macropus* can be identified with a putative novel *Alphacoronavirus* infection over periods of up to 11 weeks, extension of the period of persistent infection from about three weeks (scenario 2) to five weeks (scenario 3) markedly increased the probability of viral maintenance to almost one.
Table 2-4. Probability of viral persistence based on varying periods of infection in one and two group models in six scenarios. The one-group model (with a single infectious period) was only used in scenario 1, while the two-group model (with both transient and persistent infectious periods) was used in scenarios from 2 to 6. Scenarios differed in the infectious period estimate used: Scenario 1 and 2 used estimates from the CMR analyses (reciprocal of recovery rates (γ), which are shown in table 2-2), whereas scenarios 3-6 explored a series of hypothetical persistently infectious periods. The infectious period for transient infections was constant for all two-group scenarios (based on 1/γt), but the infectious period for persistent infections was varied. Specifically, Scenario 2 used 1/γp from the CMR analyses for persistently infectious period, whereas in scenarios 3 to 6, we assumed 5, 7, 9, and 11 weeks of persistently infectious periods respectively.

<table>
<thead>
<tr>
<th>Scenario Number</th>
<th>One-group model</th>
<th>Two-group model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transiently infectious period in weeks</td>
<td>1.774 (1/γ)</td>
<td>1.165 (1/γt) 1.165 (1/γt) 1.165 (1/γt) 1.165 (1/γt)</td>
</tr>
<tr>
<td>Persistently infectious period in weeks</td>
<td>2.982 (1/γp)</td>
<td>5 7 9 11</td>
</tr>
<tr>
<td>Probability of viral persistence</td>
<td>0.430</td>
<td>0.508 0.999 1 1 1</td>
</tr>
</tbody>
</table>

2.5. DISCUSSION

This study analyzed CMR data of a species of Australian bats (M. macropus) with a putative novel Alphacoronavirus infection in order to decide whether it is appropriate to divide bats into persistently-infected bats and transiently-infected bats, and to determine whether viral maintenance is improved by the differentiation of bats into these groups. Grouping of bats into persistently infectious bats and transiently infected bats was supported by the CMR multistate model selection processes (Table 2-3). By exploring various scenarios in epidemic models, we found that population-level viral persistence was marginally more probable when infectious periods were heterogeneous (bats were either transiently- or persistently-infected; Table 2-4). Accordingly, the two-group model was found to be actually more likely than the one-group model.
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(Figure 2-2). In addition to a previous study that suggested persistent infection of coronavirus in bats in North America through the small number of bats sampled (Dominguez et al. 2007), our study provides much validity by using larger number of bats sampled (Smith 2015), although the sample size is still not large enough to provide robust estimates.

Further understanding of the potential and likelihood of within-host persistent infections is important to understand spillover of bat-borne viruses. Persistent infections contribute not only in maintaining viruses in a population but also in triggering pulses of virus shedding. Temporally synchronized stressors such as food shortage and reproduction may weaken the immune system of bats, facilitating persistent infections in infectious bats (Plowright et al. 2015). Thus, those temporally synchronized stressors can cause viral shedding from persistently infectious bats during a limited period, leading to pulses of viral shedding. Because spillover of bat-borne viruses has been associated with pulses of viral shedding from bats (Plowright et al. 2015), the finding of persistent infection in bats contributes in understanding spillover mechanisms of bat-borne viruses.

Maternity roosts were assumed to play an important role in maintaining coronavirus at the population level (Gloza-Rausch et al. 2008). In coronavirus transmission within a population of *Myotis*, an epidemic peak was observed at the formation of the maternity colony and another peak was observed as newborn pups lost their passive immunity (Drexler et al. 2011). Transmission of viruses during the breeding season is more readily facilitated, compared with other times due to frequent contact among individuals within a maternity roost (Drexler et al. 2011, Dietrich et al. 2015, Smith 2015). Fluctuating recapture rates and coronavirus prevalence are presumed to be related to the
Persistent infection of coronavirus changing ethology of bats during the breeding season. Therefore, CMR data covering both breeding seasons and non-breeding seasons are required to investigate how coronaviruses can be maintained in a bat population even if maternal roosts do not facilitate viral transmission.

These findings need to be treated with some caution, because the low sample size limits our confidence in the results of modelling. For example, the recapture rates were markedly different from recapturing occasion to occasion (Figure 2-1 A), as only one bat was sampled at the fifth recapturing occasion (Smith 2015). Additionally, the CMR data included only coronavirus RNA detections, without detections of coronavirus antibodies. A SARS coronavirus crude antigen ELISA was not successful in detecting antibodies against the putative novel Alphacoronavirus in these bats (Smith 2015). Thus, the lack of seroprevalence in the CMR data limited the accuracy of the estimated duration of recovery. Nevertheless, the CMR data deserve intensive analyses because longitudinal sampling of individual bats for infectious diseases is difficult to achieve and allows estimation of epidemiological parameters. The CMR study in Smith’s study (Smith 2015) had relatively high recapture rates. Finding a lifting hole of a bridge, which was used as the bats’ colony, allowed the author to capture bats that roosted with a high affinity to their colony. Fluctuating recapture rates across capturing occasions in the CMR data, which might be associated with trap-shy and adaptive methods of capturing, highlighted the difficulty of recapturing specific bats during the entire sampling period.

Persistent infections have been hypothesized as a mechanism for viral persistence, not only in coronaviruses (Dominguez et al. 2007), but also in other bat-borne viruses, for example filoviruses and henipaviruses, despite lack of direct evidence (Plowright et al.
CHAPTER 2.  

2016). It was also hypothesized that a small portion of super-long-shedder bats with a long infectious period could maintain Hendra virus in a bat population (Plowright et al. 2011). Although further comprehensive datasets are needed to understand the effects of persistent infection, this study shows the value of longitudinal individual data from bats with viral infections to elucidate transmission dynamics of bat-borne viruses, underscoring the need of individual bat tracking data with infection states to improve our understanding of infection dynamics of bat-borne viruses.
CHAPTER 3

3. The Effects of Maternally-derived Immunity on Infection dynamics in Seasonally Breeding Wildlife: a Case Study of Hendra virus Epidemics in Australian flying foxes (Pteropus spp.)

3.1. ABSTRACT

The growing number of outbreaks of emerging infectious diseases originating from wildlife has increased interest in understanding the dynamics of the diseases in their wildlife reservoir hosts. Here, I explore how the population structure, influenced by seasonal breeding and maternally-derived immunity, affects the infection dynamics by using a system describing Hendra virus infection in Australian Pteropus bats (fruit bats or flying foxes). I used deterministic epidemic models to simulate transient epidemics following viral introduction into an infection-free population with a variety of timings within a year and with a range of different levels of pre-existing herd immunity. The modelling results indicated that the effect of maternal immunity on the epidemics is attributable to the dispersed timing of supply of susceptible hosts into the population, not only from births but also from loss of maternally-derived immunity. The dispersed timing delays the timing of epidemic peaks away from birth pulses, and it also increases the likelihood of viral invasion and persistence in the population. Overall, the simulation results showed that maternal antibodies could have a substantial influence on the dynamics of wildlife epidemics, particularly in seasonally breeding species.
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3.2. INTRODUCTION

Seasonal behaviour of wildlife hosts—including aggregation, reproduction, and associated physiological variations— influences infection dynamics (Altizer et al. 2006). Among those seasonally varying factors, seasonal breeding has been demonstrated to be implicated in pathogen persistence and epidemic patterns because births are a source of supply of susceptible individuals. Peel et al. (2014) stressed that seasonal breeding results in a population requiring a much larger size to maintain a pathogen. Hayman (2015) showed that a frequency of birth pulses within a year could be a major factor determining virus persistence. Begon et al. (2009) emphasised that seasonal breeding can drive the timing of recurrent epidemics in a wildlife population. All of these effects of seasonal breeding on the infection dynamics are caused by seasonally varying population size and seasonally varying proportion of susceptible hosts (Altizer et al. 2004).

Births from susceptible mothers and immigration of susceptible individuals are not the only source of supply of susceptible individuals. Newborns of immune mothers may obtain protection via maternally-derived antibodies, which then wane over a given period (Keeling and Rohani 2008), with these juveniles then entering the susceptible pool. Supply of susceptible hosts through seasonal breeding thus could be significantly modified if immune mothers confer waning passive immunity to newborns. The addition of maternally-derived immunity to seasonal breeding means that the supply of susceptible hosts comes from two sources (in addition to immigration): births to susceptible mothers and loss of maternally-derived immunity. The effects of the passive immunity on infection dynamics in seasonally breeding animals warrant further investigation.
Bats (Order: Chiroptera) have been identified as natural reservoirs of many threatening emerging infectious diseases of public health concern (Calisher et al. 2006, Luis et al. 2013). While bats do not appear to suffer from many of these viruses, the fatality rates resulting from the viruses are often high in other mammalian species (Calisher et al. 2006). For example, Hendra virus is maintained in its reservoir hosts, flying foxes (Genus: Pteropus), in Australia. The virus spills over from flying foxes to horses and from horses to humans, causing serious clinical symptoms and death in horses and humans (Field et al. 2007). Understanding the epidemiology of the virus in flying foxes may assist in reducing the public health consequences of Hendra virus. Also, the understanding could be extended to other infectious diseases in seasonally breeding wildlife that confer maternally-derived immunity.

Surveillance of spillover events showed evident temporal patterns (Plowright et al. 2015). Seasonal clustering of Hendra virus spillover incidents in the southern subtropics in eastern Australia has been observed from June to September (Plowright et al. 2015). Although a variety of conditions implicated with bats, horses, virus, and environment must be met for spillover to occur (Plowright et al. 2015), seasonal breeding and associated maternally-derived immunity are candidates that may contribute to the temporal clustering of the spillover events.

Although the mechanisms driving Hendra virus spillover have not yet been fully elucidated, high spillover risk is associated with pulses of virus shedding (Plowright et al. 2015). Two hypotheses have been advanced to explain pulses of virus shedding: ‘transient epidemics’ and ‘episodic shedding’ from persistently infected bats, although
available empirical evidence is unable to distinguish between these two alternative mechanisms (Plowright et al. 2015). The transient epidemic hypothesis depends on the among-host transmission and is deeply related to population dynamics, whereas the hypothesis of episodic shedding depends on the within-host immune response and is not explicitly affected by population dynamics (Plowright et al. 2015). As a result, this chapter, which concentrates on seasonally fluctuating population structure, assumes the hypothesis of transient epidemics.

Garnier et al. (2014) found that maternal transfer of immunity in seasonally breeding wildlife has the effect of delaying recurrent epidemics in a multi-year timescale. However, how the delay would affect the timing of epidemics within a year requires further investigation. This chapter investigates whether seasonal breeding and maternally-derived immunity can cluster the timing of epidemic peaks in a specific season with any timing of viral introduction. If flying foxes have a seasonally different movement pattern, it might contribute to the seasonal clustering of spillover events by introducing the virus during a certain season. However, flying foxes that frequently move between colonies are expected to be able to introduce the virus any time in a year (Roberts et al. 2012b).

The timing of loss of maternally-derived immunity can be significantly important in determining modelling outcomes. To evaluate the effect of the timing on epidemics, this chapter models the transfer of individuals from maternally immune stage to susceptible stage by using two different methods: an exponential distribution and a gamma distribution. Most epidemic models have used exponential distributions, which is the classical method to transfer individuals between stages, being implicit in a simple
differential equation SIR (susceptible-infectious-immune) model with constant rate parameters (Keeling and Rohani 2008). Although the exponential distribution has widely been used for the sake of computational ease, Wearing et al. (2005) indicated that models with exponential distributions might be unrealistic in representing many time delayed processes. Instead, Wearing et al. (2005) suggested a gamma distribution as an alternative. By using the two methods, this chapter shows how significantly modelling results can be affected by the method by which loss of maternally-derived immunity is modelled.

To investigate how the seasonal breeding and transfer of maternal antibodies affect the likelihood of viral persistence or fadeout and the timing of epidemic peaks, I simulated an SIR model, an MSIR (maternally immune-susceptible-infectious-immune) model with exponentially distributed periods of maternally-derived immunity, and an MSIR model with a gamma-distributed delay of maternally-derived immunity. All models included seasonal birth pulses. The models are based on Hendra virus epidemics in a population of flying foxes. I investigated how the epidemic pattern changed depending on a variety of herd immunities and time lags between a seasonal birth pulse and a viral introduction.

3.3. METHODS

3.3.1. Biology relevant to model structure

Four species of flying foxes have been potentially implicated with Hendra virus infection in Australia: the black flying fox (Pteropus alecto), the grey-headed flying fox (P. poliocephalus), the little red flying fox (P. scapulatus), and the spectacled flying fox (P. conspicillatus) (Young et al. 1996). In south-east Queensland and northern New
CHAPTER 3.

South Wales, where most Hendra virus spillover events have occurred, colonies often consist of a mixture of grey-headed flying foxes and black flying foxes (Hall and Richards 2000). Although serological surveys have shown that grey-headed flying foxes also produce an antibody response, black flying foxes appear more likely to be driving spillover events (Smith et al. 2014, Field et al. 2015), which suggests more importance on the investigation of Hendra virus transmission dynamics in the populations of black flying foxes than in the other species. Nevertheless, the features of seasonal breeding and maternal immunity have been found to be similar from species to species of flying fox, except that the breeding season of little red flying foxes is six months out of sequence with the other three species (Martin and McIlwee 2002). Therefore, I primarily used the parameters appropriate for black flying foxes, but the findings from the models should not be limited to black flying foxes.

In a spatial perspective, flying fox colonies are patchily distributed. Individuals keep moving among colonies and form a metapopulation structure (Plowright et al. 2011). This movement may introduce Hendra virus into infection-free colonies, and viral introduction may trigger transient epidemics. At the timing of viral introduction, colonies are not necessarily immunologically naïve. As the natural reservoir hosts of Hendra virus, flying fox colonies are highly likely to have been previously exposed to the virus (Plowright et al. 2011). Partial immunity of a population affects the duration and size of an epidemic caused by the viral introduction (Pulliam et al. 2007). I therefore designed models to simulate the Hendra virus dynamics in a colony of flying foxes within this broader context of a metapopulation in eastern Australia, assuming that a proportion of bats in a colony had previously been exposed to Hendra virus and thereby was partially immune.
### 3.3.2. Model structure

Assuming the hypothesis of transient epidemics, this chapter is based on an SIR deterministic compartmental model (Plowright et al. 2011). The SIR model was simulated with annual birth pulses. In addition to the SIR model, an MSIR model was built by adding a maternally immune (M) compartment to the SIR model. Maternally immune (M) newborns become susceptible at the rate $\delta$. Susceptible (S) bats become infected at the rate $\beta S I$ (i.e. assuming density-dependent transmission). Bats are infectious (I) before recovery at rate $\gamma$ and, once recovered (R), remain immune for life (Plowright et al. 2011). These SIR dynamics constitute one of three probable scenarios for Hendra virus persistence outlined in Plowright et al. (2016). Hendra virus infection does not cause clinical disease in its reservoir hosts, flying foxes (Halpin et al. 2001), so infected bats are assumed to have the same mortality rate as susceptible and recovered bats. Models were framed using ordinary differential equations (ODEs) and numerically integrated using the deSolve package (Soetaert et al. 2010) in R (R Core Team 2016). The models were simulated for eight years, and ran with a daily time step.

Simulation of seasonal birth pulses and maternally-derived immunity required an age-structured model of the bat population. The age-structure consisted of sexually immature juvenile bats (denoted by the subscript i) and sexually mature adult bats (subscript m). In the MSIR model, juveniles became adults, which could breed two years after their birth (at rate $\varepsilon$) (Wang et al. 2013). The proportion of juveniles in the total population ($\eta$) was on average 0.24 (McIlwee and Martin 2002). Four epidemic compartments for two age groups comprised a total of eight stages (see Appendix 3-1 for ODE for each stage). Although maternally immune adults are not expected to exist
in nature, I added this stage for modelling consistency. (The exponential distribution of periods of maternally-derived immunity means that some individuals stay in maternally immune compartment longer than the period from birth to becoming an adult. With the parameter values used, so few maternally immune adults remained that they had an insignificant effect on the results). I assumed an age-independent annual mortality rate \((\mu)\) of 16\% (McIlwee and Martin 2002). The mortality rate \((\mu)\) and birth rate were independent of population density and were chosen so that the population size remained constant. Bats born to immune female adults \((R_m)\) and any born to maternally immune female adults \((M_m)\) were assumed to be maternally immune \((M_i)\) in the MSIR model, whereas all newborns were assumed to be susceptible \((S_i)\) in the SIR model.

Seasonally pulsed birthing was modelled with a periodic Gaussian function (PGF) (Peel et al. 2014, Hayman 2015):

\[
b(t) = \kappa \sqrt{\frac{s}{\pi}} e^{-s \cos^2(\pi t - \varphi)},\]

where \(\kappa\) controls the magnitude of the birth pulse, \(s\) determines synchrony of birth pulse, and \(\varphi\) determines the timing of birth pulse. This function allows births to take place exclusively in a certain period within a year, with no births outside this period (Peel et al. 2014, Hayman 2015). The scaling parameter \((\kappa)\) was used so that the total population size was stable inter-annually.

At 27ºS in eastern Australia, young of black flying foxes are mostly born in October and November (Vardon and Tidemann 1998), but it appears that the seasonal breeding pattern is plastic (McIlwee and Martin 2002, Hall and Richards 2000). Instead of black flying foxes, grey-headed flying foxes \((P.\ poliocephalus)\) were used to explore the effects of seasonal birth pulses on Hendra virus transmission dynamics because grey-headed flying foxes show rigidly defined breeding seasonality, which does not seem to
be significantly affected by spatiotemporal factors (Martin and McIlwee 2002). I set $s = 130$, representing 95% of births within one month (Peel et al. 2014). Additionally, I simulated an SIR model, using a constant birth rate ($b$) that was same as the mortality rate ($\mu$). The results of this SIR model with a constant birth rate provided the number of bats in each compartment in an endemic equilibrium state, which was used to interpret the results of SIR and MSIR models with seasonal birth pulses.

The transmission mode of Hendra virus in flying foxes has not been discovered, although it has been hypothesised that a combination of frequency and density dependence characterises the transmission (Plowright et al. 2015). However, this chapter assumed density-dependent transmission, which is affected by population size, whereas frequency-dependent transmission is not affected by population size. With regard to epidemiological parameters of Hendra virus, I assumed a Hendra virus transmission rate ($\beta$) of 0.0000476 and a recovery rate ($\gamma$) of $1/7$ days$^{-1}$, which were estimated mean values from Plowright et al. (2011) (Table 3-1). The loss rate of maternally-derived immunity ($\delta$) was assumed as $1/255$ days$^{-1}$, informed by an experimental study on eight newborn black flying foxes born to seropositive bats (Epstein et al. 2013).
### Table 3-1. Model parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value</th>
<th>Unit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transmission rate</td>
<td>$\beta$</td>
<td>0.0000476</td>
<td>Per capita per day</td>
<td>(Plowright et al. 2011)</td>
</tr>
<tr>
<td>Recovery rate</td>
<td>$\gamma$</td>
<td>1/7</td>
<td>Per capita per day</td>
<td>(Plowright et al. 2011)</td>
</tr>
<tr>
<td>Mortality rate</td>
<td>$\mu$</td>
<td>1/7</td>
<td>Per capita per year</td>
<td>(McIlwee and Martin 2002)</td>
</tr>
<tr>
<td>Maternally-derived immunity losing rate</td>
<td>$\delta$</td>
<td>1/255</td>
<td>Per capita per day</td>
<td>(Epstein et al. 2013)</td>
</tr>
<tr>
<td>Ageing rate</td>
<td>$\alpha$</td>
<td>1/2</td>
<td>Per capita per year</td>
<td>(Wang et al. 2013)</td>
</tr>
<tr>
<td>Scalar to control birth rate</td>
<td>$\kappa$</td>
<td>0.00159</td>
<td>Per adult per day</td>
<td>This study</td>
</tr>
<tr>
<td>Annual birth pulse synchrony</td>
<td>$s$</td>
<td>130</td>
<td>-</td>
<td>(Peel et al. 2014)</td>
</tr>
<tr>
<td>Timing of birth pulse</td>
<td>$\phi$</td>
<td>1/2</td>
<td>-</td>
<td>This study</td>
</tr>
<tr>
<td>Constant birth rate</td>
<td>$b$</td>
<td>1/7</td>
<td>Per capita per year</td>
<td>(McIlwee and Martin 2002)</td>
</tr>
<tr>
<td>Initial herd immunity</td>
<td>HI</td>
<td>0~1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Days from the last birth pulse to viral introduction</td>
<td>DB</td>
<td>0~365</td>
<td>day</td>
<td></td>
</tr>
<tr>
<td>Colony size</td>
<td>N</td>
<td>10000</td>
<td>capita</td>
<td>(Plowright et al. 2011)</td>
</tr>
<tr>
<td>Average proportion of juvenile bats in total bats</td>
<td>$\eta$</td>
<td>0.23</td>
<td>-</td>
<td>(McIlwee and Martin 2002)</td>
</tr>
<tr>
<td>Gamma-distribution parameter</td>
<td>$g$</td>
<td>10</td>
<td></td>
<td>Arbitrarily assumed</td>
</tr>
</tbody>
</table>
3.3.3. Gamma-distributed periods of maternally-derived immunity

The MSIR model with exponentially distributed periods of maternally-derived immunity (hereafter referred to as exponential MSIR model) assumed that the number of maternally immune hosts decreased exponentially since births (Appendix 3-2). In addition to the exponential MSIR model, an MSIR model with a gamma-distributed delay of maternally-derived immunity (hereafter referred to as gamma MSIR model) was simulated. The gamma MSIR model used a gamma distribution in transferring maternally immune juveniles to susceptible juveniles. Gamma-distributed maternally immune durations were modelled by dividing the maternally immune stage into multiple numbers (g) of sub-stages, where gamma-distributed periods of maternal immunity had a mean of g and variance of 1/g (Wearing et al. 2005) (See Appendix 3-1 for equations). As the gamma distribution parameter (g) increases, the loss rate of maternally immune individuals shifts from an exponential decay to a fixed duration of maternal immunity. Although the period of maternally-derived immunity against Hendra virus in black flying foxes has been estimated to be 255 days (Epstein et al. 2013), the loss trend of the immunity against the virus is not known. Thus, I arbitrarily assumed the gamma distribution parameter (g=10).

3.3.4. Initial states of models

Population size (N) was assumed to be 10000, which is within the range of probable sizes of flying fox colonies (Plowright et al. 2011). Initial herd immunity (HI), which was the proportion of immune individuals in a colony at the moment of viral introduction, was applied to the models from 0% to 100%. To investigate the time lag between seasonal births and viral introductions, I defined a parameter (DB), which was the period in days from the last birth pulse before a viral introduction to the viral introduction. Because birth pulses were seasonal and were annually repeated at the same
time every year, the range of DB was between 0 and 365 days. While the number of adult hosts was constant over time, as the age of juveniles was modelled by exponential distribution (Hayman 2015), the number of juvenile hosts fluctuated over time due to annual birth pulses (Appendix 3-3). The number of juvenile hosts at the initiation of simulations was multiplied by a scaling parameter \( \omega \). Depending on DB, the scaling parameter \( \omega \) was a number between 0.7 (the proportion of the minimum juveniles to the average juveniles) and 1.3 (the proportion of the maximum juveniles to the average juveniles). How the initial numbers of individuals in each stage were determined is shown in Table 3-2.

3.3.5. Pseudo-extinction

Viral persistence, which is an essentially stochastic process, should normally be analysed by using stochastic models. However, insights with much less computational effort can be gained from deterministic models with identification of a cut-off to determine viral persistence (Gilligan and van den Bosch 2008). I used pseudo-extinction, in which the number of infectious bats dropping below a cut-off was assumed to be a proxy for viral extinction in a population (Hanson and Stark 2012). If the sum of infectious juveniles (\( I_j \)) and infectious adults (\( I_m \)) dropped below one in the population, I assumed that the infection failed to persist and the simulations were stopped. I applied varying conditions of initial herd immunities (HI) and days from the last birth pulse before viral introduction until viral introduction (DB), because HI could be an important factor in determining magnitude and duration of an epidemic (Plowright et al. 2011) and because DB might affect the probability of viral persistence in seasonally breeding wildlife (Peel et al. 2014). Because viral extinction is likely to occur in the first few post-epidemic troughs (King et al. 2009), the period of simulations
was limited to eight years, which was long enough to include the first few epidemics and following troughs.

Table 3-2. Initial numbers of individuals in each stage. Symbols are defined in Table 3-1. To set the initial number of each stage in SIR model, I, first, divided the bats into juveniles (i), $\eta*N$, and adults (m), $(1-\eta)*N$. Juveniles and adults, then, were divided into susceptible and immune individuals by using HI. Ten infectious adults (Im) were assumed to be introduced into an infection-free population. The scaling parameter $\omega$ was multiplied to all stages in the juvenile group to make the juvenile number fluctuate depending on DB. To set the initial number of each stage in MSIR models, maternally immune adult (Mm) was not assumed at the initiation of simulation. Juveniles were divided into susceptible juveniles (Si), $(1-HI)*\eta*N$, and maternally immune (Mi) or immune juveniles (Ri), HI*\eta*N. Then, the numbers of maternally immune juveniles (Mi) and immune juveniles (Ri) were assumed to be same, and therefore immune juveniles (Ri) were $(1/2)*HI*\eta*N$. Also, the scaling parameter $\omega$ was multiplied to all stages in the juvenile group depending on DB.

<table>
<thead>
<tr>
<th></th>
<th>Sexually immature juvenile (i)</th>
<th>Sexually mature adult (m)</th>
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<tbody>
<tr>
<td><strong>SIR model</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Susceptible (S)</td>
<td>$(1-HI)<em>\eta</em>N*\omega$</td>
<td>$(1-HI)*(1-\eta)*N$</td>
</tr>
<tr>
<td>Infectious (I)</td>
<td>0</td>
<td>10</td>
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<tr>
<td>Immune (R)</td>
<td>$HI*\eta<em>N</em>\omega$</td>
<td>$HI*(1-\eta)*N$</td>
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<tr>
<td><strong>MSIR models</strong></td>
<td></td>
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<tr>
<td>Maternally immune (M)</td>
<td>$(1/2)<em>HI</em>\eta<em>N</em>\omega$</td>
<td>0</td>
</tr>
<tr>
<td>Susceptible (S)</td>
<td>$(1-HI)<em>\eta</em>N*\omega$</td>
<td>$(1-HI)*(1-\eta)*N$</td>
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<tr>
<td>Infectious (I)</td>
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<tr>
<td>Immune (R)</td>
<td>$(1/2)<em>HI</em>\eta<em>N</em>\omega$</td>
<td>$HI*(1-\eta)*N$</td>
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3.4. RESULTS

3.4.1. Timing of epidemic peaks

Figure 3-1 shows how the timing of epidemic peaks was affected by different HI (initial herd immunities) and different DB (days since the last birth pulse before viral introduction until viral introduction) in the SIR model, in the exponential MSIR model.
and the gamma MSIR model, respectively. Overall, maternally-derived immunity had the effect of delaying the timings of epidemic peaks. When the timing of epidemic peaks was observed in terms of months within one year, the SIR model showed seasonally clustered epidemic peaks, but this feature was weakened in the exponential MSIR model, and was hardly observed in the gamma MSIR model (compare Figure 3-1 A-1, B-1, and C-1). For a majority of the parameter space, the results from the SIR model and the MSIR models were similar to each other. This was because the effect of HI on epidemics was predominant, compared to the effect of maternally-derived immunity. However, differences occurred when HI was similar to the herd immunity of an endemic equilibrium state in a SIR model that was simulated with a constant birth rate ($b=1/7$ year$^{-1}$). In this range of HI, the epidemic peaks mostly appeared within one year after viral introduction in the SIR model with birth pulses (Appendix 3-4). In the exponential MSIR model, the epidemic peaks were delayed compared to the epidemics in the SIR model and showed a broader spectrum of the timings than in the SIR model. In gamma MSIR model, after the viral introduction, the epidemics took a relatively fixed period to reach their peak, and the periods until epidemic peaks were delayed compared to the epidemics in the exponential MSIR model.

In the exponential MSIR model overall, epidemic patterns appeared to be more affected by seasonal birth pulses than maternally-derived immunity. In the exponential MSIR model, the number of maternally immune bats decreased by as much as 1/255 of remaining maternally immune bats every day. As a result, most juvenile bats lost their maternal immunity not 255 days after their births but soon after their births (Figure 3-2). When HI was high and thus maternally immune newborns were more common than susceptible newborns, the temporal trend of epidemics could have been expected to be markedly changed at 255 days after a birth pulse (the period of maternally-derived
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immunity) rather than at birth pulses. However, the number of infectious individuals began increasing at the timing of birth pulses. This phenomenon was attributable to the method of modelling the loss of maternally-derived immunity.

In comparison, the gamma MSIR model showed a more enhanced effect of maternally-derived immunity. On the other hand, in the gamma MSIR model, loss of maternally-derived immunity was focused overall on 255 days after births (Figure 3-2). Therefore, the impact of maternally-derived immunity in determining epidemic pattern was much higher than in the exponential MSIR model. Also, the two relatively separate timings made the supply of susceptible individuals to be overall steady throughout the year, decreasing the degree of seasonal clustering of epidemic peaks. Another reason that birth pulses had a stronger impact on epidemics than loss of maternally-derived immunity was that the temporal synchrony of birth pulses was higher than the temporal synchrony of loss of maternally-derived immunity. Although more susceptible hosts were supplied from loss of maternally-derived immunity than from birth pulses, the tighter span of birth pulses relative to the loss of maternally-derived immunity had a high impact on epidemic patterns.
Figure 3-1. The timing of epidemic peaks in months. (A), (B), and (C) show the simulation results of SIR model, exponential MSIR model, and gamma MSIR model, respectively. The peak of the birth pulse was set at November 1. The horizontal axes show the initial herd immunity HI at the timing of viral introduction. The vertical axis shows the month when the virus was introduced, with the shading indicating the month in which epidemics reached their peak. In the right panels, (A-1), (B-1), and (C-1) show the expansion of the horizontal axes for the range 0.55 to 0.8. White parts represent a failure of an epidemic to take-off.
Figure 3-2. The daily rates of supply of susceptible bats from births and from losses of maternally-derived immunity and the fluctuating number of susceptible hosts in exponential and gamma MSIR models. (A) MSIR model with exponential distribution. (B) MSIR model with gamma distribution (gamma parameter, g=10). This figure includes five years since viral introduction. The numbers of susceptible individuals supplied from loss of maternal immunity per year (929 for exponential distribution and 1027 for gamma distribution) were far higher than the numbers of susceptible individuals supplied from births per year (365 for exponential distribution and 323 for gamma distribution). MatAb denotes maternally-derived antibodies. In this example, the days from the last birth pulse before viral introduction until the viral introduction into a colony (DB) was 10, and initial herd immunity (HI) of the population was 0.71.
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3.4.2. Viral fadeout and persistence

In the SIR model, viral persistence was not observed for any combinations of HI and DB. However, in the exponential MSIR model and the gamma MSIR model, viral persistence occurred, depending on HI and DB. In the MSIR models, viral persistence could be achieved by three mechanisms: escaping initial fadeout, epidemic fadeout, or endemic fadeout.

First, initial fadeout means that an introduced virus disappears without a take-off of an epidemic, and occurred when the time from viral introduction until the introduction of new susceptible hosts via the birth pulse was long (Figure 3-3B-3).

Second, when the birth pulse occurred during a period of increasing prevalence, virus prevalence was likely to fall beneath the pseudo-extinction threshold. The birth pulse accelerated the increase of prevalence by supplying susceptible hosts, and resulted in high-impact epidemics and then deep troughs. Consequently, epidemic fadeout was caused (Figure 3-3 A-2, A-3, and B-4) (Lloyd-Smith et al. 2005a). On the contrary, when the birth pulse occurred during a period of declining prevalence, the virus was likely to persist because the following epidemic peak was not massively high, and therefore the trough following the epidemic was not deep enough to fall beneath the pseudo-extinction threshold. This effect arose because, in the MSIR models, susceptible bats were introduced via births and via loss of maternally-derived immunity, resulting in a more evenly distributed supply of susceptible bats, as compared with the SIR model.
Figure 3-3. Time series analyses of MSIR models. (A) MSIR model with exponential distribution in left panels and (B) MSIR model with gamma distribution in right panels. These example simulations show the numbers of individuals in each compartment in the two MSIR models with seasonal birth pulses and with loss of maternally-derived immunity. Initial herd immunity (HI) was 0.71, and days from the last birth pulse before viral introduction until viral introduction (DB) were 10, 100, 200, and 300 days from top to bottom, respectively. Grey vertical bars represent the timing of annual birth pulses. (A-1) and (B-1) were obtained from the same simulations in Figure 3-2.
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Finally, if epidemic fadeout did not occur, then, infection persisted indefinitely in these deterministic models (Figure 3-3 A-1, A-4, B-1, and B-2). However, this would not be expected in a stochastic equivalent. The relatively deep troughs between peaks of fluctuating prevalence would make indefinite viral persistence highly unlikely due to endemic fadeout, which is the extinction of a disease owing to random fluctuations in the number of infected individuals in an endemic equilibrium state (Lloyd-Smith et al. 2005a). The overall low prevalence was attributable to the assumed lifelong immunity and a high lifespan-to-infectious period ratio (7 years to 7 days). Consequently, most epidemics in populations of black flying foxes could be expected to be transient, given the assumptions I made.

Figure 3-4. Effects of initial herd immunity (HI) and days from birth pulse to viral introduction (DB) on post-epidemic troughs in MSIR model. The numbers of infected individuals at troughs following major epidemics in (A) exponential MSIR model and in (B) gamma MSIR model. White part represents viral fadeout. Note that viral persistence was possible in more cases in gamma MSIR model than exponential MSIR model.
Additionally, I observed the initial herd immunity (HI) and the timing of viral introduction (DB) that allowed viral persistence. The highest numbers of infected individuals at the troughs following epidemic peaks were found in two parts of the parameter space: top and bottom (Figure 3-4). The top part was induced when a birth pulse fell on decreasing prevalence a short time after viral introduction (Figure 3-3A-4), while the bottom part was caused when a birth pulse fell on decreasing prevalence after epidemic peaks (Figure 3-3A-1 and B-1). Compared to the exponential MSIR model, the gamma MSIR model showed more combinations of DB and HI that allowed viral persistence (Figure 3-4). This effect arose because gamma-distributed periods of maternally-derived immunity resulted in the relatively steady supply of susceptible hosts throughout the year. Thus, the steady supply caused less intense epidemics and less deep troughs.

3.5. DISCUSSION

Modelling results showed that loss of maternally-derived immunity disperses the timing of supply of susceptible individuals. The dispersed timing causes an increase in the likelihood of viral persistence and shifts the timing of epidemic peaks further away from the timing of a birth pulse. However, these conclusions need to be interpreted with caution, because the modelling results are contingent on the assumptions made. Nevertheless, this chapter demonstrated that the effect of maternally-derived immunity in seasonally breeding is not negligible and should be considered to improve our understanding of infection dynamics in wildlife host populations.
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Plowright et al. (2008a) sampled serostatus of little red flying foxes and suggested that seasonality associated with birthing and mating might lead to epidemics of Hendra virus in flying foxes from the perspective of immunology and ecology. Maternally-derived immunity in little red flying foxes was identified, and it was hypothesised that the maternally-derived immunity would significantly enhance the virus persistence in flying foxes (Plowright et al. 2008a). Here, by using theoretical modelling, I demonstrated that the dispersed timing of supply of susceptible hosts due to waning passive immunity could contribute to increasing the likelihood of the virus persistence in a population of flying foxes.

The time span of supply of susceptible individuals into a population and time span of existence of infected individuals in a population are important factors in determining virus maintenance. A long-infected state can maintain the infection even when the supply of susceptible individuals is discrete (for example, seasonal birth pulses), while a continuous supply of susceptible individuals helps the population maintain the infection even when the infectious period is short. An example of the first case is the study of George et al. (2011), which demonstrated that hibernation slowed the metabolism of rabies virus infected big brown bats (Eptesicus fuscus), and maintained their infected state until susceptible newborns were supplied through a birth pulse. An example of the second case is the Hayman (2015) study, which suggested that filoviruses can persist in a bat population with bi-annual breeding, but not annual breeding. The finding supports the hypothesis that the dispersed timing of the supply of susceptible hosts into a population has a substantial effect on maintaining an infection in host populations. This chapter also belongs to the second case. The transgenerational transfer of antibodies from a mother to her offspring divided the timing of supply of susceptible hosts into
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multiple occasions, as the multiple number of birth pulses in a year did in Hayman (2015)’s study.

Understanding the mechanisms that cause seasonal fluctuation of prevalence may help predict the high-risk season of spillover. For example, Altizer et al. (2004) showed that understanding of the seasonality of Mycoplasma gallisepticum in wild house finches (Carpodacus mexicanus) could improve the prediction of outbreaks of the disease. Hendra virus in flying fox pooled urine samples shows a consistent and strong winter peak in virus excretion in southern Queensland and central and northern New South Wales (Field et al. 2015). The winter peak coincides with the high spillover risk season in subtropical regions (Plowright et al. 2015). To date, incomplete understanding of Hendra virus epidemiology and flying fox ecology has limited the capacity of models to generate accurate predictions of the timing of high spillover risk. Nevertheless, the framework suggested in this study should be helpful to predict the timing of pulses of virus shedding, based on the hypothesis that the pulses are caused by transient epidemics (Plowright et al. 2015).

Because flying foxes are natural reservoir hosts of Hendra virus (Halpin et al. 2000), Hendra virus must be maintained in flying fox populations. However, seasonal birth pulses make it more difficult for the virus to persist in a population than does a constant birth rate (Altizer et al. 2006) (see Appendix 3-5). Furthermore, when the annual birth pulse is tight, such as flying foxes, the disadvantage is further intensified (Peel et al. 2014). The seasonal breeding of flying foxes requires the existence of mechanisms that overcome or offset the disadvantageous conditions for viral persistence in flying fox populations. My modelling results imply that maternally-derived immunity can play a
role in mitigating those adverse conditions by dispersing the timing of supply of susceptible hosts into the populations.

Wearing et al. (2005) demonstrated that infectious disease modelling predictions are significantly affected by whether the infectious period and latent period are modelled using an exponential distribution or a gamma distribution. While maternally-derived antibodies are expected to decay exponentially, the loss of maternal immunity (defined by a certain threshold) and the rate of transition to the susceptible class could also be modelled using a gamma distribution. This chapter showed that gamma-distributed periods of maternally-derived immunity could generate significantly different modelling results compared to exponentially distributed periods. Because the functional form of the loss of maternally-derived immunity against Hendra virus or maternal immunity against other viruses is unknown, this study arbitrarily chose a gamma distribution parameter (g=10) for the gamma MSIR model (see Appendix 3-2). The results of the gamma MSIR model should not be interpreted as demonstrating a more accurate prediction than the exponential MSIR model, merely that substantially different modelling results can be generated depending on whether the loss of maternally-derived immunity is exponentially or gamma-distributed. Therefore, understanding of the functional form of the loss of maternally-derived immunity against emerging infectious diseases with age is expected to help improve prediction of the disease outbreaks.

Although this study modelled epidemics in a single population, results should be considered in the context of metapopulation structure, because flying foxes form metapopulations that might be important in Hendra virus maintenance mechanisms (Plowright et al. 2011). An effect of the seasonality of epidemics in metapopulations is
that seasonal forcing of transmission may cause epidemics in each population to be synchronised (Grassly and Fraser 2006). Synchronised epidemics are likely to increase the probability of viral fadeout at the level of a metapopulation. Viral introduction would be less likely to occur if epidemics in populations become extinct at similar timing. It is therefore necessary to examine the effects of the seasonality of Hendra virus on the synchrony of epidemics in the metapopulation models of flying foxes.

Despite growing interest in the infection dynamics of emerging viruses in their wildlife reservoirs (Daszak et al. 2000), the effect of wildlife behavioural characteristics on epidemic patterns requires further understanding. By incorporating maternally-derived immunity into generic epidemic models, I have provided a framework to study epidemics in seasonally breeding wildlife species. However, the timing of epidemic peaks or viral persistence is sensitive to demographic and virus-related parameters (Begon et al. 2009). This study depended on plausible assumptions rather than verified facts, and thereby the modelling results showed the effects of maternally-derived immunity on the timing of epidemic peaks rather than the prediction of the actual timing of epidemic peaks. Caution must be exercised in generalising conclusions concerning the effect of maternally-derived immunity, given specific circumstances for each disease and host population structure.

**3.6. CONCLUSION**

My models capture some features of seasonal infection dynamics in the system of Hendra virus infection in flying foxes. Loss of maternally-derived immunity in seasonally breeding wildlife has the effect of dispersing the timing of supply of susceptible hosts from births and from loss of the passive immunity. The dispersed
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timing results in lengthening the period from viral introduction until epidemic peaks. The dispersed timing also causes low-impact epidemics and subsequently shallow troughs, and thereby increases the likelihood of avoiding epidemic fadeout. The different methods of modelling loss of maternally-derived immunity may lead to substantially different prediction of the likelihood of viral persistence, which requires more cautionary modelling to improve the accuracy of modelling outcomes. Although additional refinements, particularly a more thorough understanding of the mechanism that drives pulses of virus shedding from wildlife reservoir hosts, could improve this study, the significance of seasonal factors in determining the epidemic patterns of this and other wildlife disease seems clear. Consequently, it appears plausible that the factors may also play an important role in disease emergence from wildlife reservoir populations.
CHAPTER 4

4. The Persistence of a SIR disease in a Metapopulation: Hendra virus

Epidemics in Australian flying foxes (Pteropus spp.)

4.1. ABSTRACT

Continuous outbreaks of emerging infectious diseases originating from bat populations have increased the need to understand the dynamics of viral infection in reservoir populations. Understanding how emerging viruses persist in the bat populations is a fundamental step to understanding the processes by which viruses are transmitted from the reservoir hosts to target hosts. Hendra virus, which has caused fatal infections in horses and humans in eastern Australia since 1994, spills over from its natural reservoir hosts, Pteropus bats (colloquially known as flying foxes). Although it is still unclear how this virus can persist in flying fox populations, the metapopulation structure of flying foxes may play a critical role in Hendra virus dynamics in flying fox populations. Here, I examine whether a metapopulation consisting of flying fox colonies that are smaller than the critical community size can maintain Hendra virus. Mathematical models were used to simulate a cycle, in which viral extinction and recolonisation were repeated in a single colony within a metapopulation. Given estimated flying fox immigration rates, the simulation results showed that recolonisation occurred more frequently than extinction, which indicated that infection would not go extinct in the metapopulation. Consequently, this chapter suggests that a collection of transient
epidemics of Hendra virus in numerous colonies of flying foxes in Australia can support long-term persistence of the virus at the metapopulation level.

4.2. INTRODUCTION

Bats are sources for emerging zoonotic diseases that are serious threats to human public health globally. Among bat-borne viruses, Hendra virus (Genus Henipavirus) has raised serious public health concerns in eastern Australia. Spillover of Hendra virus from bats of the genus *Pteropus* (colloquially known as flying foxes or fruit bats) has led to high mortality rates in horses, with onward infection to humans by horses, also resulting in high mortality (Halpin et al. 2000). These spillovers of Hendra virus have stimulated research effort to elucidate how the virus persists in its natural reservoir hosts, flying foxes (Breed et al. 2011, Plowright et al. 2011, Wang et al. 2013). Because long-term maintenance of a virus is a fundamental difference between reservoir hosts and target hosts, the understanding of the maintenance mechanism is a fundamental step to understand the whole system of the reservoir-target system of emerging infectious diseases (Haydon et al. 2002).

The dynamics of Hendra virus in flying foxes are not fully understood, and researchers have proposed different mechanisms for virus maintenance in flying fox populations. Plowright et al. (2011) stressed the importance of metapopulation structure for Hendra virus maintenance in flying fox populations. Breed et al. (2011) suggested, from serosurveillance evidence, that a spectacled flying fox (*P. conspicillatus*) population was endemically infected with Hendra virus. Wang et al. (2013) proposed that recrudescent infection contributes to the maintenance of Hendra virus in flying fox populations. Although all these arguments are based on reasonable data and inferences,
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they cannot be confirmed because current empirical data are inadequate to provide conclusive evidence (Plowright et al. 2016). Among the arguments, this chapter explores the dynamics of Hendra virus based on research by Plowright et al. (2011). The authors emphasised that Hendra virus spillover or maintenance is deeply implicated with the metapopulation structure of flying foxes, in which various forms of epidemics occur depending on the metapopulation dynamics.

Bats often form spatially discrete but frequently interacting colonies, establishing a metapopulation structure (Calisher et al. 2006). The metapopulation structure of bats has been studied to elucidate the maintenance mechanism of not only Hendra virus but also rabies virus and filoviruses (Blackwood et al. 2013, Hayman 2015). The role of metapopulation dynamics in viral maintenance is closely related to the rescue effect, which is defined as transmission between colonies that acts to re-infect colonies where the infection has gone extinct (Keeling 2000). The effect contributes to preventing a local extinction from extending into global one (Hagenaars et al. 2004). If Hendra virus causes an acute immunising infection (Plowright et al. 2011), the maintenance of Hendra virus in a single population become less likely due to a short infectious period and high herd immunity. In that case, virus maintenance could become more dependent on the rescue effect provided by the metapopulation structure (Metcalf et al. 2013). Therefore, it needs to assess the relative frequency of movements of flying foxes among colonies compared to the infectious period, in order to estimate the impact of rescue effect (Cross et al. 2005). Also, it needs to investigate whether the fluctuating herd immunity depending on viral extinction and recolonisation is maintained at a certain level to allow global maintenance of infections.
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Virus maintenance in a metapopulation structure could be easily achieved if some populations were larger than critical community size (CCS), which is the threshold populations size above which pathogen persistence is ensured (Bartlett 1957). This situation was described as ‘mainland-island’ model, in which mainland and island represent populations larger than CCS and populations smaller than CCS, respectively (Grenfell and Harwood 1997, Hanski and Gilpin 1997). However, even in exceptionally large population sizes of grey-headed flying foxes (P. poliocephalus), Hendra virus could be expected to become extinct within 10 years with the assumption of a seven-day infectious period and seasonal breeding (Peel et al. 2014). Thus, we need to investigate whether a metapopulation consisting of only colonies smaller than CCS can maintain Hendra virus persistence. This situation can be described as ‘island-island’ model. In an ‘island-island’ model, global persistence would be substantially assisted by the rescue effect that may enable an ensemble of transient epidemics to maintain global persistence (Plowright et al. 2011).

In a population smaller than CCS, the infection disappears in one of three ways; initial, epidemic, or endemic fadeout. If viral introduction does not trigger an epidemic, the situation could be called ‘initial fadeout’. Given the occurrence of an epidemic, the number of infectious individuals may drop sufficiently in the trough following the initial epidemic peak that the infection disappears, resulting in ‘epidemic fadeout’ (Lloyd-Smith et al. 2005a). If the infection survives through the trough, then it would last indefinitely in a deterministic model. In a stochastic model, however, the infection may undergo ‘endemic fadeout’ due to random fluctuation of the number of infectious individuals (Lloyd-Smith et al. 2005a).
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Three hypotheses for Hendra virus dynamics have been suggested: susceptible-infectious-immune (SIR), susceptible-infectious-immune-susceptible (SIRS), and susceptible-infectious-latent-infectious (SILI) dynamics (Plowright et al. 2016). SIR and SIRS models assume that infected bats excrete virus and that once excretion ceases, the bat recovers from infection. Resistance in the SIR model is life long, whereas the SIRS model assumes that recovered individuals may later return to susceptible status. In SILI dynamics, the virus is not cleared following infection, and bats stimulated by stressors may excrete virus occasionally (Plowright et al. 2015). Current empirical data are unable to distinguish between these three dynamics (Plowright et al. 2016). As described above, given the short period of Hendra virus excretion in bats, SIR dynamics are not consistent with Hendra virus persistence in a single colony of flying foxes, requiring metapopulation dynamics for long-term persistence (Plowright et al. 2011, Plowright et al. 2015). With SILI dynamics, the within-host immune response allows viral persistence in a single population, without the necessity of invoking a metapopulation structure (Breed et al. 2011, Wang et al. 2013). A SIRS disease may persist in a single colony, as Foley et al. (1999) showed that feline enteric coronavirus could persist in cat populations as long as the population was over 5 animals. As a conservative assumption, this study is based on SIR dynamics, which are less conducive to virus maintenance than SIRS dynamics.

Hayman (2016) emphasised the importance of a three-level multiscale approach in understanding bat virus dynamics: considering within-host dynamics, colony-scale dynamics, and metapopulation-scale dynamics. The persistence of Hendra virus in flying foxes has also been explored in multiple levels. In terms of within-host dynamics, Wang et al. (2013) stated that recrudescent infection supports Hendra virus persistence. The infectivity may become reactivated after the latent state based on SILI dynamics.
CHAPTER 4.
(Plowright et al. 2016). In terms of metapopulation-scale dynamics, Plowright et al. (2011) suggested that transient epidemics following the migration of bats might play a key role in maintaining Hendra virus in flying foxes. These different levels of studies for bat-borne virus persistence are also observed in rabies virus. Blackwood et al. (2013) found that rabies virus persistence was not possible in a single colony, and frequent movements between colonies were essential for the persistence at the level of metapopulation dynamics. On the other hand, George et al. (2011) suggested, at the individual level, that the incubation period plays a vital role in maintaining rabies virus in bat populations. Current knowledge cannot confirm which processes enabling virus maintenance are predominant for bat virus persistence (Plowright et al. 2016).

The lack of understanding of the transmission mode of Hendra virus led me to investigate Hendra virus transmission dynamics in both density -dependent and frequency -dependent transmission modes in this chapter. Although it has been suggested that Hendra virus might be transmitted via a combination of the two transmission modes in flying foxes (Plowright et al. 2015), the details of the effects of each mode in transmission dynamics have remained obscure. The particular importance of transmission mode becomes more evident when contemplating reduction in population size as a strategy to control infectious diseases. Modelling studies have demonstrated that culling of wildlife is unlikely to control infectious diseases with frequency-dependent transmission (McCallum et al. 2001, Wasserberg et al. 2009, Beeton and McCallum 2011), because frequency-dependent transmission allows pathogens to invade even small-sized populations (McCallum et al. 2001). Thus, we need to fully understand the transmission mode of Hendra virus before reduction of flying fox populations is considered as a management strategy for Hendra virus in
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flying foxes. If SILI dynamics are more predominant than SIR or SIRS dynamics, then reducing population size in itself would have no direct consequences for spillover risk.

Plowright et al. (2011) generated important findings on the effects of metapopulation structures of flying foxes on Hendra virus dynamics. The authors showed how extrinsic factors such as anthropogenic land use changes might affect the metapopulation dynamics, modifying the virus dynamics to facilitate Hendra virus spillover. They emphasised the importance of an ensemble of epidemic types for the virus maintenance in the metapopulation. Nevertheless, numerical evidence is needed to show the suitability of flying fox metapopulations for Hendra virus maintenance. For this purpose, this chapter models a single population in the context of a metapopulation structure to closely observe the virus extinction and recolonisation in the colony. The model is used to estimate the periods of an infected state and the periods of an infection-free state of the colony. The two kinds of periods are utilised in the strict-sense Levins metapopulation model (Levins 1969) to determine whether long-term virus maintenance is plausible in the flying fox metapopulation.

4.3. METHODS

4.3.1. A cycle consisting of three stages

The circular structure of Hendra virus extinction and recolonization consisted of three stages (Figure 4-1). In Stage 1, an infectious bat was introduced into an infection-free colony, which had been previously exposed to the virus and thus was partially immune. The introduction of an infectious bat simulated a rescue effect, possibly triggering an epidemic. Following this virus introduction, the virus could disappear from the population due to initial, epidemic, or endemic fadeout. The herd immunity at the point
of viral fadeout was recorded. In Stage 2, the herd immunity of the population decreased during the infection-free state, as immune bats died and were replaced by susceptible young. In Stage 3, I estimated the duration of the infection-free state, defined as from when the infection disappeared in the population until an infectious individual from other infected populations immigrated into the infection-free population. The stages were specifically designed to answer the following three questions, given a certain set of parameter values: 1) How long are the periods from recolonisation to extinction? 2) How fast does herd immunity decline in an infection-free population? 3) How long are the periods between viral extinction and recolonisation?

Figure 4-1. A cycle of viral recolonisation and extinction in a single colony in the context of a metapopulation. This cycle was repeatedly simulated to estimate the periods of infected state and the periods of infection-free state in stochastic models.

4.3.1.1. Model development

No new infectious bats were assumed to migrate into the colony while the colony was infected. The cycle was simulated in population sizes of 1,000 to 150,000 to simulate the broad range of colony sizes of flying fox colonies (Department of the Environment and Energy 2013). I iterated this cycle 1000 times and discarded the first 200 iterations to remove the effect of the arbitrary choice of the initial herd immunity (HI=0.5). From
those remaining 800 iterations, I obtained the distribution of the periods of the infected state. The stochastic simulations were implemented using GillespieSSA (Gillespie stochastic simulation algorithm) (Gillespie 2007, Pineda-Krch 2008). The time of events and which event would occur were stochastically determined by the previous events with their transition rates (Keeling and Rohani 2008, Lu et al. 2013). I used R package ‘GillespieSSA’ to run the stochastic models (Pineda-Krch 2010). All models were simulated in R (R Core Team 2016).

Table 4-1. Model parameters and their values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value</th>
<th>Unit</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density-dependent transmission rate</td>
<td>( \beta )</td>
<td>0.0000476</td>
<td>Per capita per day</td>
<td>(Plowright et al. 2011)</td>
</tr>
<tr>
<td>Frequency-dependent transmission rate</td>
<td>( \beta' )</td>
<td>0.476</td>
<td>Per capita per day</td>
<td>This study, (Plowright et al. 2011)</td>
</tr>
<tr>
<td>Recovery rate</td>
<td>( \gamma )</td>
<td>1/7</td>
<td>Per day</td>
<td>(Plowright et al. 2011)</td>
</tr>
<tr>
<td>Mortality rate</td>
<td>( \mu )</td>
<td>1/7</td>
<td>Per year</td>
<td>(Martin and McIlwee 2002, Tidemann and Nelson 2011)</td>
</tr>
<tr>
<td>Birth rate</td>
<td>( b )</td>
<td>1/7</td>
<td>Per year</td>
<td>(Martin and McIlwee 2002, Tidemann and Nelson 2011)</td>
</tr>
<tr>
<td>Movement rate of flying foxes between colonies</td>
<td>( m )</td>
<td>1/9</td>
<td>Per day</td>
<td>(Roberts et al. 2012b)</td>
</tr>
<tr>
<td>Prevalence</td>
<td>Prev</td>
<td>0.0017</td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td>Colony size</td>
<td>( N )</td>
<td>1,000–150,000</td>
<td>Individuals</td>
<td>This study</td>
</tr>
</tbody>
</table>
Stage 1 estimated the duration of the infected state of a population following viral introduction into a population. The duration of the infected state was defined as the time in days from the viral introduction until the number of infectious bats became zero. This stage was simulated for up to two years, or as long as the infectious hosts existed. If the infection persisted for two years, it was defined as ‘viral persistence’. If the maximum number of infectious hosts did not exceed 50 and the infection disappeared in 50 days, it was defined as initial fadeout. Because of the very low number of infectious hosts in an endemic equilibrium state, given plausible parameter values, it was not appropriate to differentiate between epidemic fadeout and endemic fadeout. Both fadeouts were defined to be viral extinction within two years after an epidemic.

Table 4-2. Events, changes and rates used for density and frequency dependent transmission models. The models were simulated stochastically by using GillespieSSA (Gillespie stochastic simulation algorithm) method.

<table>
<thead>
<tr>
<th>Event</th>
<th>Change</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth</td>
<td>S→S+1, I→I+1, R→R+1</td>
<td>b(S + I + R)</td>
</tr>
<tr>
<td>Death</td>
<td>S→S-1, I→I-1, R→R-1</td>
<td>μS, μI, μR</td>
</tr>
<tr>
<td>Density-dependent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transmission</td>
<td>S→S-1, I→I+1</td>
<td>βSI</td>
</tr>
<tr>
<td>Frequency-dependent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transmission</td>
<td>S→S-1, I→I+1</td>
<td>βSI/N</td>
</tr>
<tr>
<td>Recovery</td>
<td>I→I-1, R→R+1</td>
<td>γI</td>
</tr>
</tbody>
</table>

SIR dynamics were stochastically simulated using GillespieSSA, and both density-dependent transmission rate (β) and frequency-dependent transmission rate (β’) were explored (Table 4-1). Susceptible (S) individuals become infectious (I) at the rate βSI in
a density-dependent transmission model or $\beta SI/N$ in a frequency-dependent transmission model (Table 4-2). The frequency-dependent transmission rate was set to be equivalent to the density-dependent transmission rate in a population size of 10,000, which is an approximately average colony size of flying foxes (Department of the Environment and Energy 2013). Individuals were assumed to be infectious until recovery at rate $\gamma$, $1/(\text{infectious period})$ and survived as immune (R) for life. The transmission rates and recovery rate were referred from the estimated values in Plowright et al. (2011).

4.1.1.2. Stage 2 – Infection-free state

Stage 2 modelled the declining herd immunity during infection-free state after virus extinction (end of Stage 1) until the next introduction of an infectious individual (Stage 3). Stage 2 was devised to estimate the herd immunity to be used in Stage 3. To calculate the decreasing herd immunity, I used an equation; $HI' = (HI)(1 - \mu)^d$, where $HI'$, $HI$, $\mu$, and $d$, respectively, denote decreasing herd immunity with the course of time, herd immunity at the moment of virus extinction, daily mortality rate, and days. This equation was calculated deterministically. Assuming lifelong immunity based on SIR dynamics, a reduction in the number of immune bats was only caused by the replacement following the death of susceptible or immune adults with birth of susceptible newborns. Immigrating and emigrating bats were assumed not to affect the herd immunity. I assumed that the colony size remained constant, with dying animals being replaced with newborns and equal numbers of immigrants and emigrants.
4.1.1.3. Stage 3 – Immigration of an infectious bat into an infection-free population

Stage 3 estimated the duration of time between viral extinction until viral reintroduction via immigration of an infectious bat into an infection-free colony. In the process of estimating the duration, I chose the parameter values to generate the longest possible duration, under the least favourable conditions for the virus to be maintained. Two factors were required to estimate the duration. First, it was necessary to assess the movement rate (m) of flying foxes among colonies. This rate was assumed from the mean movement rate of grey-headed flying foxes (P. poliocephalus) among colonies between the mean rate (m=1/9 days) and the median rate (1/2 days) observed by Roberts et al. (2012b). Second, I considered the prevalence (Prev) in colonies from which bats migrated into the infection-free colony. This source prevalence (Prev=0.0017) was obtained from the endemic equilibrium of the SIR dynamics in Stage 1, in which a colony size of 10,000 was supposed. This estimated prevalence is much lower than the prevalence (0.03) observed by Edson et al. (2015), which found an overall mean prevalence across more than 1400 individual P. alecto samples. However, Hendra virus prevalence showed strong seasonal fluctuation (Field et al. 2015). Thus, I used the estimated prevalence of 0.0017 to consider the low prevalence between the peaks of prevalence, which is a difficult situation for flying fox populations to maintain Hendra virus. I assumed that immigration and emigration occurred at the same rates, keeping the colony size constant. This stage was stochastically simulated by using GillespieSSA method.

In order to calculate the period until at least one infectious bat migrated into an infection-free colony from other infected colonies, I first calculated the probability (R) that no infectious bats migrated into an infection-free colony per day. For this purpose, I
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used the equation: \( R = (1 - P_{rev})^{m \times N} \). Then, I calculated the number of days until the probability of the migration of at least one bat exceeded 0.95. For this purpose, I used the equation: \( d = \frac{\log(1 - 0.95)}{\log R} \). This equation was obtained from \( R' \geq 0.95 = 1 - R^d \), where \( R' \) represented a probability that at least one infectious bat migrated from other infected colonies to an infection-free colony during a period of \( d \). Details of the equations are shown in Appendix 4-1.

4.1.2. Levins metapopulation model

The strict-sense Levins metapopulation model (Levins 1969) was used to assess the metapopulation structure required to support long-term maintenance of Hendra virus. In the Levins metapopulation model, a colony was simply defined as being either infection-free or infected (Keeling and Rohani 2008). The estimated periods of infected state and infection-free state were used in the Levins metapopulation model. The fraction of occupied patches, denoted by \( P \), is the probability that a particular host patch is infected by the disease (Foley et al. 1999), and the dynamics are given by \( \frac{dP}{dt} = cP(1 - P) - eP \), where \( c \) and \( e \) are the colonization rate and the per-colony viral extinction rate, respectively. I estimated \( c \) and \( e \) as a reciprocal of the mean periods of infected state from the repetition of the cycle and as a reciprocal of the mean periods of infection-free state from stage 1, respectively. The Levins metapopulation equilibrium value of \( P \) is given by \( \hat{P} = 1 - \frac{e}{c} \) (Foley et al. 1999). If \( \hat{P} > 0 \), then the strict-sense Levins metapopulation would not go extinct across all patches (Foley et al. 1999), which indicates that Hendra virus would persist within the metapopulation of flying foxes in the long-term.
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4.2. RESULTS

4.2.1. Repetition of the cycle

In the process of repeated viral recolonisation and extinction, extinction (marking the end of Stage 1) occurred in either of three forms: initial, epidemic, or endemic fadeout, unless viral persistence occurred without extinction for two years. A considerable portion of viral introductions did not bring about a substantial increase in the number of infectious hosts and failed to trigger an epidemic, resulting in initial fadeout (Figure 4-2). A relatively small portion of viral introductions succeeded in triggering epidemics, leading to epidemic fadeout, endemic fadeout or viral persistence. The low number of infectious individuals in the endemic equilibrium state made epidemic fadeout and endemic fadeout difficult to distinguish in these simulations. Figure 4-3 shows that a single long-term infection and a series of short-term infections occurred alternately, to maintain the herd immunity within a certain range. Long-term infection leading to epidemic or endemic fadeout produced a large increase in herd immunity, while short-term infection causing initial fadeout resulted in a slight decrease in herd immunity. As a result, the periods of infected state consisted of a low frequency of long-term infections and high frequency of short-term infections, and thereby the variance in the periods of the infected state was wide.
Hendra virus persistence

Figure 4-2. The relation between the frequency of viral fadeout or persistence and the proportion of viral fadeout or persistence for the period of infected state. In density-dependent transmission (A) and frequency-dependent transmission (B), the frequency of each viral fadeout and persistence (A-1 and B-1) was characterised by a wide range of colony sizes and the proportion of each viral fadeout and persistence for the period of infected state (A-2 and B-2) was characterised by a wide range of colony sizes. Epidemic and endemic fadeouts could not be differentiated from the simulation results. Horizontal axis is log-scaled. Note that the duration of simulation was two years so that the viral persistence was set to be two years although the period of viral persistence was expected to be much longer than two years.
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Figure 4-3. Repetition of the cycle of viral extinction and recolonisation in a colony. These plots show a random example of serial repetition of the cycle with density-dependent transmission (top panel) and frequency-dependent transmission (bottom panel) in a colony of 10,000 bats. In the repetition, an infectious host was introduced into a colony, and the duration of infected state and herd immunities at viral fadeout were tracked. A series of initial fadeouts that gradually decreased the herd immunity and an epidemic or endemic fadeout that increased the herd immunity abruptly appeared alternately and led the herd immunity to fluctuate within a certain range.
Figure 4-4. The mean periods of infected state and the mean periods of infection-free state. The 8000 iterations of viral recolonisation and extinction in the cycle of three stages were used to estimate the periods. Mean periods of infected state and infection-free state for each colony size are shown. The transmission mode makes a difference in period of infected state, but the mode does not make a difference in period of infection-free state.

The mean length of the periods in the infected state increased as the colony size increased, but the length was more substantially affected by colony size in density-dependent transmission than frequency-dependent transmission (Figure 4-4). The mean length of the periods in the infection-free state decreased regardless of the transmission mode. Except for the markedly small-sized colonies, the mean length of the periods of the infected state was longer than the mean periods of infection-free state for both density-dependent and frequency-dependent transmission.
Figure 4-5. The equilibrium value of fraction of occupied patches ($\hat{P}$) in the strict-sense Levins metapopulation model. If $\hat{P} > 0$, then persistence would be expected, and if $\hat{P} < 0$, then persistence would not be expected.

4.2.2. Levins metapopulation model

The colonisation rate ($c$) was overall higher than the extinction rate ($e$). Consequently, the Levins metapopulation equilibrium value, $\hat{P}$, was higher than zero and overall close to 1, except for the small-sized colonies with density-dependent transmission (Figure 4-5). The modelling result predicted that extinction of Hendra virus in the metapopulation was highly unlikely. Therefore, given relatively regular immigration of infectious bats from outside a colony, it was predicted that, at the chosen parameters, Hendra virus could be indefinitely maintained in a metapopulation of flying foxes that did not include colonies sufficiently large to be able to maintain persistent endemic infection.
4.3. DISCUSSION

There has been a hypothesis that a metapopulation of flying foxes can enable long-term persistence of Hendra virus. The significance of this study is that it provides modelling validation for the hypothesis that has been proposed but not established, provided Hendra virus is an SIR disease, with the chosen parameters. The modelling results showed that a colony of flying foxes was estimated to be in an infected state longer than in an infection-free state, given that infectious flying foxes reasonably regularly immigrated into the colony from other infected colonies. The results also reveal that a collection of transient epidemics can ensure global persistence, even without colonies larger than CCS.

Plowright (2007) stated that the balance between extinction and recolonisation is critical for the maintenance of Hendra virus in flying foxes, based on the Levins effect (Hanski 1999). By employing the Levins metapopulation model (Levins 1969), this chapter showed that the recolonisation rate was higher than the extinction rate, which meant that the periods of infected state were longer than the periods of infection-free state in colonies. In accordance with Plowright et al. (2011), the chapter provides further modelling evidence supporting the suggestion that, if Hendra virus is an SIR disease, metapopulation dynamics may be significant in maintaining Hendra virus.

This chapter improved the understanding of the maintenance mechanism of Hendra virus based metapopulation dynamics as explored in Plowright et al. (2011). While these authors simulated many colonies in metapopulations to show comprehensively the role of metapopulation structure on virus dynamics, I modelled a single colony in the context of a metapopulation to examine specifically the events occurring in a colony.
within a metapopulation. The modelling results in this chapter showed how the virus fadeout or persistence is determined by the basic epidemiological factors such as population size or transmission modes and emphasise the importance of accurate identification of transmission mode for managing wildlife diseases.

Whether Hendra virus dynamics is governed by SIR, SIRS, or SILI dynamics, or a combination of the three dynamics remains unclear (Plowright et al. 2016). Nevertheless, if we suppose SIR dynamics and exclude reactivation of the virus, metapopulation dynamics are essential to understand Hendra virus persistence because individual colonies of flying foxes do not appear to be larger than CCS (Peel et al. 2014). Appendix 4-2 shows that a single colony comprising of more than 100,000 flying foxes is necessary to support endemic persistence of the virus, for longer than two years. A flying fox colony of such a large size is unlikely to exist in natural conditions, except in certain circumstances for short periods of time (Department of the Environment and Energy 2013). Flying foxes modulate their colony size flexibly while seeking seasonally and interannually varying feed sources (Eby 1991, Hall and Richards 2000, Tait et al. 2014) and the population size fluctuates due to seasonal breeding (Peel et al. 2014). As a result, even if a colony was above the CCS, it would be unlikely to maintain such a large population size for an extended period. The large CCS for Hendra virus infection in flying foxes is mainly attributable to the short infectious period of Hendra virus (Plowright et al. 2011) (estimated mean period of seven days, although there is a possibility that these bats may stay persistently infected, see Plowright et al. (2016)). The short infectious period leaves a small number of infected individuals in endemic equilibrium, so that the infection becomes vulnerable to stochastic extinction (that is, endemic fadeout). Given a herd immunity at viral introduction (HI=0.5), an infectious period of approximately 36.5 days would be required to have a 50%
probability of maintaining Hendra virus infection in a single population of 10000 seasonally breeding grey-headed flying foxes (Peel et al. 2014).

In a metapopulation, a relatively high movement rate compared to a recovery rate is required for infectious individuals to be able to have a high probability of spreading the infection to other populations while they are infectious (Cross et al. 2005). Consequently, a low movement rate inhibits spread of infection. On the other hand, a movement rate that is too high reduces heterogeneity of spatial structure and may result in synchronised epidemics in each population (Jesse et al. 2008). Synchronised epidemics reduce the rescue effect because when a colony loses infection, it would be more likely to be surrounded by infection-free colonies that lose infection at the same time. As a result, it has been found that persistence is maximised at an intermediate level of movement rate in a metapopulation structure (Grenfell and Harwood 1997).

Also in the case of Hendra virus maintenance, a combination of various forms of epidemics is important to maintain asynchronous epidemics (Plowright et al. 2011). The availability of more realistic data on flying fox distributions could allow a quantitative analysis of the effect of synchrony on the persistence of Hendra virus in a metapopulation (Tran-Thi et al. 2016).

The metapopulation structure of flying foxes is related not only to viral persistence but also to spillover risk. Increasing numbers of flying foxes in urban areas (Tait et al. 2014) may have two opposing implications for spillover risk of Hendra virus. On the one hand, overall flying foxes moved more frequently between nearby colonies than between distant colonies (Roberts et al. 2012b). Colonies in urban areas tend to be closer to each other than are colonies in rural areas (Department of the Environment and
CHAPTER 4.
Energy 2013), which would likely result in higher inter-colony movement rates for urban bats than for rural bats. Consequently, the frequent introduction of infectious bats into infection-free colonies in urban areas appears to reduce spillover risk, because the short period in the infection-free state would result in less diminishment of herd immunity, with viral introduction triggering small-impact epidemics. This dynamic would suggest that urban colonies are at lower risk of Hendra virus. On the other hand, urban bats may move less frequently than rural bats, because closer locations between roosting sites and feeding sites and stable sources of feed in urban areas decrease the necessity for movement (Plowright et al. 2011). Such decreased movement of urban bats might facilitate high-impact epidemics by allowing more time for herd immunity to decline in infection-free colonies. It is not certain how increasing bats in urban areas affect epidemic impact with the two contradicting hypotheses. More detailed observations of flying fox movements in urban areas, as compared with rural areas, are required to better predict the effects of increasing urban colonies on Hendra virus spillover risk.

4.4. CONCLUSION
This chapter explored Hendra virus persistence in a metapopulation of flying foxes by using mathematical models, and provided modelling validation for the hypothesis that the metapopulation of flying foxes is a critical factor for Hendra virus to be maintained in their populations. The frequent movement of individual bats among nearby colonies supports frequent epidemics in each colony. Hendra virus maintenance in its reservoir populations may rely on a collection of epidemics in numerous colonies that are smaller than CCS. This chapter found that the recolonisation rate was higher than the extinction rate, indicating that long-term persistence of Hendra virus in a metapopulation of flying
foxes is likely. The long-term persistence could be possible mainly because the high movement rate of flying foxes between colonies introduces infectious bats into infection-free colonies soon after the infection becomes extinct in the colonies. Insufficient knowledge of flying fox spatial distributions requires the use of a simplified metapopulation model in this chapter. If more realistic data of flying fox distributions become available, more detailed metapopulation models could examine how synchrony of epidemics might affect persistence of the virus.
CHAPTER 5

5. Modelling Culling and Colony Dispersal as Strategies to Manage Zoonotic Bat Viruses

5.1. ABSTRACT

Frequent outbreaks of emerging infectious diseases originating from wildlife have raised the need to understand viral dynamics in wildlife populations. Wildlife species are often observed to inhabit in the form of metapopulation structure, and understanding of metapopulation dynamics is therefore required to manage wildlife diseases appropriately. Here, I explore epidemic dynamic models in metapopulation structures inspired by Hendra virus transmission in flying foxes. Culling or dispersal of flying foxes may change their metapopulation structure, affecting Hendra virus transmission. I simulate rather hypothetical scenarios (uniform metapopulation network scenarios) to understand the effects of each metapopulation-related factors on the probability of epidemic occurrence and relatively more realistic scenarios (culling and dispersal scenarios) to estimate the effects of plausible population management on the probability. The modelling results showed that increased epidemic occurrence is stimulated by the reduced numbers of major sized colonies, and the effects of number of colonies in a metapopulation and number of bats in each colony are diluted by strong spatial coupling among colonies. Despite a conservative approach of employing modelling framework that created the most favourable scenarios for population management strategies to be effective, culling or dispersal were not efficient means to
reduce Hendra virus epidemics, and rather the actions might be counter-productive. Thus, the situations in which the culling and dispersal can be effectively implemented to reduce the spillover risk of Hendra virus are quite limited and unlikely.

5.2. INTRODUCTION

The emergence of zoonotic diseases from wildlife has threatened human public health. Frequent outbreaks of fatal infectious diseases emanating from wildlife have increased interest in controlling infectious diseases in their reservoir populations before the aetiologic agents spillover (Daszak et al. 2000, Wobeser 2002). A possible strategy to control the spillover risk is to manage the population structure of wildlife (Smith and Cheeseman 2002, McCallum 2016). Culling has been used in attempts to reduce risk of spillover of zoonotic diseases originating from wildlife that have threatened human or livestock health (Wobeser 2002). However, the strategy might result in counterproductive outcomes, if it was implemented without an accurate assessment of the outcomes. For example, the culling of badgers has not been as successful as expected in managing the spillover of cattle tuberculosis from badgers to British cattle. Although TB incidence in cattle was reduced in areas where badgers were culled, the social and spatial disturbances of the badger group caused by the culling of badgers led to increased incidence in cattle in adjoining areas (Donnelly et al. 2006). Modelling studies could be effectively used to predict probable outcomes and to evaluate and guide wildlife population control programmes (McCallum 2016). In particular, modelling studies enable the investigation of a broader range of scenarios than is possible with experimental or empirical studies alone (Beeton and McCallum 2011).
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One example of a system for which wildlife management actions have been proposed as a result of an emerging infectious disease is that of Hendra virus in flying foxes. In 1994, the first identified outbreak of the previously unknown Hendra virus in horses and humans was reported in eastern Australia (Rogers 1994). A few years later pteropid bats (commonly known as flying foxes) were identified as natural reservoir hosts of Hendra virus (Young et al. 1996). All four flying fox species found in Australia – the black flying fox (*Pteropus alecto*), grey-headed flying fox (*P. poliocephalus*), little red flying fox (*P. scapulatus*), and spectacled flying fox (*P. conspicillatus*) – can be infected with Hendra virus (Field et al. 2011). However, in subtropical eastern Australia, the black flying fox appears to play a particularly important role as a reservoir for Hendra virus (Smith et al. 2014, Field et al. 2015). Hendra virus infection spills over from flying foxes to horses and from horses to humans. In both the spillover hosts it has a high case mortality (Field et al. 2007). The spillover events of Hendra virus have occurred mostly in the east coast of Queensland and northeast New South Wales during austral winter, and the frequency of spillover peaked in 2011 (Plowright et al. 2015). Spillover of Hendra virus appears to be facilitated by pulses of virus shedding, and two hypotheses have been suggested as the mechanism generating the pulses (Plowright et al. 2015). First, the pulses good be caused by ‘transient epidemics’, when the virus is transmitted among bat colonies (Plowright et al. 2015). Second, the pulses could be generated by ‘episodic shedding’ from persistently infected bats, when the immune system of individual bats is weakened by stressors (Plowright et al. 2015). The identification of the reservoir hosts of Hendra virus has led to questions as to whether population management strategies for flying foxes can reduce transmission dynamics of Hendra virus under each hypothesis, and ultimately how this may affect the spillover risk of Hendra virus.
Culling and dispersal have been commonly considered as population management strategies for flying foxes in Australia, usually more motivated by reasons other than Hendra virus spillover (Roberts et al. 2011, Florens 2015). Culling flying foxes through shooting or electrocution has been considered and intermittently implemented mainly by fruit growers to protect their orchards, which are food sources for flying foxes (Garnett et al. 1999). People living in close proximity to large flying fox colonies may be discontented because of their displeasing odour, noise, and droppings. Dispersal of colonies has been proposed and implemented as a way to alleviate these concerns (Roberts 2006). Even though such management of flying fox populations may be effective for these intended purposes, these management actions might lead to changes in the spillover risk of Hendra virus. The changed spatial structure of flying fox populations may be more likely to facilitate spillover than the spatial structure before the actions. There is therefore a need to understand how different spatial structures of flying fox populations affect Hendra virus transmission dynamics.

Here, I explore dynamic models inspired by Hendra virus transmission in flying foxes. The ultimate objective is to understand how culling and dispersal of flying foxes may affect the spillover risk of Hendra virus. However, this chapter investigates how culling and dispersal of flying foxes may affect the pulses of virus shedding in flying foxes. Because spillover events involve multilevel interactions between pathogens, reservoir hosts, target hosts, and the environment, understanding of all these factors is required to predict the spillover risk (Plowright et al. 2015). However, current knowledge of Hendra virus infection in flying foxes is insufficient to predict the spillover risk through each of these levels. As a result, this chapter investigates how culling and dispersal might affect the pulses of virus shedding in flying foxes, which it is likely to influence spillover risk (Plowright et al. 2015).
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Flying foxes in Australia form metapopulations, in which flying foxes live in colonies and move among those colonies periodically (Plowright et al. 2011). Based on the hypothesis of transient epidemics, the metapopulation structure of flying foxes may play a role in stimulating Hendra virus spillover. Plowright et al. (2011) suggested urbanisation of flying foxes may have changed metapopulation structure, contributing to an increase in Hendra virus spillover risk. Hendra virus may have a short infectious period followed by a long-lasting immunity following SIR (susceptible-infectious-immune) dynamics (Plowright et al. 2016). If this is the case, reduced movement of flying foxes would allow infection-free colonies to have a longer time in which herd immunity decreases until re-introduction of infectious flying foxes from other colonies. The decreased herd immunity would result in higher-impact epidemics, which is implicated with a higher risk of spillover (Plowright et al. 2011). Therefore, plans for culling and colony dispersal should consider the metapopulation structure of flying foxes to predict how these management actions may affect the spillover risk of Hendra virus.

This chapter consists of two parts. In the first part, I simulate hypothetically designed uniform metapopulation network scenarios to explore the effects of metapopulation-related factors (number of colonies in a metapopulation, number of bats in each colony, and distance among colonies) on the probability of epidemic occurrence. The results of this part would not only help improve our knowledge of metapopulation dynamics but also help interpret the results of the second part. In the second part, I assess the relative effect of various culling and dispersal scenarios on the probability of epidemic occurrence in a model inspired by a metapopulation of black flying foxes in south-east
Queensland, Australia. Because of the lack of knowledge of Hendra virus infection in flying foxes, this study had to rely on several assumptions that were probable but not validated. Therefore, this chapter compares the relative effects of these scenarios on the probability of epidemic occurrence rather than to aiming to predict quantitatively the spillover risk of Hendra virus.

5.3. METHODS

5.3.1. Baseline model framework

The model is based on the most suitable possible conditions for culling to be a viable means of controlling a virus with flying foxes as a reservoir. Plowright et al. (2016) suggested three possible hypotheses driving Hendra virus dynamics: SIR (susceptible-infectious-immune) dynamics, SIRS (susceptible-infectious-immune-susceptible) dynamics, and SILI (susceptible-infectious-latent-infectious) dynamics. The first two of these can be characterised as “transient epidemic” dynamics, whereas the last can be characterised as “episodic shedding”. I assumed that there were ‘transient epidemics’ because these are likely to be influenced by the decrease in population size, whereas pulses driven by episodic shedding are less likely to be influenced by reduction of population size. I further assumed density-dependent transmission, which means that transmission will decrease when population size is reduced, whereas frequency-dependent transmission does not decrease with reduction of population size (McCallum et al. 2001). Models in this chapter used SIR dynamics because the hypothesis of ‘transient epidemics’ can be simulated appropriately with SIR dynamics. Susceptible (S) individuals become infectious at the rate (βSI). Bats are infectious (I) before recovery at rate γ and survive as immune (R) for life (Table 5-1).
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Assuming that management actions take place on very short timescale, I did not model virus dynamics whilst the actions were in process, but instead, I modelled transient epidemics in the changed metapopulation structures following the completion of culling or dispersal. The situation was that infectious hosts were introduced into a colony in an infection-free metapopulation that was previously exposed to the virus and partially immune. I assumed that only one colony received infectious hosts as migrants to simulate how different metapopulation structures in various scenarios affected the spread of epidemics within the metapopulation. The number of infectious hosts introduced into the metapopulation (1/700 of the baseline colony size) was selected to ensure that appropriate level of transmission events took place in each scenario to allow comparison among scenarios. The colony to which infectious bats were introduced was determined at random, weighted by colony sizes. The pre-existing immunity (HI) was assumed to be 0.5 at the moment of viral introduction (equivalent to Hendra virus seroprevalence rates commonly observed in *P. poliocephalus* (Field 2005, Peel et al. 2014). The outcome of models was the probability of epidemic occurrence. If the number of infectious bats in any colony in a metapopulation exceeded three times the number of bats initially introduced, I defined an epidemic as having occurred.

Simulation of the model was implemented stochastically using Gillespie’s Direct Method (Gillespie 1977, Keeling and Rohani 2008). All events, with their corresponding change rates, were used to determine the time of the next event and which event would occur (Lu et al. 2013) (Table 5-2). All stochastic models were implemented using R package “GillespieSSA” (Pineda-Krch 2010) in R programming language (R Core Team 2016). The 1000 iterations were used to generate the outcomes.
Table 5-1. Model parameters.

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epidemiological and demographic parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transmission rate (β)</td>
<td>0.0000476</td>
<td>(Plowright et al. 2011)</td>
</tr>
<tr>
<td>Recovery rate (γ)</td>
<td>1/7 day</td>
<td>(Plowright et al. 2011)</td>
</tr>
<tr>
<td>Mortality rate (μ)</td>
<td>1/7 year</td>
<td>(McIlwae and Martin 2002)</td>
</tr>
<tr>
<td>Birth rate (b)</td>
<td>1/7 year</td>
<td>(McIlwae and Martin 2002)</td>
</tr>
<tr>
<td>Proportion of immune bats in populations at viral introduction (HI)</td>
<td>0.5</td>
<td>(Field 2005)</td>
</tr>
<tr>
<td><strong>Sunshine Coast metapopulation parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total colony number</td>
<td>16 colonies</td>
<td>(Towsey 2017)</td>
</tr>
<tr>
<td>Number of urban colonies</td>
<td>8 colonies</td>
<td>(Towsey 2017)</td>
</tr>
<tr>
<td>Number of rural colonies</td>
<td>8 colonies</td>
<td>(Towsey 2017)</td>
</tr>
<tr>
<td>Connectivity parameter (c)</td>
<td>0.08367</td>
<td>From this study</td>
</tr>
<tr>
<td>Baseline colony size (N)</td>
<td>4000, 7000, or</td>
<td>(Department of the Environment and Energy 2013)</td>
</tr>
<tr>
<td></td>
<td>10000 bats</td>
<td></td>
</tr>
</tbody>
</table>

Table 5-2. Events, changes and rates used for stochastic simulations.

<table>
<thead>
<tr>
<th>Event</th>
<th>Change</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth</td>
<td>S→S+1, I→I+1, R→R+1</td>
<td>b(S + I + R)</td>
</tr>
<tr>
<td>Death</td>
<td>S→S-1, I→I-1, R→R-1</td>
<td>μS, μI, μR</td>
</tr>
<tr>
<td>Transmission</td>
<td>S→S-1, I→I+1</td>
<td>βSI</td>
</tr>
<tr>
<td>Recovery</td>
<td>I→I-1, R→R+1</td>
<td>γI</td>
</tr>
<tr>
<td>Movement of an infected bat from colony i to colony j</td>
<td>I_i→I_i-1, I_j→I_j+1</td>
<td>∑ j m_ij I_i</td>
</tr>
</tbody>
</table>
5.3.2. **Sunshine Coast metapopulation**

I modelled a metapopulation based on the population data of black flying foxes in Sunshine Coast, Queensland, Australia. I focussed on black flying foxes for two reasons. First, black flying foxes and spectacled flying foxes have been more responsible for the transmission to horses than grey-headed flying foxes and little red flying foxes (Smith et al. 2014, Field et al. 2015). Second, the distribution of black flying foxes encompasses south-east Queensland and northeast New South Wales, where most Hendra virus spillover events have occurred, whereas the distribution of spectacled flying fox is limited to north Queensland (Hall and Richards 2000).

Satellite tracking data of black flying foxes in the Sunshine Coast region of south-east Queensland from “Movebank” was used to design a realistic baseline metapopulation structure (Towsey 2017). I used the data to determine the spatial layout of colonies in the model and to obtain the data to estimate the movement rate of bats between colonies. The tracking data included a total 1,852 tracking events consisting of ten individual black flying foxes from April 2015 to May 2016 (Towsey 2017). Each tracking event showed the duration of a stay in a certain latitudinal and longitudinal location (Towsey 2017). The data did not contain biological data of bats such as age and sex (Towsey 2017). The data were filtered to leave only relocating movements between successive roosting sites from one night to the next, and to exclude commuting movements between roosting sites and feeding sites and among feeding sites, because this study assumed that transmission occur only at roosting sites, not at feeding sites. After the filtration, the dataset contained the movement data of black flying foxes among 16 roosting sites in a metapopulation (hereafter referred to as Sunshine Coast metapopulation) (Figure 5-1). Because the tracking data did not include numbers of bats in each colony, I assumed that three levels of colony sizes of 7000, and 10000, which
were within the range of colony sizes of black flying foxes in Sunshine Coast region (Department of the Environment and Energy 2013). The Sunshine Coast metapopulation was assumed to consist of either of these two levels of colony size.

Figure 5-1. Colonies of black flying foxes (Pteropus alecto) around Sunshine Coast, Queensland, Australia. The 16 colonies consist of eight urban colonies and eight rural colonies. Note that urban colonies are located more closely each other than rural colonies.
The metapopulation modelling of black flying foxes required estimation of how frequently the bats migrate between colonies. I used the movement data of black flying foxes from “Movebank” to obtain an equation to estimate movement rates as a function of distance (Towsey 2017). Based on the overall tendency of movement data of black flying foxes in the Sunshine Coast metapopulation, I assumed that bats move between any pair of colonies at a rate inversely exponentially proportional to the distance between the two colonies (similar to the assumption made by Plowright et al. 2011). Thus, movement rate \( m_{ij} \) from colony i to colony j was estimated by using an equation, 
\[
m_{ij} = e^{(-cD_{ij})},
\]
where \( D_{ij} \) is a distance between two colonies i and j, and c is a connectivity parameter that controls how movements of bats between colonies drops off with increasing distance (Plowright et al. 2011). I substituted the mean movement rate (1/13.5 days\(^{-1}\)) and the mean distance among colonies (31.1 km) assessed from the satellite tracking data (Towsey 2017) into the equation and calculated the connectivity parameter (c=0.08367).

Urban colonies are of greater importance to management because the increasing number of flying foxes in urban areas in recent years has exacerbated conflicts with humans (Plowright et al. 2011, Tait et al. 2014, Edson et al. 2015). The grouping of bat colonies into urban and rural colonies could improve the analyses of culling and dispersal effects. Because there is a tendency for urban colonies to be located more closely to each other than rural colonies (Department of the Environment and Energy 2013), more closely located colonies were defined as urban colonies and more separated colonies were defined as rural colonies. The 16 colonies in the Sunshine Coast metapopulation were grouped into eight urban colonies and eight rural colonies (Figure 5-1). Although
all rural colonies were not necessarily located in rural regions, the grouping of colonies was determined by the relative distance to other colonies based on the general trend.

5.3.3. **Uniform metapopulation network scenarios**

Hendra virus dynamics was first simulated with uniform metapopulation network scenarios before various culling and dispersal scenarios were simulated. These scenarios were designed to explore the effect of each explanatory variable (colony number, colony size, and distance among colonies) on Hendra virus epidemics separately. Hereafter, colony number indicates the number of colonies in a metapopulation and colony size means the number of bats in each colony. These uniform metapopulation network scenarios assumed equal distances among colonies and same colony size and colony number in each simulation. Three levels of distance among colonies (1, 20, 50 kilometres), five levels of colony size (4000, 6000, 8000, 10000 and 12000) and five levels of colony number (2, 4, 8, 12, and 16) were applied in the uniform metapopulation network scenarios.

In uniform metapopulation network scenarios, in addition to the probability of epidemic occurrence, I recorded two extra outcomes: the probability of diffusion and the probability of rescue. The initially introduced infectious individuals tended to migrate from the initially infected colony to other infection-free colonies to balance prevalence in each colony in the metapopulation, and the migration was expected to inhibit the triggering of an epidemic in the colony. Masuda (2010) defined this situation as diffusion. Here, diffusion was defined as having occurred if there was a decrease in the number of infectious individuals in an initially infected colony and there was an increase in the sum of the number of infectious individuals in other colonies after 0.1
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days since the introduction of infectious individuals into a colony. Because the
diffusion effect influenced the dynamics as soon as infectious individuals were
introduced, the diffusion effect was measured during such a short period before an event
of rescue occurred. On the other hand, even though infection became extinct in a
colony, a rescue effect might allow the colony to be re-infected through migration of
infectious hosts from other colonies (Grenfell et al. 2001). In this chapter, conditional
upon an epidemic having occurred, rescue was defined as having occurred if the number
of infectious individuals in the initially infected colony became zero anytime between
the immigration of infectious individuals and the epidemic peak.

5.3.4. Culling scenarios
Culling could be implemented in numerous ways. I chose to investigate two factors:
whether colonies were culled in urban areas or rural areas; and whether colony number
was reduced or colony size was reduced. Considering these two factors, I modelled the
culling scenarios 1 to 3, in which colony number was reduced and modelled culling
scenarios 4 to 6 in which colony size was reduced. Each of these scenarios was
implemented with five differing degrees of intensity.

For scenarios 1 to 3 (reducing colony number) scenarios were as follows:

1/ colonies removed in random order.

2/ colonies close to each other successively removed.

3/ colonies as far away as possible from each other successively removed.

For scenarios 4 to 6 (reducing colony size) scenarios were as follows:

4/ cull the same proportion of bats in urban colonies and rural colonies.
5/ cull a proportion of bats in only urban colonies.  

6/ cull a proportion of bats in only rural colonies.  

Details of scenarios are described in Table 5-3.

5.3.5. *Dispersal scenarios*

The increased urban presence of flying foxes has led to requests for dispersal of colonies (Edson et al. 2015). As a result, all dispersal scenarios modelled dispersal of urban colonies, leaving rural colonies unaffected. I assumed that all bats in the selected colony were dispersed, eliminating the colony from the metapopulation (analogous to the roosting vegetation being removed). After dispersal, dispersed bats could either form new colonies or good combine into existing colonies. The newly formed colonies or the existing colonies joined could either be in rural or urban areas. Additionally, I considered that, where possible, dispersed flying foxes did not move far from their original sites, and they kept trying to reclaim their original sites (Roberts and Eby 2013). Considering these two factors and their response, I modelled three scenarios of bat behaviour following colony dispersal:

1/ Dispersed bats from urban colonies established new colonies nearby their original sites, making a little change in the metapopulation structure. However, dispersed bats may move more frequently than before dispersal because of their attempts to reclaim their original sites. I applied a range of increased connectivity parameters.

2/ Dispersed bats from urban colonies established new colonies in rural areas. The distances between newly formed rural colonies and existing colonies were determined
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by randomly selecting distances from a rural colony to other colonies. Urban colonies to be dispersed were randomly selected.

3/ Dispersed bats from urban colonies combine into existing colonies, not forming new colonies. The number of bats moving into each unaffected colony was inversely exponentially proportional to the distances between a dispersed colony and unaffected colonies.

Details of scenarios are described in Table 5-3.
Table 5-3. Description of culling and dispersal scenarios. The first level of actions in all scenarios (baseline model) is identical for all scenarios. The baseline model includes eight urban colonies and eight rural colonies. The number of bats in each colony was identical in the baseline model, and the number was either of 6000 or 10000. In dispersal scenario 1, the connectivity among colonies is inversely proportional to the connectivity parameter.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Control strategy</th>
<th>Location of colonies</th>
<th>Intensity of action</th>
<th>Remaining colony number after culling</th>
<th>Remaining proportion of bats in colonies after culling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>Reduction of colony number</td>
<td>All</td>
<td></td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>All</td>
<td></td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>All</td>
<td></td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>Reduction of colony size</td>
<td>Urban</td>
<td></td>
<td>1</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rural</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>Urban</td>
<td></td>
<td>1</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rural</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>Urban</td>
<td></td>
<td>1</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rural</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Control strategy</th>
<th>Location of colonies</th>
<th>Intensity of action</th>
<th>Connectivity parameter (c)</th>
<th>Remaining colony number after dispersal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Increasing connectivity</td>
<td>All</td>
<td></td>
<td>c/1</td>
<td>c/2</td>
</tr>
<tr>
<td>2</td>
<td>Removal of urban colonies and establishment of new colonies in rural areas</td>
<td>Urban</td>
<td></td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>Joining of dispersed urban bats to existing colonies</td>
<td>Urban</td>
<td></td>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>

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5.4. RESULTS

5.4.1. Uniform metapopulation network scenarios

The simulations of uniform metapopulation network scenarios showed that the probability of epidemic occurrence was proportional to colony size and inversely proportional to colony number (Figure 5-2 top panels). Given the density-dependent transmission, it was predictable that larger colonies would provide infectious bats initially introduced with a higher chance of infecting sufficient susceptible individuals to trigger an epidemic (Keeling and Rohani 2008). The assumption that the same number of infectious bats was introduced, regardless of the total number of bats in the metapopulation meant that a high number of colonies led to a lower proportion of infectious hosts to the total bats in the metapopulation. When an epidemic was in its early onset phase in the initially infected colony, the lower proportion of infectious hosts to the total bats in the metapopulation caused faster migration of infectious hosts from the initially infected colony to other infection-free colonies. The migration of infectious hosts inhibited the occurrence of an epidemic in the initially infected colony by leaving too few infectious hosts to trigger an epidemic.

The effects of colony size and colony number on the probability of epidemic occurrence were more pronounced when the distance among colonies was large than when the distance among colonies was small (Figure 5-2, top panels). The small distance among colonies that resulted in more frequent movements of hosts made the effects of colony size and colony number less prominent compared to distance, whereas the large distance among colonies that led to less frequent movements of individuals made the consequences of the number and size of colonies more pronounced compared to distance. In addition to the different variances of the probability of epidemic occurrence depending on distances, the distance among colonies showed that metapopulations
Culling and Dispersal

Consisting of small colonies generated the highest probability of epidemic occurrence in a short distance, but metapopulations composed of large colonies generated the highest probability of epidemic occurrence in a long distance. These two conflicting results of distance among colonies were related to the diffusion effect (Masuda 2010), which decreased the probability of epidemic occurrence and with rescue effect (Brown and Kodricbrown 1977) that increased the probability of epidemic occurrence.

A stronger diffusion effect was observed with large colony size, with high colony number, and with short distance among colonies because more movements of bats among colonies facilitated faster diffusion of infectious hosts from the initially infected colony to other infection-free colonies (Figure 5-2 middle panels). In addition to the diffusion effect, a rescue effect was observed in the uniform metapopulation network scenarios. A stronger rescue effect was observed with high colony number, and with short distance among colonies because more movements of bats among colonies resulted in more opportunities for infection-free colonies to be migrated by infectious bats (Figure 5-2 bottom panels). The probability of rescue events was higher in metapopulations consisting of small colony size because viral extinction more frequently occurred in small colonies than in large colonies.
Figure 5-2. Epidemics in scenarios of hypothetical metapopulations. Results are shown for uniform metapopulation network scenarios in which all colonies are of equal size and are the same distance away from each other. Shading in top panels shows the probability of epidemic occurrence (the proportion of iterations in which epidemics occurred, relative to the total iterations) in the stochastic simulations. Shading in middle panels shows the probability of diffusion. Shading in bottom panels shows the probability of rescue. I explored three different distances among colonies (columns: 1, 20, and 50 km apart), differing colony numbers (vertical axis) and differing colony sizes (horizontal axis).
When the distance among colonies was long, there was a low level of diffusion and rescue effects. As a result, the probability of epidemic occurrence was determined overwhelmingly by colony size and colony number. On the other hand, when the distance among colonies was short, there was a high level of diffusion and rescue effects. As a result, the effects of colony size and colony number on the probabilities of epidemic occurrence were diluted by relatively stronger diffusion and rescue effects. The higher probability of diffusion in metapopulations consisting of large colonies reduced the probability of epidemic occurrence in metapopulations composed of large colonies. Additionally, the higher probability of rescue in metapopulations consisting of small colonies increased the probability of epidemic occurrence in metapopulations consisting of large colonies. As a result, the diffusion and rescue effects contributed to making the variance of the probabilities of epidemic occurrence lower in metapopulations consisting of highly connected colonies.

5.4.2. Culling scenarios

Reduction of colony number (scenarios 1 to 3) was found to be counter-productive in reducing the probability of epidemic occurrence, and in most cases significantly increased the probability of epidemics (Figure 5-3 top panels). Relative to no management action, culling colonies starting with those closest together (scenario 2) showed the greatest increase in the probability of an epidemic and this effect increased as the number of colonies culled increased. This was because more distant colonies were less affected by the diffusion effect. Culling colonies in random order (scenario 1) showed a similar trend, whereas culling colonies by starting with those farthest apart (scenario 3) showed a non-linear trend. This was because the effect of closer distance among colonies due to the removal distant colonies outweighed the effect of decreased colony number.
Figure 5-3. Probability of epidemics in the culling scenarios. Top panels show the probability of epidemic occurrence in scenario 1 to 3, in which colony number was reduced. Bottom panels show the probability of epidemic occurrence in scenario 1 to 3, in which colony size was reduced. I explored the probability of epidemic occurrence with two different colony sizes (7000, 10000) and five levels of culling (horizontal axis). The first level of culling had no culling action and the baseline metapopulation was simulated, so that the very similar probabilities of epidemic occurrence were observed.

In the scenarios in which the number of colonies remained the same, the probability of an epidemic occurring decreased when colony sizes were reduced (scenario 4-6) (Figure 5-3 bottom panels). This effect was less marked when the size of urban colonies was reduced (scenario 5) in comparison with reduction of the size of rural colonies (scenario
6). This was because the diffusion effect had a lower impact when closely located colonies had fewer bats.

The probability of epidemic occurrence from culling colonies in random order (scenario 1) was intermediate between the probabilities from the scenario 2 and 3, while the probability from the reducing colony size in both urban colonies (scenario 4) was lower than the average of the probabilities from the scenario 5 and 6. This was because the removal of bats in both urban colonies and rural colonies in scenario 4 left all colonies too small for migrating infectious bats to trigger an epidemic, while introduction of infectious bats to the un-culled colonies may have a relatively high probability of triggering an epidemic in scenario 5 and 6.

In uniform metapopulation network scenarios, when metapopulations consisted of small colonies, the probability of epidemic occurrence was higher with short distances between colonies than with long distances between colonies (Figure 5-3 top panels). However, culling scenarios showed that in metapopulations consisting of small colonies the probability of epidemic occurrence was lower with the overall higher connectivity among colonies (scenario 3 and 6) than with the overall lower connectivity among colonies (scenario 2 and 5). The increased connectivity among colonies in scenario 3 and 6 still was not highly influential and still colonies in the Sunshine Coast metapopulations were located not very close to each other, compared to the 1 km distance among colonies in the uniform metapopulation network scenarios. Nevertheless, the differences in the probabilities of epidemic occurrence based on the changed connectivity among colonies diminished, as the colony size decreased.
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5.4.3. Dispersal scenarios

The change in colony number or colony size had much stronger impact on the epidemic occurrence than the change in the connectivity among colonies in the idealised Sunshine Coast metapopulation. (Figure 5-4). In dispersal scenario 3, increased colony size and decreased colony number resulted in a massive increase in the probability of epidemic occurrence. On the other hand, changes of connectivity (scenario 1) and changes of colony location (scenario 2) had a marginal effect on the probability of epidemic occurrence. In dispersal scenario 1, increased movements of bats among colonies resulted in the higher diffusion effect, which caused a decrease in the probability of epidemic occurrence. In dispersal scenario 2, the removal of urban colonies and new establishment of rural colonies decreased the overall movement rate of bats among colonies in the metapopulation, thereby decreasing the diffusion effect.

Figure 5-4. Probability of epidemics in dispersal scenarios. Results show the probability of epidemic occurrence in dispersal scenario 1 to 3. I explored the
probability of epidemic occurrence with two different colony sizes (7000 and 10000) and three levels of culling (horizontal axis). The first level of dispersal had no dispersal.

Another significant finding from the Sunshine Coast metapopulation was that overall connectivity of colonies in the Sunshine Coast metapopulation was so high that epidemics were synchronised across all colonies in the metapopulation (Figure 5-5). Once an epidemic occurred in a colony, every colony in the metapopulation showed a similar pattern of epidemics in all culling and dispersal scenarios.

Figure 5-5. Synchrony of epidemics in the metapopulation of black flying foxes in Sunshine Coast, Queensland, Australia. The plots show the number of infectious individuals in each colony in the Sunshine Coast metapopulations. The third level of dispersal scenario 2 generated these example stochastic simulations. The colony sizes were 7000 (left panel) and 10000 (right panel). The parameters used in the Sunshine Coast metapopulation models resulted in this high synchrony of epidemics among colonies.
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5.5. DISCUSSION

This chapter explored the viral dynamics in various metapopulation structures when infectious hosts are introduced into a subpopulation in an infection-free metapopulation. After I had found how colony number, colony size, and distance among colonies affected the probability of epidemic occurrence, this chapter showed how the findings from the hypothetical scenarios could be used to interpret the simulation results of more realistic culling and dispersal scenarios. This modelling study explored how different spatial structures of wildlife populations affect the likelihood of epidemics occurring, not the prevalence, which has been the outcome of previous modelling studies that have explored wildlife population management (McCallum 2016).

The models were constructed using the most favourable possible assumptions for culling to be effective. The first key assumption is a density-dependent transmission mode of Hendra virus among flying foxes. Plowright et al. (2015) suggested that Hendra virus transmission in flying foxes can be density-dependent or frequency-dependent, depending on specific circumstances. Reduction in population size is expected to reduce transmission with density-dependent transmission, but not with frequency-dependent transmission (McCallum et al. 2001). If frequency-dependent transmission is predominant for Hendra virus in flying foxes, culling to reduce colony size would be ineffective in reducing Hendra virus transmission. The second key assumption is that pulses of viral shedding are generated by transient epidemics. If flying foxes are persistently infected and episodically excrete the virus based on episodic shedding, changed metapopulation structures caused by culling or dispersal would not directly affect infection dynamics. Instead, the viral reactivation and shedding from persistently infected bats could be stimulated by stress that the management activities may bring to flying foxes (Plowright et al. 2016).
Given these assumptions, reducing colony size was found to be effective in decreasing the probability of epidemic occurrence, whereas reducing colony number increased the probability of epidemics. Culling of 50% of the entire bat population, however, resulted in only approximately 50% reduction in epidemic risk. Culling of 50% of the bat populations would have substantial effects on ecosystem services (Fujita and Tuttle 1991). Culling would have to be maintained annually to balance population recovery via increased recruitment due to long-distance and frequent movements of flying foxes (Roberts et al. 2012b). In comparison with other strategies to reduce spillover risk such as vaccination of a proportion of the horse populations (Peel et al. 2016) and property risk mitigation practices to reduce the contacts between horses and flying fox bodily fluids (Manyweathers et al. 2017a), culling is likely to be less effective. Dispersal does not seem to be an appropriate alternative for culling in terms of reducing Hendra virus spillover. The most likely reactions of dispersed bats (scenario 1 and 3) did not predict a reduced probability of epidemics. Rather, scenario 3, which simulated decreased colony number and increased colony size, predicted a substantially increased probability of epidemic occurrence. This finding suggests that the metapopulation structure changed by dispersal actions may result in increased Hendra virus spillover risk.

Even with these most favourable assumptions, the culling and dispersal scenarios often generated counter-productive outcomes. Therefore, management of Hendra virus spillover through population management of flying foxes appears unlikely to be realistic. Additionally, this study explored the Hendra virus transmission only within black flying fox populations without considering the transmission from black flying foxes to horses. Because spillover is the transmission from reservoir hosts to target
hosts, the spillover risk of Hendra virus would be affected not only by virus shedding but also by exposure of susceptible horses. Virus shedding from flying foxes is important, but in itself is not enough to represent the spillover risk.

An effect of high movement rate on the epidemic spread can be found in a relation with a recovery rate in metapopulation dynamics (Cross et al. 2005). If a recovery rate is too high compared to the movement rate, infectious individuals would have few opportunities to spread the infection to other colonies. The likely high recovery rate (1/7 per day) (i.e. the short infectious period) of Hendra virus in flying foxes (Plowright et al. 2011) limits the ability of an epidemic to spread across a metapopulation. Nevertheless, the Sunshine Coast metapopulation model generated high synchrony among epidemics in each colony, and the epidemics spread across the metapopulation very successfully (Figure 5-5). The movement rate was sufficiently high to overcome the effect of the short infectious period allowing epidemics to spread and even to occur synchronously. Nevertheless, because the epidemic spread is highly sensitive to the infectious period, more accurate estimation of the infectious period would facilitate improved estimation of the extent of epidemic spread.

Some level of diffusion is required to facilitate the epidemic spread (Masuda 2010), but the estimated movement rate of black flying foxes was much higher than the degree of diffusion that is required for the epidemic spread, and significantly decreased the probability of occurrence of epidemics. The frequent movements also increased the likelihood that the rescue effect would increase the probability of epidemic occurrence, but the impact of rescue effect was secondary because the rescue effect would be
evident only when there was a viral extinction in some colonies while other colonies were retaining the virus.

There are requirements for culling to be successful in managing wildlife diseases, but the requirements do not appear to be satisfied for Hendra virus in flying foxes. First, selective culling (culling of only infected individuals) was proved to be more efficient in managing wildlife diseases than non-selective culling (Gross and Miller 2001, Wolfe et al. 2004). However, selective culling is not possible for flying foxes because there of the large number of flying foxes in Australia and because there is no method to detect Hendra virus in flying foxes instantly. Non-selective culling of bats can be counterproductive in wildlife disease management. For example, rabies seroprevalence in vampire bats (*Desmodus rotundus*) (Streicker et al. 2012b) and Marburg virus prevalence in Egyptian fruit bats (*Rousettus aegyptiacus*) (Amman et al. 2014) increased after the procedures of non-selective culling because naïve juveniles replaced immune bats. Second, selective culling not only based on infection state but also based on age group could be effective in bat diseases. Streicker et al. (2012b) found that preferentially culling adult vampire bats resulted in the increased transmission of rabies virus because the proportion of susceptible individuals was lower in adults than in juveniles. Third, localised culling is unlikely to generate long-lasting effects, because a region where bats are culled tends to be replenished by bats from nearby regions (Martin and McIlwee 2002). Blackwood et al. (2013) emphasised that geographic coordination of culling efforts of vampire bats would be required to manage rabies virus transmissions in the highly mobile bats and that localised culling would end up attracting bats from nearby regions to the culled regions. The problem of localised culling has also been suggested as an issue for Hendra virus in flying foxes. McIlwee and Martin (2002) described the culled regions as “pteropucidal black hole” that
continually draws in bats from afield. However, culling of flying foxes in vast regions does not seem to be logistically affordable, ecologically suitable, and ethically permissible.

Apart from disease-related issues, culling of flying foxes is not appropriate in the ecological perspective. First, grey-headed flying foxes and spectacled flying foxes are listed as “vulnerable” under National status (Environment Protection and Biodiversity Conservation Act) owing to a recent decrease in their numbers (Department of the Environment and Energy 2012). Second, it has been emphasised that flying foxes that pollinate and disperse seeds are ecologically and economically important to maintain healthy forests (Fujita and Tuttle 1991, Olival 2015). Third, flying foxes also have a low reproductive rate and a relatively long lifespan, indicating that they have a low rate of intrinsic population growth. Population reduction caused by culling would be likely to produce long-lasting effects on flying foxes. (McIlwee and Martin 2002). Therefore, it should be noted that culling of flying foxes has significant potentials to damage ecosystem.

Dispersal may be a better alternative than culling in managing flying fox populations in that dispersal brings about less ecological and ethical concerns than culling does. Regarding reduction of the spillover risk of Hendra virus, dispersal is not expected to be as effective as culling. Dispersal does not reduce the number of bats, which is the fundamental principle by which culling may be able to manage wildlife diseases (Wobeser 2002, McCallum 2016). Nevertheless, dispersal actions that move flying foxes from where locations where there are many horses to locations with few horses could be employed to decrease the spillover risk by reducing contacts between flying
foxes and horses. However, the large daily foraging ranges of flying foxes (Roberts et al. 2012b) may make this impractical. The modelling outcomes in this chapter suggested how the probability of Hendra virus epidemic occurrence may be reduced by changing the metapopulation structure of flying foxes. However, it is unlikely that we can manipulate the colony location or metapopulation structure of flying foxes. It is almost impossible to direct dispersed bats into the places we want them to be. Previous dispersal actions have shown that dispersed flying foxes have a propensity to return to their original roosting sites. Even if dispersed bats form new colonies, their location cannot easily be predicted (Roberts et al. 2011, Roberts and Eby 2013). Additionally, dispersal has the possibility of facilitating the spread of the disease, because dispersal actions seem to increase the movements of flying foxes. Especially in austral summer, when the prevalence is low (Field et al. 2015), disease-free populations may be infected by the increased movement of flying foxes that can promote the introduction of the virus into the populations. Therefore, although dispersal of flying foxes is more acceptable than culling, in that it does not reduce the population size, dispersal is unlikely to be used to improve the situations regarding management of Hendra virus transmission.

5.6. CONCLUSION

This chapter explored epidemic dynamics in the metapopulation structures by using an idealised model of Hendra virus epidemics in a metapopulation of black flying foxes. The exploration of the uniform metapopulation network scenarios demonstrated that the probability of epidemic occurrence was higher with smaller colony number and with larger colony size, and the frequent movements of flying foxes among colonies diminished the effects of colony number and size on the probability of epidemic
occurrence. The diminishment was attributable to diffusion and rescue that are facilitated by frequent movements of individuals among subpopulations in metapopulation structure. In the model based on the Sunshine Coast metapopulation, the probability of epidemic occurrence was more substantially affected when culling or dispersal caused changes in the colony size or colony number than in the distance among colonies. It should be noted that the modelling results need to be mindful of the assumptions that may be pivotal to the results.
CHAPTER 6

6. General Discussion

6.1. INTRODUCTION

This thesis explored the transmission dynamics of bat-borne viruses within Australian bat populations. The chapters explored two overarching themes. The first theme was how the bat viruses are maintained in their natural reservoir hosts. I investigated the roles of persistent infection, maternally-derived immunity, and metapopulation structure in the maintenance of the viruses. The second theme was how epidemics are caused in the bat populations. More specifically, I modelled seasonal breeding and metapopulation structure to observe their effects on triggering epidemics. I assumed that among-host virus ecology, rather than within-host virus ecology, is more deeply involved in Hendra virus maintenance and epidemics. Thus, in this thesis, compartmental epidemic models were employed to investigate the effects of the bat population dynamics on the viral transmission dynamics.

Although this thesis was framed around exploration of the dynamics of Hendra virus and coronavirus in Australian bats, the applicability of results from this thesis is not limited to these two viruses in the bats. Rather, this thesis aims at investigating the ecology and epidemiology of emerging viruses in their reservoir host populations by using the system of the two viruses in the bat populations as an example. Thus, the findings from this thesis should be applicable to a broad scale of emerging infectious
diseases. Hendra virus and coronavirus infections in Australian bats are appropriate examples with which to research more general questions concerning the transmission dynamics of emerging viruses. Outbreaks of emerging infectious diseases have been more frequent in developing countries than in developed countries. Hendra virus spillover in Australia is the one of infrequent examples of emerging zoonitic diseases occurring in a region with good surveillance and research effort (Jones et al. 2008). In comparison to disease outbreaks that have occurred in developing regions, more data were available to conduct modelling studies, although parameterising and testing detailed models have been constrained by lack of detailed data. Additionally, bats have been identified as critically important wildlife reservoirs species because there have been successive outbreaks of fatal outbreaks of zoonotic viruses originating from bats such as SARS (severe acute respiratory syndrome), Ebola, Marburg, and Nipah viruses (Dobson 2005). Hendra virus and coronavirus in Australian bats are therefore important case studies to investigate emerging zoonotic diseases in general.

In this chapter, I discuss my findings in this context of advancing knowledge of pathogen dynamics in reservoir populations of zoonotic diseases. Then, I discuss management strategies for emerging infectious diseases originating from wildlife before the difficulties of modelling wildlife diseases are discussed. Finally, I suggest the topics that should direct further research.

6.2. RESERVOIRS OF EMERGING INFECTIOUS DISEASES

Emerging infectious diseases of livestock or humans almost inevitably involve pathogens that are capable of infecting multiple host species (Woolhouse et al. 2005). A
pathogen that is already established in a species may jump over the species barrier to infect another species and result in an outbreak of an emerging infectious disease (Parrish et al. 2008). The species involved in emerging infectious diseases can be classified into three categories: natural reservoir host, transmission host, and spillover host (Bean et al. 2013). Natural reservoir hosts are often one or more wildlife species that maintain the pathogen indefinitely in their populations. Transmission hosts are animals that are infected by natural reservoir hosts and then in turn infect spillover hosts. Transmission hosts are similar to vectors in that both are infected before they infect other species of hosts. However, transmission hosts are differentiated from vectors. Vectors should contact a host species multiple times in their lifetime to accomplish transmission, and the contact improves the fitness of vector while reducing the fitness of another species (Wilson et al. 2017), but a single contact may be enough for transmission hosts to infect spillover hosts, and the infection mostly aggravates the fitness not only of spillover hosts but also of transmission host (Bean et al. 2013). Last, spillover hosts are infected by transmission hosts and may present with severe symptoms and high mortality rates (Bean et al. 2013). Multi-host systems of emerging infectious diseases encompass multifaceted topics (Plowright et al. 2008b), among which this thesis concentrated on the transmission within natural reservoir host populations, without considering transmission hosts or spillover hosts.

In this section, I discuss the two main roles of reservoir hosts in emerging infectious diseases - maintenance of emerging pathogens and sources of infection to other species, which have been the main research topic of this thesis. Before discussion of the two roles, the identification of reservoir populations is first discussed to provide underlying information.
6.2.1. Identification of reservoir populations

Detecting the emergence of a novel infectious disease requires some steps. First, it is necessary to identify the infectious agent. Then, the agent needs to be isolated from its natural reservoir hosts. In the case of coronavirus, after the pandemic of SARS in 2013, the causal agent was identified as SARS coronavirus (family Coronaviridae) (Marra et al. 2003). Then, species of bats were found to be the reservoir hosts of coronaviruses closely related to those responsible for the SARS outbreak (Li et al. 2005), and SARS coronavirus was isolated from Himalayan palm civets (Paguma larvata) (Guan et al. 2003). Subsequently, it was found that humans were infected with SARS coronavirus from Himalayan palm civet that were infected from bats (Perlman and Netland 2009). In the case of Hendra virus, following a sudden outbreak of an acute respiratory syndrome in horses in Brisbane suburb, Australia in 1994, the causal agent was identified and named as Hendra virus (family Paramyxoviridae) (Halpin et al. 1999). Later, the virus was isolated from its natural reservoir, pteropid bats (colloquially known as flying foxes or fruit bats) (Halpin et al. 2000). The identification of reservoir of emerging infectious diseases is not an easy task because each emerging infectious disease has its particular form of the target-reservoir system.

Identification of the reservoir hosts is a basic step in researching emerging infectious diseases, and appropriate control measures for the diseases can be expected only when the reservoir of the diseases was accurately identified (Viana et al. 2014). A reservoir can be defined as “ecological system in which the infectious agent survives indefinitely” (Ashford 1997). According to this definition, reservoir hosts of Hendra virus are black (Pteropus alecto), spectacled (P. conspicillatus), little red (P. scapulatus), and grey-headed flying foxes (P. poliocephalus) (Young et al. 1996). However, the definition of reservoir has been further developed to mean populations in which a pathogen can be
indefinitely maintained and from which infection is transmitted to the target population”, emphasising the reservoir as a source of infections for a ‘target’ population that is the population of concern or interest to us (Haydon et al. 2002, Viana et al. 2014). According to this more specified definition of reservoirs, black flying foxes seem to be reservoirs of Hendra virus because spatial and serological analyses showed that the two species of flying foxes are more likely to be associated with Hendra virus spillover than the other two species (Smith et al. 2014, Field et al. 2015), and the regions of spillover events are more relevant to the distribution of black flying foxes than spectacled flying foxes (Hall and Richards 2000, Field et al. 2011). This understanding of the reservoir-target system of Hendra virus suggests that research to identify the spillover mechanism of the virus should be focused on black flying foxes, while research to discover the maintenance mechanism of the virus in its reservoir populations needs to consider all of the four species.

6.2.2. Reservoir populations: maintenance of a pathogen

Assuming density-dependent transmission, population size has been considered as a major determinant of pathogen maintenance. The critical community size (CCS) has been commonly been regarded as the threshold population size necessary for pathogen persistence (Keeling and Grenfell 1997). However, CCS is often not appropriate to indicate the population threshold for disease persistence because it supposes a single well-mixed population, which is rare in natural systems (Viana et al. 2014). Consequently, to understand the maintenance of pathogens in reservoir population structures, this thesis modelled a variety of qualifications on the basic single well-mixed population (e.g. individually different immune response, seasonally fluctuating population size, and multiple populations interconnected together).
In natural systems, susceptibility to infection and tolerance of infection may vary substantially between individuals (Hawley and Altizer 2011), and this variation may change the capability of populations to maintain a pathogen. For example, a portion of individuals in a population may function as ‘superspreaders’ of transmission, and they may play a more substantial role in transmitting and maintaining a pathogen than the rest of individuals in the population (Lloyd-Smith et al. 2005b). It has been emphasised that a small fraction of ‘super-long-shedders’ could play a critical role in transmission dynamics of Hendra virus in its reservoir hosts, flying foxes (Plowright et al. 2011). Chapter 2 showed that persistently infectious bats might function as super-long-shedders by having a longer infectious period than transiently infectious bats, and this improved the probability of coronavirus maintenance in the population. CCS could be reduced by the existence of super-long-shedders. The identification of super-long-shedders in their populations would provide information that could help control of emerging infectious diseases, if intervention could be focused on individuals more responsible for virus maintenance.

A factor that critically affects disease persistence in metapopulations is the rescue effect (Metcalf et al. 2013). However, not all metapopulation structures need the rescue effect for persistence. In the ‘mainland-island’ model (Grenfell and Harwood 1997), infections are indefinitely maintained in a portion of large-sized populations, and this metapopulation structure does not need the rescue effect for disease persistence. However, if there are no large-sized populations that are endemically infected, the rescue effect is a key for pathogen maintenance in metapopulations. This situation was named as ‘island-island’ model in Chapter 4. Without ‘mainlands’ larger than CCS, the
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rescue effect allows an aggregation of ‘islands’ smaller than CCS to be able to ensure disease persistence at the level of a metapopulation.

The ‘island-island’ model is likely to be particularly relevant for Hendra virus persistence in flying fox populations because seasonal breeding of flying foxes means that an extremely large ‘mainland’ is required for Hendra virus persistence. However, colonies with such a population size do not seem to exist (Peel et al. 2014). In Chapter 4, the modelling results of repeated colonisation and extinction of Hendra virus in a colony showed that persistence of Hendra virus is possible in a metapopulation of flying foxes, given repeated immigration of infectious bats on a relatively regular basis. Persistence could be achieved because the immigration of infectious bats into populations was more frequent than the extinction of infection in populations. Nevertheless, in order to maintain disease persistence, an ‘island-island’ model should meet a condition that epidemics in populations are not synchronised. Strong coupling among populations can lead to synchronised epidemics in populations, and the synchronisation may result in the extinction of infections in metapopulations (Keeling 2000). Populations become an infection-free state at a similar time, and infection-free populations encompassed by infection-free populations cannot benefit from the rescue effect (Grenfell and Harwood 1997). Therefore, if the epidemics are synchronised, then the ‘rescue effect’ would not be effective (Hagenaars et al. 2004). On the other hand, if the coupling is too weak, then an infection in one population would rarely spread the infection to other populations (Jesse et al. 2008). Weak coupling would result in a low proportion of infected populations in the metapopulation, lowering the probability of pathogen persistence in the metapopulation as a whole. In the case of Hendra virus infection, Hendra virus epidemics in a metapopulation of flying foxes tend to be synchronised due to frequent movement of flying foxes among colonies. Although this
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high synchrony of epidemics suggests a low probability of persistence in local metapopulations, persistence in the whole flying fox population of Australia would be likely due to the widespread geographic distribution of flying fox colonies (Roberts et al. 2012a).

6.2.3. Reservoir populations: sources of infection for target populations

Disease invasion and persistence have been important issues in disease ecology and epidemiology (Gilligan and van den Bosch 2008). In target-reservoir systems, invasion can be thought as a more important issue for targets because invasion may lead to transient but fatal epidemics that bring about public health concerns. Persistence can be thought as a more important issue for reservoirs because disease persistence is their key characteristic. Compared to persistence, invasion might be of less significance for reservoirs because the reservoir continuously harbours infections in its metapopulations. However, invasion can be also an important issue in reservoirs in that invasion of individual populations within the wider metapopulation may trigger pulses of virus shedding that may facilitate spillover.

It should be noted that spillover is an event related to reservoir hosts, target hosts, and even environmental factors. This thesis that explored only reservoir hosts did not aim at directly understanding or predicting spillover but aimed at describing virus shedding from bats, which is one of enabling conditions for spillover (Plowright et al. 2015). Thus, the findings in this thesis would help indirectly estimate the spillover risk in the reservoir perspective. Migration of infectious bats between colonies may trigger transient epidemics, generating pulses of virus shedding and subsequently spillover events (Plowright et al. 2011). In this thesis, it was shown that whether migration of
infectious hosts into a population would lead to invasion depends on qualities of the population.

First, seasonal breeding of reservoir hosts can be one of common features wildlife reservoir hosts that determine the qualities of populations. Chapter 3 explored how a population of black flying foxes fluctuates in terms of population size and herd immunity due to seasonal breeding and following maternally-derived immunity against Hendra virus. The chapter showed that seasonal breeding of black flying foxes was capable of causing highly clustered timings of epidemic peaks when maternally-derived immunity was not assumed. Addition of maternally-derived immunity was demonstrated to dilute the effect of seasonal breeding on temporally clustering the epidemic peaks, but the level of dilution was dependent on how the loss of maternally-derived immunity was calculated. The simulations showed that the interaction of seasonal breeding and maternally-derived immunity plays a role in creating seasonally fluctuating prevalence. This modelling evidence of the prevalence pattern is in accordance with the observation of Hendra virus prevalence from urine samples of flying foxes, which also showed seasonally fluctuating prevalence (Field et al. 2015).

Second, herd immunity of a population is a factor that needs to be considered for invasion in reservoir populations (Pulliam et al. 2007). In a target-reservoir system, a target population is often completely susceptible because pathogens are occasionally introduced and often disappear after local chains of transmission. On the other hand, a reservoir population is often partially susceptible because infection exists indefinitely, maintaining the disease in the reservoir populations. Thus, invasion in reservoir
populations should be considered with a variety of levels of herd immunity, as in Chapter 4.

Last, migration of infectious bats among colonies would result in various forms of epidemics in terms of duration and magnitude of epidemics in the metapopulation structure. The ensemble of a variety of epidemics contributes to maintaining the virus and to facilitate invasion (Plowright et al. 2011). In Chapter 3 and 4, various forms of epidemics were observed. Viral introduction leading to initial fadeout fails to trigger an epidemic, but the initial fadeout is significant in that the decreased herd immunity due to initial fadeout means that future viral introductions have an increasing probability of causing an epidemic. Viral introduction leading to epidemic fadeout does not produce a long-lasting infection, but it causes a high impact epidemic, bringing about pulses of virus shedding and possibly high spillover risk. Although viral introduction leading to endemic fadeout does not cause a high-impact epidemic, it produces a long-lasting infection, playing a crucial role in viral maintenance. Migration of infectious bats allows herd immunity of bat colonies in a metapopulation structure to fluctuate within a certain range, and the fluctuating herd immunity allows viral introduction to result in various forms of epidemics.

The rescue effect was found to facilitate not only disease persistence but also epidemic occurrence in metapopulations. Chapter 5 showed that the rescue effect increases the likelihood of epidemic occurrence in a metapopulation. In a single population structure, once an infection failed to trigger an epidemic in a population and disappeared, there would no more chance that an epidemic may occur. However, in a metapopulation structure, even if an infection disappears in a population, the rescue effect introduces the
infection again into the population, providing another chance of epidemic occurrence. In addition to sufficiently low coupling decreasing the probability of epidemic occurrence because infrequent movement of individuals dampens the spread of epidemics across subpopulations (Masuda 2010), low coupling represses the epidemic occurrence due to a low rescue effect. Sufficiently high coupling inhibits the epidemic occurrence due to the high diffusion effect. Thus, intermediate coupling is most likely to lead to the occurrence of epidemics in a metapopulation structure. This is similar to the conclusion that an intermediate level of coupling was best for disease persistence (Keeling 2000).

6.3. MANAGEMENT OF EMERGING INFECTIOUS DISEASE: TARGET-RESERVOIR SYSTEM

Researching emerging infectious diseases ultimately needs to provide useful information for improvement of public health. For this reason, I discuss the findings in this thesis in terms of management of emerging infectious diseases. It is critical to clarify what we can know and what we cannot know from the models we simulated. Due to many poorly understood aspects of Hendra virus dynamics, it is not desirable to directly apply the findings from the models in this thesis. Instead, the models should be understood in relation to the specific assumptions made up. Therefore, it is not advisable to directly apply these theoretical models to design specific management policies, rather the models should be used to understand Hendra virus dynamics, forming a basis on which to plan proper management strategies. In addition, models for successful wildlife management should include not only pathogen transmission dynamics but also human factors such as people's perception of the risk of the infection (Decker et al. 2007). Here, I discuss management strategies in terms of three parts of a
6.3.1. Target control

In the target-reservoir system, emerging pathogens are often transmitted from their natural reservoir hosts of wildlife to spillover hosts of humans via transmission hosts that are domestic animals (Bean et al. 2013). This route of transmission emphasises the significance of domestic animals in controlling outbreaks of emerging zoonotic diseases in humans, and targeted control focused on domestic animals can sometimes be enough to prevent outbreaks. In the case of Hendra virus, horses are transmission hosts that are infected by flying foxes before horses infect humans. The target control can be achieved by the horse vaccine (Broder et al. 2016), which has been developed and proved to be effective (Peel et al. 2016). However, development of an effective vaccine does not ensure widespread use of the vaccine. Vaccine acceptance has always been challenged by people who question, and sometimes refuse, vaccines (Larson et al. 2014). Some horse owners are reluctant to use the Hendra virus vaccine for horses because they are concerned about vaccination costs and perceived safety issues, especially in pregnant mares (Kung et al. 2013). To resolve this issue, it is important to build the trust between veterinarians and horse owners to persuade horse owners to accept the vaccinations (Manyweathers et al. 2017b).

6.3.2. Blocking tactics

In addition to vaccinating horses, blocking the contact between flying foxes and horses can be an effective control measure to prevent Hendra virus spillover. Blocking has been recommended by the government for non-vaccinating horse owners.
(Manyweathers et al. 2017a). Particularly because it does not appear to be a long temporal gap between the excretion of Hendra virus from flying foxes and the transmission to horses (Martin et al. 2015), confinement of horses in a virus-free environment could be a more effective measure to prevent spillover. Although no significant difference in exposure risk has been found due to the density of horses or individual horse movement behaviour, research to investigate horse behaviour and landscape utilisation is required to find out efficient measures to block the contact between flying foxes and horses (Smith et al. 2014, Field et al. 2016).

This thesis exclusively explored the transmission within bat populations, and thus target control or blocking tactics was not explored in depth. Nevertheless, the findings in this thesis can provide suggestions for target control or blocking tactics. For example, seasonal clustering of epidemic peaks due to seasonal breeding of flying foxes can signify the high spillover risk season. Accordingly, horse owners can plan a vaccination schedule so that their horses become resistant to Hendra virus infection before the high spillover risk season. Or, during the high risk spillover season, horse owners can pay more attention to the management of their horses to prevent the contact between flying foxes and horses. Prediction of the high spillover season is expected to save effort and cost consumed for prevention of spillover by concentrating the effort and cost on the more needed situations.

### 6.3.3. Reservoir control

Although reservoir control can be a more fundamental solution to prevent spillover and improve public health than target control or blocking tactics, reservoir control is often more difficult than the other two methods. This is because reservoir hosts are often
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more intractable wildlife, while target hosts are often relatively controllable domestic animals (Bean et al. 2013). Consequently, the range of methods to control wildlife reservoir hosts tends to be more limited than the range of measures available to manage domestic animals. Also, because wildlife is a part of natural ecosystems, reservoir hosts often have roles to play in maintaining healthy ecosystems (Gortazar et al. 2014), meaning that there may be deleterious effects of managing wildlife populations.

A commonly used strategy to control wildlife reservoir to prevent the spillover of emerging infectious diseases has been to reduce the population size by culling wildlife populations (Smith and Cheeseman 2002). Culling of flying foxes in Australia has also been considered as a strategy to prevent Hendra virus spillover (Olival 2015). Research, including my results in Chapter 5, suggests that culling of flying fox populations is unlikely to be effective as a management strategy for Hendra and similar bat borne viruses (Florens 2015). The role of flying foxes in the ecosystem so critical that culling at the level necessary to control Hendra virus, even given the most favourable assumptions for culling to be successful, would be likely to lead to unacceptable consequences for the ecosystem as a whole.

Despite these difficulties of wildlife reservoir control, there is the need to pay attention to reservoir control for management of emerging infectious diseases. Control does not necessarily mean active intervention that changes the current state of viral dynamics in reservoir populations. Rather, in a broader sense, control can have the objective of eliminating negative effects imposed by humans on reservoir populations. The elimination may help reservoir populations restore their original state of viral dynamics, in which spillover had not occurred in the past. For example, anthropogenic
transformations of bat habitats in Australia have been suggested as leading to Hendra virus spillover (Plowright et al. 2011). As the results in Chapter 5 showed, the spatial distribution of flying foxes affects the probability of epidemic occurrence, which might be implicated with spillover risk. Also, stress caused by colony disturbance may increase the spillover risk (Plowright et al. 2016). Thus, removing negative anthropogenic activities is an important method of controlling reservoir populations and to reduce the spillover risk.

6.3.4. Conservation and management: One Health

Emerging zoonotic diseases provide a relevant system to emphasise the principles of “One Health” because the diseases are often involved with wildlife, domestic animals, humans, and the environment, all of which are highly interconnected, affecting each other (Thompson 2013). The management of bat-borne Hendra virus should also be researched in terms of One Health. We should plan the management of the disease by not narrowly focusing on the wildlife reservoir hosts but by comprehensively recognising the various interrelated factors. Focusing management actions only on the reservoir hosts (such as culling bats) can be expected to have a temporary effect at best and is likely to aggravate the situations in the long run. While prevention of diseases originating from wildlife and conservation of disease-carrying wildlife may appear to be in conflict. However, in view of One Health, conservation of wildlife and prevention of diseases are not contradicting values. In the long run, to conserve wildlife is an appropriate method to prevent emergences of novel viruses from wildlife because the factors driving the spillover of emerging viruses from wildlife reservoir hosts are often threats to the reservoir host populations (Breed et al. 2006). Consequently, the desirable direction of ongoing approaches to managing emerging infectious diseases is to allow the ecosystem to function naturally and to keep its healthy state.
6.4. MODELLING WITH INSUFFICIENT INFORMATION

Models need to be based on existing parameter estimates, together with estimates of their variance, but it is not common that all necessary knowledge is offered to build an accurate model. In particular, modelling wildlife diseases often encounters the difficulty of poor data compared to livestock or human diseases (McCallum 2016). Uncertainty regarding the structure of the system and parameter estimates limits the ability of the models to generate accurate outcomes (Russell et al. 2017). I suggest three approaches to tackle this challenge to build appropriately designed models.

First, it is to make the best use of available data. When we build a model, the first task is to define its purpose (Evans et al. 2013). Between the two main purposes of prediction and understanding (Keeling and Rohani 2008), understanding is more likely to be achieved than prediction, when available data is meagre. Predictive models must consider various aspects to produce accurate and reliable results, and thus the models often need abundant information to build a complicated model. By contrast, models for comprehension avoid complexity to elucidate the mechanisms by which the causes and effects work in the model. Thus, a model aiming understanding can achieve its purpose with relatively less information. This thesis has overall focused on improving our understanding transmission of bat viruses, in particular coronavirus and Hendra virus. For example, although Chapter 5 used parameters appropriate for the specific system of black flying foxes (P. alecto) and Hendra virus, and addressed the specific of the effect of culling and dispersal on spillover, it also addressed more general issues of understanding viral dynamics in various metapopulation structures.
Second, it is to infer assumptions. As all models are only as good as their underlying assumptions (Evans et al. 2013) and so using appropriate assumptions is a precondition for creating a good model. Models intrinsically need assumptions to describe the simplified imitation of the real world. Parts that do not significantly affect the results of the model should be simplified or omitted. Although this simplified situation can be too ideal and unrealistic, it does not undermine the integrity of modelling as long as idiosyncrasy does not affect the mechanism that the models are aimed at discovering (Vynnycky and White 2010). In addition to the fundamental necessity of assumptions for simplification in modelling, assumptions can be used to fill unknown but essential parts for modelling. In this case, the logic of which assumption would be chosen is critical to maintaining the soundness of the modelling, and the logic must depend on the aim of the models. In this thesis, the key parts of Hendra virus dynamics depended on the assumptions to fill gaps in knowledge. Hendra virus dynamics are not fully understood, but logical inference has suggested SIR (susceptible-infectious-immune), SIRS (susceptible-infectious-immune-susceptible), and SILI (susceptible-infectious-latent-infectious) dynamics (Plowright et al. 2016). The mechanism generating pulses of Hendra virus shedding has been proposed as ‘transient epidemics’ or ‘episodic shedding’ (Plowright et al. 2015). Because the aim of the models in this thesis was to explore transmission dynamics among individuals rather than the immune response within individuals, the models assumed population dynamics-related assumptions such as SIR dynamics and transient epidemics. Episodic shedding could occur when the immune system of bats is weakened by stressors, whereas transient epidemics could occur when the population structure of bat populations temporarily changes to favourable conditions for transmission (Plowright et al. 2015). Thus, this thesis, when exploring the effects of population structure on viral transmission dynamics, should assume transient epidemics, not episodic shedding. It should be noted that the models in
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this thesis did not include all possibilities of the mechanisms triggering Hendra virus epidemics. Also, the models employed density-dependent transmission rather than frequency-dependent transmission because the models explored how the changes in population size affected transmission. As a result, the models have a limitation that the modelling results should be interpreted only in situations in which the assumptions can be accepted as appropriate. In some cases, it may be necessary to explore a range of plausible assumptions and to investigate how sensitive the modelling results are to the different sets of assumptions. Chapter 3 used exponential and gamma distributions to shift individuals from the maternally immune stage to the susceptible stage (Wearing et al. 2005). By doing so, the models could help explore the possible range of the effect of maternal immunity.

Finally, it is important to implement interdisciplinary research to improve data quality in the longer term. While construction and parameterisation of models rely on the findings from fieldwork or experimental studies, modelling studies can also direct the next phase of empirical studies in directions so that the most critical data can be obtained. The study units of emerging infectious diseases are often so vast and complex (e.g. ecosystem or metapopulation) that the scale of the study units is too large for empirical studies to cover (Heesterbeek et al. 2015). Models are capable of encompassing many aspects of emerging infectious diseases in virtual systems and can integrate the findings from a number of individual empirical studies into a systematic model to show where information gaps occur. This approach to tackling the paucity of wildlife diseases data cannot be a direct and instant solution, but a collaboration of models, fieldworks, and experiments is a crucial part of efficient research of wildlife infectious diseases (Plowright et al. 2008b, Restif et al. 2012).
6.5. FURTHER RESEARCH RECOMMENDATIONS

Poor data cause challenges to studying emerging infectious diseases originating from wildlife reservoirs (McCallum 2016). Consequently, the suggestions for further research are mainly directed at indicating the parts that need more data and information on wildlife disease ecology.

Searches for probable new zoonotic viruses in their reservoir populations are required to counteract the threats of emerging disease to humans (Daszak et al. 2000). Pandemics of SARS and MERS (Middle East respiratory syndrome) have raised the necessity for surveillance of coronavirus in bat reservoir populations (Hilgenfeld and Peiris 2013). The monitoring of coronavirus in bats can help understand transmission dynamics of coronavirus in bat populations, and the surveillance provides basic information about how to counter coronaviruses that may emerge. More specifically, the identification of persistent infection of coronavirus in this thesis (Chapter 2) can be followed by research that explores what factors are related to the differentiation of infectious bats into persistently and transiently infectious bats. Verification of the factors related to the differentiation could proceed by finding factors that covary with persistent infection. The verification will need individual bat tracking data including a wider variety of biological and ecological factors than the data used in Chapter 2.

As it was previously mentioned, the identification of the target-reservoir system of emerging infectious diseases is a fundamental part to understand the dynamics of these diseases. Hendra virus is known to be maintained in four flying fox species (*P. alecto*, *P. conspicillatus*, *P. scapulatus*, and *P. poliocephalus*) (Young et al. 1996). Of these, *P.
CHAPTER 6.

*alecto* and *P. conspicillatus* were found to be more strongly implicated in Hendra virus transmission to its target hosts, horses (Smith et al. 2014, Field et al. 2015). However, the Hendra virus target-reservoir system is not fully understood. The extent to which each species is involved in the overall maintenance of Hendra virus requires further investigation. Spatiotemporal prevalence and seroprevalence for each species are expected to help provide information on this yet unknown area.

The transmission mode of Hendra virus in flying foxes needs to be more clearly defined between density-dependent transmission and frequency-dependent transmission. Definition of transmission mode is one of the most fundamental part in building epidemic models. Nevertheless, whether Hendra virus transmission is density-dependent or frequency-dependent has not been determined. In Chapter 3 and 5, density-dependent transmission was assumed to concentrate on observing the effects of population size on Hendra virus epidemics, while in Chapter 4, both density and frequency-dependent transmissions were modelled to see if Hendra virus persistence can be ensured in both transmission modes. Plowright et al. (2015) suggested that both transmission modes are engaged in Hendra virus transmission within flying fox populations and the predominance of each transmission mode depends on specific situations, in which the virus is transmitted. Hendra virus transmission could therefore be more accurately predicted by a model that simulates the two separate situations in which each transmission mode is applied. Considering the critical importance of the transmission mode in managing infectious diseases by reducing host population size (McCallum et al. 2001), a model combining two modes of transmission is expected to be useful to predict the outcome of flying fox population management plans accurately.
General Discussion

Colony structure of flying foxes needs to be studied to design more accurate models. The models of Hendra virus dynamics in this thesis assumed that all bats are homogeneously mixed to have the same contact rate and transmission rate. However, activities related to mating, breeding, and nurturing tend to make bats stay more closely and contact more often with particular groups of age and sex (Hall and Richards 2000). As a maternity colony of *Myotis myotis* bats has been found to play a critical role in the amplification of the coronavirus (Drexler et al. 2011), the understanding of flying fox colony structure can help find ‘hot spots’ for transmissions.

Understanding of flying fox ecology is expected to improve plans for the management of flying fox populations. In particular, it is necessary to investigate the factors that determine where bats establish their colonies. This necessity originates from the flying fox population management strategy of dispersal. Dispersing flying foxes away from human residential areas has been commonly considered as a strategy to resolve conflicts between flying foxes and humans, but how it might be possible to control where the dispersed bats move to has not been well understood (Roberts and Eby 2013). Without this understanding, flying foxes would be dispersed only to generate temporary benefits or only to move the conflict to another human dwelling area (Roberts et al. 2011). There have been studies showing that feed sources are an important factor in determining bat roosting areas. Feed sources might be the cause of increasing trend of bats occurring in urban or peri-urban regions (Plowright et al. 2011). It was also suggested that the bat migration pattern could be predicted by Eucalypt phenology (Giles et al. 2016). Thus, management of bat feed resources could be essential to manage the locations of where dispersed bats move to. Nevertheless, the bats’ behaviour that determines their colony location has not been understood sufficiently that dispersed bats can be managed to form their colonies at where we want. More thorough understanding of the factors that
CHAPTER 6. 

determine the location of flying fox colonies may be developed by investigating the 
relation between the spatiotemporal pattern of their feed resources and their movement 
patterns. Furthermore, it is necessary to examine whether the establishment of feed 
sources for flying foxes in rural regions can draw flying foxes from urban regions to 
rural regions, reducing conflicts between flying foxes and humans in urban areas. 

6.6. CONCLUSION 

Disease is defined as any harmful deviation from the “normal” structural or functional 
state of an organism, and a diseased organism commonly exhibits signs or symptoms 
indicative of its “abnormal” state (Scarpelli and William 2017). “Treatment” is to allow 
an individual to recover from an abnormal state and return to a normal state. As a result, 
treatment depends on the understanding of abnormality, which relies on the knowledge 
of normality. This is why it is taught in the order of physiology (normal), pathology 
(abnormal), and internal medicine (treatment). 

This concept can also be applied into an ecosystem, where novel infectious diseases are 
emerging. In a normal state, viruses are contained in their natural reservoir hosts 
without spilling over, and the viruses do not tend to harm other species as well as 
reservoir species that have co-evolved with the viruses for a long period (Calisher et al. 
2006). In an abnormal state, any factors that disturb the normal state result in the 
spillover of the viruses to infect other species that had not been previously infected by 
the viruses and are often fatally infected (Bean et al. 2013). The recent increase in 
outbreaks of emerging infectious diseases could be attributable to anthropogenic 
activities that impair normal states of viral dynamics in wildlife populations (Daszak et 
al. 2000). Then, we can treat the problems brought by emerging infectious diseases by
changing the abnormal state back to the normal state. Consequently, investigation of the factors causing spillover occurs should be based on the understanding of the factors related to the transmission of viruses within their natural reservoir hosts, and it has to be followed by research for how to manage the ecosystem in order to reduce the spillover.

In many cases of emerging infectious diseases, not much information has been accumulated, because only recently have zoonotic diseases attracted substantial scientific and popular attention (Morens et al. 2008). Even the “normal” state of emerging viruses in their natural reservoir hosts has not been much studied and discovered. For this reason, the majority of this thesis was devoted to understanding of Hendra virus and coronavirus transmission within their natural reservoir hosts without considering any abnormally imposed external factors. Factors that disturb viral dynamics in reservoir hosts to facilitate spillover or predicting the efficacy of wildlife management to prevent spillover have been secondary interests of this thesis. Thus, the chapters concentrated on the conditions of intrinsic factors of reservoir populations such as persistent infection, seasonality, metapopulation structure, or spatial distribution rather than abnormal effects of external factors such as anthropogenic modification or climate change. Although Chapter 5 considered management strategies of flying foxes, the primary goal of the chapter was to improve our understanding of the effects of metapopulation dynamics on Hendra virus epidemics. The foundational information of the normality in this thesis provides the basis for other research exploring the abnormality and the treatment.


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APPENDICES

APPENDIX 0-1. CAPTURE-MARK-RECAPTURE DATA

Table A2-1. Detection of a putative novel Alphacoronaviruses in a 52 Myotis macropus from a Capture-Mark-Recapture study. This table was modified with permission from Smith (Smith 2015).

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</tr>
<tr>
<td>49 Female</td>
<td>Adult</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>50 Male</td>
<td>Unknown</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>51 Male</td>
<td>Unknown</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>52 Female</td>
<td>Sub-adult</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>
Appendices

APPENDIX 0-2. R CODE A1

Analyses of Capture-Mark-Recapture data of *Myotis macropus* using R2OpenBUGS package in R. This R code analyzed model number 3 in table 2-3. The output of this R code generated the estimates or ranges of all infection type in Table 2-2.

```r
# text file
"E:/all.txt"

2 1 0 0 0 0 0 2 2
1 2 2 0 0 0 2 0 2
0 2 2 0 1 0 0 0 0
0 2 0 0 0 1 1 2
0 0 2 2 1 0 1 1 0
0 0 0 2 0 0 0 0 2
0 0 0 2 0 0 0 0 2
2 0 0 0 0 0 0 1 0
0 2 1 0 0 0 0 1 0
0 2 1 0 0 0 1 0 0
0 2 1 1 0 0 1 1 0
0 2 1 0 1 0 0 0 0
0 2 0 0 0 0 1 1
0 0 2 1 1 0 1 1 0
0 0 2 0 1 0 0 0 0
1 0 2 0 0 0 0 0 0
0 1 2 0 0 0 0 0 0
0 0 0 1 2 0 0 0 1
1 0 0 1 0 0 0 0 2
0 1 1 0 0 0 0 0 2
0 1 1 0 0 0 0 0 2
0 1 0 1 1 0 0 0 2
0 0 1 1 1 0 0 0 2

## Without grouping of bats into two groups based on frequency of coronavirus detection

library(R2OpenBUGS)

# Import data

CH <- as.matrix(read.table(file = "E:/all.txt", sep = " "))

n.occasions<-dim(CH)[2]

#Compute vector with occasion of first capture

f<-numeric()

for(i in 1:dim(CH)[1]){f[i]<- min(which(CH[i,]!=0))}

#Recode CH matrix: note, a 0 is not allowed by OpenBUGS!

rCH<-CH #Recoded CH

rCH[rCH==0]<-3

# Specify model in BUGS language

sink("E:/corona_multistate_all.txt")
cat("model {

# Parameters:

# phiA: survival probability at Negative
```

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# phiB: survival probability at Positive
# psiAB: transition probability from Negative to Positive
# psiBA: transition probability from Positive to Negative
# pA: recapture probability at Negative
# pB: recapture probability at Positive

# States (S):
# 1 alive Negative
# 2 alive Positive
# 3 dead
# Observations (O):
# 1 seen Negative
# 2 seen Positive
# 3 not seen

# Priors and constraints
for (t in 1:(n.occasions - 1)){
  phiA[t] <- mean.phi[1]
  phiB[t] <- mean.phi[2]
  psiAB[t] <- mean.psi[1]
  psiBA[t] <- mean.psi[2]
  p[t] <- mean.p
}
mean.p ~ dunif(0, 1)  # Priors for mean state-spec. recapture
for (u in 1:2){
  mean.phi[u] ~ dunif(0, 1)  # Priors for mean state-spec. survival
  mean.psi[u] ~ dunif(0, 1)  # Priors for mean transitions
}

# Define state-transition and observation matrices
for (i in 1:nind){
  # Define probabilities of state S(t+1) given S(t)
  for (t in f[i]:(n.occasions - 1)){
    ps[1,i,t,1] <- phiA[t] * (1 - psiAB[t])
    ps[1,i,t,2] <- phiA[t] * psiAB[t]
    ps[1,i,t,3] <- 1 - phiA[t]
    ps[2,i,t,1] <- phiB[t] * psiBA[t]
    ps[2,i,t,2] <- phiB[t] * (1 - psiBA[t])
    ps[2,i,t,3] <- 1 - phiB[t]
    ps[3,i,t,1] <- 0
    ps[3,i,t,2] <- 0
    ps[3,i,t,3] <- 1
  }
  # Likelihood
  for (i in 1:nind){
    # Define latent state at first capture
    z[i,f[i]] <- y[i,f[i]]
    for (t in (f[i]+1):n.occasions){

  # Define probabilities of O(t) given S(t)
  po[1,i,t,1] <- p[t]
  po[1,i,t,2] <- 0
  po[1,i,t,3] <- 1 - p[t]
  po[2,i,t,1] <- 0
  po[2,i,t,2] <- p[t]
  po[2,i,t,3] <- 1 - p[t]
  po[3,i,t,1] <- 0
  po[3,i,t,2] <- 0
  po[3,i,t,3] <- 1
  }
  # t
  }
  # i

  # Define state-transition and observation matrices
  for (i in 1:nind){
    # Define probabilities of state S(t+1) given S(t)
    for (t in f[i]:(n.occasions - 1)){
      ps[1,i,t,1] <- phiA[t] * (1 - psiAB[t])
      ps[1,i,t,2] <- phiA[t] * psiAB[t]
      ps[1,i,t,3] <- 1 - phiA[t]
      ps[2,i,t,1] <- phiB[t] * psiBA[t]
      ps[2,i,t,2] <- phiB[t] * (1 - psiBA[t])
      ps[2,i,t,3] <- 1 - phiB[t]
      ps[3,i,t,1] <- 0
      ps[3,i,t,2] <- 0
      ps[3,i,t,3] <- 1
    }
    # Likelihood
    for (i in 1:nind){
      # Define latent state at first capture
      z[i,f[i]] <- y[i,f[i]]
      for (t in (f[i]+1):n.occasions){

  # Define probabilities of O(t) given S(t)
  po[1,i,t,1] <- p[t]
  po[1,i,t,2] <- 0
  po[1,i,t,3] <- 1 - p[t]
  po[2,i,t,1] <- 0
  po[2,i,t,2] <- p[t]
  po[2,i,t,3] <- 1 - p[t]
  po[3,i,t,1] <- 0
  po[3,i,t,2] <- 0
  po[3,i,t,3] <- 1
  }
  # t
  }
  # i

  # Define state-transition and observation matrices
  for (i in 1:nind){
    # Define probabilities of state S(t+1) given S(t)
    for (t in f[i]:(n.occasions - 1)){
      ps[1,i,t,1] <- phiA[t] * (1 - psiAB[t])
      ps[1,i,t,2] <- phiA[t] * psiAB[t]
      ps[1,i,t,3] <- 1 - phiA[t]
      ps[2,i,t,1] <- phiB[t] * psiBA[t]
      ps[2,i,t,2] <- phiB[t] * (1 - psiBA[t])
      ps[2,i,t,3] <- 1 - phiB[t]
      ps[3,i,t,1] <- 0
      ps[3,i,t,2] <- 0
      ps[3,i,t,3] <- 1
    }
    # Likelihood
    for (i in 1:nind){
      # Define latent state at first capture
      z[i,f[i]] <- y[i,f[i]]
      for (t in (f[i]+1):n.occasions){

  # Define probabilities of O(t) given S(t)
  po[1,i,t,1] <- p[t]
  po[1,i,t,2] <- 0
  po[1,i,t,3] <- 1 - p[t]
  po[2,i,t,1] <- 0
  po[2,i,t,2] <- p[t]
  po[2,i,t,3] <- 1 - p[t]
  po[3,i,t,1] <- 0
  po[3,i,t,2] <- 0
  po[3,i,t,3] <- 1
  }
  # t
  }
  # i

  # Define state-transition and observation matrices
  for (i in 1:nind){
    # Define probabilities of state S(t+1) given S(t)
    for (t in f[i]:(n.occasions - 1)){
      ps[1,i,t,1] <- phiA[t] * (1 - psiAB[t])
      ps[1,i,t,2] <- phiA[t] * psiAB[t]
      ps[1,i,t,3] <- 1 - phiA[t]
      ps[2,i,t,1] <- phiB[t] * psiBA[t]
      ps[2,i,t,2] <- phiB[t] * (1 - psiBA[t])
      ps[2,i,t,3] <- 1 - phiB[t]
      ps[3,i,t,1] <- 0
      ps[3,i,t,2] <- 0
      ps[3,i,t,3] <- 1
    }
    # Likelihood
    for (i in 1:nind){
      # Define latent state at first capture
      z[i,f[i]] <- y[i,f[i]]
      for (t in (f[i]+1):n.occasions){

  # Define probabilities of O(t) given S(t)
  po[1,i,t,1] <- p[t]
  po[1,i,t,2] <- 0
  po[1,i,t,3] <- 1 - p[t]
  po[2,i,t,1] <- 0
  po[2,i,t,2] <- p[t]
  po[2,i,t,3] <- 1 - p[t]
  po[3,i,t,1] <- 0
  po[3,i,t,2] <- 0
  po[3,i,t,3] <- 1
  }
  # t
  }
  # i
Appendices

# State process: draw S(t) given S(t-1)

\[
z[i,t] \sim \text{dcat}(ps[z[i,t-1], i, t-1])
\]

# Observation process: draw O(t) given S(t)

\[
y[i,t] \sim \text{dcat}(po[z[i,t], i, t-1])
\]

# Function to create known latent states z

\[
\text{known.state.ms} < - \text{function}(ms, notseen)\{
\text{state} <- ms
\text{state}[\text{state==notseen}] <- NA
\text{for} \ (i \ \text{in} \ 1:\text{dim}(ms)[1])\{
\text{m} <- \text{min(\text{which}(\!\text{is.na}(\text{state}[i])))}
\text{state}[i,m] <- NA
\}
\text{return}(\text{state})
\}
\]

# Function to create initial values for unknown z

\[
\text{ms.init.z} < - \text{function}(ch, f)\{
\text{for} \ (i \ \text{in} \ 1:\text{dim}(ch)[1])\{\text{ch}[i,1:f[i]] <- NA\}
\text{states} <- \text{max}(ch, \text{na.rm} = \text{TRUE})
\text{known.states} <- 1:(\text{states}-1)
\text{v} <- \text{which}(\text{ch}==\text{known.states})
\text{ch}[v] <- \text{sample}(\text{known.states}, \text{length}(v), \text{replace} = \text{TRUE})
\text{ch}[-v] <- \text{NA}
\text{return}(\text{ch})
\}\]

# Bundle data

\[
\text{bugs.data} <- \text{list}(y = \text{rCH}, f = f, n.occasions = \text{dim(rCH)}[2], nind = \text{dim(rCH)}[1], z = \text{known.state.ms}(\text{rCH}, 3))
\]

# Initial values

\[
\text{inits} <- \text{function}()\{\text{list}(\text{mean.phi} = \text{runif}(2, 0, 1), \text{mean.psi} = \text{runif}(2, 0, 1), \text{mean.p} = \text{runif}(2, 0, 1), z = \text{ms.init.z}(\text{rCH}, f))\}
\]

# Parameters monitored

\[
\text{parameters} <- \text{c}(\text{"mean.phi"}, \text{"mean.psi"}, \text{"mean.p"})
\]

# MCMC settings

\[
\text{ni} <- 10000
\text{nt} <- 6
\text{nb} <- 1000
\text{nc} <- 3
\]

# Call OpenBUGS from R

\[
\text{multistate.total} <- \text{bugs}(\text{data=bugs.data, inits=inits, parameters.to.save=parameters, n.iter=ni, model.file = "E:/corona_multistate_all.txt", n.chains=nc, n.burnin=nb, n.thin=nt, debug=TRUE)}
\text{print(multistate.total, digits=4)}
APPENDIX 0-3. R CODE A2

Analyses of Capture-Mark-Recapture data of *Myotis macropus* using R2OpenBUGS package in R. This R code analyzed model number 1 in table 2-3. The output of this R code generated the estimates or ranges of persistent and transient infection types in Table 2-2.

```r
# text file

"E:/persistent.txt"
2 1 0 0 0 0 0 2 2
1 2 2 0 0 0 2 0 2
0 2 2 0 1 0 0 0 0
0 2 0 0 0 0 1 1 2
0 0 2 2 1 0 1 0 2
0 0 0 2 0 0 0 2 2
0 0 0 2 0 0 0 2 2

"E:/transient.txt"
2 0 0 0 0 0 1 0
0 2 1 0 0 0 1 0
0 2 1 0 0 0 1 0
0 2 1 1 0 0 1 0
0 2 1 0 1 0 0 0
0 2 0 0 0 0 0 1 1
0 0 2 1 1 0 1 1 0
0 0 2 1 0 1 0 0 0
1 0 2 0 0 0 0 0 0
0 1 2 0 0 0 0 0 0
0 0 0 1 2 0 0 0 1
1 0 0 1 0 0 0 0 2
0 1 1 0 0 0 0 0 2
0 1 1 1 0 0 0 0 2
0 1 0 1 1 0 0 0 2
```

library(R2OpenBUGS)

```
CHm<-as.matrix(read.table("E:/persistent.txt")) #bats with multiple detections

CHs<-as.matrix(read.table(file = "E:/transient.txt", sep = " ")) #bats with single detection

# Merge capture-histories by row

CH <- rbind(CHm, CHs)

group<-c(rep(1,dim(CHm)[1]),rep(2,dim(CHs)[1]))

n.occasions<-dim(CH)[2]

#Compute vector with occasion of first capture

f<-numeric()

for(i in 1:dim(CH)[1]){f[i]<-min(which(CH[i,]!=0))}

#Recode CH matrix: note, a 0 is not allowed by OpenBUGS!
```
Appendices

# l=seen negative, 2= positive, 3= not seen
rCH<-CH #Recoded CH
rCH[rCH==0]<-3

# Specify model in BUGS language
sink("E:/corona_multistate_persistent+transient.txt")
cat("#

model {

  # -------------------------------
  # Parameters:
  # phiA: survival probability at Negative
  # phiB: survival probability at Positive
  # psiAB: transition probability from Negative to Positive
  # psiBA: transition probability from Positive to Negative
  # p: recapture probability

  # -------------------------------
  # States (S):
  # 1 alive Negative
  # 2 alive Positive
  # 3 dead
  # Observations (O):
  # 1 seen Negative
  # 2 seen Positive
  # 3 not seen
  # -------------------------------

  for (u in 1:g){
    mean.p.g[u] ~ dunif(0, 1) # Priors for mean state-spec. recapture
  }

  for (u in 1:g){
    for (v in 1:2){
      mean.phi.g[v,u] ~ dunif(0, 1) # Priors for mean state-spec. survival
      mean.psi.g[v,u] ~ dunif(0, 1) # Priors for mean transitions
    }
  }

  # Define state-transition and observation matrices
  for (i in 1:nind){
    for (t in f[i]:(n.occasions-1)){
      ps[1,i,t,1] <- phiA[i,t] * (1-psiAB[i,t])
      ps[1,i,t,2] <- phiA[i,t] * psiAB[i,t]
      ps[1,i,t,3] <- 1 - phiA[i,t]
      ps[2,i,t,1] <- phiB[i,t] * psiBA[i,t]
      ps[2,i,t,2] <- phiB[i,t] * (1-psiBA[i,t])
      ps[2,i,t,3] <- 1 - phiB[i,t]
      ps[3,i,t,1] <- 0
    }
  }

  phiA[i,t] <- mean.phi.g[1,group[i]]
  phiB[i,t] <- mean.phi.g[2,group[i]]
  psiAB[i,t] <- mean.psi.g[1,group[i]]
  psiBA[i,t] <- mean.psi.g[2,group[i]]
  p[i,t] <- mean.p.g[group[i]]

  for (u in 1:g){
    for (v in 1:2){
      mean.psi.g[v,u] ~ dunif(0, 1) # Priors for mean state-spec. survival
    }
  }

  for (u in 1:g){
    for (v in 1:2){
      mean.phi.g[v,u] ~ dunif(0, 1) # Priors for mean state-spec. survival
    }
  }

  # Priors and constraints
  for (i in 1:nind){
    for (t in 1:(n.occasions-1)){
      # Equations here
    }
  }

  # Equations here

  # Equations here

  # Equations here

  # Equations here
ps[3,i,t,2] <- 0
ps[3,i,t,3] <- 1

# Define probabilities of O(t)
given S(t)
po[1,i,t,1] <- p[i,t]
po[1,i,t,2] <- 0
po[1,i,t,3] <- 1-p[i,t]
po[2,i,t,1] <- 0
po[2,i,t,2] <- p[i,t]
po[2,i,t,3] <- 1-p[i,t]
po[3,i,t,1] <- 0
po[3,i,t,2] <- 0
po[3,i,t,3] <- 1

Likelihood
for (i in 1:nind){

# Define latent state at first capture
z[i,f[i]] <- y[i,f[i]]
for (t in (f[i]+1):n.occasions){

# State process: draw S(t) given S(t-1)
    z[i,t] ~ dcat(ps[z[i,t-1], i, t-1,])

# Observation process: draw O(t) given S(t)
y[i,t] ~ dcat(po[z[i,t], i, t-1,])
}
}

# Function to create known latent states z
known.state.ms <- function(ms, notseen){
    # notseen: label for 'not seen?
    state <- ms
    state[state==notseen] <- NA
    for (i in 1:dim(ms)[1]){ m <-
        min(which(!is.na(state[i,])))
    state[i,m] <- NA
    }
    return(state)
}

# Function to create initial values for unknown z
ms.init.z <- function(ch, f){
    for (i in 1:dim(ch)[1]){ch[i,1:f[i]] <- NA
        states <- max(ch, na.rm = TRUE)
        known.states <- 1:(states-1)
        v <- which(ch==states)
        ch[-v] <- NA
        ch[v] <- sample(known.states, length(v), replace = TRUE)
    return(ch)
}

# Bundle data
bugs.data <- list(y = rCH, f = f,
n.occasions = dim(rCH)[2], nind =
dim(rCH)[1], z = known.state.ms(rCH,
g=length(unique(unique(group))),group=g)

# Initial values
inits <- function(){list(mean.phi.g =
    runif(2, 0, 1), mean.psi.g =
    runif(2, 0, 1), mean.p.g =
    runif(2, 0, 1), z = ms.init.z(rCH, f))}

# Parameters monitored
parameters <- c("mean.phi.g", "mean.psi.g", "mean.p.g")

# MCMC settings
ni <- 10000
Appendices

nt <- 6
nb <- 1000
nc <- 3

# Call OpenBUGS from R

multistate.single.multiple<- bugs(bugs.data, inits, parameters, "E:/corona_multistate_persistent+transient.txt", n.chains=nc, n.thin=nt, n.iter=ni, n.burnin=nb, debug=TRUE)

print(multistate.single.multiple, digits=3)
APPENDIX 0-4. PREVALENCE OF A PUTATIVE NOVEL ALPHACORONAVIRUSES IN 52 MYOTIS MACROPUS

Capturing of *M. macropus* was not performed from week 9 to 11. We used an equation, \( \ln(I(t)) = \ln(I(0)) + \Lambda t \), to calculate the grow rate of the epidemic (\( \Lambda \)) (Vynnycky and White 2010). \( I(t) \) and \( I(0) \) represent the number of infectious individuals at time \( t \) and the number of infectious individuals at the start. We assumed that week 7 was the start of the epidemic, and we assumed that week 12 as time \( t \). Thus, we calculated the growth rate from week 7 to week 12. The number of infectious individuals was calculated by multiplying the prevalence of each week with the population size of 86.

\[
\ln\left(\frac{11}{25} \times 86\right) = \ln\left(\frac{1}{12} \times 86\right) + \Lambda \times (12 - 7)
\]

As a result, \( \Lambda = 0.3328 \).

*Figure A2-1. Prevalence of a putative novel Alphacoronaviruses in a 52 Myotis macropus from a Capture-Mark-Recapture study (Smith 2015).*
Simulation of epidemic models that contained scenario 1 to 6. The simulation of this R code generated probabilities of viral maintenance within the study population in scenario 1 to 6. R code S1 and S2 generated parameter values that were used in R code A3.

```r
library(deSolve)
library(mc2d)
week=12 # number of weeks
N=10000 # number of iteration
n=86 # number of individuals
prev=.278571 # mean prevalence from the CMR data
prop<- 7/23 # proportion of persistently infected bats
t=seq(0,week,by=1)

########################################################
SIRS model for 1 group model
SIRS1<- function(t, state, par) {
  with(as.list(c(state, par)),{
    dS<- -beta*S*I+omega*R-mu*S
    dI<- beta*S*I-gamma*I-m1*I
    dR<- +gamma*I-omega*R-mu*R
    floor(S)
    floor(I)
    floor(R)
    return(list(c(dS, dI, dR)))
  })
}
state<-c(S=n*(1-prev), I=n*prev, R=0) # Initial number of bats in two group models
# Scenario 1 one-group
inf.sta1<-numeric() # number of infected bats at the end of simulation
persist1<-numeric() # the probability of viral persistence
for (i in 1:N){
```

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par<-c(beta= 0.0104,# transmission rate
gamma=rpert(1,.3236,.5638,.8031 ), # recovery rate
omega=0.08333 , #immunity loosing rate
mu=rpert(1,0,1-.9848,1-.9268),  # mortality rate of uninfected infected bats
mi=rpert(1,0,1-.9731,1-.8866) # mortality rate of infected infected bats
)
out<-ode(y=state, times=t, func=SIRS1, parms=par)
inf.sta1[i]<-floor(out[week,3]) #the number of infected ones in the stabilized state
persist1[i] <- if (inf.sta1[i] <= 1) 0 else 1 # infected less than 1 or larger than 1
out1<-out #ode simulation result of scenario 1

#SIRS model for 2 group model
SIRS2<- function(t, state, par) {
  with(as.list(c(state, par)),{
    dS<- -beta*S*(Ip+It)+omega*R-mut*S*(1-f)-mup*S*f
    dIp<- f*beta*S*(Ip+It)-gamma.p*Ip-mip*Ip
    dIt<- (1-f)*beta*S*(Ip+It)-gamma.t*It-mit*It
    dR<- gamma.p*Ip+gamma.t*It-omega*R-mut*R*(1-f)-mup*R*f
    floor(S)
    floor(Ip)
    floor(It)
    floor(R)
    return(list(c(dS, dIp, dIt, dR)))
  })
}
state<-c(S=n*(1-prev), Ip=n*prev*prop,It=n*prev*(1-prop), R=0) #Initial number of bats in two group models

#Scenario 2: two group
inf.sta2<-numeric() # number of infected bats at the end of simulation
persist2<-numeric() # the probability of viral persistence
for (i in 1:N){
  par<-c(bbeta= 0.0104, gamma.p=rpert(1,0.121, 0.3354,0.6518 ),
gamma.t=rpert(1,0.4985, 0.8582,0.9943 ),
omega= 0.0833333,
mup=rpert(1,0,1-.9848,1-.9268), mip=rpert(1,0,1-.9731,1-.8866),
}
Appendices

mut=rpert(1,0,1-.9848,1-.9268), mit=rpert(1,0,1-.9731,1-.8866), f=7/23)
out<-ode(y=state, times=t, func=SIRS2, parms=par)
out.mod<-matrix(NA, ncol=4, nrow=week+1)
inf.sta2[i]<-floor(out.mod[week,3]) #the number of infected ones in the stabilized state
persist2[i]<-if (inf.sta2[i] <= 1) 0 else 1 # infected less than 1 or larger than 12.9815 2.9815
out2<-out #ode simulation result of scenario 2

#Scenario 3: 5 weeks of persistent infectious period
inf.sta3<-numeric()
persist3<-numeric()
for (i in 1:N){
  par<-c(beta= 0.0104, gamma.p=1/5, gamma.t=rpert(1,0.4985,0.9983,1-0.9983,1-0.7183),
    mip=rpert(1,1-1,1-.9748,1-.8711), mut=rpert(1,0,1-.9834,1-.9185),
    mit=rpert(1,1-1,1-.9834,1-.9185), f= 7/23)
  out<-ode(y=state, times=t, func=SIRS2, parms=par)
  out.mod<-matrix(NA, ncol=4, nrow=week+1)
  inf.sta3[i]<-floor(out.mod[week,3]) #the number of infected ones in the stabilized state
  persist3[i]<-if (inf.sta3[i] <= 1) 0 else 1 # infected less than 1 or larger than 1
}
out3<-out #ode simulation result of scenario 3

#Scenario 4: 7 weeks of persistent infectious period
inf.sta4<-numeric()
persist4<-numeric()
for (i in 1:N){
  par<-c(beta=0.0104, gamma.p=1/7, gamma.t=rpert(1,0.4985,0.8582,0.9943),
    ,omega=0.0833333, mup=rpert(1,1-0.9983,1-0.9383,1-0.7183),
    mip=rpert(1,1-1,1-.9748,1-.8711), mut=rpert(1,0,1-.9834,1-.9185),
    mit=rpert(1,1-1,1-.9834,1-.9185), f= 7/23)
  out<-ode(y=state, times=t, func=SIRS2, parms=par)
  out.mod<-matrix(NA, ncol=4, nrow=week+1)
  inf.sta4[i]<-floor(out.mod[week,3]) #the number of infected ones in the stabilized state
  persist4[i]<-if (inf.sta4[i] <= 1) 0 else 1 # infected less than 1 or larger than 1
}
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out.mod[,1]<-out[,1];out.mod[,2]<-out[,2];out.mod[,3]<-
out[,3]+out[,4];out.mod[,4]<-out[,5]

inf.sta4[i]<-floor(out.mod[week,3]) #the number of infected ones in the
stabilized state

persist4[i] <- if (inf.sta4[i] <= 1) 0 else 1 # infected less than 1 or
larger than 1

out4<-out #ode simulation result of scenario 4

#Scenario 5: 9 weeks of persistent infectious period
inf.sta5<-numeric()
persist5<-numeric()
for (i in 1:N){
  par<-c(beta=0.0104, gamma.p=1/9, gamma.t=rpert(1,0.4985,
  ,0.9943 ), omega=0.0833333, mup=rpert(1,1-0.9983,1-
  0.9383, 1-0.7183),
  mip=rpert(1,1-1,1-.9748,1-.8711), mut=rpert(1,0,1-.9834,1-.9185),
  mit=rpert(1,1-1, 1-.9834,1-.9185), f= 7/23)

  out<-ode(y=state, times=t, func=SIRS2, parms=par)
  out.mod<-matrix(NA, ncol=4, nrow=week+1)
  out.mod[,1]<-out[,1];out.mod[,2]<-out[,2];out.mod[,3]<-
  out[,3]+out[,4];out.mod[,4]<-out[,5]

  inf.sta5[i]<-floor(out.mod[week,3]) #the number of infected ones in the
  stabilized state

  persist5[i] <- if (inf.sta5[i] <= 1) 0 else 1 # infected less than 1 or
  larger than 1
}

out5<-out #ode simulation result of scenario 5

#Scenario 6: 11 weeks of persistent infectious period
inf.sta6<-numeric()
persist6<-numeric()
for (i in 1:N){
  par<-c(beta=0.0104, gamma.p=1/11, gamma.t=rpert(1,0.4985,
  ,0.9943 ), omega=0.0833333, mup=rpert(1,1-0.9983,1-
  0.9383, 1-0.7183),
  mip=rpert(1,1-1,1-.9748,1-.8711), mut=rpert(1,0,1-.9834,1-.9185),
  mit=rpert(1,1-1, 1-.9834,1-.9185), f= 7/23)

  out<-ode(y=state, times=t, func=SIRS2, parms=par)
  out.mod<-matrix(NA, ncol=4, nrow=week+1)
  out.mod[,1]<-out[,1];out.mod[,2]<-out[,2];out.mod[,3]<-
  out[,3]+out[,4];out.mod[,4]<-out[,5]

  inf.sta6[i]<-floor(out.mod[week,3]) #the number of infected ones in the
  stabilized state
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```r
persist6[i] <- if (inf.sta6[i] <= 1) 0 else 1 # infected less than 1 or larger than 1
}
out6<-out #ode simulation result of scenario 6

##########################################################################
#the probability of viral persistence
p1<-sum(persist1)/length(persist1)
p2<-sum(persist2)/length(persist2)
p3<-sum(persist3)/length(persist3)
p4<-sum(persist4)/length(persist4)
p5<-sum(persist5)/length(persist5)
p6<-sum(persist6)/length(persist6)
p<-c(p1,p2,p3,p4,p5,p6);p
```
APPENDIX 0-6. ODES (ORDINARY DIFFERENTIAL EQUATIONS)

- SIR model equations

\[
\frac{dS_i}{dt} = -\varepsilon S_i + b(t)(S_m + I_m + R_m) - \beta S_i(I_i + I_m) - \mu S_i \\
\frac{dI_i}{dt} = -\varepsilon I_i + \beta S_i(I_i + I_m) - \gamma I_i - \mu I_i \\
\frac{dR_i}{dt} = -\varepsilon R_i + \gamma I_i - \mu R_i \\
\frac{dS_m}{dt} = \varepsilon S_i - \beta S_m(I_i + I_m) - \mu S_m \\
\frac{dI_m}{dt} = \varepsilon I_i + \beta S_m(I_i + I_m) - \gamma I_m - \mu I_m \\
\frac{dR_m}{dt} = \varepsilon R_i + \gamma I_m - \mu R_m
\]

- MSIR model equations

\[
\frac{dM_i}{dt} = -\varepsilon M_i + b(t)(M_m + R_m) - \delta M_i - \mu M_i \\
\frac{dS_i}{dt} = -\varepsilon S_i + b(t)(S_m + I_m) - \beta S_i(I_i + I_m) + \delta M_i - \mu S_i \\
\frac{dI_i}{dt} = -\varepsilon I_i + \beta S_i(I_i + I_m) - \gamma I_i - \mu I_i \\
\frac{dR_i}{dt} = -\varepsilon R_i + \gamma I_i - \mu R_i \\
\frac{dM_m}{dt} = \varepsilon M_i - \delta M_m - \mu M_m \\
\frac{dS_m}{dt} = \varepsilon S_i - \beta S_m(I_i + I_m) + \delta M_m - \mu S_m
\]
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\[ \frac{dl_m}{dt} = \epsilon I_i + \beta S_m(I_i + I_m) - \gamma I_m - \mu I_m \]

\[ \frac{dR_m}{dt} = \epsilon R_i + \gamma I_m - \mu R_m \]

- gamma MSIR model equations (gamma distribution parameter, \( g = 10 \))

\[ \frac{dM_1^1}{dt} = b(t)(M_m + R_m) - \delta g M_1^1 - \mu M_1^1 \]

\[ \frac{dM_1^2}{dt} = -\delta g M_1^2 + \delta g M_1^1 - \mu M_1^2 \]

\[ \frac{dM_1^3}{dt} = -\delta g M_1^3 + \delta g M_1^2 - \mu M_1^3 \]

\[ \frac{dM_1^4}{dt} = -\delta g M_1^4 + \delta g M_1^3 - \mu M_1^4 \]

\[ \frac{dM_1^5}{dt} = -\delta g M_1^5 + \delta g M_1^4 - \mu M_1^5 \]

\[ \frac{dM_1^6}{dt} = -\delta g M_1^6 + \delta g M_1^5 - \mu M_1^6 \]

\[ \frac{dM_1^7}{dt} = -\delta g M_1^7 + \delta g M_1^6 - \mu M_1^7 \]

\[ \frac{dM_1^8}{dt} = -\delta g M_1^8 + \delta g M_1^7 - \mu M_1^8 \]

\[ \frac{dM_1^9}{dt} = -\delta g M_1^9 + \delta g M_1^8 - \mu M_1^9 \]

\[ \frac{dM_1^{10}}{dt} = -\epsilon M_1^{10} - \delta g M_1^{10} + \delta g M_1^9 - \mu M_1^{10} \]

\[ \frac{dS_i}{dt} = -\epsilon S_i + b(t)(S_m + I_m) - \beta S_i(I_i + I_m) + \delta g M_1^{10} - \mu S_i \]

\[ \frac{dl_i}{dt} = -\epsilon I_i + \beta S_i(I_i + I_m) - \gamma I_i - \mu I_i \]

\[ \frac{dR_i}{dt} = -\epsilon R_i + \gamma I_i - \mu R_i \]
\[
\frac{dM_m}{dt} = \varepsilon M_i^{10} - \delta M_m - \mu M_m
\]

\[
\frac{dS_m}{dt} = \varepsilon S_i - \beta S_m (I_i + I_m) + \delta M_m - \mu S_m
\]

\[
\frac{dI_m}{dt} = \varepsilon I_i + \beta S_m (I_i + I_m) - \gamma I_m - \mu I_m
\]

\[
\frac{dR_m}{dt} = \varepsilon R_i + \gamma I_m - \mu R_m
\]
Figure A3-1. Number of maternally immune hosts by different numbers of compartments (g, gamma distribution parameter) of maternally immune stage. Three years since viral introduction are shown. Irrespective of the value of g, the mean duration of the maternally-derived immune period is 255 days. When g=1, the distribution of the maternally immune period is exponential, but as g increases the maternally immune period becomes closer to a constant length. The herd immunity at viral introduction (HI) was 0.71 and the virus was introduced at the timing of peak of a birth pulse.
Figure A3-2. Seasonal fluctuations in population sizes in each age group. Horizontal axis is time in days for two years and vertical axis is the number of individuals. Vertical grey bars represent birth pulses.
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APPENDIX 0-9. TIMING OF EPIDEMIC PEAKS IN DAYS

How long it took for epidemics to reach their peaks since viral introduction in days was observed in three models: SIR model, exponential MSIR model, and gamma MSIR model.
Figure A3-3. The timing of epidemic peaks in days since viral introduction. (A), (B), and (C) show the result of SIR model, exponential MSIR model, and gamma MSIR model, respectively. The horizontal axes show the initial herd immunity HI at the timing of viral introduction. The vertical axis shows the number of days after the birth pulse when the virus was introduced, and the shading shows how long after the introduction of the virus the epidemics reached their peaks in days. In the right panels, (A-1), (B-1), and (C-1) show the expansion of the horizontal axes for the range 0.55 to 0.8. White parts represent a failure of an epidemic to take-off.
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APPENDIX 0-10. TIMING OF EPIDEMIC PEAKS IN THE SIR MODEL WITH A CONSTANT BIRTH RATE

Comparing the SIR model with birth pulses and another SIR model with a constant birth rate (b=1/7 per year per capita), I found that viral fadeout was more likely to be avoided in the SIR model with the constant birth rate than in the SIR model with birth pulses, supposing the same number of births over a year in both models. In terms of initial fadeout, the conditions in which epidemics occurred were marginally more in the SIR model with the constant birth rate than the conditions in the SIR model with birth pulses (Compare Figure 3-1 and Figure A3-4). The difference in viral persistence between the two models was more evident in terms of epidemic fadeout. Epidemics could avoid epidemic fadeout in a considerable portion of HI in the SIR model with the constant birth rate, while the avoidance did not occur in any combinations of HI and DB in the SIR model with birth pulses. Comparing Figure 3-4 to Figure A3-4, it was observed that the number of cases of viral persistence avoiding epidemic fadeout and the number of infectious individuals at troughs were similar each other in the SIR model with the constant birth rate and the MSIR models. This observation indicates that the addition of maternally-derived immunity to seasonal birth pulses had an effect to dilute the effect of seasonal birth pulses.
Figure A3-4. Timing of epidemic peaks in the SIR with birth pulses and the number of infectious individuals at troughs. Although y-axis is meaningless in this simulation with a constant birth rate, it remains to help compare this result with Figure 3-1. The x-axis shows initial herd immunity HI, with the right panels showing an expansion of the x-axis for the range 0.55 to 0.8. In (A), the y-axis shows the number of days after the birth pulse when the virus was introduced, and the shading shows how long after the introduction of the virus the epidemics reached their peaks in days. In (B), the y-axis shows the month when the virus was introduced, with the shading showing the month in which epidemics reached their peak. White parts represent failure of an epidemic to
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take-off. (C) shows the cases in which epidemics avoided epidemic fadeout to reach an endemic equilibrium state.
APPENDIX 0-11. EQUATIONS CALCULATING THE PERIOD OF
MIGRATION OF AN INFECTIOUS BAT IN STAGE 3

I devised two equations to calculate the period until an infectious bat migrates into an infection-free colony from other infected colonies. The two are;

\[ R = (1 - P)^{m*N}, \]  
\[ d = \frac{\log(1 - 0.95)}{\log R}, \]

in which the result of the first equation was used to calculate the second equation.

The first equation calculates the probability (R) that no infectious bats migrate into an infection-free colony per day. (1 - P (prevalence)) is the probability that a bat migrating from other infected colonies to an infection-free colony is not an infectious bat. To apply this probability for all immigrating bats into the infection-free colony per day, (1 - P) was raised to the power of the daily number of the immigrating bats, m*N, where m and N denote the daily rate of migration (movement rate) and colony size, respectively.

The second equation was devised to calculate the period of days (d) that at least one infectious bat migrates into an infection-free colony from other infected colonies. For this purpose, I should estimate the probability (R') that at least one infectious bat migrates from other infected colonies to an infection-free colony during a period of time, based on R calculated from the first equation. R' exponentially decreases with the course of time, and R' \( \geq 0.95 \) was assumed to assure the migration of at least one infectious bat. The processes to obtain the second equation is like below;

\[ R' \geq 0.95 = 1 - R^d \]
\[ R^d = 1 - 0.95 \]

\[ \log R^d = \log(1 - 0.95) \]

\[ d = \frac{\log(1 - 0.95)}{\log R} \]
APPENDIX 0-12. MAGNITUDE AND PERIOD OF EPIDEMICS IN
THE DENSITY-DEPENDENT TRANSMISSION MODEL AND IN
THE FREQUENCY-DEPENDENT TRANSMISSION MODEL

In addition to modelling the cycle consisting of three stages, I modelled epidemics following viral introduction into infection-free populations. This modelling was used to explore the effects of colony size (N) and herd immunity at viral introduction (HI) on magnitude and duration of epidemics. The epidemic magnitude was defined as the proportion of the maximum number of infected bats to the colony size (N), and epidemic duration was defined as the time in days from the viral introduction until the number of infectious bats became zero. Each combination of a range of colony sizes and herd immunities at viral introduction was simulated once for density-dependent transmission and once for frequency-dependent transmission over a two-year timeframe. In the model, two years were sufficient to encompass a complete epidemic peak and a subsequent trough. Figure A4-1 shows the magnitude and duration of epidemics as a function of transmission modes, colony size (N), and herd immunity at viral introduction (HI). The magnitude of epidemics (as a proportion of the total colony size) increased with increasing colony size (N) in the density-dependent transmission model, whereas epidemic magnitude was constant regardless of colony size (N) in the frequency-dependent transmission model. This is in accord with an epidemiological principle that force of infection increases with increasing population size in density dependence, while the force of infection does not change with population size in frequency dependence (Vynnycky and White 2010).
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Viral fadeout can be classified into three forms by the concept of net reproduction number ($R_n$), which means an average number of secondary infectious individuals resulting from one infectious individual in a partially susceptible population (Vynnycky and White 2010). When $R_n<1$, epidemic did not occur (initial fadeout). When $R_n>1$, epidemics could occur, and the epidemic patterns were different between when $R_n$ was considerably higher than 1 and when $R_n$ was slightly higher than 1. In the former, viral fadeout occurred at the deep trough following the high-impact epidemic (epidemic fadeout), whereas in the latter the infection survived the trough and lasted until the infection became extinct due to random fluctuations in the number of infected individuals (endemic fadeout). Additionally, the continuously high number of infected individuals in large-sized colonies excluded the possibility of endemic fadeout in density-dependent transmission mode.
Figure A4-1. Magnitude and period of epidemics in the density-dependent transmission model and the frequency-dependent transmission model. I used stochastic simulations to characterise the epidemic magnitude and duration with a range of different combinations of colony sizes (N) and herd immunities at viral introduction (HI). (A) the magnitude of the epidemic in density-dependent transmission. (B) the magnitude of the epidemic in frequency-dependent transmission. (C) viral fadeout and persistence in density-dependent transmission. (D) viral fadeout and persistence in frequency-dependent transmission. Both magnitudes of epidemic and duration of the infected state were shown in log-scale. The red lines represent R_n (net reproduction number) = 1. Above the red lines, R_n<1, and below the red lines, R_n>1. In (C) and (D), the plots could be divided into four parts: initial fadeout, epidemic fadeout, endemic fadeout, and viral persistence. First, initial fadeout was observed in mostly blue parts. Initial fadeout means the disappearance of infection in a population without an epidemic. Second, epidemic fadeout was observed in mostly yellow parts. Epidemic fadeout means extinction of disease at the trough following an epidemic. Third, endemic fadeout was observed in mostly on the border between initial fadeout and epidemic fadeout.
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Endemic fadeout means extinction of disease due to random fluctuation after it reaches an endemic equilibrium state. Last, in the density-dependent transmission model, continuous viral persistence was observed in mostly red parts. Except for high HI, the viral persistence was ensured in large-sized colonies by overcoming epidemic and endemic fadeout.
REFERENCES IN APPENDICES

Smith, C. S. 2015. Australian bat coronaviruses. The University of Queensland, Brisbane, QLD, Australia.