DISSERTATION

AN ECO-EPIDEMIOLOGICAL APPROACH TO MANAGEMENT OF TUBERCULOSIS IN FREE-RANGING AND CAPTIVE WILDLIFE

Submitted by

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ABSTRACT

AN ECO-EPIDEMIOLOGICAL APPROACH TO MANAGEMENT OF TUBERCULOSIS IN FREE-RANGING AND CAPTIVE WILDLIFE

Tuberculosis (TB) is a disease of global importance affecting millions of humans, livestock, and wildlife. Control and eventual eradication of TB depends on dedicated management actions for all species. Accurately diagnosing TB can be challenging in wildlife species, for which validated tests may be unavailable or of limited sensitivity or specificity. Managing TB in wildlife poses additional difficulties, requiring considerable time and resources to implement at an appropriately broad scale. Each unique ecosystem where TB occurs requires management interventions designed to meet the area's conservation, ecological, social, and financial needs. In this dissertation, I explored the diagnosis and management of tuberculosis in wildlife in three different settings: free-ranging European badgers (*Meles meles*) in Ireland, working African elephants (*Loxodonta africana*) in Zimbabwe, and captive African and Asian elephants (*Elephas maximus*) in North America. Badgers are a reservoir of bovine TB in Ireland, while captive elephants around the world are at risk of TB from their human handlers. Badgers have historically been managed by culling, but there is a current transitioning to vaccination as the primary management tool. In contrast, captive elephants in high-resource settings are typically treated for TB upon diagnosis, although this option may be limited in low-income countries.

The first objective of this research was to assess the impact of environmental factors in management of TB over three different studies. I explored how biotic and abiotic factors influence trapping success of badgers being managed for bovine TB in Ireland. In a second study of badgers, I estimated density of a population undergoing vaccination in relation to environmental variables and prior management history. Underlying badger density is an important driver in the TB disease dynamics between cattle and badgers, and can be used in predictions about and assessment of outcomes under

vaccination. Finally, I examined potential risk factors for TB seropositive status in working African elephants in Zimbabwe, and identified unique potential exposures from the environment.

The second objective of this dissertation was to study the performance of diagnostic tests in a novel setting and interpret the results in the context of exposures within the ecosystem. This study employed two serological tests, STAT-PAK and DPP, for the first time in working African elephants in a range country. I interpreted the results suggestive of exposure to mycobacteria in some elephants based on possible interactions with the complex community of humans, livestock, wildlife, and mycobacteria.

The third objective of this dissertation was to develop recommendations for TB management programs based on surveys, capture data, and consideration of individual, population, and community factors. The results from our badger trapping study in Ireland formed the basis of suggested conditions under which vaccine delivery can be increased, because captures are most likely. We used mark-recapture data to estimate badger density in a vaccination area, which adds an important dimension to the Irish TB management program that includes badgers and cattle. Population density is an important factor in pathogen transmission and estimating density using these methods may be a priority for other wildlife populations being managed for TB. Our study of TB treatment in elephants provided a compilation of empirical data for elephant managers and veterinarians to inform clinical decision making. It also underscores the need for improved diagnostics to more confidently identify when animals are no longer infectious. For working African elephants, we documented other wildlife species with host potential on and around facilities, and considered these as possible sources for mycobacterial transmission. Our management guidelines for TB prevention specifically include measures to reduce direct and indirect contact with potential host species.

Management of TB across humans and animal species remains a challenging prospect. A One Health approach that incorporates data and techniques across disciplines to build a complete picture of disease control is ideal for TB in wildlife. I drew from ecology and epidemiology to implement a holistic approach to diagnosing and managing TB in species of conservation concern, provide insight into the

challenges of diagnosing and managing TB in free-ranging and captive wildlife, describe the benefits of a transdisciplinary approach, and expose areas in need of further research.

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My parents, Liz and Marc Rosen, have been staunch supporters from afar during my three degrees and 14 years at Colorado State. I promise this is the last one.

I am deeply indebted to a small group of wonderful friends who have supported me throughout the program. Laura Backus never failed to listen or make me laugh, and has been my trusted confidant through everything, even from a distance. Janine Meuffels, despite being on the other side of the world, is a true kindred spirit whose friendship and infectious good nature brightened my days. Greta Krafsur has been my friend, study buddy, and supporter for more than a decade at CSU. Stacey Elmore helped give me perspective, encouragement on all fronts, and most importantly, a reality check when needed. Elizabeth Sagen has been a voice of reason and running partner through difficult days.

Finally, this work could not have been completed without Greg Foggin, who has given me unflinching support from land and sea all over the world. His genuine good heart, relentlessly positive spirit, and unwavering belief in me have carried me through the most challenging days of this journey. I am beyond grateful to have him in my life.

DEDICATION

This dissertation is dedicated to the memory of my grandfather, Sidney Mudd,

for understanding my love of animals, encouraging me to find the answers I didn't know,

believing in my ability to succeed in dark times, and teaching me that "it can be done."

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CHAPTER 1: INTRODUCTION

Tuberculosis (TB) is a disease of global importance affecting humans, livestock, and wildlife (de Lisle et al. 2001; Kyu et al. 2017). It is most frequently caused by the bacteria *Mycobacterium tuberculosis (M. tuberculosis)* in humans and *Mycobacterium bovis (M. bovis)* in livestock and wildlife (Gordon and Behr 2015). *M. bovis* is also a zoonotic pathogen of humans (Cosivi et al. 1995), while *M. tuberculosis* is increasingly emerging in captive and free-ranging wildlife at the human-wildlife interface (Michel et al. 2013; Miller and Olea-Popelka 2013). Several countries have TB control programs in livestock, but these are often hampered by the presence of wildlife reservoirs (de Lisle et al. 2002). Successful control of TB in any species will require a coordinated effort to control the disease in all host species (Miller and Olea-Popelka 2013).

Disease management is desirable for maintaining livestock health, preventing zoonotic disease in humans, and benefitting wildlife conservation (Wobeser 2002). Diagnosis is the first step to managing the disease at all scales, from identifying disease in individuals to monitoring populations (Maas et al. 2013). Validating diagnostic tests for wildlife species, however, proves challenging when known positive or negative samples are often difficult to acquire or otherwise in short supply (Cousins and Florisson 2005). Once a diagnosis is confirmed, management of TB takes various forms depending on local factors. Prevention is key where the disease is not present, while eradication may be a goal for established disease, and control is often desirable in endemic areas (Wobeser 1994). Approaches for controlling established disease differ between free-ranging and captive wildlife populations. Free-ranging populations may be manipulated through culling or widescale vaccination efforts, while endangered or valuable captive wildlife such as elephants can feasibly be treated for TB (de Lisle et al. 2002; Fitzgerald and Kaneene 2013). Both free-ranging and captive wildlife can benefit from modification of human activities that increase TB risk for animals (Fitzgerald and Kaneene 2013).

The goal of my dissertation was to use principles from epidemiology and ecology to analyze field data to improve our understanding of diagnosis and management of TB in wildlife. Each system contains

a unique group of hosts, mycobacterial pathogens, and environmental conditions that call for different management strategies adapted to the circumstances. In chapter 2, I review literature pertaining to common diagnostic assays and management strategies for TB in both captive and free-ranging wildlife around the world. Chapters 3–6 describe studies of wildlife species at risk of TB in three different settings (Figure 1.1): free-ranging European badgers (*Meles meles*) in Ireland, where they are a reservoir for *M. bovis* and hinder eradication of bovine TB from cattle; working African elephants (*Loxodonta africana*) in Zimbabwe that interact with humans in this high TB (*M. tuberculosis*) burden area; and captive African and Asian elephants (*Elephas maximus*) in North America, where they continue to become infected with *M. tuberculosis* despite routine testing.

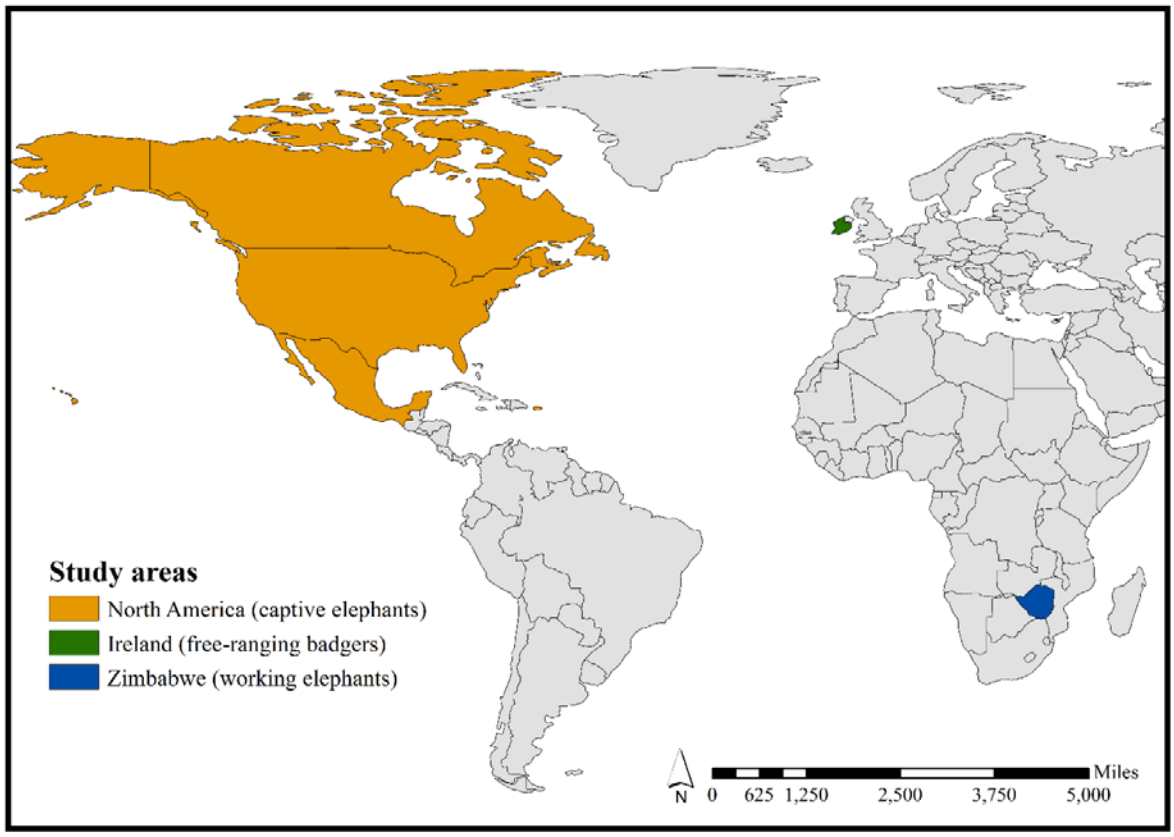

Figure 1.1: Map of the world showing each of the study areas in this dissertation: Ireland, where free-ranging badgers were studied (Chapters 3 and 5); Zimbabwe, where working African elephants were studied (Chapter 4); and North America, where captive Asian and African elephants were studied (Chapter 6).

The studies in Ireland were designed to use badger capture data from the Kilkenny Vaccine Trial in County Kilkenny to provide wildlife managers with new information to support the country's move to replace badger culling with vaccination as a control strategy for *M. bovis*. My objectives were to investigate environmental factors that maximize badger trapping success, and to estimate the density of badgers within a vaccination area as a baseline. Chapter 3 is a manuscript published in the European Journal of Wildlife Research, describing biotic and abiotic factors associated with successful trapping of badgers as part of an intramuscular vaccination program used in Ireland's bovine TB management. Improving trapping success is vital for delivery of vaccines. Chapter 5 is a manuscript describing the density of badgers within the Kilkenny vaccination area in Ireland, using spatially explicit capture-recapture methods. Badger density is an important driver of transmission dynamics among badgers and cattle. The density estimates will be used in conjunction with data from cattle for a coordinated approach to assessing the success of vaccination in controlling TB.

The objective of the study in Zimbabwe was to use survey data from working African elephants at ecotourism facilities in the Victoria Falls area of Zimbabwe to assess potential exposure to *M. tuberculosis* from humans in this high human TB burden area. Chapter 4 is a manuscript published in Transboundary and Emerging Diseases, describing TB serological status of these working elephants and potential risk factors for seropositive status. This study is the first to examine TB in African elephants in a range country, where facility conditions and local community ecology differ considerably from those of either working Asian elephants in range countries or captive elephants in zoos and private collections.

Two surveys were distributed to elephant facilities in North America: one survey covered demographics, management, and potential risk factors for TB (caused by *M. tuberculosis*) in captive elephants, and the other covered results from diagnostic assays for TB and protocols from elephants treated for TB. Chapter 6 is a manuscript describing TB treatment protocols, serum drug concentrations, and adverse effects associated with TB treatment in elephants in North America. This chapter is the first compilation of treatment protocols for TB in a wildlife species, which have previously been limited to a

various case reports. Finally, chapter 7 contains final conclusions about the contributions of this dissertation to the field of TB research and directions for future research.

CHAPTER 2: A REVIEW OF DIAGNOSIS AND MANAGEMENT OF TUBERCULOSIS IN FREE-RANGING AND CAPTIVE WILDLIFE

2.1 Introduction

A rapidly changing world poses many threats to wildlife conservation. The increasing human population and expansion of agriculture lead to continued encroachment on wildlife habitat and changes in land use (Foley et al. 2005). Alteration of global climate promotes disease emergence and spread to new areas (Daszak et al. 2001; Cunningham et al. 2017). Human activity may also result in increased stress on wildlife (Baker et al. 2013), which can compromise immune function (Cohen and Williamson 1991), making animals more susceptible to disease (Hofer and East 2012). Globalization has resulted in rapid movement of people, animals, and pathogens around the world, with major potential consequences for human and animal health (Daszak et al. 2001). Increasing interactions among humans, livestock, and wildlife have real risks for increased pathogen transmission across species (Daszak et al. 2001).

Disease is a ubiquitous process in organisms of all types, and a normal constituent of ecosystems (Delahay et al. 2009). Changing conditions may create a perfect storm of risk factors that allow for disease to occur at a scale that threatens wildlife populations. Sylvatic plague, caused by the bacteria *Yersinia pestis*, and canine distemper virus wreaked havoc on black-footed ferret (*Mustela nigripes*) populations and brought the species to the brink of extinction (Santymire et al. 2014). Devil facial tumor disease, a newly emerging disease caused by a transmissible tumor, has led to a precipitous decline of Tasmanian devils (*Sarcophilus harrisii*), resulting in a loss of top-down control via alterations in trophic cascades (Hollings et al. 2014). North American bats are being decimated by white nose syndrome (Frick et al. 2010), a devastating disease resulting from infection with the fungus *Pseudogymnoascus destructans* (Minnis and Lindner 2013). Mass mortality events and species declines need not occur only from emerging infectious diseases, which tend to capture public interest and funding priority (Wiethoelter et al. 2015), but under the right confluence of circumstances, enzootic pathogens also have the ability to devastate wild animal populations. A staggering 200,000 critically endangered saiga antelope (*Saiga*

tatarica) – 62% of the global population – died off in 3 weeks as a result of opportunistic *Pasteurella multocida* overgrowth, precipitated by high humidity and higher-than-typical ambient temperature (Kock et al. 2018). Disease can also play a more insidious role in population declines outside of mass mortality events, by altering population dynamics or behavior (Preece et al. 2017), or contributing to host mortality and sterility (Grogan et al. 2018).

Management intervention for diseases of wildlife may be motivated by the intrinsic value of wildlife, threats to beneficial wildlife populations (particularly populations with value for harvest or tourism), or the need to control diseases in wildlife that threaten human or livestock health (Wobeser 1994; de Garine-Wichatitsky et al. 2013). Controlling animal diseases can confer significant economic benefits through increased opportunities for trade; for example, African livestock exports are limited by endemic diseases in these countries, which have served as a barrier to entering profitable international markets (Kock 2005). Study of wildlife diseases is becoming increasingly relevant in both wildlife conservation and various health fields with the resurgence of the concept of One Health (Cunningham et al. 2017). One Health stipulates an inherent connection between animal, human, and environmental health, and underscores the importance of a multidisciplinary approach to studying diseases (Osburn et al. 2009; Cumming and Cumming 2015). The concept of "One Medicine," or a link between human and veterinary medical sciences, was once more widely acknowledged, and is again being embraced by newer generations of professionals across health disciplines (Kahn et al. 2007). One Health has roots in both epidemiology and ecology (Cumming and Cumming 2015). Epidemiology has a foundation of investigating disease determinants associated with so-called epidemiologic triad of host, agent, and environment (Thrusfield 2005), while disease ecology is based on interactions at all biological scales, from individual to ecosystem, involved in disease (Tompkins et al. 2011). Wildlife disease research and management can benefit from a more holistic approach that considers human and domestic animal health in addition to wildlife health, as well as their shared environments and pathogens (Gebreyes et al. 2014).

2.2 Management of Wildlife Diseases

2.2.1 Mathematical Principles

Underlying infectious disease management are basic mathematical principles of disease dynamics and population biology. All infectious diseases have a basic reproductive rate, R_0, which describes the average number of secondary cases produced by a primary case in a susceptible population, assuming homogeneous mixing of hosts (Anderson and May 1991). R_0 dictates the behavior and persistence of a pathogen in the host population where $R_0 > 1$ is required to maintain a disease in a population and $R_0 < 1$ will result in the disease dying out (Anderson 1991). This behavior is based on the assumption of intraspecific transmission within a single host species; in multi-host systems, interspecific transmission can "top up" R_0, creating a more complex dynamic (Nugent 2011). In such a system, pathogens persist in maintenance or reservoir hosts without transmission from other sources (Thrusfield 2005), and maintenance hosts can transmit pathogens to non-maintenance hosts in spillover transmission events (Nugent 2011). Spillover hosts have the potential to transmit pathogens back to the maintenance host species, a process which is called spillback (Nugent 2011).

Host populations can be defined into simple states of susceptible, infected, and recovered or resistant (Figure 2.1), and a variety of mathematical interpretations of disease dynamics can be assembled using so-called SIR models, as originally described by Kermack and McKendrick (1927; 1932; 1933). Deterministic SIR models provide a theoretical background to understand population-level disease dynamics and the influence of different management interventions on each disease class.

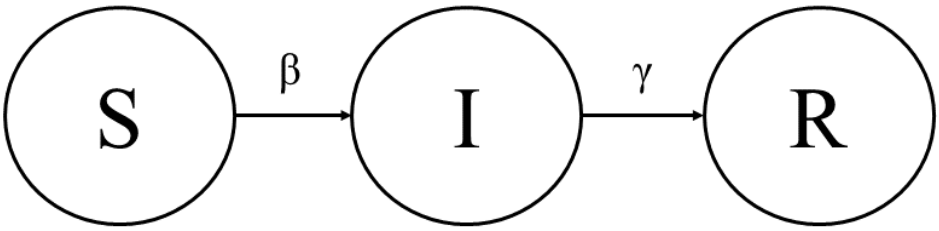

Figure 2.1: Basic population model of disease, including susceptible (*S*), infected (*I*), and recovered/resistant (*R*) classes. The transition from *S* to *I* is governed by the transmission rate, β, and the transition from *I* to *R* is governed by the recovery rate, γ. Different disease control strategies aim to decrease or increase the populations within certain classes.

In the basic SIR model example in Figure 2.1, modified from Anderson and May (1979) assuming density-dependent transmission and excluding mortality, the model compartments are governed as follows:

$$\frac{dS}{dt} = -\beta SI \tag{2.1}$$

where S is the susceptible class, I is the infected class, and β is the transmission coefficient, and

$$\frac{dI}{dt} = \beta SI - \gamma I \tag{2.2}$$

$$\frac{dR}{dt} = \gamma I \tag{2.3}$$

where R is the resistant/recovered class, and γ is the recovery rate.

The SIR framework provides a starting point for understanding and analyzing population dynamics of disease and the potential impact of management interventions, but has limitations that are not reflective of the complexity of natural systems. SIR models are based on the tenuous assumption of homogeneous mixing of individuals, which is unsupported by natural human and animal behaviors and spatial distribution (Anderson and May 1991). Basic SIR models are deterministic, and do not account for individual heterogeneity in factors such as immunity or bacterial shedding intensity. Individual-based models can be used to incorporate stochasticity into disease simulation models (e.g., Smith et al. 2001; Shirley et al. 2003; Ramsey 2007). SIR models can be modified to include disease latency, maternal antibodies (Anderson and May 1991), and demography (e.g., age class, sex, births, and deaths; see Anderson and Trewhella 1985; Smith et al. 2001; Shirley et al. 2003; Graham et al. 2013; Brooks-Pollock and Wood 2015; Abdou et al. 2016). Lastly, basic SIR models are designed for a single host, but can be modified to include multiple hosts (Cox et al. 2005; Brooks-Pollock and Wood 2015). Substantially more complex variations of the simple SIR structure can be constructed to more accurately represent transmission dynamics for a specific system, but doing so expands the number of parameters and model equations required to define the system. Empirical data to quantify model parameters may be sparse or non-existent for the population of interest.

Different disease management strategies manipulate populations of different classes to influence the transitions between different states, most importantly the transmission coefficient; under the assumption of density-dependence, the contact rate of individuals increases with host density, as does the per capita force of infection (Begon et al. 2002) Disease management takes the form of four major categories: prevention, control, eradication, and *laissez-faire*, or doing nothing (Wobeser 2002). This last option has been applied in many situations where disease management is not feasible or justifiable in wildlife (Wobeser 2002; Gortazar et al. 2015). Prevention of disease is often important for wildlife because diseases are difficult to eradicate once established (Wobeser 1994). Eradication of diseases may be desirable, but is not always an attainable goal (Wobeser 1994). Thus disease control, which acknowledges some level of ongoing commitment to disease management, is extremely important to successful management of disease in wildlife (Wobeser 1994).

Disease control may be further subdivided into six categories: management of the disease agent and/or vector, manipulation of the host population, treatment, immunization, environmental modification, and influencing human activities (Wobeser 1994). Directly addressing the disease agent and/or its vector, modifying the environment or human activities, and treating animals all decrease the *I* class within the SIR framework. Managing the host population may reduce the *I* class in the case of selective culling or fertility control, or both *S* and *I* classes if general depopulation is used. Immunization transitions animals from the *S* class to the *R* class.

2.2.2 Unintended Ecological Consequences

Disease control is intended to benefit wildlife populations, but wildlife managers must consider unintended ecological consequences of control programs in their long-term management strategies. Management approaches that promote survival (e.g., vaccination and treatment) might be considered more benign than other approaches (e.g., culling), but complex host and pathogen communities are susceptible to altered dynamics when diseases are controlled.

At the pathogen level, Lloyd-Smith (2013) suggests that pathogen eradication results in a vacated niche, which may be filled by other pathogens as a form of competitive release. This phenomenon has been observed in the recent rise of peste des petits ruminants (PPR) in the wake of rinderpest eradication (Banyard et al. 2014). Vaccination against rinderpest virus, a relative of the PPR virus, ended once the disease was globally eradicated; PPR has become more prevalent as hosts lose the cross-protective benefits of rinderpest vaccination (Banyard et al. 2014; Libeau et al. 2014).

Interventions may have opposite effects at the levels of individual host and host population. African buffalo coinfected with helminths and *Mycobacterium bovis*, the causative agent of bovine tuberculosis (TB), treated with an anthelmintic experienced reduced mortality from TB, which is beneficial to the individual (Ezenwa and Jolles 2015). The population level effect, however, was harmful, because surviving infected buffalo continued shedding *M. bovis* and infecting herd members, resulting in an eightfold increase in R_0 in the host population (Ezenwa and Jolles 2015).

At the community level, when disease acts to regulate the population, increased survival of one species may have negative consequences for other species. Simulation models of vaccinating lions against canine distemper virus showed negative impacts on survival and increased likelihood of local extinction for cheetahs (Chauvenet et al. 2011). Management intended to promote conservation of one threatened species could compromise conservation of other threatened species within the community (Chauvenet et al. 2011).

2.3 Disease at the Wildlife-Livestock-Human Interface

Complex interactions between species are often pertinent in diseases of wildlife. Contact between livestock and wildlife creates an important interface in pathogen transmission, as seen in Texas, where cattle (*Bos taurus*) died of wildebeest-associated malignant catarrhal fever – a form of the disease not typically found in the United States – after being on a mixed-use operation with exotic game, including wildebeest (USDA APHIS 2008). Keeping wildlife in captivity, such as in game ranching or zoological institutions, can also result in increased opportunities for transmission given close contacts between

multiple species, including humans. An outbreak occurred at a zoo after an Asian elephant (*Elephas maximus*) infected with *Mycobacterium tuberculosis* transmitted the bacteria to a chimpanzee (*Pan troglodytes*) and several (human) zoo staff (Stephens et al. 2013). Wildlife are implicated in the epidemiology of most zoonoses (Kruse et al. 2004), making the human-wildlife interface one with major risks for human health.

Human activities can also indirectly influence disease dynamics in wildlife. Settlers in nineteenth century Africa brought cattle with them, evidently infected with rinderpest, which spread throughout the continent in the following decades (Mack 1970). The naïve cattle and wildlife of Africa were subsequently devastated, with an estimated 80–90% of African buffalo (*Syncerus caffer*), eland (*Taurotragus oryx*), giraffe (*Giraffa* spp.), wildebeest (*Connochaetes* spp.), kudu (*Tragelaphus* spp.), and antelope perishing, and approximately 2.5 million cattle lost in South Africa alone (Mack 1970). Disease threats can also result when extenuating circumstances disrupt normal preventive measures. Political unrest in Zimbabwe led to a dramatic reduction in vaccination of domestic dogs (*Canis familiaris*) against rabies; canine rabies incidence subsequently increased, resulting in spillover to wildlife reservoirs and establishment of enzootic rabies in jackals (Lawrence et al. 1980).

The economic and epidemiological consequences of the wildlife-livestock-human interface in disease transmission have contributed to increasing interest in managing disease in free-ranging wildlife. However, diagnosing and managing disease in wildlife can be particularly challenging, as effective diagnostic tools are often lacking, and management options tend to be limited and/or resource-intensive.

2.4 Tuberculosis as a Case Study of Disease Management

2.4.1 Background

Tuberculosis (TB) is a useful case study for exploring the challenges of disease management at the wildlife-livestock-human interface. TB is one of the oldest known diseases of humans and animals, with written references to its existence in humans (Steele and Ranney 1958) and captive wildlife (Iyer 1937) from thousands of years ago, and evidence of its presence in livestock and free-ranging wildlife

dating from prehistory (Rothschild and Laub 2006; Rothschild and Martin 2006). Most infected humans develop latent TB, without clinical signs of disease (Rengarajan et al. 2015), but ~10% of infected individuals develop active clinical disease that includes non-specific signs such as fever and cough (American Thoracic Society 2000). TB in cattle has a chronic progression without distinct stages (Waters et al. 2015). TB is classically characterized by development of caseonecrotic granulomas (Waters et al. 2015), formed by T cells and macrophages to contain mycobacteria (American Thoracic Society 2000). Transmission is most frequently by aerosol, but can also occur by ingestion of contaminated animal products, feed, or water, as well as indirectly by contact with fomites (Waters et al. 2015).

TB remains a global health threat despite decades of control efforts (Kyu et al. 2017). Approximately one quarter of the world's human population is infected with *Mycobacterium tuberculosis*, the causative agent of human TB (World Health Organization 2018). The burden of TB in wildlife has not been formally estimated, but the burden in cattle worldwide is estimated to be > 50 million head infected, costing billions of dollars annually (Waters et al. 2012). TB control costs to governments and producers include testing costs, labor, indemnity payments, depopulation, and repopulation (Wolf 2005). The economic impact of TB control programs is considerable: for example, the European Union spends an estimated €537 million controlling human TB (Diel et al. 2014), while the UK spends £91 million annually on bovine TB control (Department for Environment, Food and Rural Affairs 2011). Controlling TB does have considerable economic returns (Amanfu 2006), as demonstrated by the United States bovine TB eradication program from 1917–1962, which is estimated to have cost approximately $258 million, but resulted in $3.2 billion (in 1918 dollars) of benefits for the livestock sector alone (Olmstead and Rhode 2004). Other benefits of control relate to trade: states with accredited TB free status are not subject to testing, movement, or quarantine restrictions that other states may impose on animal exports (Wolf 2005).

Species in the *M. tuberculosis* complex (MTBC) cause TB in a variety of species. TB in humans is most frequently the result of infection with *M. tuberculosis*, while TB in animals is often the result of infection with *M. bovis* (Gordon and Behr 2015). The MTBC also includes the human-adapted strains *M.*

africanum and *M. canetti* (Gordon and Behr 2015). The animal-adapted strains are named for the host species in which they were first or most frequently isolated, but may be found in other host species (Gordon and Behr 2015); these include *M. caprae*, found in domestic goats (*Capra aegagrus hircus*), *M. microti*, found in field voles (*Microtus agrestis*; Gordon and Behr 2015), *M. pinnipedi*, found in marine mammals (Cousins et al. 2003) and the as yet unnamed "Dassie bacillus" of rock hyrax (*Procavia capensis*; Smith 1960). The past decade has seen three new members added to the animal-adapted MTBC strains: *M. mungi*, found in banded mongoose (*Mungos mungo*; Alexander et al. 2010); *M. orygis*, found in oryxes (*Oryx* spp.; van Ingen et al. 2012), and *M. suricattae*, found in meerkats (*Suricata suricatta*; Parsons et al. 2013). Other mycobacteria (known as non-tuberculous mycobacteria [NTM], mycobacteria other than tuberculous mycobacteria [MOTT], or atypical mycobacteria) can cause clinical disease, but not the classic lesions of TB (American Thoracic Society 1997).

2.4.2 Tuberculosis at the Wildlife-Livestock-Human Interface

It is important to note the zoonotic and reverse zoonotic potential of some MTBC members. *M. bovis* is primarily an animal pathogen, but it does cause zoonotic TB cases in humans – the true burden of which is likely underestimated due to a lack of testing and reporting (Cosivi et al. 1998; Olea-Popelka et al. 2017). Zoonotic TB is most prevalent in developing countries, in those handling or consuming contaminated products such as unpasteurized milk, and in agricultural workers (Cosivi et al. 1998). Those infected with human immunodeficiency virus (HIV) are particularly at risk, as TB is the most commonly found co-infection with HIV (Cosivi et al. 1998). *M. tuberculosis* and *M. bovis* may be an occupational hazards to zoo staff and veterinarians (Dalovisio et al. 1992; Davis 2001) and *M. bovis* a recreational risk to hunters (Wilkins et al. 2003). Other MTBC species can also infect humans, as has been reported for *M. pinnipedii* (Kiers et al. 2008), *M. orygis* (Dawson et al. 2012), and even archeological evidence for an MTBC ancestor from seals over 1,000 years ago (Bos et al. 2014).

The human-wildlife interface has been a source of reverse zoonoses for some time, as *M. tuberculosis* has long been reported in captive wildlife (Fox 1923). While *M. bovis* has a wide host range

and affects a large variety of species (de Lisle et al. 2001), some non-domestic ungulates appear to be more susceptible to *M. tuberculosis* than their domestic counterparts (Lomme et al. 1976). *M. tuberculosis* infection in elephants, particularly Asian elephants (*Elephas maximus*), has become problematic in zoos and other captive facilities (Mikota et al. 2000; Mikota and Maslow 2011), and new outbreaks continue to emerge despite surveillance (Vogelnest et al. 2015; Zlot et al. 2015; Ghielmetti et al. 2017; Simpson et al. 2017). *M. tuberculosis* is increasingly recognized as a pathogen in free-ranging wildlife as well (Alexander et al. 2002; Michel et al. 2013). Case reports from free-ranging Asian and African elephants (*Loxodonta africana*) raise conservation concerns about this human pathogen becoming endemic in threatened wildlife populations (Obanda et al. 2013; Perera et al. 2014; Chandranaik et al. 2017; Zachariah et al. 2017). *M. tuberculosis* appears to be spilling over into wildlife in areas of high human TB prevalence, with potential for spillback to humans (Michel et al. 2013; Zachariah et al. 2017), highlighting the risks of pathogen transmission at species interfaces.

The livestock-wildlife interface can result in transmission of *M. bovis* to wildlife species, and wildlife reservoirs may be established (Miller and Sweeney 2013), with the potential for spillback to livestock (Fitzgerald and Kaneene 2013). Wildlife maintenance hosts can maintain the agent independently, and so have the potential to reinfect livestock, which makes them problematic for eradication efforts in livestock (de Lisle et al. 2001). Current global TB hotspots involve cattle and at least one free-ranging wildlife species, varying by location. North American wildlife TB hosts include white-tailed deer (*Odocoileus virginianus*) in Michigan in the US (Schmitt et al. 1997), and wood bison (*Bison bison athabascae*) in Wood Bison National Park and elk (*Cervus elaphus*) and white-tailed deer in Riding Mountain National Park in Canada (Wobeser 2009). European wildlife TB hosts include European badgers (*Meles meles*) in Ireland and the UK (de Lisle et al. 2001), and wild boar (*Sus scrofa*), red deer (*Cervus elaphus*), and fallow deer (*Dama dama*) in the Iberian peninsula (Gortázar et al. 2015). African wildlife TB hosts include African buffalo in South Africa, and lechwe (*Kobus leche*) in Zambia (Fitzgerald and Kaneene 2013). Brushtail possums (*Trichosurus vulpecula*) are maintenance hosts in New

Zealand (de Lisle et al. 2001). Eradication of TB requires management from all hosts, but eradication from an established, free-ranging wildlife host is extremely difficult (Michel et al. 2006).

Managing wildlife for TB becomes more complex when taking into account conservation of protected reservoir species (de Lisle et al. 2001), as lethal control of diseases in indigenous free-ranging wildlife is often contentious (Bengis et al. 2002). Badgers are a protected species in Ireland and the UK, therefore management for TB by culling them has been controversial due to concerns about conservation and welfare (White and Whiting 2000). In south Asia, *M. orygis* is emerging at the wildlife-livestock-human interface, with infections reported in captive spotted deer (*Axis axis*) and blue bull (Thapa et al. 2015), and a free-ranging greater one-horned rhinoceros (*Rhinoceros unicornis*) in Nepal (Thapa et al. 2016), cattle and rhesus monkeys *(Macaca mulatta*) in Bangladesh (Rahim et al. 2017), and a woman from India who infected a cow (Dawson et al. 2012). The burden of this pathogen in wildlife is unclear (Thapa et al. 2017), but greater one-horned rhinoceros and other protected species such as Asian elephants and Bengal tigers (*Panthera tigris*) may be at risk (Thapa et al. 2016). Culling iconic protected species is unlikely to be a culturally acceptable proposal.

One of the most complex systems of TB in free-ranging wildlife is Kruger National Park (KNP) in South Africa, home to an ongoing epidemic of bovine TB. Cattle around the park are assumed to be the source of *M. bovis* in African buffalo within the park, first detected in 1990 (Bengis et al. 1996). TB subsequently spread to other species in the park, including cheetah (*Acinonyux jubatus*), lion (*Panthera leo*), chacma baboon (*Papio ursinus*; Keet et al. 1996), greater kudu (Keet et al. 2001), common warthog (*Phacochoerus aethiopicus*), leopard (*Panthera pardus*; Renwick et al. 2007), and black rhinoceros (*Diceros bicornis*; Miller et al. 2017). The population-level effects of TB go beyond mortality from TB infection; for instance, buffalo in high prevalence herds have worse body condition and increased fecal endoparasite egg counts (Caron et al. 2003). Bovine TB in KNP has resulted in restricted movement of animals outside the park for national and international translocation efforts, which challenges conservation of endangered species (Michel et al. 2006) and financial viability of the park itself (Geoghegan 2012). *M. bovis* has continued moving through wildlife in the park to buffalo in neighboring

Gonarezhou National Park in Zimbabwe, highlighting the need for disease control (de Garine-Wichatitsky et al. 2010; Caron et al. 2016).

2.5 Diagnosis of Tuberculosis in Wildlife

The first step in disease management is to determine what disease is affecting the population. Once identified, any simulation modeling using an SIR framework for predictions depends in part on realistic estimates of the membership of each disease class (e.g., an estimate of prevalence to determine the size of the *I* class). Correctly identifying individuals as infected or not is crucial to developing an accurate depiction of the disease dynamics. Diagnostic test characteristics such as sensitivity (the ability of a test to correctly classify infected animals) and specificity (the abilty of a test to correctly classify uninfected animals; Dohoo et al. 2009) are informative in understanding how reliable prevalence estimates may be. Some diagnostic modalities have the ability to inform estimates of exposure (e.g., antibody tests that suggest immune response due to exposure), while others can inform estimates of infection (e.g., culture, which shows growth of live organisms).

Disease diagnosis may not be straightforward in wildlife given a lack of established diagnostic tests for non-domestic species. Validation of diagnostic tests according to the World Organisation for Animal Health (OIE) requires a specific test purpose, type of diagnostic specimen, and target species (Maas et al. 2013). Acquiring known positive and negative samples from wildlife species poses serious difficulties for test development and validation (Cousins and Florisson 2005). For some wildlife species, it may be difficult to test sufficient numbers of animals to estimate sensitivity and specificity (Cousins and Florisson 2005). Published sensitivity and specificity estimates may not reflect these values under all circumstances, as testing generally occurs under less than ideal circumstances (Cousins and Florisson 2005). Test development is unlikely to be a profitable enterprise for many wildlife species.

When tests are available, additional practical constraints may limit their use. Animals must first be available for testing, which can make antemortem diagnosis of free-ranging wildlife difficult or impossible in certain scenarios (Wobeser 1994). Large and highly mobile species may require physical

capture and chemical immobilization for handling and collection of sample for testing (Wobeser 1994). Immobilization drugs, capture crews, and capture equipment (e.g., traps, helicopters) all add to the cost of the investigation. In cases of mortality events, finding carcasses may be difficult (Wobeser 1994), particularly before decomposition has advanced to the point of excluding some diagnostic modalities.Diagnosing TB as early as possible, particularly in captive wildlife, is desirable as infected animals become infectious before developing clinical signs (de la Rua-Domenech et al. 2006). A wide variety of tests have been developed to address the needs for diagnosing TB. These modalities approach diagnosis from three pathways: direct detection of TB organisms or the pathological response to them, humoral immune response to infection, or cell-mediated immune response to infection (Cousins and Florisson 2005). Commonly used methods for TB screening and diagnosis in wildlife species are described below, but this list is not exhaustive, and some assays that have been used less frequently are not discussed.

2.5.1 Direct Detection

Direct antemortem confirmation of infection with TB is desirable, particularly in captive animals where treatment might be applied. Even establishing whether disease is present may not be straightforward, as clinical signs of TB are rarely seen in wildlife (Montali et al. 2001). Most clinical signs are non-specific, with weight loss most frequently reported (Lécu and Ball 2011). Cough and dyspnea, if present, are signs of end-stage disease (Lécu and Ball 2011). Several diagnostic methods for direct detection of TB organisms have been developed, with culture being the most important.

2.5.1.1 Culture

Mycobacterial culture is considered the gold standard diagnostic for confirmation of TB (de Lisle et al. 2002), as it demonstrates the presence of live mycobacteria within the host. Culture for mycobacteria should be performed in suspected TB cases because of its improved sensitivity over microscopy, and for species identification, genotyping, and drug susceptibility testing if treatment is to be

pursued (American Thoracic Society 2000). Culture can be used as a postmortem diagnostic to confirm infection in free-ranging or captive wildlife, but may also be used as an antemortem diagnostic in captive wildlife (Montali et al. 2001). Collecting antemortem samples for culture generally involves a veterinary procedure under sedation (Hermes et al. 2018) to obtain sputum, gastric lavage, and bronchial lavage (Thoen et al. 2006), or specialized training to perform unsedated, as with elephant trunk washes (Mikota, Lyashchenko, et al. 2015). Culture has several major limitations: it may take up to eight weeks for results, appropriate antemortem samples may be difficult to collect from wildlife, and antemortem samples may be contaminated and result in bacterial or fungal overgrowth (Mikota, Lyashchenko, et al. 2015). Animals shed mycobacteria intermittently, which necessitates repeated sampling for antemortem culture (Mikota, Lyashchenko, et al. 2015) and contributes to suboptimal test sensitivity.

2.5.1.2 Direct Microscopy

Samples may also be evaluated by direct microscopic examination for more rapid preliminary results. Mycobacteria have a cell wall containing mycolic acid, which can uptake dyes, and such acid-fast bacilli (AFB) can be identified using stains such as Ziehl-Neelsen or Kinyoun (Thoen et al. 2009). However, other acid-fast organisms, including NTM and *Nocardia* spp., can be confused for tuberculous mycobacteria, and further workup is required (Lécu and Ball 2011; Mikota, Lyashchenko, et al. 2015). A lack of AFB does not rule out the possibility of TB, as lesions may have few organisms present (de Lisle et al. 2002) and approximately 5000 bacilli/ml are required for positive identification (Lécu and Ball 2011).

2.5.1.3 Gross Pathology and Histopathology

Some species develop characteristic TB lesions that can be used to make a presumptive postmortem diagnosis, but lesions in wildlife vary considerably by species in appearance and distribution (de Lisle et al. 2002). Traditional granulomatous lesions are yellow, have a caseous core, and may be calcified in late infections (Adugna et al. 2014). Generalized lesions in carnivores are distinctly different

than those in herbivores and omnivores, appearing proliferative or cavitational, with no abscessation, caseation, or calcification (de Lisle et al. 2002). Lesions are typically associated with the route of transmission: animals infected via respiratory route most commonly have lesions in the lungs (e.g., brushtail possum, European badgers) and thoracic lymph nodes (e.g., lechwe, African buffalo), while animals infected orally most commonly have lesions in the head lymph nodes (e.g., greater kudu, warthog) and the mesenteric and peripheral lymph nodes (e.g., lion, leopard; de Lisle et al. 2002).

Histopathology is useful for examining suspected TB lesions, particularly if tissues are not suitable for culture (de Lisle et al. 2002). Classic lesions in cattle have central necrosis with mineralization, a surrounding granulomatous response with macrophages, and an outer capsule of fibrous connective tissue, but lesions in wildlife vary (de Lisle et al. 2002). Histopathology can be used to exclude a TB diagnosis for another differential diagnosis (de Lisle et al. 2002), but alone it is not specific enough to definitively diagnose TB (Cousins and Florisson 2005).

2.5.1.4 Radiography and Computed Tomography

Radiography or computed tomography studies can be a useful non-invasive tool for determining whether pulmonary lesions characteristic of TB are present in some species, including carnivores, marine mammals, and non-human primates (Morris et al. 1996; de Lisle et al. 2001; Jurczynski et al. 2011). Calcified lesions may not appear on radiographs if relatively small (Capuano et al. 2003). Detecting extrapulmonary TB lesions is more challenging, and radiological signs such as splenomegaly or mesenteric lymphadenopathy are non-specific (Bushmitz et al. 2009). Radiological methods require the appropriate equipment, anesthesia for proper immobilization, and are not suitable for species with a large thoracic cage, such as ungulates > 250 kg (Lécu and Ball 2011).

2.5.1.5 Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) testing of biological samples can detect small amounts of mycobacteria without the long wait required with culture (de Lisle et al. 2002). PCR has been widely used

in wildlife species to differentiate MTBC mycobacteria from NTM (Maas et al. 2013). PCR can be used on fresh samples, cultured bacteria, or formalin-fixed tissues if fresh tissue is not available for culture (de Lisle et al. 2002). Its use on suspect samples rather than cultured samples requires a higher bacterial load (Maas et al. 2013), as inhibitors may be present in biological samples and impair PCR detection (de Lisle et al. 2002; Lécu and Ball 2011). One drawback to PCR is that it amplifies any genetic material present, which includes live bacteria, dead bacteria, and extracellular DNA (Rogers et al. 2010), and does not confirm the presence of live organisms as in culture. PCR for TB diagnosis has been used in studies of free-ranging African ungulates (Cleaveland et al. 2005), and captive non-human primates (Alfonso et al. 2004), carnivores (Miller 2008), camelids and ungulates (Pate et al. 2006), pinnipeds (Kriz et al. 2018), and small mammals, rodents, and lagomorphs (Miller and Lyashchenko 2015).

2.5.1.6 Genotyping

Genotyping of mycobacteria is a continually evolving field, with numerous methods available to differentiate strains. IS*6110* restriction fragment length polymorphism (RFLP) is the oldest commonly used genotyping method, and is based on fingerprinting repetitive DNA elements known as IS*6110* in MTBC species (van Embden et al. 1993). RFLP is a labor- and time-intensive process that takes weeks for culture and DNA purification (Supply et al. 2006). Spacer oligotyping, or spoligotyping, is a technique that exploits DNA polymorphism in the direct repeat locus of MTBC bacteria (Kamerbeek et al. 1997). Spoligotyping is easy to perform, and can be used on clinical specimens or fixed tissues for more rapid results than culture (Kamerbeek et al. 1997). Mycobacterial interspersed repetitive unit–variable number of tandem repeats (MIRU-VNTR) (Frothingham and Meeker-O'Connell 1998; Supply et al. 2006) also makes use of polymorphisms, and can be applied up to 24 loci (Roetzer et al. 2013). MIRU-VNTR is faster than IS*6110* RFLP (Supply et al. 2006) and can be performed on killed bacteria, which minimizes biohazards to laboratory personnel (Frothingham and Meeker-O'Connell 1998). Whole genome sequencing (WGS) provides detail on all genetic information rather than specific polymorphic

targets (Roetzer et al. 2013), and is superior to other genotyping methods for differentiating between relapse and re-infection in TB cases (Bryant et al. 2013).

Genotyping is particularly valuable as an epidemiological tool for investigating outbreaks and understanding transmission patterns (de Lisle et al. 2002), as well as identifying laboratory cross-contamination (Frothingham and Meeker-O'Connell 1998). Genotyping methods are often used in combination: for example, an investigation in zoo elephants used MIRU-VNTR, spoligotyping, and WGS to determine the source of infection and route of transmission among animals (Ghielmetti et al. 2017). WGS is increasingly used in multi-host epidemiological investigations of outbreaks in free-ranging wildlife and cattle to determine strains involved, directionality of transmission, and persistence (Biek et al. 2012; Trewby et al. 2015; Glaser et al. 2016; Crispell et al. 2017).

2.5.2 Humoral Immune Response Detection

The humoral response allows for assessment of TB status from a blood sample, but the immune response of many species to mycobacterial infection is not well described (Chambers 2013). The serological response is generally only elevated in the latter part of the disease (Cousins and Florisson 2005; Maas et al. 2013; Bezos et al. 2014), although elephants have an unusually robust antibody response that can be detected years before positive cultures (Greenwald et al. 2009). Humoral antibody tests are generally considered poor indicators of TB infection (Cousins and Florisson 2005), as the humoral response may be absent in early infection (Bezos et al. 2014). The main drawback to serological assays is that they are an indirect measure of disease status, with the potential for cross-reaction, and may represent exposure or past infection but cannot be interpreted to indicate a current infection (Gilbert et al. 2013).

2.5.2.1 Immunochromatographic Assays

Immunochromatographic assays are simple tests that result in a color change when antigen-antibody binding occurs (Sakamuri et al. 2015). Disposable point-of-care commercial tests have been

developed for wildlife; these tests are relatively inexpensive, simple to use, can be performed patient-side, and deliver results within 15–20 minutes, and are valuable for use in diagnosis of captive and free-ranging wildlife (Lyashchenko et al. 2008). The rapid test or VetTB STAT-PAK test was adapted for a variety of species (Lyashchenko et al. 2008), including elephants (ElephantTB STAT-PAK; Lyashchenko et al. 2006; Greenwald et al. 2009; Lyashchenko et al. 2012), primates (Lyashchenko et al. 2007 PrimaTB STAT-PAK), badgers (BrockTB STAT-PAK; Chambers et al. 2008), and cervids (CervidTB STAT-PAK; Gowtage-Sequeira et al. 2009). STAT-PAK testing of elephants was a mainstay of annual TB testing of elephants when previously mandatory testing was required by the US Department of Agriculture (United States Animal Health Association 2010).

The STAT-PAK was known to result in false positive reactions, likely due to NTM infections (Lacasse et al. 2007; Greenwald et al. 2009), and has been discontinued. It was replaced by the more specific Dual-Path Platform (DPP) VetTB assay, which uses the same antigens (ESAT-6, CFP10, and MPB83) as the STAT-PAK but two nitrocellulose strips to independently deliver the test sample and reagent (Greenwald et al. 2009; Lyashchenko et al. 2012). DPP results can be quantified using an optical reader device (Greenwald et al. 2009). DPP has been used in elephants (Greenwald et al. 2009; Lyashchenko et al. 2012; McGee et al. 2014; Yakubu et al. 2016), lions (Miller et al. 2012), fallow deer (Boadella et al. 2012), wild boar (Che'Amat et al. 2015), warthogs (Miller et al. 2015), and banded mongoose (Brüns et al. 2016).

2.5.2.2 Enzyme-linked Immunosorbent Assay (ELISA)

The enzyme-linked immunosorbent assay (ELISA) or enzyme immunoassay (EIA) is a laboratory test that detects antibodies to a specific MTBC antigen(s) (Sakamuri et al. 2015). ELISA assays have been developed or modified in a number of wildlife species, including in elephants to screen for *M. tuberculosis* (Larsen et al. 2000), and to detect *M. bovis* in farmed red deer (Griffin et al. 1994), badgers (Kampfer et al. 2003; Aznar et al. 2014), lions (Cleaveland et al. 2005), wild boar (Aurtenetxe et al. 2008; Boadella et al. 2011), fallow deer (Boadella et al. 2012), and warthogs (Roos et al. 2016). However,

results must be interpreted with caution when ELISAs have not been validated for the individual species tested (Maas et al. 2013). ELISA testing has been used in Spain for immunosurveillance of wild boar to MTBC bacteria (Chambers 2013), but has been found to be poorly sensitive in other wildlife species, limiting its utility (de Lisle et al. 2002). It is also unsuitable in resource-poor settings where laboratory facilities and/or testing funds are insufficient (Sakamuri et al. 2015).

2.5.2.3 Multiantigen Print Immunoassay (MAPIA)

The multiantigen print immunoassay (MAPIA) is a laboratory test to immobilize a cocktail of serum antigens onto nitrocellulose membranes (Lyashchenko et al. 2000). MAPIA is a more cost-effective and reproducible method with greater responses than observed in ELISA assays (Lyashchenko et al. 2000). MAPIA binds serum IgG antibodies using 12 mycobacterial antigens, which may include recombinant ESAT-6, CFP10, Mtb8, Mtb48MPB64, MPB59, MPB70, MPB83, α-crystallin [Acr1], and 38 kDa protein; protein fusions TBF10, CFP10/ESAT-6, and Acr1/MPB83; and native antigens bovine protein purified derivative [PPD-B], and *M. bovis* culture filtrate [MBCF] (Lyashchenko et al. 2008). MAPIA has been useful in characterizing species-specific responses to mycobacterial antigens; for instance, red deer infected with *M. avium* subsp. *paratuberculosis* registered as false positives on the CervidTB STAT-PAK and cross-reacted with MPB83 on MAPIA (Buddle et al. 2010), while ESAT-6 is strongly recognized in sera from culture-positive elephants (Lyashchenko et al. 2006). One advantage of this test is its ability to quantify the animal's antigen recognition patterns (Lyashchenko et al. 2006; Lyashchenko et al. 2008), which may be valuable for monitoring antibody response during treatment (Lyashchenko et al. 2006; Duncan et al. 2009). It can also be used as a confirmatory test, given its use of antigens specific to the MTBC (Lyashchenko et al. 2006).

2.5.3 Cellular Immune Response Detection

Tests of cellular immunity can be used in some species (e.g., cattle) to diagnose animals earlier than antibody assays (de la Rua-Domenech et al. 2006). During mycobacterial infections, a T-helper type

1 (Th1) response occurs and results in production of cytokines, in particular interferon-γ (de la Rua-Domenech et al. 2006; Adugna et al. 2014). Tests of cellular immunity are based on the reactions of these Th1 cells, either in their release of interferon-γ or a delayed hypersensitivity response from Th1 memory cells when stimulated by tuberculin, a crude antigen mixture of mycobacterial extracts (Tizard 2009).

2.5.3.1 Tuberculin Skin Test (TST)

The tuberculin skin test (TST; also known as the Mantoux test or intradermal test) is commonly used as a routine screening diagnostic test in humans and cattle around the world (Cousins and Florisson 2005). Intradermal testing is simple and inexpensive to perform, and detects a delayed-type hypersensitivity reaction in infected animals (Monaghan et al. 1994). To perform the test, bovine PPD is inoculated intradermally in either the caudal fold at the base of the tail (for a caudal fold test [CFT]) or the mid-cervical region (for a single intradermal cervical test [SIT]; Buddle et al. 2015). The injection site is assessed for an increase in skin thickness 72 hours post-inoculation, and any increase in skin thickness is considered a positive response (Buddle et al. 2015). The CFT is used as a routine screening test in cattle in North America and throughout the southern hemisphere, while the SIT is used in cattle in continental Europe (Buddle et al. 2015). The single intradermal comparative cervical test (SICCT), an alternative approach to increase specificity, uses PPD-B and avian PPD from *M. avium*, inoculated in the mid-cervical region approximately 12.5 cm apart (Buddle et al. 2015). Skin thickness, as measured with calipers, is assessed pre- and 72 hours post-inoculation, and increases are compared between the avian and bovine PPD sites (Buddle et al. 2015). One advantage of the SICCT is higher specificity as compared to the CFT or SIT based on the ability to better determine whether a response is due to infection with *M. bovis* or NTM such as the *M. avium* complex (de Lisle et al. 2002), as a larger reaction would be expected at the corresponding PPD site (Palmer et al. 2015).

TST is used as a screening test in certain taxa in captivity, including cervids and other ungulates, as well as primates, with some species-specific modifications (Miller and Lyashchenko 2015). Captive deer in the US are tested using SIT, and suspects or reactors re-tested using SICCT (Palmer et al. 2015).

Primates are screened using TST, although in these species the test is generally performed with mammalian old tuberculin (MOT; Chambers 2013) on the upper eyelid (Miller and Lyashchenko 2015). The test does present complications when used in free-ranging wildlife, as animals must be immobilized twice over a period of three days to both administer the tuberculin and then evaluate the test site. TST has been applied in free-ranging African buffalo in southern Africa (le Roex et al. 2015), but capture and testing requires significant effort and cost.

Drawbacks to TST are subjectivity in measuring the response (Sakamuri et al. 2015), uncertainty in optimal dosing of tuberculin depending on species (de Lisle et al. 2002), and variable testing frequency, type and site of tuberculin administration, and interpretation guidelines among species (Miller and Lyashchenko 2015). NTM can cause cross-reaction, and TST has been found to be of poor diagnostic value in camelids (Miller and Lyashchenko 2015) and elephants (Mikota, Lyashchenko, et al. 2015). In some animals, anergy or stress may result in a false negative test (Schiller et al. 2010).

2.5.3.2 Interferon-γ (IFN-γ) Assay

The interferon-γ assay (also known as IFN-γ or interferon gamma release assay [IGRA]) is another useful diagnostic technique that has been used as an ancillary test to the TST in cattle (Bezos et al. 2014). Blood samples are incubated in the presence of test antigens – usually PPD-A and PPD-B, and a negative control – to stimulate release of IFN-γ, which is then quantified using an ELISA (de la Rua-Domenech et al. 2006). IFN-γ, similar to the SICCT, can differentiate between tuberculous and NTM infections based on the response to either bovine or avian antigens (de la Rua-Domenech et al. 2006). IFN-γ testing is not as cost-effective as serology (Schiller et al. 2010), and requires a laboratory setup, which is less feasible in low-resource settings (Sakamuri et al. 2015). The assay is also ineffective in anergic animals (de la Rua-Domenech et al. 2006).

The commercial IFN-γ assay developed for cattle, BOVIGAM (Wood and Jones 2001), has also been used in free-ranging buffalo (Michel et al. 2011) and wood bison (Koller-Jones et al. 2006). Another commercial assay, PRIMAGAM, was developed for use in non-human primates (Lerche et al. 2008). The

QuantiFERON-TB Gold (In-Tube Method) stimulation platform (to replace PPDs in the BOVIGAM) has been used in buffalo (Parsons et al. 2011), chacma baboons (Parsons et al. 2009), and black rhinoceros (Miller et al. 2017), and along with the IFN-γ ELISA in lions (Sylvester et al. 2017). Assays specific to elephants have also been developed (Angkawanish et al. 2013; Paudel et al. 2016).

2.6 Control of Tuberculosis in Wildlife

Understanding management strategies for TB within the SIR framework requires revision of the model to a modified SEIR model (Figure 2.2), again modified from Anderson and May (1979), which incorporates an additional class representing exposed animals. The *E* class then represents animals with latent TB infection, where no shedding occurs (Ernst 2012). In this model, the *R* class represents vaccinated animals rather than those that have acquired immunity from natural infection, and for this reason, the transition occurs from animals in the *S* class.

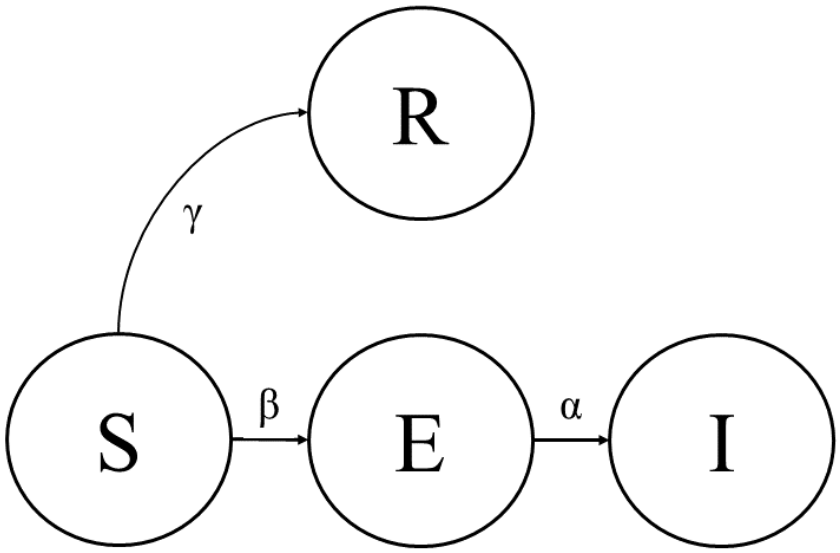

Figure 2.2: A modified SEIR model describing a basic system of tuberculosis for management purposes, representing susceptible (*S*), exposed (*E*), infected (*I*), and recovered/resistant/vaccinated (*R*) classes. The transition from *S* to *E* is governed by the transmission rate, β; the transition from *E* to *I* is governed by the rate of disease progression, α; and the transition from S to R is governed by the vaccination rate, γ.

The SEIR model in Figure 2.2 is governed by the following equations:

$$\frac{dS}{dt} = -\beta SI - \gamma S \tag{2.4}$$

where S is the susceptible class, I is the infected class, β is the transmission coefficient, and γ is the vaccination rate, and

$$\frac{dE}{dt} = \beta SI - \alpha E \tag{2.5}$$

$$\frac{dI}{dt} = \alpha E \tag{2.6}$$

$$\frac{dR}{dt} = \gamma S \tag{2.7}$$

where E is the exposed class, R is the vaccinated class, and α is the rate of progression to active TB,

Management of TB in wildlife is typically undertaken to mitigate transmission to livestock or to protect valuable wildlife species (de Lisle et al. 2002). A *laissez-faire* approach has been demonstrably harmful in some cases: for instance, the devastating foot-and-mouth disease outbreak in the UK necessitated that veterinarians suspend routine cattle TB testing, and as a result, TB prevalence increased in both cattle and badgers (Woodroffe et al. 2006). Effective management of TB includes components of all active forms of disease management: prevention, eradication, and control (Figure 2.3). The strategies used or proposed for TB control in wildlife include culling, fertility control, immunization, treatment, and alteration of human activities (Figure 2.3). The strategies that are feasible and used frequently differ by species and location, and between free-ranging and captive wildlife populations.

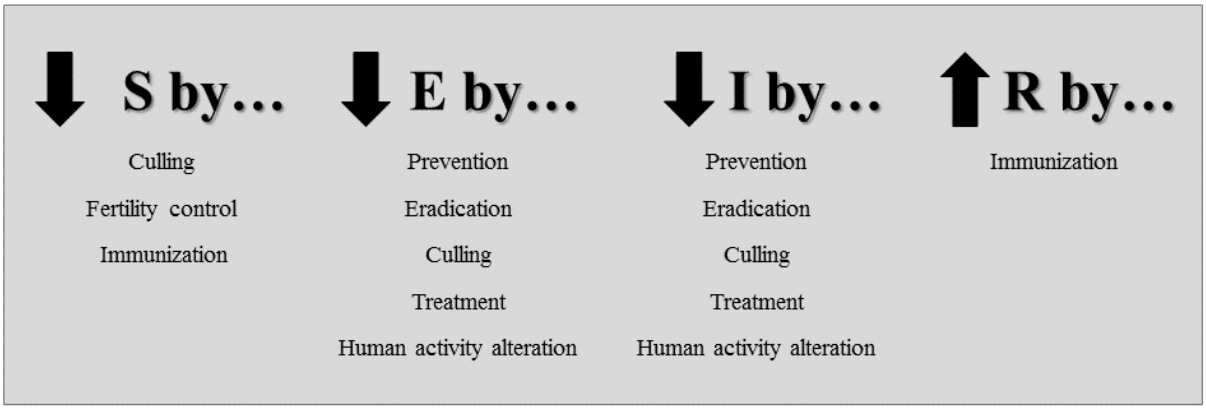

Figure 2.3: Tuberculosis management strategies addressing susceptible (*S*), exposed (*E*), infected (*I*), and recovered/resistant (*R*) classes.

2.6.1 Prevention

Areas that are currently free of TB in free-ranging wildlife have disease prevention as their ultimate goal (de Garine-Wichatitsky et al. 2013; Michel et al. 2015). Prevention is intended to disallow transmission between susceptible and infected individuals in populations, often by limiting contact. If there is no opportunity for contact between susceptible and infected animals, the product βSI in equations 2.4 and 2.5 is 0, and no transition occurs. TB prevention in captive wildlife is dependent on adequate post-arrival quarantine procedures and routine surveillance (Montali et al. 2001), and in free-ranging wildlife, is partially dependent on management and movement of livestock, given the risk of transmission from infected herds. A short- to medium-term strategy for containing diseases and preventing transmission between infected and uninfected populations is the use of barriers (Bengis et al. 2002). Fencing to separate livestock and free-ranging wildlife is an important management strategy that has been applied widely across Africa, but has considerable ecological impacts by limiting movements of free-ranging wildlife (Ferguson and Hanks 2012). Fencing is expensive to purchase and place, and requires ongoing maintenance (Ferguson and Hanks 2012), but is unlikely to be completely eliminated in areas where negative impacts have been observed.

Translocation of wildlife is also a risk factor for movement of pathogens. Movement of zoo animals and free-ranging wildlife in other countries sometimes requires certification of the animals' freedom from TB, but there is no consistent protocol in place for how certification of TB-free status for individual animals is made (Lécu and Ball 2011; Maas et al. 2013). Pre-transport TB testing of captive wildlife is recommended to minimize potential exposure at the facility (Bushmitz et al. 2009), but all potentially susceptible animals should be quarantined until a TB test has been performed (de Lisle et al. 2001). The risks of translocation were demonstrated in an outbreak of TB at a private South African game reserve after introduction of free-ranging wildlife that were not TB tested (Hlokwe et al. 2016). African buffalo are the only wildlife species in South Africa that require TB testing prior to translocation, but these practices would be beneficial in all susceptible host species to limit movement of infected animals into TB-free areas (Hlokwe et al. 2016).

2.6.2 Eradication

Countries may seek to eradicate bovine TB to facilitate trade of livestock with high standards of animal health and food safety (Reviriego Gordejo and Vermeersch 2006). Complete eradication is an ideal but typically elusive long-term management goal once TB is present (Bengis et al. 2002). Eradication in the strictest sense results in extinction of the *E* and *I* classes, with the entire population free of disease or infection. Instead, the goal may be to reduce the *I* class to a negligible number rather than zero, as with the OIE's definitions regarding countries' TB status: to qualify as a country officially free from TB (OTF) in bovids, TB must be a notifiable disease; a surveillance program testing all herds must be in place, with at least 99.8% of herds (representing at least 99.9% of bovids in the country) uninfected; ante- and postmortem surveillance and regulatory measures for early detection must be in place; and imported bovids and semen must meet strict criteria to minimize the likelihood of disease transmission (World Organisation for Animal Health 2017). Similar regulations are defined for OTF certification for captive cervids (World Organisation for Animal Health 2017). Eradication in captive wildlife facilities is similarly difficult, and requires testing throughout the collection to identify infected animals (de Lisle et al. 2001).

Eradication at the national scale has had limited success and is highly dependent on country-specific circumstances. Australia was successful in eradicating TB from cattle, in part due to the fact that there was no native wildlife reservoir (More et al. 2015). Feral water buffalo (*Bubalus bubalis*) were culled extensively during the eradication process (Cousins and Roberts 2001; Radunz 2006); more extreme control measures like this are generally only accepted in feral or non-native species (Bengis et al. 2002). The United States has dramatically reduced its cattle TB over the last century from 5% to <0.001%, and is considered a major success in TB control (Schiller et al. 2010). A persistent reservoir of white-tailed deer and TB in imported cattle from Mexico have thwarted complete eradication in the US, however (Schiller et al. 2010). Ireland, the United Kingdom, and New Zealand are working towards eradication but are met with similar challenges with wildlife reservoirs (Schiller et al. 2010). Disease eradication is most feasible on islands (Kaneko et al. 2000), where host populations are limited and

movements of incoming livestock and wildlife can be more strictly controlled than in countries sharing borders with other countries where TB prevalence and control efforts may vary considerably (Acevedo et al. 2013).

2.6.3 Control

2.6.3.1 Culling

Culling host populations is the most practical method for controlling TB in free-ranging wildlife, and culling animals with clinical disease or reactive test results is the most effective approach in captive wildlife (de Lisle et al. 2001). Culling has varying levels of social acceptability based on the setting, and may be problematic in species of conservation concern. For instance, there has been considerable public opposition to culling of badgers in the UK, where they are a native protected species (White and Whiting 2000) with intrinsic value (O'Brien et al. 2011). Interference in badger culling operations is lower in Ireland, while compliance and participation from landowners is higher (Sheridan 2011). Proactive culling to reduce populations of certain species, such as African buffalo, may be beneficial but socially unpalatable (Renwick et al. 2007). In contrast, culling is less controversial in non-native brushtail possums in New Zealand, where dairy and beef exports are of great importance to the economy (O'Brien et al. 2011). Opposition to the use of the chemical toxicant sodium monofluoroacetate (1080) for lethal control of possums has centered on ecological effects of environmental contamination (Eason et al. 1992) rather than conservation concerns. Public acceptance of culling may also be improved when it has had noticeable positive outcomes for disease control. Simulation modeling of outcomes prior to implementing these management strategies may be valuable, as models of targeted removal of white-tailed deer in Michigan in the US did not result in reduced TB prevalence (Cosgrove et al. 2012).

The theory behind culling is based on principles of density-dependence and R_0; for a disease to be maintained endemically in a population ($R_0 > 1$), the population of susceptible animals, K, must exceed a threshold density, K_T,

$$K_T = \frac{(\sigma + a)(\alpha + a)}{\beta\sigma} \tag{2.8}$$

where β is the transmission coefficient, $\frac{1}{\sigma}$ is the average latent period, α is the death rate of infected animals, and a is the average per capita birth rate (Anderson et al. 1981). If a constant quota of animals, Λ, is to be removed to maintain a set population density, and $K_T > \frac{1}{2}K$, then it is possible to control density below K_T if $\Lambda > \Lambda_T$,

$$\Lambda_T = rK_T\frac{(1 - K_T)}{K} \tag{2.9}$$

where r is the per capita population growth rate (Anderson et al. 1981). The population cannot be maintained stably by quota culling if $K_T < \frac{1}{2}K$, however (Anderson et al. 1981). The other approach for culling involves a constant effort, and in this case, the population can be maintained below K_T if

$$\frac{\Delta b}{r} = 1 - \frac{1}{R_0} \tag{2.10}$$

where Δb is additional mortality due to culling (Anderson et al. 1981). The goal of such widescale removals is to reduce β such that remaining susceptible animals are partly protected from infection (Swinton et al. 1997).

Culling may include both susceptible and infected individuals, resulting in decreased population densities, or can be selective to specifically target infected animals. The scale of culling varies by species and setting. For invasive brushtail possums, removing both susceptible and infected individuals is acceptable, so widescale removal efforts using 1080 have been implemented (Eason et al. 1992). Culling has shifted to a "systematic overkill" policy to reduce possum numbers far below the calculated threshold for disease persistence, to more rapidly eliminate *M. bovis* from the possum population (O'Brien et al. 2011). More targeted culling of infected animals occurs in other landscapes such as Hluhluwe-iMfolozi Park, South Africa, where African buffalo are mass captured and tested using TST, and reactors are culled under a trial test and cull program (le Roex et al. 2015). A selective culling program has also been

proposed for wood bison in Canada (Shury et al. 2015). Such intensive targeted programs have significant costs and are therefore only feasible in certain settings with adequate investment (Geoghegan 2012).

The outcomes of culling have been variable depending on the setting. The success of culling in brushtail possums is indicated by a > 92% reduction in the number of infected herds during intensive culling operations from 1994–2009 (O'Brien et al. 2011). However, conflicting findings from the UK and Ireland have been a source of controversy in management of badgers for TB. In the UK, the Randomized Badger Culling Trial (RBCT) employed both reactive culling (localized culling to reduce TB in cattle but minimizing badgers culled) and proactive culling (widespread, repeated culling) of badgers (Donnelly et al. 2003). The results showed increased TB risk in cattle in reactively culled areas, and lower TB risk in cattle in proactively culled areas, with an increased TB risk in cattle in the areas surrounding proactive culling (Donnelly et al. 2005). These results have been proposed to be due to a social perturbation effect, in which culling disrupts badger territories and social behavior, increasing ranging behavior and interspecific contact with cattle (Woodroffe et al. 2006). The Easy Offaly Badger Research Project in Ireland found lower TB incidence in cattle herds where badgers were removed (Ó Máirtín et al. 1998; Eves 1999), and the Four Areas Project found that TB incidence in cattle was lower in areas of proactive than reactive badger culling (Griffin et al. 2005). The conflicting results between the UK and Ireland led to divergent opinions on management practices; scientists in the UK concluded that badger culling was not a viable strategy for bovine TB control (Bourne et al. 2007), while Ireland implemented a national badger culling program (Good and Duignan 2017).

The differences in the results from the RBCT and the Irish studies have been hypothesized to occur due to various differences in each countries' cattle or badger populations, or study implementation (O'Connor et al. 2012). Larger cattle herd sizes in the UK may have allowed for more infection persistence, and the R_0 value in cattle may differ between the UK and Ireland (O'Connor et al. 2012). Higher badger density in the UK may have resulted in increased perturbation from culling, prevalence in badgers may have been increasing in the UK, or previous culling in the UK may have contributed to badger social instability (O'Connor et al. 2012). Study implementation also differed between countries: a

larger proportion of land in the UK went un-culled and may have created a source-sink dynamic; the period of culling was less consistent in the UK; badgers in the UK were trapped using a less effective method than in Ireland, meaning residual populations may have existed; and badger movements back into study areas in the UK were less restricted by natural barriers (O'Connor et al. 2012).

2.6.3.2 Fertility Control

Fertility control has been explored as an alternative method of controlling free-ranging host populations, by reducing population density by limiting the birth rate rather than through increased mortality rates as in culling. Fertility control is less effective than culling at reducing population density in the short term, and only removes susceptible animals from joining the population (Swinton et al. 1997; Tuyttens and Macdonald 1998), and is therefore unsuitable for controlling outbreaks in naïve or captive populations. It has been proposed as a control strategy in brushtail possums (Ramsey et al. 2006; Ramsey 2007) and badgers (Swinton et al. 1997; White et al. 1997; Tuyttens and Macdonald 1998). Simulation modeling studies in badgers have had varying results: one suggested that fertility control would not be effective alone, but could be effective if combined with culling (White et al. 1997), while another found that fertility control could be as effective as culling if used in combination with vaccination or as a standalone measure if deployed as a permanent contraceptive (Smith and Cheeseman 2002). Suppressing reproductive behavior can reduce contacts between individuals and will reduce population density over time, albeit more slowly than with culling (Killian et al. 2007). However, fertility control may have complex effects on population dynamics, social behavior, and β (Caley and Ramsey 2001).

There are several approaches to controlling fertility in wild populations, but all have drawbacks: surgical sterilization would not be practical on a wide scale (Tuyttens and Macdonald 1998); developing effective and safe fertility control agents is likely to be a high cost endeavor (White et al. 1997); and abortifacients have logistical and welfare concerns (Tuyttens and Macdonald 1998). Field trials have been conducted in brushtail possums, where fertility control has been shown unlikely to negatively impact their social behavior (Ramsey 2007). The effectiveness for disease control is less clear, with evidence for

increase of pathogen transmission in one study (Caley and Ramsey 2001), but reduced contact and pathogen transmission rates in sterilized populations in another (Ramsey et al. 2006; Ramsey 2007). A management program involving multiple approaches may be more efficient than a single control strategy; one study found that an initial brushtail possum cull combined with BCG vaccination and a fertility control vaccine with at least 50% efficacy applied every 3 years was the most cost-effective option for local eradication modeled (Ramsey and Efford 2010).

2.6.3.3 Immunization

Immunization is becoming an increasingly relevant approach to TB control in certain countries, particularly Ireland, the UK, Spain, and New Zealand (Waters et al. 2012). The goal of immunization is to reduce individuals in the S class by transitioning them to the R class, or to lower β. The success of any vaccination program hinges on the ability to induce herd immunity, the principle that once a significant proportion of the population is vaccinated, there are not enough susceptible individuals to maintain an $R_0 \geq 1$, and unvaccinated individuals are protected from infection (Anderson and May 1985). The level of vaccination required to eradicate a pathogen is defined by $p > 1 - \left(\frac{1}{R_0}\right)$, where p is the proportion of hosts that must be vaccinated (Anderson and May 1982); however, this value is also dependent on the efficacy of the vaccine. Population density and prevalence of infection influence the success of wildlife vaccination programs (Abdou et al. 2016).

The only available vaccine against *M. tuberculosis* and *M. bovis* is the Bacille Calmette-Guerin (BCG) vaccine, which is derived from an attenuated *M. bovis* strain (Andersen and Doherty 2005). BCG has been used in humans since 1921, with billions of doses delivered, but only has a protective effect of 50% against TB infection (Colditz 1994; Brewer 2000). Individuals without active TB who are vaccinated are significantly more likely to react on TST, therefore infected and vaccinated individuals cannot be differentiated using TST (Wang 2002). The European Union has prohibited vaccination of cattle against TB (EU Council Directive 78/52/EEC) for this reason, and captive wildlife are also typically not

vaccinated to allow for routine TST testing (OIE 2009). Development of a differentiating infected from vaccinated animals (DIVA) test and DIVA vaccine for use in livestock and wildlife is underway (Waters et al. 2012).

BCG vaccination of free-ranging wildlife is allowable. Public attitudes towards vaccination of wildlife vary, but it is generally seen as acceptable to favorable (Warren et al. 2013; Enticott 2015) and less controversial than culling. Vaccine efficacy varies widely by species. A study of African buffalo found that intramuscular BCG vaccination conferred no protection from infection or disease (de Klerk et al. 2010). Oral BCG vaccination did not result in protection from infection, but did result in reduced disease severity in brushtail possums (Aldwell et al. 2003), white-tailed deer (Nol et al. 2008), and wild boar (Garrido et al. 2011). In badgers, oral BCG vaccination reduced seroconversion, disease, and the number of lesions (Gormley et al. 2017), while intramuscular BCG vaccination resulted in a decreased risk of infection (Carter et al. 2012) and reduced disease severity and bacterial shedding when administered at 10 times the human dose (Lesellier et al. 2011). The intent of vaccination against TB using BCG is not to eliminate new infections, but to lessen morbidity and mortality and most importantly, limit transmission (Wobeser 2002).

The nature of BCG alters the structure of the SEIR model and adds complexity by lending the *R* class several special characteristics. First, unlike in the traditional SIR model, animals do not recover from disease (i.e., the *I* class) and gain immunity, but rather move directly from the *S* class to the *R* class via vaccination. Second, in the case of TB, the *R* class is not a true resistant class, as BCG does not deliver sterilizing immunity that completely protects from infection, but reduces the severity of disease (Aldwell et al. 2003; Nol et al. 2008; Garrido et al. 2011; Lesellier et al. 2011). Vaccinated animals effectively function as a second class of susceptibles, and infected vaccinated animals have decreased shedding, resulting in a lower transmission coefficient associated with this class. Third, assuming that BCG does not provide lifelong immunity, vaccinated individuals will return to the *S* class once immunity wanes.

These characteristics could be modeled with a modified SEI model (Figure 2.4). Here, in lieu of an *R* class, the other disease classes are modeled separately based on vaccination status, with the subscript *v* indicating vaccinated animals. There are two transmission coefficients, β and η, to represent the different bacterial shedding rates of unvaccinated and vaccinated infected animals. The rate of disease progression, α, is also defined differently based on vaccination status. Vaccinated animals can also transition back to the unvaccinated susceptible class with loss of immunity over time, δ.

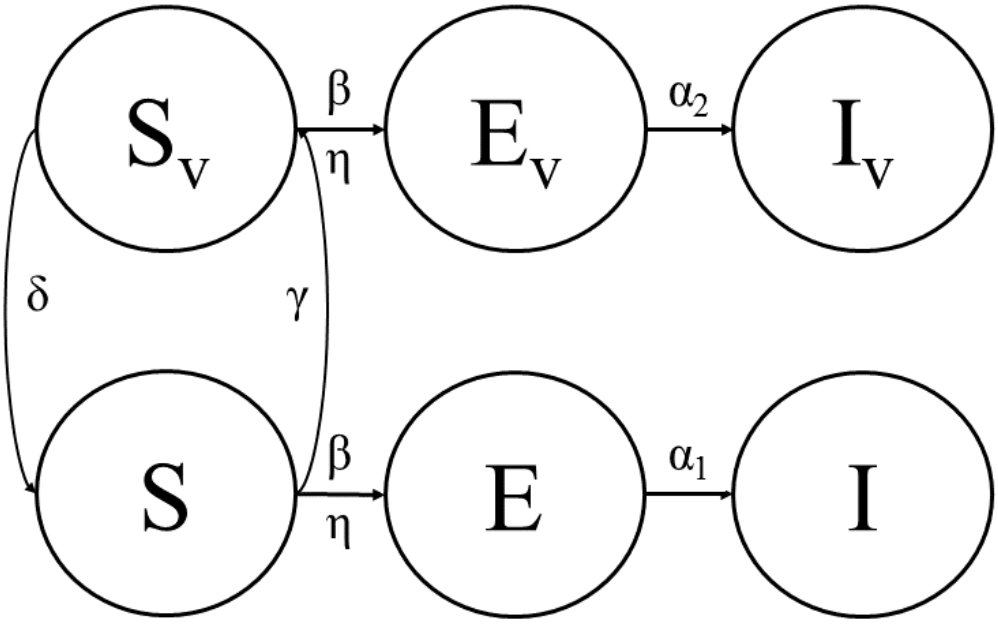

Figure 2.4: A modified SEI model based on vaccination against tuberculosis in wildlife, representing susceptible (*S*), exposed (*E*), and infected (*I*) classes for both unvaccinated and vaccinated animals (designated by subscript *v*). The transition from *S* to *E* classes is governed by the transmission rates from different groups, β and η; the transition from *E* to *I* is governed by the rate of disease progression, α; the transition from *S* to S_v is governed by the vaccination rate, γ, and the transition from S_v to *S* is governed by the rate of lost immunity, δ.

Equations 2.13–2.18 describe the model compartments under this scenario:

$$\frac{dS}{dt} = \delta S_v - \beta S(I + I_v) - \eta S(I + I_v) - \gamma S \tag{2.11}$$

where *S* is the unvaccinated susceptible class, S_v is the vaccinated susceptible class, *I* is the unvaccinated infected class, I_v is the unvaccinated infected class, δ is the rate of loss of immunity, β is the transmission coefficient for shedding from unvaccinated animals, η is the transmission coefficient for reduced shedding from vaccinated animals and γ is the vaccination rate, and

$$\frac{dE}{dt} = \beta S(I + I_v) + \eta S(I + I_v) - \alpha_1 E \quad (2.12)$$

$$\frac{dI}{dt} = \alpha_1 E \quad (2.13)$$

where E represents the expose unvaccinated class, α_1 represents the rate of disease progression for unvaccinated animals, and

$$\frac{dS_v}{dt} = \gamma S - \delta S_v - \beta S_v(I + I_v) - \eta S_v(I + I_v) \quad (2.14)$$

$$\frac{dE_v}{dt} = \beta S(I + I_v) + \eta S(I + I_v) - \alpha_2 E_v \quad (2.15)$$

$$\frac{dI}{dt} = \alpha_2 E_v \quad (2.16)$$

where E_v represents the exposed vaccinated class, and α_2 represents the rate of disease progression for vaccinated animals.This model set demonstrates the complexity in attempting to simulate population dynamics when using an imperfect vaccine. An additional component to consider with vaccination is how to deliver vaccines to free-ranging wildlife populations. Widescale vaccine delivery to wildlife poses several logistical challenges. Parenteral delivery requires individual capture and handling, which is time- and labor-intensive (Wobeser 2002). Despite these challenges, intrasmuscular vaccination has been used in trials in Ireland (Aznar et al. 2014) and the UK (Brown et al. 2013; O'Connor 2016) using BadgerBCG, which has been licensed specifically for use in badgers (Brown et al. 2013). Oral vaccination programs using widespread dispersal of vaccine baits are the most commonly implemented wildlife vaccination strategy, and have been used successfully to control rabies in carnivores in North America and Europe (Sterner et al. 2009; Freuling et al. 2013). Oral vaccines have unique considerations: they must be able to survive the gut and induce immunity when ingested, not be pathogenic if ingested by non-target species, and be stable and remain immunogenic in the environment (Wobeser 2002). Implicit safety concerns with use of oral vaccines include reversion to virulence, oncogenecity, excretion from vaccinated animals, and public health risks (Stöhr and Meslin 1997). Consumption of baits by non-target

species, especially cattle, is a particularly relevant concern, as oral BCG vaccine can result in positive TST in cattle (Buddle et al. 2005). An ideal DIVA vaccine alternative would not produce disease in hosts or non-target species, provide long-lasting immunity and protection against all local TB strains, be incapable of reversion to virulence, and be inexpensive and stable (Wobeser 2002). Improved vaccine options are an area of ongoing research (Buddle et al. 2013).

2.6.3.4 Treatment

Treatment of TB is designed to transition individuals from the I class back to the susceptible class rather than removing them from the population. This can be shown in a simple SEI model (Figure 2.5), where the only difference from the SEI component of the model in Figure 2.3 is the addition of a transition, ε, that represents cure of infected animals and return to the *S* class.

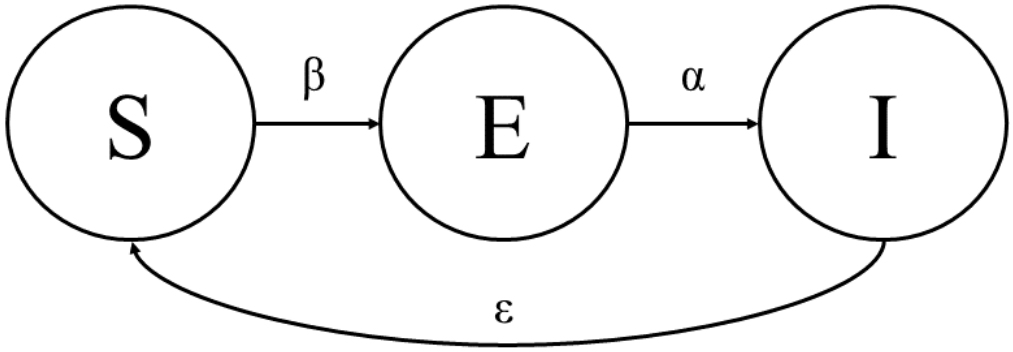

Figure 2.5: A modified SEI model based on treatment of tuberculosis in wildlife, representing susceptible (*S*), exposed (*E*), and infected (*I*) classes. The transition from *S* to *E* classes is governed by the transmission rate, β; the transition from *E* to *I* is governed by the rate of disease progression, α; and the transition from *I* to *S* is governed by the cure rate, ε.

The model in figure 2.5 can be represented by the following model set:

$$\frac{dS}{dt} = \varepsilon I - \beta SI \tag{2.17}$$

where *S* is the susceptible class, *I* is the infected class, ε is the cure rate, and β is the transmission coefficient, and

$$\frac{dE}{dt} = \beta SI - \alpha E \tag{2.18}$$

$$\frac{dI}{dt} = \alpha E - \varepsilon I \tag{2.19}$$

where E is the exposed class, and α is the rate of disease progression.

While on a mathematical level treatment is a simple intervention to reduce the number of infected animals, in practice it is difficult to implement. TB treatment in elephants involves a costly (e.g., $20,000-60,000/elephant/year; Dumonceaux and Mikota 2006; Mikota 2008; Simpson et al. 2017) multi-drug regimen administered over a long period of time, and is therefore not a practical option for free-ranging wildlife. Captive collections with rare species or charismatic megafauna may choose to administer treatment to infected animals rather than euthanize (de Lisle et al. 2001), but treatment of clinical TB in laboratory non-human primates is not recommended (Montali et al. 2001). Infected animals pose a disease risk to other animals within the collection, and a potential public health risk to employees. Some outbreaks in zoos have involved multiple species (Lewerin et al. 2005), although the transmission routes in these cases were not always clear. There have been reported cases of TB in human handlers of elephants and rhinoceros with TB (Murphree 2011; Miller and Olea-Popelka 2013), but the risk of transmission is considered low for those with brief contact (e.g., members of the public viewing animals; Michalak et al. 1998).

Successful treatment of infected animals in captivity has been recorded in elephants (Mikota et al. 2001; Vogelnest et al. 2015), black rhinoceros (Mann et al. 1981; Duncan et al. 2009), bongo (*Tragelaphus eurycerus*; Auclair et al. 2002), Arabian oryx (*Oryx leucoryx*; Greth et al. 1994), chimpanzees (Stephens et al. 2013), and Matschie's tree kangaroo (*Dendrolagus matschiei*; Miller and Lyashchenko 2015). Treatment of non-domestic species is fraught with difficulties in determining appropriate drug doses, which are extrapolated from human recommendations, length of treatment, and delivery routes (Stakeholders Task Force on Management and Research Priorities of Tuberculosis in Elephants 2017). Drugs most commonly used in treatment of animal TB include isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), ethambutol (EMB), amikacin, and levofloxacin (Bush et al. 1990; Greth et al. 1994; Auclair et al. 2002; Stakeholders Task Force on Management and Research Priorities of

Tuberculosis in Elephants 2017). The mainstays of treatment for elephants are INH, RIF, PZA, and EMB, and general guidelines for treatment protocols have been published for elephants (Stakeholders Task Force on Management and Research Priorities of Tuberculosis in Elephants 2017). Drugs may be administered orally directly or disseminated in feed or water, but oral delivery may be complicated by poor acceptance (Auclair et al. 2002). Some rectal formulations have been developed for elephants (Maslow, Mikota, Zhu, Isaza, et al. 2005; Zhu et al. 2005), and amikacin, EMB, and INH were delivered intramuscularly or subcutaneously in bongo (Auclair et al. 2002). Some species may have sensitivities to drugs, and fatal drug reactions have been reported, as with INH toxicity in Bactrian camels (Bush et al. 1990). Adverse effects of treatment are not uncommon and may be severe, resulting in interruption of treatment and risking promotion of drug resistance (Dumonceaux et al. 2011). In worst case scenarios, uncertainty over antemortem diagnostic results to confidently determine disease status, adverse drug effects, and public health concerns may make euthanasia the only viable option (Bush et al. 1990; Dumonceaux et al. 2011).

2.6.3.5 Alteration of Human Activity

Altering human activities or enterprises that modify the natural landscape can be used to control TB in wildlife, often by reducing β by removing factors that promote clusters of artificially high animal density. Effecting changes or garnering support for enforcing changes in human activities to mitigate diseases in free-ranging wildlife can prove extremely challenging, however. As an example, badgers in the UK have been shown to visit farmyards and exploit resources from feed troughs, sheds, haystacks, and other areas, and to defecate and urinate onto cattle feed; these activities represent clear opportunities for pathogen transmission (Garnett et al. 2002). Simple exclusion measures such as aluminum sheet gates and fencing are highly effective at preventing badger entry into feed storage and cattle housing areas, but in one study, willingness of farmers to deploy these measures (which were purchased and installed at no cost to them) was highly variable (Judge et al. 2011). In Michigan, white-tailed deer tend to congregate at artificially high densities due to feeding and baiting practices, which aids in transmission (Schmitt et al.

1997). A majority of local stakeholders support the TB eradication goal, in principle (Dorn and Mertig 2005). However, when asked about specific strategies for managing TB in deer in this area – banning baiting and feeding and reducing deer numbers – those in opposition outweighed those in support for all proposed interventions (Dorn and Mertig 2005). Surveys in Spain showed more encouraging results, with some potential interventions – separation of wildlife and livestock access to waterholes, increased testing of cattle on farms with a positive TB case, and removing gut-piles after hunting – ranked as practical by various stakeholder groups (Cowie et al. 2015). Interestingly, stopping supplemental feeding was considered the most effective potential intervention by experts, yet was not considered practical by any stakeholder groups (Cowie et al. 2015).

Introducing short-term management changes to control small TB outbreaks may be more socially acceptable than long-term interventions. A TB outbreak in white-tailed deer in Minnesota was contained by using a multifaceted approach: increased hunting opportunities, permits for private landowners to remove unlimited numbers of deer from their property as long as carcasses were tested for TB, and a feeding ban with strong enforcement (Carstensen et al. 2011). This outbreak appears to have been a spillover event that was managed aggressively with good compliance from the public, and the disease did not become established as in Michigan (Carstensen et al. 2011). A TB outbreak in a troop of chacma baboons was associated with the troop sleeping in an abandoned building, which facilitated accumulation of feces and other potentially infectious material, and aerosol transmission among troop members sleeping at high density in an enclosed space (Keet et al. 2000). This outbreak resolved without further intervention after access to the building was restricted and the baboons returned to sleeping in trees (Keet et al. 2000).

2.7 Conclusions

Virtually all of earth's ecosystems have been modified by human activity (Vitousek et al. 1997), which has complex and variable impacts on disease prevalence in wildlife (Brearley et al. 2013). Wildlife diseases impact not only individuals and populations, but through their interactions with other species,

wildlife disease impacts communities and ecosystems as well (Tompkins et al. 2011). TB has an extraordinary global burden in millions of wildlife, livestock, and humans (Waters et al. 2012; World Health Organization 2016). Although veterinary TB control is a daunting task, it is essential to livestock, wildlife, and human health. The World Health Organization End TB Strategy aims for a 90% reduction in human TB deaths and 80% reduction in TB incidence by 2030 (World Health Organization 2016). This ambitious plan cannot succeed without a One Health approach, and must address both zoonotic TB in humans and bovine and human TB in animals (World Health Organization et al. 2017). However, disease managers are often fragmented across agencies, including public health officials for human disease, agricultural officials for livestock disease, and wildlife or natural resource officials for wildlife disease. An integration of data and strategies among these groups is required to fully explore the multi-host dynamics of TB (Gebreyes et al. 2014; Thirunavukkarasu et al. 2017). Ignoring or underestimating interspecific transmission and the environmental component of One Health are likely to be hampering TB control. This gives rise to the first objective of this dissertation: to assess the impact of environmental factors affecting wildlife populations and TB management strategies. Three studies compose the contributions to this objective: exploring weather and other environmental factors in trapping success of European badgers being managed for bovine TB; estimating population density of badgers, relative to habitat variables, within a BCG vaccination area; and identifying potential risk factors for TB seropositive status in working African elephants stemming from demography, intra- and interspecific contact.

Estimating the burden of TB in a population and the impact of management in reducing TB depends on the accuracy of diagnostic tools. Diagnostic modalities for TB have advanced considerably in the last decades, and improved assays are continually being developed (Schiller et al. 2010; Chambers 2013). The uncertainty that remains in diagnostic testing for TB has troubling implications for successful disease control. TB cannot be controlled without identifying both actively infected individuals and latently infected individuals, who have the potential to develop active disease and transmit bacteria. While treatment is possible, cure is difficult to define without being able to confidently determine a change in infection state. Tests for TB exposure or infection have variable sensitivity and specificity in different

wildlife species (Maas et al. 2013), and the predictive value of positive or negative test results is dependent on population prevalence (Dohoo et al. 2009), making interpretation subject to local prevalence. Management decisions depend on understanding test results in light of the local disease scenario, which leads to the second objective of this dissertation: to study the performance of diagnostic tests in a novel setting and interpret the results in the context of exposures within the ecosystem. This study employed two serological tests, STAT-PAK and DPP, for the first time in working African elephants in a range country with a complex community of humans, livestock, wildlife, and mycobacteria.

The diversity in wildlife TB settings necessitates a management approach tailored to the local conditions and host communities (Johnson et al. 2015). The varying success of a management strategy applied in the same species in relatively similar habitats – for instance, culling badgers in England compared to Ireland – points to additional ecological components that play a role in disease dynamics. The biology and ecology of the host species involved, host species immune responses, and the community ecology of hosts and mycobacterial pathogens are all features that should be considered. Feasibility and social acceptance of implementing proposed interventions, as well as financial resources are other factors that vary widely around the world but have considerable influence on management success. Control of TB in wildlife is evolving as options for vaccination become more available and feasible (Buddle et al. 2011), but the long-term effects of newer control strategies for wildlife such as BCG vaccination remain unclear. The challenges of adapting management to local ecology and needs form the basis of the final objective of this dissertation: developing recommendations for TB management programs based on surveys, capture data, and consideration of individual, population, and community factors. This objective comprises the studies listed above, as well as a retrospective compilation of treatment data for captive elephants in North America. The results of these studies using prevention, culling, vaccination, and treatment are framed within the context of the long-term success of each locality's management goals and extenuating circumstances.

TB is a complex multi-host–pathogen system that requires a management approach tailored to its unique local dynamics. Managing diseases in the context of an integrated One Health approach that

incorporates human, animal, pathogen, and environmental factors is necessary to fully address complex interactions (Osburn et al. 2009; Cumming and Cumming 2015). TB has far-reaching impacts beyond health, including livestock productivity, producer livelihoods, international trade, and wildlife conservation (Geoghegan 2012); therefore, its effective control has tremendous economic and social benefits (Olmstead and Rhode 2004). Controlling TB at the wildlife-livestock-human interface must occur through a coordinated interdisciplinary approach to be successful (de Garine-Wichatitsky et al. 2013; Miller and Olea-Popelka 2013).The projects in this dissertation represent highly collaborative efforts across disciplines and borders to improve our understanding of TB management in wildlife.

CHAPTER 3: WEATHER INFLUENCES TRAPPING SUCCESS FOR TUBERCULOSIS MANAGEMENT IN EUROPEAN BADGERS (*MELES MELES*)[1]

3.1 Summary

European badgers (*Meles meles*) in Ireland and the United Kingdom are a reservoir for *Mycobacterium bovis*, the causative agent of bovine tuberculosis (TB). A number of interventions have been evaluated in attempts to control bovine TB within badger populations, many of which rely on the capture of badgers. One strategy being implemented within Ireland is intramuscular vaccination using Bacille Calmette Guerin (BCG), as an alternative to badger culling. The success of vaccination as a disease control strategy depends on the ability to capture badgers and administer vaccines, thus trapping success is crucial to effectively vaccinate the population (maximize vaccine coverage). A field vaccine trial was conducted in County Kilkenny, Ireland, from 2010–2013. We used data from this trial to evaluate the association between weather (precipitation and temperature data), badger sett characteristics, and badger trapping success. Approximately 10% of capture efforts resulted in a badger capture. Our results indicate that badger captures were highest in drizzle, rain, and heavy rain weather conditions, and when minimum temperatures ranged from 3–8 °C. Badger captures were highest at main setts (large burrow systems) and when sett activity scores were high (qualitative classes 4 or 5). Using local precipitation and temperature data in conjunction with observed sett characteristics provides wildlife managers with guidelines to optimize trapping success. Implementing capture operations under optimal conditions should increase the trapping success of badgers and allow for increased delivery of vaccines to manage bovine TB.

[1] Originally published as: Martin LER, Byrne AW, O'Keeffe J, Miller MA, Olea-Popelka FJ. 2017. Weather influences trapping success for tuberculosis management in European badgers (*Meles meles*). European Journal of Wildlife Research 63:30. doi: https://doi.org/10.1007/s10344-017-1089-2. Statistical models in Appendix D.

3.2 Introduction

Managing endemic diseases in free-ranging wildlife relies on interventions intended to control or eradicate the disease (Wobeser 2002). When controlling disease, vaccination of wildlife is considered under certain circumstances, particularly in offering protection against diseases that threaten livestock production or wildlife species of conservation concern (Cross et al. 2007). Vaccination programs may be useful in the control of wildlife diseases such as bovine tuberculosis (TB). Bovine TB affects livestock and a number of wildlife species around the world, and is an important disease of cattle in the Republic of Ireland (More and Good 2015). The European badger (*Meles meles*) is an important wildlife reservoir implicated in bovine TB persistence, which hampers disease eradication efforts. In Ireland, badgers are listed as a protected species under the Irish Wildlife Act, thus their conservation is an important consideration in disease management (Byrne, Sleeman, et al. 2012). Bovine TB management in Ireland has included targeted culling of badgers in areas of cattle TB outbreaks (O'Keeffe 2006). Culling is not a long-term sustainable management strategy of a protected species, thus vaccination of badgers against bovine TB has been proposed as an alternative. Vaccination has the dual benefits of controlling disease without reducing badger populations, while lessening the morbidity and mortality in badgers due to TB disease. The Bacillus Calmette-Guérin (BCG) vaccine has been shown to be safe (Lesellier et al. 2006) and efficacious (Chambers et al. 2011; Lesellier et al. 2011) in badgers. However, a considerable portion of a given badger population must be immunized for vaccination to effectively replace culling as a management strategy (Wilkinson et al. 2004; Byrne, O'Keeffe, et al. 2012).

An intramuscular BCG vaccine is currently available for use in badgers, which requires capture and handling for administration. Maximizing trapping success will therefore be crucial to an effective vaccination program. Live-capture rates are low among carnivores. Capture success of red foxes (*Vulpes vulpes*) has been reported in the range of 1.5–1.8 captures/100 trap-nights (Baker et al. 2001) or 0–3.4 captures/100 trap-nights (Ruette et al. 2003), and for stone martens (*Martes foina*) and pine martens (*Martes martes*), 0.6–7.8 captures/100 trap-nights (Ruette et al. 2003). Badgers are nocturnal and fossorial, which further reduces capture opportunities. A study in Atlantic Spain showed that a minimum

of 40 traps had to be set per sampling period per sett to trap 93.18% of adult badgers (Acevedo et al. 2014). Byrne et al. (2012) showed mean badger trappability during a vaccine trial in County Kilkenny, Ireland to be 34–35% across the population, with variation by season and age-class. Examining specific factors that improve trapping success of badgers can provide wildlife managers with information to optimize trapping efforts and, in doing so, guide wise allocations of resources to protect badgers and livestock from TB.

Trapping success is influenced by factors related to trapping methods, environmental variables, and species characteristics (Pawlina and Proulx 1999; Tuyttens et al. 1999). Few mammal trapping studies have examined the role of weather. Temperature and time since rainfall affected trapping success of Valley pocket gophers (Cox and Hunt 1992), and rainfall and lower temperatures were positively correlated with captures of Western Red-backed voles (Maguire 1999). In the United Kingdom (UK), temperature and precipitation have been shown to influence the efficiency of cage-trapping badgers (Noonan et al. 2015). However, that study in the UK was conducted over a small area with a discrete, systematically trapped population using bait and a different trap style than that employed in Ireland. Thus, the objective of this study was to examine the effect of local weather (temperature and precipitation), season, as well as sett type and sett activity, on badger trapping success in disease management in Ireland.

3.3 Methods

3.3.1 Study Area

This study used data from the Kilkenny Vaccine Trial; the study area and capture protocol have been previously described by Byrne et al. (2012). Briefly, the study area is approximately 755 km^2 located in the northwest of County Kilkenny, Ireland, and encompasses pasture land with extensive hedgerows (Figure 3.1). This area was divided into three zones (A, B, and C) for different treatments of the vaccine trial.

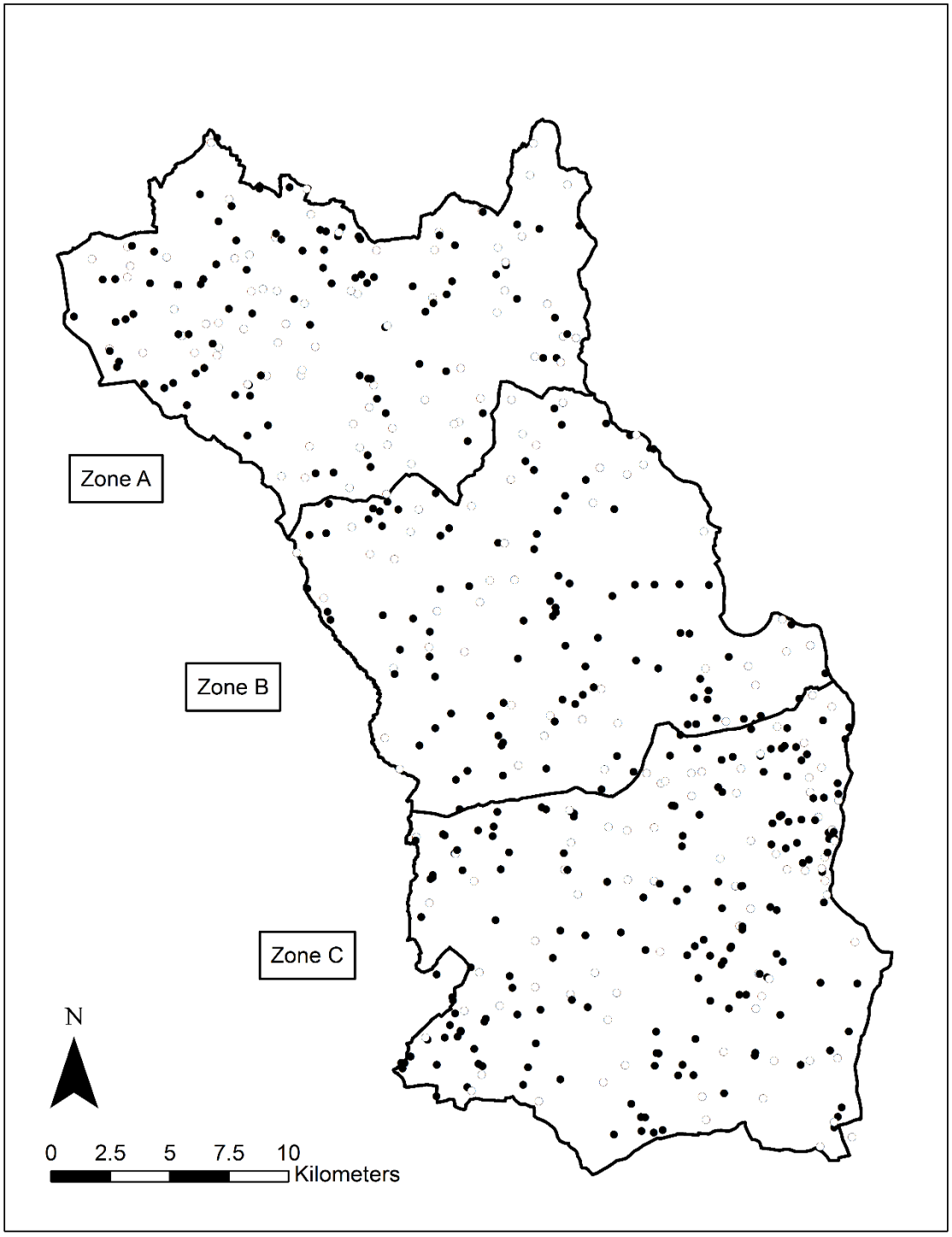

Figure 3.1: Map of badger vaccine trial area and zones with County Kilkenny, Ireland. Black dots are main setts and white dots are non-main setts where trapping occurred from 2010–2013.

3.3.2 Capture Protocol

Badgers were captured in six sessions from February 2010 – December 2013. Trapping occurred during all months of the year, but most heavily from March–June and October–November and infrequently during July–August. Badgers were captured using stopped wire restraints (for details on these restraints and their impact on trapped badgers see: Byrne et al. 2015b). In each zone, active setts were targeted each week for trapping, and 1–35 restraints were laid at each sett. The number of restraints laid was subjectively determined by signs of activity, number of openings available, and badger passes or paths available for restraints to be set. Setts were classified as main setts (those used for breeding and over-wintering; Smal 1995) or non-main setts. Starting in February 2011, at the beginning of the week, setts were assigned activity scores ranging from 0–5 (Table 3.1). Trapping was conducted at setts with activity scores ≥ 3. Trapping occurred at selected setts over a period of 8 nights. A sett-night is defined as all trapping effort at one sett over one trap night. All traps were checked daily prior to 12 pm.

Table 3.1: Standardized badger sett activity scoring system used in Ireland.

Score	Description
0	No activity; openings closed or partly closed by clay, leaves, etc. No fresh paths or tracks are visible leading to, or between openings. Old existing paths are overgrown and beginning to disappear. Grass or scrub growing around openings and on the spoil heap.
1	Beginning of movement; first signs of activity, with paths leading to the openings. Openings however are not cleaned out. Could not be sure if paths are badger or non-target species.
2	Possible badger activity; distinctive paths leading to openings. Openings may or may not be cleaned out. No fresh spoil heap. Possible footprint. Impression is that the badger is travelling around different setts rather than setting up home.
3	Badger activity; opening cleaned out but spoil heap not fresh. Paths can be visibly seen leading to and from openings. Bottom of opening is clean where animal is moving in and out of the sett.
4	Definite badger activity; paths can be clearly seen leading to and between openings. Openings cleaned out with fresh spoil heaps, possibly with or without bedding. Bottom and side of openings are clean and shiny in appearance where badger is moving in and out of the sett. Fresh latrine pits in the vicinity of the openings.
5	Definite badger activity; as above at number 4 but at a main sett.

In each zone, minimum and maximum temperatures were recorded using a maximum/minimum thermometer (AgriHealth, Monaghan, Ireland) for each 24 hour period from morning to the time when traps were checked the following morning. Precipitation scores were assigned on a scale of 1–6, where 1 = dry, 2 = drizzle, 3 = light rain, 4 = rain, 5 = heavy rain, and 6 = snow. A single precipitation score,

representative of conditions overnight when badgers were expected to be active, was assigned by the field staff for each 24-hour period.

3.3.3 Statistical Analyses

Descriptive statistics (counts, frequencies, proportions, means and standard deviations) were calculated to evaluate the data distribution. The temperature data revealed a non-linear relationship between minimum and maximum temperature and badger trapping success. To further explore this relationship, minimum and maximum temperatures were separated into four categories based on quartiles. Logistic regression modeling was used to assess trapping success, which was considered as a binary event (badger captured or not) in a given sett/night.

Univariable logistic regression models were used to assess the association between variables (sett type, sett activity score, precipitation score, minimum and maximum temperature categories, number of traps per sett, season, year, and zone) and whether or not a badger was captured. All variables found to be significant (using a significance threshold of $p < 0.15$) in the univariable analysis were considered for inclusion in a multivariable model. Continuous variables were assessed for collinearity using pairwise Pearson correlation, and categorical variables were assessed for collinearity using Spearman correlation. When collinearity was detected in a pair of variables, only the variable with higher correlation to badger trapping success was retained.

A series of ten logistic regression candidate models were developed using variables from the univariable analysis. Two-way interaction terms between weather and season were also investigated in some candidate models. Using a full fixed effects logistic regression model, model fit was assessed using the Hosmer-Lemeshow goodness-of-fit test and a receiver operating characteristic curve. To control for lack of independence from repeated observations within setts, the candidate model set was tested using three-level mixed logistic regression models with setts nested within zones. An information-theoretic approach with Akaike's Information Criterion (AIC) was used to perform model selection (Burnham and Anderson 2002). The model with the lowest AIC (and highest weight) was considered the preferred

model, and models with ΔAIC < 2 were considered equally parsimonious. The candidate model set is shown in Table 3.2. All candidate models contained season, year, and number of traps set as controls. To ensure comparability of AIC values by using the same number of observations for all models (Burnham and Anderson 2002), model selection was performed on a subset of the data (13,860 of 19,196 total sett-nights) for which no covariate data was missing. All analyses were performed in Stata 13.1 (StataCorp 2013).

Table 3.2: Candidate models and model statistics for mixed effects logistic regression models of badger trapping success.

Model	LL	K	AIC	ΔAIC	w_i
Main Activity Weather MinTemp Traps Season Year	-4028.453	20	8096.906	0	0.79
Activity Weather MinTemp Traps Season Year	-4031.012	19	8100.025	3.12	0.17
Main Weather MinTemp Traps Season Year	-4134.651	18	8305.301	208.40	0.00
Main Activity Weather Traps Season Year	-4034.823	17	8103.646	6.74	0.03
Activity Weather Traps Season Year	-4037.215	16	8106.429	9.52	0.01
Main Activity MinTemp Traps Season Year	-4039.411	15	8108.821	11.92	0.00
Activity MinTemp Traps Season Year	-4041.986	14	8111.971	15.07	0.00
Main Activity Traps Season Year	-4051.036	12	8126.073	29.17	0.00
Main Activity Weather MinTemp Traps Season Year Weather*Season	-4024.328	30	8108.656	11.75	0.00
Activity Weather MinTemp Traps Season Year Weather*Season	-4026.878	29	8111.756	14.85	0.00

K: number of parameters; LL: log likelihood; AIC: Akaike's information criterion; ΔAICc: difference in AIC from minimum AIC model; w_i: Akaike weight

3.4 Results

Trapping occurred at 561 unique setts over 19,196 sett-nights from 2010-2013. The number of trapping events at main and non-main setts was approximately equal, with 9,316 trap-nights at main setts and 9,880 at non-main setts. Number of restraints per sett ranged from 1–35 (mean 7.8, SD 3.3). Sett activity was not quantified in 2010 or early 2011 (before February). Of the 14,272 sett-nights where sett activity was recorded, 54.5% were scored 3, 24.2% were scored 4, and 21.3% were scored 5. Using observer precipitation scores, there were 7,526 sett-nights with dry weather, 2,721 sett-nights with drizzle, 2,848 sett-nights with light rain, 3,274 sett-nights with rain, 1,941 sett-nights with heavy rain, and 262 sett-nights with snow. Minimum temperature ranged from -12 to 16 °C (mean 5 °C, SD 4.5). Maximum temperature ranged from -3 to 30 °C (mean 12.4 °C, SD 4.9). The majority of sett-nights (17,394/19,196; 91%) did not result in capture of any badgers. A total of 1,802 sett-nights resulted in trapping success;

most successful sett-nights (1,623) resulted in one badger captured, but 167 sett-nights resulted in two badgers captured and 12 sett-nights resulted in three badgers captured. The overall trapping success rate was 9.5 badgers/100 sett-nights.

Univariable logistic regression analysis indicated significant (at $p < 0.15$) differences in badger trapping success for sett type, sett activity, precipitation score, minimum temperature category, maximum temperature category, number of traps set, season, and year (Table 3.3). Minimum and maximum temperature were highly correlated, and minimum temperature was retained for inclusion in multivariable models based on its higher correlation with badger trapping success. Analysis of Spearman correlation coefficients for categorical variables did not show strong correlations ($\rho > 0.7$).

Table 3.3: Results from univariable logistic regression analysis of sett characteristics, weather variables, and other predictors of badger trapping success in Co. Kilkenny, Ireland 2010–2013.

Variable	Category	OR	95% CI	p-value
Main	Non-main	REF	–	–
	Main	1.79	1.62–1.97	< 0.001
Sett activity	3	REF	–	–
	4	2.64	2.28–3.06	< 0.001
	5	4.56	3.97–5.24	< 0.001
Weather	1 (dry)	REF	–	–
	2 (drizzle)	1.37	1.18–1.59	< 0.001
	3 (light rain)	1.17	1.00–1.36	0.041
	4 (rain)	1.52	1.33–1.74	< 0.001
	5 (heavy rain)	1.63	1.39–1.91	< 0.001
	6 (snow)	0.70	0.41–1.18	0.178
Minimum temperature	≤ 2 °C	REF	–	–
	3–5 °C	1.36	1.19–1.55	< 0.001
	6–8 °C	1.08	0.94–1.23	0.26
	≥ 9 °C	0.74	0.63–0.86	< 0.001
Maximum temperature	≤ 9 °C	REF	–	–
	10–12 °C	1.07	0.94–1.22	0.28
	13–16 °C	0.88	0.77–1.00	0.049
	≥ 17 °C	0.62	0.53–0.72	< 0.001
No. Traps	N/A	1.13	1.12–1.15	< 0.001
Season	Spring	REF	–	–
	Summer	0.47	0.38–0.57	< 0.001
	Autumn	1.01	0.89–1.14	0.92
	Winter	1.36	1.20–1.53	< 0.001
Year	2010	REF	–	–
	2011	1.14	1.00–1.31	0.06
	2012	1.06	0.92–1.22	0.42
	2013	0.92	0.80–1.07	0.29

REF = reference

The full logistic regression model had adequate performance under the ROC curve (AUC = 0.71), and the Hosmer-Lemeshow goodness-of-fit test did not reveal a lack of fit in the model (p = 0.70). AIC of the mixed effects logistic regression models revealed a single most parsimonious model, the full model containing sett type, sett activity, precipitation score, and minimum temperature (Table 3.2, Figure 3.2). When controlling for the combined effect of all other variables, there was a difference in badger trapping success between sett types, with captures more likely to occur at main setts than non-main sett (OR = 1.23, 95% CI 1.03–1.48). Badger captures were more likely at setts with activity scores of 4 (OR = 2.58, 95% CI 2.18–3.06) and 5 (OR = 3.04, 95% CI 2.54–3.64), compared to setts with an activity score of 3. Captures were only marginally higher at setts with activity scores of 5 compared to 4 (OR = 1.18, 95% CI 0.95–1.46). Captures were more likely in drizzle (OR = 1.20, 95% CI 1.00–1.43), light rain (OR = 1.08, 95% CI 0.89–1.3), rain (OR = 1.29, 95% CI 1.29–1.53), and heavy rain (OR = 1.47, 95% CI 1.21–1.79), compared to dry weather. Captures were less likely in snow than dry weather (OR = 0.41, 95% CI 0.13–1.31). Badger captures were more likely in minimum temperatures of 3–5 °C (OR = 1.33, 95% CI 1.12–1.59), 6–8 °C (OR = 1.20, 95% CI 1.01–1.43), and ≥ 9 °C (OR = 1.05, 95% CI = 0.86–1.30) compared to minimum temperatures ≤ 2 °C. As the number of restraints set increased by 1, the odds of capturing a badger increased 6% (OR = 1.06, 95% CI 1.04–1.08). Badger captures were more likely in spring (OR = 1.78, 95% CI 1.38–2.31), autumn (OR = 1.9, 95% CI 1.46–2.42), and winter (OR = 2.4, 95% CI 1.81–3.13), compared to summer. Badger trapping success varied little in 2012 (OR = 0.98, 95% CI 0.84–1.14) and 2013 (OR = 0.98, 95% CI 0.83–1.13) compared to 2011.

3.5 Discussion

In this study, the proportion of trapping events resulting in capturing at least one badger was low with < 10% of sett-nights resulting in a badger captured, highlighting the importance of determining factors associated with trapping success for implementing a vaccination program. Our results indicate that sett type, sett activity, weather, number of restraints set, and season all influence badger trapping success.

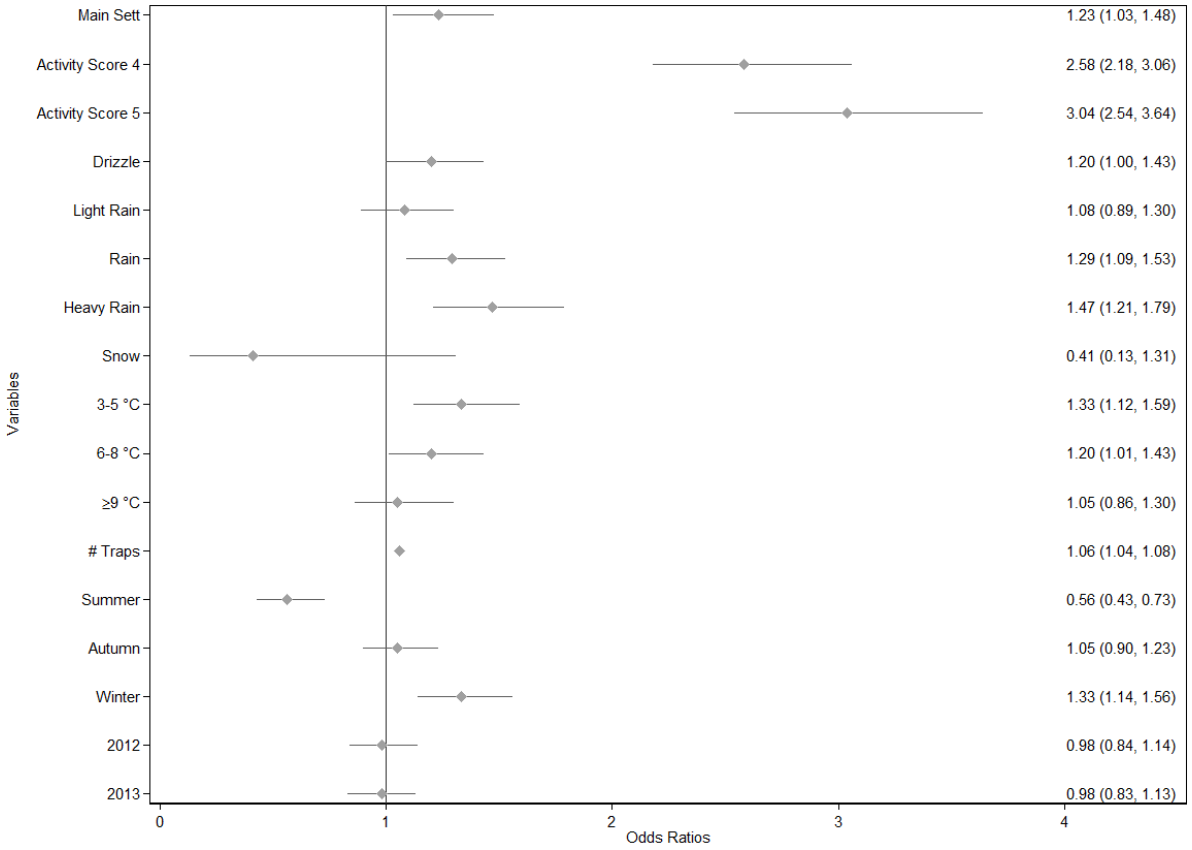

Figure 3.2: Forest plot of odds ratios and associated 95% confidence intervals for variables in the top logistic regression model of badger capture success in County Kilkenny, Ireland. Values for odds ratios and 95% confidence intervals are reported on the right side of the plot. Reference groups for variables (given in parentheses) are as follows: non-main sett (sett type); activity score 3 (sett activity score); dry (precipitation); ≤ 2 °C (minimum temperature); spring (season); 2011 (year)

Sett characteristics proved to be important predictors of trapping success. Badger captures were more likely at main setts than non-main setts (Byrne et al. 2013a). Main setts are typically larger and more active than non-main setts (Smal 1995), and therefore have more badgers present or more entrances available for trapping (Byrne et al. 2013a). Trapping success also increased marginally with each additional restraint set, which is related to some degree to the number of sett entrances and paths available for trapping. Badger captures were more likely at setts with higher activity scores, and previous work has shown sett activity can provide a crude indication of badger presence (Wilson et al. 2003). Although the relationship between trapping success and sett activity was not surprising, the magnitude of the differences in capture odds among activity scores was notable. It is clear that setts with a score of 4 or 5

should be prioritized for maximizing trapping success, given odds ratios 2.6–3 times higher for scores of 4 or 5 as compared to 3. Sett activity was the most important factor (highest odds ratios) contributing to badger captures in this study.

Anecdotal reports in Ireland indicated badger captures might increase after nights with rain or wind. Our analysis supports this idea, with all but one category of rainy weather associated with increases in badger captures. Two factors may be driving the relationship between rainfall and trapping success. First, earthworms are an important food source for badgers and are reported in numerous studies of badger diet in Ireland (reviewed by Byrne, Sleeman, et al. 2012) and elsewhere (reviewed by Goszczyński et al. 2000). Badgers are likely to emerge after rainfall to take advantage of emerging earthworms, an abundant and easily obtained protein source. Elliott et al. (2015) reported that badgers in a study site in Ireland tended to make smaller foraging trips during wet weather. This was interpreted to mean that food resources (primarily earthworms) were more plentiful, and thus badgers did not have to forage as far from their setts to find prey. Indeed, recently research from Ireland has found that badger body mass is significantly, and positively, affected by precipitation (Byrne et al. 2015). Badgers appear to gain weight in the months following above long-term average rainfall, indicating that badgers are indeed gaining physiologically through increased access to food resources (Byrne et al. 2015). The second hypothesis relates to badgers having a strong sense of smell and typically being wary of human scent, some to the point of failing to emerge from a sett overnight if the sett has been visited by a person during the day (Roper 2010). Rain may reduce human scent outside setts and around restraints, making badgers less wary of emerging from setts or approaching restraints. Frequent rainfall in Ireland offers ample opportunities for improved trapping success.

Badgers were most likely to be captured when minimum temperatures were in the 3–8 °C range, and during winter compared to other seasons. The 3–8 °C temperature range comprised the interquartile range of the observed minimum temperature data in our study, indicating a preference for cooler and moderate temperatures. This observation is supported by anecdotal evidence from field staff, who reported poor perceived trapping success in warm weather and weather cold enough to produce frost or

snow. Badgers do not hibernate in Ireland, but their activity levels are known to decrease during winter in northern latitudes, consistent with a type of torpor or winter lethargy (Fowler and Racey 1988; Roper 2010; Newman et al. 2011). It may be difficult for badgers to forage in frozen soils. Badgers experience a net negative energy balance and increased heat loss (Fowler and Racey 1988) despite a decrease in resting metabolic rate (McClune et al. 2015), with weight dramatically decreasing during winter months (Byrne et al. 2015). Badgers may reside outside of the sett and sleep above ground during high temperatures (Roper 2010). Badgers may become less trappable during warm nights when they are away from their setts, as stopped restraints are predominantly set near sett entrances (Byrne et al. 2013b).

In a study of badgers in Wytham Woods, England, time-lagged temperature and precipitation contributed to trapping efficiency (Noonan et al. 2015). Noonan et al. (2015) also found a non-significant positive effect of rain on trapping efficiency during summer months, but a significant negative effect during spring trapping. Our data did not provide strong support for models with an interaction between weather and season. Season has been shown to affect badger trappability elsewhere (Tuyttens et al. 1999; Byrne, O'Keeffe, et al. 2012; Byrne et al. 2013a). Badgers in our study were most likely to be captured in winter, followed by autumn and spring. This finding is in agreement with prior studies in Ireland that found more badgers captured in autumn/winter (Byrne, O'Keeffe, et al. 2012) or winter/spring (Byrne et al. 2013a), and with field staff perceptions of optimal badger trapping during November–March. In England, trappability was found to be lowest in autumn (Tuyttens et al. 1999), with some evidence of low trappability in winter from another study (Wilson et al. 2003). It is likely that the differences in seasonal trappability between Britain and Ireland relate to the predominant trap used (stopped restraints in Ireland and cage traps in Britain; Byrne, O'Keeffe, et al. 2012) and how these traps operate. Restraints rely on the ability to go undetected by the badger, and are placed near sites through which badgers are known to move (sett entrances and along runs; Byrne et al. 2013a). In Ireland, restraints are not used very frequently during summer months, as vegetation growth can obscure badger setts (Byrne et al. 2013b; Byrne et al. 2013a). Restraints have been specifically designed to capture adults, not cubs (Sleeman et al. 2009), and in the spring, cubs may emerge from setts and disturb restraints, preventing adults from being

captured. In contrast, cage traps are baited (typically with peanuts), so badgers are drawn towards the trap and can be placed away from badger setts, for example near border latrines.

Trapping a significant proportion of badger populations is essential for reaching herd immunity via vaccination, the threshold where the infection is unable to establish in the susceptible population (Begon 2009). The basic reproductive ratio of a disease (R_0) must be less than one for disease eradication, although the entire population need not be vaccinated to achieve this (Begon 2009). The exact number of badgers that must be vaccinated to induce herd immunity is currently unknown. Simulations of an oral vaccination strategy based on data from England suggested at least 40% of badgers needed to be immunized annually to eradicate TB (Wilkinson et al. 2004), and benefits of vaccination are most likely with long-term annual vaccination programs (Hardstaff et al. 2013). Another simulation study using data from Ireland suggested that vaccination success depends on population density and prevalence of TB in badgers (Abdou et al. 2016). Trapping efforts must be prioritized at setts and under conditions most likely to yield badger captures.

We conclude that efforts should be concentrated on main setts and setts with activity scores of 4 or 5, and under rainy weather conditions when minimum temperatures range from 3–8 °C. The factors investigated in this study are advantageous in that all can be determined easily and inexpensively, allowing managers to make informed decisions about when and where to prioritize trapping efforts. Tailoring capture operations to optimal conditions should increase the trapping success of badgers and allow for increased vaccine delivery. An effective badger vaccination program will move Ireland closer to the goal of bovine TB eradication.

CHAPTER 4: TUBERCULOSIS SEROSURVEILLANCE AND MANAGEMENT PRACTICES OF CAPTIVE AFRICAN ELEPHANTS (*LOXODONTA AFRICANA*) IN THE KAVANGO-ZAMBEZI TRANSFRONTIER CONSERVATION AREA[2]

4.1 Summary

Transfrontier conservation areas represent an international effort to encourage conservation and sustainable development. Their success faces a number of challenges, including disease management in wildlife, livestock, and humans. Tuberculosis (TB) affects humans and a multitude of non-human animal species and is of particular concern in sub-Saharan Africa. The Kavango-Zambezi Transfrontier Conservation Area encompasses five countries, including Zimbabwe, and is home to the largest contiguous population of free-ranging elephants in Africa. Elephants are known to be susceptible to TB, thus understanding TB status, exposure, and transmission risks to and from elephants in this area is of interest for both conservation and human health. To assess risk factors for TB seroprevalence, a questionnaire was used to collect data regarding elephant management at four ecotourism facilities offering elephant-back tourist rides in the Victoria Falls area of Zimbabwe. Thirty-five working African elephants were screened for *Mycobacterium tuberculosis* complex antibodies using the ElephantTB Stat-Pak and the DPP VetTB Assay for elephants. Six of 35 elephants (17.1%) were seropositive. The risk factor most important for seropositive status was time in captivity. This is the first study to assess TB seroprevalence and risk factors in working African elephants in their home range. Our findings will provide a foundation to develop guidelines to protect the health of captive and free-ranging elephants in the southern African context, as well as elephant handlers through simple interventions. Minimizing exposure through shared feed with other wildlife, routine TB testing of elephant handlers, and regular serological screening of elephants are recommended as preventive measures.

[2] Originally published as: Rosen LE, Hanyire TG, Dawson J, Foggin CM, Michel AL, Huyvaert KP, Miller M, Olea-Popelka FJ. 2018. Tuberculosis serosurveillance and management practices of captive African elephants (*Loxodonta africana*) in the Kavango-Zambezi Transfrontier Conservation Area. Transboundary and Emerging Diseases 65:e344–354. Statistical models in Appendix D.

4.2 Introduction

Transfrontier conservation areas (TFCAs) in Africa incorporate large tracts of adjacent private, communal, and public land between neighboring countries and represent a broad-scale approach to improving biodiversity conservation and socioeconomic development of local communities (Hanks 2003). One goal of TFCAs is to increase connectivity of an otherwise fragmented landscape for wildlife, while boosting nature-based tourism (Osofsky et al. 2008). An inherent challenge in the transboundary landscape of TFCAs is disease management, given movement and interfaces among wildlife, livestock, and humans in countries with varying disease prevalence and management strategies (Osofsky et al. 2008). A variety of diseases, including zoonoses, have been identified as threats to human and animal health within TFCAs (Cumming 2011). Movement of diseases within TFCAs is a major concern: for example, work by Caron et al. (2016) has documented the spread of bovine tuberculosis (TB) and other diseases in African buffalo (*Syncerus caffer*) within the Great Limpopo TFCA.

Sub-Saharan African countries are particularly affected by TB in livestock, wildlife, and humans (Corbett et al. 2006; de Garine-Wichatitsky et al. 2013). Human TB, caused by *Mycobacterium tuberculosis* (*M. tuberculosis*), has high prevalence in the region as a result of high incidence of human immunodeficiency virus (HIV) and limited healthcare infrastructure and disease control measures (Corbett et al. 2006; World Health Organization 2015). Sub-Saharan African countries feature prominently in the high-burden country lists for TB, TB/HIV coinfection, and multidrug-resistant (MDR) TB (World Health Organization 2016). Angola, Botswana, Namibia, Zambia, and Zimbabwe all appear on at least one of these three lists, and both Angola and Zimbabwe appear on the lists for high TB, TB/HIV, and MDR-TB burden (World Health Organization 2016). These five countries contribute land to the Kavango-Zambezi (KAZA) TFCA (Figure 4.1), the largest proposed TFCA at approximately 520,000 km^2 (Anonymous 2014).

The KAZA TFCA supports the largest contiguous population of African elephants in the world (*Loxodonta africana*) (Van Aarde et al. 2006), concentrated in Botswana and Zimbabwe (Anonymous 2014). Threats to conservation of elephants include disease epidemics, human-elephant conflict, and

illegal killing for ivory (Sukumar 2003). Illegal killing of elephants for ivory has driven a steep decline in African elephant populations (Wittemyer et al. 2014), with overall population declines of 8% per year (Chase et al. 2016). Elephants are among the top-ranked species that visitors are interested in viewing (Lindsey et al. 2007), and the loss of elephants due to poaching results in an estimated $25 million of lost tourism revenue to African countries annually (Naidoo et al. 2016). Declining elephant numbers appear to drive tourism losses rather than vice versa (Naidoo et al. 2016), thus further losses of elephants will only exacerbate tourism losses and concomitant economic impacts.

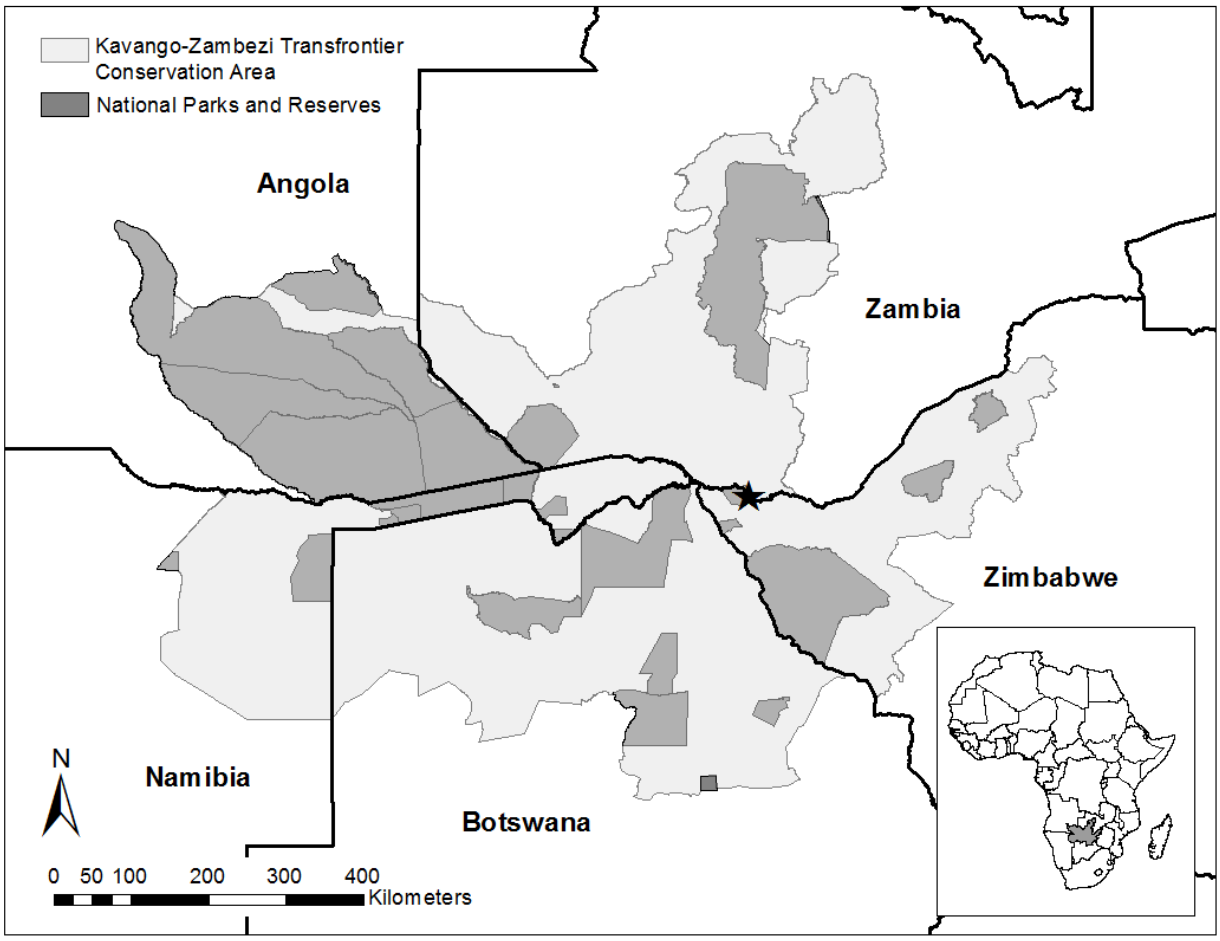

Figure 4.1. Map of the Kavango-Zambezi Transfrontier Conservation Area. The black star indicates Victoria Falls, Zimbabwe.

Investing in the sustainable conservation of elephants is therefore of economic benefit to range countries in addition to promoting biodiversity. As a result, diseases affecting elephants, including TB,

are of particular interest within the TFCA. Elephants in zoological collections around the world have been diagnosed with TB (Mikota et al. 2001; Lewerin et al. 2005; Vogelnest et al. 2015; Zlot et al. 2015), primarily caused by *M. tuberculosis* or, more rarely, *M. bovis* (Mikota and Maslow 2011). Infections with zoonotic pathogens transmitted from humans to animals (reverse zoonosis or zooanthroponosis) are more frequently reported in captive animals than their wild counterparts (Epstein and Price 2009). A case of TB caused by *M. tuberculosis* has been documented in a wild African elephant in Kenya, and, although the source of that animal's infection could not be definitively traced, it had known extended prior contact with humans (Obanda et al. 2013). In 2016, an elephant was found dead in Kruger National Park and, at necropsy, evident TB lesions were found in the lungs with subsequent culture and identification of *M. tuberculosis* (M. Miller, pers. comm. 2017). Spillover of *M. tuberculosis* from humans has been documented in free-ranging and captive wildlife in South Africa (Michel et al. 2013).

Elephants are used as working animals in logging camps or tourist resorts in some countries where they are native (Sukumar 2003). Working elephants have close contact with humans, providing a potential interface for bidirectional pathogen transmission. Tuberculosis infection or seroconversion has been documented in working Asian elephants (*Elephas maximus*) in Thailand (Angkawanish et al. 2010; Angkawanish et al. 2013), India (Verma-Kumar et al. 2012), Malaysia (Ong et al. 2013; Yakubu et al. 2016), Laos (Lassausaie et al. 2014), and Nepal (Mikota, Gairhe, et al. 2015). In contrast, the health of working African elephants has only been studied in the contexts of stress response (Millspaugh et al. 2007) and animal welfare governance (Duffy and Moore 2011), not their risk of TB or other diseases. Transmission of *M. tuberculosis* has been documented both from humans to elephants (Michalak et al. 1998) and vice versa (Murphree 2011), thus the interface between humans and working elephants presents a risk for pathogen transmission to both species.

Approximately 50–60 captive African elephants are used in ecotourism facilities in the KAZA TFCA for the purpose of elephant-back safaris, and may therefore be at higher risk of exposure to *M. tuberculosis* than their wild counterparts. Standard practices at these facilities allow elephants to freely forage during the day, with the potential for contact with wild elephants. Thus, these captive elephants

represent an important human-wildlife interface scenario, having contact with both humans in a high-burden *M. tuberculosis* area as well as an important population of free-ranging African elephants.

The goals of this study were twofold: 1) to screen this population of elephants to determine the seroprevalence of TB antibodies and 2) to explore associations between elephant demographics and management characteristics (risk factors) and seropositivity among captive African elephants in the Victoria Falls area of Zimbabwe in the KAZA TFCA. Based on our findings, management recommendations are provided to mitigate risk of *M. tuberculosis* exposure to captive elephants, as well as free-ranging wildlife and humans.

4.3 Materials and Methods

4.3.1 Study Population and Potential Risk Factors Questionnaire

Four ecotourism facilities offering elephant-back safaris in the Victoria Falls area of Zimbabwe consented to participate in the study. To maintain confidentiality of participating facilities, each facility was designated as A, B, C, or D. A questionnaire was developed to gather information about elephant demographics and management through in-person interviews, and survey responses were used to define potential risk factors for seropositive status. The survey (Appendix A) included 45 closed- and open-ended questions regarding the working elephants, including questions about: (1) demographics (sex, age, birthplace); (2) breeding status (sexual maturity, pregnancies, and births); (3) management style (free vs. protected contact with handlers); (4) social structure (housing, social grouping, contact with wild elephants); (5) public contact; and (6) staff numbers, type (full-time, part-time, or volunteer), and TB testing frequency. Staff at each facility were interviewed in English, Tonga, Ndebele, or Shona during September–October 2014. All survey responses were recorded in English. Follow-up interviews to clarify responses were conducted in English in February 2016.

4.3.2 Serologic Testing

Seroprevalence was determined by testing elephant blood samples for antibodies to *Mycobacterium tuberculosis* complex (MTBC) bacteria using two serologic assays used for elephants. A total of 35 African elephants were sampled at the four facilities in September 2014. Five animals were not sampled because they could not be sampled safely. Blood samples were collected from the auricular or saphenous vein, and allowed to clot at room temperature before testing, then centrifuged to separate serum. Each serum sample was tested using two serologic tests for MTBC antibodies that have been validated for elephants, the ElephantTB STAT-PAK® (no longer commercially available; Chembio Diagnostic Systems, Inc., Medford, NY, USA) and the DPP® VetTB Assay for Elephants (Chembio Diagnostic Systems, Inc., Medford, NY, USA), according to manufacturer's instructions. STAT-PAK detects antibodies to ESAT-6, CFP10, and MPB83 (Lyashchenko et al. 2006) and DPP detects antibodies to MPB83 and a CFP10/ESAT-6 fusion (Lyashchenko et al. 2012). Each STAT-PAK and DPP test result was interpreted as reactive (clearly visible test line), suspect (a faint color change response), or negative (no visible test line). During January 2015, blood samples were collected as part of another study from a subset of eight of the original 35 elephants, and STAT-PAK and DPP tests were repeated. In February 2016, 10 of the original elephants were tested again using STAT-PAK. If the STAT-PAK was reactive on this occasion, the sample was also tested using DPP. For each sampling period, STAT-PAK and DPP results were interpreted in parallel, such that if an elephant had any reactive result to either the STAT-PAK or DPP, it was considered seropositive.

All serological testing was performed with the written consent of elephant owners. Approval for this study was obtained from the animal ethics committee at the University of Pretoria, the Department of Livestock and Veterinary Services, and the Division of Research, Diagnostics and Technical Services in the Zimbabwe Ministry of Agriculture, Mechanisation and Irrigation Development.

4.3.3 Statistical Analysis

Descriptive statistics including counts, frequencies, 95% confidence intervals, mean, standard deviation, and ranges were calculated to assess seropositivity and the data distribution for potential risk factors. For each facility, seroprevalence was calculated as the proportion of seropositive elephants among the total number of elephants tested during each sampling period. For the risk factor analysis, a group of factors/characteristics from the survey data were selected based on potential to serve as biological risk factors. Risk factors were assessed as categorical variables, and included the following: sex, time in captivity (< 15 years or ≥ 15 years), overnight contact with other elephants (yes or no), contact with wild elephants (yes or no), birthplace (captive or wild-born), shared feed with wildlife (yes or no), and facility (A, B, C, or D). To evaluate potential associations between data collected under different survey questions, correlation between variables was assessed using Spearman's correlation, with correlation values of $\rho \geq 0.6$ being considered correlated. When a strong correlation between variables was found, the decision about which of the variables to use for the risk factor analysis was made based on biological plausibility. For the risk analysis, a candidate model set of 6 models was built using a simple univariable logistic regression model for each factor and an intercept-only model to represent a null model; serological status (positive or negative) was the outcome variable in this analysis. Akaike's Information Criterion adjusted for small sample sizes (AICc) was calculated for each model and was used to select the best model where the highest-ranked model carries the lowest AICc value and highest Akaike weight (Burnham and Anderson 2002). All analyses were performed using the AICcmodavg package (Mazerolle 2016) in R 3.3.2 (R Core Team 2016).

4.4 Results

4.4.1 Survey Results

The number of elephants at each facility ranged from 2–17 throughout the study. The sex ratio of sampled elephants was approximately equal (17 females, 18 males). The mean reported age of the elephants was 22.0 years (SD 11.4, range 4–41 years), and 51% of the population sampled had reached

breeding age. However, some ages were approximate, because ages for wild-born elephants were estimated and the majority (83%) of elephants were wild-born. The average estimated time under human management was 13 years, but actual time under human management for older animals was greater given that history at any previous facility may be unknown.

Wild-born elephants at the facilities originated from within Zimbabwe. Elephants were routinely used for morning and evening rides for tourists, lasting approximately 45–60 minutes each. In addition, tourists had the opportunity to feed, touch, and take photos with the elephants for periods of 5–25 minutes. During the day, elephants were allowed to forage on the facilities' property under the supervision of staff. Wildlife within the Victoria Falls National Park had access to the facilities (Figure 4.2), and free foraging during the day could bring working elephants into contact with species including wild elephants, buffalo, greater kudu (*Tragelaphus strepsiceros*), impala (*Aepyceros melampus*), bushbuck (*Tragelaphus scriptus*), waterbuck (*Kobus ellipsiprymnus*), common duiker (*Sylvicapra grimmia*), common warthogs (*Phacochoerus africanus*), Burchell's zebra (*Equus quagga burchellii*), giraffe (*Giraffa camelopardalis*), banded mongoose (*Mungos mungo*), chacma baboons (*Papio ursinus*), and vervet monkeys (*Chlorocebus pygerythrus*).

Figure 4.2. Warthogs and an elephant feeding from the same trough at an ecotourism facility in Zimbabwe.

Elephants at all four facilities were kept in conditions that allowed for natural breeding. All facilities had at least one breeding bull elephant, and three facilities had at least one previous known elephant pregnancy. Three facilities used gonadotropin-releasing hormone (GnRH) vaccine to prevent musth and aggressive behavior in bulls. Elephants at two facilities were permitted overnight contact with other elephants in the herd, while overnight contact was restricted at the other two facilities. There were no indoor elephant enclosures at any of the facilities. During the day when foraging, elephants had direct and close contact with the other elephants and caretakers. In addition to foraging, the elephants' diet was supplemented with one or more of the following items: additional browse, local hay (with molasses or salt additives), commercial game cubes, green bana grass (*Pennisetum* hybrid), commercial horse feed, or vegetables. At three facilities, warthogs were reported to have contact with elephant feed or were observed in close contact with elephants, especially during feeding in stable areas, including sharing game cubes and vegetables from feeding containers (Figure 4.2). Baboons and mongoose were also reported or observed to have contact with elephant feed. Potential contact with other wildlife was indirect through shared foraging areas. At three facilities, contact with wild elephants was possible but discouraged.

Elephants at all facilities were managed using unprotected contact. Handlers were not assigned to work with the same individual elephants but rotated through the herd. Full-time elephant handlers ranged from 8–14 per facility; part-time or volunteer staff were not used. Staff testing for TB was not required at any facility.

4.4.2 Serological Test Results

Thirty-five elephants were tested with both STAT-PAK and DPP (Table 4.1) during the first round of sampling in September 2014. Six elephants were reactive to STAT-PAK (17.1% seroprevalence, 95% CI: 4.7–29.6%), but none were reactive to DPP. In the second round of sampling during January 2015, eight of the 35 elephants were re-tested using both STAT-PAK and DPP. All eight elephants had been seronegative during the first round of sampling, and all STAT-PAK and DPP tests were non-reactive during the second round of sampling. In the third round of sampling during February 2016, 10 of the 35

elephants were re-tested using STAT-PAK. Two of these elephants had been seropositive in the first round of sampling, and an additional four elephants had also been tested during the second round of sampling. Nine of the tested elephants had a non-reactive STAT-PAK, and one was reactive. The reactive elephant, which had previously had a reactive STAT-PAK in the first round of sampling, was tested using DPP and that result was negative. In total, six out of 35 (17.1%; 95% CI 4.7–29.6%) elephants were classified as TB seropositive based on parallel interpretation of STAT-PAK and DPP results. One of these elephants was classified as seropositive during two sampling sessions. All other elephants were classified as TB seronegative.

Table 4.1. Seroprevalence of *Mycobacterium tuberculosis* complex (MTBC) antibodies in captive elephant facilities in Zimbabwe across sampling periods.

	First sampling (Sept. 2014)			Second sampling (Jan. 2015)			Third sampling (Feb. 2016)		
Facility	# elephants	# screened	# seropositive (%)	# elephants	# screened	# seropositive (%)	# elephants	# screened	# seropositive (%)
A	16	12	1 (8.3)	17	4	0 (0.0)	17	5	0 (0.0)
B	4	4	0 (0.0)	2	1	0 (0.0)	2	0	0 (0.0)
C	9	8	3 (37.5)	4	0	0 (0.0)	3	2	0 (0.0)
D	11	11	2 (18.2)	11	3	0 (0.0)	11	3	1 (33.3)
Total	**40**	**35**	**6 (17.1)**	**34**	**8**	**0 (0.0)**	**33**	**10**	**1 (10.0)**

4.4.3 Risk Factors

Proportions of seropositive elephants and associated 95% confidence intervals, calculated for each risk factor, are shown in Figure 4.3. Strong correlations were noted between time in captivity and birthplace ($\rho = 0.67$), and between contact with wild elephants and facility ($\rho = 0.86$). Based on their perceived biological relevance, time in captivity and contact with wild elephants were included in the logistic regression analyses and models were ranked using Akaike weights (Table 4.2). The top-ranked model ($w = 0.41$) was for time in captivity, with elephants that had spent a shorter time in captivity (<15 years) having higher seroprevalence (36.4%) compared to elephants that spent >15 years in captivity (8.3%). The intercept-only model ranked second ($w = 0.18$) suggesting some model selection uncertainty, and the model for shared feed with warthogs or other wildlife ($w = 0.13$) ranked third, with elephants sharing feed with wildlife having higher seroprevalence (19.4% compared to 0%). Models for contact

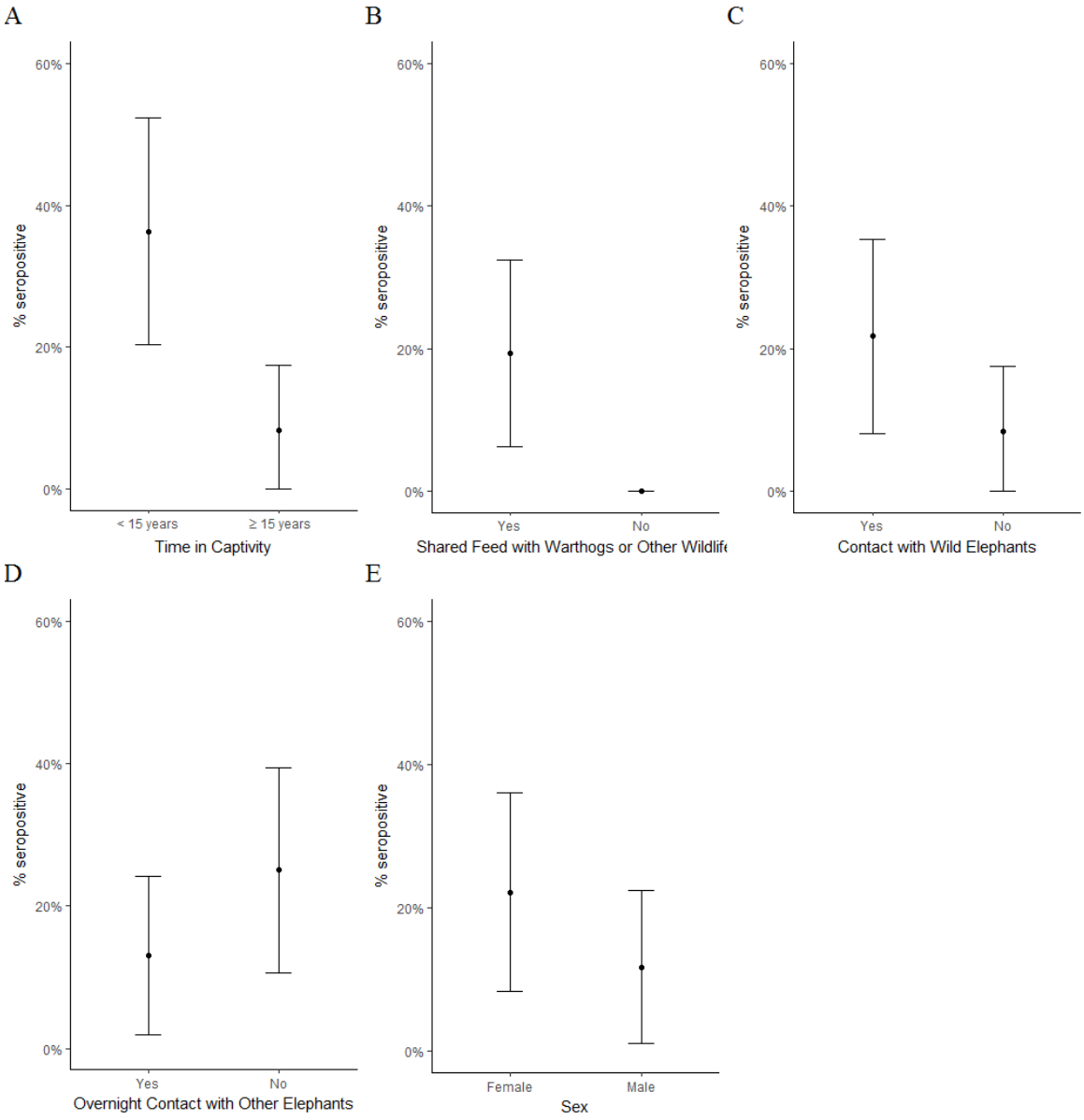

Figure 4.3. Proportions of captive African elephants that were *Mycobacterium tuberculosis* complex (MTBC) seropositive, reported for five potential risk factors: A) time in captivity, B) shared feed with warthogs or other wildlife, C) contact with wild elephants, D) overnight contact with other elephants, and E) sex. Error bars indicate 95% confidence intervals.

Table 4.2. Candidate logistic regression models for *Mycobacterium tuberculosis* complex (MTBC) seropositivity in captive elephants in Zimbabwe.

Model	**LL**	**K**	**AICc**	**ΔAICc**	***w***
Time in captivity	-14.09	2	32.56	0	0.41
Intercept only	-16.04	1	34.19	1.63	0.18
Shared feed	-15.23	2	34.84	2.27	0.13
Contact with wild elephants	-15.48	2	35.34	2.78	0.10
Overnight contact with elephants	-15.65	2	35.68	3.12	0.09
Sex	-15.69	2	35.76	3.20	0.08

K: number of parameters; LL: log likelihood; AICc: Akaike's information criterion; ΔAICc: difference in AICc from minimum AICc model; w_i: Akaike weight

with wild elephants, overnight contact with elephants, and sex ranked approximately equally (w = 0.08–0.10). For these variables, seroprevalence was higher in elephants that had contact with wild elephants (21.7% compared to 8.3%), no overnight contact with other captive elephants (25% compared to 13%), or were female (22% compared to 11%).

4.5 Discussion

This study provides the first assessment of TB seroprevalence in captive African elephants in a range country and investigates factors associated with positive serological responses. At the facilities under study, 17.1% of 35 elephants tested were seropositive during the first round of sampling. Seroprevalence in later sampling sessions, where a subset of elephants were tested, ranged from 0–10%. In addition, this study describes management of captive African elephants in Zimbabwe and explores potential factors associated with TB seropositive status. The risk factor most strongly associated with seropositive status was time in captivity. Models for contact with wild elephants, sex, and overnight contact with other captive elephants carried less weight.

It is important to note that this study only screened elephants for antibody responses to MTBC antigens, thus, a seropositive result cannot be considered equivalent to a diagnosis of clinical TB. Accurate and affordable tests for diagnosing mycobacterial infection are needed for elephants and other non-domestic species. However, serological testing provides a valuable starting point for screening elephants potentially at risk for MTBC exposure. Other immunoassays, such as the interferon gamma release assay, for detecting TB in elephants have been developed and show promise for antemortem diagnosis (Angkawanish et al. 2013; Paudel et al. 2016). However, these assays require special processing of blood samples and a laboratory with the expertise to run ELISAs, which can be a significant constraint to using these tests in areas with limited resources. While mycobacterial culture is considered the gold standard for TB diagnosis in elephants (Mikota and Maslow 2011), there are also major challenges to obtaining samples for culture among elephant populations under human care in range countries. First, elephants must be trained to perform a trunk wash procedure to collect samples for culture (Mikota and

Maslow 2011). Trunk wash training is not a routine procedure at ecotourism facilities in Africa, and requires specialized training of both elephants and their handlers. This training is under way at the four study facilities in order to be able to perform trunk washes in the future. Mycobacterial culture is also time-consuming, requires sample submission to a laboratory capable of growing and differentiating mycobacterial species, and may have a reduced sensitivity because samples frequently can be contaminated. Because elephants constantly interact with their environment using their trunks, trunk wash samples in culture are prone to overgrowth of environmental microorganisms (Mikota and Maslow 2011). At ecotourism facilities, where elephants are housed outdoors in natural habitat, environmental contamination is more likely to interfere with culture.

In lieu of culture, serology allows for simplified and expedited routine testing of elephants that may be at risk of TB. Blood samples can be obtained more easily than trunk washes, tests can be performed on site in the field, and results are obtained quickly (within approximately 20 minutes). STAT-PAK and DPP have high reported sensitivity (100%) and specificity (95% and 100%, respectively) in isolated studies in captive elephants in the US and Europe (Greenwald et al. 2009). However, interpretation of test results must take into consideration differences in host species, local factors (e.g., presence of cross-reacting non-tuberculous mycobacteria), and true disease prevalence (Dohoo et al. 2009). Serologic tests may not conclusively identify infected elephants; for example, captive Asian elephants in Thailand that were confirmed to have *M. tuberculosis* infection had one or more negative STAT-PAK tests, including an individual that died of severe clinical disease hours after testing (Angkawanish et al. 2010). Species differences between Asian and African elephants may influence serologic test performance, given that only the immune response of Asian elephants to TB has been characterized in other studies (Landolfi et al. 2010; Landolfi et al. 2014). Conversely, STAT-PAK false positives are known to occur in cases of non-tuberculous mycobacterial infection (Lacasse et al. 2007; Greenwald et al. 2009) and with chronic inflammatory conditions like arthritis (Greenwald et al. 2009). Infection with non-tuberculous mycobacteria may alter the antibody profile that elephants produce (Lyashchenko et al. 2012). The elephants in this study may be more likely than zoo elephants to be

exposed to both tuberculous mycobacteria, due to their location in a high TB burden country, and non-tuberculous mycobacteria, given their outdoor housing and foraging behavior. These factors should be taken into consideration when interpreting serological test results, which can still provide important information that, when used in combination with other diagnostics, can inform prevention and control strategies for reducing the risk of MTBC transmission.

Interestingly, all seropositive elephants in this study were reactive to STAT-PAK but not DPP. The STAT-PAK positive results could indicate early infections, as STAT-PAK uses a larger volume of serum than DPP (30 µl compared to 5 µl; Greenwald et al. 2009), and therefore has a lower threshold of detection. Based on the lower specificity of STAT-PAK compared to DPP, these results could also indicate false positive STAT-PAK results in this population, possibly as the result of factors such as non-tuberculous mycobacteria or inflammatory conditions. One elephant that was seropositive during the first round of testing became seronegative on the third round of testing. In this case, the elephant may have cleared a TB infection, as was hypothesized for elephants with intermittent borderline reactive DPP results after exposure to a TB-infected elephant (Vogelnest et al. 2015). In addition, there was one seroreactive elephant that remained seroreactive on the third round of testing; although DPP was nonreactive, this finding may increase suspicion of a true MTBC infection.

In humans, a positive serological test result without accompanying clinical disease or detection of *M. tuberculosis* is interpreted as a latent infection, but it is unclear if elephant serological responses should be interpreted similarly (Ong et al. 2013). Given their apparent predilection toward strong humoral responses to mycobacterial infection, elephants may become persistently seropositive years before *M. tuberculosis* or *M. bovis* are isolated from trunk wash culture samples (Greenwald et al. 2009; Lyashchenko et al. 2012; Vogelnest et al. 2015). Increased monitoring with DPP (as STAT-PAK is no longer available) can show persistent reactions or those of increasing intensity that are suggestive of active TB disease (Vogelnest et al. 2015). Elephants with positive serological tests results should be monitored for clinical signs associated with TB, and subjected to increased TB diagnostic testing such as more frequent serological assays and trunk wash cultures.

Potential risk factors in this population may differ from other elephant populations where epidemiological studies have identified risk factors because of contrasts in environment and elephant management practices. For example, working Asian elephants with an assigned mahout (elephant handler) have an increased risk of TB seropositivity (Yakubu et al. 2016), but elephants at the African facilities do not have assigned handlers. Close and prolonged contact between humans and elephants, such as spending hours indoors with infected elephants (Zlot et al. 2015), or cleaning barns and aerosolization during pressure washing (Miller and Olea-Popelka 2013) have been implicated in human cases of TB at animal facilities. However, indoor elephant housing does not exist at the facilities in this study, nor are pressure washers in use. Determining risk factors for transmission of TB among elephants and between elephants and humans at ecotourism facilities is crucial to enacting preventive measures to protect both elephants and humans.

In this analysis, time in captivity was the most important variable associated with higher risk for seropositive status in elephants based on AICc weight. The association of seropositive status with a shorter time in captivity is somewhat counterintuitive, as increased time in captivity would be expected to be associated with increased human exposure and therefore risk of TB. This may be in part due to the correlation observed between age and time in captivity, as elephants that have spent less time in captivity are generally young. Young elephants could be more curious in exploring the environment with their trunks, leading to an increased exposure to environmental organisms. Young elephants or those that have spent less time in captivity may be handled more for training purposes, and increased handling time could increase risk of exposure to human TB if handlers are infected. One major potential factor not explored in this study is the TB status of elephant handlers, and TB transmission is most likely to occur from humans to elephants in high TB burden countries (Lassausaie et al. 2014). Alternatively, seropositive results may have been more likely in elephants that have been exposed or infected but have not had time to observe a waning humoral response. In cattle, antibody responses to mycobacteria can wane over time (Waters et al. 2010), thus if older elephants or those in captivity for longer were exposed to mycobacteria in the distant past, they may no longer have a robust antibody response.

The null or intercept only model, ranked second, serves as a comparison point for the models evaluated in this study. That the intercept-only model was highly ranked and all of the models were within 3-4 AICc units of each other suggests that there is some model selection uncertainty and that other factors that were not evaluated may be involved. With the relatively small sample size in our study, it was not possible to adequately investigate multivariable logistic regression models that could better reflect the multifactorial etiology of TB and allow for potential compounding or interactive effects among different factors. Nonetheless, the relative Akaike weights of the evaluated models for risk factors may allow for prioritization of variables to explore in future studies.

Interactions with other wildlife through shared feed may also be important in elephant seropositive status. All seropositive elephants were housed at facilities that reported having warthogs or other wildlife around their stables. At one facility in this study, a banded mongoose colony appeared to use a hay barn as a semi-permanent refuge. Warthogs are known spillover hosts of TB (Renwick et al. 2007), and wildlife having access to stored food may be a factor worth further investigation, given the importance of shared feed in other ecosystems in which TB is present and transmitted across species. For instance, European badgers (*Meles meles*) are known to consume cattle feed, and indirect contact through shared feed or direct contact during feeding may be important for *M. bovis* transmission between badgers and cattle (Garnett et al. 2002). In North America, supplemental feeding of wildlife is considered a risk factor for *M. bovis* transmission (Miller and Sweeney 2013), and indirect transmission through feed sharing has been documented from experimentally infected white-tailed deer (*Odocoileus hemionus*) to cattle (Palmer et al. 2004). Securing food from free-ranging wildlife and preventing wildlife contact while elephants are feeding would reduce the potential risk of pathogen transmission via shared feed.

Seroprevalence was higher among elephants that had contact with wild elephants. It is unknown whether wild elephants in the area are infected with mycobacteria and could serve as a source of infection to captive elephants. However, this finding has important conservation implications, as seropositive captive elephants have the potential to transmit pathogens when in contact with wild elephants. Although contact between captive and wild elephants is discouraged, interactions between them have occurred.

Such interactions have included captive elephants mating with wild elephants, captive elephants taken into a wild herd, and an orphaned wild-born elephant being adopted into a captive herd. These interactions demonstrate the potential for close contact between captive and free-ranging populations that could put elephants at risk for pathogen transmission.

The remaining factors carried less weight in terms of importance to seropositive status indicating that they are less important in shaping seroprevalence. Seroprevalence was higher among elephants without overnight contact with other captive elephants. Again, this finding is somewhat counterintuitive, as higher seroprevalence might be expected in elephants with overnight contact, given the opportunity for transmission via direct contact. This finding may be associated with other management differences between facilities that contribute to variation in risk but were not measured in this study. Seroprevalence was higher among female elephants (22.2%) than males (11.8%), which is in contrast to trends reported in working Asian elephants (Lassausaie et al. 2014; Yakubu et al. 2016). Most of the male elephants in this study were housed at facilities that reported using GnRH vaccine to suppress musth. High testosterone levels during musth may result in immunosuppression (Sukumar 2003), thus suppressing musth might indirectly benefit bull elephant immunity.

The captive elephants at the facilities under study are present in a unique community of mycobacteria and other wildlife. Humans in these communities are hosts to *M. tuberculosis*, and *M. bovis* is present in cattle in neighboring Zambia (Phiri 2006; Munyeme et al. 2009; Munyeme et al. 2010; Muma et al. 2013). An emergent MTBC species, *M. mungi* (Alexander et al. 2010), has been identified in banded mongoose in northwest Zimbabwe (Alexander et al. 2016). Banded mongoose in South Africa were recently found to be hosts of *M. bovis* (Brüns et al. 2016), and nearly all of the other wildlife species reportedly seen on the facilities are known natural hosts of *M. bovis* or other tuberculous mycobacteria (Mukundan et al. 2015). A variety of non-tuberculous mycobacteria have been cultured from tissues of South African wildlife species, including elephants (Botha et al. 2013), and non-tuberculous mycobacteria can induce cross-reaction to MTBC antigens (Gcebe and Hlokwe 2017). Relatively little is known about movement of mycobacteria among humans, wildlife, and domestic species, especially in southern Africa.

Deceased working elephants and, when possible, other susceptible wildlife hosts should have a post-mortem examination conducted and any tuberculous lesions cultured for mycobacteria. Post-mortem examinations are a critical surveillance tool; however, implementation may be limited by finding fresh carcasses and the logistics associated with sampling large animals in field settings.

Better understanding the dynamics of infection among and between species will be crucial to effectively preventing and controlling diseases within animal populations within TFCAs. In addition, ecotourism facilities in the area can take steps to limit risks to their elephants and staff. An important first step would be implementation of routine TB screening for elephant handlers at the time of hiring and annually, allowing timely treatment of TB infected handlers. Elephants should be screened for TB using serologic testing with the DPP; elephants reactive on serology should receive increased monitoring for TB and increased testing frequency using serologic tests or culture. Additionally, other screening and diagnostic tests (e.g., interferon gamma assay and mycobacterial culture) should be evaluated and considered to continuing monitoring this population of African elephants in the KAZA TFCA. Securing feed sources from other wildlife would eliminate a known route for TB transmission. Continuing to explore potential risk factors, including others we did not evaluate, is warranted in future studies. Elephant facility owners should be proactive in working with local public health authorities, veterinarians, employees, and other stakeholders in creating an informed management plan to mitigate risk of pathogen transmission to or from humans, elephants, and other wildlife.

CHAPTER 5: ESTIMATING DENSITY OF EUROPEAN BADGERS (*MELES MELES*) IN IRELAND UNDER BOVINE TUBERCULOSIS MANAGEMENT

5.1 Summary

Diseases shared between livestock and wildlife, such as bovine tuberculosis, are challenging to manage. Ireland is one of many countries with a bovine tuberculosis control program, which has been hampered by the presence of a wildlife reservoir, the European badger (*Meles meles*). Badgers have historically been culled for tuberculosis management, but are now being vaccinated as part of the country's eradication plan. Badger population density is an important factor in rates of tuberculosis in cattle, therefore determining the effectiveness of vaccination as a control strategy depends on the underlying density of badger populations. We implemented spatially explicit capture-recapture models using data from a multi-year vaccination trial in County Kilkenny, Ireland, to estimate badger population density according to environmental and management covariates. We found that soil drainage determined badger density, with an estimated 0.8 badgers/km^2 in the moderately drained soil present in most of our study area (range 0.49–1.24 badgers/km^2 for all soil types). We also found evidence of considerable trap-wariness, with the baseline probability of capture decreasing from 0.141 in naïve badgers to 0.045 in previously captured badgers. The magnitude of the behavioral response to trapping we observed has important implications for management using vaccination. Our results provide a baseline density estimate at the start of the vaccination program, and a framework for use in estimating badger densities elsewhere and over time as disease management continues. Monitoring host population density is important for understanding the ecological effects of disease management programs and evaluating the success of management interventions.

5.2 Introduction

The interface between livestock and wildlife inherently creates a pathway for pathogen transmission in both directions (Bengis et al. 2002), and diseases shared between livestock and wildlife are a source of human-wildlife conflict (Woodroffe et al. 2005). These diseases can have dramatic socioeconomic impacts from lost livestock production, and may create concerns for wildlife conservation as well (Cleaveland et al. 2001; Gortázar et al. 2007; Caron et al. 2013). Livestock-wildlife interactions are increasingly relevant as growing global demand for meat protein increases livestock production (Tilman and Clark 2014).

A multi-host approach is required to manage diseases at the livestock-wildlife interface, involving control measures in both livestock and wildlife populations combined with prevention of transmission (Miller et al. 2013). Once a disease has been introduced into a wildlife population, disease control in wildlife typically takes the form of host population control or vaccination (Gortazar et al. 2015). Reducing population density of a host species is a common disease control method, based on the general principle that infectious diseases often function as density-dependent processes (Wobeser 1994). However, vaccination has the benefits of being non-destructive to the target species and typically being a more socially acceptable management strategy, although delivery at a broad spatial scale is challenging (Gortazar et al. 2015). Disease management programs should include a measurement of efficacy to assess progress towards achieving the program's objectives (Wobeser 1994).

Countries around the world have programs aimed at controlling or eradicating bovine tuberculosis (TB), a disease of economic importance and zoonotic potential that affects livestock and wildlife (Thoen et al. 2006). Bovine TB, caused by *Mycobacterium bovis*, is particularly relevant in Ireland, one of the world's largest beef exporters (Bord Bia 2017), where annual costs of managing the disease may exceed €60 million (Abernethy et al. 2013). Ireland has actively managed TB in cattle since 1950, with rigorous annual test and slaughter regulations currently in place, resulting in considerable reduction but not eradication of the disease (Good 2006). Failure to eradicate TB in cattle despite strict testing points to a

reservoir for infection; in this setting, the European badger (*Meles meles*) is considered the wildlife reservoir for bovine TB (Noonan et al. 1975).

Historically, badgers have been culled locally in response to TB "breakdowns" (outbreaks) in cattle if an introduced cattle source or residual infection from a prior breakdown could not be identified, with the goal of reducing badger density (Good and Duignan 2017). Badger removal has been shown to reduce TB incidence in cattle in Ireland during two major field trials, the East Offaly Project (Ó Máirtín et al. 1998; Eves 1999) and the Four Area Project (Griffin et al. 2005). The status of badgers as a protected species in Ireland necessitates a more sustainable approach to disease management, such as vaccination, going forward. The goal of a vaccination program in badgers is to maintain a badger population that is more resistant to bovine TB, and limit the transmission of *M. bovis* between cattle and badgers (and *vice versa*) . Experiments using the Bacille Calmette-Guerin (BCG) vaccine have showed it to be safe and able to reduce TB severity and progression in badgers (Lesellier et al. 2006; Lesellier et al. 2011; Perrett et al. 2018), but unable to offer complete protection (Buddle et al. 2011), and the duration of immunity under field conditions is unknown. From 2009–2013, a large-scale trial was conducted in County Kilkenny, Ireland, to assess the use of BCG in badgers as an alternative to culling (Aznar et al. 2011). This initial trial had favorable results and led to a second experiment across six counties (More and Good 2015). Vaccination of badgers has been officially included in Ireland's bovine TB eradication plan (Good and Duignan 2017) as a result of these trials, but determining the efficacy of vaccination in managing TB levels in cattle will require evaluation of important confounding factors.

One such factor may be the density of badgers. If *M. bovis* is transmitted in a density-dependent manner, high badger density areas may be considered at higher risk for TB breakdowns in cattle. Areas of Ireland where TB has been prevalent in cattle have often undergone repeated badger culling operations, drastically reducing badger densities. Low TB incidence in cattle in these areas may be an indirect result of low badger density, and badger density must be quantified to address its role in TB in cattle. Badgers can be expected to become more abundant as culling is reduced under the vaccination program, potentially increasing the risk of TB in cattle if vaccination of badgers is not effective in the long term.

Badgers are challenging to capture (Byrne, O'Keeffe, et al. 2012; Martin et al. 2017), as they are nocturnal animals and also spend a significant portion of time underground in shared burrow systems called setts (Roper 2010). Badger sett densities have been estimated using a variety of methods (Byrne, Acevedo, et al. 2014), but actual badger densities have been more difficult to estimate from capture data. Simulation models of badger management in Ireland showed that the success of vaccination depends in part on population density and the proportion of the population infected (Abdou et al. 2016), making density estimates valuable in predicting the performance of vaccination as a management strategy. Accounting for local badger density as a potential driver of cattle TB risk will be important for evaluating the efficacy of the vaccination program, and understanding changing disease dynamics as management evolves.

Spatially explicit capture-recapture (SECR) methods provide an estimate of population density unbiased by edge effects and imperfect detection (Efford 2017a). This is advantageous for use in badgers, where trappability is known to be low (Byrne, O'Keeffe, et al. 2012) and movements can occur over a large spatial scale (Byrne, Quinn, et al. 2014). SECR methods are applicable in studies where individuals can be uniquely identified at known locations (Efford 2017b). Physically capturing badgers for vaccination has allowed for marking of individuals via passive integrated transponder (PIT) tags and tattooing, providing a dataset suitable for SECR analysis. SECR methods have been applied to trends in frequency-dependent disease (Lazenby et al. 2018), and could be applied similarly to density-dependent dynamics in badgers over time.

Our objective was to use SECR methods to analyze available capture-mark-recapture data from a multi-year vaccination trial and develop an estimate of density for European badgers in County Kilkenny, Ireland. Our analysis provides a framework for estimating badger density in vaccination areas, which will allow for assessment of vaccine success in reducing cattle TB rates and influence on badger population dynamics over time.

5.3 Methods

5.3.1 Field Study Protocol

The study site is located in northwest County Kilkenny, Ireland (Figure 5.1). During the Four Area Project from 1997–2002, sections of County Kilkenny were designated as removal areas, where badgers were systematically removed from all known setts; buffer areas surrounding removal areas where natural barriers to badger migration did not exist and additional badgers were removed; and reference areas where badgers were not removed (Griffin et al. 1999). The study methods for the vaccine trial have been fully described by Byrne et al. (2012). Briefly, setts were assessed for signs of activity as described by Martin et al. (2017). Each week approximately 30 active setts throughout the study area were trapped. Trapping was generally conducted in two week blocks, where up to 35 traps were laid at sett entrances and along paths four nights each week. Most traps used were stopped wire restraints designed specifically for adult badgers, with occasional use of cage traps. Traps were checked the following morning by 12 PM. If a badger was captured, it was immobilized using a combination of 10 mg/kg ketamine and 0.1 mg/kg medetomidine, then weighed, sexed, and had age class (cub, juvenile, adult, or old) determined by tooth wear. Each badger received a PIT tag subcutaneously, and had the last four digits of its unique number tattooed in the inguinal region. Each badger was injected with BCG vaccine or placebo intramuscularly and then released. All badger captures were conducted under license from the Irish Department of Health and Children, and the study was approved by the University College Dublin animal ethics committee. Badgers were trapped at 569 setts throughout the Kilkenny study area over a period of 749 trapping nights from 2009–2013. Each sett was trapped for a total of 1–22 weeks throughout the study period. A total of 2295 captures of 1166 individual badgers were recorded.

6.3.2 Density Estimation

SECR methods (Appendix D) estimate maximum likelihood using data from capture locations and marked animals (Borchers and Efford 2008), and require files for trap layout and captures (Efford 2017a). A spatiotemporal capture history is constructed from these files within the secr package in R

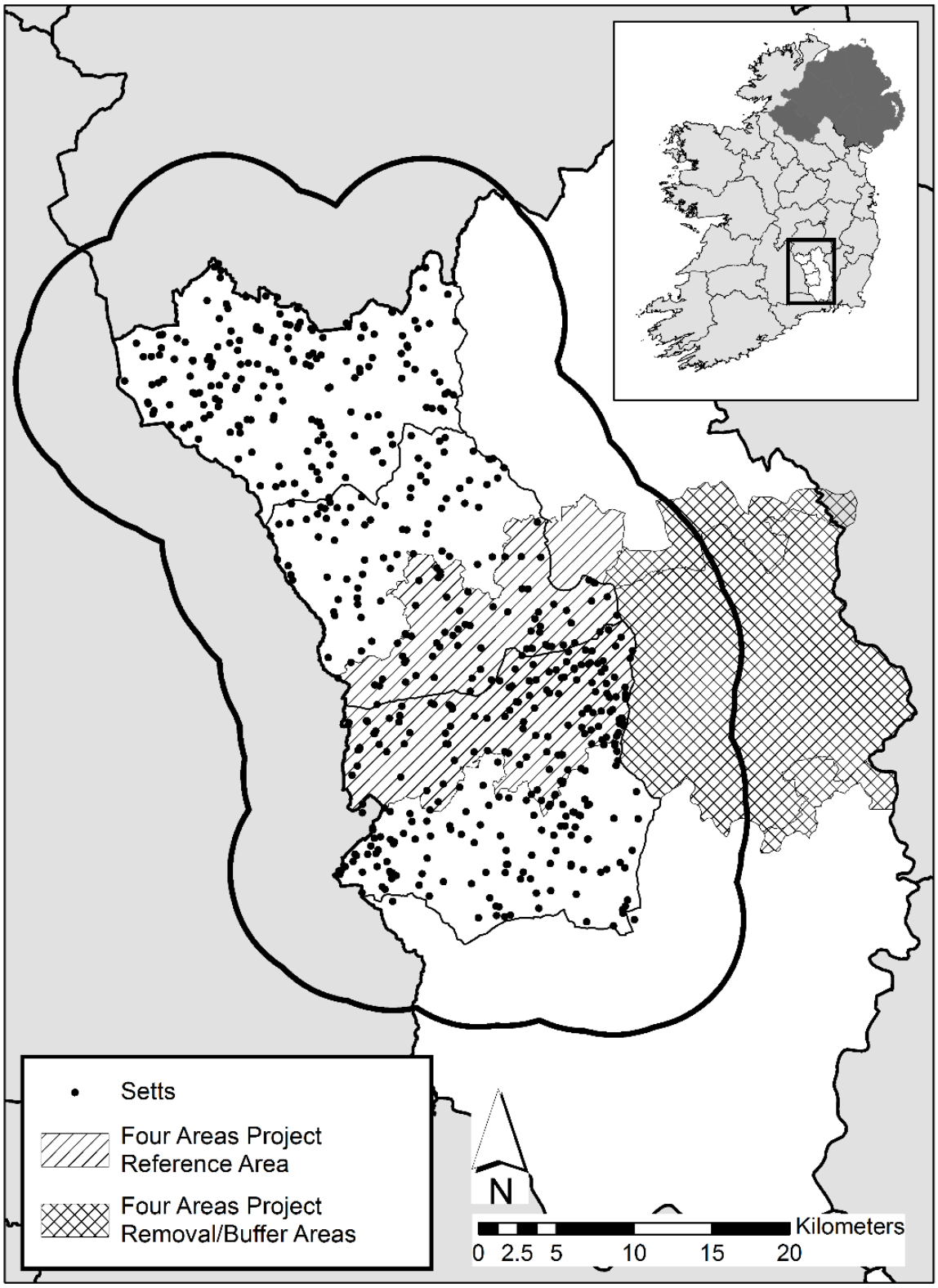

Figure 5.1: Map of the Kilkenny Vaccine Trial study area in County Kilkenny, Ireland, and setts where trapping occurred from 2009–2013. The extent of the 7 km buffer used to construct habitat masks for SECR models is shown around the study area. Also shown are the extent of removal, buffer, and reference areas from the Four Area Project.

(R Core Team 2017). The spatial modeling is then based on a habitat mask structured from habitat within a certain buffer of the detectors (Efford 2017c).

A variable number of traps (1–35 restraints and/or cage traps) were laid at known badger setts during trapping efforts, but individual trap locations were not recorded. We classified the cluster of traps at a given sett as a "multi" detector type (i.e., a multi-catch trap) (Efford 2017d) in our models and used the sett Cartesian coordinates as the coordinates for the detector. We divided our dataset into multiple sessions to better meet assumptions of population closure within a trapping period. Sessions are assumed to be independent and recaptures are only considered based on captures within a session rather than the entire study period (Efford et al. 2009; Efford 2017e). Session length determines the number of recaptures recorded, and must be balanced to be long enough to accumulate adequate recaptures to perform an analysis but short enough to assume a stable population. We determined session length by looking at the percentage of setts each week that had been trapped in the previous week, and starting a new session in weeks where $\leq 50\%$ of setts had been trapped in the previous week to minimize dependence between sessions, with a minimum session length of 10 weeks. We accounted for varying effort in trapping (Efford et al. 2013) within each session by recording a binary usage history of whether each sett was trapped on each occasion (trap night). We created a trap layout file for each session detailing all detectors (setts) used, their Cartesian coordinates, and their usage within the session. Badgers were uniquely identified by PIT tag number, or tattoo number if the full PIT tag number was unavailable or the tattoo was not matched to a PIT tag. We assigned each badger to a binary age class based on the recorded age class: juvenile, which included cubs and juvenile badgers, or adult, which included adults and "old" badgers. Our capture file included the badger's identifier, the session and occasion of capture, the detector (sett) at which the badger was captured, and its age class at that capture.

We chose to maximize the full likelihood to obtain density estimates directly (as opposed to derived estimates when maximizing the conditional likelihood), which allowed us to model heterogeneity in density according to habitat covariates (Borchers and Efford 2008). We estimated three parameters in multi-session models by maximizing the full likelihood using a Newton-Raphson optimization algorithm:

density (D), the intercept of the detection function (g0), and the spatial scale of the detection function (σ). We modeled a hazard rate detection function (Efford 2017d) as it has a long tail, which can accommodate occasional large-scale movements, consistent with what has been observed in badgers in Ireland (Byrne, Quinn, et al. 2014).

We hypothesized that the observation model parameters (g0 and σ) might vary by three factors (Efford 2017b): a learned behavioral response (b) (Borchers and Efford 2008; Efford 2017d), resulting in a step change after the first detection (Borchers and Efford 2008); a site-specific learned response (bk), resulting in trap-happy or trap-shy behavior relative to a specific detector (*sensu* Royle et al. 2011); and individual heterogeneity (hcov), modeled here as a two-class hybrid mixture model (Borchers and Efford 2008; Efford 2017f) of age class. If class is known for all individuals, as it was in our case, the mixture model is equivalent to a covariate (Efford 2017f). We hypothesized that learned responses would reflect trap-shy behavior (Roper 2010; Byrne, O'Keeffe, et al. 2012), and that juvenile badgers might range farther than adult badgers as a result of dispersal. Models fitted by maximizing the full likelihood can only handle a single individual-level covariate in the finite mixture model (Efford 2017f), so we opted to include age class, which has important management considerations: early vaccination of young badgers hopefully provides protection against TB before they are exposed, and badgers of a particular age class can be targeted by using different trapping methods (cage traps for young badgers, and stopped restraints for adult badgers).

Initial test models were fitted using a mask with a buffer size of 4988 m ($4\sigma_{\text{half-normal}}$ calculated using the RPSV function, using the largest σ value from all sessions), but retrospective checks of buffer size using the suggest.buffer() function and examination of plots of varying buffer width produced using the esa.plot() function indicated a larger buffer was needed (Efford 2017c). Long-tailed detection functions such as the hazard rate, which we used, require a large buffer for stable density estimates (Efford 2017c). A buffer of 7000 m was adequate based on visual inspection of a plot generated using esa.plot() from a fitted model using a hazard rate detection function. The final habitat mask was defined by buffering traps by 7000 m (Figure 5.1), with grid points spaced at 800 m across the mask.

We classified land within the habitat mask according to different covariates: Four AreasProject land classification, land cover, soil drainage, and soil texture (Figure 5.2). We used a shapefile of the Four Area Project study area to classify land as removal (which included the Four Area Project buffer areas), reference, or neither. We classified land cover based on the CORINE Land Cover 2012 database (European Union 2018). For simplicity, categories were collapsed into “artificial,” “arable/crop,” “pasture,” “forest,” “shrubs,” “peat,” and “water.” We used existing soil drainage categories “excessively,” “well,” “moderately,” “imperfectly,” “poor,” and “other” from the Irish National Soil dataset. We used exisiting soil texture categories “alluvial,” “coarse loamy,” “fine loamy,” “loamy,” and “peat.” Areas that were categorized as “artificial” (i.e., human development) or water were excluded as non-habitat from the habitat mask. Spatial files used to create the habitat mask were generated using ArcMap 10.4.2 (Environmental Systems Research Institute, Redlands, CA).

We hypothesized that density might vary by land classification during the Four Area Project, land cover, soil drainage, or soil texture. We hypothesized that in models using Four Area Project land classification, density would be lowest in former removal areas because many badgers had been previously removed, highest in reference areas because culling was limited, and intermediate in areas outside the Four Area Project management areas. We hypothesized that in land cover models, density would be highest in forest and pasture areas because these would be associated with preferred badger habitat of field edges (Byrne, O’Keeffe, et al. 2012). Soil drainage is an important characteristic for badger sett-building preferences, and we hypothesized that density would increase as soil drainage improved based on preferences for well-drained soils (Byrne, Acevedo, et al. 2014). Soil texture has also been associated with badger sett preferences (Byrne, Sleeman, et al. 2012), and we hypothesized higher densities in areas of loamy soils over peaty soils to correspond to trends in badger sett building (Byrne, Sleeman, et al. 2012).

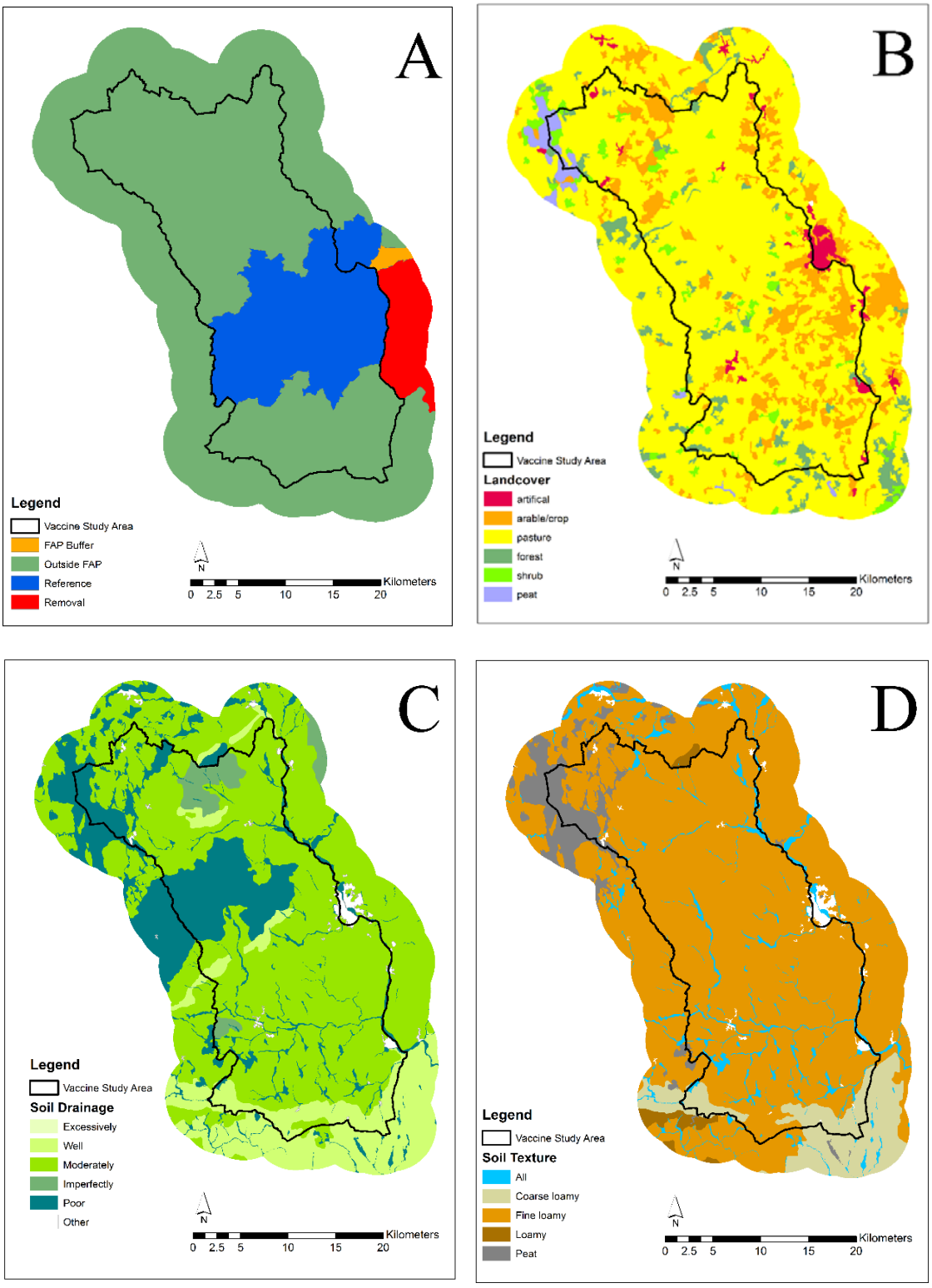

Figure 5.2: Maps of covariates of interest within the Kilkenny study area and a composite 7 km habitat mask buffer. Actual habitat masks were constructed at lower resolution, using points spaced 800 m apart. A) Land classification during the Four Area Project, B) collapsed land use categories using CORINE landcover data, C) soil drainage, D) soil texture.

We developed an a priori SECR model set and ranked candidate models using Akaike's information criterion corrected for small sample size (AICc; Burnham and Anderson 2002), using differences in AICc scores between the top-ranked model and other models, and AICc weights. We used model averaging if the top model did not receive ≥ 0.9 of the model weight (Burnham and Anderson 2002). All analyses were performed in R 3.4.3 (R Core Team 2017). We manipulated spatial files using package rgdal 1.2-16 (Bivand et al. 2017), performed SECR analysis using package secr 3.1.3 (Efford 2017b), and generated certain figures using package ggplot2 2.2.1 (Wickham 2009).

5.4 Results

5.4.1 Live Captures

The badgers captured were approximately equally distributed by sex (592 females, 574 males). Aprpoximately 80% of captures (443 females, 465 males) were categorized as adults at their last capture. The remaining badgers were categorized as juveniles (149 females, 109 males). The number of captures per badger ranged from 1–9; 568 badgers (48.7%) were recaptured at least once. The 749 capture nights in our dataset were divided into 13 independent sessions; each session contained 49–68 trap nights or occasions. The number of captures per badger within sessions ranged from 1–4, and there were 0–52 spatial recaptures (0–20.3% total captures) per session. Distance moved between recaptures within sessions (Figure 5.3) ranged from 0 (recaptured at the same sett) to 10.8 km; the mean distance was 621.3 m (SD 1370.4 m).

5.4.2 Density Estimate

A single model was selected as the top model, with 94.0% of the AIC weight (Table 5.2). This model included density varying by soil drainage (Figure 5.4), a behavioral trap response for g0, and the finite mixture model for σ. The estimated densities from the top model (Figure 5.5) were 0.80 badgers/km^2 (95% CI 0.68–0.93) in moderately drained soils, with estimates of 0.62 badgers/km^2 (95%

CI 0.50–0.77) in poorly drained soils and 1.24 badgers/km^2 (95% CI 0.97–1.60) in imperfectly drained soils, and estimates of 0.49 badgers/km^2 (95% CI 0.35–0.69) in well drained soils and 0.80 badgers/km^2 (95% CI 0.68–0.93) in excessively drained soils. The estimated g0 was 0.141 (95% CI 0.110–0.179), and with the behavioral response, 0.046 (95% CI 0.038–0.055). The estimated σ was 442.6 m (95% CI 392.5–499.1) for adults and 444.2 m (95% CI 382.7–515.6) for juveniles. The mixture proportion was adults = 0.80 (95% CI 0.76–0.84) and juveniles = 0.20 (95% CI = 0.16–0.24).

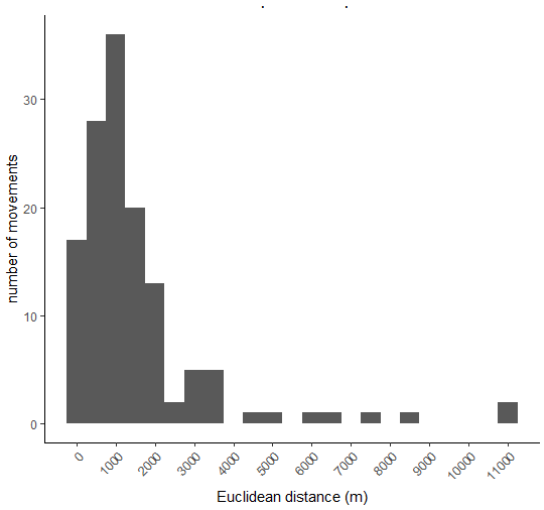

Figure 5.3: Distances moved during spatial recapture events.

Table 5.2: Candidate model set and results of AICc model selection. The log likelihood (-2LL), Akaike's information criterion value corrected for small sample size (AICc), number of parameters (K), and Akaike weight (*w*) are presented for each model.

Model	Log likelihood	AICc	ΔAICc	K	*w*
D~soil drainage, g0~b, σ~h2	-9581.287	19186.73	0	12	0.940
D~Four Area class, g0~b, σ ~h2	-9587.104	19192.3	5.57	9	0.058
D~soil drainage, g0~bk, σ ~h2	-9587.592	19199.34	12.61	12	0.000
D~land cover, g0~b, σ~h2	-9591.734	19205.6	18.σ87	11	0.000
D~Four Area class, g0~bk, σ ~h2	-9599.914	19217.92	31.19	9	0.000
D~soil texture, g0~b, σ~h2	-9596.75	19219.69	32.96	13	0.000
D~land cover, g0~bk, σ ~h2	-9600.242	19222.62	35.89	11	0.000
D~soil texture, g0~bk, σ~h2	-9602.891	19225.78	39.05	10	0.000
D~1, g0~1, σ~1	-9623.359	19256.75	70.02	5	0.000
D~1, g0~1, σ~h2	-9623.353	19258.75	72.02	5	0.000

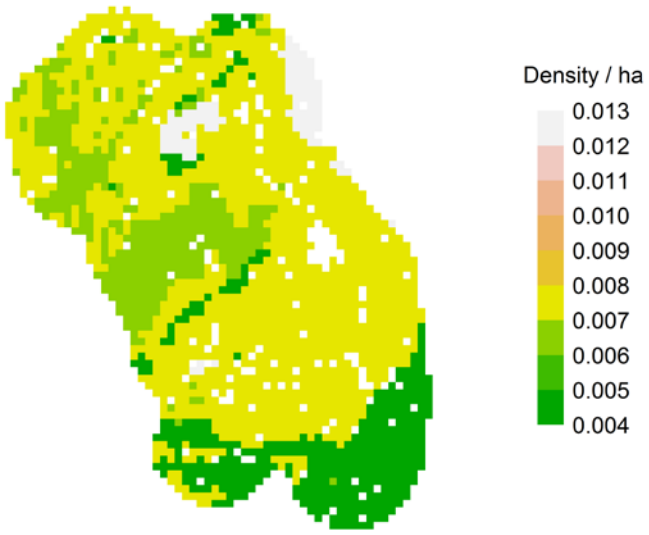

Figure 5.4: Density surface created from the top model of density varying by soil drainage, with estimated badgers/ha.

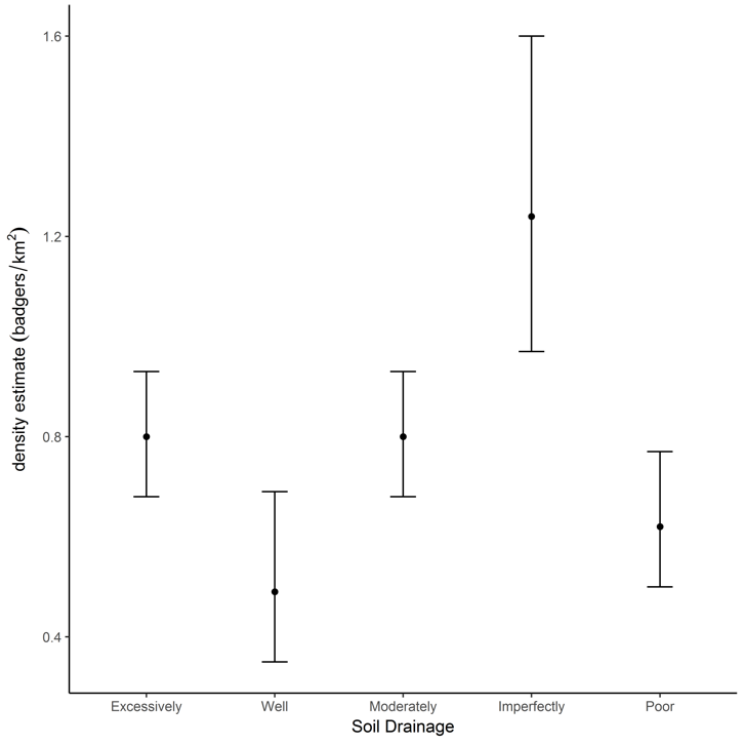

Figure 5.5: Density estimates by soil drainage and 95% confidence intervals for badgers/km^2 from the top model.

5.5 Discussion

Our study is the first spatially explicit estimate of badger density in Ireland using empirical capture data. We examined the effect of behavioral, habitat, and management predictors on badger density and detection probability in a SECR analysis. Our best model indicated a density of 0.80 badgers/km^2 in the effective sampling area within and around County Kilkenny, with densities ranging from 0.49–1.24 badgers/km^2 among different soil drainage quality. Detection probability was defined by a considerable behavioral response to trapping and neglible differences in the spatial scale of detection between age classes.

Our results provide insight into the state of badger population management at the outset of the vaccination program. The Department of Agriculture's Wildlife Unit had culled badgers to reduce densities from > 2 badgers/km^2 to 0.2–0.5 badgers/km^2 on 28% of the country's agricultural land (Good and Duignan 2017). Prior crude estimates of density in the Kilkenny area, using non-spatial methods subject to edge effect bias, were approximately 0.5 badgers/km^2 (J. O'Keeffe, unpublished data). Previous studies predicted main sett density in this area at ~0.2 main setts/km^2 (Byrne, Acevedo, et al. 2014), combined with a crude mean group size estimate of 4.1 based on pooling prior studies throughout Ireland (Byrne, O'Keeffe, et al. 2012) for a rough estimate of 0.82 badgers/km^2, which is remarkably close to our estimate of 0.80 badgers/km^2 for most of the land in the study area. Using SECR methods to estimate density may be a more sensitive method to evaluate changes under the vaccination program if mean group size increases rather than the number of main setts increasing.

Our top model was based on soil drainage, which is an important characteristic for badger sett-building (Roper 2010; Byrne, Acevedo, et al. 2014) and correlated with soil type in the national soil dataset. Badgers tend to avoid wet and poorly draining areas (O'Corry-Crowe et al. 1996; Roper 2010) and prefer dryer soils that are easy to dig out (Hammond et al. 2001). Soil characteristics were also important in a SECR study of American badgers (*Taxidea taxus*), where density varied by soil depth (Gould and Harrison 2017). Our density estimates are reasonable for this area but did not scale exactly as expected with soil drainage. The lowest badger density occurred in well-drained soils, while the highest

badger density occurred in imperfectly drained soils. The estimate for imperfectly drained soils has a larger confidence interval than our other estimates (Figure 5.3), and the study area contained only small areas of this type of soil (Figure 5.2), and therefore should be interpreted with some caution. We assumed that soil drainage and other covariates were measured without error, but some degree of measurement error may have influenced our results. The lower estimate for well-drained soils is counterintuitive based on findings from previous studies and field observations where these soils are preferred for badger setts. Exploring this relationship using capture data from the other vaccination areas would be worthwhile, to better understand trends in badger density according to soil properties.

Our top model indicated a marked trap response that affected detection probability. The baseline g0 estimate was 0.141, or a 14.1% probability of detecting a badger on a single occasion using a trap placed at its home range center. With the negative behavioral response, g0 is estimated to be 0.045, or about one third of the naïve estimate. This trap-wary response is supported by results from our descriptive analysis, which showed that while a small proportion of individuals were repeatedly recaptured, more than half of badgers were never recaptured during the study. Our analysis was limited by the fact that we used data collected from management activities rather than a study designed for spatial recapture analysis. Live-trapping generally results in fewer captures than non-invasive methods such as camera trapping or hair snares (e.g., De Bondi et al. 2010), and clearly a behavioral response impacted our ability to recapture badgers. Despite these limitations, we captured a large number of badgers over the course of the trial, and the precision of density estimates increases with > 20 recaptures (Efford et al. 2004),.

Our observation model included a hybrid mixture model to describe σ. Mixture models may represent individual heterogeneity other than the covariate of interest, which can make biological interpretation challenging (Efford 2017f), but we hoped to account for heterogeneity in movement patterns if present. Our model had a 4:1 ratio between the two classes (adults: juveniles). We expected to have mostly adults in our dataset as trapping using stopped restraints is targeted towards capturing adults. Our model results indicate the spatial scale of detection for juveniles to be virtually identical to that for adults. Other studies have found sex differences in badger movements, including smaller home range

sizes for females and “super-ranging” behavior by a minority of males that may be related to mating (Gaughran et al. 2018). Ranging behavior has ramifications for intra- and interspecific transmission based on increased contact with other individuals (Gaughran et al. 2018), so sex may be an informative covariate to explore in future models of badger density.

The scale of σ was smaller than expected. Movements ≤ 1 km were observed only 43% of the time in one previous study, with mean distance 2.6 km (Byrne, Quinn, et al. 2014). This finding may be an artifact of the trapping scheme and badger behavior. Badger trapping is specifically targeted at active setts rather than with traps randomly distributed throughout the landscape. Badgers have varying degrees of site fidelity to their setts (Stewart et al. 1999; Byrne, Quinn, et al. 2014), but badgers in our study were often recaptured at the same sett, which is not useful in a SECR framework that depends on spatial recaptures. Our σ estimates may be biased low by these non-spatial recaptures. While an altered sampling scheme might have increased the number of spatial recaptures, it would not have been feasible to conduct a live-trapping study over the same spatial and temporal scale outside of management purposes. Our inference about the top model is only in relation to the other candidate models examined our model set (Burnham and Anderson 2002). Other environmental or demographic factors that we did not test, such as sex and availability of preferred food sources, may play a role in badger densities. We think valuable inference can still be made from our data.

Our findings have serious implications for TB management in badgers. Badger trappability is generally low (Byrne, O’Keeffe, et al. 2012), but the trap-shy behavioral response quantified in our model suggests a ~70% decrease in the baseline capture probability of captured badgers compared to naïve badgers. The current intramuscular formulation used in vaccine trials, BadgerBCG, has an unknown duration of immunity and is recommended for annual use (Brown et al. 2013). A parenteral vaccine used as part of the eradication program must deliver long-lasting immunity if a majority of previously captured badgers are unlikely to be recaptured to be revaccinated. The use of an oral BCG vaccine would ameliorate the problem of capture for vaccine delivery, although it raises other concerns about consumption of baits by non-target species, particularly cattle. Vaccination of cattle is currently

prohibited in the European Union (EU Council Directive 78/52/EEC), and consuming oral BCG vaccine can result in positive tuberculin skin test in cattle (Buddle et al. 2005). A DIVA (differentiating vaccinated from infected animals) vaccine and test are needed against TB but none are currently available. Despite these limitations, initial trials in badgers have demonstrated oral BCG vaccine efficacy (Chambers et al. 2017; Gormley et al. 2017), safety (Perrett et al. 2018), and bait palatability (Gowtage et al. 2017).

Vaccination is becoming increasingly relevant as the management strategy of choice against TB in a number of systems (Buddle et al. 2013; Chambers et al. 2014), and other intramuscular BCG vaccination programs for badgers are underway in England and Wales (Brown et al. 2013; O'Connor 2016). Understanding social changes that result from reduced culling and increased badger survival due to vaccination will be an important priority going forward. The social perturbation associated with culling badgers in the UK (Tuyttens et al. 2000; Woodroffe et al. 2006) has not been observed in badgers in Ireland (O'Connor et al. 2012). Social alterations that may result from vaccination remain to be seen. Vaccination also has long-term consequences on population dynamics by increasing survival, which may lead to higher population density (Tuyttens and Macdonald 1998), as will the reduction of culling. Monitoring density of populations undergoing vaccination is important to fully understand the ecological impacts of the management program. Future models could use diagnostic test data to define disease status in the finite mixture model covariate within secr, to estimate the density and movements of susceptible compared to infected badgers, and estimate the ratio of susceptible:infected badgers. Models using empirical data can inform management decisions within an adaptive framework management (Walters and Holling 1990) in response to disease intervention outcomes.

Another consideration for changing disease dynamics under vaccination is the effect of BCG's limited efficacy. Vaccinated badgers have shown decreased disease severity, progression, and bacterial shedding in laboratory studies (Chambers et al. 2011; Lesellier et al. 2011), and vaccinated free-ranging badgers have a reduced risk of infection (Carter et al. 2012). Encouraging preliminary results from the Kilkenny vaccine area showed a decrease in seropositive and clinically diseased badgers (Good and

Duignan 2017). Vaccinated badgers may maintain a less severe infection for a longer period of time and have reduced mortality compared to unvaccinated infected badgers, and the consequences of these factors for direct transmission are unclear. The role of environmental transmission has not been well-explored in TB, but both *M. bovis* and the BCG strain can persist in soil (Young et al. 2005), which could lead to increased environmental contamination from extended low-level bacterial shedding. Further exploring the changing badger population under vaccination, and the potential role of the environment will help to elucidate the complex transmission of *M. bovis* in this system.

Our density estimates will be used in conjunction with cattle TB data to assess the impact of badger vaccination on bovine TB in Ireland. Including both hosts in a coordinated management approach is best for understanding and controlling the dynamics of a disease with multiple hosts. This study provides a framework for estimating wildlife population density in other vaccination areas, and over time as new data are collected from field operations to assess population-level impacts of vaccination. SECR models can also quantify behavioral responses – positive or negative – to trapping, which may influence managers' decision-making about trapping objectives. Our study demonstrates the value of estimating density and detection probability of animals in disease management operations. Monitoring density of marked or uniquely identifiable individuals is a valuable tool for evaluating progress and success of disease management interventions, particularly when density-dependent transmission occurs.

CHAPTER 6: SURVEY OF ANTITUBERCULOSIS DRUG ADMINISTRATION AND ADVERSE EFFECTS IN ELEPHANTS IN NORTH AMERICA

6.1 Summary

Tuberculosis, caused by *Mycobacterium tuberculosis* (*M. tb*), is a disease causing morbidity and mortality in elephants (*Elephas maximus* and *Loxodonta africana*) as well as free-ranging individuals. Elephants in North America, diagnosed with tuberculosis, are often treated with anti-tuberculosis drugs, unlike livestock species, which has necessitated the development of treatment guidelines adapted from recommendations for humans. There are few published reports describing empirical treatment, which may be complicated by poor patient compliance, interruptions in drug administration, and adverse effects. A survey of elephants in North America was conducted to compile information on treatment protocols, including drugs, dosages, and routes of administration, serum drug concentrations, and adverse effects of anti-tuberculosis treatment. Responses were received from 182 elephants, 12 of which were treated prophylactically or therapeutically with anti-tuberculosis drugs. Treatment protocols varied among elephants, and included various combinations of isoniazid, rifampin, pyrazinamide, ethambutol, enrofloxacin, levofloxacin, and ethionamide. Serum drug concentrations also varied considerably among and within individuals. Facility staff reported five elephants (5/12; 42%) that exhibited clinical signs that may have been associated with anti-tuberculosis drugs or treatment procedures. Anorexia, decreased water intake, constipation, depression, ataxia, limb paresis, and tremors were among the signs observed. Most adverse effects were reported to be moderate or severe, resulting in interruption of the treatment. The results from this survey provide veterinarians and elephant managers with valuable historical data to make informed clinical management decisions regarding anti-tuberculosis therapy in elephants.

6.2 Introduction

Infection with *Mycobacterium tuberculosis* (*M. tb*) is a growing disease concern for elephants. More than sixty elephants in the United States have been confirmed with tuberculosis (TB) since 1994, with a median point prevalence of 5.1% in Asian elephants (*Elephas maximus*) from 1997–2011 (Feldman et al. 2013). Despite ongoing research, diagnosis and treatment of TB in elephants remain a challenge for veterinarians and animal managers (Mikota et al. 2001; Maslow, Mikota, Zhu, Isaza, et al. 2005). The large size, complex anatomy, and unique physiological features of elephants confound application of commonly used methods to assess TB status. Adding to the diagnostic challenge is the fact that elephants can be infected with *M. tb* for years without showing any clinical signs of disease (Greenwald et al. 2009; Lyashchenko et al. 2012).

As long-lived charismatic megafauna, elephants diagnosed with TB can be and often are treated for the disease. Veterinarians and elephant managers play an essential role in ensuring the health and welfare of these species, although knowledge gaps regarding effective treatment of TB still exist. The decision to treat elephants is contrary to management of TB in most species, such as cattle, which are typically culled if reactive to indirect screening tests or found to be infected. The lack of precedent for treating TB in animals has required the development of treatment guidelines extrapolated from human TB treatment protocols (United States Animal Health Association 2010). Treatment of TB in elephants commonly uses combination therapy with the first line drugs isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and ethambutol (EMB) (Maslow, Mikota, Zhu, Isaza, et al. 2005; Dumonceaux et al. 2011; Vogelnest et al. 2015; Stakeholders Task Force on Management and Research Priorities of Tuberculosis in Elephants 2017). However, determining appropriate doses for elephants may be complicated by inability to accurately determine body weight of some individuals in low-resource settings (Fowler and Mikota 2006; Mikota and Maslow 2011; Brock et al. 2014). Pharmacokinetic studies of anti-TB drugs in elephants have been conducted for INH (Maslow, Mikota, Zhu, Isaza, et al. 2005; Egelund et al. 2016), EMB (Maslow, Mikota, Zhu, Riddle, et al. 2005), PZA (Zhu et al. 2005), RIF (Peloquin et al. 2006; Egelund et al. 2014; Egelund et al. 2016), and all four in conjunction (Brock et al. 2014), using

small numbers of elephants. However these studies did not evaluate treatment efficacy; therefore the therapeutic drug serum levels for effective treatment in elephants are still unknown and are currently directly extrapolated from recommended human levels known to be curative (Maslow, Mikota, Zhu, Isaza, et al. 2005; United States Animal Health Association 2010).

Other factors complicate appropriate treatment of TB in elephants in addition to determining anti-TB drug dosages. Elephants may refuse to ingest medications (Lyashchenko et al. 2006; Simpson et al. 2017), expel orally administered drugs or hold them in their mouths for prolonged periods of time without swallowing (Brock et al. 2014). Some drugs may be administered rectally to avoid problems with oral administration, but RIF does not reach recommended serum levels when administered rectally (Peloquin et al. 2006), and EMB results in mucosal irritation and expulsion of the drug (Maslow, Mikota, Zhu, Riddle, et al. 2005). In addition, administration of oral or rectal medications requires trained elephants and staff to ensure compliance (Stakeholders Task Force on Management and Research Priorities of Tuberculosis in Elephants 2017). Oral clearance (defined as the apparent volume of distribution in the systemic compartment divided by the first order absorption rate constant) (Zhu et al. 2005) or the presence of food in the gastrointestinal tract may influence drug absorption and make it difficult to reach targeted serum concentrations (Maslow, Mikota, Zhu, Isaza, et al. 2005; Zhu et al. 2005). Similarly, dose reduction to ameliorate adverse effects from treatment may compromise the ability to reach desired serum levels (United States Animal Health Association 2010).

Achieving effective drug serum concentrations must be balanced with the individual elephant's response to therapy and adverse effects from treatment. The criteria for effective drug serum levels may vary according to the goal of therapy and individual elephant. Since determining "cure" is difficult even in humans with TB, an achievable goal may be to sustain a consistent drug serum concentration that stops mycobacterial shedding in elephants without adverse effects. Since effective drug levels are unknown for elephants, targeted drug serum concentrations extrapolated from humans have been used. However, the guidelines for treatment of TB in elephants (developed under the auspices of the United States Animal Health Association) cited anecdotal evidence that some elephants could not tolerate anti-TB drugs at the

doses recommended to achieve targeted drug serum concentrations recommended for humans (United States Animal Health Association 2010). First-line anti-TB drugs have a variety of adverse effects in humans, including hepatitis, gastrointestinal intolerance, central nervous system toxicity, elevated liver enzymes, drug fever, rash, arthralgia, peripheral neuropathy, and optic neuritis (Yee et al. 2003; Zumla et al. 2015). Adverse effects documented in elephant case reports include inappetence (Mikota et al. 2001; Dumonceaux and Mikota 2006; Lyashchenko et al. 2006; Dumonceaux et al. 2011; Vogelnest et al. 2015; Simpson et al. 2017), depression/listlessness (Mikota et al. 2001; Lyashchenko et al. 2006; Dumonceaux et al. 2011; Simpson et al. 2017), epiphora (Dumonceaux and Mikota 2006; Dumonceaux et al. 2011; Vogelnest et al. 2015), blepharitis (Dumonceaux and Mikota 2006; Dumonceaux et al. 2011), hepatitis/liver toxicity (Mikota et al. 2001; Dumonceaux et al. 2011), diarrhea (Vogelnest et al. 2015), anemia (Dumonceaux et al. 2011), pica (Mikota et al. 2001), leukopenia (Mikota et al. 2001), trunk paralysis (Dumonceaux et al. 2011), elevated lactic dehydrogenase (Mikota et al. 2001), and stiff/sore limbs (Dumonceaux and Mikota 2006) associated with anti-TB treatment. The decision to deviate from or interrupt a treatment plan can interfere with consistent therapy but may result when elephants demonstrate adverse effects (Lyashchenko et al. 2006; Dumonceaux et al. 2011; Vogelnest et al. 2015; Egelund et al. 2016). For example, one case report described two elephants with poor tolerance for daily INH, although dosing every other day was tolerated (Simpson et al. 2017). Because INH is recommended for use in all elephants being treated for TB, acceptance of this drug with minimal adverse effects is crucial to complete therapy (Stakeholders Task Force on Management and Research Priorities of Tuberculosis in Elephants 2017). Incomplete therapy may have serious consequences, including disease recurrence (Lyashchenko et al. 2006), emergence of multi-drug resistance (Dumonceaux et al. 2011), infection of herdmates due to continued exposure (Mikota and Maslow 2011), public health risk to elephant handlers, and euthanasia of the affected elephant (Lyashchenko et al. 2006; Dumonceaux et al. 2011).

There is a paucity of literature on the response of elephants to TB treatment, except for information that can be gleaned from individual case reports (Dumonceaux et al. 2011; Vogelnest et al. 2015; Egelund et al. 2016). The objective of this study was to report the drugs, dosages, routes of

administration, serum drug concentrations during therapy, and adverse effects from a larger pool of elephants under TB treatment to establish a baseline for the North American captive elephant population. The results from this survey can be used to inform future clinical decision-making and research priorities on this important zoonotic disease.

6.3 Methods

A cross-sectional survey study was conducted to evaluate the diagnosis and treatment of TB in elephants in North America. The population for distribution of the surveys included elephants listed in the Association of Zoos and Aquariums' 2010 Asian (Keele 2010) and 2011 African (Olson 2011) elephant studbooks alive on January 1, 2010, for which contact information was available (365 animals). In order to preserve confidentiality, elephants were assigned a random code and questions were designed to avoid information that could be used to identify individuals.

The survey was available on-line from June 2013 through November 2014 to maximize response rate. A link to the survey was distributed via professional listserves, including the American Association of Zoo Veterinarians, Association of Zoos and Aquariums elephant group, Elephant Managers Association, and direct contact with veterinarians and elephant managers at individual facilities. Follow-up reminders were sent through the same listserves and to individual veterinarians.

Data were collected using an online survey website (SurveyMonkey©, San Mateo, California 94403, USA). Two surveys were distributed: one on potential risk factors for TB, along with general information about history of TB and TB treatment (Appendix B), and one on TB diagnostic test results and treatment protocols (Appendix C). The risk factor survey included demographic information (species, age, and sex); if a response was recorded for an elephant in both surveys, the demographic information was applied to the responses associated with this elephant in the diagnostics and treatment survey. The treatment portion of the diagnostics and treatment survey had 17 questions, including drop-down lists and fill-in fields. For treated elephants, fields for a list of commonly used anti-TB drugs (INH, RIF, EMB, PZA, fluoroquinolones, and amikacin) were presented and details on drug dosage (mg/kg),

frequency, route of administration, drug formulation, total doses received, duration, and purpose (prophylactic or therapeutic) of treatment were requested. Serum drug level values were also requested if they had been performed. If common adverse effects associated with anti-TB therapy had been observed, respondents were asked to record their severity, duration of single episodes, recurrence, and whether these caused an interruption in treatment. The survey specifically asked about the following adverse effects: decreased appetite/anorexia, decreased water intake, constipation, diarrhea, bloat, colic, ptyalism, depression/lethargy, agitation, generalized weakness, paresis of the limbs, tremors/muscle fasciculations, incoordination/ataxia, and photophobia. All adverse effects were subjective qualitative observations reported by the facility staff and not based on standardized criteria.

6.4 Results

There were survey responses for 182 elephants, 162 of which indicated that the elephant had not been treated with anti-TB drugs. Four elephants had unknown treatment status, three surveys had no response to this question regarding treatment, and 13 elephants were reported to have been treated with anti-TB drugs at some point in their lifetime. One of these 13 elephants was reported to have been treated with isoniazid, but no further information was provided; the following results are based on data from the other 12 elephants. These 12 elephants came from five different facilities. Eight of these elephants also had demographic information available. All eight were Asian elephants, seven females and one male, ranging in age from 15–48 yr. The ages listed were the elephants' ages at the time of the survey, not the time of anti-TB treatment. One treated elephant died in 2013, but cause of death was not reported.

Of the 12 elephants treated for TB, eight were treated prophylactically (one for a herd history of TB, and seven for a reactive serological test result) and five were treated therapeutically after a positive *M. tb* culture (Table 1). One elephant was treated both prophylactically and therapeutically. The most frequently used drugs were INH (n = 12), PZA (n = 5), and enrofloxacin (n = 4). In some cases, the same individual was treated with a different dosage, route, or formulation of the same drug; this is shown by

different rows for the same elephant in Table 6.1. A summary of the drugs, dosages, and frequencies and routes of administration are summarized Table 6.2.

Twelve treated elephants had information about serum drug levels reported. Serum drug levels were reported for elephants treated with INH, PZA, RIF, EMB, and enrofloxacin (Table 6.3). Isoniazid serum levels from elephant 7 may have been affected by collecting samples in hot weather; INH dissipates quickly after blood collection and samples should be kept on ice (Mikota and Maslow 2011). The results in Table 6.3 show the large variability in serum drug concentrations at various time points, and even at the same time point when measured repeatedly in the same individual.

Information about adverse effects attributed to TB treatment was reported for seven of the twelve treated elephants. No side effects were observed in two elephants. The other five of the seven elephants with survey responses to this question experienced adverse effects. Each of these elephants experienced two to five adverse effects during treatment, with at least one adverse effect being reported by facility staff as moderate or severe (Table 6.4). For four of the five elephants, at least one adverse effect resulted in interruption of treatment.

The most commonly reported adverse effect from these five elephants was anorexia (n = 4). Single episodes were reported to last three days to one week (n = 2) or two weeks or longer (n = 1). Two elephants experienced multiple episodes of anorexia during treatment. Other commonly reported adverse signs were decreased water intake (n = 3) and depression/lethargy (n = 3). Single episodes of decreased water intake were reported to last three days to one week (n = 2), and sometimes occurred in multiple episodes (n = 2). Single episodes of depression/lethargy lasted two weeks or longer (n = 2), and occurred multiple times in one elephant. Two elephants were observed to have incoordination/ataxia. A single episode lasted two weeks or longer (n = 1) but did not recur. One elephant became constipated, lasting for three days to one week, and had multiple episodes. One elephant had tremors/muscle fasciculations, single episodes of which lasted for less than 24 hours. No elephants were reported to experience other signs listed in the survey such as diarrhea, bloat, colic, ptyalism, discolored feces, agitation, generalized weakness, paresis of the trunk, or photophobia.

Table 6.1: Complete prophylactic and therapeutic antimycobacterial treatment protocols used in elephants included in this survey.

Elephant[ab]	Drug[cd]	Dose range (mg/kg)	Frequency[g]	Route[h]	Form	Duration (mo.)	Interrupted treatment	Complications
1[P]	INH	3–6	SID	RE	Powder	10–12	No	None
	RIF	10–11	SID	OD	Powder	10–12	No	None
	PZA	NR	SID	RE	Powder	0–3	No	None
2[P,T]	INH	NR	TIW	RE	Powder	4–6	No	Mild
		NR	TIW	OD	Powder	10–12	Yes	Moderate
	RIF	NR	TIW	OD	Powder	10–12	Yes	No
	EMB	NR	TIW	OD	Powder	4–6	No	Moderate
3[P]	INH	1–5	TIW	RE	Powder	0–6	No	None
	PZA	20–31	TIW	RE	Powder	0–6	No	None
4[T]	INH	2–6	TIW	RE	Powder/tablet	0–3	No	Moderate-severe
	PZA	30–40	TIW	RE	Powder	0–18	Yes	None-moderate
5[T]	INH	UNK[f]	SID	RE	Powder	4–6	Yes	Severe
		UNK	TIW	RE	Powder	22–24	Yes	Severe
	PZA	UNK	SID	RE	Powder	4–6	Yes	Severe
		UNK	TIW	RE	Powder	22–24	Yes	Severe
	ENRO	NR	SID	RE	Powder	4–6	Yes	Severe
6[T]	INH	2–5	TIW	RE	Powder	0–9	No	None
7[T]	INH	5–6	SID	RS	Powder	0–3	Yes	Moderate
		5–10	EOD	RS	Powder	0–9	Yes	No
	PZA	29–30	SID	RS	Powder	0–3	Yes	Moderate
	Fluoroquinolone[e]	5–6	SID	OF	Powder	10–12	Yes	None
8[P]	INH	4–5	SID	OF	Powder	10–12	No	None
	ENRO	9–10	SID	OF	Powder	10–12	No	None
9[P]	INH	4–5	SID	OF	Powder	10–12	No	None
	ENRO	9–10	SID	OF	Powder	10–12	No	None
10[P]	INH	4–5	SID	OF	Powder	7–9	No	None
	ENRO	9–10	SID	OF	Powder	7–9	No	None
11[P]	INH	4–5	SID	OF	Powder	10–12	No	None
	ENRO	9–10	SID	OF	Powder	10–12	No	None
12[T]	RIF	9–10	SID	OD	Powder	16–18	No	None
	Ethionamide	27–28	EOD	OD	Powder	0–3	Yes	Yes
	LEVO	19	SID	OD	Powder	0–3	No	None

[a] P = prophylactic treatment, T = therapeutic treatment, NR = not reported.

[b] Elephant 13 was treated with isoniazid, but no other data regarding dosing or frequency were provided.

[c] INH = isoniazid; RIF = rifampin; PZA = pyrazinamide; EMB = ethambutol; ENRO = enrofloxacin; LEVO = levofloxacin.

[d] Drugs are reported in multiple rows if there were multiple routes of administration or dosing intervals reported.
[e] drug not specified.
[f] UNK = unknown (entered by respondent).
[g] EOD = every other day; SID = once daily: TIW = three times/week.
[h] OD = orally, directly in mouth; OF = orally, on food; RE = rectal enema; RS = rectal suppository.

Table 6.2: Summary of antituberculosis drugs, dosages, frequency and route of administration in elephants.

Drug[a]	Dosage[b] (mg/kg)	Frequency[c]	Route[d]	Did complications occur?
INH	1–10	SID–TIW	RE, RS	Yes
INH	4–5	SID	OF	No
PZA	20–40	SID–TIW	RE, RS	Yes
RIF	9–11	SID–TIW	OD	Yes
EMB	NR	TIW	OD	Yes
Ethionamide	27–28	EOD	OD	No
ENRO	NR	SID	RE	Yes
ENRO	9–10	SID	OF	No
LEVO	19	SID	OD	No

[a] INH = isoniazid; RIF = rifampin; PZA = pyrazinamide; EMB = ethambutol; ENRO = enrofloxacin; LEVO = levofloxacin.
[b] NR = not reported.
[c] EOD = every other day; SID = once daily: TIW = three times/week.
[d] OD = orally, directly in mouth; OF = orally, on food; RE = rectal enema; RS = rectal suppository.

Table 6.3: Summary of serum drug concentrations in elephants treated prophylactically or therapeutically for *M. tuberculosis* infection.

Elephant	Drug	Dose range (mg/kg)	Route[a]	Time after administration (hr)	Serum drug level range (µg/ml)	No. Samples
1	INH	3–4	RE	1, 2, 4, other	0–1	12
	INH	4–5	RE	2, 4, other	0–1	6
	RIF	10–11	OD	2, 4	2–3; 3–4; 4–5	6
				Other	0–1	3
2	INH	3–4	OD	0.25, 0.5, 1, 2, 4, other	0–1	6
	INH	4–5	OD	0.25	0–1; 2–3	2
				0.5	0–1; 4–5	2
				1	0–1; 3–4	2
				1.5, 2, 3, 4, other	0–1	11
	INH	7–8	RE	0.25, 0.5, 1	3–4	3
	INH	10–11	RE	0.25	10–11	1
				0.5	6–7	1
				1	4–5	1
	INH	12–13	RE	0.25	3–4	1
				0.5	0–1	1
				1	2–3	1
	EMB	30–31	OD	0.25, 4	0–1	2
				0.5	4–5	1
				1	7–8	1
				2	3–4	1
	RIF	6–7	OD	1, 2	0–1	3
				4	2–3; 3–4	3
				Other	0–1; 2–3	6
3	INH	3–4	RE	0.25	5–6	1
				0.5	2–3	1
				1, 2, 4	3–4	2
	INH	5–6	RE	0.25, 0.5	2–3	1
				1	0–1; 2–3; 3–4; 4–5	5
				2	0–1; 2–3; 3–4; 4–5	5
				4	2–3	1
				Other	0–1	2
	PZA	20–21	RE	0.25	14–15	1
				0.5	0–1	1
				1	9–10	1
				2, 4	10–11	2
	PZA	30–31	RE	0.25, 0.5	0–1	2
				1	4–5; 6–7; 7–8; 9–10; 22–23	5
				2	7–8; 9–10; 11–12; 13–14; 20–21	5
				4	9–10	1
				Other	0–1; 8–9	2
4	INH	5–6	RE	0.5	6–7	1
				2	2–3	1
				4	2–3; 3–4	1
				Other	0–1; 2–3	4
	PZA	30–31	RE	0.25	19–20	1
				0.5, 1	26–28	2
				2	25–26	1
	PZA	39–40	RE	0.25	21–22	1
				0.5, 2	30–33	2
				1	21–22	1

6	INH	4–5	RE	0.25	2–3; 3–4; 7–8	3
				0.5, 1	2–3; 3–4	6
7	INH	10–11	RE	0.25	3–4; 7–8; 11–12	3
				0.5	3–4; 7–8; 10–11	3
				1	2–3; 5–6; 8–9	3
				Other	4–5; 5–6; 8–9; 10–11	4
	PZA	30–31	RE	0.5	30–33; 38–40	2
				1	26–28; 30–33	2
				1.5	25–26; 26–28	2
				2	24–25	1
8	ENRO	9–10	OF	2	2–3	1
	INH	4–5	OF	2	0–1	1
9	ENRO	9–10	OF	2	2–3	1
	INH	4–5	OF	2	2–3	1
10	ENRO	4–5	OF	2	3–4	1
	INH	4–5	OF	2	3–4	1
11	ENRO	10–11	OF	2	3–4	1
	INH	4–5	OF	2	0–1	1
12	RIF	9–10	OD	1	3–4	1
				2	5–6	1
				3	8–9	1
				4	9–10	1

[a] INH = isoniazid; RIF = rifampin; EMB = ethambutol; PZA = pyrazinamide; ENRO = enrofloxacin.
[b] OD = orally, directly in mouth; OF = orally, on food; RE = rectal enema

Table 6.4: Adverse effects, severity, and treatment interruption associated with antimycobacterial therapy in elephants.

Elephant	**Adverse effect**	**Severity**	**Treatment interruption**
2	Decreased appetite/anorexia	Severe	Yes
	Decreased water intake	Severe	Yes
4	Decreased appetite/anorexia	Severe	Yes
	Decreased water intake	Severe	No
	Constipation	Severe	Yes
	Depression/lethargy	Moderate	Yes
	Incoordination/ataxia	Mild	NR[a]
5	Decreased appetite/anorexia	Severe	Yes
	Depression/lethargy	Moderate	Yes
	Paresis/weakness of the limbs	Moderate	Yes
	Incoordination/ataxia	Moderate	Yes
7	Decreased appetite/anorexia	Moderate	Yes
	Decreased water intake	Moderate	Yes
	Depression/lethargy	Moderate	Yes
12	Discolored urine	Moderate	No
	Tremors/muscle fasciculations	Mild	No

[a] NR = not reported

6.5 Discussion

The results from this study represent the collection of treatment protocols, serum drug concentrations, and adverse effects from twelve elephants receiving TB treatment. Treatment of TB in elephants requires a significant commitment, both financially ($20,000–$60,000 per elephant) (Dumonceaux and Mikota 2006; Mikota 2008; Simpson et al. 2017) and in terms of time and effort to medicate and monitor an elephant for 12–18 months. These survey data provide a baseline of information for future research that can be used to develop treatment recommendations based on elephant data.

It is not surprising that the responses for elephants treated for TB were all from Asian elephants, as in North America, TB is reported almost exclusively in this species rather than African elephants (*Loxodonta africana*) (Feldman et al. 2013). While the reported ages of the elephants do not correspond to their age at treatment (to protect confidentiality), most individuals with age data were older (> 30 yr), as would be expected in a chronic disease like TB. Most elephants in this survey had been treated on a prophylactic basis, which has been described anecdotally (Simpson et al. 2017), using recommendations in published guidelines (United States Animal Health Association 2010; Stakeholders Task Force on Management and Research Priorities of Tuberculosis in Elephants 2017).

The 12 cases presented here demonstrate the variability in treatment plans for TB in elephants. The drugs used to treat an individual elephant depends on feasible routes of administration, individual compliance, and occurrence of adverse effects. Drug dosages and frequency of administration also may need to be altered until an acceptable regimen is found that achieves desired targeted serum drug levels, elephant compliance, and minimal adverse effects, while accomplishing treatment objectives (i.e., cessation of shedding, arrest of disease progression) (Zlot et al. 2015). Even then, elephants may need to be retreated if the first round of therapy does not achieve the therapeutic goal (Lyashchenko et al. 2006; Mikota and Maslow 2011; Simpson et al. 2017).

The high variability in reported drug levels includes numerous concentrations below the published recommended serum concentrations for humans: 3–5 µg/ml for isoniazid, 8–24 µg/ml for rifampin, 20–60 µg/ml for pyrazinamide, and 2–5 µg/ml ethambutol (United States Animal Health

Association 2010). At least some of these lower levels are likely an artifact of measurements taken at times other than when drug concentrations were expected to be maximized (Stakeholders Task Force on Management and Research Priorities of Tuberculosis in Elephants 2017). Peak drug concentrations are reported to vary by individual elephant (Brock et al. 2014), thus even a measurement taken at the recommended time (Stakeholders Task Force on Management and Research Priorities of Tuberculosis in Elephants 2017) may not represent that individual's peak drug concentration. Humans undergoing TB treatment also demonstrate similar variation in serum drug levels, with factors like age, sex, and genetics being consistently associated with higher or lower concentrations of certain drugs (McIlleron et al. 2006). In addition, drug penetration into infected tissues is influenced by pharmacokinetics and pharmacodynamics within lesions (Dartois 2014), which depend on factors like pH, active transport mechanisms, protein binding, and solubility (Prideaux et al. 2015). The high variability in serum drug concentrations, even within individuals, underscores the importance of individual therapeutic drug monitoring in elephants under treatment to determine appropriate dosing to achieve serum concentrations assumed to be effective for antimicrobial activity (Brock et al. 2014). Simpson et al. (2017) describe changing a treatment protocol using EMB to enrofloxacin after monitoring serum concentrations, presumably due to inadequate EMB concentrations. Therapeutic drug monitoring is particularly crucial when modifying a treatment protocol as required by individual tolerance. It is important to keep in mind, however, that although cessation of bacterial shedding has been anecdotally reported, the lack of markers of disease progression in elephants limits studies on efficacy of the human-based target drug levels currently recommended (Stakeholders Task Force on Management and Research Priorities of Tuberculosis in Elephants 2017).

Adverse effects of treatment are a serious concern for elephant welfare and ability to maintain an effective TB treatment plan. In humans, adverse effects from treatment resulted in termination of use of at least one drug in 23% of patients in one study (Schaberg et al. 1996), and in another, 14% of defaulters from TB treatment gave medication side effects as their reason for discontinuing treatment (Tekle et al. 2002). Adverse effects of anti-TB treatment can be severe in some other animals as well; in Bactrian

camels (*Camelus bactrianus*), treatment with INH caused bone marrow toxicity resulting in mortalities (Bush et al. 1990). The survey responses in this study provide information about the severity and duration of adverse effects encountered during TB treatment of elephants. The burden of adverse effects reported in this study was substantial; elephants experienced multiple adverse effects, at least one of which was considered moderate or severe, and most of which resulted in interruption of treatment. Interruptions in treatment are suboptimal not only for a disease requiring lengthy treatment but for minimizing the risk of selecting for drug-resistant bacteria (World Health Organization and Stop TB Initiative 2010). Elephants being treated for TB should be closely monitored for adverse effects associated with treatment, and treatment plans should be adaptable to account for the elephant's ability to tolerate drugs and interruptions that may prolong the treatment period.

There were several limitations in the survey employed in this study. The survey was designed to protect confidentiality and avoid identifying individual elephants, which inherently limited the amount of information collected. The survey was directed towards attending veterinarians who could review medical records to provide responses to the survey questions, but respondents were not identified to maintain confidentiality. Information collected about treatment protocols did not extend to which drug doses were administered together or for exactly how long, exact drug formulations, or the sequence of therapy. The dates when therapy occurred were not requested to protect confidentiality since individual treated elephants may be identified by when treatment was administered. There were also no data collected on treatment outcomes since elephants may have been in various stages of treatment, and including animals at various times in or after treatment may bias results if insufficient time has passed. Other limitations are related to the information collected on adverse effects. Adverse effects reported were based on subjective observations, although respondents were able to provide information about the duration and repetition of episodes. It is possible that some of the observed side effects attributed to treatment were in fact age-related changes in behavior, or a result of comorbid conditions. The results reported here still represent a baseline of multiple facets of treating TB in elephants, and provide a foundation for assessing future TB treatment data.

The treatment protocols, serum drug concentrations, and adverse effects reported for the 12 elephants in this study represent a valuable addition to understanding the clinical picture of elephant TB. The unique challenges of treating a chronic disease in elephants, where high individual variability has been observed, demand flexibility and adaptation of treatment guidelines. Providing elephant managers and veterinarians with information on what protocols have been tried, their effectiveness in achieving desired serum drug concentrations, and the adverse effects encountered allows for more informed clinical management of treatment for TB in elephants. Similar information, as well as treatment outcomes, should be published for elephants treated under the most current recommendations so that they may be adapted for best practices.

CHAPTER 7: CONCLUSIONS AND FUTURE DIRECTIONS IN WILDLIFE TUBERCULOSIS

Management of TB across humans and non-human animal species remains a challenging prospect. A One Health approach that incorporates data and techniques across disciplines to build a more complete picture of disease control is ideal for TB in wildlife. Both ecology and epidemiology can offer insight into the dynamics of infectious diseases in populations (Anderson 1991). My coauthors and I drew from ecology and epidemiology to implement a holistic approach to improving TB diagnosis and management in species of conservation concern: European badgers, and African and Asian elephants. Our results provide insight into the challenges of diagnosing and managing TB in free-ranging and captive wildlife, the benefits of a transdisciplinary approach, and areas in need of further investigation.

7.1 One Health and the Role of the Environment

The human-captive wildlife-free-ranging wildlife interface is rich with opportunities for pathogen transmission, with consequences for both human health and for conservation of free-ranging wildlife. Studies in Asia have revealed a troubling picture of TB exposure in working Asian elephants and their handlers (Angkawanish et al. 2010; Verma-Kumar et al. 2012; Ong et al. 2013; Paudel et al. 2014; Mikota, Gairhe, et al. 2015; Yakubu et al. 2016), with recent evidence of spillover into free-ranging elephants (Perera et al. 2014; Chandranaik et al. 2017; Zachariah et al. 2017). A case report of TB in a free-ranging African elephant in Kenya (Obanda et al. 2013) and evidence of *M. tuberculosis* in other southern African wildlife (Michel et al. 2013) highlight the risk to free-ranging wildlife throughout Africa. Human influences on ecosystems drive the level of disease risk at human-animal interfaces (Hassell et al. 2016). Our study in Zimbabwe provides support for the growing body of evidence of TB at high-risk human-wildlife interfaces in crucial conservation areas (Chapter 4; Rosen et al. 2018). The risks of this type of interface extend beyond TB to other pathogens; it is an ideal candidate for an integrated One Health approach involving screening of human handlers, monitoring of captive wildlife, and surveillance of free-ranging wildlife populations.

The environmental component of One Health is underrecognized, and therefore underutilized (Barrett and Bouley 2015), and the environmental component of the epidemiologic triad is often underinvestigated. Health research can benefit from introducing aspects of systems thinking from ecology, such as non-linear relationships (Zinsstag et al. 2011). Our studies in Ireland integrated environmental factors including precipitation, ambient temperature, and soil characteristics into assessment of the bovine TB management program (Chapter 3; Martin et al. 2017). Identifying conditions that are associated with desirable outcomes – trapping badgers – provides managers with data-based recommendations for focusing field efforts and resources to maximize impact. Similarly, exploring ecological drivers of badger density in conjunction with mark-recapture data can inform future predictive modeling of population densities across the country's varying landscape where trapping data may not be available (Chapter 5).

Environmental factors and transmission routes should be considered in TB and other infectious disease research as they can alter models of disease dynamics if, for instance, infectious pathogens can persist in the environment for relatively long periods of time (Almberg et al. 2011). Environmental transmission also impedes wildlife disease management by adding a potential source of infection that is challenging to eliminate (Miller et al. 2004; Miller et al. 2006). *M. bovis* has been shown to persist in soil, and even the BCG strain used in vaccines can remain viable in soil for months (Williams and Hoy 1930; Duffield and Young 1985; Young et al. 2005; Fine et al. 2011; Ghodbane et al. 2014; Barbier et al. 2017). Environmental contamination can result from unexpected sources: for instance, earthworms can disseminate *M. bovis* from cattle feces into soils and shed the bacteria for several days after moving to soils previously free of *M. bovis* (Barbier et al. 2016). Predation on earthworms by host species such as badgers could also represent an additional transmission pathway for *M. bovis* (Barbier et al. 2016). Pathogen sources outside of other vertebrate hosts need better incorporation into models of disease dynamics and research objectives.

7.2 The Role of Imperfect Diagnostics in Management

Diagnostic accuracy is critical for appropriately informing management decisions for culling, vaccination, and treatment strategies. The dynamics of theoretical SIR models will not accurately reflect those of a population if disease status is defined incorrectly. Test-and-cull schemes of cattle and wildlife depend on the ability to identify infected animals to cull. A vaccine superior to BCG, such as a more efficacious vaccine with DIVA capacity, may hold the key to controlling TB in wildlife and livestock, and ultimately eradication of the disease. Such a vaccine would depend on a reliable diagnostic test to differentiate vaccinated animals from infected animals to allow selective removal. Our elephant treatment study highlighted how the shortcomings of available diagnostic assays for elephants – with no method for confidently determining current infection status or cessation of bacterial shedding – impede the definition of successful treatment. All management interventions rely, in part, on diagnostic test results to characterize success in reducing the TB burden in wildlife.

Making inference from diagnostic tests forms the basis for our understanding of disease incidence and prevalence in populations. Test characteristics such as sensitivity and specificity give an indication of the test's performance, but not how reliably a test result under field conditions can be taken as a reflection of the animal's true disease state (Dohoo et al. 2009). The negative and positive predictive values of test results are governed by sensitivity, specificity, and population prevalence (Dohoo et al. 2009), which makes test interpretation dependent on the underlying true prevalence in the population under study. Other mitigating circumstances, especially from the underappreciated environmental component of One Health, need to be considered when interpreting diagnostic test results. For example, environmental NTM infections can result in cross-reaction on diagnostic tests for wildlife, including the TST (Gcebe and Hlokwe 2017) and STAT-PAK (Greenwald et al. 2009). Our elephant serology study addressed the potential role of NTM and *M. mungi*, a newly emerging MTBC strain, in positive serological test results. We recognized that positive test results could be due to exposure to *M. tuberculosis* from humans, *M. bovis* from animal hosts, potentially *M. mungi* (another MTBC species present in the local ecosystem), or

NTM exposure (Chapter 4; Rosen et al. 2018) Test results should be evaluated with locally occurring MTBC and NTM species in mind rather than a single species of interest.

7.3 Recommendations to Inform Management Using Field Data and Local Ecology

TB has been studied and managed for decades, but unique features of each ecosystem can subtly alter the dynamics and management requirements (Miller and Olea-Popelka 2013). Bovine TB impacts badgers and cattle in the UK and Ireland, but similar management approaches through culling have had different outcomes in each country(O'Connor et al. 2012). In the UK, badger culling was believed to have "no meaningful contribution to cattle TB control" (Bourne et al. 2007) given observed increases in cattle TB after culling in some areas (Donnelly et al. 2005), while culling was shown to be effective in reducing cattle TB in Ireland (Ó Máirtín et al. 1998; Griffin et al. 2005; Olea-Popelka et al. 2009). White-tailed deer in Michigan are a persistent reservoir for *M. bovis*, while an outbreak in deer in Minnesota was successfully controlled and eliminated (Carstensen et al. 2011). Management programs would do well to consider the full host community, environment, and social factors in proposed control strategies.

One method for incorporating local ecology and conditions into TB research is by building a complete picture of the host community, as well as highlighting differences from other related TB systems that may be important drivers of transmission. For instance, elephant management practices at the ecotourism facilities in our study in Zimbabwe are markedly different from those used for other populations of captive elephants. Practices such as humans and elephants sharing indoor facility airspace (Zlot et al. 2015), using pressure washers (Miller and Olea-Popelka 2013), and having an assigned handler (Lassausaie et al. 2014) were identified as risk factors in studies from other populations, but are not relevant in our study because none of these practices are in place at any of the facilities. Studies of TB in working Asian elephants have tended to focus on human-elephant contact (Ong et al. 2013; Lassausaie et al. 2014; Yakubu et al. 2016), without consideration of other wildlife or livestock hosts that may be involved in transmission. We documented other wildlife species with host potential, including warthogs (Woodford 1982) and banded mongoose (Alexander et al. 2010; Brüns et al. 2016), on and around

facilities, and considered these as possible sources for mycobacterial transmission in working African elephants. Our management guidelines for TB prevention specifically include measures to reduce direct and indirect contact with these potential host species (Chapter 4; Rosen et al. 2018).

Social factors can also be important drivers and obstacles to management programs. Elephants' size, longevity, and conservation status make them unsuitable as experimental research animals; TB management has been solely informed by the response of animals to natural infections. Privacy concerns in North American elephant facilities have limited data sharing about elephant TB. Our study of TB treatment in elephants provided a compilation of empirical data for elephant managers and veterinarians to use to inform clinical decision making. We found considerable variation in drug protocols and serum concentrations used to determine appropriate dosing, and a substantial burden of moderate or severe adverse effects (Chapter 6). These findings underscore the difficulty in treating elephants in a standardized fashion, and the need for improved diagnostics to more confidently identify when animals are no longer infectious.

All management activities need metrics to determine progress in disease control. Our study of badger density in Ireland supports the Department of Agriculture, Fisheries and the Marine's integrated program for TB management of cattle and badgers (O'Keeffe 2006; Good and Duignan 2017). One arm of this program involves badger vaccination as a management strategy (Good and Duignan 2017), which currently employs an intramuscular BCG vaccine. An oral vaccine formulation has the potential to reduce the labor intensity of vaccine delivery, which could prove valuable for effectively protecting elusive species such as badgers, or for vaccinating multiple target species, as in southern Africa. Until an oral vaccine is available for use in free-ranging wildlife, intramuscular vaccination will continue in Ireland, which requires a high rate of trapping success. Our results on weather and activity scores allow managers to best use resources by prioritizing field efforts under conditions when badger trapping is most likely to be successful. We also provided an estimate of badger density in one vaccination trial area derived from mark-recapture data, which may be more sensitive to subtle changes in density than estimates based on sett densities. Our results provide a baseline for using SECR models to assess badger densities over time

in an adaptive management framework (Walters and Holling 1990) that allows for the eradication program to be refined as new information becomes available. Our models can also be combined with culture data from badger mortalities to estimate densities of badger in different disease classes. Incorporating these badger population data with cattle testing results and slaughter surveillance can better inform understanding of the complex multi-host system of TB and accelerate progress towards eradication. These models could also be adapted for use in other free-ranging species undergoing disease management where host population density is important to estimate.

Our results from Ireland highlight the difficulty in managing free-ranging wildlife using vaccines. The proportion of the population that must be vaccinated to eradicate a pathogen is $p > 1 - \left(\frac{1}{R_0}\right)$ (Anderson and May 1985), but in reality this number is higher in the case of TB in because BCG does not offer complete immunity. The low trapping success for badgers is a major obstacle to delivering enough vaccine to induce herd immunity. However, Ireland's transition to vaccination has been designed as a non-inferiority approach, with the goal of demonstrating that a new intervention (BCG) is "not unacceptably worse" than culling, the current intervention (Schumi and Wittes 2011). Preliminary results from the trial have been positive, indicating that vaccination is not worse than culling for controlling TB in cattle, and the capturing effort for vaccine is not more resource intensive than for culling operations (Good and Duignan 2017). Our results will be used to assess the role of badger density as a confounder in cattle TB rates and the success of badger vaccination in controlling TB in cattle. The intramuscular BCG vaccine program may not be sufficient for TB eradication, but will promote badger conservation compared to culling in continued management efforts.

BCG vaccine programs for free-ranging wildlife do have long-term impacts that must be considered as these plans are implemented. Vaccinated animals are not completely protected from infection and are therefore not truly resistant to infection as is assumed in a traditional SIR framework. Data from vaccinated badgers suggests lower levels of seroconversion and clinical disease in the field (Good and Duignan 2017), and laboratory studies found decreased bacterial shedding (Lesellier et al.

2011). It is unclear how increased survival but decreased transmission (β) associated with vaccination will affect TB prevalence in free-ranging populations. New vaccine development holds promise for an option with higher efficacy and ideally, DIVA capacity that would allow for use in both livestock and wildlife. Vaccination is becoming the future of TB management in free-ranging wildlife (Buddle et al. 2013), and closely studying populations where vaccination is being implemented will be informative to understanding the modified transmission dynamics under vaccine programs.

In conclusion, my dissertation adds to our collective knowledge about the ecology and epidemiology of TB in wildlife. I integrated often-overlooked environmental aspects into my research to provide a broader scope beyond host and pathogen. My work underscores the limitations of current diagnostic tests and the need for interpretation in context of the local ecosystem. Lastly, I demonstrated the value of large and small datesets from field studies to conduct applied research that directly informs a variety of wildlife disease management strategies.

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2014 Victoria Falls Elephant TB Risk Assessment Survey

Section I. Individual Elephant Information

1. Elephant identification

Country location: _____

Facility Location: _____

LocStat: _____

Elephant Unique Identification Number
(Please use **either** the VFWT's or the Owner's number and remain consistent):

_____

Name (if applicable): _____

2. What is the gender of this elephant? Please circle one.

Male
Female

3. What year was this elephant born?

Year: _____

Other (please specify)
_____

4. Has this elephant reached puberty? Please circle one.

Yes
No
Other (please specify):
_____

5. Has this elephant reached breeding age?

Yes
No
Other (please specify):
_____

6. Was this elephant wildborn?

Yes
No
Other (please specify):
_____

7. How long has this elephant been managed by humans?

_____

8. Has this elephant ever been kept in a natural breeding situation (i.e., housed with a breedingaged elephant of the opposite sex)?

Yes
No
Unknown
Other (please specify):
_____
_____
_____
_____
_____
_____

Note: For questions 9-11, if this is a female elephant, answer only questions related to pregnancies and live births; If this is a male elephant, answer only question related to breeding bull.

9. In female elephants of breeding age, how many pregnancies have resulted?

By natural breeding: 0 1 2 3 4 5 6

Other (please specify)
_____

10. How many live births have occurred? Please circle one.

0, 1, 2, 3, 4, 5, 6

Other (please specify)

_____

_____

11. Has this male elephant ever been used as a breeding bull?

By natural service: Yes No Unknown
Other (please specify)

_____

_____

Section II. Elephant management

12. How is this elephant currently managed?

Unprotected physical contact handlers only

Unprotected physical contact with multiple people

Protected contact – only handled when there is a barrier or restraints (chains)

Combination of unprotected and protected contact

Other (please specify)

_____

_____

_____

_____

13. Has the management of this elephant changed during the past 5 years?

Yes
No
Unknown

Other (please specify)

_____

_____

_____

_____

14. If the management of this elephant has changed, how has the management of this elephant changed in the last 5 years?

_____
_____
_____
_____
_____

15. How many elephants are housed in separate enclosures or barns (i.e., no physical contact) from this elephant? Please circle one.

0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 10+

Other (please specify)

_____
_____
_____

16. What is the approximate size of the current outdoor enclosure for this elephant (please specify in either square meters or hectares)?

_____
_____

17. Has the size of the outdoor enclosure for this elephant changed during the last 5 years?

Yes
No
Unknown

Other (please specify)

_____
_____
_____

18. How has the size of the outdoor enclosure changed during the last 5 years?
Unchanged
Increased in size
Decreased in size
Unknown
Other (please specify)

_____
_____

19. How many elephants are in the outdoor enclosure with this individual (including this individual)? Please circle one.

1, 2, 3, 4, 5, 6, 7, 8, 9, 10,

Other (please specify)

_____

_____

20. How many of the elephants in the outdoor enclosure are over 30 years of age (including this individual)?

1, 2, 3, 4, 5, 6, 7, 8, 9, 10,

Other (please specify)

_____

_____

21. Have any new elephants been added to the outdoor enclosure in the last 5 years?

Yes
No
Unknown

Other (please specify)

_____

_____

_____

22. What is the typical social grouping for this individual elephant when it is in the outdoor enclosure? Please circle one.

Housed individually
Housed with a single elephant (adult)
Housed with more than one elephant (all adult group)
Housed with more than one elephant (mixed age group cow/calves)

Other (please specify)

_____

_____

_____

23. Has at least one elephant moved into the facility during the last 5 years?

Yes
No
Unknown

Other (please specify)

_____
_____
_____

24. Has the elephant(s) that moved been in physical contact with this individual elephant?

Yes
No
Unknown

Other (please specify)

_____
_____
_____

25. Has this elephant traveled away from its home facility during the last 5 years?

Yes
No
Unknown

Other (please specify)

_____
_____
_____

26. Does this elephant share an indoor enclosure or shelter with other elephants?

Yes
No
Unknown
Other (please specify)

_____
_____
_____

27. Is the elephant allowed to free-range (with handlers in attendance) during the day for foraging?

Yes
No
Unknown

Other (please specify)

_____
_____
_____

28. Does this elephant come into contact with wild elephant when free-ranging?

Never
Very infrequently
Sometimes
Frequently

Please specify an approximate number of encounters with wild elephants in the past month.

_____/ month

29. If this is a female elephant, has pregnancy occurred as a result of this female elephant interactions with wild elephants?

Yes
No
Unknown

Other (please specify)

_____
_____
_____

30. How is this elephant kept at night?

Chained
Penned singly
Penned with others

31. How many hours a day is the animal kept confined or restrained in an enclosure?

_____ **hours.**

32. When confined or restrained in an enclosure, or chained at night, can this elephant touch other elephant?

Yes. If yes, how many other elephants? _____

No

33. Does this elephant have access to the feed troughs of other elephant?

Yes
No
Unknown

Other (please specify)

_____
_____
_____

34. Does this elephant share water trough(s) with other elephant?

Yes
No
Unknown

Other (please specify)

_____
_____
_____

35. What is the origin of supplementary feed supplied to your elephant?

Commercial bagged feed

Fresh browse or hay cut in an area that other wildlife, including elephant, has access to

Dried, baled hay

Vegetables and other fresh feedstuffs which may have been extensively handled by persons not associated with the elephant facility

Section III. Elephant-Human interaction

36. Does this elephant share an indoor enclosure or shelter with people?

Yes
No
Unknown

Other (please specify)

_____
_____
_____

37. How many fulltime staff (trainers, handlers, keepers) are currently employed to provide daily care at the facility for elephants?

- As Main handlers (every day)

1–3, 4–6, 7–10, 10+

Other (please specify)

_____
_____

- As Back-up handlers (sometimes)

1–3, 4–6, 7–10, 10+

Other (please specify)

_____
_____

38. What is the average duration of staff direct contact (in hours) per day with this elephant?

(Direct contact is defined as interaction within a distance of less than 5 meters between the elephant and the person).

0–1/2 hour, 1–2, 3–5, 5–8, 8+

Other (please specify)

_____
_____

39. What is the average duration of public contact (in hours) for each contact? Please circle one.

0–1/2 hour, 1–2, 3–4, 5–6, 6+

This elephant does not have public contact

Other (please specify)

_____

_____

40. Approximately how many different members of the public touch and / or ride this elephant per month (average)?

_____ **people**

41. How many part-time staff (1 staff=20 hours/week) are currently employed to provide daily elephant care at this facility?

1–3, 4–6, 7–10, 10+

Other (please specify)

_____

_____

42. How many volunteers (1 volunteer = average 5 hours/week) are used to assist with elephant care duties at this facility?

1–3, 4–6, 7–10, 10+

Other (please specify)

_____

_____

43. How often do you require paid elephant staff to be tested for tuberculosis?

At the time of hiring
Semiannually (every 6 months)
Annually
Only if an elephant has a suspect/positive test for tuberculosis
Never
Other (please specify)

_____

_____

44. How often do you require volunteers involved with elephant care to be tested for tuberculosis?

At the time of initial volunteering

Semiannually (every 6 months)

Annually

Only when an elephant has a suspect/positive test for TB

Never

Other (please specify)

_____

_____

Section IV. Past Management

Please describe any management practices that changed during the last 5 years for this elephant.

Section V. Tests results

Sample type obtained	Sample collection date	Sample processed date	DPP Result

Sample type obtained	Sample collection date	Sample processed date	INF gamma Result

Sample type obtained	Sample collection date	Sample processed date	Culture Result

APPENDIX B: RISK FACTORS SURVEY FOR NORTH AMERICAN ELEPHANTS

1.

***1. Enter elephant ID code**

***2. What is the gender of this elephant?**

- ◯ Male
- ◯ Female
- ◯ Other (please specify)

***3. What is the species of this elephant?**

- ◯ Asian
- ◯ African

***4. Has this elephant ever been housed with an elephant of the opposite species (African-Asian)?**

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

***5. What year was this elephant born?**

***6. Was this elephant born in the U.S.?**

- ◯ Yes
- ◯ No
- ◯ Other (please specify)

2.

7. Did this elephant receive milk replacer as the primary source of milk for more than one month of its neonatal life?

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

***8. Has this elephant lived in a non-U.S. facility outside of its range country for more than one year?**

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

3.

9. If the elephant resided outside the country for more than a year, in what country was the facility located?

4.

***10. Was this elephant wild-born?**

- ◯ Yes
- ◯ No
- ◯ Other (please specify)

5.

***11. If wild born, what is the country of origin?**

- ◯ India
- ◯ Thailand
- ◯ Vietnam
- ◯ Sri Lanka
- ◯ Myanmar
- ◯ Burma
- ◯ South Africa
- ◯ Namibia
- ◯ Zimbabwe
- ◯ Mozambique
- ◯ Swaziland
- ◯ Botswana
- ◯ Zambia
- ◯ Tanzania
- ◯ Kenya
- ◯ Unknown

Other (please specify)

12. Has this elephant reached puberty/breeding age?

- ◯ Yes
- ◯ No
- ◯ Other (please specify)

6.

13. Has this elephant been housed in a natural breeding situation (i.e., housed with a breeding-aged elephant of the opposite sex)? [If female elephant, answer only questions related to pregnancies and live births; if male elephant, answer only question related to breeding bull]

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

14. In female elephants of breeding age, how many pregnancies have resulted?

	0	1	2	3	4
By natural breeding	◯	◯	◯	◯	◯
By artificial insemination	◯	◯	◯	◯	◯

Other (please specify)

15. How many live births have occurred?

- ◯ 0
- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5
- ◯ 6

Other (please specify)

16. Has this male elephant every been used as a breeding bull?

	Yes	No	Unknown
By natural service	◯	◯	◯
For artificial insemination	◯	◯	◯

Other (please specify)

7.

17. How is this elephant currently managed?

- [ ] Free contact
- [ ] Protected contact
- [ ] Combination of free and protected contact
- [ ] Other (please specify)

18. Has the management of this elephant changed during the past 5 years?

- [ ] Yes
- [ ] No
- [ ] Unknown
- [ ] Other (please specify)

8.

19. If it has changed, how has the management of this elephant changed in the last 5 years?

20. How many elephants are housed in separate enclosures or barns (i.e., no physical contact) from this elephant?

- ◯ 0
- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5
- ◯ 6
- ◯ 7
- ◯ 8
- ◯ 9
- ◯ 10
- ◯ 10+

Other (please specify)

21. What is the approximate size of the current outdoor enclosure for this elephant (please specify in either square feet or acres)?

22. Has the size of the outdoor enclosure for this elephant changed during the last 5 years?

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

9.

23. How has the size of the outdoor enclosure changed during the last 5 years?

- ◯ Unchanged
- ◯ Increased in size
- ◯ Decreased in size
- ◯ Unknown
- ◯ Other (please specify)

24. How many elephants are in the outdoor enclosure with this individual (including this individual)?

- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5
- ◯ 6
- ◯ 7
- ◯ 8
- ◯ 9
- ◯ 10
- ◯ Other (please specify)

25. How many of the elephants in the outdoor enclosure are over 30 years of age (including this individual)?

- ◯ 0
- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5
- ◯ Other (please specify)

26. Have any new elephants been added to the outdoor enclosure in the last 5 years?

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

27. What is the typical social grouping for this individual elephant when it is in the outdoor enclosure?

- ☐ Housed individually
- ☐ Housed with a single elephant (adult)
- ☐ Housed with more than one elephant (all adult group)
- ☐ Housed with more than one elephant (mixed age group - cow/calves)
- ☐ Other (please specify)

28. What is the predominant type(s) of substrate in the outdoor enclosure currently housing this elephant? (Check all that apply.)

- ☐ Natural substrate (ex. grass, dirt, sand)
- ☐ Decomposed granite (DG)
- ☐ Manmade material (ex. concrete, asphalt)
- ☐ Other (please specify)

29. Has the type of outdoor substrate changed for this elephant during the last 5 years?

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

30. What is the total approximate size (in square feet) of the current indoor facility housing this elephant?

31. Has the size of the indoor holding facility changed in the last 5 years?

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

10.

32. How has the size of the indoor holding facility changed?

- ◯ Unchanged
- ◯ Increased in size
- ◯ Decreased in size
- ◯ Other (please specify)

33. How many elephants are housed in the current indoor holding facility with this elephant (including this individual)?

- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5
- ◯ 6
- ◯ 7
- ◯ 8
- ◯ 9
- ◯ 10
- ◯ 11
- ◯ 12
- ◯ Other (please specify)

34. Has the number of elephants housed in the indoor holding facility changed in the last 5 years?

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

35. How has the number of elephants housed in the indoor holding facility changed in the last 5 years?

- ◯ Increased number of elephants housed during the last 5 years
- ◯ Decreased number of elephants housed during the last 5 years
- ◯ Unchanged
- ◯ Unknown
- ◯ Other (please specify)

36. How many elephants are typically in physical contact (with or without a barrier) with this elephant while housed indoors?

- ◯ 0
- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5
- ◯ Other (please specify)

37. How is this elephant typically housed when indoors?

- [ ] Individual stall/room - no physical contact with other elephants
- [ ] Individual stall/room - physical contact through a barrier
- [ ] Shared stall/room - physical contact with other elephants
- [ ] Common indoor area - open room used to house multiple elephants
- [ ] Other (please specify)

38. What is the average number of hours per 24 hour period that this elephant currently spends indoors in the summer?

- ◯ 0-3
- ◯ 4-6
- ◯ 7-9
- ◯ 10-12
- ◯ 13-15
- ◯ 16-18
- ◯ 19-22

Other (please specify)

39. What is the average number of hours per 24 hour period that this elephant currently spend indoors during the winter?

- ◯ 0-3
- ◯ 4-6
- ◯ 7-9
- ◯ 10-12
- ◯ 13-15
- ◯ 16-19
- ◯ 20-22

Other (please specify)

40. Does the average amount of time indoors change based on herd dynamics/management?

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

41. Has the amount of time housed indoors changed during the last 5 years for this individual elephant?

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

11.

42. How has the amount of time spent indoors changed over the last 5 years for this elephant?

43. What type of material is used as the substrate for the indoor enclosure for this elephant (excluding bedding materials)? (Check all that apply.)

- [ ] Natural substrate (ex. sand, dirt)
- [ ] Decomposed granite (DG)
- [ ] Manmade material (ex. concrete)
- [ ] Unknown
- [ ] Other (please specify)

44. Has the type of indoor substrate changed for this elephant during the last 5 years?

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

45. What type of ventilation is currently used in the indoor holding facility for this elephant? (Check all that apply.)

- [ ] Fans
- [ ] Ceiling vents
- [ ] Large doors
- [ ] Open walls
- [ ] Other (please specify)

46. Has the ventilation system of the indoor holding area changed in the last 5 years?

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

47. What cleaning procedures are currently used in indoor holding facility for this elephant? (Check all that apply.)

- ☐ Low pressure hosing
- ☐ High pressure hosing
- ☐ High pressure sprayer
- ☐ Manual removal
- ☐ Other (please specify)

48. Have the cleaning procedures in the indoor facilities changed during the past 5 years?

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

49. How have the cleaning procedures changed in the last 5 years?

50. Is a cleaning product used to disinfect the indoor facilities?

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

12.

51. How often is/are the cleaning product(s) used?

- [ ] 7 days/week (daily)
- [ ] 2-6 days/week
- [ ] 1 day/week (weekly)
- [ ] 2-3 days/month
- [ ] 1 day/month (monthly)
- [ ] As needed
- [ ] Cleaning products are not used
- [ ] Other (please specify)

52. Has at least one elephant moved into the facility during the last 5 years?

- [ ] Yes
- [ ] No
- [ ] Unknown
- [ ] Other (please specify)

13.

53. Has the elephant(s) that moved been in physical contact with this individual elephant?

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ No new elephants have moved into the facility
- ◯ Other (please specify)

54. Has this elephant traveled away from its home facility during the last 5 years?

- ◯ Yes
- ◯ No
- ◯ Unknown

Other (please specify)

14.

55. How many times has the elephant left the facility during the last 5 years?

***56. Has this elephant had public contact during the last 5 years (i.e. been within 25 feet of public, touched, ridden)?**

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

15.

***57. What is the average duration of public contact (in minutes) for each contact?**

- ◯ 0-15
- ◯ 15-30
- ◯ 30-45
- ◯ 45-60
- ◯ 60+
- ◯ This elephant does not have public contact
- ◯ Other (please specify)

***58. Has this elephant ever shared the same indoor holding facility (barn) with a TB culture positive elephant?**

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

59. How long ago did they share the same indoor holding space?

- ☐ Never
- ☐ Currently share same indoor holding facility
- ☐ 0-5 years
- ☐ 6-10 years
- ☐ 11-15 years
- ☐ 16-20 years
- ☐ 21-25 years
- ☐ 25+ years
- ☐ Unknown
- ☐ Other (please specify)

***60. Has this elephant ever shared the same outdoor enclosure with a TB culture positive elephant?**

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

61. How long ago did this elephant share the same outdoor enclosure with the TB culture positive elephant?

- ◯ Never
- ◯ Currently shares an outdoor enclosure with a TB culture positive elephant
- ◯ 0-5 years
- ◯ 6-10 years
- ◯ 11-15 years
- ◯ 16-20 years
- ◯ 25+ years
- ◯ Unknown
- ◯ Other (please specify)

***62. Has this elephant ever been at the same facility but in a separate enclosure (not in physical contact) with a TB culture positive elephant?**

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

16.

63. How long ago did this elephant share the same facility (but not same enclosure) as a TB culture positive elephant?

- [ ] Never
- [ ] Currently shares same facility with a TB culture positive elephant
- [ ] 0-5 years
- [ ] 6-10 years
- [ ] 11-15 years
- [ ] 16-20 years
- [ ] 21-25 years
- [ ] 25+ years
- [ ] Unknown
- [ ] Other (please specify)

***64. Has this elephant ever had a positive Mycobacterium tuberculosis culture from any bodily fluid or tissue sample?**

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Not tested
- ◯ Other (please specify)

***65. Has this elephant ever had a reactive result in an experimental ELISA test?**

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Not tested
- ◯ Other (please specify)

***66. Has this elephant ever had a reactive result in the ElephantTB STAT-PAK test?**

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Not tested
- ◯ Other (please specify)

67. Has this elephant ever had a reactive result in the MAPIA?

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Not tested
- ◯ Other (please specify)

***68. How often are trunk wash cultures currently performed for mycobacterial testing on this elephant?**

- [ ] Annually
- [ ] Semiannually (every 6 months)
- [ ] Quarterly (every 3 months)
- [ ] Monthly
- [ ] Never
- [ ] Other (please specify)

69. Has this elephant ever been treated with antimycobacterial drugs? (Check all that apply.)

	Yes	No	Unknown	Not applicable
Due to exposure to a TB culture positive elephant	☐	☐	☐	☐
Due to a reactive serologic test from this elephant	☐	☐	☐	☐
Due to a positive culture result from this elephant	☐	☐	☐	☐
Recurrence of TB post-treatment	☐	☐	☐	☐

Other (please specify)

70. Has this elephant ever had a positive TB culture post-treatment?

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Not applicable - not treated
- ◯ Other (please specify)

17.

***71. Have you answered additional questions pertaining to general herd management and TB history for your facility when entering information for another elephant at the same facility using this survey (if so, you will be able to skip the next section)?**

- ◯ Yes
- ◯ No

Other (please specify)

18.

72. How many elephants currently reside at the same facility with this elephant (including this individual)?

- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5
- ◯ 6
- ◯ 7
- ◯ 8
- ◯ 9
- ◯ 10
- ◯ 12
- ◯ 13
- ◯ 14
- ◯ 15
- ◯ 16
- ◯ 17
- ◯ 18
- ◯ 19
- ◯ 20
- ◯ 20+

Other (please specify)

73. How many elephants residing at the facility are currently over the age of 30 years (including this elephant)?

- ◯ 0
- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5
- ◯ 6
- ◯ 7
- ◯ 8
- ◯ 9
- ◯ 10
- ◯ 10+

Other (please specify)

74. Has the number of elephants residing at the facility changed over the past 5 years?

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

19.

75. How has the number of elephants residing at the facility changed over the last 5 years?

- ◯ Increased in number
- ◯ Decreased in number
- ◯ Number has varied over the last 5 years
- ◯ Unchanged
- ◯ Unknown

Other (please specify)

76. Does this facility house both Asian and African elephants?

- ◯ Yes
- ◯ No

20.

***77. In what region is the elephant facility permanently located?**

- ◯ Northeast (ME, NH, MA, CN, VT, NY, PA, NJ, DE, MD, PA)
- ◯ Mid-Atlantic (VA, NC, SC, WV, TN, KY)
- ◯ Southeast (FL, AL, MS, GA)
- ◯ Midwest (OH, IN, MI, WI, MN, IL, IA, MO)
- ◯ South (AR, LA, TX, NM)
- ◯ Southwest (CA, AZ, NV)
- ◯ Plains states (ND, SD, NE, KS, OK)
- ◯ West (MT, WY, CO, ID, UT)
- ◯ Northwest (WA, OR, AK)
- ◯ Hawaii
- ◯ Other (please specify)

21.

***78. How many full-time staff (trainers, handlers, keepers) are currently employed to provide daily care at the facility for elephants?**

- ◯ 1-4
- ◯ 5-8
- ◯ 9-12
- ◯ 13-16
- ◯ 17-20
- ◯ 20+

Other (please specify)

***79. How many part-time staff (1 staff=20 hours/week) are currently employed to provide daily elephant care at this facility?**

- ◯ 0-4
- ◯ 5-8
- ◯ 9-12
- ◯ 13-16
- ◯ 17-20
- ◯ 20+

Other (please specify)

80. How many volunteers (1 volunteer = average 5 hours/week) are used to assist with elephant care duties at this facility?

- ◯ 0-4
- ◯ 5-8
- ◯ 9-12
- ◯ 12+

***81. Has the full-time elephant staff changed over the last 5 years?**

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

82. Has the part-time elephant staff changed over the past 5 years?

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

***83. How often do you require paid elephant staff to have tuberculin skin tests?**

- ☐ At the time of hiring
- ☐ Semiannually (every 6 months)
- ☐ Annually
- ☐ Only if an elephant has a suspect/positive test for TB
- ☐ Never
- ☐ Other (please specify)

84. How often do you require volunteers involved with elephant care to have a tuberculin skin test?

- [ ] At the time of initial volunteering
- [ ] Semiannually (every 6 months)
- [ ] Annually
- [ ] Only when an elephant has a suspect/positive test for TB
- [ ] Never
- [ ] Other (please specify)

22.

***85. Has there been a case (dead or alive) of an elephant with TB (Mycobacterium tuberculosis complex organisms) cultured from any bodily fluid or tissue at this facility during any time in the past or currently?**

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

23.

***86. How many cases of elephant TB have occurred at this facility?**

- ◯ 0
- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5
- ◯ 6
- ◯ Unknown
- ◯ Other (please specify)

87. How many years ago did the case(s) occur? (Check all that apply.)

- ☐ Never had a TB case
- ☐ 0-5 years
- ☐ 6-10 years
- ☐ 11-15 years
- ☐ 16-20 years
- ☐ 20-25 years
- ☐ 25+ years
- ☐ Unknown
- ☐ Other (please specify)

88. Which type of TB was cultured? (Check all that apply.)

- [ ] Never had a TB case
- [ ] Mycobacterium tuberculosis
- [ ] Mycobacterium bovis
- [ ] Other mycobacterium species
- [ ] Unknown
- [ ] Other (please specify)

89. What sample(s) was the organism isolated from? (Check all that apply.)

- [ ] Never had a TB case
- [ ] Trunk wash
- [ ] Trunk swab
- [ ] Necropsy tissue - identify tissue in other
- [ ] Other diagnostic sample - identify in other
- [ ] Unknown
- [ ] Other (please specify)

90. What was the outcome of the culture positive elephant(s)? (Check all that apply.)

- [ ] Never had a TB case
- [ ] Treated and alive
- [ ] Treated and died/euthanized
- [ ] Died
- [ ] Euthanized
- [ ] Unknown
- [ ] Other (please specify)

APPENDIX C: DIAGNOSTICS AND TREATMENT SURVEY FOR NORTH AMERICAN ELEPHANTS

1. ELEPHANT TB DIAGNOSTIC TESTING HISTORY

***1. Please enter elephant ID code**

2. Please record all trunk wash culture information available for this elephant from the medical records, starting with the most current.

	Month trunk wash performed	Year trunk wash performed	Laboratory performing culture	Final result of culture
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				

30				
31				
32				
33				
34				
35				
36				
37				
38				
39				
40				
41				
42				
43				
44				
45				
46				
47				
48				
49				
50				
51				
52				
53				
54				
55				
56				
57				
58				
59				
60				

Other (please specify)

3. Please record all GenProbe information available from the medical records for this elephant, starting with the most current.

	Month sample collected	Year sample collected	Laboratory where test performed	Final result
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				

Other (please specify)

4. Please record all ElephantTB STAT-PAK information available from the medical records for this elephant, starting with the most current.

	Month sample collected	Year sample collected	Laboratory where test performed	Final results
1				
2				
3				
4				
5				
6				
8				
9				
10				

Other (please specify)

5. Please record all DPP (dual path platform) information available from the medical records for this elephant, starting with the most current.

	Month sample collected	Year sample collected	Laboratory where test performed	Final result
1				
2				
3				
4				
5				

Other (please specify)

6. Please record all ELISA information available from the medical records for this elephant, starting with the most current.

	Month sample collected	Year sample collected	Laboratory where test performed	Final result
1				
2				
3				
4				
5				

Other (please specify)

7. Please record all tuberculin skin test information available from the medical records for this elephant, starting with the most current.

	Month sample collected	Year sample collected	Tuberculin used	Final result	Injection site
1					
2					
3					
4					
5					

Other (please specify)

8. Please record all genotyping/spoligotyping information available from the medical records for this elephant, starting with the most current.

	Month sample collected	Year sample collected	Laboratory where test performed	Final result-designate strain
1				
2				
3				
4				
5				

Other (please specify)

2. ELEPHANT TB TREATMENT HISTORY

9. Has this elephant ever been treated with antimycobacterial drugs?

- ◯ Yes
- ◯ No
- ◯ Unknown
- ◯ Other (please specify)

3. ELEPHANT TB TREATMENT HISTORY

10. If this elephant has been treated with isoniazid, please provide the following information from the medical records.

	Dosage (mg/kg)	Frequency	Route	Drug formulation	Total full doses received	Duration of entire treatment (including interruptions)	Did treatment interruptions occur	Complications associated wit medication
1								
2								
3								
4								
5								

Other (please specify)

11. If this elephant has been treated with rifampin, please provide the following information from the medical records.

	Dosage (mg/kg)	Frequency	Route	Drug formulation	Total full doses received	Duration of entire treatment (including interruptions)	Did treatment interruptions occur	Complications associated wit medication
1								
2								
3								
4								
5								

Other (please specify)

12. If this elephant has been treated with ethambutol, please provide the following information from the medical records.

	Dosage (mg/kg)	Frequency	Route	Drug formulation	Total full doses received	Duration of entire treatment (including interruptions)	Did treatment interruptions occur	Complications associated wit medication
1								
2								
3								
4								
5								

Other (please specify)

13. If this elephant has been treated with pyrazinamide, please provide the following information from the medical records.

	Dosage (mg/kg)	Frequency	Route	Drug formulation	Total full doses received	Duration of entire treatment (including interruptions)	Did treatment interruptions occur	Complications associated wit medications
1								
2								
3								
4								
5								

14. If this elephant has been treated with a fluroquinolone (ex. enrofloxacin, ciprofloxacin), please provide the following information from the medical records.

	Dosage (mg/kg)	Frequency	Route	Drug formulation	Total # of full doses received	Duration of entire treatment (including interruptions)	Did treatment interruptions occur	Complications associated wit medication
1								
2								
3								

Other (please specify)

15. if this elephant has been treated with amikacin, please provide the following information from the medical records.

	Dosage (mg/kg)	Frequency	Route	Drug formulation	Total # fulld doses received	Duration of entire treatment (including interrruptions)	Did treatment interruptions occur	Complications associated wit medication
1								
2								
3								

Other (please specify)

4. SERUM BLOOD LEVELS

16. If serum blood levels were performed, please provide the following information from the medical records.

	Drug	Dosage (mg/kg)	Route of administration	Time serum collected post-administration	Serum drug level achieved (ug/ml)
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					

Other (please specify)

5. ADVERSE EFFECTS OF TREATMENT

17. If any adverse effects of antimycobacterial drug treatment were observed, please provide the following information from the medical records.

	Severity of clinical sign	Duration of single episode to resolve	Did clinical sign interrupt treatment?	Did multiple episodes of clinical sign occur?
Decreased appetite/anorexia				
Decreased water intake				
Discolored urine (ex. red-tinged)				
Discolored feces (ex. blood, melena)				
Constipation				
Loose feces/diarrhea				
Bloat				
Colic				
Drooling/excessive salivation				
Depression/lethargy				
Agitation				
Generalized weakness				
Paresis/weakness of the trunk				
Paresis/weakness of the limb(s)				
Tremors/muscle fasciculations				
Incoordination/ataxia				
Photophobia/light sensitivity				
Other:				

Other (please specify)

Chapter 3

Univariable logistic regression models

Adapted from Czepiel 2002, Dohoo et al. 2009, and Ott and Longnecker 2010.

The univariable logistic regression models in this chapter are defined as

$$\ln \frac{p(x)}{1-p(x)} = \beta_0 + \beta_1 x \tag{1}$$

where $\ln \frac{p(x)}{1-p(x)}$ is the log of the odds of capturing a badger, β_0 is the intercept where the value of the predictor is 0, β_1 is the regression coefficient for the predictor variable, and x is the value of the predictor variable.

The error terms take on two values:

$$\text{if } Y = 1 \text{ then } \varepsilon = 1 - \left(\beta_0 + \sum \beta_n x_n\right) \tag{2}$$

$$\text{if } Y = 0 \text{ then } \varepsilon = -\left(\beta_0 + \sum \beta_n x_n\right) \tag{3}$$

The likelihood function expresses the values of β in terms of the known values of $\boldsymbol{y}$, a vector of the observed counts of successes:

$$L(\beta|\boldsymbol{y}) = \prod_{i=1}^{N} \frac{n_i!}{y_i!\,(n_i - y_i)!} \pi_i^{y_i} \left(1 - \pi_i\right)^{n_i - y_i} \tag{4}$$

where N is the total number of individuals, n_i is the total number of observations for individual i, π_i is the probability of success for any given observation in the i^{th} individual, $\pi_i^{y_i}$ is the probability of y_i successes, and $(1-\pi_i)^{n_i - y_i}$ is the probability of $n_i - y_i$ failures.

The predictor variables in this analysis were as follows:

Predictor	Type	Definition
Sett type	categorical	main or non-main sett as defined by Department of Agriculture records
Sett activity score	categorical	score 0-5 assigned by field staff
Precipitation score	categorical	score 1-6 representing dry, drizzle, light rain, rain, heavy rain, snow
Minimum temperature	categorical	$\leq 2°C$; $3-5°C$; $6-8°C$; $\geq 9°C$
Maximum temperature	categorical	$\leq 9°C$; $10-12°C$; $13-16°C$; $\geq 17°C$
Traps per sett	continuous	number of traps laid at sett entrances on night of trapping
Season	categorical	season when trapping occurred (spring = Mar-May, summer = Jun-Aug, autumn = Sept-Nov, winter = Dec-Feb)
Year	categorical	year of trapping (2010-2013)
Zone	categorical	vaccination zone (A, B, or C) within study area

Multivariable logistic regression models

The multivariable logistic regression models in this chapter are defined as

$$\ln \frac{p(x)}{1-p(x)} = \beta_0 + \beta_1 x_1 + \ldots + \beta_n x_n \tag{5}$$

1

where $\ln \frac{p(x)}{1-p(x)}$ is the log of the odds of capturing a badger, β_0 is the intercept where the value of all predictors is 0, β_1 is the regression coefficient for predictor variable 1 with the values of the other predictors held constant, x_1 is the value of predictor variable 1, β_n is the regression coefficient for the n^{th} predictor variable with the values of the other predictors held constant, and x_n is the value of the n^{th} predictor variable. The likelihood function is defined as in equation 4. The predictor variables in this analysis include sett type, sett activity score, precipitation score, minimum temperature, traps per sett, season, and year from the table above, and an interaction term between weather and season.

Chapter 4

Univariable logistic regression models

The univariable logistic regression models in this chapter are defined as in equation 1, where $\ln \frac{p(x)}{1-p(x)}$ is the log of the odds of having a positive serological test result, β_0 is the intercept where the value of the predictor is 0, β_1 is the regression coefficient for the predictor variable, and x is the value of the predictor variable. The likelihood function is defined as in equation 4. The predictor variables in this analysis were as follows:

Predictor	Type	Definition
Time in captivity	binary	< 15 years or ≥ 15 years
Shared feed with wildlife	binary	yes or no
Contact with wild elephants	binary	yes or no
Overnight contact with other captive elephants	binary	yes or no
Sex	binary	male or female

Chapter 5

Spatially explicit capture-recapture models

Adapted from Borchers and Efford 2008.

The R package secr fit spatially explicit capture-recapture models to estimate animal density. These models estimate density of a closed population by placing K traps in an area containing animals with home ranges that have fixed centers.The period of time preceding a trap check is a trapping occasion. Animals are initially assumed to be at equal risk of capture.
Traps are in place for S trapping occasions. The kth trap has Cartesian coordinates $\mathbf{x}_k$ and locations of traps in the study is $\mathbf{x} = (\mathbf{x_1}, ..., \mathbf{x}_K)$. The number of uniquely identified animals is n. $\boldsymbol{X}$ is considered as the animal's home range center.
If animal i was captured on any of S occasions, $\omega_i = 1$; otherwise, $\omega_i = 0$. If animal i was captured in trap k on occasion s $(s = 1, ..., S)$, $\omega_{is} = k$; otherwise, $\omega_i = 0$. The ith animal's capture location history is $\omega_i = (\omega_{i\mathbf{1}}, ..., \omega_{iS})$.
The probability of an animal with home range center $\boldsymbol{X}$ being caught in trap k on occasion s, where $\boldsymbol{\theta}$ is the capture probability parameter vector, is $p_{ks}(\mathbf{X}; \boldsymbol{\theta})$. The probability that an animals is caught in any of K traps on occasion s is $p_{.s}(\mathbf{X}; \boldsymbol{\theta})$, and the probability that an animal was ever caught over S occasions is $p_{.}(\boldsymbol{X}; \boldsymbol{\theta})$ such that $p_{.}(\mathbf{X_i}; \boldsymbol{\theta}) = \Pr(\omega_i = 1 | \mathbf{X_i}; \boldsymbol{\theta})$.

State Model of Animal Home Range Distribution

Adapted from Borchers and Efford 2008 and Efford et al. 2009.

The analysis in chapter 5 is based on maximizing the likelihood below, where given n badgers captured and their associated captures histories $\omega_1, ..., \omega_n$,

$$L(\phi, \boldsymbol{\theta}|n, \omega_1, ..., \omega_n) = \Pr(n|\phi, \boldsymbol{\theta}) \Pr(\omega_1, ..., \omega_n|n, \boldsymbol{\theta}, \phi) \tag{6}$$

where $\boldsymbol{\theta}$ is the vector of detection function parameters and ϕ is a vector of parameters of the inhomogeneous Poisson process that governs density and distribution of badgers. Modeling an inhomogeneous Poisson process allows density to vary by different habitat covariates of interest. The inhomogeneous Poisson process has a rate parameter $D(\boldsymbol{X}; \phi)$ and its associated parameter vector ϕ. Based on an assumption of independent captures between badgers, the marginal distribution for n is based on a Poisson process with the rate parameter $\lambda(\phi, \boldsymbol{\theta})$ resulting from integration of the Poisson process with a probability of being caught at least once:

$$\lambda(\phi, \boldsymbol{\theta}) = \int_{R^2} D(\boldsymbol{X}; \phi) p_\cdot(\boldsymbol{X}; \boldsymbol{\theta}) d\boldsymbol{X} \tag{7}$$

Omitting parameter vectors for readability, the conditional distribution for $\omega_1, ..., \omega_n$ given n is:

$$\Pr(\omega_1, ..., \omega_n|n, \phi, \boldsymbol{\theta}) \equiv \Pr(\omega_1, ..., \omega_n|\omega_1 > 0, ..., \omega_n > 0, \phi, \boldsymbol{\theta}) \equiv \binom{n}{n_1, ..., n_C} \prod_{i=1}^{n} \Pr(\omega_i|\omega_i > 0, \phi, \boldsymbol{\theta}) \tag{8}$$

where $n_1, ..., n_C$ are the frequencies of observation for each of C observed capture histories, $\binom{n}{n_1, ..., n_C}$ is the associated multinomial coefficient, and $\Pr(\omega_i|\omega_i > 0, \phi, \boldsymbol{\theta}) = \int_{R^2} \Pr(\omega_i|\omega_i > 0, \boldsymbol{\theta}, \boldsymbol{X}) f(\boldsymbol{X}|\omega_i > 0, \phi, \boldsymbol{\theta}) d\boldsymbol{X}$ is the probability of observing capture history ω_i for badger i, given that it was captured.
The terms inside the integral can be expressed in terms of the capture probability function $p_{ks}|(\mathbf{X}; \boldsymbol{\theta})$and the inhomogeneous Poisson process rate $D(\boldsymbol{X}; \phi)$. The first term in the integral, the probability of observing capture history ω_i for badger i, given that its home range center is $\boldsymbol{X}$ and that it was captured, again omitting $\boldsymbol{\theta}$ for readability, is:

$$\Pr(\omega_i|\omega_i > 0, \boldsymbol{X}) = p_\cdot(\boldsymbol{X})^{-1} \prod_{s=1}^{S} \prod_{k=1}^{K} p_{ks}(\boldsymbol{X})^{\delta_k(w_{is})} [1 - p_{\cdot s}(\boldsymbol{X})]^{1 - \delta_\cdot(\omega_{is})} \tag{9}$$

where $\delta_k(w_{is}) = 1$ if $\omega_{is} = k$ and is 0 otherwise, $\delta_\cdot(\omega_{is}) = 1$ if $\delta_k(w_{is}) > 0$ for any $k = 1, ..., K$ and is 0 otherwise. Assuming independence of capture between occasions,

$$p_\cdot(\boldsymbol{X}) = 1 - \prod_{s=1}^{S} [1 - p_{\cdot s}(\boldsymbol{X})] \tag{10}$$

The second term in the integral, the conditional density of home range centers given an animal is captured is expressed as:

$$f(\boldsymbol{X}|\omega_i > 0, \phi, \boldsymbol{\theta}) = \frac{D(\boldsymbol{X}; \phi) p_\cdot(\boldsymbol{X}; \boldsymbol{\theta})}{\int_{R^2} D(\boldsymbol{X}; \phi) p_\cdot(\boldsymbol{X}; \boldsymbol{\theta}) d\boldsymbol{X}} = \frac{D(\boldsymbol{X}; \phi) p_\cdot(\boldsymbol{X}; \boldsymbol{\theta})}{\lambda(\phi, \boldsymbol{\theta})} \tag{11}$$

Model parameters $\boldsymbol{\theta}$ and ϕ can be estimated by maximizing the likelihood in equation 6, and evaluation of $D(\boldsymbol{X}; \phi)$ at the maximum likelihood estimate $\hat{\phi}$ returns an estimate of the density surface. The mean value of $D(\boldsymbol{X}; \hat{\phi})$ over an area is the maximum likelihood estimate of the mean animal density in the area.

Observation Model of Detection Probability

The probability of an animal being captured at least once over S occasions depends on the distances $d_k(\mathbf{X})$ to each of K traps is:

$$p_\cdot(\mathbf{X}; \boldsymbol{\theta}) = 1 - \prod_{s} \prod_{k} \{1 - p_s(d_k(\mathbf{X}); \boldsymbol{\theta})\} \tag{12}$$

where p_s is the detection function. In chapter 5, detection functions were modeled as hazard rates, which take the form:

$$p_s = g_0 \left[1 - e \left\{ - \left(\frac{d}{\sigma} \right)^{-b} \right\} \right] \tag{13}$$

where d is the distance between an animal's home range center and the detector, and b is a shape function. The parameters in $\boldsymbol{\theta}$ are g_0, σ, and b. The parameter g_0 is the probability of detection at a detector at the center of a home range, σ is the spatial scale of detection, and b is an additional shape parameter specific to the hazard rate function. When b is fixed at a large value, the hazard rate function is similar to a step function where $p_s(d) \approx 0$ when $d > \sigma$.
The multiple traps laid around a sett are considered as a multi-catch trap, which uses a competing risks hazard-rate form:

$$p_{ks} = \frac{h\left(d_k\left(\boldsymbol{X}\right)\right)}{h.\left(\boldsymbol{X}\right)} \left[1 - e^{-h.(X)} \right] \tag{14}$$

where $h\left(d_k\left(\boldsymbol{X}\right)\right) = -\ln\left\{1 - p_s\left(d_k\left(\boldsymbol{X};\boldsymbol{\theta}\right)\right)\right\}$ and $h.\left(\boldsymbol{X}\right) = \sum_{k=1}^{K} h\left(d_k(\boldsymbol{X})\right)$.

Model Extensions

Density Covariates

Different models included habitat covariates as predictors of density. The predictors in this analysis were:

Predictor	Type	Definition	Source
Four Areas Project land classification	categorical	Removal (includes both removal and buffer zones), reference, or outside the Four Areas Project study areas	Official records from Centre for Veterinary Epidemiology and Risk Analysis, University College Dublin
Land cover	categorical	Artificial, arable/crop, pasture, forest, shrubs, peat, water	European Union CORINE Land Cover 2012 database
Soil drainage	categorical	Excessively, well, moderately, imperfectly, poor, other	Irish Soil Information System Dataset
Soil texture	categorical	Coarse loamy, fine loamy, loamy, peat, alluvial	Irish Soil Information System Dataset

Sessions

Adapted from Efford et al. 2009 and Efford 2017a.

Capture data from the course of the study was split into sessions, which are treated as independent sampling blocks in which the population is closed to demographic changes. A combined multisession likelihood, or the product of within-session likelihoods, is used to model variation between sessions.

Varying Effort

Adapted from Efford et al. 2013 and Efford 2015.

To account for varying effort or usage (e.g., setting traps at only a subset of setts on a given night), a usage history is constructed for each occasion within a session at each trap. A binary indicator variable represented whether or not traps were laid at a sett on a given night. The model below describes the detection (δ_{sk}, y_{sk}) of an animal with home range center $\boldsymbol{X}$ at multi-catch detector k on occasion s, assuming effort T_{sk}:

$$\delta_{sk} \sim \text{Bernoulli}\left(p_{sk}\right) \tag{15}$$

where δ_{sk} is a binary indicator variable for the presence of an animal on occasion s and binary detector k. In equation 15, p_{sk} is defined as:

$$p_{sk}\boldsymbol{X} = \left[1 - e^{h_{\cdot s}(X)}\right] \frac{h_{sk}(\boldsymbol{X})}{h_{\cdot s}(\boldsymbol{X})} \tag{16}$$

The individual hazard $h_{sk}(\boldsymbol{X})$ in equation 16 is defined as:

$$h_{sk}(\boldsymbol{X}) = \frac{-T_{sk}}{T_0 \ln\left[1 - g\left(d_k(\boldsymbol{X})\right)\right]} \tag{17}$$

The probability of detection for an individual declines with the distance $d_k(X)$ between a detector k and the home range center coordinates $\boldsymbol{X} = (x, y)$. In the spatial detection function $g\left(d_k(\boldsymbol{X}); \boldsymbol{\theta}\right)$, $g(\cdot)$ is defined so that when $d_k = 0$, its intercept is a nonspatial scale parameter, g_0. The $g(\cdot)$ function is used to model per capita detection probability, p_{sk}, which is used to calculate the probability of observing each δ_{sk}. The probability of observing a given detection history is the product of the sequence of such probabilities, assuming independence between occasions. The full likelihood includes the model of the probability of each detection history, depending on the parameter vector $\boldsymbol{\theta}$, with a spatial point process model for the distribution of home range centers, depending on parameter vector $\boldsymbol{\phi}$, and maximization of the likelihood gives an estimate of $\boldsymbol{\theta}$.

Behavioral Response

Adapted from Borchers and Efford 2008 and Efford 2017c.

The automatically generated "b" predictor variable in the package secr invokes an M_b model describing a behavioral response to trapping is based on h_0 as a function of index b_{si}, which is 1 if badger i has been captured prior to occasion s and 0 otherwise.

Site-Specific Behavioral Response

Adapted from Royle et al 2011 and Efford 2017c.

The automatically generated "bk" predictor variable in the package secr invokes an M_{bk} model describing a site-specific behavioral response to trapping is based on a model of individual Bernoulli trials, $y_{i,j,k}$, where i represents the individual badger, j represents the trap, and k represents the sample period. The capture probability of encounter $p_{i,j,k}$ is:

$$\text{cloglog}\left(p_{i,j,k}\right) = \log\left(\lambda_0\right) - b1 \times d_{i,j}^2 + b2 \times x_{i,j,k} \tag{18}$$

where $d_{i,j}$ is the distance between the animal's activity center and the trap location, and $b1 = \frac{1}{\sigma^2}$.

Individual Heterogeneity

Adapted from Borchers and Efford 2008, Efford 2017b, and Efford 2017c.

The automatically generated "hcov" predictor variable in the package secr invokes a hybrid mixture model with two or three latent classes. If the class of all individuals in the model is known, the model is equivalent to a covariate. To model additional individual heterogeneity, a 2-class hybrid mixture model for age class was used to model σ. The age class variable is defined $u = 1, 2$ to index the subpopulations (juvenile and adult), and the class frequencies are n_0, n_1. The different populations have different capture probabilities, which can be modeled by making either the intercept $h_0(\cdot)$ or the scale parameter of $g(\cdot)$ dependent on u. The product of the Poisson term and the expression for λ must be split over detected individuals and include a multinomial term for the observed distribution over classes:
Including u in equation 6 results in a Poisson mixture model:

$$L(\boldsymbol{\theta}, \psi, \phi) \propto \frac{\lambda^n e^{-\lambda}}{n!} \prod_{i=1}^{n_0} \sum_{u=1}^{2} \int \frac{\Pr\left\{\omega_i | \boldsymbol{X}, \theta_u\right\}}{p_\cdot\left\{\boldsymbol{X}, \theta_u\right\}} f(\boldsymbol{X}, u | \delta_\cdot > 0) d\boldsymbol{X}$$

$$\times \prod_{i=n_0+1}^{n} \int \frac{\Pr\left(\omega_i | \boldsymbol{X}, \theta_{u_i}\right)}{p.\left(\boldsymbol{X}, \theta_{u_i}\right)} f'(\boldsymbol{X}, u | \delta. > 0; u_i) d\boldsymbol{X} \tag{19}$$

$$\times \binom{n-n_0}{n_1, n_2} \prod_{u=1}^{2} \left[\frac{\lambda_u}{\lambda}\right]^{n_u}$$

where $\lambda_u = \psi_u \int D(\boldsymbol{X}) p.(\boldsymbol{X}, \theta_u) d\boldsymbol{X}$, the multinomial coefficient $\binom{n-n_0}{n_1, n_2}$ is a constant that can be omitted, and $f'(\cdot)$ is the probability density of $\boldsymbol{X}$ for a given u_i:

$$f'(\boldsymbol{X} | \delta. > 0; u_i) = \frac{D(\boldsymbol{X}) p.(\boldsymbol{X}, \theta_{u_i})}{\int D(\boldsymbol{X}) p.(\boldsymbol{X}, \theta_{u_i}) d\boldsymbol{X}} \tag{20}$$

The likelihood in equation 18 conditions on the number of known-class animals detected, and assumes that the probability of class being recorded is not dependent on class, and that recording class occurs without error.

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LIST OF ACRONYMS AND ABBREVIATIONS

AFB	acid-fast bacilli
AIC	Akaike's information criterion
AICc	Akaike's information criterion corrected for small sample size
BCG	Bacille Calmette-Guérin
CI	confidence interval
CFP10	10 kDa culture filtrate protein
CFT	caudal fold test
CORINE	Coordination of Information on the Environment
DIVA	differentiating infected from vaccinated animals
DPP	Dual-Path Platform
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
EMB	ethambutol
ESAT-6	6 kDa early secretory antigenic target
HIV	human immunodeficiency virus
IS*6110*	insertion sequence 6110
KAZA	Kavango-Zambezi
KNP	Kruger National Park
IFN	interferon
INH	isoniazid
LL	log likelihood
MAPIA	multiantigen print immunoassay
MBCF	*Mycobacterium bovis* culture filtrate
MDR-TB	multi-drug-resistant tuberculosis
MIRU-VNTR	mycobacterial interspersed repetitive unit–variable number tandem repeat
MPB	protein purified from *M. bovis* BCG
MTBC	*Mycobacterium tuberculosis* complex
MOT	mammalian old tuberculin
MOTT	mycobacteria other than tuberculous mycobacteria
NTM	non-tuberculous mycobacteria
OIE	World Organisation for Animal Health (Office International des Epizooties)
OR	odds ratio
OTF	officially tuberculosis free
PCR	polymerase chain reaction
PPD	purified protein derivative
PIT	passive integrated transponder
PPR	peste des petits ruminants
PZA	pyrazinamide
RBCT	Randomized Badger Culling Trial
RIF	rifampin
RFLP	restriction fragment length polymorphism
RPSV	root pooled spatial variance
SD	standard deviation

SECR	spatially explicit capture-recapture
SIR	susceptible-infected-recovered/resistant
SIT	single intradermal cervical test
SICCT	single intradermal comparative cervical test
TFCA	transfrontier conservation area
TB	tuberculosis
TST	tuberculin skin test
WGS	whole genome sequencing