Disease Control through Fertility Control: Explorations in Two Urban Systems

DISSERTATION

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By

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Abstract

In many areas, wildlife populations have increased substantially in their local density because of a loss of natural controls or some artificially supplemented resource. These populations are often managed to avoid harmful effects on other wildlife species and human-wildlife conflicts. Many species are managed using lethal population reduction, but in those that are resistant to these means or where the method is unpalatable due to public concern, fertility control is becoming increasingly common. This method seeks to reduce the population size of some target problem species by capturing, sterilizing, and releasing individuals back into their habitat. Fertility control is often paired with vaccination programs because each has synergistic effects. Sterilization reduces the population size, making it easier to achieve a higher vaccination proportions for herd immunity. However, these programs have uncertain effects on both the basic biology, population demographics, and disease epidemiology. The current literature makes strongly countered species-specific conclusions. It is also unclear if fertility control is an effective method at reducing the population size in an economically viable way, compared to lethal removal.

Here I use computer simulations, cross sectional surveys, and long-term monitoring of two populations, the street dogs (*Canis lupus familiaris*) of Rajasthan, India, and the raccoons (*Procyon lotor*) of the Columbus Zoo and

Aquarium, to investigate what impact fertility control makes on the populations it targets. In Chapter 2, I exposed replicate simulated populations to various control schemes to see which most lowered the population size and increased vaccination coverage. In Chapter 3, I report the results of surveys of dogs from several real world Indian cities with varied histories of fertility control for several diseases. In Chapters 4 and 5, I report the results of a randomized control study on raccoons, which measured differences in parasite load and survival among control, vaccinated and vaccinated/sterilized individuals.

My work demonstrates that fertility control programs can be more effective than lethal control, although the methods used to locate sexually intact individuals for treatment can significantly affect the results. In Chapter 3, I found that intact dogs living in cities with more fertility control had significantly lower prevalence of several diseases compared to those dogs living in cities with less fertility control. This is especially significant because the interventions only vaccinated against rabies, meaning that the fertility control affected local disease epidemiology. This indicates that the sterilization program buffered treated individuals' ability to resist or spread disease enough to lower exposure to non-treated individuals. I found that sterilization and vaccination in raccoons did not affect the apparent monthly survival rates, but lowered parasite prevalence in males. However, female raccoon parasite prevalence was negatively affected by sterilization. I suggest that the sterilization method used does not eliminate hormone production, causing females to increase the length or intensity of their reproductive seasons. As a whole, this work highlights the importance of understanding the secondary effects of intervention policies. I show that altering reproductive behavior can cause dramatic changes to population dynamics and epidemiology.

Dedication

Dedicated to Phillip, I hope you enjoy the chase, but please don’t catch the antelopes.

Acknowledgments

This work is the sum effort of an enormous support network and I could not have hoped to execute these studies without them. Ian M Hamilton and Stanley D Gehrt gave me direction with the freedom to follow my passion. Jack F Reece and the ABC team at Help in Suffering were a beacon of calm in a sea of chaos. The entire veterinary staff at the Columbus Zoo and Aquarium were incredibly supportive and invested a tremendous amount of time and resources into this study. Rebecca Garabed and Liza Comita have provided invaluable support. Jonathan Hall and Anil K. Chhangani helped to spark my initial research interests.

My family and my partner Kellen have been by my side throughout all my wanderings.

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Publications

Yoak, A.J., Reece, J.F., Gehrt, S.D., & Hamilton, I.M. In Press. A survey of street dog gastro-intestinal helminthes in Jaipur and Jodhpur. Indian Veterinary Journal.

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Fields of Study

Major Field: Evolution, Ecology and Organismal Biology

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

Anthropogenic impacts on the environment do not always result in the decline or eradication of affected species; conversely, some have adapted well to fragmented landscapes (Bradley & Altizer 2007, Gehrt *et al.* 2002, Hughes & Macdonald 2013). As these species' population sizes dramatically increase, wildlife managers come under additional pressure to intervene because these problem wildlife can predate on less abundant species (Barton & Roth 2007) or spread species-jumping pathogens (Reece 2007). Traditionally, these interventions take the form of lethal depopulation and attempt to depress population density (Sillero-Zubiri *et al.* 2009). However, by removing individuals, lethal control interrupts the social and territorial relationships between remaining individuals which may increase the spread of disease (Donelly *et al.* 2006, Vicente *et al.* 2007). The lowered density in depopulated areas could also draw in individuals from outside the treatment area at the landscape scale (Killian *et al.* 2007). Both public ethical concern and resistance to depopulation by certain species has caused biologists to pursue fertility control as an alternative method (Killian *et al.* 2007).

Animal Birth Control (ABC) programs use varied methods, but generally seek to slowly lower the density of target species by reducing their reproductive rate (Killian *et al.* 2007, Kirkpatrick *et al.* 2011, Gray & Cameron 2010, Reece 2007). "Success" for ABC programs is normally defined as a reduction in the population size, but some programs use this decline only as a means to eliminate some particularly problematic pathogen such as rabies with concurrent vaccination (Reece 2007). In zoos, fertility control is becoming increasingly common, as they struggle to house species with high reproduction rates or costly requirements (Kirkpatrick *et al.* 2011). Advances in the use of the injectable sterilizing vaccines against gonadotropin-releasing hormone (GnRH) and porcine zona pellucida (PZP) have produced promising results with species such as white tailed deer (*Odocoileus virginianus,* Rutberg *et al.* 2004), African elephants (*Loxodonta africana,* Delsink *et al.* 2006), and American black bears (*Ursus americana,* Lane *et al.* 2007). In areas without the resources for new techniques or in species where they have not been tested, surgical sterilization is still common. This process can either entail complete sterilization, where the ovaries/testes are removed by spay/castration, or functional sterilization, where egg/sperm are blocked from exit by tubal ligation/vasectomy. Functional sterilization leaves hormone production intact and is the preferred method when managers want hormone-linked behaviors (such as territoriality) to be maintained (Bromley & Gese 2001) and spay/castration is preferred when managers what to eliminate those behaviors (Reece 2005)

The domestic dog, *Canis lupus familiaris,* is the most abundant large mammal with at least 700 million individuals worldwide (Hughes & Macdonald 2013) and is responsible for 55,000 human deaths each year from rabies (Knobel *et al.* 2005). While vaccination and licensing laws in the developed world have eliminated canine rabies and keep their populations in check, dogs in developing nations have little to no oversight on breeding and rabies vaccination is sporadic (Butcher 1999, Hughes & Macdonald 2013, Reece 2007). In India, street dogs make up a large proportion of the urban dog population, and the temporal pattern of attacks on humans is strongly associated with the period after the breeding season when bitches are caring for litters (Reece *et al.* 2013). ABC programs targeting street dog rabies in Indian cities have eliminated human rabies cases (Reece *et* *al.* 2007) and lowered the bite rates (Reece *et al.* 2013) in the areas they operate, but not all municipalities have invested in these programs (Yoak *et al.* 2014).

In the eastern US, the role of an urban mesopredator and scavenger is filled by the common raccoon (*Procyon lotor*) whose populations can become very dense (Rosatte *et al.* 2007, Smith & Engeman 2002) and carry a large number of diseases (Berentsen *et al.* 2013, Hirsch *et al.* 2013, Junge *et al.* 2007, Page *et al.* 2008). Raccoons are hunted in some areas of the US (Hodges *et al.* 2000), but in many areas their populations are unmanaged, leading to expensive damage to crops (Beasley & Rhodes Jr 2008), heavy predation on already threatened wildlife (Barton & Roth 2007), and disease spillover (Gavin *et al.* 2002, LoGuidice 2003). When raccoons are managed though lethal depopulation, the population returns to the same, if not higher, population density in a short time period (~1 year) after the intervention concludes (Rosatte *et al.* 2007). There is disagreement on whether the repopulation occurs because of local reproduction (Rosatte *et al.* 2007) or dispersal into the treated zone from outside the treatment area (Barton & Roth 2007). Zoos in the US have a substantial incentive to manage the raccoon population on their grounds because their captive animals come from broad taxonomic distributions with variable susceptibility to raccoon pathogens. Because of their resistance to depopulation and the concern about their pathogens some zoos have begun fertility control programs targeting raccoons on their grounds (Myers *et al.* 2004).

The research presented here investigates the effects that fertility control has on population disease dynamics as well as population size and demographics in two species of urban carnivore: the domestic dog (*Canis lupus familiaris*) and common raccoon (*Procyon lotor*). These studies seek to increase both the basic and applied knowledge surrounding fertility control by better understanding how the structure of ABC programs influences their effectiveness and how individuals and populations respond to sterilization. A better understanding of how to control wildlife populations using sterilization programs could give managers a powerful tool to minimize human-wildlife conflicts.

In chapter two, I produced a spatially explicit agent based model of the female dog population in Jaipur, India, which modeled the seasonality of breeding, variable dog movement, stochastic death, and various dog population capture and control method strategies. Using this model, we tested which methods best reduced the dog population size and favorably altered age demographics by directly contrasting fertility control and lethal control. Using a survey of sterile dog locations around the city to inform fertility control produces the lowest population size, highest sterilization percentage, and lowest proportion of the population under three months old.

In chapter three, I investigated three cities in Rajasthan, India for the prevalence of several canine pathogens in their sexually intact street dog population. The three locations varied in their history of fertility control implementation; ranging from none, to 7 years, to 17 years of ABC. For six of ten diseases examined, there was a trend toward healthier dogs in cities with more ABC. For two diseases, there was no effect of location and tick infestations were more prevalent in ABC cities. Because this study only focuses on the sexually intact dogs living each city, it establishes that ABC programs can have spillover effects even for non-targeted individuals and non-vaccinated diseases.

In chapter four, I examined the effect of vaccination and sterilization on the apparent survival of raccoons living on the grounds of the Columbus Zoo and Aquarium. Individuals were split into three treatment groups; a group which was sterilized and vaccinated, a group which was vaccinated, and a control group. We were not able to discern between a Barker Robust Design model that included the effect of vaccination and sterilization from a null model that predicts no difference between treatment groups.

In chapter five, I describe the effects of the treatment groups from the previous chapter on gastrointestinal helminths. Male raccoons that received any treatment at all had significantly lower parasite loads compared to control males, likely because the treatment included an anti-helminthic drug. Females that were sterilized, however, had significantly higher parasite compared to both other female treatment groups, possibly because the sterilization method caused changes to female behavior.

Chapter 2: Optimizing street dog control programs using agent based models.

ABTRACT

Street dog populations in the developing world are managed by a patchwork of local practitioners, government programs, and non-governmental organizations with varied effectiveness. Lethal removal is still practiced commonly while competing vaccination and fertility control methods have begun to be adopted. Identifying which method provides the most cost effective management is needed to inform dog population managers. Here we describe an agent-based model with which we sought to simulate the population of street dogs in Jaipur, a typical Indian city, then apply various lethal and fertility control methodologies to identify the best options. This spatially explicit model includes accurate temporal and demographic details of street dog populations in order to replicate real world systems. Control regimes for both lethal and fertility control methods that focus their efforts around the city were tested for their effects on population size, age structure, vaccination coverage, as well as the number of dogs that are handled. Models were run for 15 years to show the long term effects of any intervention. We show that fertility control outperforms lethal removal programs at reducing the population size while vaccinating a significant proportion of the population. Lethal programs skewed population demographics towards younger dogs. We also show that targeting city districts with high percentages of unsterilized dogs yields better

results than targeting districts with high numbers of unsterilized dogs or randomly targeting districts.

INTRODUCTION

Domestic dogs are the most common large carnivore with a global population of 700 million (Hughes & Macdonald 2013). Street dog population densities in urban environments can be high with estimates ranging from 88 to 250/km^2 (Matter *et al.* 2000, Townsend *et al.* 2013). Higher densities of dogs, compared to other predators, are possible because of human tolerance and supplementation of their diets with leftovers, refuse, and offal (Butler & du Toit 2002). Controlling their populations in developing nations is an important issue, as they spread rabies and other zoonoses, threaten local wildlife, and often live with low quality of life (Hughes & Macdonald 2013, Lacerda *et al. 2009,* Totton *et al.* 2011, Yoak *et al.* 2014). Dogs in natural areas have been shown to create artificial edge effects in intact forest ranges by disrupting wildlife behavior and land use (Lacerda *et al.* 2009). Street dogs regularly come in contact with wildlife (Butler *et al.* 2004) and commonly carry zoonotic and sylvatic diseases such as leishmaniasis (Ashford *et al.* 1998), canine distemper, canine parvovirus, and ehrlichiosis (Butler *et al.* 2004, Yoak *et al.*2014). In India alone, canine rabies kills 20,000 people each year, with patients predominately coming from the lowest income classes (Sudarshan *et al.* 2007) and dog-transmitted cystic echinococcosis costs the nation $212.35 million per year (Singh *et al.* 2014). Disease spillover from domestic dog populations into wildlife populations has been confirmed for multiple events and this risk could be eliminated or at least mitigated by reducing local dog density (Cleaveland *et al.* 2000).

Historically, lethal removal has been the preferred method of canine control; more recently however, it has been shown to be less effective and more expensive over time compared to birth control programs (WHO 2005). Additionally, simulations of lethal removal's effect on disease progression in wildlife show that it may increase infection intensity and spillover risk (Choisy & Rohani 2006). Canine control programs using trap-neuter-vaccination-release have already arisen in an attempt to curtail dog population sizes and reduce the incidence of rabies in those areas where lethal control was already culturally prohibited (Reece 2007). These programs catch free-roaming dogs on the street, neuter and vaccinate them against rabies, then return sterilized individuals back to the point of capture within a few days (Reece & Chawla 2006). A focus on female dogs is commonly used by these animal birth control (ABC) programs, as any reduction of the intact female population has a much greater effect on reproduction compared to spreading effort across both sexes (WHO/WSPA 1990). These programs have produced effective results; in Jaipur, capital of the state of Rajasthan, India, the Help in Suffering (HIS) ABC program has brought the local human rabies incidence to zero and reduced the dog population by at least 28% (Reece and Chawla 2006). As ABC programs are begun in other Indian cities (Totton *et al.* 2011), there is increasing need for rigorous data collection methods to compare success between regions. However, long term programs to monitor the effectiveness of intervention present a challenge to small ABC programs, either from a lack of funds or if the benefits for starting one are unclear.

Our goal was to build a demographically accurate and spatially explicit model of a free roaming dog population to investigate how to best maximize the effect of intervention. We seek to produce collection methods that keep the costs of intervention for NGOs low while reducing dog population sizes. We are investigating how altering the search methods used by control programs impacts their efficacy and how intervention affects dog population demographics.

To simulate the effects of different survey methods on dog control programs, we developed an agent-based model (ABM) of the female dog population of Jaipur, India. ABMs have been used address large scale ecological management questions in general (Bonnell *et al.* 2010) and canid behavior in particular (Belsare & Gompper 2014, Pitt *et al.* 2003). To standardize the presentation of processes and results we use the updated Overview, Design concepts, and Details (ODD) protocol for describing ABMs (Grimm *et al.* 2010). We produced a model dog population that matches the demographics of northwest India's street dogs (Hiby *et al.* 2011) as well as their locally seasonal breeding (Chawla and Reece 2002) and daily movement patterns (unpublished data from J Reece). Evaluating how vastly different resource intensive management policies affect the same population over a long period of time in the real world would clearly be impossible and highlights the value of modeling in dog management. A spatially discrete ABM, as opposed to more traditional modeling approaches, captures the variation in dog movement and survival while better accounting for variations in dog density across the city. Using this ABM, we investigated a) the relative effects of lethal control and fertility control and b) the effects of various strategies used by control programs to target dogs for either lethal or fertility control on resulting dog demographics. These iterations are carried out without having to invest any of the capital that limits experimentation in this system.

METHODS

We initially developed a spatially explicit model of the dog population of Jaipur, India that realistically matched its seasonal and demographic traits. Then, various search strategies for both lethal and fertility control methods were tested

Model Description

This model was created using Netlogo v5.04 (Wilensky 1999). A map of Jaipur was rasterized using GIMP 2 photo editing software to produce a background landscape. Both packages are open source and are available free for download from http://ccl.northwestern.edu/netlogo/ and http://www.gimp.org/ respectively. Models were run using the Ohio Supercomputer Center's assistance (www.osc.edu).

Purpose

The purpose of this model is to test the effect that altering capture protocols has on ABC and lethal removal management programs on street dog populations; program effectiveness is measured by the number of surgeries/euthanasia, population demographics, final population size, and for ABC methods, the sterilization/vaccination percentage achieved.

Entities, state variables, and scales

In our model we included dog agents and ABC capture team agents, as well as their interactions with the landscape. The environment is made up of a 256 x 356 square cell grid, where each cell is roughly a 50 m x 50 m area of Jaipur. A rasterized map was produced using Google Earth and imported into the model. As HIS personnel split the city into more manageable areas when investigating the real world population, we assigned cells to one of 18 zones for use in the surveillance process. These city zones were based on groupings of real world neighborhoods in Jaipur and show some variation in size and shape (Fig 1). Agents are described in table 1.

Table 1. Agent parameters for dogs and ABC capture (dog control) teams.

Agent	Parameter	Meaning	Default Value or Range	Reference
Dog	L	Location in the city	-	
	M	The number of patches a dog moves each day	Multinomial distribution (see text)	Derived from unpublished data from Reece
	λ_a	Adult dog yearly survival rate	0.8	Reece *et al.* 2008
	λ_j	Juvenile dog (< 1 y) yearly survival rate	0.25	Reece *et al.* 2008
	P	Average number of puppies produced during each reproductive event	2.82	Chawla & Reece 2002
	R	Chance of reproduction per day	.0004 - .0075 (Varies seasonally)	Derived from Chawla & Reece 2002
ABC Capture Team	L	Location in the city	-	-

The main agents are dogs, representing individual bitches that make up the whole female street dog population. Dog agents have several state variables specific to each dog: age, sterilization status, movement pattern, and location.

In those models that examine the effects of intervention, a second agent class representing the dog control capture team is included. The capture team agent's only state variables are the search method being used and location.

Patches, or cells, exist in square grid and form the landscape of which the city is formed. In these patches, dogs and capture team agents carry out their actions. Each patch is assigned a zone number depending on where it falls in the map of Jaipur. To represent heterogeneity in habitat quality throughout the city, each zone has a carrying capacity that is drawn each model run from a uniform distribution +/- 20% from the mean carrying capacity. This mean carrying capacity was calculated to allow the model dog population size to be equivalent to the real-world Jaipur's known population size (Hiby *et al.* 2011) on the model's map.

A single time step is equivalent to one day. Dogs move, breed, and die; ABC vans spawn and sterilize each time step. All versions of the model includes a five year "burn-in" where the population of dogs runs with no intervention to reduce the effects of initial parameters.

Process Overview and Scheduling

To initialize the model, the city map is imported (Fig. 1) and an initial population of 25,000 female dogs is created. We parameterized the model by altering breeding success at a set local density to have a natural stable population of approximately 25,000 females in the non-intervention model. This figure is modified from the population estimate from Jaipur (Hiby *et al.* 2011) and a 1:1 sex ratio (Reece and Chawla 2006) while accounting for growth in the city's human population. Dog agents are randomly seeded around the map within the city boundaries and their age distribution matches published information (Reece *et al.* 2008).

Dogs can be seen as the black (sexually intact) and grey (sterilized) dots. A control van can be seen as the grey car. Shades of the background environment indicate different zones

Figure 1. The model area seeded with agents.

A diagram of the model processes can be found in figure 2. Each time step, dogs move, then potentially breed and die, then update their state variables. Next, a survey may be performed that determines the location of each dog, both sexually intact and neutered. This survey is only performed once per year. Next, two capture team agents have an 88% chance to spawn in a manner matching the particular search method being used. This spawn rate matches the real world days off from holidays, weather, or mechanical problems (J. Reece, pers comm). The different models are as follows:

- Under the *non-intervention* model, the dog capture teams never spawn and no dogs are ever altered, simulating the natural population size and structure with no intervention.
- The *spatially-random ABC* model places two dog capture teams in two randomly derived locations around the city map and they sterilize dog agents.
- The *informed-absolute ABC* model places two dog control teams in two semi-random locations around the city with the chance of being spawned in a certain zone being weighted by the absolute number of dogs in that zone compared to other zones. For example, if a three zone city had 100, 50, and 40 intact bitches in each zone respectively; each day until the next survey is completed, it would choose to move to the first zone one 52.6% of the time. The second zone would be selected 26.3% of the time and the third zone 21.1% of the time, focusing effort on areas that have higher numbers of intact females. The generalized equation is as follows:

$$Probability\ to\ choose\ Zone\ X = \frac{N_{dogs\ in\ zone\ x}}{N_{dogs\ in\ all\ zones}}$$

- The *informed-percentage ABC* model places two dog control teams in two semi-random locations similar to the informed-absolute method, only using the percentage of unsterilized dogs in the zones instead of the absolute number of them to weight the chance of spawning in each zone. This weighting produces a greater chance of ABC vans spawning in zones with high proportions of intact individuals.

- The *spatially-random lethal* model is identical to the spatially-random ABC method, only instead of sterilizing dog agents, these control teams euthanize them instead.
- The *informed-absolute lethal* model is identical to the informed-absolute ABC method, but like the other lethal method, euthanizes dogs instead.

There is no informed-percentage lethal model as there are no sterilizations performed during lethal models to inform the capture team's selection of a location.

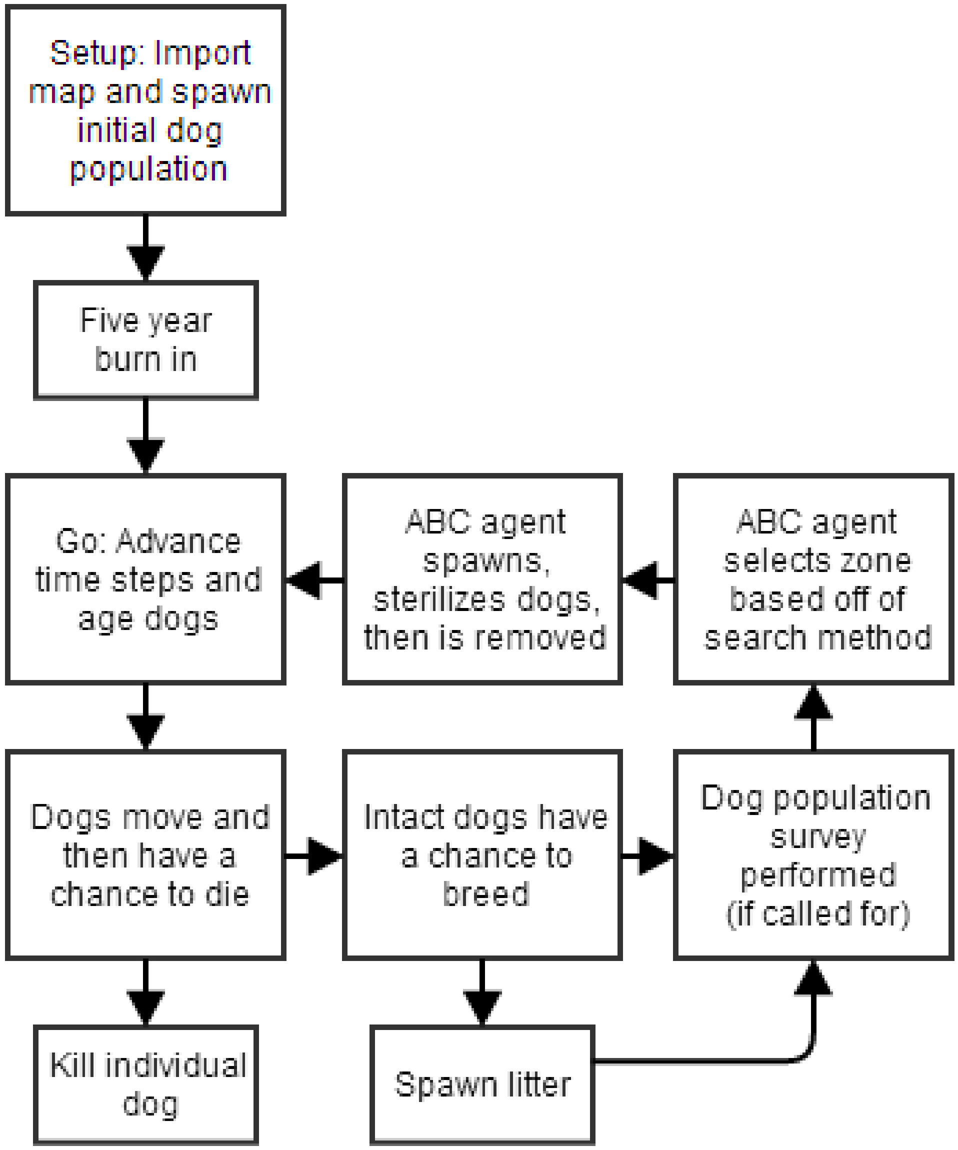

Figure 2. Flowchart depicting processes in the spatially explicit, agent based model of bitches in Jaipur.

Design concepts

Basic Principles

Emergence: The overall and zone-specific dog populations, sterilization percentages, and age structures emerge from the processes of ABC treatment, mortality, and reproduction. The number of surgeries or euthanasia performed emerges from the interplay between population size and ABC or lethal removal treatment.

Adaptation: Dog agents limit their reproduction if their local dog density is higher than the zone's carrying capacity in the breeding submodel. Capture teams under the informed-absolute and informed-percentage search methods used (sometimes outdated) information from the last city-wide survey about the location of intact dogs to choose where to spawn each time step.

Objectives: The general objective of intervention regimes was to reduce the dog population size, have a high sterilization/vaccination percentage, and to do so in as few actions as possible. However, these objectives are analyzed only at the end of each computational run and do not impact any decisions by dogs or capture teams during a run.

Interaction: The only interaction between dogs was indirect limiting of breeding success based on local population density. Capture teams in ABC models collected dogs in a small radius and altered their sexually intact status to neutered. In lethal removal models, capture teams collect dogs in an identical manner to the ABC model; however, instead of altering sexually-intact status, they remove the dog agent from the landscape.

Stochasticity: Daily dog movements, survival, reproduction, and dog collection figures each introduced stochastic effects into the model. Each time step, the order each dog performed its actions was randomized to avoid any unintended benefits to first–acting individuals.

Collectives: For purposes of the surveys, the dogs within in a single zone are collectively analyzed to produce the figures on percentage and absolute number of intact dogs. Collection team agents use the same information (the yearly survey of dog locations) but make their decisions about where to go independently of one another.

Observation: The seasonal breeding causes a variation of +/- 1000 dogs in the model within a year, so to account for this variation, an average of the daily population size, percentage of the population under 3 months, and sterilization percentage from the past year is produced each time step. These year-long averages are the figures used for analysis of their respective variables.

Initialization

25,000 dog agents are spawned randomly around the city map and are given a randomized age structure matching published information (Reece *et al.* 2008, Hiby *et al.* 2011). Before any data recording or ABC treatments are begun, a "burn in" period of 5 years is run to remove any effects of the initial population characteristics.

Input Data

The main model input is the distribution of city zones. These are taken from the real-world locations of neighborhoods used for local government management.

Submodels

Movement: Each step, dogs selected a random direction then moved forward a number of patches drawn from a multinomial distribution calculated from the real-world movements of Jaipur dogs. Unpublished data from Reece's work (*et al.* unpublished data) on the fecundity of street dog included data on where dogs were captured both an initial and second time, and how much time had passed between the two events. Using this information, km moved per day was calculated for 256 dogs and then the data summarized by a multinomial distribution with 31% of individuals not moving at all. For individuals who do move, the distance is calculated from a random lognormal distribution with a mean of -3.40 and standard-deviation of 1.86. Any negative movement distances were altered to 0. Each dog recalculates their movement each day. These movement rates capture the variability in dog territory size and movement patterns in Jaipur.

Mortality: Individual dogs over one year old and within the city boundaries have a chance of natural death corresponding to the published $\lambda_a = 0.70$ of sterilized bitches older than one year in Jaipur city (Reece *et al.* 2008) This survival estimate produced expected lifespans that closely matched the published 3.8 years expected lifespan at a year old. Puppies and juveniles have a substantially lower yearly survival in the real world and we found that the published juvenile $\lambda_j = 0.25$ produced the expected 1.3 years expected lifespan at birth (Reece *et al.* 2008). Individuals that have wandered out of the city into the surrounding countryside have a substantially lower survival rate to mimic the paucity of food and other important canine resources outside the city.

Breeding: Each intact bitch over eight months old had a seasonally adjusted chance of reproducing and immediately whelping a litter. The seasonal rate is taken from the percentage of bitches that were pregnant at the time of capture by the ABC program (Chawla and Reece 2002). Each successful reproductive event produces a small number of female puppies with a normal distribution ($\mu = 2.81$, $\sigma = 1$). This matches the mean of 5.62 fetuses per pregnant bitch in Jaipur, (this figure did not discern the sex of the fetus) while keeping some natural variation (Chawla and Reece 2002).

Abandonment: Each day, a small number of dogs ($\mu = 2$, $\sigma = 1$, rounded to nearest non-negative integer) with an age of 1.5 years were created randomly around the city, representing the abandonment of pet dogs after they have passed being a puppy. No figures are available due to its rare nature, but this is a significant point of concern for ABC programs (J Reece, pers comm).

Sterilization: In models with ABC, dog control agents each collected several ($\mu = 6$, $\sigma = 2$, rounded to nearest integer) of the nearest sexually intact dogs and altered their status to sterilized, eliminating their ability to reproduce. This figure matched the average dogs collected by HIS in Jaipur by each van. Dog control agents then disappeared.

Euthanasia: In those models which used lethal control, the dog agents which have been selected by dog control agents are removed entirely. Dog control agents then disappeared.

STATISTICAL ANALYSIS

The model produced information on the dog population, sterilization percentage, and number of surgeries performed. Each dog management regime was repeated for 40 trials to ensure we detected stochastic variation in the model outcomes but did not create false significance through over-repetition. A local sensitivity analysis was performed to assess the effects of minor variations (±5%) of parameters on model results. While some parameter variation had significant effects on model results, each dog management regime was similarly affected and none changed the ordinal ranking of each regime's efficacy (Appendix A). Each simulation was run using a Linux OS based super-computing center (www.osc.edu).

The comparisons between control regimes was assessed using one way ANOVA with a *post –hoc* Tukey's test using SPSS v22 statistical software (IBM Corporation). All figures were created using Sigma Plot 12.5 (Systat Software).

RESULTS

Effect on population size

The method of dog collection showed significant impacts on the dog population size ($F= 188.506$, $df_{num}=5$, $df_{denum}=234$, $p < 0.05$) and the results can be seen in Fig 3a. Informed-percentage ABC reduced the dog population compared to the non-intervention model more than any other method (followed by informed-absolute ABC, spatially-random ABC, then both lethal control methods. The two lethal control models showed no significant difference from each other.

Effect on puppy percentage

The method of dog collection showed significant differences in the proportion of the population under 3 months of age (puppies, Fig. 3b). Informed-percentage ABC achieved the lowest puppy percentage compared to all other models ($F = 312.370$, $df_{num}=5$, $df_{denum}=234$, $p< 0.005$). All ABC models significantly lowered the puppy percentage compared to the non-intervention model while all lethal control models raised it. There were significant differences between all methods except the two lethal methods.

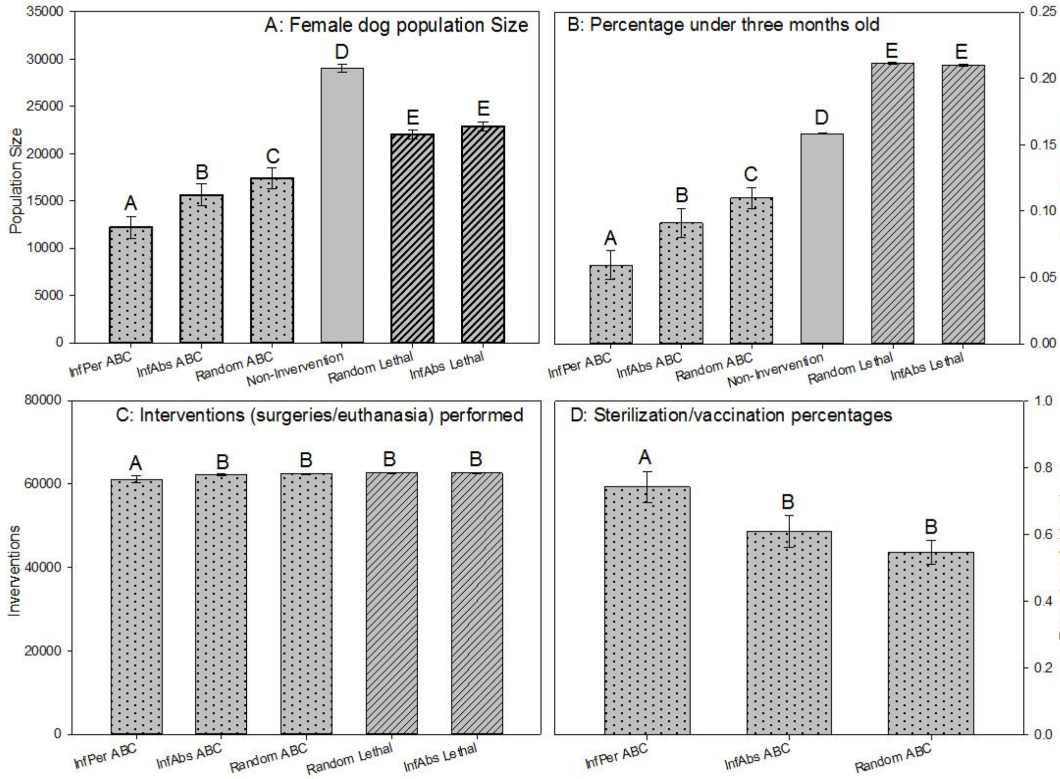

Figure 3. The mean female dog population size, percentage under three months of age, the number of interventions performed, and sterilization/vaccination percentages for each model's treatments.

ABC-based models are stimpled, Lethal-control is striped, and non-intervention is solid. Significance groupings are denoted by matching letters.

Effect on number of interventions performed

The five models with some level of intervention (either surgeries or euthanasia) showed little difference in the number of interventions performed (Fig. 3c). The informed-percentage model performed significantly fewer interventions ($F = 10.863$, $df_{num}=4$, $df_{denum}=195$, $p < 0.05$) than all other models but as this difference in interventions amounts to under a 0.5% decrease, it is likely not ecologically relevant.

Effect on sterilization/vaccination percentage

When comparing the three ABC models (Fig 3d), the informed-percentage ABC method produced significantly higher sterilization/vaccination proportions compared to either the informed-absolute or spatially random methods ($F = 20.686$, $df_{num}=2$, $df_{denum}=117$, $p < 0.05$). These other ABC models had no significant differences in sterilization/vaccination percentage.

Effect on age demographics

Each model produced significantly different age distributions. The non-intervention model had a life expectancy of 1.16 years at birth and 3.68 at one year and all three ABC methods had increased life expectancies with the informed-percentage ABC having the highest at 2.99 years at birth and 3.82 years at one year. Both lethal methods lowered life expectancy to ~0.65 years at birth and ~3.25 years at one year.

Validation

A local sensitivity analysis for adult survival rate, juvenile survival rate, daily chance to spawn control agents, average number of dogs abandoned per day, average control agent bag limit, maximum inter-zone variability on carrying capacity, and baseline carrying capacity was performed for both absolute informed ABC and Lethal models. The results for the effect of each parameter on dog population sizes and number of interventions performed can be seen in supplementary tables 1 and 2 in appendix A, respectively. We utilized a pattern-oriented modeling approach to validate this population (Grimm & Railsback 2005) Our ABC model achievements in the decline of the population size matched the reported decline in the real-world population in both the timespan and severity of the decline. Any lethal control programs in similar environments are generally not performed with significant monitoring effort so validation is difficult.

DISCUSSION

This model demonstrates the benefits in both scientific and economic terms that ecological modeling can bring to veterinarians working with street dogs. Enormous efforts are undertaken every day by animal welfare organizations, public health departments, and veterinarians but the paucity of rigorous scientific data often produced limits their ability to influence donors, scientists, and governments to invest in their work. A yearly survey for sexually intact dogs spread around the city can help focus ABC efforts, and bring significantly better management results. For dog population managers, this means a more effective program through an increase in the percentage of the population sterilized and vaccinated against diseases, notably rabies.

Perhaps most importantly, the informed-percentage ABC method achieved significantly lower population sizes, higher sterilization/vaccinations percentages, and lower percentages of the population under three months old compared to all other methods tested. This effect showed the greater efficacy of the informed-percentage method at targeting collection efforts in those areas that warrant them the most. The greater sterilization numbers caused a faster reduction in the population size, potentially reducing the number of surgeries that must be performed in the future to maintain a lower population.

All three ABC-based models outperformed the two lethal removal-based models at population reduction and demographic shifting while utilizing near equivalent numbers of interventions (surgeries or euthanasia). This effect is likely due to ABC limiting a population's ability to increase in size by increasing the proportion of sterilized individuals that are still competing for local resources but lack the ability to use them for reproduction. Lethal control quickly reduces the population, but once depressed densities are reached, it still has a large ability to "bounce back" because intact bitches can quickly replace removed dogs. The magnitude of effort required for an effective lethal control program is greater than those programs utilizing ABC methods, making them the favorable method in areas where the pool of available resources for an undertaking is small.

As ABC programs normally vaccinate each sterilized dog before release and improvements in vaccine's protective period in street dogs mean each will likely be protected for its entire lifetime, eliminating the need for a second capture to re-vaccinate (Coyne *et al.* 2001). Rabies incidence in street dog populations has been shown to be more sensitive to an increase in the vaccination percentage than a reduction in the population size (Morters *et al.* 2013). Therefore, the higher rate of sterilization (and thus, vaccination) in the informed-percentage ABC capture method (66.73%) than in the spatially-random ABC (52.93%) and informed-absolute ABC methods (58.70%) may be an especially important epidemiologically .

As puppies play a larger role in the spread of disease because of their naïve immune system, they are a key section of the population (Fontanarrosa *et al.* 2006). Both of our lethal control models reduced the dog population by ~5,500 total dogs but they actually increased the absolute number of puppies in the city by ~250 compared to the non-intervention model, resulting in a dramatic rise in the proportion of puppies.

Lethal control programs, both in the real world (Killian *et al.*2007) and in our model here, target individuals of all ages but are replaced by only young individuals, driving the population's age distribution downward. This suggests lethal control results in a trade off between the benefits of reducing the population size and the potential costs of these shifted demographics potentially increasing disease spread. This trade off does not arise under non-lethal control, where both population size and proportion of puppies are reduced relative to the non-intervention model.

Parameterization of the model used data from Jaipur when possible (Table 1). We do not suggest that the behavior of model agents are perfect representations of the behavior of individual dogs, but rather accurate simulations of the dog population as a whole. With a greater understanding of street dog population demography and the impacts of ABC programs, it will be easier to accurately predict the state of a dog population before, during, and after an intervention.

The actual costs to perform a lethal control program are unavailable but would likely mean lower per dog price compared to the ABC methods. However, this price does not include the human rabies costs (deaths, post exposure treatments) and it is important to remember that a reduction in the expenses in dog control do not necessarily results in lowered costs for the city as a whole. The cost of performing this yearly survey is estimated to be $106.39 (₹6,580), including the gas and salaries of the veterinary technicians involved required to fully survey Jaipur city (Pers comm J Reece). As the current approximate price per capture, surgery, and vaccination is $10.07 (₹ 623) (Per comm J Reece) any potential benefit to changing dog collection methods would only need to reduce the number of surgeries by 11 to be economically viable for an ABC program. Our results show a slight, but statistically significant, difference in the number of surgeries performed after 15 years of informed-absolute ABC intervention and the lowered population size and higher sterilization/vaccination figures point to an even more significant potential future reduction. As the cost of a survey to determine the location of intact dogs is comparatively small compared to the yearly operating budgets of most programs and utilizes freely available information and programs, we highly recommend its inclusion in any ABC program.

Chapter 3: Disease control through fertility control: Secondary benefits of animal birth control in Indian street dogs.

ABTRACT

We sought to (1) survey street dogs for a wide range of diseases in three cities in Rajasthan, India and (2) evaluate the links between the health of non-treated dogs and both the presence and duration of animal birth control (ABC) programs.

Viral and bacterial disease prevalences were assessed in 240 sexually intact street dogs from Jaipur, Jodhpur, and Sawai Madhopur cities in September 2012. Those individuals and 50 additional dogs were surveyed for the prevalence of ticks and given body condition scores. The presence of an ABC program was associated with significantly lowered prevalence of open wounds likely caused by fighting, infectious canine hepatitis (ICH), *Ehrlichia canis*, *Leptospira interrogans* serovars, flea infestations, and higher overall body condition scores. Dogs in cities with ABC programs had significantly higher prevalences of Brown Dog Tick (*Rhipicephalus sanguineus*) infestations. Canine Distemper Virus (CDV), Canine Parvovirus (CPV), and *Brucella canis* prevalence was not significantly different between cities. This study is the first to demonstrate the health benefits of ABC on non-vaccinated non-treated diseases.

INTRODUCTION

Street dogs are common in the developing world, and often live with little or no veterinary care, consuming refuse and feces to survive (Butler and du Toit 2002, Butler *et al.* 2004, Reece *et al.* 2008). India's large population of street dogs originates from a unique combination of local tolerance, abundant food, shelter, breeding opportunities, and ineffective dog control policies (Reece 2007). Compounding the issue, vulture populations on the Indian subcontinent have undergone dramatic (>95%) declines and carcasses that would normally be consumed by vultures now supplement dog diets (Markandya *et al.* 2008). Surveys of Indian dog population sizes are scarce, but at one rubbish dump in Rajasthan, the population has increased from 60 dogs in 1992 to 1200 individuals nine years later (unpublished results in Prakash *et al.* 2003). Dog bites account for 90% of the human post exposure rabies treatments and 96.5% of the 20,000 human rabies deaths in India (Knobel *et al.* 2005, Kale *et al.* 2006). Canine and rabies control are both being addressed by canine animal birth control (ABC) and vaccination programs in urban centers to reduce canine reproductive capability and the progression of rabies (Reece 2007). Several Indian cities began to use ABC programs in the early 1990s as an alternative to culling strategies (including conscious electrocution, beatings, and strychnine poisoning, Reece 2007) which may, counter-intuitively, increase rabies incidence. By disrupting canine social structures, culling often leads to increased fighting and rabies transmission opportunities (Donnelly *et al.* 2006, Reece & Chawla 2006, Killian *et al.* 2007). ABC programs seek to produce a socially stable, but declining, street dog population through ovariohysterectomy and castration of a majority of dogs and is advocated by all major international dog control organizations (WHO 2004, ICAM 2007).

When sterilized in this manner, normal canine hormonal cycling is halted, decreasing conspecific aggression, breeding behavior, and aggregation around bitches in estrus, behaviors that are high-risk factors for disease transmission (Killian *et al*. 2007). ABC programs in Jaipur and Jodhpur, two cities in the northwestern Indian state of Rajasthan, have been highly effective in reducing the street dog population and rabies incidence (Reece & Chawla 2006, Totton *et al*. 2010b). Additionally, dogs which have previously been treated by an ABC program have significantly superior health compared to their sexually intact neighbors in the same city (Totton *et al*. 2011). This effect was attributed to the behavioral changes and lowered energy requirements of sterilized dogs compared to intact dogs which must care for pups and maintain territories (O'Farrell & Peachey 1990, Totton *et al*. 2011).

This study seeks to demonstrate if treated dog's behavioral changes, reduction in dog density, and lowered energy requirements caused by sterilization substantially affect the cycling of common canine diseases. The ABC programs in this survey vaccinate solely against rabies, so our reported disease prevalences are not affected by direct vaccination or treatments of these diseases. Since this work only examines sexually intact dogs that have not been through an ABC program, it highlights the benefits of having sterilized neighbors where other investigators report the benefits to the individual dog that is sterilized (Totton *et al*. 2011). This also illustrates health benefits for proximate humans and wildlife susceptible to similar disease pressures. Additionally, this study is the first to survey multiple diseases over several different localities with large numbers of dogs

MATERIALS AND METHODS

Study Area

A cross sectional survey of canid disease was performed in three different Indian cities, Jaipur (26°55' N, 75°49' E), Jodhpur (26°17' N, 73°01' E), and Sawai Madhopur (25°59' N, 76°22' E). All three cities are within Rajasthan, the arid northwestern state of India (Fig 4). Jaipur is a large city with an estimated 36,580 dogs (Hiby *et al* 2011) and has been serviced by the Help in Suffering ABC program since 1994 (Reece & Chawla 2006). Jodhpur is another large city with an estimated 24,853 dogs (Hiby *et al.* 2011) that has been serviced by the Marwar Animal Protection Trust ABC program since 2004. Although the proportion varies yearly, roughly 80% of bitches are sterilized in these cities (Hiby *et al.* 2011). The third location, Sawai Madhopur, is a smaller city on the edge of Ranthambore National Park and has never had any ABC or large rabies vaccination program. No rigorous estimate of dog population numbers in Sawai Madhopur has been made, though if estimates of dogs/human ratios in another small Rajasthani city with limited ABC, Jaisalmer, holds, a rough estimate of ~4500 dogs is reached (Census 2011, Hiby *et al.* 2011).

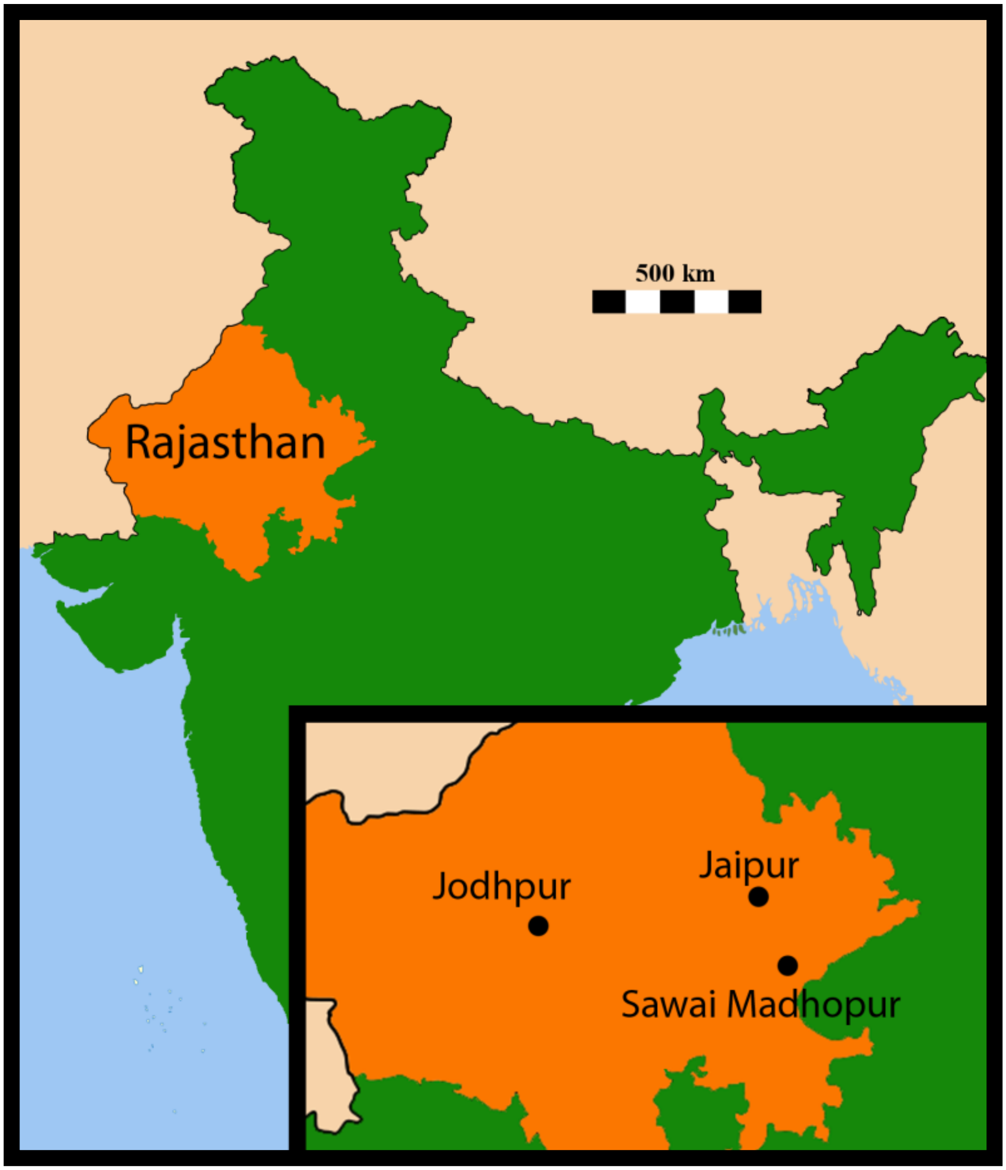

Figure 4. The study cities of Jaipur, Jodhpur, and Sawai Madhopur in the state of Rajasthan, India.

Survey Procedures

Dogs in Jaipur and Jodhpur were collected by their respective ABC programs, Help In Suffering and the Marwar Animal Protection Trust, and sampled immediately prior to sterilization. In Sawai Madhopur, dogs were hand caught in the early morning and restrained for sampling and rabies vaccination. Dogs were selected for sampling solely on the basis they were on the street with unrestrained movement (unchained/unfenced).

Blood was tested for the presence of immunoglobulin G for canine distemper virus (CDV), canine parvovirus (CPV), *Leptospira interrogans* serovars, *Brucella canis*, *Ehrlichia canis*, and infectious canine hepatitis (ICH) using commercially available ELISA testing kits (Immunocomb® kits, BioGal Labs, Kibbutz Galed, Israel, 19240). The *L. interrogans* test looked for serovars *canicola, icterohemorrhagiae copenhagi, icterohemorrhagiae RGA, pomona,* and *grippotyphosa* but did not differentiate between them. Additionally, immunoglobulin M values were gathered for canine distemper virus and canine parvovirus, providing information about what disease stage an individual dog is currently experiencing (susceptible, active infection, gaining immunity, immune, immune re-exposed). The test specificity and sensitivity information is available online and is proofed for dogs (BioGal 2011, see Supplementary Table 3 in Appendix B).

Dogs were given a brief physical examination to provide a body condition score (based off a simplified scale from Totton *et al*. 2011) and checked for the presence of ticks, fleas, and open wounds. The body condition scores (1-4) corresponds to weight classes of emaciated (1), underweight (2), healthy weight (3), and obese (4). In analysis, body conditions of 3 and 4 were combined because of the absence of obese body condition dogs. Before release, Sawai Madhopur dogs were vaccinated against rabies (ImRab 3®, Merial Ltd, Duluth, GA, 30096).

Statistical Analysis

The observed relative risk and 95% confidence intervals were calculated for each disease and compared between cities. Relative risk was derived using binary presence/absence of disease. Body conditions scores and the relationship of host traits (e.g. age, sex, location, concurrent secondary infections, fight wounds) were analyzed for significant relationships to infection status using Fisher's exact test. For traits other than sample source location, dogs from each city were pooled together for analysis. Bonferroni's correction was used to account for multiple tests.

RESULTS

The prevalence of diseases and body condition scores, the significance of the pairwise comparisons between cities, and the total effect of location in predicting disease prevalence are shown in Table 2. There were significant effects of location on body condition scores, tick, flea, fight wound, *E. canis*, *Leptospira* serovars, and infectious canine hepatitis presence. In the case of each of these diseases, with the exception of ticks, the direction of the effect showed a positive influence of ABC on dog health.

Location had a significant effect ($p = 0.0001$) on the proportion of dogs currently infested with ticks (all identified to be in the *R. sanguineus* species complex). In pairwise comparisons for ticks, Jaipur and Jodhpur differed significantly from Sawai Madhopur (both $p = 0.0001$) but did not differ from each other ($p = 0.131$).

We found similar results for body condition, with the fewest number of low body condition dogs (emaciated) and the highest body condition (healthy weight) dogs in the ABC cities compared to the non-ABC city. For each of the other diseases for which location was a significant predictor, significant pairwise comparisons show lowered disease prevalence as the duration of ABC increased.

Other conditions important to dog health were observed but not comprehensively evaluated because of their rarity. Several dogs presented with suspected *Babesia canis/gibsoni* and *Leishmania* sp. infections but no confirmatory test was locally available. Some dogs presented with canine transmissible venereal tumors, however as many early stage tumors can easily go undiagnosed, it was not recorded for this survey.

Table 2. The prevalence (and number surveyed) of body condition scores and diseases in the three study cities of Jaipur (17 y of ABC), Jodhpur (7 y of ABC), and Sawai Madhopur (no ABC). When there are significant differences between cities it is indicated by * and the superscript indicates statistically significant groupings by using pairwise comparisons.

-		More ABC		Less ABC
Disease		Jaipur	Jodhpur	Sawai Madhopur
Body Condition Score*		n = 106 ^{A}	n = 83 ^{AB}	n = 101 ^{B}
	1 (Emaciated)	22.65%	42.17%	45.54%
	2 (Low Weight)	48.11%	45.78%	37.62%
	3 (Healthy)	29.95%	12.05%	16.83%
Ticks*		n = 120 ^{A}	n = 83 ^{A}	n = 48 ^{B}
		53.33%	63.86%	25.00%
Fleas*		n = 120 ^{A}	n = 83 ^{A}	n = 64 ^{B}
		4.17%	1.20%	26.56%
Fight Wounds*		n = 153 ^{A}	n = 88 ^{A}	n = 102 ^{B}
		3.92%	6.83%	25.49%
CPV		n = 100	n = 78	n = 60
	Susceptible	14.00%	24.36%	18.33%
	Infected	1.00%	3.85%	6.67%
	Immune	85.00%	71.79	75.00%
CDV		n = 100	n = 78	n = 60
	Susceptible	34.00%	33.33%	15.00%
	Infected	28.00%	30.77%	36.67%
	Immune	38.00%	35.90%	48.33%
*Ehrlichia canis**		n = 100 ^{A}	n = 79 ^{B}	n = 60 ^{C}
		45.00%	58.23%	76.67%
Leptospira serovars*		n = 100 ^{A}	n = 77 ^{A}	n = 58 ^{A}
		12.00%	7.79%	39.66%

Table 2: Continued

Disease	More ABC Less ABC Jaipur	Jodhpur	Sawai Madhopur
Infectious Canine Hepatitis*	n = 100 [A]	n = 78 [A]	n = 60 [B]
	74.00%	92.31%	96.67%
Brucella canis	n = 100	n = 79	n = 58
	10.00%	5.06%	3.45%

DISCUSSION

We found that ABC areas exhibited lower prevalence of disease in unsterilized and untreated dogs for seven (body condition score, fleas, fight wounds, CDV, *E. canis*, Leptospira serovars, and ICH) of the ten conditions. CPV and *B. canis* seroprevalence did not differ significantly among cities. The *E. canis* prevalences in Jaipur differed significantly from both Jodhpur and Sawai Madhopur. The ABC program in Jaipur has operated for 17 years, in contrast to 7 years in Jodhpur and not at all in Sawai Madhopur. Thus, for these conditions, prevalence is also affected by the duration of ABC. The association between the presence of ABC programs and a lower prevalence of infection may result from the ABC-driven decline of the dog population size as well as the behavioral and immunological changes. Importantly, ABC programs appear to influence disease dynamics in the entire dog population, and not just treated dogs, since this survey only included sexually intact dogs who had not undergone sterilization and vaccination. We argue that untreated dogs were less likely to be exposed to infection because neighboring ABC-treated dogs were healthier (Totton *et al.* 2011), more capable of resisting infection, and less likely to transmit disease.

While most of the disorders in this survey are unlikely to be affected by climatic conditions, leptospirosis in India is one that may be heavily influenced by the availability of other principal hosts (particularly rodents) and high rainfall levels (Venkataraman and Nedunchelliyan 1992). The yearly average rainfall for Jaipur, Jodhpur, and Sawai Madhopur is 60, 30, and 75 cm, respectively (Singh *et al.* 1974). While all three cities had experienced a moderately heavy monsoon season in the months prior to this survey, the mildly damper conditions in Sawai Madhopur may cause higher numbers of Leptospira infections, unrelated to ABC coverage. Therefore, we recommend additional investigation with methods that differentiate between *Leptospira* serovars.

We found significantly fewer open wounds in the cities with ABC coverage. Based on concurrent behavioral observations and wound morphology (A.J. Yoak and J.F. Reece, pers. obs.), we suggest that the majority of these are received by fighting with other dogs. As the sterilization programs in Jaipur and Jodhpur halts hormonal cycling in bitches, they do not enter estrus and thus do not elicit competition and congregation over breeding opportunities. Breeding associated fighting was seen nearly every day of the week-long survey in Sawai Madhopur and never in the other cities (surveys were performed during the established breeding season). This decrease in incidental biting may substantially lower the rabies transmission rate by reducing pre-symptomatic transmission, benefiting dogs, humans, and wildlife (Killian *et al.* 2007).

Dogs from ABC cities had higher prevalences of *R. sanguineus* ticks. This may be because ABC centers facilitate the transfer of ticks by housing dogs in close contact and there may be differences in the availability of alternate hosts or environmental conditions between cities. Protocols to mitigate the risk of tick spread (use of cypermethrin spray) should be implemented in ABC programs as suggested by Totton (*et al.* 2011). Nevertheless, dogs in ABC cities still had significantly lower prevalence of infection by the tick-borne spirochaete bacterium *E. canis*, which suggests that factors other than tick density influence ehrlichiosis in these dogs.

The dogs sampled here may represent a subset of the more catchable dogs, although this is difficult to assess. As our study cities are not perfectly comparable with respect to their dog population size, human demographics, or environmental conditions, the claim that ABC will lower the prevalence of non-targeted diseases in street dog populations must be tempered. However, we would predict that many of the differences between cities would expect to result in greater disease prevalences in ABC cities (e.g., greater dog populations mean a larger pool of susceptible individuals), in contrast with our findings. We suggest that by sterilizing and vaccinating against rabies, ABC programs yield a healthier and more stable street dog population with lowered disease prevalence. This has significant impacts for developing nations like India, where dogs live in close contact with both humans and endangered wildlife.

CONCLUSION

Dogs in cities with ABC programs showed a significantly lower prevalence of several diseases important to both dog and wildlife health. Only tick prevalence was higher in ABC cities; and we echo others' calls to include an ectoparasite control procedure in ABC program design (Totton *et al.* 2011). Even so, untreated dogs in these ABC cities were still significantly healthier than those in the city not undergoing ABC.

Chapter 4: Short term survival of raccoons is unaltered by a wildlife fertility control program

Andrew J. Yoak, Gwen Myers, Michael Barrie, Randall Junge, Barbara Wolfe, Priya Bapodra, Matthew R. O'Connor, Stanley D. Gehrt, Ian M. Hamilton

ABSTRACT

Fertility control programs are increasingly important in wildlife management practices. Because sterilization limits the ability for treated individuals to allocate energy towards reproduction away from other potential energy allocations (e.g. fighting disease), there is some evidence that fertility control can increase survival of targeted individuals. Here we present a randomized control study that investigates the survival of wild raccoons living on grounds of a large suburban zoo. Treatments were split into three groups varying in the application of sterilization and vaccines to assess the effect of each independently. Monthly survival rates were assessed using a Barker Robust Design model with 3.5 years of capture data. We found equivalent support for two models in which survival was influenced by treatment group and a model in which all treatment groups had equivalent survival rates (AICc = 1886.51 and 1886.44, respectively). Apparent monthly survival was high (95%+) for all groups. This

study provides no support for any substantial survival gains to sterilized or vaccinated raccoons.

INTRODUCTION

Animal birth control (ABC, also called trap-neuter-release) is often heralded as a solution to wildlife overpopulation, especially for some species that flourish in ecosystems heavily modified by anthropogenic change (Garside *et al.* 2014, Killian et al 2007, Yoak *et al.* 2014). Generally, ABC programs collect animals from a target species, sometimes specifically selecting females as their sterilization more significantly affects population growth than males (Reece 2007), and neuter them either by complete sterilization (castration/spay) or functional sterilization (vasectomy/tubal ligation). Often, these programs are combined with a vaccination protocol targeting a specific disease of concern in an attempt to reduce its prevalence as the population size is lowered. The choice of sterilization method is important, as maintaining normal hormone production would not alter potentially key behaviors (territory maintenance, reproduction associated behaviors, etc.) that, depending on the species, may be best eliminated (Bromley & Gese 2001b). Using fertility control over more traditional practices like culling can be highly controversial (Killian *et al.* 2007) and any treatment's success can be dramatically affected by a multitude of species-specific factors (Caughley *et al.* 1992). For example, fertility control schemes with cats in the USA have been largely ineffective (Anderson *et al.* 2004) but ABC in Indian street dogs has been remarkably successful (Reece 2007). Other non-surgical methods have controlled insect populations using inherited sterility by genetic modification (Gemmell *et al.* 2013) and for limiting reproduction in a broad range of wildlife species using contraceptive vaccines (Kirkpatrick *et al.* 2011).

When individuals in the population have their health buffered by fertility control, it can have a beneficial effect on survival for both treated and non-treated members of the population (reviewed by Gray & Cameron 2010). Sexually intact dogs living in cities with ABC programs had lowered prevalence of several viral and bacterial pathogens compared to similar dogs living in ABC-free cities. (Yoak *et al.* 2014). If an intervention reduces the prevalence of the targeted disease, for instance the immuno-suppressive canine distemper virus, it may increase the ability to resist other secondary non-targeted diseases. Additionally, if ABC limits energy expenditure on reproduction, those resources could be shifted towards normal homeostasis (Totton *et al.* 2011).

Raccoons (*Procyon lotor*) are a widely distributed and abundant mesopredator in the United States. They can have significant ecological impacts and are important in the disease dynamics of several serious pathogens, particularly rabies (Gehrt 2003, Rossatte *et al.* 2006, Hirsch *et al.* 2013). In zoos, raccoon predation on collection animals is of some concern, but the majority of the threat is perceived to be from the pathogens they carry (Junge *et al.* 2007), most notably *Baylisascaris procyonis* (Kazacos *et al.* 1991), canine distemper virus (Appel *et al.* 1994), and rabies (Slavinski *et al.* 2012). In zoos, lethal removal of raccoons is a common practice (Pers Obs, A. Yoak) despite minimal evidence to support its effectiveness. To our knowledge, there have been no published reports outlining methods or results of this management approach. Outside of zoos, two separate experimental lethal removals of raccoons intended to reduce the predation rates on sea turtles (several species - Ratnaswamy et al. 1997, *Caretta caretta* - Barton & Roth 2007) resulted in minimal gains to turtle nest success. Rosatte (*et al.* 2007) showed recolonization of the depopulated areas occurs quickly (< 1 year) and mostly occurred not because of invasion from the surrounding non-culled areas, but from local reproduction by surviving residents.

In an attempt to reduce the risks associated with raccoons on zoo grounds, the Columbus Zoo and Aquarium began a Raccoon Health Program (RHP) in 2002 (Myers *et al.* 2004). Initially, the program followed a standard trap-neuter-vaccinate-release protocol, but in 2011, the RHP was restructured to a randomized control trial with more rigorous data collection. As this was the first program of its kind for raccoons, it was unclear how wild raccoons would respond to vaccination and sterilization. Here we describe this new RHP study as it investigates the effects that sterilization and vaccination have on raccoon survival by creating three treatment groups: a control (non-intervention), a vaccine-only group (who were given several vaccines against common pathogens, anti-parasitics, and care for injuries), and a vaccine-and-sterilization group (who received all the treatments of the vaccine-only group but were also sterilized using tubal ligation and vasectomy). We predict raccoon survival of vaccine-and-sterilization individuals will be higher than vaccine-only individuals whose survival will be higher than the control group. We hypothesize that vaccination directly protects against pathogens, antiparasite treatment directly protects against parasites, and sterilization results in both reduced energetic expenditure to reproductive activities and, potentially, increased ability to resist infections.

METHODS

Ethics statement

The methods we employ in this study were approved by the Columbus Zoo animal care committee and follow guidelines set by the American Society of Mammalogists (Gannon *et al.* 2007).

Study area

This research was performed on the grounds of The Columbus Zoo and Aquarium, a semi-urban zoological park (40. 156° N, 83.118° W; Fig 5). The zoo covers 0.465 km^2 and an additional 0.174 km^2 expansion was added during the final year of this study. The area around the zoo is predominantly housing developments and young mixed-oak forest with a large river running along the western edge.

Capture Methods

Live-trapping targeting raccoons began in Oct. 2011 and ended Sept. 2014 with yearly winter breaks (approximately 3-4 months) in collection effort. Twenty-nine Tomahawk traps (Tomahawk Live-Trap Co., Tomahawk, WI, USA) spaced at ~100 m intervals (Fig. 5) were placed around zoo grounds out of view of visitor areas and 23 capture sessions were performed, each consisting of seven consecutive trap-nights. Raccoons are nocturnal and active mostly at night (Gehrt 2003) so traps were opened in the evening (18:30-20:30), set with wet cat food (9Lives, Del Monte Foods, San Fransisco, USA), and closed in the morning (6:00-8:00). Incidentally captured feral cats were taken to the Delaware Country Humane Society and non-target native species were released.

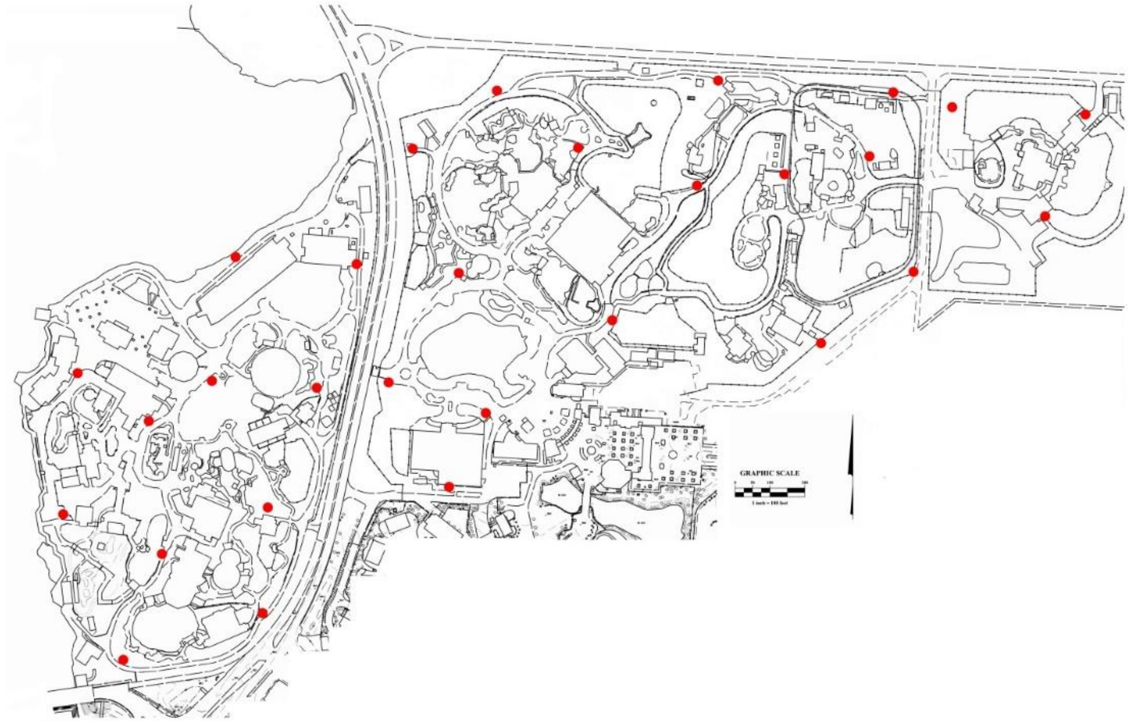

Figure 5. The grounds of the Columbus Zoo and Aquarium and the locations of raccoon traps.

Upon capture, animals were anesthetized with 4-5 mg/kg of tiletamine/zolazepam (Telazol) (Kreeger & Arnemo 2007) then, if undergoing surgical procedures, intubated and maintained using 1-1.5% isoflurane gas. Using a low dose of Telazol allowed raccoons to quickly recover and minimized the impact of trap-induced stress on future trapping success (Gehrt *et al.* 2001) and allowed veterinary staff more control on sedation. New individuals were randomly assigned to either "Control", "Vaccine-only", or "Vaccine-and-Sterilization" treatment groups. All individuals were given an ear tag, intra-scapular PIT tag, rabies vaccine (IMRAB 3, Merial), treatment for trap injuries, and when recovered fully from anesthetic, released at the area they were trapped. Animals in the control group were given no additional treatment. Animals in the vaccine-only group were given vaccines that protected against ten of the most common viral and bacterial raccoon pathogens (Feline rhinotracheitis, feline calicivirus, panleukopenia, canine distemper, canine adenovirus type 2, canine cornonavirus, canine parainfluenza, canine parvovirus, and the *Canicola* and *Icterohaemorragiae* serovars of *Leptospira*) as well as palliative care against any natural injuries (open wounds, broken teeth, etc.), moxidectin against intestinal parasites, and frontline plus spray (fipronil and s-methoprene) against ticks and fleas. Vaccine-and-Sterilization individuals received those same additional treatments but were also sterilized by either tubal ligation or vasectomy. This sterilization technique maintains normal hormone production and was selected in an attempt to limit behavioral differences between groups.

Raccoons captured before the 2011 season were treated with a non-standardized treatment protocol most resembling the vaccine-and-sterilization group because they received some vaccines and were sterilized with tubal ligation and vasectomy. To ensure consistent methods, these individuals were considered part of a separately analyzed "Original Program" group.

Mark-Recapture Analysis

We estimated treatment group survival using a Barker Robust Design structure model (Kendall *et al.* 2013) in program MARK (White & Burnham 1990) v8.0. This hybrid type model combines the model structure of Pollack's (1982) robust design, (allowing for small, closed, secondary capture periods within the normal open sessions) and the increased observation types (dead recoveries and non-trap sightings) possible in Barker's (1997) model. Thus, we are able to more accurately estimate survival by using information that would have been excluded from other models. For a description of how program MARK assesses survival, please see figure 6 and box 1.

Box 1. This information is a summarization of Kendall's (*et al.* 2013) model. A Barker Robust Design model (fig 6) in Program MARK uses a capture history format characterized by a series of secondary captures (each a night of trapping, l_T) which are grouped together into single primary sessions (T). Within a primary session, the population is considered closed with no death. This assumption is obviously inaccurate, however it greatly increases the accuracy of the model's estimations and, when secondary periods cover a short time span, this assumption is only a minor alteration. The probability of capturing individuals is informed by nightly capture sessions, which allows both the probability of being available for capture (a_x) and the probability of capturing individuals given they are available for capture (p_x).

Between primary capture periods, marked individuals can be sighted alive by some auxiliary event or their body can be recovered. This allows survival (S_x) to be assessed separately from individual's fidelity to the capture area (F_x).

For example, a capture history for a 3 night capture session repeated twice would have the following format):

LLL D LLL D

With L representing potential capture by the regular capture protocol and D representing an opportunity for either an auxiliary observation outside the normal capture program or the recovery of a carcass. An individual capture history of:

100 2 111 1

would represent an individual captured during the first night of the first session (100), seen sometime between capture sessions (2), captured every night of the second capture session (111), and whose carcass was recovered after the second session (1).

The Barker Robust Design model creates estimates of:

S (probability of survival from the previous time period),

r (probability that a deceased individual will be recovered),

R (probability that a surviving individual is detected by auxiliary observation),

R' (probability that an individual is reported by auxiliary observation while alive then later dies in that same time period),

a' (probability that an individual is available for detection, contingent upon it being previously unavailable in the prior time period),

a''(probability that an individual is available for detection, contingent upon it being previously available in the prior time period),

F (probability than an individual stays in the the study area given it survives the prior time period),

p (probability that an individual is captured when it was available and alive)

and **fo** (the probability that an individual is seen by auxiliary observation when it is available and alive).

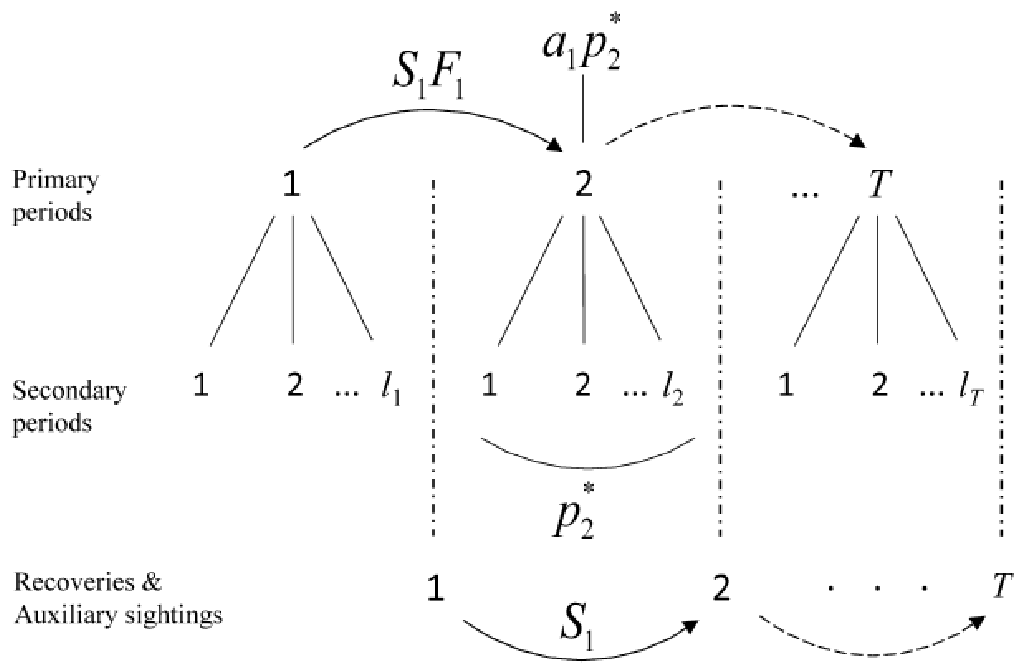

Figure 6. A Barker Robust Design model structure (see box 1 for parameter description).

Several model structures were run, all focusing on survival variation between treatment groups. Other model parameters that were likely not affected by any of our treatments (i.e. the probability individuals are available to be detected and the probability of detection) were considered equivalent values across treatment group divisions. The survival parameter was tested for the effects of treatment type (original program, control, vaccine-only, and vaccine-and-sterilization), sex, a two season (winter/not winter) yearly variation (adapted from Troyer *et al.* 2014), and the interactive effects between each. The "winter" time period consists of the 3-4 month halt in trapping effort and "not winter" is the remainder of the year. A parameterization of the effect of treatment group type in which pre-2011 original program raccoons were combined with the full treatment (because of similarities between these two groups) was also investigated. Model selection was performed using Akaike's information criterion with a correction for finite sample sizes (AIC_C).

RESULTS

We captured 111 individual raccoons in 263 secondary sessions over 153 capture nights (4437 trap-nights). Two individuals were immediately euthanized due to serious injury and were excluded from analysis. Thirty-two raccoons were placed in the control group, 31 into the vaccine-only group, and 30 into the vaccine-and-sterilization group. Eighteen raccoons from the original program were recaptured throughout the course of this study. Three deceased marked individuals, all from the vaccine-group, were recovered on grounds by zoo staff.

The ten models with the lowest AIC_c values are shown in table 3. The model which posited survival was affected by the three treatment groups (in which original program raccoons were pooled into full treatment) and a null model (wherein all treatment groups have equivalent survival) were indistinguishable from each other using AIC_c values. The next two most parsimonious models, in which survival was affected by the season and by sex, were less supported, however ΔAIC_c was still under 2. The apparent monthly survival rates when treatment groups are pooled together and when they are split into three groups (when original treatment individuals are grouped into vaccine-and-treatment) are all over 95%. These values are shown in figure 7a and 7b, respectively.

Table 3 Model comparison for Barker Robust Design model structure analysis of raccoon survival rates.

Variable definitions can be found in box 1. Model parameterizations were performed investigating the effect of our four treatment groups (original program, control, vaccine-only, and vaccine-and-sterilization), three reduced treatment groups (wherein original program is combined with vaccine-and-sterilization), sex, and a two-season (winter/not winter) variable; interactions between each of the variables were also performed AIC_c is Akaike's Information Criterion corrected for small samples sizes, ΔAIC_c is the difference between the current and top-ranked model, model weight is the probability that this model is the best fit, model likelihood is the relative support for the model, parameters is the number of parameters in the model, deviance is model deviance. (.) following a parameter indicates that the value is constant.

Model	AIC_c	ΔAIC_c	Model Weight	Model Likelihood	Parameters	Deviance
S(.) + r(.) + R(.) + R'(.) + a'(.)+ a''(.) + F (.) + p(.) +f_0(.)	1886.44	0.00	0.291	1.00	8	1433.63
S(3 treatment groups) + r(.) + R(.) + R'(.) + a'(.)+ a''(.) + F (.) + p(.) +f_0(.)	1886.51	0.09	0.281	0.97	10	1429.40
S(season) + r(.) + R(.) + R'(.) + a'(.)+ a''(.) + F (.) + p(.) +f_0(.)	1888.13	1.70	0.124	0.42	9	1433.19
S(sex) + r(.) + R(.) + R'(.) + a'(.)+ a''(.) + F (.) + p(.) +f_0(.)	1888.34	1.90	0.112	0.39	9	1433.39
S(4 treatment groups) + r(.) + R(.) + R'(.) + a'(.)+ a''(.) + F (.) + p(.) +f_0(.)	1888.68	2.24	0.088	0.33	11	1429.40
S(3 treatment group x sex) + r(.) + R(.) + R'(.) + a'(.)+ a''(.) + F (.) + p(.) +f_0(.)	1889.57	3.13	0.056	0.21	13	1425.88
S(season x sex) + r(.) + R(.) + R'(.) + a'(.)+ a''(.) + F (.) + p(.) +f_0(.)	1889.93	3.49	0.047	0.17	11	1430.65
S(season x 3 treatment group) + r(.) + R(.) + R'(.) + a'(.)+ a''(.) + F (.) + p(.) +f_0(.)	1890.03	3.59	0.044	0.16	13	1426.34
Continued below						

Table 3 Continued

S(4 treatment group x sex) + r(.) + R(.) + R'(.) + a'(.)+ a''(.) + F (.) + p(.) +f_o(.)	1894.05	7.61	0.006	0.02	15	1425.88
S(season x 4 treatment group) + r(.) + R(.) + R'(.) + a'(.)+ a''(.) + F (.) + p(.) +f_o(.)	1894.51	8.07	0.005	0.01	15	1426.34

DISCUSSION

As common as raccoons are in the urban landscape, there are surprisingly large gaps in the knowledge base about their ecology and behavior (Gehrt 2003). Our goal was to track individuals over their lifespan to measure the impact intervention policies had on their demographics. Although we hypothesized that raccoon survival would benefit from this intervention (because of reduced disease load and reduced allocation of energy to reproduction), we found that apparent survival rates were not strongly affected by functional sterilization (tubal ligation and vasectomy), at least on this time scale (3.5 years). The difference in AICc values for a null model, in which survival does not vary with sex, season, or treatment, and a model in which survival was affected by treatment was close to zero. For the model in which survival differences among treatments were included, vaccine-and-sterilization individuals had the highest rate of survival, and vaccine only individuals the lowest, but, overall, survival was generally high (Figure 7).

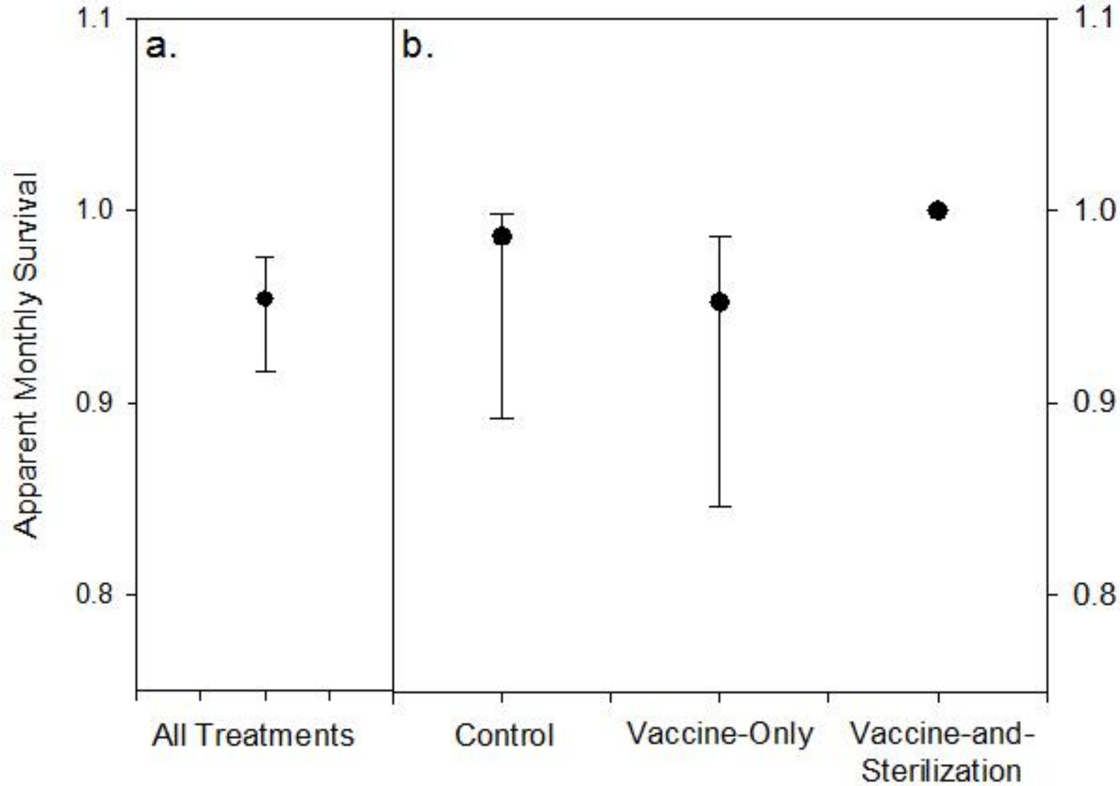

Figure 7. Apparent monthly raccoon survival for the top two ranked models a. no difference between treatments and b. survival differing by treatment group (when the original program group is combined with vaccine-and-sterilization) with 95% confidence intervals.

Fertility control (employing various methods) has increased the survival rate of horses (Turner & Kirkpatrick 2002), rabbits (Williams *et al.* 2007), and coyotes (for 2 of three study years, Bromley & Gese 2001b). Sterilization that reduces all reproductive behavior is predicted to decrease the transmission of *Brucella abortus* in bison (*Bison bison*) as most infections occur because of contact with post-parturient material and from mother to calf through milk (Miller *et al.* 2004). In captive mice, the method of sterilization employed (complete vs. functional) did not influence the effectiveness of reducing population growth rates (Chambers *et al.* 1999), suggesting that there were not additional increases or decreases in mortality associated with the type of fertility control. How this might translate to a wild environment where disease and behavioral differences could substantially alter survival is unclear. Other studies have found fertility control can have potentially negative effects on survival. For example, tubal ligated brushtail possums (*Trichosurus vulpecula*) had increased *Leptospira interrogans* serovar *balcanica* transmission rates compared to non-treated populations because of higher contact rates as the hormonally normal female continuously copulated to become pregnant (Caley & Ramsey 2001). Our study does not support either positive or negative influence of tubal ligation on apparent survival, but if higher contact rates and subsequent infection prevalence were predominantly in low-mortality diseases, it would not be detected here.

While one model in which season affected survival had a ΔAICc under 2, this was the only high ranking model that utilized survival. Others have found no seasonal survival trend (Troyer *et al.* 2014). This raccoon population does have substantial food supplements from human trash which could either better prepare them for winter hardship or sustain them throughout it. The slightly lower survival rates for vaccine-only raccoons, seen in Fig. 7b, may also illustrate some of the peculiar conditions experienced by this raccoon population. For example, the vaccine-only raccoon P219 was killed after entering the lion enclosure and unknown individuals have been reported to be killed by alligators in the past; these are likely not common causes of death for Ohio raccoons. This study is the first to measure survival in a population of raccoons living on zoo grounds but it appears they are not significantly different from populations living in other areas (Troyer *et al.* 2014).

Our methods likely have little impact on herd immunity as only 2/3 of individuals in the program received vaccinations and it can be assumed that other unknown raccoons either on zoo grounds or on its' periphery play a role in disease dynamics. Schubert (*et al.* 1998) found that a canine distemper virus vaccination program focusing on raccoons reduced the probability that individuals were infected with the virus; however, reducing this burden did not increase local raccoon abundance, indicating that distemper was not a significant limit to population growth. Canine distemper has been cited as a major source of mortality in unharvested and high-density populations (Riley *et al.* 1998), both of which apply to our study location. This would be expected to produce an increase in raccoon survival because of reduced disease-related mortality (not only against distemper) but our results do not support this effect.

In summary, these results do not suggest sterilization, vaccination, anti-helminthic treatments, or palliative care increase the survival of treated individuals as predicted over the time scale of this study. Population managers should not be concerned that by implementing a fertility control intervention there will be negative effects, at least for survival rate, on raccoon enrolled in the program.

ACKNOWLEDGEMENTS

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Chapter 5: A wild raccoon health program's effects on parasite load

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Abstract

Zoos represent a risky environment for the spread of pathogens as exotic animals from disparate locations mix with native wildlife living on the zoo grounds. Keepers typically mitigate the risks of disease spillover by removing local species that represent the most serious threats; however these eradications are rarely complete. When these species repopulate, it is often done rapidly and the resulting population can have lowered group immunocompetency or skewed demographics that may increase the probability or severity of outbreaks. A potential alternative to lethal depopulation is a vaccination and sterilization campaign to produce a smaller, potentially healthier population by surgically limiting reproduction and protecting against targeted diseases. However, these fertility control programs focus mostly on post-intervention abundance, rather than the direct consequences of fertility control on the health of targeted individuals. Here we describe the results of a randomized control trial that measures the effect of vaccination and sterilization on parasite prevalence in common raccoons (*Procyon lotor*) living on the grounds of a large suburban zoo. We predicted lowered parasite prevalence in those groups which received vaccination or vaccination and sterilization (vasectomy or tubal

ligation) compared to controls. We found that vaccination, but not sterilization, reduced parasite prevalence in males. However, females who had been vaccinated and concurrently sterilized had higher parasite loads compared to control females. We suggest that tubal ligation may increase contact rates and facilitate parasite transmission. This suggests that the method of sterilization and sex being targeted may have an important role in determining the success of interventions.

INTRODUCTION

Zoos are important locations for conservation science and endangered species recovery. In addition, there are often resident populations of locally common wildlife living on zoo grounds. Interactions between these resident species and zoo collection animals may be of concern because disease spillover events from wildlife to collection animals have occurred in rabbits (Sato *et al.* 2002), ratites (Kazacos *et al.* 1991), multiple large cat species (Nagao *et al.* 2011, Appel *et al.* 1994), and many others.

Fertility control has been explored as a potential method of problem wildlife species management (Killian *et al.* 2007) by seeking to reduce both the absolute number of individuals and, in conjunction with vaccination schemes, the disease prevalence in a target population. Fertility control utilizing surgical intervention can be categorized as complete (removal of gonads, i.e. castration and spay) or functional (maintenance of normal hormone production, i.e. tubal ligation and vasectomy) sterilizations. These programs are often performed along-side vaccination programs to target specific diseases of concern (Killian *et al.* 2007).

Fertility control programs have been found to impact disease (Totton *et al.* 2011, Yoak *et al.* 2014). Reece and Chawla (2006) showed a significant reduction in the free-roaming dog (*Canis lupus familiaris*) population and complete elimination of human rabies deaths in a densely populated Indian city over eight years by using large scale dog vaccination and complete sterilization of bitches. Totton (*et al.* 2011) showed that completely sterilized free roaming dogs had higher body conditions compared to their sexually intact conspecifics in the same location, which they suggest is due to a lack of engagement in reproductive behaviors (territory maintenance, fetal investment, increased contact rates, etc.) which allocate energetic resources away from fighting pathogens. Further, dogs living in cities with high sterilization proportions were significantly healthier compared to sexually intact dogs living in other cities with lower sterilization proportions (Yoak *et al.* 2014). These individuals who have higher overall body condition and health should shed fewer parasites even if they become infected (Ezenwa 2004). In contrast, functional sterilization though tubal ligation, which renders females sexually active but sterile, may actually increase disease prevalence of sexually transmitted or contact-dependent diseases by increasing contact rates because of constant hormonal cycling (Caley & Ramsey 2001).

In zoos in Eastern North America, a common species of concern, is the Northern raccoon (*Procyon lotor*). Raccoons are susceptible to many common, highly infectious diseases including canine distemper virus (CDV) (Cranfield *et al.* 1984), feline parvovirus, canine adenovirus type-1, *Leptospira* serovars (Junge *et al.* 2007, Jardine *et al.* 2011), rabies (Berentsen *et al.* 2013) and *Baylisascaris procyonis* helminths (Page *et al.* 2008). *B. procyonis* can cause potentially life threatening disease in humans (Gavin *et al.* 2002), placing park visitors at risk as well. Raccoons are often considered “unwelcome” in other nations (García *et al.* 2011) as they have a high propensity to invade outside of their natural range and wherever they populate, tend to predate on native wildlife (Barton & Roth 2007, García *et al.* 2011, Ikeda *et al.* 2004) and spread pathogens (Ikeda *et al.* 2004, LoGiudice

2003). Both inside and outside of zoos, issues with raccoon populations have been traditionally addressed by lethal removal, however these depopulation programs sometimes fail to halt the issue of concern (Ratnaswamy *et al.* 1997) and the treated areas are repopulated quickly (<1 year, Rosatte *et al.* 2007). Barton and Roth (2007) found that lethal depopulation achieved a male:female skew of 10:1. They suggested this was driven by male-biased dispersal from other areas potentially bringing diseases not previously present in the focal area, in opposition to Rosatte (*et al.* 2007), who found that local reproduction by remnant raccoons was a more important driver of repopulation. Lethal removal can actually increase both the prevalence of contact dependent diseases and the absolute number of infected individuals by increasing the birth rate in a depressed density population (Choisy & Rohani 2006).

Because of the threats that raccoons and their pathogens pose and instigated by known contact events between raccoons and captive animals, the Columbus Zoo and Aquarium began a Raccoon Heath Program (RHP) in 2002 which attempts to lower the raccoon population size while buffering their health against disease through vaccination and functional sterilization (Myers *et al.* 2004). This sterilization method maintains normal hormone production, in contrast to ovariohysterectomy or castration, was chosen to minimize any hormonally driven change in raccoon behavior. In 2011, we introduced a randomized control study that measures the impacts of sterilization and vaccination on raccoon health by splitting the population into varying treatment groups: control (the non-intervention group), vaccine-only (who received a series of vaccines and anti-parasitic drugs), and vaccine-and-sterilization (which were functionally sterilized in addition to receiving the vaccine-only group's treatments). We predicted that both vaccine-only and vaccine-and-sterilization treatments would exhibit lower parasite loads than controls groups, because of the benefits of anti-parasitic drug treatment. We predicted that parasite loads would be further reduced in the vaccine-and-sterilization treatment females because these animals can allocate more of their energy to resisting infection. This beneficial effect of energy allocation should not be present in vaccine-and-sterilization male, which still undergo spermatogenesis and engage in reproductive behaviors. As such, we predicted there would be no difference between the two male intervention groups.

METHODS

Ethics statement

All methods presented here were approved by the Columbus Zoo animal care committee (protocol approved 7/5/2011) and follow guidelines set by the American Society of Mammalogists (Gannon & Sikes 2007)

Study area

The Columbus Zoo and Aquarium is a large suburban zoological park outside of Columbus, OH, USA (40. 156° N, 83.118° W) with 10,000+ captive animals on grounds and covers approximately 0.465 km^2. An additional 0.174 km^2 expansion was added during the final year of this study, converting a grassland to mimic an African savannah complete with exotic fauna. The surrounding area is mixed oak forest, housing developments, and the park abuts a large river.

Study Species

The raccoon is a common mesopredator that has successfully adapted to living around human development. Raccoon behavior is complex and shows considerable variation over temporal and spatial scales (Gehrt & Frizell 1998, Pitt *et al.* 2008, Prange *et al.* 2011). The breeding season occurs during the spring (Mar – May) and during this time period males shift their territories to increase contacts with females (Gehrt & Fritzell

1998). During the breeding season, larger, more dominant males consort with many females while most females consort with one male (Gehrt & Fritzell 1999). Adult female raccoons are the sole caretakers of young and have high (~95%) yearly pregnancy rates (Asanto *et al.* 2003) and will continue estrous late into the season if the first pregnancy fails or the female is not impregnated (Gehrt & Fritzell 1996). Male territories have been found by some (Fritzell 1978) but it is typically observed that raccoons occupy overlapping home ranges, occasionally forming coalitions that can then quickly dissolve (Gehrt & Fritzell 1998, Prange *et al.* 2011). Raccoons, although previously thought to be nearly entirely solitary, have recently been shown to contain complex social networks that are unaffected by genetic relatedness (Prange *et al.* 2011, Hirsch *et al.* 2013).

Capture Methods

Raccoons were trapped for the final seven days of each of the following 23 months: Oct and Nov 2011, Mar-Oct 2012, Apr-Sept 2013, and Mar-Sept 2014. Trapping was halted for winter months (on average for 3-4 months) when low temperatures reduced raccoon activity and to minimize impacts on raccoon health. In total, the trapping period consisted of 23 sessions, utilizing 29 Tomahawk live traps spaced evenly around the zoo at approximately 100 m apart (see fig 5). Traps were set in the evening between 18:30-20:30 and closed in the morning between 6:00-8:00. Non-target native species were immediately released and feral cats were taken to the Delaware County Humane Society.

Upon capture, animals were anesthetized with Telazol and if new to the program, were randomly assigned to one of three treatment groups (described below). All individuals received an ID ear tag, intra-scapular PIT tag, rabies vaccine (IMRAB 3 TF, Merial), and were then released within 12 hours at their original trap location. The rabies vaccine likely had no impact on survival as the raccoon variant rabies strain was not present in central Ohio at the time of this study (Berentsen *et al.* 2013). All individuals received supportive care for any injuries sustained because of the trap.

Raccoons were assigned to one of three treatment groups: control, vaccine-only, and vaccine-and-sterilization. Control group raccoons received no additional treatment. Vaccine-only animals received three additional vaccines against the diseases of most concern: A combination feline rhinotracheitis-calci-panleukopenia vaccine (Merial), a canine distemper-adenovirus type 2-coronavirus-parainfluenza-parvovirus vaccine with a *Leptospira canicola-icterohaemorrhagiae* bacterin (Recombitek C6, Merial). Additionally, this group was administered a weight-specific dose of the anti-helminthic drug moxidectin (ProHeart6, Fort Dodge), sprayed with tick and flea preventatives fipronil and S-methoprene (Frontline Plus, Merial), and palliative care even for non-trap induced wounds (broken teeth, old lacerations). Vaccine-and-sterilization individuals received the same treatments as the vaccine-only treatment, but in addition, were sterilized by either tubal ligation or vasectomy (where appropriate). Both vaccine-only and vaccine-and-sterilization individuals' vaccines were re-administered if it had been over a year since the last booster, following veterinary advice.

Parasite Collection and Testing

While animals were anesthetized, fecal samples were taken rectally and individuals were thoroughly searched for fleas and ticks. Some individuals' colons did not contain enough fecal material for testing so samples were not collected at every capture. Samples were tested in the Columbus Zoo diagnostic lab within 24 hours by centrifugal float and smear tests for the presence of intestinal parasites. Infestation intensity measurement methods varied throughout the study, so we utilize only presence or absence here.

Identification to genus was made, when possible, based on egg shape and a single egg was enough to confirm infection.

Statistical Analysis

Differences between groups were assessed using generalized estimating equations (GEE) in SPSS v22 (IBM Corporation) with raccoon ID as a subject variable and the days since the individual's first treatment began as within-subject variable. This method allows multiple captures from some individuals to not overly influence the results. To ensure any differences between groups were not influenced by non-random initial differences in parasite load, presence of parasites at the initial capture (before any treatment had been given) were compared between sexes and treatment groups. To test for differences among treatments, we used only recaptures (i.e. captures after the individual entered the program). Treatment group, sex, and season were included as fixed effects in the model, along with the interaction of sex and treatment group. Seasonality of infection was investigated using a two season variable: wherein "Spring" captures included Mar-June and "Fall" included July-Nov (adapted from Mitchell *et al.* 1999). We built separate models for the following response variables: presence/absence of both any parasite and each parasite species individually (using a binomial logistic model) and the absolute number of individual intestinal helminth species (using a Poisson log-link model).

RESULTS

Initial differences between groups

There were significant differences at the initial capture between the sexes for *Toxocara* and between treatment groups for *Trichuris* infection. We did not find significant differences between treatment groups, sex, or season for parasite presence of any other species, or for parasite species abundance (see Suplementary Table 4 in Appendix C).

Ectoparasites

We did not find significant effects of treatment group, sex, season, or the interaction of sex and treatment group on the presence of ectoparasites (ticks and fleas) (Table 4).

Gastrointestinal Helminths

There was a significant effect of the interaction between sex and treatment groups on endoparasite presence and helminth species count. Vaccine-only and vaccine-and-sterilization males had significantly lower general parasite prevalence compared to control males (Fig 8). The vaccine-and-sterilization group females had significantly higher general parasite load compared to vaccine-only and control females (Fig 8).

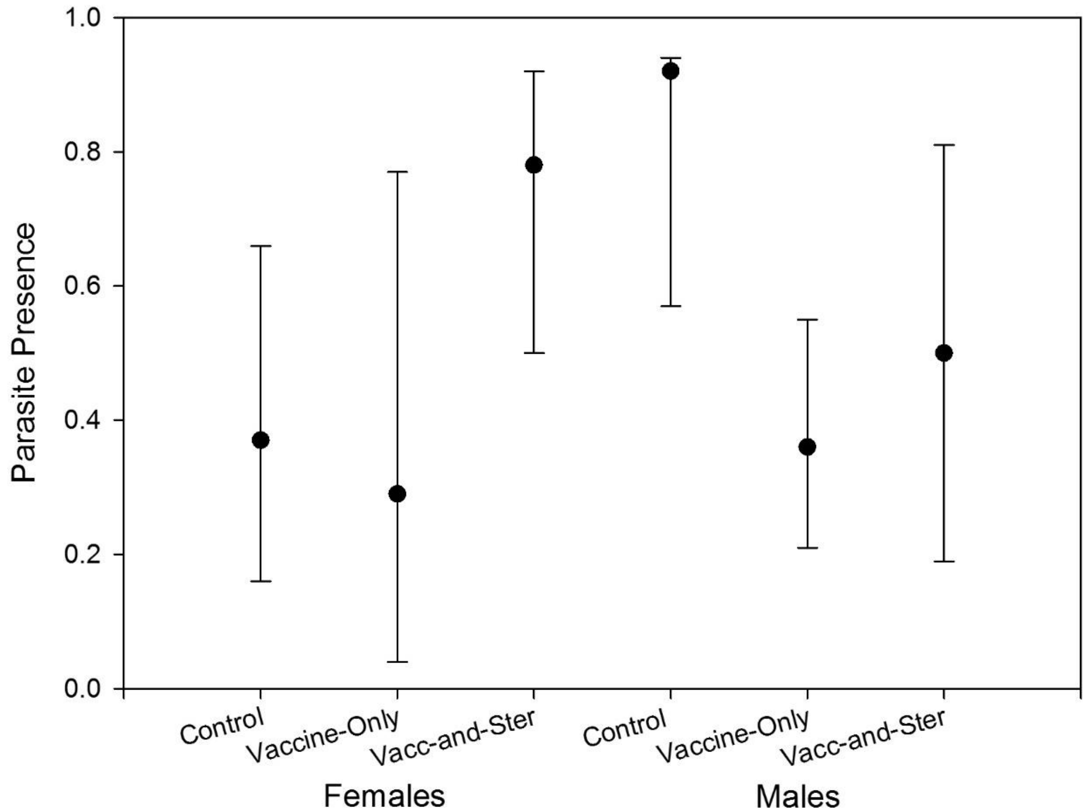

Figure 8. The presence of any gastointestinal parasites in study raccoons by sex and treatment type with 95% C.I.

Baylisascaris procyonis presence was significantly affected by the season (Fig 9a), with higher disease load in the fall (EMM: 0.29, CI: 0.14-0.51) compared to spring (EMM: 0.08, CI: 0.03-0.22), but not raccoon sex or treatment group (Table 4, Fig 9b, and Fig 9c). For the other individual parasite species, there were no significant effects of season, sex, treatment group, or the interaction between treatment group and sex on probability of infection. Some calculations of treatment group by sex and treatment group could not be performed because the low prevalence of infection caused incomplete separation of the data.

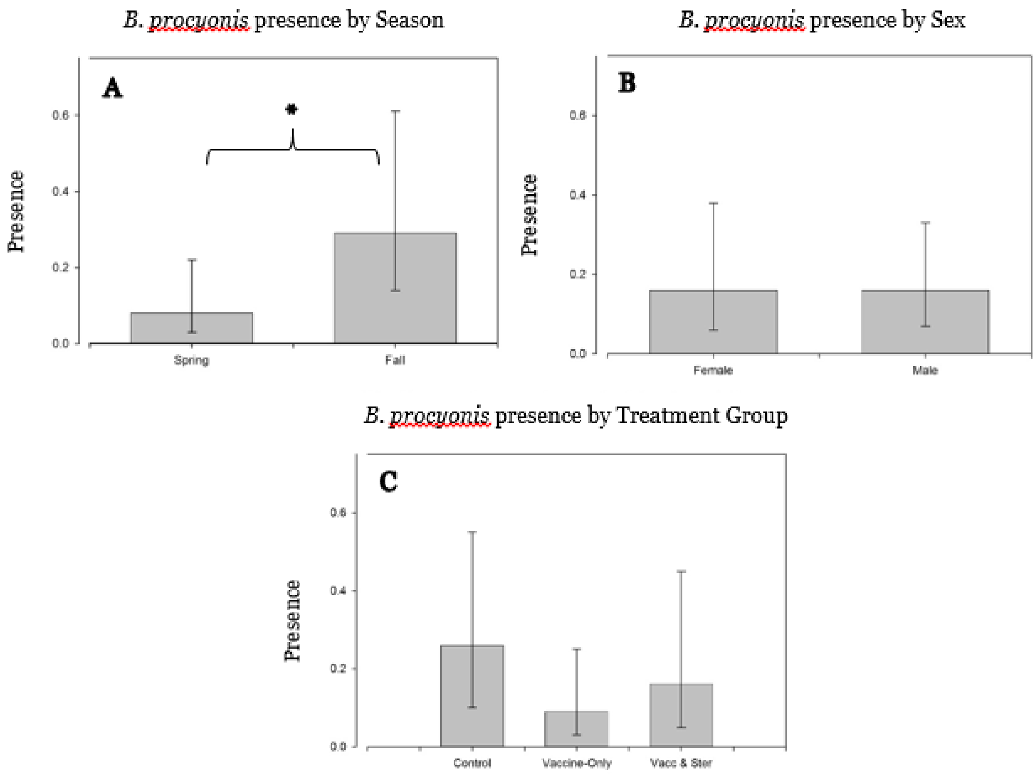

Figure 9. The effect of season, sex, and treatment group on *Baylisascaris procyonis* presence with 95% CI

Table 4 The effects of seasonality, sex, treatment group, and the interaction between treatment group and sex on gastrointestinal helminth prevalence and abundance, as well as ectoparasite load for all captures past the initial treatment date.

	Season (df=1)	**Sex (df=1)**	**Treatment Group (df = 2)**	**Treatment Group + Sex (df =2)**
Parasite Type Count	χ^2=1.992, p=0.158	χ^2=0.443, p=0.506	χ^2=3.918, p=0.141	χ^2=13.932, p=0.001
Any Intestinal Parasite Presence	χ^2=0.177, p=0.674	χ^2=0.991, p=0.320	χ^2=4.591, p=0.101	χ^2=6.911, p=0.032
Ancyclostoma‡	χ^2=0.508, p=0.476	χ^2=0.390, p=0.533	**N/A**	**N/A**
Baylisascaris	χ^2=4.150, p=0.042	χ^2=0.001, p=0.971	χ^2=2.098, p=0.350	χ^2=5.137, p=0.077
Capillaria‡	χ^2=0.655, p=0.418	χ^2=0.038, p=0.846	**N/A**	**N/A**
Coccidia†	χ^2=0.094, p=0.759	χ^2=0.845, p=0.358	χ^2=1.723, p=0.423	**N/A**
Trichuris‡	χ^2=0.616 p=0.433	χ^2=0.225, p=0.635	**N/A**	**N/A**
Any Ectoparasite	χ^2=0.010, p=0.921	χ^2=2.009, p=0.156	χ^2=0.712, p=0.700	χ^2=4.393, p=0.111

†indicates the Treatment group + Sex interaction was removed because prevalence was so low the data were quasi-separated. ‡Treatment group and Treatment group+sex were removed because of low prevalence causing quasi-separation.

DISCUSSION

Here we demonstrate that at minimum, a vaccination/anti-parasitic treatment is effective in reducing both the overall occurrence and species abundance of intestinal parasite infection in male raccoons. It is unsurprising that there are no differences in parasite load between the two higher intervention male treatment groups because vaccine-and-sterilization males differed from vaccine-only males in the additional burden of undergoing a surgical procedure but should not differ substantially in behavior (Bromley and Gese 2001b). Vasectomies do not affect spermatogenesis in male domestic cattle over the short term (20 weeks, Amann 1962) however it appears to slow spermatogenesis over the a longer time period (20+ years) in humans (Xiang *et al.* 2013). It is unclear to what effect vasectomy has on spermatogenesis at the time scale relevant to our study (1 month – 3.5 years), but if a reduction occurs, it may release a non-insignificant amount of energy for alternative use (Thomsen *et al.* 2006). At least for male parasite load, we find support for our hypothesis that vaccination/anti-helminthic treatment has a beneficial effect and that sterilization has no effect. Additional work should be performed to determine the effect of vasectomy on raccoon spermatogenesis.

For females, however, we did not find a difference between control and vaccine-only groups and found higher parasite loads in the vaccine-and-sterilization group. Whereas our vaccine-only females could become pregnant and then should then cease their reproductive behavior (Gehrt & Fritzell 1996), tubal ligated females may have continuously expressed courtship and copulation behaviors, resulting in increased breeding season length for these females. As a result, diseases may have increased transmission rates because of changes to reproductive contacts (Caley & Ramsey 2001) or because a greater allostatic load would increase immunosuppression (Eberhardt *et al.*

2013). Female brushtail possums (*Trichosurus vulpecula*), a species with similar ecology, that underwent tubal ligation showed significantly higher transmission rates of a contact dependent pathogen (*Leptospira interrogans* serovar *balcanica*) than unsterilized controls (Caley and Ramsey 2001).

Raccoon helminth epidemiology is complex, and while our treatments have a significant effect on prevalence, there are numerous other factors that may influence parasite load (Wright and Gompper 2005). As the anti-helminthic treatments discussed here only clear the digestive tract of helminths of current infestations and provide minimal future protection (Pers Communication, Gehrt and Prange), re-infection is assumed to be an important factor in our treated groups. Gastro-intestinal parasite epidemiology is complex, especially considering parasite species have varying life history characteristics and that anti-helminthic treatments have produced mixed results (Foster *et al.* 2006). Experimentally increasing raccoon contact rates using food supplementation increased the overall prevalence and abundance of gastrointestinal helminths, mostly those parasite species that are directly transmitted between hosts (Wright & Gompper 2005).

Wildlife fertility control has been shown to increase the overall health of both the targeted individuals (Totten *et al.* 2011) and neighboring conspecifics (Yoak *et al.* 2014). Immunosuppressed or energetically depleted individuals suffer higher mortality rates from infection and shed significantly more parasite eggs when infected (Ezenwa 2004). Here we show raccoon parasite load is measurably affected by supportive care and vaccination. However, we did not find a beneficial effect of sterilization on parasite load. Male parasite load is more positively influenced by anti-helminthic and vaccine treatment while females are most dramatically and negatively affected by tubal ligation. Rigorous zoo raccoon management plans that incorporate anti-helminthic and vaccination treatments may see benefits from a lowered force of infection from their resident native wildlife to both visitors and collection animals. Future studies should evaluate the effects of complete gonadectomy, which may reduce behaviors that increase potentially disease spreading contacts and decrease reproduction-related stressors.

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Chapter 6: Conclusion

Wildlife fertility control is being expanded to new species as its effects become better understood and the barriers to entry are lowered (Grey & Cameron 2010, Killian *et al.* 2007). The method of sterilization and specific traits of the species it is applied to appears to have a large effect on the success or failure of any population control program (Caley & Ramsey 2001, Kirkpatrick *et al.* 2011, Reece 2007). Here, I've sought to explore the effects of two fertility control programs in two disparate species.

In chapter 2, I found that street dog fertility control outperformed lethal control program at reducing the population size in an agent based model of a street dog population. Additionally, ABC did not produce the potentially negative skew toward a younger population that occurs in lethal programs, while simultaneously vaccinating a high proportion of the population. In chapter 3, we explored the effect that past investment into ABC programs by three Indian cities had on disease prevalence in sexually intact street dogs. There was a general trend towards lowered prevalence in those cities with longer ABC programs, showing that ABC enhanced the overall health of even non-treated dogs. Chapters 4 and 5 reported the results of a sterilization and vaccination program in a raccoon population living on grounds of a large zoo. We found minimal effects on survival rates, but sex-dependent differences on general parasite presence. Males benefited from treatment, but sterilized females experienced a negative effect. This result highlights how influential the sterilization method can be and due to differences in reproductive behavior between sexes, how the sexes experience sterilization differently.

We were able to examine the effect of fertility control on both the targeted individuals (in raccoons) and their conspecific neighbors (in dogs) by utilizing diverse research methods. This body of work examined many facets of fertility control with both modeling and real-world studies.

Canine fertility control is well established and an effective method for controlling dog populations in areas where they are not strictly managed by private individuals (Reece & Chawla 2006, WHO/WSPA 1990). This work confirms and expands on the current literature of dog population management (Reece & Chawla 2006, Totton *et al.* 2011, Yoak *et al.* 2014) by demonstrating explicitly the beneficial results of fertility control over lethal control when applied to the same population and creates a tool that can be used to test other hypotheses of disease ecology or demographic shifts (Chapter 2).

Raccoon fertility control may be a viable option for zoo managers, but should be combined with a vaccination program targeting specific diseases of concern (Myers *et al.* 2004, Schubert *et al.* 1998). Other studies have demonstrated that tubal ligation has significant effects on the behavior of females (Caley & Ramsey 2001) and chapter 5 seems to confirm this in female raccoons. Because of these potentially negative behavioral side effects, complete sterilization should be investigated as an alternative.

References

Ashford, D.A., David, J.R., Freire, M., David, R., Sherlock, I., Eulálio, M., Sampaio, D.P., & Badaro, R., 1998. Studies on control of viscera leishmaniasis: impact of dog control on canine and human visceral leishmaniasis in Jacobina, Bahia, Brazil. *Journal of Tropical Medicine and Hygiene*, 59(1), 53-57.

Amann, R.P., 1962. Reproductive capacity of dairy bulls. III. The effect of ejaculation frequency, unilateral vasectomy, and age on spermatogenesis. *American Journal of Anatomy*, 110(1):49-67.

Anderson, M.C., Martin, B.J., & Roemer, G.W., 2004. Use of matrix population models to estimate the efficacy of euthanasia versus trap-neuter-return for management of free-roaming cats. *Journal of the American Veterinary Medical Association*, 225(12):1871-1876.

Appel, M.J., Yates, R.A, Foley, G.L., Bernstein, J.J., Santinelli, S., Spelman, L.H., Miller, L.D., Arp, L.H., Anderson, M., & Barr, M., 1994. Canine distemper epizootic in lions, tigers, and leopards in North America. *Journal of Veterinary Diagnostic Investigations*, 6:277-288.

Asano, M., Matoba, Y., Ikeda, T., Suzuki, M., Asakawa, M., & Ohtaishi, N., 2003. Reproductive characteristics of the feral raccoon (*Procyon lotor*) in Hokkaido, Japan. *Theriogenology*, 65(3):369-373.

Barton, B.T & Roth, J.D., 2007. Raccoon removal on sea turtle nesting beaches. *Journal of Wildlife Management*, 71(4): 1234-1237.

Beasley, J.C. & Rhodes Jr., O.E., 2008. Relationship between raccoon abundance and crop damage. *Human-Wildlife Interactions*, 2(2):248-259.

Belsare, A.V. & Gompper, M.E., 2014. A model-based approach for investigation and mitigation of disease spillover risks to wildlife: dogs, foxes, and canine distemper in central India. *Ecological Modeling*, 296, 102-112.

Berentsen, A.R., Dunbar, M.R., Fitzpatrick, C.E., & Walter, W.D., 2013. Spatial ecology of urban raccoons in Northeastern Ohio: Implications for oral rabies vaccination. *USDA National Wildlife Research Center-Staff Publications,* Paper 1447.

Bonnell, T.R., Sengupta, R.R., Chapman, C.A., & Goldberg, T.L., 2010. An agent-based model of red colobus resources and disease implicates key resource sites as hot spots of disease transmission. *Ecological Modeling,* 221(20), 2491-2500.

Bradley, C.A. & Altizer, S., 2007. Urbanization and the ecology of wildlife diseases. *TRENDS in Ecology and Evolution*, 22(2):95-102.

Bromley, C.A & Gese, E.M., 2001b. Effects of sterilization on territory fidelity and maintenance, pair bonds, and survival rates of free-ranging coyotes. *Canadian Journal of Zoology*, 79:386-392.

Burnham, K.P., 1993. A theory for combined analysis of ring recovery and recapture data. In: *Marked Individuals in the Study of Bird Population*. Eds: Lebreton, J.D. and North, P.M: 193-213.

Butcher, R., 1999. Stray dogs - a worldwide problem. *Journal of Small Animal Practice,* 40(9):458-459.

Butler, J.R.A. & du Toit, J.T., 2002. Diet of free-ranging domestic dogs (*Canis familiaris*) in rural Zimbabwe: implications for wild scavengers on the periphery of wildlife reserves. *Animal Conservation*, 5(1), 29-37.

Butler, J.R.A., du Toit, J.T., & Bingham, J., 2004. Free-ranging domestic dogs (*Canis familiaris*) as predators and prey in rural Zimbabwe: threats of competition and disease to large wild carnivores. *Biological Conservation,* 115(3), 369-378.

Caley, P. & Ramsey, D., 2001. Estimating disease transmission in wildlife, with emphasis on leptospirosis and bovine tuberculosis in possums, and effects of fertility control. *Journal of Applied Ecology*, 38:1362-1370.

Caughley, G., Pech, R., & Grice, D., 1992. Effect of fertility control on a population's productivity. *Wildlife Research*, 19:623-627.

Chambers, L.K., Singleton, G.R., & Hinds, L.A., 1999. Fertility control of wild mouse populations: the effects of hormonal competence and an imposed level of sterility. *Wildlife Research*, 26(5):579-591.

Chawla S, Reece, J., 2002. Timing of oestrus and reproductive behavior in Indian street dogs. *The Veterinary Record,* 150, 450-451.

Choisy, M. & Rohani, P., 2006. Harvesting can increase severity of wildlife disease epidemics. *Proceedings of the Royal Society: Biological Sciences,* 273(1597), 2025-2034.

Coyne M.J., Burr, J.H.H., Yule, T.D., Harding, M.J., Tresnan, D.B., & McGavin, D., 2001. Duration of immunity in dogs after vaccination or naturally acquired infection. *The Veterinary Record*, 149, 509-515.

Cleaveland, S., Appel, M.G.J., Chalmers, W.S.K., Chillingworth, C., Kaare, M., & Dye, C., 2000. Serological and demographic evidence for domestic dogs as a source of canine distemper virus infection for Serengeti wildlife. *Veterinary Microbiology*, 72(3-4), 217-227.

Cranfield, M.R., Barker, I.K., Mehren,K.G., & Rapley, W.A., 1984. Canine distemper in wild raccoons (*Procyon lotor*) at the Metropolitan Toronto Zoo. *The Canadian Veterinary Journal,* 25(2):63-66.

Delsink, A.K., van Altena, J.J., Grobler, D., Bertschinger, H., Kirkpatrick, J., & Slotow, R., 2006. Regulation of a small, discrete African elephant population through immunocontraception in the Makalali Conservancy, Limpopo, South Africa. *South African Journal of Science*, 102:402-405.

Donnelly, C.A., Woodroffe, R., Cox, D.R., Bourne, F.J., Cheeseman, C.L., Clifton-Hadley, R.S., Wei, G., Gettinby, G., Gilks, P., Jenkins, H., Johnston, W.T., Le Fevre, A.M., McInerney, J.P., & Morrison W.I., 2006. Positive and negative effects of widespread badger culling on tuberculosis in cattle. *Nature*, 439:843-846.

Eberhardt, A.T., Costa, S.A., Marini, M.R., Racca, A., Baldi, C.J., Robles, M.R., Moreno, P.G., & Beldomenico, P.M., 2013. Parasitism and physiological trade-offs in stressed capybaras. *PLoS ONE*, 8(7):e70382.

Ezenwa, V.O., 2004. Interactions among host diet, nutritional status and gastrointestinal parasite infection in wild bovids. *International Journal of Parasitology*, 34:535-542.

Fontanarrosa, M.F., Vezzani, D., Basabe, J., & Eiras, D.F., 2006. An epidemiological study of gastrointestinal parasites of dogs from Southern Greater Buenos Aires (Argentina): age, gender, breed, mixed infections, and seasonal patterns. *Veterinary Parasitology*, 136(3-4), 283-295.

Foster, G.W. Cunningham, M.W., Kinsella, J.M., McLaughlin, G., Forrester, D.J., 2006. Gastrointestinal helminths of free-ranging Florida panthers (*Puma concolor coryi*) and the efficacy of the current antihelmenthic treatment protocol. *Journal of Wildlife Diseases*. 42(2):402-406.

Frey, S.N., Majors, S., Conover, M.R., Messmer, T.A., & Mitchell, D.L., 2003. Effect of predator control on ring-necked pheasant populations. *Wildlife Society Bulletin*, 31(3):727-735.

Fritzell, E.K., 1978. Aspects of raccoon (*Procyon lotor*) social organization. *Canadian Journal of Zoology*, 56:260-271.

Gannon, W.L. & Sikes, R.S., 2007. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *Journal of Mammalogy*, 88(3):809-823.

García, J.T., García, F.J., Alda, F., González, J.L., Aramburu, M.J., Cortés, Y., Prieto, B., Pliego, B., Pérez, M., Herrera, J., García-Román, L., 2012. Recent invasion and status of the raccoon (*Procyon lotor*) in Spain. *Biological Invasions*, 14(7):1305-1310.

Garside, D, Gebril,, A, Alsaadi, M, & Ferror, V.A., 2014. Fertility Control in Wildlife: Review of Current Status, Including Novel and Future Technologies. In: *Reproductive sciences in Animal Conservation*. Eds. Holt, W.V., Brown, J.L., & Comizzoli, P.

Gavin, P.J., Kazacos, K.R., Tan, T.Q., Brinkman, W.B., Byrd, S.E., Davis, A.T., Mets, M.B., & Shulman, S.T., 2002. Neural larva migrans caused by the raccoon roundworm *Baylisascaris procyonis*. *Pediatric Infectious Disease Journal*, 21(10):971-975.

Gehrt, S.D., 2003. Raccoons and allies. 611-633. In*: Wild Mammals of North America: biology, management and conservation*. Second Edition. Eds: Feldhamer, G.A., Thompson, B.C., & Chapman, J.A.,

Gehrt, S., & Fritzell, E.K., 1996. Second estrus and late litters in raccoons. *Journal of Mammalogy*, 77(2):388-393.

Gehrt, S.D. & Fritzell E.K., 1998. Resource distribution, female home range dispersion and male spatial interactions: group structure in a solitary carnivore. *Animal Behavior*, 55:1211-1227.

Gerhrt, S.D. & Fritzell , E.K., 1999. Behavioral aspects of the raccoon mating system: determinants of consortship success. *Animal Behavior,* 57(3):593-601.

Gehrt, S.D., Huberg Jr, G.F., & Ellis, J.A., 2002. Long-term population trends of raccoons in Illinois. *Wildlife Society Bulletin*, 30(2):457-463.

Gehrt, S.D., Hungerford, L.L., & Hatten, S., 2001. Drug effects on recaptures of raccoons. *Wildlife Society Bulletin*, 29(3):833-837.

Gemmell, N.J., Jalilzadeh, A., Didham, R.K., Soboleva, T., & Tompkins, D.M., 2013. The Trojan female technique: a novel, effective and humane approach for pest population control. *Proceedings of the Royal Society B*. 280: 20132549

Goymann, W. & Wingfield, J.C., 2004. Allostatic load, social status and stress hormones: the costs of social status matter. *Animal Behavior*, 67(3):591-602.

Gray, M.E. & Cameron, E.Z., 2010. Does contraceptive treatment in wildlife result in side effects? A review of quantitative and anecdotal evidence. *Reproduction*, 139:45-55.

Grimm, V., Berger, U., DeAngelis, D.L., Polhill, J.G., Giske, J., & Railsback, S.F., 2010. The ODD protocol: A review and first update. *Ecological Modeling*, 210, 2760-2768.

Grimm, V. & Railsback, S.F., 2005. *Individual-based modeling and ecology*. Princeton University press.

Hiby, L., Reece, J., Wright, R., Jaisinghani, R., Singh, B., & Hiby, E., 2011. A mark-resight survey method to estimate the roaming dog population in three cities in Rajasthan, India. *BMC Veterinary Research*, 7(1), 46.

Hirsch, B.T., Prange, S., Hauver, S.A., & Gehrt, S.D., 2013. Raccoon social networks and the potential for disease transmission. *PLoS ONE,* 8(10): e75830.

Hodges, K.M.,Chamberlain, M.J., & Leopold, B.D., 2000. Effects of summer hunting on ranging behavior of adult raccoons in central Mississippi. *Journal of Wildlife Management,* 64(1):194-198.

Hughes, J. & Macdonald, D.W., 2005. A review of the interactions between free roaming domestic dogs and wildlife. *Biological Conservation*, 157, 341-351.

ICAM (International Companion Animal Management), 2007. *Humane dog population management guidance*. Available online at http://www.wsava.org/PDF/2008/Misc/AWC_ICAM_Coalition.pdf

Ikeda, T., Asano, M., Matoba, Y., & Abe, G., 2004. Present status of invasive alien raccoon and its impact in Japan. *Global Environmental Research*, 8(2):125-131.

Indian Census Data, 2011. Available online at http://www.census2011.co.in/ . Accessed April 2nd, 2012.

Jardine, C., Lindsay, L.R., Nicholson, V.M., Ojkic, D., & Prescott, J.F., 2011. Longitudinal study on the seroprevalence of Avian Influenza, Leptospirosis, and Tularemia in an urban population of Raccoon (*Procyon lotor*) in Ontario, Canada. *Vector-Borne and Zoonotic Diseases*, 11(1):37-42.

Junge, R., Bauman, K., King, M., and Gompper, M., 2007. A serologic assessment of exposure to viral pathogens and *Leptospira* in an urban raccoon (*Procyon lotor*) population inhabiting a large zoological park. *Journal of Zoo and Wildlife Medicine*, 38(1): 18-26.

Kale, K.M., Wadhva, S.K., Aswar, N.R., & Vasudeo, N.D., 2006. Dog bites in children. *Indian Journal of Community Medicine*, 31(1): 24-25.

Kazacos, K.R., Fitzgerald, S.D., & Reed, W.M., 1991. *Baylisascaris procyonis* as a cause of cerebrospinal nematodiasis in ratites. *Journal of Zoo and Wildlife Medicine,* 22(4):460-465

Kendall, W.L., Pollock, K.H., and Brownie, C., 1995. A likelihood-based approach to capture-recapture estimation of demographic parameters under the robust design. *Biometrics*, 51:293-308.

Kendall, W.L., Barker, R.J., White, G.C., Lindberg, M.S., Langtimm, C.A., & Peñaloza, C.L., 2013. Combining dead recovery, auxiliary observations, and robust design data to estimate demographic parameters from marked individuals. *Methods in Ecology and Evolution*, 4:828-835.

Killian, G., Fagerstone, K., Kreeger, T., Miller, L., & Rhyan, J., 2007. Management strategies for addressing wildlife disease transmission: the case for fertility control. In: Nolte, D.L., Arjo, W.M., Stalman, D.H. (Eds.), *Proceedings of the 12th Wildlife Damage Management Conference,* 265-271.

Kirkpatrick, J.F., Lyda, R.O., & Frank, K.M., 2011. Contraceptive vaccines for wildlife: a review. American *Journal of Reproductive Immunology,* 66:40-50.

Knobel, D.L., Cleaveland, S., Coleman, P.G., Fevre, E.M., Meltzer, M.I., Miranda, M.E.G., Shaw, A., Zinsstag, J., & Meslin, F.X., 2005. Re-evaluating the burden of rabies in Africa and Asia. *Bulletin of the World Health Organization,* 83(5):360-368.

Lacerda, A.C.R.,Tomas, W.M., & Marinho-Filho, J., 2009. Domestic dogs as an edge effect in the Brasília National Park, Brazil: interactions with native mammals. *Animal Conservation*, 15(5), 477-487.

Lane V.M., Liu I.K.M., Casey K., Van Leeuwen E.M.G., Flanagan D.R., Murata K., Munro C., 2007. Inoculation of female American black bears (*Ursus americanus*) with partially purified porcine zona pellucida limits cub production. *Reproduction, Fertility and Development*, 19:617–625.

LoGiudice, K., 2003. Trophically transmitted parasites and the conservation of small populations: Raccoon roundworm and the Imperiled Allegheny woodrat. *Conservation Biology,* 17(1):258-266.

Markandya, A., Taylor, T., Longo, A., Murty, M.N., Murty, S., & Dhava, K., 2008. Counting the cost of vulture decline - An appraisal of the human health and other benefits of vultures in India. *Ecological Economics,* 67:194-204.

Matter, H.C., Wandeler, A.I., Neuenschwander, B.E., Harischandra, L., & Meslin, F.X., 2000. Study of the dog population and the rabies control activies in the Mirigama area of Sri Lanka. *Acta tropica,* 75(1), 95-108.

Miller, L.A., Rhyan, J.C., & Drew, M., 2004. Contraception of bison by GnRH vaccine: A possible means of decreasing transmission of Brucellosis in bison. *Journal of Wildlife Diseases,* 40(4):725-730.

Mitchell, M.A., Hungerford, L.L. Nixon, C., Esker, T., Sullivan, J., Koerkenmeier, R., & Dubey, J.P., 1999. Serological survey for selected infectious disease agents in raccoons from Illinois. *Journal of Wildlife Diseases,* 35(2):347-355.

Morters, M.K., Restif, O., Hampson, K., Cleaveland, S., Wood, J.L.N., & Conlan, A.J.K., 2013. Evidence-based control of canine rabies: a critical review of population density reduction. *Journal of Animal Ecology,* 82(1), 6-14.

Myers, G.E., Barrie, M., and Rentier, M., 2004. An alternative to euthanasia for population control of nuisance wildlife. *Proceedings American Association of Zoo Veterinarians, American Association of Wildlife Veterinarians, Wildlife Disease Association: health and conservation of captive and free-ranging wildlife: joint conference,* San Diego, California, August 28-September 3, 2004. American Association of Zoo Veterinarians.

Nagao, Y., Nishio, Y., Shimoda, H., Tamaru, S., Shimojima, M., Goto, M., Une, Y., Sato, A., Ikebe, Y., and Maeda, K., 2011. "An outbreak of canine distemper virus in tigers (*Panthera tigris*): possible transmission from wild animals to zoo animals" *The Journal of Veterinary Medical Science,* 74(6): 699-705.

Nieilsen, C.L.R., & Nielsen C.K., 2007. Multiple paternity and relatedness in southern Illinois raccoons (*Procyon lotor*). *Journal of Mammalogy,* 88(2): 441-447.

Page, L.K., Gehrt, S.D., & Robinson, N.P., 2008. Land-use effects on prevalence of raccoon roundworm (*Baylisascaris procyonis*). *Journal of Wildlife Diseases*, 44(3):594-599.

Pitt, J.A., Larivière, S., & Messier, F., 2008. Social organization and group formation of raccoons at the edge of their distribution. *Journal of Mammalogy*, 89(3):646-653.

Prange, S., Gehrt, S. D., & Hauver, S., 2011. Frequency and duration of contacts between free-ranging raccoons: uncovering a hidden social system. *Journal of Mammalogy*, *92*(6), 1331-1342.

Oaks J.L., Gilbert M., Virani, M.Z., Watson, R.T., Meteyer, C.U., Rideout B.A., Shivaprasad, H.L., Ahmed, S., Chaudry, M.J.I., Arshad, M., Mahmood, S., Ali, A., & Khan, A., 2004. Diclofenac residues as the cause of the vulture population decline in Pakistan. *Nature*, 427: 630-633.

Pitt, W, C., Box, P.W., & Knowlton, F.F., 2003. An individual-based model of canid populations: modeling territoriality and social structure. *Ecological Modeling,* 166(1-2), 109-121.

Prakash, V., Pain, D.J., Cunningham, A.A., Donald, P.F., Prakash, N., Verma, A., Gargi, R., Sivakumar, S., & Rahmani A.R., 2003. Catastrophic collapse of Indian white-back *Gyps bengalensis* and long-billed *Gyps indicus* vulture populations. *Biological Conservation*, 109:381-390.

Ratnaswamy, M.J., & Warren, R.J., 1998. Removing raccoons to protect sea turtle nests: are there implications for ecosystem management? *The Wildlife Society Bulletin*, 26:846-850.

Ratnaswamy, M.J., Warren, R.J., Kramer, M.T., & Adam, J.D., 1997. Comparisons of lethal and nonlethal techniques to reduce raccoon depredations of sea turtle nests. *Journal of Wildlife Management*, 21:368-376.

Reece, J.F., 2005. Dogs and dog control in developing countries. In: *The state of the animals III*. Eds: Salem, D.J., & Rowan, A.N., 55-64.

Reece, J.F., 2007. Rabies in India: an ABC approach to combating the disease in street dogs. T*he Veterinary Record*, 161, 292-293

Reece, J.F., Chawla, S.K., Hiby, E.F., & Hiby, L.R., 2008. Fecundity and longevity of roaming dogs in Jaipur. *BMC Veterinary research*, 4(1), 6.

Reece, J.F. & Chawla, S.K., 2006. Control of rabies in Jaipur, India, by the sterilization and vaccination of neighborhood dogs. *The Veterinary Record*, 159, 379-383.

Reece, J.F., Chawla, S.K., & Hiby, A.R., 2013. Decline in human dog-bite cases during a street dog sterilization programme in Jaipur, India. *The Veterinary Record*, 172(18):473.

Riley, S.P.D., Hadidian, J., & Manski, D.A., 1998. Population density, survival, and rabies in raccoons in an urban national park. *Canadian Journal of Zoology*, 76:1153-1164.

Rutberg, A.T., Naugle, R.E., Thiele, L.A., & Liu, I.K.M., 2004. Effects of immunocontraception on a suburban population of white-tailed deer *Odocoileus virginianus*. *Biological Conservation*, 166(2):243-250.

Sato, H., Furuoka, H., & Kamiya, H., 2002. First outbreak of *Baylisascaris procyonis* larva migrans in rabbits in Japan. *Parasitology International*, 51:105-108.

Schubert, C.A., Barker, I.K., Rosatte, R.C., MacInnes, C.D., & Nudds, T.D., 1998. Effect of canine distemper on an urban raccoon population: an experiment. *Ecological Applications*, 8(2):379-387.

Shi, D., Wan, X., Davis, S.A., Pech, R.P., & Zhang, Z.,2002. Simulation of lethal control and fertility control in a demographic model for Brandt's Vole *Microtus brandi*. *Journal of Applied Ecology* 39:337-348.

Smith, G.C., & Cheeseman, C.L. 2002. A mathematical model for the control of diseases in wildlife populations: culling, vaccination, and fertility control. *Ecological Modeling*, 150:45-53.

Singh, B.B., Dhand, N.K., Ghatak, S., & Gill, J.P.S., 2014. Economic losses due to cystic echinococcosis in India: need for urgent action to control the disease. *Preventive Veterinary Medicine*, 113, 1-12.

Singh, G., Joshi, R.D., Chopra, S.K., & Singh, A.B., 1974. Late quaternary history of vegetation and climate of the Rajasthan Desert, India. *Philosophical Transactions of the Royal Society of London. Series B, Biological*. 267(889):467-501.

Sillero-Zubiri, C., Sukumar, R., & Treves, A., 2009. Living with wildlife: the roots of conflict and the solution. In: *Key Topics in Conservation Biology*. Eds: MacDonald, D. & Service, K.

Slavinski, S., Humberg, L., Lowney, M., Simon, R., Calvanese, N., Bregman, B., Kass, D., & Oleszko, W., 2012. Trap-vaccinate-release program to control raccoon rabies, New York, USA. *Emerging Infectious Diseases*, 18(7):1170-1172.

Smith, H.T. & Engeman, R.M., An extraordinary raccoon, *Procyon lotor*; density at an urban park. *Canadian Field–Naturalist*, 116(4):636-639.

Sudarshan, M.K., Madhusudana, S.N., Mahendra, B.J., Rao, N.S.N., Ashwath Narayana, D.H., Abdul Rahman, S., Meslin, F.X., Lobo, D., & Ravikumar, K., 2007. Assessing the burden of human rabies in India: results of a national multi-center epidemiological survey. *International Journal of Infectious Diseases,* **11**(1), 29-35.

Thomsen, R., Soltis, J., Matsubara, M., Matsubayashi, K., Onuma, M., & Takenaka, O., 2006. How costly are ejaculates for Japanese macaques? *Primates*, 47:272-274.

Torrence, M.E., Jenkins, S.R., & Glickman, L.T., 1992. Epidemiology of raccoon rabies in Virginia, 1984 to 1989. *Journal of Wildlife Diseases,* 28(3): 369-376.

Totton, S.C, Wandeler, A.I., Gartley, C.J., Kachhawaha, S., Suman, M., Ribble, C.S., Rosatte, R.C., & McEwen, S.A., 2010a. Assessing reproductive patterns and disorders in free-ranging dogs in Jodhpur, India to optimize a population control program. *Theriogenology*, **74**:1115-1120.

Totton, S.C., Wandeler, A.I., Zinsstag, J., Bauch, C.T., Ribble, C.S., Rosatte, R.C., & McEwen, S.A., 2010b. Stray dog population demographics in Jodhpur, India following a population control/rabies vaccination program. *Preventive Veterinary Medicine*, **97**: 51-57.

Totton, S.C., Wandeler, A.I., Ribbe, C.S., Rosatte, R.C., & McEwen, S.A., 2011. Stray dog population health in Jodhpur, India in the wake of an animal birth control (ABC) program. *Preventative Veterinary Medicine*, 98(2), 215-220.

Townsend, S.E., Sumantra, I.P., Pudjiatmoko, Bagus, G.N., Brum E, Cleaveland, S., Crafter, S., Dewi, A.P.M,. Dharm, D.M.N, Dushoff, J., Girardi, J., Gunata, I.K., Hiby, E.F., Kalalo, C., Knobel, D.L., Mardiana, I.W., Putra, A.A.G., Schoonman, L., Scott-Orr., H., Shand, M., Sukanadi, I.W., Suseno, P.P., Haydon, D.T., Hampson, K., 2013. Designing programs for eliminating canine rabies from islands: Bali, Indonesia as a case study. *PLoS Neglected Tropical Diseases*, **7**(8): 2372.

Turner, A. & Kirkpatrick, J.F., 2002. Effects of immunocontraception on population, longevity and body condition in wild mares (*Equus caballus*). *Reproduction*, 60:187-195.

Troyer, E.M., Cameron Devitt, S.E., Sunquist, M.E., Goswami, V.R., and Oli, M.K., 2014. Survival, recruitment, and population growth rate of an important mesopredator,: the Northern Raccoon. *PLoS ONE*, 9(6):e98535.

Vanak, A.T. & Gompper, M.E., 2009. Dietary niche separation between sympatric free-ranging domestic dogs and Indian foxes in central India. *Journal of Mammalogy*, **90**(5):1058-1065.

Venkataraman, K.S. & Nedunchelliyan, S., 1992. Epidemiology of an outbreak of leptospirosis in man and dog. *Comparative Immunology, Microbiology, and Infectious Diseases*, 15(4):243-247.

Vicente, J., Delahay, R.J., Walker, N.J., & Cheeseman, C.L., 2007. Social organization and movement influence the incidence of bovine tuberculosis in an undisturbed high-density badger *Meles meles* population. *Journal of Animal Ecology*, 76:348-360.

Williams, C.K., Davey, C.C., Moore, R.J., Hinds, L.A., Silvers, L.E., Kerr, P.J., French, N., Hood, G.M., Pech, R.P., & Krebs, C.J., 2007. Population responses to sterility imposed on female European rabbits. *Journal of Applied Ecology*, 44:291-301.

Wright, A.N. & Gompper, M.E., 2005. Altered parasite assemblages in raccoons in response to manipulated resources availability. *Oecologia*, 144:148-156.

WHO, 2004. Expert consultation on Rabies. *First Report*: *WHO Technical Report Series* 931. Geneva, World Health Organization.

WHO, 1988. Report of a WHO consultation on dog ecology studies related to dog rabies control. , 22-25.

WHO/WSPA., 1990. Guidelines for dog population management. *Geneva: World Health Organization.*

WHO, 2005. WHO expert consultation on Rabies. *Technical Report Series 931*.

Wilensky, U. 1999. Netlogo, 1999. *Center for connected Learning and Computer-Based Modeling, Northwestern Unviersity. Evanston, IL.*

Xiang, Y., Luo, P., Cao, Y., & Yang, Z-W., 2013. Long-term effect of vasectomy on spermatogenesis in men: a morphometric study. *Asian Journal of Andrology*, 13:434-436.

Yoak, A.J., Reece, J.F., Gehrt, S.D., Hamilton, I.M., 2014. Disease control through fertility control: Secondary benefits of animal birth control in Indian street dogs. *Preventive Veterinary Medicine,* 113(1), 152-156.

Appendix A: Model Local Sensitivity Analysis (Chapter 2)

Table 5. Local sensitivity analysis of the relative effect on dog population size for Informed-Absolute ABC and Informed-Absolute Lethal control methods

Parameter	Meaning of Parameter	Reference Value	Reference Source	Quality of Knowledge	ABC S-	ABC S+	Lethal S-	Lethal S+
A Surv	Adult yearly survival rate	0.7	Reece *et al.* 2008	5	14.57	13.45	18.28	16.78
J Surv	Juvenile Yearly Survival Rate	0.25	Reece *et al.* 2008	5	24.45	25.36	21.43	21.79
Daily Chance	Daily chance of dog control agents being spawned	0.88	Unpublished data from Reece	5	16.92	14.68	19.16	19.37
Released Dogs	Average number of owned dogs abandoned each day	2	No data available	1	22.66	22.37	19.49	20.65
Bag Limit	Average number of dogs caught by each control van	6.5	Unpublished data from Reece	5	16.32	13.55	19.06	19.78
Zone Variability	Maximum percentage difference between each zone's carrying capacity	20%	No data available	2	2.03	1.96	1.94	2.11
K	Limit on density around a dog above which they cannot breed.	0.81	No data available	2	25.60	25.99	20.94	21.50

Table 6. Local sensitivity analysis of the relative effect on the number of interventions (surgeries/euthanasia) performed for Informed-Absolute ABC and Informed-Absolute Lethal control methods.

Parameter	Meaning of Parameter	Reference Value	Reference Source	Quality of Knowledge	ABC S-	ABC S+	Lethal S-	Lethal S+
A Surv	Adult yearly survival rate	0.7	Reece *et al.* 2008	5	19.950	19.348	19.988	20.016
J Surv	Juvenile Yearly Survival Rate	0.25	Reece *et al.* 2008	5	2.021	2.005	1.999	1.996
Daily Chance	Daily chance of dog control agents being spawned	0.88	Unpublished data from Reece	5	20.956	20.530	21.021	21.024
Released Dogs	Average number of owned dogs abandoned each day	2	No data available	1	20.073	19.986	19.968	19.947
Bag Limit	Average number of dogs caught by each control van	6.5	Unpublished data from Reece	5	20.943	20.009	21.074	20.943
Zone Variability	Maximum percentage difference between each zone's carrying capacity	20%	No data available	2	19.902	19.901	19.979	19.987
K	Limit on density around a dog above which they cannot breed.	0.81	No data available	2	20.227	20.078	19.994	20.035

Appendix B: Test kit sensitivity and specific (Chapter 3)

Table 7. Sensitivity and specificity of BioGal ELISA test kits

Disease	Sensitivity	Specificity
Brucella canis	98%	93%
Ehrlichia canis	100%	94.1%
Leptospira serovars	80%	60%
Infectious Canine Hepatitis	98%	86%
Canine Parvovirus		
IgM	91.4%	90.8%
IgG	997%	100%
Canine Distemper Virus		
IgM	93.1%	95.5%
IgG	95%	100%

Appendix C: Drivers of parasite presence (Chapter 5)

Table 8. The comparisons between individuals' general parasite presence based on seasonality, sex, and treatment group.

	Season (df=1)	Sex (df=1)	Treatment Group (df = 2)
Parasite Species Count	χ^2=0.491, p=0.483	χ^2=0.005, p=0.943	χ^2=1.208, p=0.537
Any Intestinal Parasite Presence	χ^2=0.014, p=0.906	χ^2=0.881, p=0.348	χ^2=0.068, p=0.967
Ancyclostoma	χ^2=0.585, p=0.444	χ^2=1.642, p=0.200	χ^2=0.493, p=0.781
Baylisascaris	χ^2=0.202, p=0.653	χ^2=0.893, p=0.345	χ^2=0.118, p=0.943
Capillaria	χ^2=0.211, p=0.646	χ^2=0.010, p=0.919	χ^2=1.966, p=0.161
Coccidia	χ^2=0.121, p=0.728	χ^2=2.238, p=0.135	χ^2=1.456, p=0.483
Toxocara	χ^2=1678.500, p=0.000	χ^2=0.520, p=0.471	χ^2=0.895, p=0.639
Trichuris	χ^2=0.002, p=0.967	χ^2=0.192, p=0.662	χ^2=1521.612, p=0.000
Any Ectoparasite	χ^2=0.227, p=0.634	χ^2=1.290, p=0.256	χ^2=1.446, p=0.485