THESIS

DOG DEMOGRAPHY AND POPULATION ESTIMATES FOR RABIES CONTROL IN BALI, INDONESIA

Submitted by

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ABSTRACT

DOG DEMOGRAPHY AND POPULATION ESTIMATES FOR RABIES CONTROL IN BALI, INDONESIA

Rabies is a fatal zoonotic disease with global significance. At the end of 2008, rabies emerged in the Island of Bali, Indonesia, drawing international attention. As the disease became wide-spread, the government focused on island-wide mass vaccination of dogs and improving public awareness, however the local dog population is not well documented. The Center for Indonesian Veterinary Analytical Studies (CIVAS), a local non-government organization in Indonesia, and the International Livestock Research Institute (ILRI) initiated a project to explore the link between the dog population and local communities in Bali with focus on the impact of this relationship in the spread of rabies. As part of that project, the objective of the study is to (1) characterize the demographics and rabies vaccination of owned and free-roaming dogs and (2) estimate the abundance and identify factors associated with the distribution of the dogs in Bali.

The study was conducted on two dog subpopulations, owned and free-roaming dogs, in 310 banjars in Denpasar city, Gianyar district, and Karangasem district in Bali. Banjar is a subvillage structure in Bali. The sampling design was a two-stage sampling with villages as the primary sampling unit and banjars as the secondary sampling unit. Data were collected between March 2011 and March 2012. Survey of owned dogs was carried out through door to door interview of owners and photographic mark recapture was used to collect data on free-roaming dogs.

Dogs were predominantly owned and the effect of unowned dogs towards the total population was minimal. Demographically, the sex ratio was male-biased and juveniles make up 15-20% of the population. Free-roaming dogs were dominated by adults and a higher proportion of males.

There were differences in the demographics of dogs in urban and non-urban areas which should be considered when planning and implementing control programs.

Overall vaccination coverage was high (>70%), however juveniles and females have a higher likelihood of not being vaccinated. The endurance of vaccination collars should be improved to better represent the true vaccination coverage in free-roaming dogs as there is high confidence that most free-roaming dogs were actually owned dogs. Recent culling was associated with increased proportions of juveniles and a 40% higher risk of dogs not being vaccinated.

The observation of free-roaming dogs should always account for detection probability as only 20% of dogs in this study were seen at any given time. Failure to account for detection probability will result in severe underestimation of the population abundance. The human population, presence of a forest and recent culling accounted for 28% of variation in the number of owned dogs in banjars. Accordingly, the number of owned dogs and presence of rice paddies accounted for 61% of variation in the number of free-roaming dogs in banjars.

Finally, the overall and median human to dog ratios were the least biased ratios available for estimating the overall dog population, however it is a crude tool with poor precision. The overall ratio on average slightly underestimates the total population, while the median ratio slightly overestimates the total population. For rabies control purposes, overestimation is preferred over underestimation and the median human to dog ratio is recommended.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I.

INTRODUCTION

1.1. Background and Significance

Rabies is a fatal zoonotic disease with global significance. Its case fatality rate is almost 100% with only one known human survivor [1]. Approximately 55,000 people die from dog-contracted rabies every year [2], mostly in developing countries in Africa and Asia [3–5]. Over 1.74 million disability-adjusted life years (DALYs) are lost and 550 million USD spent on post-exposure prophylaxis annually in these countries [5].

Indonesia is one of the many developing Asian countries still struggling with rabies. On average, Indonesia suffers from 142 human cases yearly [6]. Rabies was first detected in the country in animals in 1884 and humans in 1894 [7]. Currently the disease is endemic in 24 of 33 provinces [6]. At the end of 2008, Indonesia suffered an outbreak that drew international attention. The first ever case of rabies was discovered in Bali, an island famous for its international tourism.

The island of Bali is located at 8^{o} 39’ 0” S and 115^{o} 13’ 0” E, east of Java, one of the main islands in the country. The island has an area of 5.634 km^2 with a total population in 2010 of 3,890,757 people [8, 9]. Tourism plays a central role in the island’s economy; every year over 2 million international tourists visit the island [10]. Main attractions of the island are its tropical climate, white sand beaches, and unique culture. Unlike most of Indonesia, which is dominated by Muslims (87.2%), over 80% of people in Bali practice the Hindu religion [9, 11]. This religious belief is reflected in every aspect of their social and cultural life, including dog ownership and management. A common saying in Bali is “you are not Balinese if you don’t have a dog”. Hence the population density of dogs in Bali is generally believed to be higher than most other regions in Indonesia.

When rabies emerged on the island, the government and public was caught unprepared. Dogs served as the main maintenance population of the disease. Initial cases were limited in the southern tip of the island and the government took immediate action in the form of local mass vaccination and culling of dogs [12]. Containment was unsuccessful. By October 2009 a total of 15 people had died from rabies [13] and in early 2010 the virus had spread to other parts of the island [12]. The abundance of susceptible dogs on the island made control very challenging. A total of 104 human cases were diagnosed as of November 2010 [12].

As the disease became wide-spread, the government focused on island-wide mass vaccination of dogs and improving public awareness. Mass vaccination is the most effective method for controlling rabies [14–17] and domestic dogs represent the majority of dogs in Bali [18]. Regrettably, the local dog population, owned and un-owned, is not well documented. Two phases of mass vaccination were carried out between September 2010 and December 2011 and with each campaign estimates of the dog population was derived (Table 1). A third phase was started in March 2012 [19].

The Balinese government received local and international help in its effort to control rabies. Nationally, the Indonesian government provided vaccines for rabies post-exposure treatment in humans [20]. International aid came from the Australian government, United States government, Food and Agriculture Organization (FAO) and the World Society for the Protection of Animals (WSPA) in the form of program funding, supplies and technical support [20–22]. Locally in Bali, a non-government organization, Bali Animal Welfare Association (BAWA), contributed significantly in the early implementation of mass vaccination of dogs [20].

The Center for Indonesian Veterinary Analytical Studies (CIVAS), a local non-government organization in Indonesia, and the International Livestock Research Institute (ILRI) initiated a project to explore the link between the dog population and local communities in Bali with focus on the impact of this relationship in the spread of rabies. Specific objectives of the project were (1) to understand the extent of rabies prevention and mitigation efforts in Bali, (2) to estimate the dog population in Bali and its dynamics, (3) to understand dog ecology in Bali and measure its contact intensity with other animals and humans, (4) to understand the social cultural relationship between dogs and the Balinese community, and (5) to develop a model for sustainable rabies prevention, control and eradication through community empowerment and behavior change. The project was funded by Canada's International Development Research Center (IDRC) and aimed to support the Balinese government in controlling rabies in dogs. This thesis is focused on the second objective mentioned above.

1.2. Research Objectives

The objective of the study is to (1) characterize the demographics and rabies vaccination of owned and free-roaming dogs and (2) estimate the abundance and identify factors associated with the distribution of the dogs in Bali. There are two subpopulations of dogs in Bali, the owned dog and free-roaming dog. Individuals in each subpopulation overlaps as un-owned dogs co-mingle with free-roaming owned dogs as illustrated in Figure 1 [18].

The proportion of un-owned dogs is estimated to be less than 5% [18], but as nearly all dogs are without identification (e.g., tag/collar), separating un-owned from free-roaming owned dogs is extremely difficult. Due to this constraint, this study is focused on both owned dogs and free-roaming dogs.

Table 1.1. Vaccination report and population estimates of dogs in Bali based on phase I (October 2010-April 2011) and phase II (May 2011-December 2011) island-wide mass vaccination campaigns.

	Phase I [23]			**Phase II** [24]		
District/ Municipality	**Initial Population Estimates ***	**Number of Vaccinated Dogs**	**Estimated Population**	**Initial Population Estimates**	**Number of Vaccinated Dogs**	**Estimated Population**
Buleleng	49,926	27,687	39,578	33,561	28,300	34,512
Karangasem	31,751	17,670	35,920	24,781	20,379	24,689
Tabanan	33,630	20,406	20,582	33,284	28,859	32,794
Badung	43,494	25,459	23,312	50,741	37,739	42,898
Denpasar	63,076	39,161	25,164	46,986	22,244	46,052
Bangli	17,232	22,025	24,411	35,775	31,975	38,605
Jembrana	20,929	19,150	20,970	25,747	21,257	25,923
Gianyar	37,630	48,293	51,443	46,548	36,278	42,176
Klungkung	10,113	-	-	4,890	3,975	4,906
TOTAL	307,783	219,851	241,281	302,043	231,155	292,196

*Based on a dog to human ratio of 1:12.5

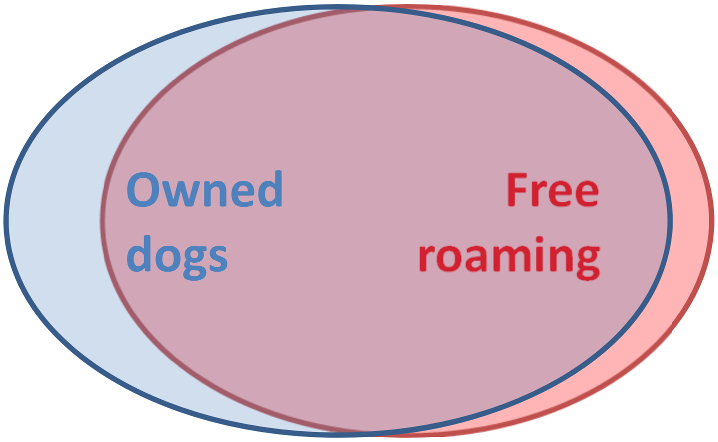

Figure 1.1. Illustration of the composition of the dog population in Bali, Indonesia.

CHAPTER II.

LITERATURE REVIEW

2.1. Epidemiology of Rabies

Rabies is a fatal neurologic disease caused by the rabies virus (RABV). The virus is a non-segmented single strand RNA virus belonging to the genotype 1 Lyssavirus genus within the Rhabdoviridae family [25]. The family consists of 12 virus species recognized by the International Committee on Taxonomy of Viruses since 2011 with an additional two new species yet to be classified [26]. Ten of the 12 recognized species originate from bats, namely the Lagos bat virus, Duvenhage virus, European bat Lyssavirus 1 & 2, Australian bat Lyssavirus, Aravan virus, Khujand virus, Irkut virus, West Caucasian bat virus and Shimoni bat virus. One species, the Mokola virus, was recovered from shrews [27]. The two new members currently proposed, Bokeloh virus and Ikoma virus, were isolated from a bat and an African civet respectively.

There are 7 major lineages of RABV; 6 from non-flying mammals and 1 predominately from bats. Non-flying mammal lineages were named after their geographical distribution, namely Indian subcontinent, Asian, Africa 2, Arctic-related, Africa 3, and Cosmopolitan [28]. The predominate bat lineage is referred to as the American Indigenous [27]. This lineage includes a few skunk and raccoon strains. Four new canine RABV clades have recently been discovered in the Middle East and North Africa [29]. The host range of RABV includes all mammalian species.

Rabies virus is transmitted primarily through the bites of rabid animals. Saliva serves as the main vehicle of transmission. Hence, contamination of any open wound by infectious saliva could cause infection [26, 30]. Infection resulting from inhalation of aerosolized virus has been recorded, but is very unlikely [26]. Iatrogenic infection could also result from the transplantation of organs from RABV infected donors [26, 31]. The incubation period of rabies in dogs ranges from 10 days to several months [32]. In humans the incubation period is generally 20 to 90 days although cases where the disease developed after only a few days or more than a year post exposure have been documented [33].

The rabies virus causes fatal encephalomyelitis in its host. After the virus enters the body, it replicates in surrounding muscle cells and neurons and ascends to the central nervous system through retrograde axonal transport in peripheral nerves [34]. The virus then spreads in the brain and to salivary glands via cranial nerves and is secreted with saliva [34]. In dogs, clinical signs including behavior change (abnormal aggression or reclusion), hypersalivation, swallowing difficulties, and sensory hypersensitization appear as a result of brain dysfunction [32, 34], however the virus can be secreted in the saliva up to 14 days before any clinical sign is apparent [35]. In humans, 80% of rabies cases manifest as the encephalitic form and 20% paralytic form. Encephalitic rabies is commonly associated with hyperexcitability, hydrophobia, hypersalivation, lacrimation, sweating, and dilated pupils [33]. Paralysis and mortality ensues with disease progression. Rabid dogs and cats die within 10 days after symptoms first appear [36]. No carrier status is known [37].

Globally, dogs are the main maintenance population for rabies transmission to humans. According to WHO, over 90% of rabies cases in humans originated from dogs [5, 38]. Only in North America and Europe are wild animals such as raccoons, skunks, foxes, bats, raccoon dogs, jackals, civets, and honey badgers the major animal hosts for rabies [39–43]. To prevent human exposure to rabies, disease control strategies should target animal hosts and maintenance populations [44]. In most cases this entails focusing efforts on the local dog population. In addition to controlling the disease at its source, the cumulative cost of this strategy is significantly lower than relying on human post-exposure treatment alone [45].

2.2. Rabies Control and Eradication Strategies

The most effective method to control rabies in dogs or canine rabies is mass vaccination [14–17]. The aim of vaccination is to develop herd immunity and reduce the basic reproductive number (R_0) of the disease to less than 1 [46, 47]. When R_0 is less than one, the disease will die out. The R_0 of rabies is generally low (<2), hence mass vaccination has a high chance of success as long as a minimum of 20-45% of the population are immune at any given time [15]. To maintain this level of coverage, a higher proportion of the population must be vaccinated to account for failure in vaccination and attrition over time due to population turnover. Factors such as demographics, behavior, and spatial characteristics play an important role in determining the minimum level of herd immunity needed [5, 15, 48]. Additionally, abundance estimation holds a key position in logistical planning of vaccination campaigns and estimation of vaccination coverage. As a general rule, an annual coverage of 70% is recommended [5, 48]. The United States, United Kingdom, Japan, Taiwan, Hong Kong, Singapore, and part of Malaysia have successfully eradicated canine rabies with mass vaccination [49].

A single parenteral injection is the preferred method for vaccinating domestic dogs, but in some cases this could become impractical when animals are evasive or difficult to handle. The vaccine commonly used is a cell-culture inactivated adjuvanted vaccine which could stand alone or be combined with vaccines for other diseases such as canine distemper, canine parvovirus, canine adenovirus type 1 and leptospira for dogs or feline panleukopenia virus, feline calicivirus and feline parvovirus for cats [5]. Researchers are also studying the possibility of incorporating chemical contraceptives with the vaccine to suppress population growth [50].

Oral rabies vaccine is an alternative for difficult to access animals, such as wildlife [5]. It was first developed in the early 1960s to control rabies in red foxes in North America [51, 52]. The first vaccines were made from a Street Alabama Dufferin (SAD) strain of rabies virus. Though effective in foxes, it failed to produce adequate immune responses in raccoons and skunks. A vaccinia-rabies recombinant vaccine (V-RG) was later developed for better efficacy in raccoons and foxes [53]. Four recognized SAD-related vaccines are ERA, SAD-Bern, SAD-B19 and Vnukova-32 [54]. Using these vaccines and a mutant of SAD (SAG2), Western Europe and Estonia successfully controlled and in some countries eliminated fox rabies [55–59]. Similarly, North America also succeeded in controlling rabies in foxes, coyotes and raccoons using oral vaccination [60, 61]. Skunks, however, still remain a problem. Recently, a human adenovirus-rabies glycoprotein recombinant vaccine was commercially produced with claims of better efficacy in raccoons and skunks [62]. Field testing in Canada and the US, however, has yet to demonstrate a significant increase in immunity in skunks [63, 64]. Some alternative vaccines researched for raccoons and skunks are canine adenovirus recombination [65] and genetically modified viruses [66].

Feral, stray and free-roaming owned dogs can be as difficult to access as wild animals. In Bali, the government still used parenteral vaccination and employed dog catchers to overcome this issue at the cost of time and manpower. However, oral vaccination is becoming a potential option. Several studies have looked into the application of oral vaccines in dogs. Classic oral vaccines had less promising results. SAG2 required 180 days to seroconvert all dogs [67]. In a recent study in India, total seroconversion ≥0.5 IU/ml was not achieved in 109 days, but all SAG2 vaccinated dogs survived a rabies challenge test [68]. In the Philippines, a field trial with SAD vaccine had identical issues of dogs failing or slow to seroconvert [69]. On the contrary, recombinant vaccines seem to perform quite well [70]. In a study using recombinant rabies-canine adenovirus type 2 vaccines, 90% of vaccinated dogs developed titers ≥0.5 IU/ml within 6 weeks and over 90% of those dogs maintained it for more than 24 months [71]. One issue with the CAV-based recombinant rabies vaccine, however, is possible inhibition from CAV antibodies in dog populations where CAV2 is prevalent [72].

2.3. Domestic Dog Ecology and Rabies

In situations where canine rabies is the main problem, better understanding of the local dog ecology is essential for effective disease control [14, 73–76]. Many rabies endemic countries, such as the Philippines [77], India [74, 78], Sri Lanka [79], Kenya [80], Madagascar [81], Mexico [82], and Tanzania [83] have conducted ecology studies on dogs for this very purpose. Ownership status and confinement properties are two characteristics commonly used to define dog population types. Dividing the population based on ownership status is most popular.

Numerous studies on dog populations are focused on owned dogs since data can be conveniently collected from owners and in most cases owned dogs represent the majority of the domestic dog population [77, 79–89]. Un-owned dogs, conversely, are difficult to access and hence rarely studied. In Antananarivo, Madagascar, a group of researchers interviewed people regarding every dog seen free-roaming to gather information on the ownership status of the dog [81].

Based on the confinement property, domestic dogs could be divided into confined and free-roaming populations. The confined population consists entirely of owned dogs, whereas free-roaming populations can be divided into 3 types: the free-ranging population of owned dogs, strays and feral dogs [90, 91]. Strays may be defined as recently lost owned dogs or abandoned-ownerless dogs that still receive food and shelter from humans (community dog) and feral dogs are dogs completely de-socialized to humans [90, 91]. Although common characteristics exist, each population is unique depending on resources made available and treatment given by humans [92, 93]. Therefore, social cultural acceptance and responsibility can significantly influence the population size and accessibility of dogs in a community.

Numerous countries with canine rabies find free roaming dogs to be a problem [94]. Many of these dogs are in fact owned dogs allowed to roam freely by their owners [76, 77, 79–81, 83–88, 95]. In a region where the population is mostly free-roaming, metapopulation size of dogs is an important predictor for rabies transmission probability. Metapopulations are spatially separated groups of individuals from the same species with limited interaction between groups [96]. In Tanzania, metapopulations of less than 150 dogs in a village were found to have low probabilities of acquiring rabies from external sources [97]. This knowledge could be utilized to identify high risk areas and focus resources, if needed, but caution must be practiced in establishing the meta-populations. For example, it is reasonable to consider dogs living in far apart rural villages as separate metapopulations, however such a clear cut definition might not be plausible in an urban setting. This once again reinforces the need to better understand the characteristics and abundance of the local dogs.

2.4. Abundance Estimation

As previously elaborated, dog abundance is an important parameter for logistical planning, evaluation of vaccination campaigns and the estimation of risk of rabies transmission between dog metapopulations. There are three measures of dog abundance: (1) the total number of animals, (2) the number of animals per unit area (absolute density) and (3) the density of animals relative to humans (relative density) [90]. Absolute density is preferable when comparing area and habitat between populations and relative density is recommended when there is a strong relationship between the number of humans and dogs [90]. The total number of animals is less useful unless it is presented together with information on the area or scope. Most literature describes absolute and relative density as measures of abundance. Dog to human ratios have been recorded to average between 1:4 and 1:10, however there was high variability even between urban and rural areas in the same region [75, 77, 81–88, 93, 98]. Dog density was also found to greatly vary from as low as 1 dog/km^2 to over 13,000 dogs/km^2 [79, 80, 88, 95, 98].

WHO recommends using questionnaires and mark-recapture/re-sight studies to estimate dog population abundance [5]. Questionnaires are feasible when residents can identify all dogs within the community. The mark-recapture method was developed primarily to estimate abundance in wildlife, but is applicable for free-roaming domestic dogs [99]. Distance sampling, another method designed for wildlife, is also an option for abundance estimation [95].

A key component in abundance estimation of animals is modeling the detection probability [100]. In most count surveys, observers will not be able to detect all animals in the area. Unless there is certainty that detection is perfect, utilizing only the count data will result in an underestimation of abundance. Methods like mark recapture and distance sampling aim to model the detection probability and use it to acquire the most efficient and reliable estimate of population abundance [100–102].

The basic concept of mark recapture is to use previous animal capture or sighting to estimate the overall capture/detection probability of animals [100–102]. This method requires at least two surveys and individual marking or identification of animals. Mark integrity and identification is an important component of mark recapture [103]. One of the very first mark recapture studies on free-roaming dogs was conducted in Baltimore, USA, using photographs to identify individuals (i.e., mark) [99]. This approach was also utilized by studies in Nepal, Japan, and most recently India [74, 98]. Paint marking and vaccination collars/spray paint from previous vaccination campaigns can be used to mark dogs [78, 95]. At its simplest, mark recapture assumes the same capture/detection probability for all animals. Realistically, the probability of capturing or detecting an animal can vary by time, behavior response and heterogeneity in the population such as age, sex, social status or home range [100–102].

In distance sampling, detection probability is modeled as a function of distance [100]. The further away an animal is from the observer, the lower the detection probability becomes. Fundamental assumptions of distance sampling are detection is perfect on the transect line or point, animals are observed at their original location, distances are measured correctly and the placement of transects are random in relation to the animals [95, 100, 103]. Childs et al. reviewed distance sampling for density estimation of dogs in the Philippines [95]. Compared to mark-recapture, distance sampling is logistically less demanding because it only requires one visit without the need to identify animals [95]. The placement of transect lines, however, needs to be random with respect to the population studied. This will be difficult with private properties.

CHAPTER III.

MATERIALS AND METHODS

3.1. Sampling Design

The study was conducted on two dog subpopulations, owned and free-roaming dogs, in 310 banjars in Denpasar city, Gianyar district, and Karangasem district in Bali, Indonesia. Banjar is a subvillage structure in Bali which could serve as a cultural structure, governmental structure or both for its people. The banjar is considered more important than the village in that it is the center of day to day activity and regulation in the community [104]. Hence biologically, variation in banjars is likely more influential on domestic dogs than at village level. The type of banjar aimed in this study is the government banjar; cultural banjars are unfeasible because sometimes there is geographical overlap. The sampling design was two-stage sampling. The primary sampling unit was villages and the secondary sampling unit was banjars. A village is typically made of 4 to 12 government banjars.

In the first stage, villages were stratified by district/city and randomly selected. Denpasar city was selected to represent urban areas, Gianyar district to represent suburban areas and Karangasem to represent rural areas in Bali. The initial primary sample size was 36 villages proportionally allocated to each district/city. Denpasar, Gianyar and Karangasem have 43, 70, and 78 villages respectively, and were allocated 8, 13, and 15 villages for sampling. The Livestock Service Office of Karangasem District requested the addition of one village into the project which resulted in the final primary sample size to be 37 villages.

Sampling in the second stage differed between owned and free-roaming dogs. All banjars in selected villages were included for the owned dog survey; meanwhile only 4 banjars per village were included in the free-roaming dog survey. The free-roaming dog survey initially encompassed all banjars in the village; however it was later reduced due to large village sizes and time constraints. Banjars involved in the free-roaming survey were selected through simple random sampling.

3.2. Data Collection

Data were collected between March 2011 and March 2012. Survey of owned dogs was carried out through door to door interview of owners. Photographic mark recapture was used to collect data on free-roaming dogs. A field team of three pre-trained veterinarians were given six days in each village to complete data collection. The team was constantly escorted by local banjar officials during the process.

3.2.1. Door to Door Survey

A census of dogs owned in the banjar was collected through their owners. Every dog-owning household identified by the local banjar official was surveyed by the field team. A representative of the household was interviewed based on a form (Appendix 1). Data collected included sex, age group, coat color, vaccination status, and confinement status of every dog owned by the household. Banjar officials are banjar members selected by the community to handle day to day businesses regarding the banjar. Due to the strong social relationship within the community, officials have personal knowledge of every member of the banjar.

3.2.2. Photographic Mark Recapture

The photographic mark recapture (PMR) method requires the collection of capture (mark/first sight) and recapture (re-sight) data of individual dogs within the study area. Unique identification of dogs relied on photographs and individual pelage patterns. None of the dogs were physically captured. Transect lines following main roads and public pathways were surveyed in each sampled banjar for 4 consecutive days. Preliminary survey suggested dogs were most active in the morning and afternoon; therefore survey times were alternated between 1600 to 1830 hours on day 1 and 3 and 0630 to 1000 hours on day 2 and 4. Observers travelled at a maximum speed of 20 kilometers per hour on transect lines using a motorcycle. All dogs seen free-roaming within a 25 meter radius of the line were photographed for individual identification. Free-roaming was defined as not being confined within an enclosure or attached to a device which restricts movement, e.g., leash or rope. The first time an individual dog was observed was defined as the initial sighting (capture) event. The second, third, or fourth time the dog was observed were considered as resighting (i.e. recapture) events. Data on coat color, age group, sex, location, presence of vaccination collar and special characteristics such as injury marks were collected from photographed dogs and used to identify individual animals (Appendix 2). Vaccination collars, which were given to dogs during government mass-vaccination campaigns, were used to infer the vaccination status of free-roaming dogs. Only dogs with collars were assumed to be vaccinated.

3.2.3. Banjar Variables

Data on human population and binary information on the presence of a market, bus terminal, public temple, school, beach, rice paddies, plantation, and forest in banjars were collected as possible predictor variables of owned dog and free-roaming dog populations. Data on the presence of dog culling activities three months prior to the study was also collected to assess possible effects of recent culling on dog population size and demographics. Additionally, information on public facilities and occupations, which was only available at the village level, was collected for post-stratification of banjars into urbanization categories.

3.3. Data Analysis

3.3.1. Banjar Post-Stratification

The main unit of sampling and analysis is banjar, however post-stratification of the urbanization category was carried out at village level due to limitations in available data. Villages were post-stratified into urban, suburban and rural stratums or hereafter referred to as the urbanization variable based on occupational and public facility records from each village. Urban villages mainly have non-agricultural occupations and more public facilities, such as markets, public transportation facilities, schools, and government offices, while rural villages have agriculture as the main occupational activity and limited public facilities [105]. Accordingly, 11 villages were post-stratified into the urban stratum, six villages in the suburban stratum and 20 villages in the rural stratum. Stratum classification was carried over to related banjars. The banjar urbanization variable is included in subsequent analysis as a confounder or possible effect modifier.

3.3.2. Demography Analysis

Owned dog data from door to door survey and individual free-roaming dog data collected from photographic mark recapture in banjars were included in the demography analysis. Variables of interest were sex, age group and, additionally for owned dogs, confinement status. Data were stratified by urbanization and most variables were presented as proportions with 95% confidence intervals. For sex, a male to female ratio was presented instead. Contingency tables between categories were explored.

Multiple logistic regression was used to separately model the likelihood of a dog being a female (sex), juvenile (age) and confined (confinement status) when applicable for both owned and observed free-roaming dogs. Predictor variables included in the modeling process were age, confinement status and recent culling for the sex model; recent culling for the age model; and age and recent culling for the confinement model. Urbanization was included as a confounder in all the models. A predictor variable was confounded if the adjusted odds ratio differed more than 10% from the crude odds ratio [106]. Model building was based on purposive selection described by Hosmer et al [107]. Univariate regressions were run for each covariate to screen for possible effects. Variables with p-values <0.25 were simultaneously included in the model. Left out covariates were individually added to the model to test for effects and covariates in the model with p-values >0.05 were subjected to possible removal. Following each model update, left out variables were again individually added to test for change of effect. After the best additive model was determined, interaction terms between variables retained were tested into the model. Excluding the first step in this method, variables or terms were added one at a time, starting from those with the greatest effect. Dropping variables or terms was also conducted one at a time, starting with those least important. Model selection was based on Akaike's Information Criteria (AIC). A delta AIC of greater than 2 was set as the threshold for adding or retaining terms. After the model was finalized, contrast statements were created to obtain odds ratios. Data manipulation and modeling were conducted in program R [108]. Contrasts and odds ratios were computed with R package ‘multcomp’ [109]. Three dogs with missing sex values were excluded from all sex-related analysis.

3.3.3. Vaccination Coverage

Rabies vaccination coverage was computed from all owned and observed free-roaming dog data. The vaccination status of owned dogs was based on owner reports, while the status in observed free-roaming dogs was inferred from the presence of a vaccination collar. Coverage was presented as a proportion with 95% confidence intervals, stratified by urbanization and cross tabulated with demographic categories sex, age group and confinement status. Multiple logistic regression with purposive selection was also used to model the likelihood of a dog not being vaccinated [107]. AIC was used to select between models. Variables included were urbanization, age, sex, recent culling, and confinement status. Contrast statements were created and odds ratios computed using R package ‘multcomp’ [109]. Data manipulation and modeling were conducted with program R [108].

3.3.4. Population Abundance

3.3.4.1. Owned dog population

The door to door survey collected data on owned dogs from 310 banjars. Seven of these banjars elected not to have dogs and were excluded from the analysis. The response variable was the number of owned dogs within each banjar. A multiple linear regression model was fitted to the response variable with human population size, urbanization category and the presence of a market, bus terminal, school, beach, rice paddies, plantation and forest included as predictor variables. Dog count and human population had lognormal distributions and were therefore logtransformed. Residuals were normally distributed and homoscedastic. Purposeful selection with AIC was used to select the model [107]. All analyses were conducted with program R [108].

3.3.4.2. Free-roaming dog population

General modeling strategy. Sight-resight data on free-roaming dogs were analyzed using program MARK version 7.1 [110]. The Huggins closed capture model was selected for the analysis because it was appropriate for the data type and supports the use of predictor variables [111, 112]. This model estimates the detection probability of animals from which population abundance could be derived. Four villages were excluded from the analysis due to missing banjar level data. The sight-resight data consisted of individually identified dogs (n =1491) encountered over four occasions (day) in 122 groups (i.e., banjars). The sighting history for each dog was written as 4 consecutive binary codes, each representing an occasion. Sight or resight of a dog was coded 1 and no sighting of the dog during an occasion was coded 0. For example, the encounter history of the first dog in the dataset was 1000, which means the dog was sighted on the first occasion, but never sighted again in subsequent occasions. Modeling efforts were focused on variation by time and two individual dog variables, sex and age. Additionally, banjar level variables, namely human population, urbanization, recent culling and the presence of a market, bus terminal, school, beach, rice paddies, plantation, and forest within the banjar were included.

Detection probability. Detection probability is the probability of an animal to be detected at a given time within the study area. The closed captures model provides two parameters for detection, p is the probability of unmarked animals to be first detected and c is the probability of identified animals to be detected again. Estimates of p would be modeled different from c if behavioral response was suspected, i.e. animals becoming more or less likely to be subsequently detected after first detection. However, behavioral responses are unlikely in this study and therefore p was set equal to c in all occasions. Detection probability was also assumed equal across banjars, i.e. unique detection probabilities for each banjar was not modeled, and possible variation in dog detection associated with banjars was modeled through banjar variables instead. Models were constructed in which detection could vary by time, age, sex, urbanization, and presence or absence of a market, bus terminal, school, beach, rice paddies, plantation and forest. Detection is hypothesized to be influenced by individual and environmental variables hence the selection of these variables. Parameters in the model were estimated using maximum likelihood with a logit link function. All possible combinations containing up to 5 variables were run, resulting in 1096 models. The multi-model inference approach was taken to select the best model based on Akaike's Information Criteria with small sample size correction (AICc). AICc cumulative weights were computed for each variable using the entire model set [113]. The optimal predictive model for detection was constructed of variables with weights of 0.5 or greater as recommended by Barbieri and Berger (2004) [114].

Abundance estimation. Abundance estimates of free-roaming dogs in banjars were derived from the optimal predictive detection model. Following Huggins (1991) notation, if captured individuals are $i = 1, \dots, n$, the vector of parameters in the model is β and the probability of animal i being captured at least once is $p_i(\beta)$, then the estimate of abundance ($\widehat{N}(\beta)$) is [111]:

$$\widehat{N}(\beta) = \sum_{i=1}^{n} p_i(\beta)^{-1}$$

Because abundance is a product of the estimated detection probability, it too has standard errors associated with each estimate. In order to account for the uncertainty in the estimates and properly model the true process variation in free-roaming dog abundance in banjars, a variance components approach was taken [115]. The total variance of the estimates of abundance is a combination of biological process variance and sampling variance. The variance components analysis allows the modeling of the biological process variance by separating it from the sampling variance [116]. Variables hypothesized to be associated with the number of free-roaming dogs in a banjar, namely human population, urbanization, recent culling, total number of owned dogs or free-roaming owned dogs, and presence of a market, bus terminal, school, beach, rice paddies, plantation and forest were included in the model as predictor variables. Process variance from regression models with single and multiple variables were compared with the intercept only model to identify which variables were important predictors. Variance components analysis of the derived parameter results in a linear regression of the derived parameter.

3.3.4.3. Total population estimates

The total population of dogs in banjars can be estimated from the count of owned dogs and abundance estimates of free-roaming dogs. However, assumptions need to be made because the ownership status of free-roaming dogs could not be determined. An article published by the Disease Investigation Center Denpasar, Bali, in 2011 estimated only 5% or less of dogs were unowned [18]. Therefore, scenarios where 0% to 50% of free-roaming dogs in banjars were assumed to be unowned were run. The number of unowned dogs was summed with the number of owned dogs in banjar to obtain estimates of the total population. The estimates were then compared to the 0% scenario to assess the influence of unowned dog proportions to the total population size. A total of 122 banjars in which owned and free-roaming dog data was available were included in the analysis.

3.3.4.3. The human to dog ratio

The human to dog ratio is an index commonly used to estimate the number of domestic dogs in a population. Overall, mean and median human to dog ratios have been reported however there is no known assessment of accuracy [2, 74, 76, 80–82, 84–88, 98]. The overall human to dog ratio is essentially the ratio estimator; it was calculated as the ratio of mean human population and mean dog population. Meanwhile, the mean and median human to dog ratios in this study was calculated as the average or median value of all human to dog ratios for each sampling unit (banjar). Owned dog counts and human population from 310 banjars were used to evaluate the accuracy of the human to dog ratios. The different measures of ratios were calculated from a sample of banjars and used to estimate the total population of owned dogs. The proportion of bias was computed by dividing the difference between the estimate and the known total population with the total population. The effect of stratification and sample size was also assessed by comparing biases from general and stratum-specific human to dog ratios and altering the sample size. Stratums used in this assessment were urban and non-urban. Suburban and rural stratums were considered similar and combined into the non-urban stratum. The sampling of banjars was simulated 1000 times for each measure of human to dog ratio. Differences between mean biases were tested with two-sample t-tests and differences between variances were tested with an F-test.

CHAPTER IV.

RESULTS

4.1. Demography

In the Door to Door survey we visited a total of 310 banjars, 10,352 dog-owning households, and collected data on 17,376 owned dogs. On average, dog-owning households had 1.68 dogs (SE 0.009). In seven banjars none of the households owned any dog. In the Photographic Mark Recapture survey, we made 2,597 observations of free-roaming dogs and identified 1,972 individuals. No dog was observed free-roaming in eight banjars. Over 70% of owned dogs were male and more than 80% were adults. In free-roaming dogs, more than 75% of observed dogs were male and over 95% were adults. Thirty nine banjars in four villages experienced dog culling (n=650) within the last three months. Twenty three of the banjars were in the urban stratum and 16 were in the rural stratum.

4.1.1. Sex Distribution

In all urbanization stratums, more males were owned and observed free-roaming than female dogs (Table 4.1). The male to female ratio in owned dogs is 1.7:1 to 3.2:1 and in observed free-roaming dogs 2.4:1 to 4.6:1. Sex ratios in both populations were lower in the urban stratum than in suburban and rural stratums, indicating a higher proportion of female dogs in urban areas. The crude and adjusted odds ratio of owning or observing a female dog is shown in Table 4.2 and 4.3 for owned dogs. In free-roaming dogs, urbanization was the only significant predictor variable, therefore only the crude odds ratio is presented (Table 4.5). In owned dogs, urbanization was found to be a confounder for the effect of confinement and culling on the odds of owning a female. Additionally, the effect of age and recent culling on the likelihood of having a female dog depended on level of confinement status.

As indicated by the sex ratio, urban owners were more likely to own female dogs than suburban (OR 1.30, 95%CI: 1.17-1.44) and rural owners (OR 1.67, 95%CI: 1.52-1.83). Similarly the odds of observing a female free-roaming dog was higher in the urban stratum compared to the suburban (OR 1.79, 95%CI: 1.30-2.45) and rural stratum (OR 1.95, 95%CI: 1.55-2.47) (Table 4.3).

In owned dogs, the male to female ratio was lower in juveniles (1.6:1) than adults (2.6:1), which translates to higher odds of owners having a female dog in juveniles than adults. Among confined dogs, the odds ratio of was 1.29 (95% CI: 1.13-1.48) and among free-roaming owned dogs the odds ratio was 1.74 (1.56-1.94). Owners in recently culled banjars were also more likely to own free-roaming females than non-culled banjars (OR 1.43, 95% CI 1.23-1.65). In confined dogs, there was no difference between recently culled and non-culled banjars (OR 1.05, 95% CI 0.93-1.19).

In observed free-roaming dogs, sex ratios by age or recent culling were not statistically different. It is worth to note only a small number of juveniles were observed in free-roaming dogs. The overall male to female ratio in adult dogs was 3.3:1 and in juveniles 4.4:1.

4.1.2. Age Distribution

The majority of dogs were reported or observed to be ≥1 year old (Table 4.1 & 4.4). About 83.5% of owned dogs and 96.6% of observed free-roaming dogs were ≥1 year old. Between the two populations, there were higher proportions of adults in observed free-roaming dogs than there was in owned dogs. The likelihood of owning or observing a juvenile dog compared to an adult is presented as crude (Table 4.2) and adjusted odds ratios (Table 4.3) for owned dogs and only crude odds ratio (Table 4.5) for observed free-roaming dogs.

In owned dogs, the odds of owning a juvenile was different by urbanization and recent culling, however there was no confounding. Odds were higher in the urban (OR 1.43, 95%CI: 1.27-1.62) and rural stratum (OR 1.37, 95%CI: 1.22-1.55) than in the suburban stratum (Table 4.2). In recently culled banjars, people were 30% more likely to own juveniles than in non-culled banjars (OR 1.34, 95% CI: 1.20-1.50). The proportion of juveniles was 5% higher in recently culled banjars (Table 4.12). In observed free-roaming dogs, the odds of observing a juvenile was similar for all.

4.1.3. Confinement of Owned Dogs

There was a marked difference in confinement practices reported for dogs in the urban stratum versus dogs in the suburban and rural stratum (Table 4.1). The proportion of free-roaming owned dogs reported was low in the urban stratum (<30%) and high in suburban and rural stratums (80-90%). Over 70% of owners in urban areas claimed to have restrained their dogs, while only 15% and 8% of dogs in suburban and rural areas reported restrain. Hypothetically, this suggests that owned dogs in suburban and rural areas would have a higher influence on the free-roaming dog population than in urban areas.

The crude and adjusted odds ratio of a dog being confined is shown in Table 4.2 and 4.3 respectively. The effect of recent culling on the confinement of dogs was confounded and interacted with urbanization. The likelihood of confinement in urban banjars was three times higher in banjars with recent culling as opposed to those without (OR 2.97, 95% CI: 2.58-3.43).

The opposite association was observed in rural banjars where owners in recently culled banjars were more likely to allow their dogs to roam (OR 0.42, 95% CI: 0.28-0.62). However, confinement of owned dogs in rural banjars were already very low to begin with (<10%, Table 4.1 and 4.12). Overall, juveniles were 35% more likely to be confined than adults (OR 1.35, 95%CI: 1.21-1.50).

4.2. Vaccination coverage

The average vaccination coverage was 83.6% in owned dogs and 30.9% in observed free-roaming dogs. Rabies vaccination reported in owned dogs was high (>70%) in the majority of groups except in juveniles (15% to 60%; Table 4.6), meanwhile vaccination coverage in observed free-roaming dogs was overall low (Table 4.9). Coverage in observed free-roaming dogs was 20% to 40%. The crude and adjusted odds ratio of not being vaccinated is shown in Table 4.7 and 4.8 for owned dogs and Table 4.10 and 4.11 for observed free-roaming dogs respectively.

In owned dogs, the effect of recent culling and confinement status on the odds of vaccination was confounded by urbanization. The effect of age and confinement status was also modified by urbanization. In adults, being in urban (OR 0.47, 95% CI: 0.39-0.56) and suburban banjars (OR 0.53, 95% CI: 0.45-0.63) reduces the risk of not being vaccinated by half compared to dogs in rural banjars. In juveniles, the risk of not being vaccinated was 10 to 70 times the risk of adults in urban, suburban and rural banjars (OR 9.85-71.06, 95% CI: 8.58-96.04). Female dogs had 36% higher chance of not being vaccinated than males (OR 1.36, 95% CI: 1.23-1.50) and free-roaming dogs were more likely to not be vaccinated than confined dogs. This was particularly large in urban (OR 2.98, 95% CI: 2.45-3.61) and suburban stratum (OR 3.60, 95% CI: 2.38-

5.42), and to a lesser magnitude in the rural stratum (OR 1.85, 95% CI: 1.44-2.38). In terms of recent culling, coverage in owned dogs was >70% in both recently culled and non-culled banjars (Table 4.12). However, owned dogs in culled banjars were 40% more likely to not be vaccinated than those in non-culled banjars (OR 1.40, 95% CI 1.22-1.61).

In observed free-roaming dogs, the effect of culling on the odds of being vaccinated was confounded and interacted with urbanization. Free-roaming dogs in recently culled urban banjars were 50% less likely to not be vaccinated than non-culled banjars (OR 0.43, 95% CI: 0.32-0.59), while in rural banjars there was no significant difference (OR 1.18, 95% CI: 0.46-3.02). Juveniles were 4 times more likely to not be vaccinated than adults (OR 3.76, 95% CI: 1.69-8.35; Table 4.8) and female dogs had higher odds if not being vaccinated than males (OR 1.56, 95% CI: 1.23-2.00).

4.3. Population Abundance

4.3.1. Owned Dog Population

The number of owned dogs in surveyed banjars (n=310) is summarized in Figure 4.1. The mean number of dogs was 56.0 (SD 46.9). The distribution was right-skewed with most banjars owning less than 100 dogs. Univariate linear regression of predictor variables on owned dog population in banjars indicated human population as the strongest predictor (R^2=0.26), while other variables explained very little variance (Table 4.12). Multiple linear regression with purposive selection concluded human population, recent culling and the presence of a forest to be significant predictors of the owned dog population (R^2=0.28, Table 4.13). Human population and the presence of a forest were positively associated with the number of owned dogs, while recent culling was negatively associated with the owned dog population in banjars. Because both the owned dog population and human population required log-transformation, the relationship is non-linear on the normal scale. Although the presence of a forest and recent culling were significantly associated with changes in the dog population size, observation of the R^2 values from both univariate and multiple linear regression shows that human population was the strongest predictor in the model.

4.3.2. Free-Roaming Dog Population

4.3.2.1. Detection Probabilities

The top 10 ranking models for detection were a combination of time (occasion), sex and presence or absence of rice paddies, school, forest, market, and bus terminal (Table 4.14). Cumulative AICc weights for each variable based on the complete model set are shown in Table 4.16. Time and sex had cumulative weights greater than 0.5, therefore the optimal predictive model for detection probability was {Intercept + time + sex} [114].

The average detection probability estimated by the model was 0.19 and process standard error 0.024 (Appendix 5). Based on these results, approximately $(1-0.19)^4 = 0.43$ (43%) of dogs were never encountered during the study. Plotting sex-specific detection probabilities by time, there was a downward trend in the probabilities (Figure 4.2). Detection was lowest at the fourth occasion in both sexes. Female dogs had higher probabilities of being detected than males although not statistically significant.

4.3.2.2. Abundance Estimation

A histogram of the estimated abundance of free-roaming dogs in banjars (n=122) is shown in Figure 4.3. The mean number of dogs per banjar was 19.8 (SD 13.6). Similar to owned dogs, the distribution was right-skewed. The majority of banjars had an estimated abundance of less than 40 free-roaming dogs.

Through variance components analysis, the abundance of free-roaming dogs could be modeled using predictor variables (Table 4.17). From univariate linear regressions, the number of owned dogs was found to be the strongest predictor variable, explaining 51% of variation. Substituting the total number of owned dogs with the number of free-roaming owned dogs did not perform as well. Univariate regression with the variable rice paddies explains 9% of variation. Recent culling and human population accounted for less than 5% of the variation in free-roaming dogs. In the end, the number of owned dogs, the presence of rice paddies and the interaction between the number of owned dogs and rice paddies was found to be the best model for estimated abundance of free-roaming dogs in banjars. This model explained 61% of variation in the abundance estimates. Model coefficients are shown in Table 4.18. The number of owned dogs was positively associated with the abundance of free-roaming dogs. However, the effect of rice paddies was dependent on the size of the owned dog population in the banjar.

4.3.3. Total Population

Of the 120 banjars included in this analysis, the total number of owned dogs was 7,467 dogs and estimated free-roaming dogs was 2,417 dogs. The combined human population of all the banjars was 108,570 people. Banjars were comprised of 26 urban banjars, 24 suburban banjars, and 70 rural banjars. Based on the 0% to 50% scenarios of free-roaming dogs being unowned, a 1% increase in free-roaming dogs assumed to be unowned contributes to an average 0.32% inflation of the total dog population (Figure 4.4). In the extreme case where 50% of free-roaming dogs were assumed to be unowned, the total population would be inflated by 16.24%.

4.3.4. The Human to Dog Ratio

In assessing the accuracy of the overall, mean and median human to dog ratios, a sample size of 50 banjars was used for the simulation. It was found that the mean ratio had a severe positive bias (34.63%), meanwhile the overall and median human to dog ratio on average had slight negative (-3.84%) and positive biases (3.67%), respectively (Table 4.19, Figure 4.5). The overall and median human to dog ratio was significantly less biased than the mean ratio (Table 4.20). The overall ratio was more precise than the median (Table 4.21), however standard deviations of both ratios were still large ($SD_{overall}$=10.63, SD_{median}=13.68). To be conservative, a positive bias (overestimation) is preferred; hence the median ratio was used in subsequent assessments of general and stratum-specific ratios and sample size (Figure 4.6).

Simulations for the general and stratum specific ratios were conducted with equal sample sizes to obtain a fair comparison. Sample sizes simulated for the general human to dog ratio was 60, 100 and 150 banjars. Accordingly, sample sizes for the stratum-specific ratio were 30, 50 and 75 banjars per stratum. Both measures of ratio had a mean bias of less than 5% for all sample sizes (Table 4.19). The general ratio was less biased in estimating the population than the stratum specific ratio in most cases (Table 4.20). Increasing the sample size reduced the bias variance for both measures. The stratum-specific ratio had smaller bias variance when total sample size was small (n=60), however was not statistically different from the general ratio at larger sample sizes.

Table 4.1. Demographics of reported owned dogs in 310 banjars by urbanization category.

Variable	Urban	Suburban	Rural
Number of Dogs (n)	6605	3501	7270
Sex (%, 95% CI)			
Overall	1.7:1	2.6:1	3.2:1
Juvenile	1.2:1	1.7:1	2.1:1
Adult	1.8:1	2.8:1	3.5:1
Restrained	1.6:1	2.2:1	3.2:1
Free-roaming	2.0:1	2.7:1	3.2:1
Age (%, 95% CI)			
Juvenile, < 1 year old	18.3 (17.4, 19.2)	12.5 (11.4, 13.6)	16.9 (16.0, 17.8)
Adult, ≥1 year old	81.7 (80.8, 82.6)	87.5 (86.4, 88.6)	83.1 (82.2, 84.0)
Confinement status (%, 95% CI)			
Confined	71.2 (70.1, 72.3)	16.1 (14.9, 17.3)	8.0 (7.4, 8.6)
Free-roaming	28.8 (27.7, 30.0)	83.9 (82.7, 85.1)	92.0 (91.4, 92.6)

Table 4.2. Odds ratios for sex, age group and confinement status in owned dogs by univariate logistic regression. Outcomes were sex=female, age group=juvenile and confinement status=confined.

Univariate analysis	Crude OR	95% Confidence Interval	
		Lower	Upper
Sex=female			
Urbanization (suburban vs rural*)	1.23	1.13	1.35
Urbanization (urban vs rural*)	1.88	1.75	2.02
Age (juvenile vs adult*)	1.58	1.45	1.72
Confinement (confined vs free-roam*)	1.62	1.51	1.73
Recent culling vs no culling*	1.48	1.35	1.61
Age group=juvenile			
Urbanization (suburban vs rural*)	0.71	0.63	0.79
Urbanization (urban vs rural*)	1.11	1.02	1.21
Recent culling vs no culling*	1.46	1.32	1.63
Confinement status=confined			
Urbanization (suburban vs rural*)	2.21	1.96	2.51
Urbanization (urban vs rural*)	28.65	25.92	31.67
Age (juvenile vs adult*)	1.35	1.25	1.47
Recent culling vs no culling*	4.23	3.87	4.61

*reference level

Table 4.3. Odds ratios for sex, age group and confinement status in owned dogs by multiple logistic regression. Regression models were fit to outcomes sex=female, age group=juvenile and confinement status=confined (Appendix 3).

Contrast Statement	OR	95% Confidence Interval	
		Lower	Upper
Sex=female			
Suburban vs Rural*	1.29	1.17	1.41
Urban vs Rural*	1.67	1.52	1.83
Urban vs Suburban*	1.30	1.17	1.44
Juvenile vs adult* if confined	1.29	1.13	1.48
Juvenile vs adult* if free-roaming	1.74	1.56	1.94
In culled vs non-culled* banjars if confined	1.05	0.93	1.19
In culled vs non-culled* banjars if free-roaming	1.43	1.23	1.65
Age group=juvenile			
Rural vs Suburban*	1.37	1.22	1.55
Urban vs Rural*	1.05	0.96	1.15
Urban vs Suburban*	1.44	1.27	1.63
Culled vs non-culled banjars*	1.34	1.20	1.50
Confinement status=confined			
Juvenile vs adult*	1.35	1.21	1.50
Culled vs non-culled* banjars in Urban	2.97	2.58	3.43
Culled vs non-culled* banjars in Rural	0.42	0.28	0.62

*reference level

Table 4.4. Demographics of free-roaming dogs observed in 4 villages and 122 banjars by urbanization category.

Variable	Urban	Suburban	Rural
Number of Dogs (n)	840	313	819
Sex (%, 95% CI)			
Overall	2.4:1	4.2:1	4.6:1
Juvenile	3.4:1	7.0:1	4.4:1
Adult	2.3:1	4.1:1	4.6:1
Age group (%, 95% CI)			
Juvenile, < 1 year old	3.0 (1.8, 4.2)	5.1 (2.7, 7.6)	3.3 (2.1, 4.5)
Adult, ≥1 year old	97.0 (95.8, 98.2)	94.9 (92.5, 97.3)	96.7 (95.5, 97.9)

Table 4.5. Odds ratios for sex and age group in observed free-roaming dogs by univariate logistic regression. Outcomes were sex=female and age group=juvenile.

Univariate analysis	Crude OR	95% Confidence Interval	
		Lower	Upper
Sex=female			
Urbanization (suburban vs rural*)	1.09	0.78	1.53
Urbanization (urban vs rural*)	1.95	1.55	2.46
Age (juvenile vs adult*)	0.75	0.40	1.41
Recent culling vs no culling*	1.74	1.30	2.32
Age group=juvenile			
Urbanization (suburban vs rural*)	1.58	0.84	2.97
Urbanization (urban vs rural*)	0.79	0.45	1.40
Recent culling vs no culling*	0.95	0.45	2.03

*reference level

Table 4.6. Breakdown of vaccination coverage in owned dogs by urbanization category and demographic variables.

Vaccination coverage	Urban	Suburban	Rural
Total (%, 95% CI)	88.8 (88.0, 89.6)	83.7 (82.5, 84.9)	78.7 (77.8, 79.6)
Sex (%, 95% CI)			
Male	90.3 (89.4, 91.2)	86.0 (84.6, 87.3)	80.6 (79.6, 81.7)
Female	86.3 (84.9, 87.7)	77.9 (75.3, 80.5)	72.7 (70.6, 74.8)
Age group (%, 95% CI)			
Juvenile, < 1 year old	57.5 (54.7, 60.3)	18.0 (14.4, 21.6)	39.8 (37.1, 42.6)
Adult, ≥1 year old	95.8 (95.3, 96.3)	93.1 (92.2, 94.0)	86.6 (85.7, 87.5)
Confinement Status (%, 95% CI)			
Restrained	90.6 (89.7, 91.4)	89.9 (87.4, 92.4)	83.0 (80.0, 86.1)
Free-roaming	84.4 (82.8, 86.1)	82.6 (81.2, 84.0)	78.4 (77.4, 79.3)

*reference level

Table 4.7. Odds ratios of not being vaccinated against rabies in owned dogs by univariate logistic regression.

Univariate analysis	Crude OR	95% Confidence Interval	
		Lower	Upper
Urbanization (suburban vs rural*)	0.72	0.65	0.80
Urbanization (urban vs rural*)	0.47	0.42	0.51
Sex (female vs male*)	1.40	1.29	1.53
Age (juvenile vs adult*)	13.59	12.38	14.93
Confinement (confined vs free-roam*)	0.47	0.43	0.52
Recent culling vs no culling	1.08	0.97	1.21

*reference level

Table 4.8. Odds ratios of not being vaccinated against rabies in owned dogs by multiple logistic regression (Appendix 5).

Contrast Statement	OR	95% Confidence Interval	
		Lower	Upper
Female vs male*	1.36	1.23	1.50
Culled vs non–culled* banjars	1.40	1.22	1.61
Juveniles vs adults* in Urban	19.47	16.17	23.44
Juveniles vs adults* in Suburban	71.06	52.58	96.04
Juveniles vs adults* in Rural	9.85	8.58	11.31
Adult dogs in Urban vs Rural*	0.47	0.39	0.56
Adult dogs in Suburban vs Rural*	0.53	0.45	0.63
Adult dogs in Urban vs Suburban*	0.88	0.71	1.10
Juveniles in Urban vs Rural*	0.93	0.74	1.16
Juveniles in Suburban vs Rural*	3.85	2.85	5.20
Juveniles in Urban vs Suburban*	0.24	0.17	0.34
Free-roaming vs confined* in Urban	2.98	2.45	3.61
Free-roaming vs confined* in Suburban	3.60	2.38	5.42
Free-roaming vs confined* in Rural	1.85	1.44	2.38

*reference level

Table 4.9. Breakdown of vaccination coverage in observed free-roaming dogs by urbanization category and demographic variables.

Vaccination coverage	Urban	Suburban	Rural
Total (%, 95% CI)	37.5 (34.2, 40.8)	21.4 (16.8, 26.0)	27.8 (24.7, 30.9)
Sex (%, 95% CI)			
Male	39.5 (35.5, 43.4)	22.9 (17.7, 28.1)	29.9 (26.4, 33.3)
Female	33.3 (27.5, 39.2)	15.0 (5.9, 24.1)	18.5 (12.2, 24.8)
Age group (%, 95% CI)			
Juvenile, < 1 year old	20.0 (4.0, 36.0)	0 (0,0)	7.4 (0, 17.5)
Adult, ≥1 year old	38.0 (34.7, 41.3)	22.6 (17.8, 27.4)	28.5 (25.4, 31.7)

Table 4.10. Odds ratios of not being vaccinated against rabies in observed free-roaming dogs by univariate logistic regression.

Univariate analysis	Crude OR	95% Confidence Interval	
		Lower	Upper
Urbanization (suburban vs rural*)	1.42	1.04	1.93
Urbanization (urban vs rural*)	0.64	0.52	0.79
Sex (female vs male*)	1.36	1.07	1.71
Age (juvenile vs adult*)	3.84	1.74	8.46
Recent culling vs no culling*	0.42	0.32	0.54

*reference level

Table 4.11. Odds ratios of not being vaccinated against rabies in observed free roaming dogs by multiple logistic regression (Appendix 5).

Contrast Statement	OR	95% Confidence Interval Lower	 Upper
Juvenile vs adult*	3.76	1.69	8.35
Female vs male*	1.56	1.23	2.00
Culled vs non-culled* Urban banjars	0.43	0.32	0.59
Culled vs non-culled* Rural banjars	1.18	0.46	3.02

*reference level

Table 4.12. Demographics and vaccination coverage in owned and observed free-roaming dogs by urbanization and the occurrence of recent culling (within 3 months prior to survey).

	Urban		Rural	
	No culling	Recently culled	No culling	Recently culled
Number of dogs				
Owned dogs	4769	1836	6577	693
Observed free-roaming dogs	613	227	794	25
Sex ratio (male:female)				
Owned dogs	1.8:1	1.5:1	3.3:1	2.3:1
Observed free-roaming dogs	2.9:1	1.9:1	4.6:1	5.3:1
Juvenile, <1 year old (%, 95% CI)				
Owned dogs	17.2 (16.2, 19.3)	21.2 (19.3, 23.1)	16.3 (15.4, 17.2)	22.1 (19.0, 25.2)
Observed free-roaming dogs	3.1 (1.7, 4.5)	2.6 (0.6, 4.7)	3.1 (1.9, 4.4)	8 (0.0, 19.4)
Confinement of owned dogs (%, 95% CI)				
Confined	65.8 (64.5,67.2)	85.2 (83.6,86.9)	8.4 (7.7, 9.1)	3.8 (2.3, 5.2)
Vaccination coverage (%, 95% CI)				
Owned dogs	89.5 (88.6, 90.3)	87.1 (85.6, 88.6)	79.6 (78.6, 80.6)	70.1 (67.3, 74.1)
Observed free-roaming dogs	32.3 (28.6, 36.0)	51.5 (45.0, 58.1)	28.0 (24.8, 31.1)	24.0 (6.0, 42.0)

Table 4.13. Univariate linear regressions of predictor variables on owned dog population in banjars. The response variable is log(dog population) (n=303).

Variables	R^2	Estimate	Std. Error	Pr(>|z|)
Log(human population)	0.26	0.74	0.07	<0.0001
Urbanization				
Suburban	0.05	0.14	0.13	0.281
Urban		0.45	0.11	<0.0001
Market	0.002	-0.13	0.17	0.449
Bus terminal	0.003	0.86	0.87	0.326
Temple	0.0007	-0.05	0.10	0.649
School	0.02	0.23	0.10	0.026
Beach	<0.0001	0.05	0.44	0.905
Rice paddies	0.0007	0.05	0.11	0.652
Plantation	0.003	-0.10	0.11	0.351
Forest	0.004	0.19	0.16	0.259
Recent culling	0.003	-0.13	0.15	0.382

Table 4.14. Coefficient estimates of the best linear regression model for owned dog population in banjars (R^2=0.28). Response variable is log(dog population) (n=303).

Parameter	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	-1.47	0.48	-3.067	0.002
Log(human population)	0.79	0.073	10.889	<0.0001
Recent culling	-0.41	0.13	-3.113	0.002
Forest	0.28	0.14	2.019	0.044

Table 4.15. Top 10 models for detection probability of free-roaming dogs (n=1096).

Model	AICc	Δ AICc	AICc Weights
Rice paddies + time + sex + school	6593.16	0	0.053
Rice paddies + time + sex	6593.26	0.100	0.050
Rice paddies + forest + time + sex	6594.18	1.015	0.032
Rice paddies + time + sex + market	6594.35	1.194	0.029
Time + sex + market	6594.45	1.294	0.028
Time + sex + school	6594.50	1.344	0.027
Time + sex	6594.59	1.429	0.026
Rice paddies + time + age + sex	6594.60	1.444	0.026
Time + sex + market + school	6594.84	1.679	0.023
Rice paddies + time + sex + bus terminal	6595.01	1.8508	0.021

Table 4.16. Variable cumulative AICc weights based on detection probability model set.

Variable	Cumulative weights
Time	1.000
Sex	0.820
Rice paddies	0.380
School	0.257
Market	0.221
Forest	0.164
Age	0.150
Bus terminal	0.134
Beach	0.124
Plantation	0.112
Temple	0.108
Human population	0.107
Urbanization	0.069

Table 4.17. Variance components analysis of abundance estimates of free-roaming dogs in banjars compared to the intercept only model (σ^2=179.75).

Predictor variables	Process variance	95% Confidence Interval		% variation explained
		LCL	UCL	
Owned dog + rice paddies + owned dog*rice paddies	69.97	49.70	100.11	61.07
Owned dog + rice paddies + recent culling	76.96	54.91	109.69	57.18
Owned dog + rice paddies + human population	77.96	55.72	110.96	56.63
Owned dog + rice paddies	78.02	55.81	110.92	56.60
Owned dog	87.47	62.80	123.93	51.34
Free-roaming owned dog	96.41	69.29	136.55	46.36
Rice paddies	163.84	120.04	228.36	8.85
Recent culling	170.62	124.89	237.99	5.08
Human population	171.42	125.63	238.88	4.63
Forest	177.89	130.43	247.80	1.03
School	178.17	130.66	248.14	0.88
Plantation	180.55	132.46	251.38	0.00
Bus terminal	181.01	132.78	252.04	-0.45
Market	181.10	132.89	252.09	-0.70
Temple	181.34	133.07	252.43	-0.75
Beach	181.58	133.22	252.79	-0.89
Urbanization	181.90	133.35	253.50	-1.02

Table 4.18. Coefficient estimates of the best linear multiple regression model for abundance estimates of free-roaming dogs in banjars (Appendix 9).

Parameter	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	8.17	1.78	4.60	<0.0001
Owned dog	0.12	0.026	4.83	<0.0001
Rice paddies	-0.99	2.71	-0.37	1.2849
Owned dog*rice paddies	0.12	0.04	3.30	<0.0001

Table 4.19. Mean and standard deviation of population estimation biases (%) from different measures of human to dog ratio (1000 simulations each).

Measures of Human to Dog Ratios	Mean Bias (%)	Standard Deviation
Overall ratio n=50	-3.84	10.63
Mean ratio n=50	34.63	10.94
Median ratio n=50	3.67	13.68
General median ratio n=60	3.25	12.52
General median ratio n=100	3.47	8.83
General median ratio n=150	3.70	6.68
Stratum-specific median ratio n=30/stratum	4.93	11.32
Stratum-specific median ratio n=50/stratum	4.90	8.51
Stratum-specific median ratio n=75/stratum	3.97	6.40

Table 4.20. Results of two sample t-tests comparing the mean of population estimation biases from different measures of human to dog ratio.

Comparison of Mean		p-value
Overall ratio n=50	Mean ratio n=50	<0.0001
Overall ratio n=50	Median ratio n=50	<0.0001
Mean ratio n=50	Median ratio n=50	<0.0001
General median ratio n=60	Stratum-specific median ratio n=30/stratum	0.0016
General median ratio n=100	Stratum-specific median ratio n=50/stratum	0.0002
General median ratio n=150	Stratum-specific median ratio n=75/stratum	0.3465

Table 4.21. Results of F-test comparing the variance of population estimation biases from different measures of human to dog ratio.

Comparison of Variance		p-value
Overall ratio n=50	Mean ratio n=50	0.3695
Overall ratio n=50	Median ratio n=50	<0.0001
Mean ratio n=50	Median ratio n=50	<0.0001
General median ratio n=60	Stratum-specific median ratio n=30/stratum	0.0014
General median ratio n=100	Stratum-specific median ratio n=50/stratum	0.2425
General median ratio n=150	Stratum-specific median ratio n=75/stratum	0.1788

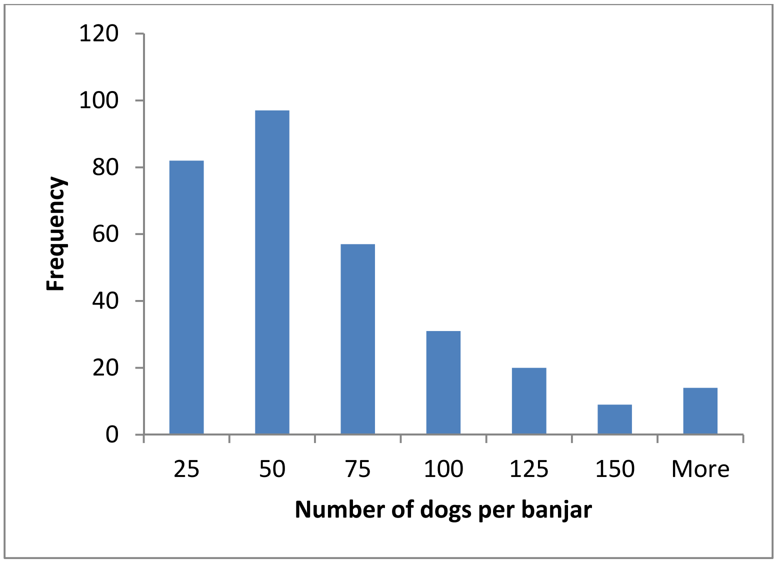

Figure 4.1. Histogram of the owned dog population size in banjars (n=310).

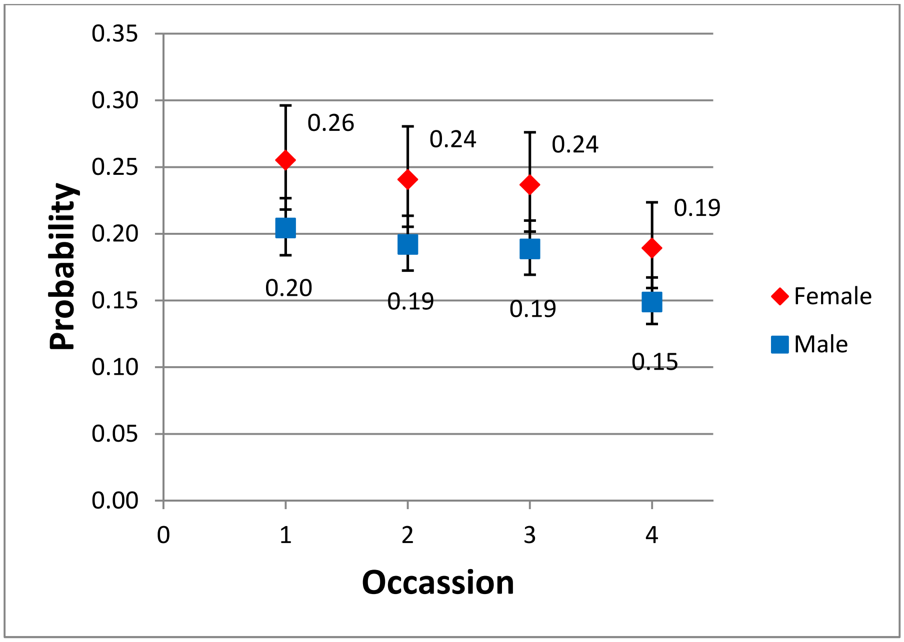

Figure 4.2. Detection probabilities of free-roaming dogs by time and sex based on best predictor model {Intercept+time+sex}.

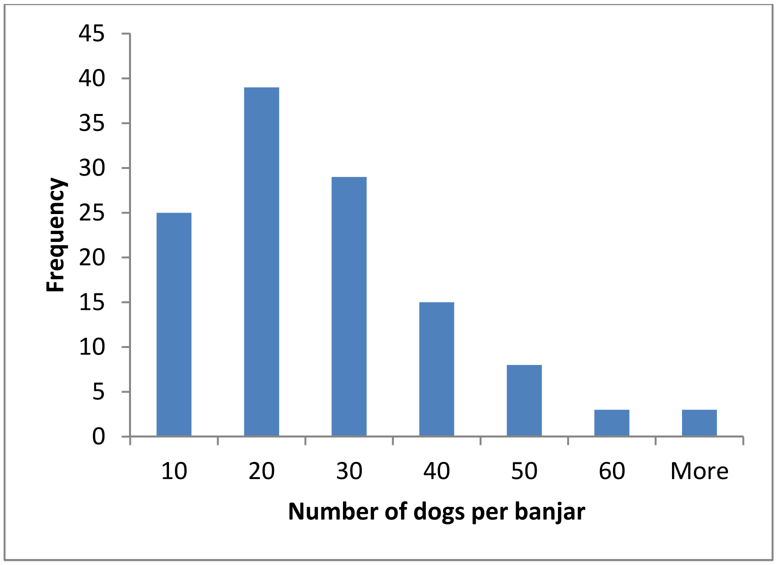

Figure 4.3. Histogram of estimated abundance of free-roaming dogs in banjars (n=122)

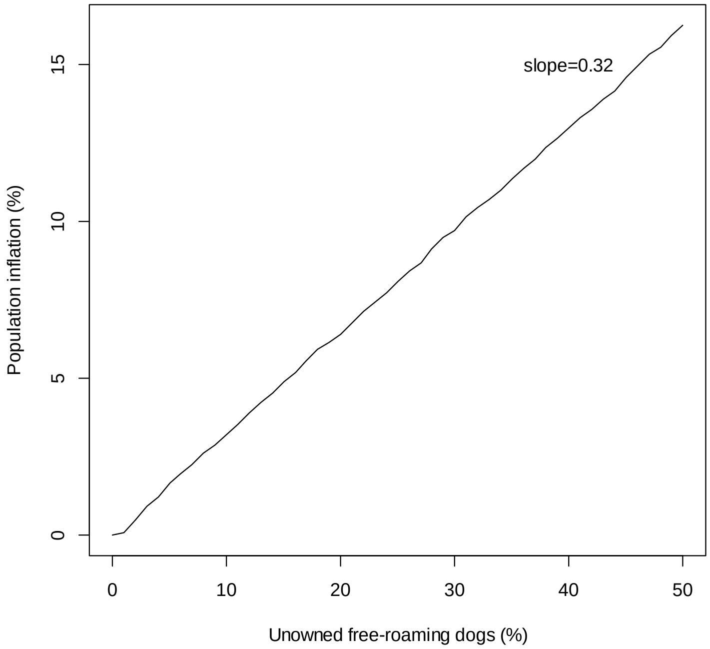

Figure 4.4. Inflation of total dog population by proportion of estimated free-roaming dogs assumed to be unowned (n=122 banjars).

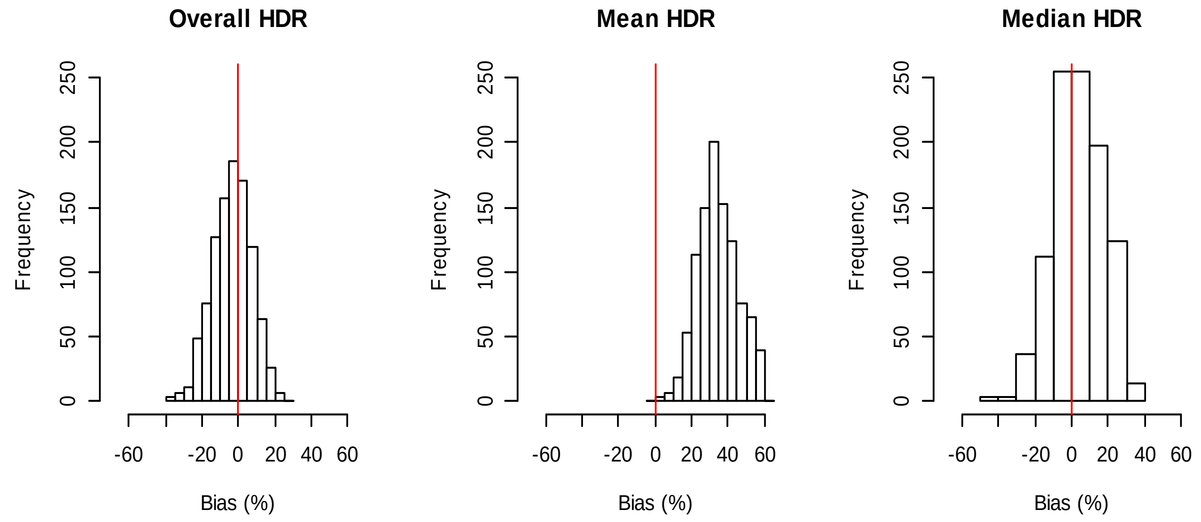

Figure 4.5. Sampling distributions of the bias in population estimation from the overall (left), mean (center) and median (right) human to dog ratio with a sample size of 50 banjars and 1000 iterations.

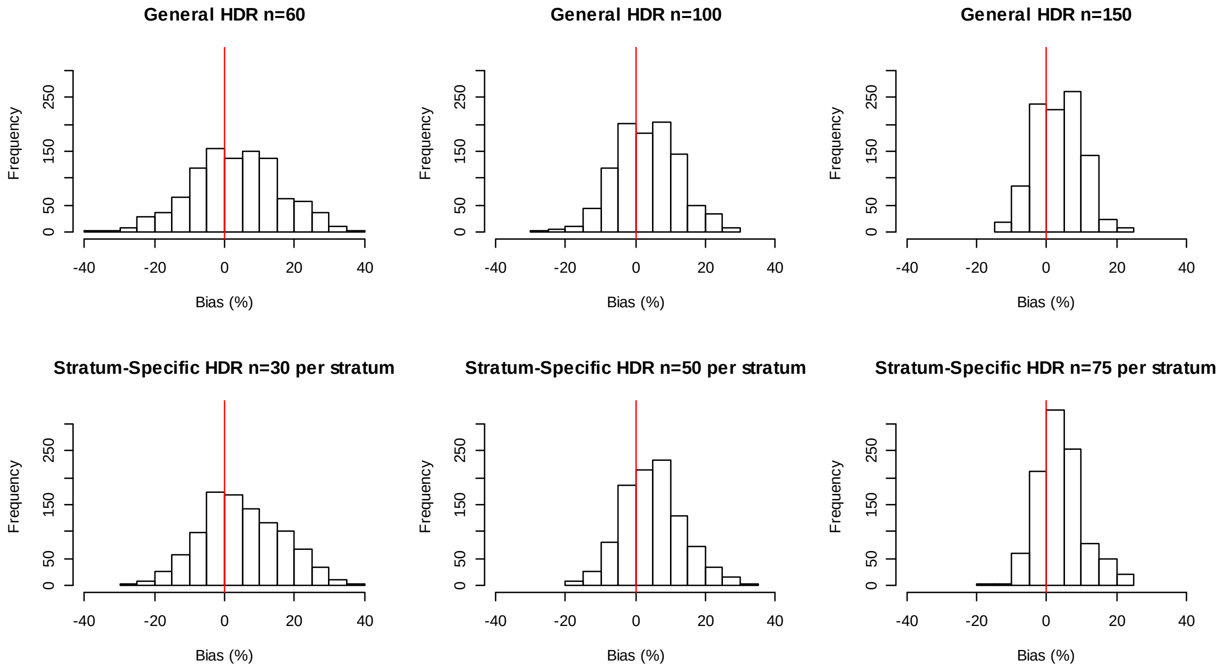

Figure 4.6. Sampling distributions of the bias in population estimation from the general (top row) and stratum-specific (bottom row) human to dog ratio with *n* number of samples and 1000 iterations. Stratums in the stratum specific human to dog ratio was urban and non-urban.

CHAPTER IV.

DISCUSSION

The Balinese identify dog ownership as part of their culture. Dogs roaming freely in and around the neighborhood were a common sight before rabies and it still is in most parts of the island. Determining the ownership status of the dogs is difficult, however the Disease Investigation Center in Denpasar estimated over 95% of dogs are owned [18]. People mainly keep dogs as guards, as do many dog-owners in other parts of the world, and male dogss are commonly preferred for this function [76, 80, 81, 84, 85, 88]. In Hindu, a religion practiced by the majority of Balinese, dogs are respected animals. Additionally, the Balinese also believe dogs can sense evil spirits and warn their owners.

The introduction of rabies on the Island in 2008 has forced changes in some communities. In the last couple of years, there was movement in some banjars to enact traditional community laws (*perarem*) which introduces stringent regulations on dog ownership or even to the extreme of not owning dogs in the banjar. Seven banjars (2%) in this study were found to not own dogs. Two banjars were Islamic communities; hence the lack of dog ownership was expected. However, the other five banjars were Balinese communities. Although in-depth interview on members of the banjar was not conducted, it is valid to suspect this absence of dog ownership was a result of banjar ruling influenced by the spread of rabies on the island. Because these were special cases where the absence of dogs was likely enforced upon the community, the banjars were excluded from most analysis aimed at modeling the dog population. Six of the banjars were also involved in the free-roaming dog survey and only one banjar was completely dog free.

4.1. Demography

The majority of owned dogs reported in this study were free-roaming, largely in suburban and rural areas. Owners had a strong preference towards males which is a common in communities where dogs are allowed to roam [78, 81, 82, 85, 88]. Male bias was stronger in adults compared to juveniles. Females were 30-75% more likely in juveniles than adults, indicating fewer females were owned as dogs mature. In rural Chile and Kenya, females have lower life expectancy than males, indicating a similar situation [80, 88]. Higher mortality and abandonment of female dogs are likely drivers of this discrepancy. Males could be more supported than females resulting in different survival rates [80]. In Madagascar, the male to female ratio in unowned dogs was found to be equal [81].

The difference between owned dogs in urban and non-urban stratums was interesting. Unlike non-urban stratums, dogs in urban areas were three times more likely to be confined or leashed. Urban owners were also 30-70% more likely to own female dogs. Government activity and outreach is strong in urban areas and a common message communicated is for owners to keep their dogs confined or leashed. This could contribute to the high proportion of dog confinement. Nonetheless reporting bias could not be ruled out. Owners could have lied, due to fear or convenience, or unintentionally misinformed interviewers because of miscommunication or lack of knowledge. There is a growing notion that dogs were confined only during the day and allowed to free roam at night. A random response survey could be implemented to reduce the risk of reporting bias, however questions need to be specific to avoid misunderstanding [117].

Observations of free-roaming dogs were dominated by adults and males. The proportions were greater than in owned dogs. Although part of observed dogs were potentially unowned, separation from free-roaming owned dogs was impossible. From the data, there is strong indication that free-roaming behavior in dogs is largely influenced by age and sex. Observations were dominated by males despite females having slightly higher detection probabilities. Similarly, very few juveniles were observed even though the detection probability of dogs was not affected by age.

4.2. Rabies vaccination

The overall vaccination coverage reported in owned dogs was very high, particularly in adults (85-95%). In juveniles, vaccination was variable and in some cases very low (15-60%). In free-roaming dogs, coverage was very low overall (20-40%) and in the few juveniles observed, it was 5% or less. Juveniles in both observed free-roaming and owned dogs were 4 to 70 times more likely of not being vaccinated compared to adults. Recent birth, growth into the adult group (≥ 1 year old) and perceived ineligibility during vaccination campaign due to young age could exacerbate the low coverage in juveniles. Studies in Mexico and Bolivia also found that young dogs were at higher risk of not being vaccinated [82, 87]. Additionally, in Mexico, the risk of not being vaccinated was higher in neighborhood dogs compared to family owned dogs [82]. In this study, juveniles only represent 15-20% of the dog population, however in some countries with very high turnover, half or more of the population were dogs less than 1 years old [76, 80, 84]. Unvaccinated young dogs are as susceptible to rabies as unvaccinated adults, therefore vaccine administration to dogs of all ages is recommended by WHO [5]. Female dogs in this study were also 40-60% more likely of not being vaccinated compared to males. Hence this is another part of the dog population in Bali which needs better outreach. In Kenya, school children were recognized to play an important role in dissemination information on rabies and bringing dogs for vaccination [80].

The disparity between vaccination coverage in owned and observed free-roaming dogs is concerning. Bias in the form of owners misreporting their dogs as vaccinated could not be ruled out. This direction of bias is more likely to occur than owners misreporting their dogs as unvaccinated and could result from intentional misreporting or due to lack of knowledge. Consequently, the vaccination coverage in owned dogs could be overestimated On the other hand, vaccination coverage in observed free-roaming dogs could have been underestimated due to misclassification bias. The use of collars to infer the vaccination status of free-roaming dogs was the only non-invasive means to do so. However, some vaccinated dogs could have lost their collar or were given temporary marking during vaccination campaigns and were misclassified as non-vaccinated. Spray paint was used to mark dangerously aggressive dogs for post-campaign evaluation. The paint disappears after two weeks and future identification is impossible. Collars from the first campaign were notorious for falling off, making future identification also impossible. Collars in ensuing campaigns were said to be improved. A two marker system could have been used to estimate the rate of collar loss, which is likely to increase with time as damage accumulates [90].

The government did report high coverage (>70%) from the first and second vaccination campaign, in which a majority of the dogs targeted were owned [20]. Since this study was conducted before and during the implementation of the second vaccination campaign, vaccination coverage is expected to be lower than what is reported from the first campaign due to population attrition. To our knowledge, none of the banjars surveyed were recently vaccinated. Hence, coverage in owned dogs, particularly adults, was likely misreported as it was very high. Additionally, a number of observed free-roaming dogs were likely misclassified into the non-vaccinated group due to lost collars and marks. Overestimation of coverage in owned dogs and underestimation of coverage in free-roaming dogs would inflate the apparent difference in vaccination coverage between the two subpopulations. Misreporting and misclassification of the vaccination status is likely unrelated to the demographics of the dogs, therefore it is non-differential misclassification. Generally, non-differential misclassification of a binary outcome biases the odds ratio towards the null [118].

High turnover rate in the population will cause faster attrition of vaccination coverage. Demographic data from the first vaccination campaign in Badung district in late 2008 shows that 30% of vaccinated dogs were less than 1 year and 50% were between 1 and 3 years old [18]. In this study, approximately 15-20% of the population was less than 1 year old (juvenile), suggesting the overall turnover rate was not too high. In order for mass vaccination to succeed in eliminating rabies, a minimum coverage of 20-45% must always be maintained [15]. Recent analysis of rabies in Bali suggests the basic reproductive number (R_0) is 1.2; therefore unless population turnover is >70%, an annual coverage of ≥70% during vaccination campaigns is sufficient to eliminate rabies from the island [119].

Oral vaccination is a promising alternative to achieve high coverage in dogs. Recent developments of recombinant vaccines seem to perform quite effectively in dogs [70]. Delivery is easy and protection is equivalent to parenteral vaccines. Demonstrating coverage, however, could be quite challenging. With conventional vaccination using parenteral injection, vaccinated animals are marked during handling and coverage is visually assessed immediately after for each banjar. Oral vaccination, however, does not give visual cues and rely on testing for seroconversion to determine coverage. Extensive laboratory capacity would be needed to achieve the same level of evaluation from oral vaccination and the laboratory capacity in Bali might not be sufficient to achieve this on a regular basis.

From a social standpoint, conventional vaccination might be a better tool in developing public awareness and responsibility as owners are typically asked to hold and handle their dogs. Additionally, the presence of collars and visual marking provides assurance to the public that dogs in a certain area have been vaccinated. This is locally and internationally important as Bali's economy relies on domestic and international tourism. Ideally there should be a time when owners would responsibly and voluntarily vaccinate their dogs against rabies; however it is not likely to happen in the near future. When parenteral injection is no longer feasible for achieving island-wide coverage, then oral vaccination might be the only option.

4.3 Recent culling

Recent culling activities were reported in 13% of banjars in this study. The government has officially stopped culling programs in 2010, however community-driven culling was suspected to still occur [20]; therefore it is likely that all culling activities observed in this study were community driven. Although culling might seem like a quick solution, there has been no record of success in eliminating rabies. The case in Flores Island, Indonesia, is one example. Approximately 70% of dogs in the initial case district was culled after rabies emerged in late 1997 and it failed to eliminate the disease [120]. In most cases, culling is counterproductive because it increases the rate of population turnover and drives people to hide or move potentially infected dogs to other areas in an attempt to save them [20, 120]. In Bali, it is suspected that people from the initial outbreak area moved their dogs to other parts of the island in fear of culling programs, which resulted in sporadic spread of the disease in the first year of the outbreak [20]. Data from this study show owners in recently culled banjars were 30% more likely to own juveniles. Knowing that juveniles have a higher risk of not being vaccinated, this could translate to more susceptible animals in the population. The higher likelihood of owned dogs in culled banjars to not be vaccinated indicates that such hypothesis might be true. Based on the abundance analysis in this study, recent culling was associated with fewer numbers of owned dogs, but was insignificant towards the number of free-roaming dogs in a banjars.

Vaccination coverage in owned dogs was >70% regardless of urbanization stratum and cull status. However, dogs in recently culled banjars had 40% higher risk of not being vaccinated. Increased proportion of juveniles and a false sense of security induced by the act of culling could have contributed to this increase in risk. In free-roaming dogs, the opposite trend was observed. Dogs observed free-roaming in recently culled banjars were twice more likely to have vaccination collars than free-roaming dogs in non-culled banjars. This is indeed a concerning phenomenon where vaccination coverage seems to be higher in recently culled banjars, as perceived from the apparent number of dogs seen with collars, while owned dogs on the contrary were less likely to be vaccinated compared to those in non-culled banjars.

4.4. Estimating the dog population

The dog population in Bali was difficult to estimate because a majority of dogs were free-roaming and separation of owned from unowned dogs was unfeasible. A method such as inquiring members of the population about every dog seen outside (free-roaming) could be done, however bias was still unavoidable [81]. The study in Madagascar concluded 11% of dogs inquired were unowned [81], while in Zimbabwe and Tanzania feral dogs were rare and estimated to be less than 1% [76, 84]. In Chile, unowned dogs were suspected to be more prevalent in urban than rural areas [88]. Eventually, the proportion of unowned dogs is area-specific depending on the culture [76] and carrying capacity of the environment. In Bali, unowned dogs were estimated to be less than 5% of the population [18]. Based on this estimation, several assumptions were made on the proportion of free-roaming dogs assumed to be unowned.

Detection probability is a large yet often overlooked problem when studying dogs that are free-roaming. In this study, only 20% of free-roaming dogs were seen at any given time. Considering the survey was conducted when dogs were assumed to be most active, the probability of detecting dogs at any other time would likely be lower. Therefore, attempts to estimate the population based only on raw counts would severely underestimate the number of dogs. This issue of detection is also important for the evaluation of vaccination campaigns. What is the capture probability of the vaccination team in a given banjar? Door to door approaches will likely capture most home-based dogs, however actively free-roaming dogs might be vaccinated at a lower rate. In this study, free-roaming owned dogs were found to have 2 to 4 times higher risks of not being vaccinated than confined dogs.

Human population was the strongest predictor of the number of owned dogs in banjars. Without taking into account other variables, the human population in banjars explains 26% of variation in the number of owned dogs through a positive relationship. In the multivariate model, the presence of a forest was also associated with larger populations of owned dogs, while recent culling was associated with less numbers of owned dogs. It is interesting to find that banjars with a forest area would have more dogs than banjars without, given everything else was held constant. There might be an unknown association between the presence of a forest and the carrying capacity of the environment or forest might be a surrogate for an unknown variable, for example human behavior related to dog ownership. Ten percent of banjars had forests and almost all were in the rural stratum. Recent culling was negatively associated with the number of owned dogs, which might indicate that some owned dogs were culled. It could also be an indication of a recent rabies incident in the area which led to the subsequent culling of dogs in the community.

In free-roaming dogs, the population of owned dogs was the strongest predictor, explaining 50% of the variation in free-roaming dog population in banjars. This was expected considering owned dogs were estimated to represent over 95% of the population. However, the number of roaming owned dogs was less predictive than the total which might suggest there was reporting bias in dog confinement status. In the multivariate model, the presence of rice paddies was a significant predictor and its effect depends on the level of owned dog population. Thirty-two percent of banjars in the study had rice paddies. Again, there might be an unknown association between the presence of rice paddies and the capacity of the banjar in supporting free-roaming dogs or rice paddies might be a surrogate for an unknown variable related to the abundance of free-roaming dogs. The number of free-roaming dogs in banjars, however, was not significantly affected by the recent culling. The variance components analysis conducted in Program MARK version 7.1, however, was only capable of fitting a linear model to the non-transformed estimates of free-roaming dogs in banjars, which is log-normal in distribution. Therefore, the linear model assumption is likely violated. Although the owned dog population as a major predictor is not expected to change, the presence of rice paddies as a predictor variable might be subject to further scrutiny.

There were some limitations related to the use of the closed captures model to estimate free-roaming dog abundance. First of all, the model assumes demographic and geographic closure [101]. Although there is high confidence of demographic closure due to short intervals between transects in each banjar (daily), geographic closure might be violated, particularly in urban banjars. The boundary of a banjar is a human concept which has no meaning to a dog. In suburban and rural areas where banjars are more spaced out, the movement of dogs between banjars is less likely. However, in urban areas where banjars are practically next to each other, there is higher chance that a surveyed banjar could be visited by dogs from neighboring banjars. Such behavior will increase the count of dogs observed in the banjar. Another assumption of the model is correct recording of the sight-resight history [101], which in the case of this study relies on correct identification of individual dogs. If a dog was misidentified and counted as two or more different individuals, then this will also inflate the count of dogs observed in the banjar. In an attempt to minimize misidentification in this study, at least 3 different photos were taken for every observed dog and identification conducted on the day of the survey. Potential violations described above will result in overestimation of the number of free-roaming dogs in a banjar.

Scenarios were tested to determine how much the assumed percentage of unowned free-roaming dogs would likely affect the total population of dogs in banjars. From the simulation, a 1% increase in free-roaming dogs assumed to be unowned only inflates the total population by 0.32%. Based on the article published by the Disease Investigation Center in Denpasar [18] and more conservative estimates, it would be reasonable to assume that 5-20% of free-roaming dogs were likely to be unowned. Even in that case, inflation of the total population is expected to be less than 7%. Hence, it can be concluded that unowned dogs have a very limited effect on the total population of dogs in Bali and the majority of dogs are likely to be owned.

The human to dog ratio is commonly used to describe and estimate the size of dog populations in many studies [121]. The majority provides estimates of overall human to dog ratio [76, 80–85, 87, 98] and one reported the median ratio [74]. One study separated human to dog ratios between cities and rural communities [86] and another by wards in Tanzania [76]. A single estimate of the overall human to dog ratio is accurate for the study area, however extrapolation should be conducted carefully. This study is unique in that human to dog ratios can be calculated for all the units. This allows for the evaluation of different measures of human to dog ratio and its accuracy in estimating the total population.

With the data available from this study, there were 310 sampling units (banjars) with high variability in human to dog ratios between units. The mean human to dog ratio performed poorly as it was sensitive to extreme values. The overall and median human to dog ratio were equally less biased, with the overall ratio slightly underestimating the total population and the median ratio slightly overestimating the total population on average. Variances in the bias were quite large in all ratios. For rabies control programs, taking a conservative approach by slightly overestimating the total population is preferred over underestimation. It is better that more effort is put into control programs and more animals vaccinated than less. In studies with limited areas and few sampling units, the median ratio might not be feasible and the overall ratio would be the only option. For estimating the population of dogs in a region through extrapolation, representative sampling is advised and median human to dog ratio recommended for conservativeness.

The general human to dog ratio and stratum-specific ratio both performed equally in terms of bias and precision. However, the general ratio was simulated by simple random sampling which is often not feasible in field settings. Stratified sampling might be a more common approach. Urbanization is the mostly used variable for stratification [121]. Sampling units from which human to dog ratios are obtained should be small enough to minimize the variance within the unit, but large enough to obtain a meaningful ratio. An appropriate unit for Bali might be a banjar or village since significant environmental factors affecting the dog population such as rice paddies or forests would generally be uniform in these units.

Conclusively, the human to dog ratio is a crude tool for estimating the total population in the case of Bali. The overall or median ratio obtained from a representative sample would provide a good estimate of the total dog population on average, however the range of bias is wide, indicating a concerning lack in precision. This is a problem because sampling is typically only conducted once. As a form of ratio estimator, the precision of the human to dog ratio depends on the strength of correlation between the human and dog population [122]. In previous analysis, the human population in banjars explained less than 30% of variation in the number of owned dogs in banjars. This likely explains the lack of precision. In the end, this index is not a reliable tool for estimating the total dog population in Bali; however it might be the only tool available. In other regions or countries where there is strong correlation between the human and dog population, the human to dog ratio might be a more reliable tool.

CHAPTER V.

CONCLUSION AND FUTURE DIRECTIONS

5.1. Conclusion

Having knowledge of the dog population is important for rabies control programs. Understanding their demographics and identifying factors associated with their abundance and distribution can help control efforts become more effective and efficient.

Through this study it has been identified that dogs in Bali are predominantly owned and the effect of unowned dogs towards the total population is minimal. Demographically, the sex ratio is male-biased and juveniles make up 15-20% of the population. Free-roaming dogs are dominated by adults and a higher proportion of males. There are differences in the demographics of dogs in urban and non-urban areas which should be considered when planning and implementing control programs. For example, urban areas have more females and a larger proportion of dogs are confined.

Overall vaccination coverage is high (>70%), however juveniles and females have a high likelihood of not being vaccinated. The endurance of vaccination collars should be improved to better represent the true vaccination coverage in free-roaming dogs as there is high confidence that most free-roaming dogs are actually owned dogs. Recent culling is associated with increased proportions of juveniles and a 40% higher risk of dogs not being vaccinated.

The observation of free-roaming dogs should always account for detection probability as only 20% of dogs in this study were seen at any given time. Failure to account for detection probability will result in severe underestimation of the population abundance. The human population, presence of a forest and recent culling account for 28% of variation in the number of owned dogs in banjars. Accordingly, the number of owned dogs and presence of rice paddies account for 61% of variation in the number of free-roaming dogs in banjars.

Finally, the overall and median human to dog ratios were the least biased measures for estimating the overall dog population. However, both are crude tools with poor precision for estimating the dog population in Bali.

5.2. Future research

Based on the results of this study, there are several directions for future research. First of all, there needs to be research on better methods for estimating the total dog population in Bali. Since it is essential, especially for rabies control program, improving current tools or developing new ones should be prioritized. Secondly, more research should be conducted to identify predictors of the dog population in local units, e.g. banjars. Programs will be logistically more efficient and insights could be gained on the ecology of the domestic dog in Bali. Finally, a study on vaccination collar attrition rate and temporary paint mark used might be beneficial to estimate the true number of vaccinated free-roaming dogs in between campaign evaluations.

5.3. Recommendation

Based on the findings from this study, there are several recommendations for future rabies control programs in Bali.

1. Rabies vaccination campaign
    a. Focusing vaccination efforts on owned dogs is sufficient to achieve adequate vaccination coverage as the impact of unowned dogs on the herd immunity of the dog population is likely minimal. In the unlikely case that up to 20% of free-roaming dogs in banjars are assumed to be unowned, inflation of the total population is expected to be less than 7%. Encouraging owners to mark their dogs will help improve estimates of this percentage since free-roaming owned dogs will then be identifiable. Regardless, even based on this model, herd immunity is largely influenced by rabies vaccination in owned dogs.
    b. With the banjar structure in Bali, collecting information on the number of owned dogs should be feasible. Human to dog ratios could be used as a quick and dirty way to provide an overall estimate of the dog population for logistical preparation of vaccination campaigns, however further fine-tuning would be necessary for campaign implementation at local levels.
    c. In addition to current efforts, more attention should be put into vaccinating owned juvenile and female dogs. These groups were less likely to roam than males; however they were also less likely to be vaccinated. When comparing the sex and age ratio of owned dogs to observed free-roaming dogs, it is apparent there was a higher ratio of male dogs free-roaming despite females having slightly higher detection probabilities and that almost all roaming dogs were adults. Thus, owned juvenile and female dogs should be more accessible during door to door campaigns and higher vaccination coverage in these groups should be achievable.

    d. Free-roaming owned dogs were 2 to 4 times less likely to be vaccinated compared to confined dogs; however increasing vaccination coverage in this group might be more challenging. Either vaccination should be timed when free-roaming owned dogs are most likely at home or owners should be encouraged to confine their dogs during vaccination campaigns in the neighborhood.
    e. Future campaigns should use more durable collars to mark vaccinated dogs. It would be beneficial for between campaign evaluations of vaccination coverage. Additionally, being able to correctly identify the rabies vaccination status of a dog would help officials better respond to dog bite cases. The number of dogs marked with collars and temporary spray paint should be recorded for better estimation of vaccination coverage between campaigns.

2. Dog culling

   Culling should be discouraged at all time because it is counterproductive to vaccination campaigns. Culling is associated with lower vaccination coverage in owned dogs. It stimulates population turnover and new dogs are likely unvaccinated. Because all culling activities recorded in this study is suspected to be community driven, rabies control programs should emphasize on educating local officials and the public on the appropriate methods to control rabies in their neighborhood, which is mass vaccination of host animals.

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APPENDICES

Appendix 1. Door to Door Questionnaire

Field From for DTD

Name of Village / District : ______________________________
Day / Date of Collection : ______________________________

No.	Name of Owner	Banjar	Address	Sex	Age Group	Coat Color	Confinement Status	Vaccination Status	Note

Enumerator:_________________________ **Validation date:** _________________________ **Signature:** ______________________________

Appendix 2. Form for Photographic Mark (Capture) Recapture Identification (PCR)

Identification Form for PCR

Name of Village / District : ___________________________

Day / Date of Collection : ___________________________ **Time** : ____________________

Survey Day No : ___________________________

Dog Code	Banjar	Village	Age Group	Sex	Coat Color	Vaccination Status	Note

***<u>Note</u>*: Vaccination status determined based on the presence of red collar and/or V tag on collar**

Enumerator:__________________________ **Validation date:**__________________________ **Signature:**

____

Appendix 3. Logistic Regression Models for Demographic Variables in Owned Dogs

Sex=Female

```
glm(formula = sex ~ uc + age + confine  + cull + age:confine + confine:cull, family = binomial(link =
logit), data = own)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.2212  -0.8595  -0.6889   1.3958   1.7634

Coefficients:
                                   Estimate Std. Error z value Pr(>|z|)
(Intercept)                        -1.31761    0.03119 -42.245  < 2e-16 ***
ucS                                 0.25256    0.04771   5.294 1.20e-07 ***
ucU                                 0.51192    0.04760  10.755  < 2e-16 ***
ageJuvenile                         0.55348    0.05568   9.941  < 2e-16 ***
confineConfined                     0.25604    0.04969   5.153 2.57e-07 ***
cull1                               0.35474    0.07466   4.752 2.02e-06 ***
ageJuvenile: confineConfined       -0.29533    0.08774  -3.366 0.000763 ***
confineConfined:cull1                       -0.30522    0.09594  -3.181 0.001466 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)
    Null deviance: 21118  on 17375  degrees of freedom
Residual deviance: 20670  on 17368  degrees of freedom
AIC: 20686
Number of Fisher Scoring iterations: 4
```

Age group=Juvenile

```
glm(formula = age ~ uc + cull, family = binomial(link = logit), data = own)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.6984  -0.6117  -0.5991  -0.5170   2.0389

Coefficients:
                  Estimate Std. Error z value Pr(>|z|)
(Intercept)      -1.62688    0.03199 -50.860  < 2e-16 ***
ucS              -0.31805    0.06027  -5.277 1.31e-07 ***
ucU               0.04563    0.04613   0.989    0.323
```

```
cull1              0.29452    0.05638   5.224 1.75e-07 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)
    Null deviance: 15587  on 17375  degrees of freedom
Residual deviance: 15501  on 17372  degrees of freedom
AIC: 15509
Number of Fisher Scoring iterations: 4
```

Confinement status=Confined

```
glm(formula = confine ~ uc + cull + age + urb_cull, family = binomial(link = logit), data = own)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-2.0601  -0.5806  -0.4080   0.5805   2.5899

Coefficients:
                   Estimate Std. Error z value Pr(>|z|)
(Intercept)        -2.44415    0.04586 -53.293  < 2e-16 ***
ucS                0.74904    0.06412  11.683  < 2e-16 ***
ucU                3.05009    0.05403  56.447  < 2e-16 ***
cull1              -0.87400    0.20482  -4.267 1.98e-05 ***
ageJuvenile        0.29900    0.05585   5.354 8.62e-08 ***
urb_cull           1.96338    0.21729   9.036  < 2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)
    Null deviance: 22192  on 17375  degrees of freedom
Residual deviance: 14732  on 17370  degrees of freedom
AIC: 14744
Number of Fisher Scoring iterations: 5
```

Appendix 4. Logistic Regression Models for Demographic Variables in Observed Free-Roaming Dogs

Sex=Female

```
glm(formula = sex ~ uc, family = binomial(link = logit), data = free)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.8404  -0.8404  -0.6266  -0.6266   1.8571

Coefficients:
                   Estimate Std. Error z value Pr(>|z|)
(Intercept)        -1.52814    0.09130 -16.738  < 2e-16 ***
ucS                 0.08909    0.17016   0.524    0.601
ucU                 0.66886    0.11854   5.642 1.68e-08 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)
    Null deviance: 2128.8  on 1968  degrees of freedom
Residual deviance: 2092.7  on 1966  degrees of freedom
AIC: 2098.7
Number of Fisher Scoring iterations: 4
```

Age group=Juvenile

```
glm(formula = age ~ 1, family = binomial(link = logit), data = free)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.2591  -0.2591  -0.2591  -0.2591   2.6119

Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  -3.3773     0.1261  -26.78   <2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)
    Null deviance: 571.25  on 1968  degrees of freedom
Residual deviance: 571.25  on 1968  degrees of freedom
AIC: 573.25
Number of Fisher Scoring iterations: 6
```

Appendix 5. Logistic Regression Models for the Odds of Owned and Observed Free-Roaming Dogs Not being Vaccinated against Rabies

Owned dog

```
glm(formula = vaccination ~ uc + age + sex + cull + confine + uc:age + uc: confine, family =
binomial(link = logit), data = own)

Deviance Residuals:
   Min      1Q   Median      3Q      Max
-2.7816   0.2120   0.3845   0.5184   2.0633

Coefficients:
                              Estimate    Std. Error z value Pr(>|z|)
(Intercept)                   -1.93912    0.04144   -46.789  < 2e-16 ***
ucS                           -0.62859    0.08270   -7.601 2.94e-14 ***
ucU                           -0.75431    0.09211   -8.189 2.63e-16 ***
ageJuvenile                    2.28772    0.07076  32.333  < 2e-16 ***
sexFemale                      0.30602    0.05195   5.891 3.84e-09 ***
cull1                          0.33667    0.07101   4.741 2.13e-06 ***
confineConfined               -0.61727    0.12845  -4.806 1.54e-06 ***
ucS: ageJuvenile               1.97587    0.16919  11.678  < 2e-16 ***
ucU: ageJuvenile               0.68092    0.11819   5.761 8.36e-09 ***
ucS: confineConfined          -0.66242    0.24603   -2.692  0.00709 **
ucU: confineConfined          -0.47363    0.16272   -2.911  0.00361 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)
   Null deviance: 15525  on 17375  degrees of freedom
Residual deviance: 11636  on 17365  degrees of freedom
AIC: 11658
Number of Fisher Scoring iterations: 6
```

Free-roaming dog

```
glm(formula = vaccination ~ uc + age + sex + cull + urb_cull, family = binomial(link = logit), data = free)

Deviance Residuals:
   Min      1Q   Median      3Q      Max
-2.2037  -1.4351   0.7329   0.8459   1.2838
```

```
Coefficients:
              Estimate Std. Error z value Pr(>|z|)
(Intercept)    0.84377    0.08182  10.312  < 2e-16 ***
ucS            0.33344    0.15978   2.087 0.036895 *
ucU           -0.25566    0.11855  -2.157 0.031035 *
AgeJuvenile    1.32475    0.40719   3.253 0.001140 **
SexFemale      0.44768    0.12452   3.595 0.000324 ***
cull1          0.16723    0.47868   0.349 0.726820
urb_cull      -1.00202    0.50483  -1.985 0.047160 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 2437.4  on 1968  degrees of freedom
Residual deviance: 2349.6  on 1962  degrees of freedom
AIC: 2363.6

Number of Fisher Scoring iterations: 4
```

Appendix 6. Beta Estimates for Detection Model

```
                  LOGIT Link Function Parameters of {Intercept+time+sex}
                                                      95% Confidence Interval
 Parameter                    Beta         Standard Error      Lower          Upper
 -------------------------  --------------  --------------  --------------  --------------
    1:B1                 -1.4541702       0.1067380       -1.6633766      -1.2449638
    2:t1                  0.3834613       0.0722767        0.2417990       0.5251237
    3:t2                  0.3060421       0.0729869        0.1629878       0.4490964
    4:t3                  0.2842970       0.0731981        0.1408287       0.4277653
    5:sex                -0.2879515       0.1094545       -0.5024823      -0.0734207
```

Appendix 7. Real Parameter Estimates for Detection Model

```
                    Real Function Parameters of {Intercept+time+sex}
Following estimates based on unstandardized individual covariate values:
        Variable   Value
        ---------  -------------
        SEX        0.8108652
        AGE         0.9651241
        COLLAR     0.2823608
                                                        95% Confidence Interval
 Parameter                  Estimate       Standard Error      Lower           Upper
 -------------------------  --------------  --------------  --------------  --------------
   1:p                  0.2134592       0.0104605        0.1936735       0.2346779
   2:p                  0.2007493       0.0100692        0.1817375       0.2212123
   3:p                  0.1972830       0.0099614        0.1784840       0.2175377
   4:p                  0.1560838       0.0086411        0.1398859       0.1737784


                    Real Function Parameters of {Intercept+time+sex} - female}
 Following estimates based on user-specified individual covariate values not standardized:
        Variable   Value
        ---------  -------------
        SEX        0.0000000
        AGE         0.0000000
        COLLAR     0.0000000
                                                        95% Confidence Interval
 Parameter                  Estimate       Standard Error      Lower           Upper
 -------------------------  --------------  --------------  --------------  --------------
   1:p                  0.2552680       0.0199303        0.2181991       0.2962480
   2:p                  0.2408308       0.0192141        0.2051991       0.2804664
   3:p                  0.2368781       0.0190122        0.2016497       0.2761323
   4:p                  0.1893608       0.0163847        0.1593093       0.2235736
```

```
                  Real Function Parameters of {Intercept+time+sex} - male}
         Following estimates based on user-specified individual covariate values not standardized:
             Variable   Value
             ---------  -------------
             SEX        1.0000000
             AGE        0.0000000
             COLLAR     0.0000000
                                                        95% Confidence Interval
 Parameter                  Estimate       Standard Error      Lower           Upper
 -------------------------  --------------  --------------  --------------  --------------
    1:p                   0.2044579       0.0108985       0.1839233       0.2266484
    2:p                   0.1921530       0.0104671       0.1724670       0.2135063
    3:p                   0.1888002       0.0103483       0.1693476       0.2099226
    4:p                   0.1490438       0.0088924       0.1324415       0.1673258
```

Appendix 8. Variance Components of Real Parameter Estimates for Detection Model

{Intercept + time + sex}

```
   Beta-hat SE(Beta-hat)
------------------------
   0.191145     0.013831

     S-hat    SE(S-hat)    S-tilde  SE(S-tilde) RMSE(S-tilde)
-----------------------------------------------------------
   0.213459    0.010461    0.211959    0.010163     0.010273
   0.200749    0.010069    0.199962    0.009813     0.009845
   0.197283    0.009961    0.196680    0.009716     0.009735
   0.156084    0.008641    0.157331    0.008509     0.008600

Naive estimate of sigma^2 = 0.0005582 with 95% CI (0.0001381 to 0.0085358)

Estimate of sigma^2 = 0.0005617 with 95% CI (0.0001417 to 0.0085393)

Estimate of sigma = 0.0236993 with 95% CI (0.0119017 to 0.0924083)

Trace of G matrix = 3.8511308
```

Appendix 9. Variance Components Analysis of Derived Parameter Abundance Model

{Intercept + owned dog + rice paddies + owned dog*rice paddies}

Bali dog

```
   Beta-hat SE(Beta-hat)
 ------------------------
   8.170819    1.776675
   0.123748    0.025636
  -0.989353    2.710119
   0.120691    0.036568

     S-hat    SE(S-hat)    S-tilde  SE(S-tilde) RMSE(S-tilde)
 ----------------------------------------------------------
  10.005781    2.618596    9.756962    2.503087    2.515424
  12.322189    3.088928   11.608122    2.904552    2.991039
  25.922741    4.419140   24.805856    3.961726    4.116152
  29.517513    4.747781   27.336775    4.169711    4.705540
  17.454835    3.651922   16.568909    3.358123    3.473018
  16.935812    3.479053   15.580466    3.224068    3.497368
  17.973858    3.825781   16.759197    3.489743    3.695092
  17.195324    3.565355   16.227545    3.289041    3.428467
  17.195324    3.565355   17.253750    3.320136    3.320650
  15.138426    3.261454   14.551588    3.046508    3.102514
   5.392157    2.080153    5.388136    2.021060    2.021064
  10.524803    2.841613   10.034628    2.696004    2.740202
   8.467906    2.450092    8.263946    2.354778    2.363595
   3.594772    1.696644    3.571413    1.664020    1.664184
   5.392157    2.080153    5.362687    2.020933    2.021148
  12.322189    3.088928   12.108292    2.907353    2.915210
  34.131136    5.020030   31.087285    4.336301    5.297975
   8.986929    2.691129    8.669930    2.568521    2.588008
  31.314899    4.904781   31.438594    4.324129    4.325898
  35.928522    5.168768   31.384479    4.417018    6.337063
  23.366015    4.375653   21.613613    3.898716    4.274448
  23.366015    4.375653   23.056114    3.915981    3.928225
  31.314899    4.904781   30.126330    4.284767    4.446563
  13.860064    3.222550   13.274394    3.017522    3.073833
  22.327970    4.067006   21.154970    3.676990    3.859557
  12.062678    2.986321   12.238330    2.823667    2.829125
  30.296047    4.954144   26.924620    4.289705    5.456015
  24.125355    4.246366   21.697103    3.800671    4.510156
  29.777024    4.815803   26.464269    4.190871    5.342073
  21.309118    4.119027   19.708296    3.708738    4.039477
  24.125355    4.246366   22.771125    3.808477    4.042083
  87.254981    8.238268   83.250999    6.853500    7.937401
  25.663230    4.348249   23.569325    3.874296    4.403931
  24.644378    4.394658   22.956710    3.915030    4.263295
  10.524803    2.841613   10.125843    2.695668    2.725031
  45.953496    6.091387   41.864039    5.012988    6.469444
  28.998490    4.614203   26.962477    4.067196    4.548344
  26.941593    4.381751   25.055908    3.903479    4.335085
```

55.440254	6.566108	48.338113	5.248088	8.830789
14.119575	3.318776	13.313183	3.096290	3.199575
7.189543	2.404487	6.936048	2.316079	2.329910
10.524803	2.841613	9.942158	2.699039	2.761211
37.226079	5.477804	34.215662	4.635550	5.527289
23.106504	4.297631	20.950957	3.835871	4.400033
10.524803	2.841613	10.164935	2.695682	2.719596
10.784315	2.951081	10.485195	2.789103	2.805097
22.587481	4.143275	20.958165	3.731610	4.071803
41.061168	5.538350	36.863942	4.692401	6.295660
31.833921	5.038546	28.077680	4.335612	5.736452
20.790095	3.959248	19.629906	3.595056	3.777627
37.725908	5.313918	34.027989	4.522950	5.842232
36.188033	5.228817	33.809983	4.486497	5.077773
43.637088	5.844071	43.549770	5.010782	5.011542
35.169182	5.270183	30.595744	4.479839	6.401975
42.099213	5.768661	38.156945	4.809543	6.218777
17.973858	3.825781	17.468994	3.497668	3.533917
23.106504	4.297631	21.843069	3.845734	4.047955
22.846992	4.220162	20.770460	3.780651	4.313387
23.366015	4.375653	21.150924	3.898274	4.483656
24.903890	4.469785	23.742144	3.971591	4.138018
33.112284	5.057511	29.095653	4.356254	5.925393
20.790095	3.959248	19.079834	3.589993	3.976561
58.535197	6.947466	50.946568	5.457192	9.347097
26.960787	4.709913	23.969159	4.119869	5.091479
60.332583	7.063021	51.893929	5.503975	10.074950
31.833921	5.038546	28.267951	4.338017	5.615562
23.106504	4.297631	21.044762	3.836164	4.355104
22.327970	4.067006	21.372617	3.678603	3.800634
12.322189	3.088928	11.782424	2.903914	2.953653
17.714347	3.738736	16.413457	3.423115	3.661971
1.797386	1.198439	1.833728	1.186829	1.187385
1.797386	1.198439	1.807514	1.186779	1.186822
3.594772	1.696644	3.608306	1.664060	1.664115
10.524803	2.841613	10.223574	2.695880	2.712658
12.322189	3.088928	11.740452	2.907220	2.964851
8.727418	2.571798	8.396241	2.462458	2.484628
5.132646	1.926441	5.004849	1.880226	1.884564
10.524803	2.841613	10.047658	2.695924	2.737823
10.265292	2.730840	9.737576	2.601972	2.654947
15.916961	3.534568	14.753555	3.265075	3.466155
5.392157	2.080153	5.217265	2.021267	2.028820
38.244930	5.432812	36.377293	4.638459	5.000338
44.415622	6.020863	39.373960	4.999660	7.100349
25.922741	4.419140	25.058795	3.976934	4.069694
42.099213	5.768661	38.415774	4.902586	6.132135
1.797386	1.198439	1.776307	1.186854	1.187042
3.335260	1.506261	3.377517	1.483351	1.483953
6.670520	2.134129	6.497294	2.070654	2.077887
3.335260	1.506261	3.295071	1.483841	1.484385
12.062678	2.986321	11.459105	2.818612	2.882512
18.733198	3.684648	17.973823	3.386981	3.471065
30.555559	5.024399	29.539991	4.367529	4.484048
19.771244	4.016685	19.592738	3.678184	3.682513
37.745102	5.607162	33.227585	4.702013	6.520497

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 21.309118   4.119027  21.469508   3.791517   3.794908
 12.581701   3.190874  11.823657   2.988368   3.083014
  5.392157   2.080153   5.319060   2.020843   2.022165
 10.524803   2.841613  10.315382   2.698228   2.706343
 14.379086   3.414756  14.174883   3.175218   3.181778
 22.846992   4.220162  21.071719   3.787337   4.182765
  5.392157   2.080153   5.239079   2.021102   2.026891
 65.205717   7.297405  60.979057   5.875824   7.238091
 18.992709   3.767049  17.916294   3.446621   3.610799
  6.930032   2.271478   6.745228   2.196492   2.204252
  7.189543   2.404487   7.171798   2.314962   2.315030
  1.797386   1.198439   1.808059   1.187019   1.187067
 10.524803   2.841613  10.160945   2.698255   2.722678
 15.657449   3.443492  14.673670   3.192517   3.340657
 20.530584   3.880146  18.999528   3.530707   3.848380
 13.600552   3.126055  12.901120   2.934940   3.017130
 15.657449   3.443492  14.696538   3.196758   3.338055
 15.397938   3.352453  14.570243   3.123393   3.231201
 12.322189   3.088928  11.623279   2.904428   2.987336
 40.301828   5.632536  35.713858   4.731512   6.590650
  1.797386   1.198439   1.963399   1.187408   1.198957
  3.335260   1.506261   3.272122   1.483668   1.485011
 12.062678   2.986321  12.041089   2.822126   2.822208
  4.613623   1.590585   4.596371   1.563485   1.563580
 25.922741   4.419140  23.358483   3.921676   4.685612
  8.986929   2.691129   8.716456   2.568432   2.582634
 48.769734   6.177531  41.504890   5.013397   8.826783
 53.143040   6.591427  47.439766   5.288933   7.778184

Naive estimate of sigma^2 = 212.3472622 with 95% CI (164.1239929 to 282.9859034)

Estimate of sigma^2 = 69.9675936 with 95% CI (49.6994858 to 100.1097056)

Estimate of sigma = 8.3646634 with 95% CI (7.0497862 to 10.0054838)

Trace of G matrix = 111.0447722
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