COMMUNITY LEVEL CANINE HEALTH ASSESSMENT: IMPLICATIONS FOR HUMAN HEALTH AND WILDLIFE CONSERVATION

By

HOU-MING LILLIAN FUNG

(Under the Direction of Nicole Lynn Gottdenker)

ABSTRACT

Domestic dogs often serve as reservoir hosts for many zoonotic and wildlife pathogens. This study evaluates the role dog health plays in infectious disease transmission, particularly in areas of low economic status. This field study evaluates the health of domestic dogs in 3 rural communities in La Chorrera, Panama. From each dog, blood and fecal samples were collected to examine associations between poverty, wildlife, and zoonotic disease infection risk. Routine hematology (complete blood counts), body condition, and fecal helminth parasites were assessed. Dogs were also tested for *Trypanosoma cruzi*, Canine Distemper Virus, and cytokine expression (Interferon-γ and Interleukin-10). This study concludes that isolated communities of lower economic status may have less healthy dogs with potentially increased risk to transmit zoonotic diseases to human and spillover to wildlife. Future directions include incorporating data collected from the field study into a transmission model to assess impact of body condition and coinfection on *T. cruzi* transmission.

Index words: Zoonoses, Chagas disease, *Trypanosoma cruzi*, Intestinal helminths, Neglected Tropical Diseases, Canine Distemper Virus

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HOU-MING LILLIAN FUNG

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By

HOU-MING LILLIAN FUNG

Major Professor: Nicole Gottdenker

Committee: Vanessa O. Ezenwa
Andrew W. Park

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
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CHAPTER ONE

LITERATURE REVIEW

Importance of understanding reservoir hosts in disease transmission dynamics

Understanding how reservoir hosts influence disease transmission is critical to understanding, predicting, and preventing the spread of infectious diseases. Frequently, disease transmission is not evenly distributed among hosts. The 20/80 rule of disease ecology, a concept where 20% of individuals in a given population are responsible for 80% of pathogens, emphasizes that there is an individual heterogeneity in disease transmission (Woolhouse et al. 1997). This heterogeneity has the potential to lead to the emergence of "superspreaders" (individuals who disproportionally transmit diseases at higher rates) and can change population disease distribution (Lloyd-Smith et al. 2005, Paull et al. 2012). Being able to target specific host populations or individuals for the control of zoonotic diseases may therefore have a positive impact on disease transmission and is often a more cost effective alternative to interventions to human populations (Molyneux et al. 2011). Effective control of schistosomiasis (*Schistosoma japonicum)* in China was achieved through removing bovine from snail-infested grasslands and therefore decreasing transmission at the reservoir-vector interface (Wang et al. 2009). With approximately 60% of human pathogens and an additional 75% of emerging pathogens being zoonoses (diseases that can be transferred from animals to humans),

animal reservoir hosts should be included for effective disease control strategies (Taylor et al. 2001).

Domestic dogs as disease reservoirs in humans and wildlife

Because of a strong interaction and connection between infectious multihost pathogens of humans, domestic animals, and wildlife (Figure 1.1), domestic animals, such as the domestic dog (*Canis familiaris*), pose a threat to humans because of their potential to spread zoonotic diseases to and spillover to wildlife populations (Daszak et al. 2000). Throughout history, human societies have used dogs for companionship, hunting, or herding, just to name a few activities (Serpell 1995). The domestic dog has even shared parasites with humans since prehistoric times. Archeoparasitologists found evidence in excavated human and animal coprolites dating back to approximately 1,100 years ago that not only showed similar pathogens found in both humans and canids, but suggested that canids may have been potentially responsible for the emergence of zoonoses in Ancestral Puebloans in present day Arizona (Fugassa et al. 2011). The domestic dog also serves as a definitive or important host for zoonotic diseases of significance in many parts of the world that impact animal and human health alike (Table 1.1). Therefore, it is important to consider dog health when controlling infectious zoonotic diseases in human public health programs.

In some regions of Latin America, such as Argentina, dogs serve as a key reservoir host for Chagas disease, a neglected, vector-borne, multihost zoonotic disease caused by the protozoal Kinetoplastid *Trypanosoma cruzi* (Gürtler et al. 1993, Gurtler et al. 1996, Barbabosa-Pliego et al. 2011). Chagas disease is a significant cause of

morbidity and mortality in Latin America, with approximately 10 million people infected and an additional 60 million people at risk. Human Chagas disease in Latin America is estimated to have a 750,000 productive life years lost, 1.2 billion dollars lost, and 670,000 disability adjusted life years (DALYs) annually (Wanderley and Correa 1995, Cubillos-Garzon et al. 2004, Tarleton et al. 2007).

Zoonotic soil transmitted helminths where dogs serve as a definitive host, particularly roundworms and hookworms, can lead to developmental disabilities and impaired cognitive function (Stephenson et al. 1993, Crompton and Nesheim 2002, Dujardin et al. 2010). A study conducted in Brazil showed that school children with moderate to heavy hookworm infection had scored lower on standardized tests than children who had lighter infections (Jardim-Botelho et al. 2008). Aberrant juvenile hookworm and roundworm migration can cause cutaneous larval migrans, or creeping eruption in humans. Transmission often occurs when juvenile hookworms enter through the skin rather than the typical oral route and larval migrans results from the worm traveling towards the intestines (Hotez et al. 2004)

In addition to being important zoonotic disease hosts, domestic dogs are also hosts for diseases transmissible to wildlife. Canine Distemper Virus (CDV) and rabies have been responsible for impacting and altering wildlife carnivore populations, sometimes almost to the point of local extinction; the extinction of the African Wild dog (*Lycaon pictus*) was partly due to the introduction of CDV, rabies and parvovirus by domestic dogs (Cleaveland and Dye 1995, Cleaveland et al. 2000, Butler et al. 2004).

CDV was also a major cause in the drastic decrease of lion populations in Africa because of its interaction with the tick-borne parasite *Babesia* (Munson et al. 2008). Additionally, canine parvovirus antibodies have been found in grey foxes, demonstrating a potential for canine parvovirus to infect wild carnivores (Appel and Summers 1995, Riley et al. 2004). Noteworthy, dogs used for assistance in hunting may pose a higher threat to wildlife populations due to the more frequent opportunities for exposure to wild carnivores (Fiorello et al. 2006). Clearly, understanding factors influencing infectious disease transmission in domestic dogs is important not only to the health management of humans but to wildlife as well .

Neglected Tropical Diseases (NTDs) disproportionally affect poor and impoverished countries, and are often lower on the priority list for funding than other diseases; in recent years, only 0.6% of overseas development assistance funds were allocated to NTDs despite 1 billion people worldwide affected by these diseases (Ehrenberg and Ault 2005, Liese et al. 2010). The interaction between infectious disease and poverty makes economic development difficult to achieve; individuals with disease burden may be not be well enough to work in order to generate income, or they lose learning capabilities that contribute to productivity (Schneider et al. 2011). Additionally, poverty levels driven by infectious diseases can lead to poverty traps, which are formed by the interaction between income levels and other variables essential for economic growth. Rather than only assuming income and disease impact being unidirectional, poverty traps suggest that there is a negative feedback cycle in that decreased income

leads to increased infectious diseases, which in turn leads to an even more decreased income potential (Bonds et al. 2010).

Latin America and the Caribbean have been identified as regions of significant importance and are disproportionately impacted by NTDs. Vector-borne pathogens and soil-transmitted helminths have been identified as the first and second major group (respectively) of poverty related neglected infectious diseases targeted by the Pan American Health Organization (Dujardin et al. 2010). Many of these pathogens, such as *Trypanosoma cruzi*, have domestic animal reservoir hosts.

Infectious diseases of domestic animals may also be influenced by human poverty; dogs and cats in impoverished Chilean regions have been found to have a higher infection rate of zoonotic diseases than dogs and cats in less impoverished areas (Lopez et al. 2009). The reservoir competence of dog hosts for NTDs may be indirectly impacted by human poverty because people of lower economic status may be less able to provide sufficient food for their pets, resulting in poor nutritional condition. A dog (or any animal host), in poor nutritional condition could potentially be unable to fight off infection, which leads to the host becoming increasingly sick and increasingly infected with more pathogens. This negative feedback could prevent a host from recovering by being caught in a vicious cycle of declining health and parasitism (Beldomenico et al. 2009a, Beldomenico et al. 2009b, Beldomenico and Begon 2010). Dog health and nutrition can play a significant role in transmission dynamics of Chagas disease. Dogs in poorer external body condition and in poorer nutritional states have been found to have increase

infectivity of *T. cruzi* to feeding vectors (Petersen et al. 2001). Ultimately, the probability that a particular host will be a good host is dependent on complex interactions between extrinsic factors (ecological conditions outside the host body) and intrinsic factors (internal mechanisms within the host body). Thus, identifying the determinants of variation in domestic dog host competence for infectious diseases of human and wildlife is a critical step in designing disease control strategies and explaining patterns of infection in nature.

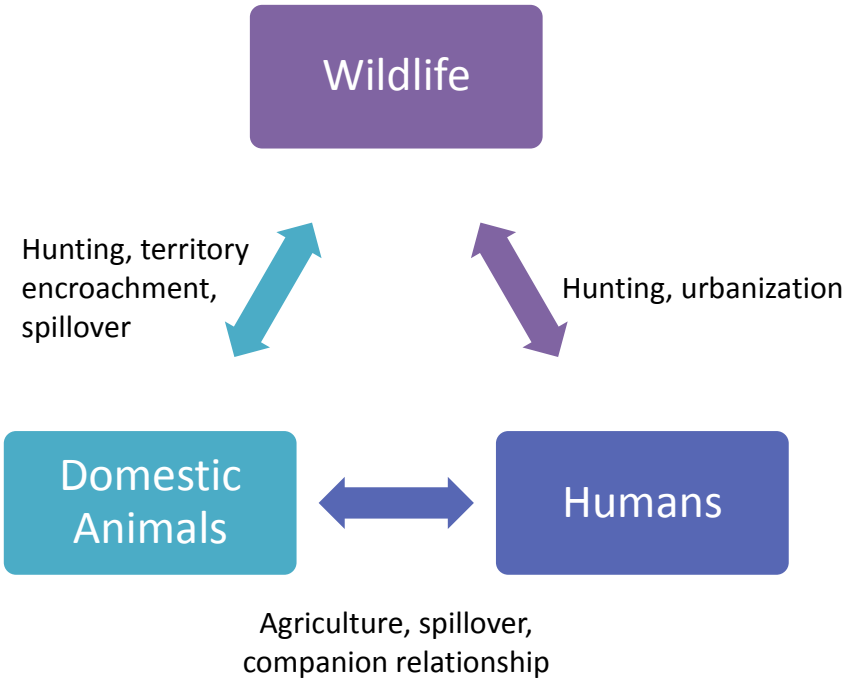

Figure 1.1. Human, domestic animal, and wildlife infectious diseases are intricately connected .

Disease/Pathogen	Pathogen type	Transmission mode	Zoonotic?	Wildlife risk?
Rabies	Virus	Direct	Y	Y
Distemper	Virus	Direct	N	Y
Scabies	Arthropod	Direct	Y	Y
Hookworm	Nematode	Direct/Indirect	Y	Y
Larval migrans	Nematode	Indirect	Y	N
Echinococchus	Cestode	Indirect	Y	Y
Chagas disease	Protozoa	Vector-borne	Y	Y
Leishmaniasis	Protozoa	Vector-borne	Y	Y
Lyme disease	Bacteria	Vector-borne	Y	Y
Erhlichia	Bacteria	Vector-borne	Y	Y

Table 1.1. List of some diseases shared between dogs, humans, and wildlife (Small 1996, Knobel et al. 2005, Carlos et al. 2007, Dantas-Torres 2007, Gurtler et al. 2007, Torgerson et al. 2009, Aydingoz and Mansur 2011, Nelson et al. 2012, Smith et al. 2012)

Because the idea of “One Health” or “One Medicine” has been gathering support in the veterinary, medical, public health, and scientific research fields alike, international collaborations are being forged between organizations such as the World Organization for Animal Health (OIE), World Health Organization (WHO), the Food and Agriculture Organization (FAO), the United Nations Children fund (UNICEF) and the World Bank with a focus placed on vector-borne zoonotic diseases (Day 2011). It is important that collaborators take advantage of these open lines of communication to make headway in controlling infectious multihost zoonotic diseases, particularly in the area of domestic animal health. By understanding the factors that influence domestic dogs health and role in disease transmission in rural tropical communities, this study contributes to guiding zoonotic disease control strategies and wildlife conservation efforts.

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CHAPTER TWO

COMMUNITY LEVEL CANINE HEALTH ASSESSMENT: IMPLICATIONS FOR HUMAN HEALTH AND WILDLIFE CONSERVATION[1]

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Abstract

Dogs play an important role in infectious disease transmission as reservoir hosts of many zoonotic and wildlife pathogens. This study evaluates the role dog health plays in infectious disease risk in dogs, particularly in areas of low economic status. This study sampled domestic dogs from 3 rural communities in La Chorrera, Panama. From each dog, blood and fecal samples were collected to examine associations between poverty, wildlife, and zoonotic disease transmission. Routine hematology (complete blood counts), body condition, and fecal helminth parasites were assessed. Dogs were also tested for *Trypanosoma cruzi* (*T. cruzi* antibody immunochromatographic dipstick), Canine Distemper Virus (serum neutralization assay), and cytokine levels (Interferon-γ and Interleukin-10) through enzyme-linked immunosorbent assays. We found a significant difference between communities and dog health, and decreased pathogen richness in associated with dog health and owner economic status. This study concludes that isolated communities of lower economic status may have less healthy dogs that have potentially increased risk of transmitting zoonotic diseases to between humans and wildlife.

Introduction

The "One Health" approach to controlling infectious diseases has been a priority for policymakers and international health organization, such as the World Health Organization. According to the "One Health" concept, because humans, domestic animals, and wildlife commonly share pathogens, research and policies regarding health and well-being need to be integrated on both human and animal fronts (Zinsstag et al. 2011). These shared, or "multihost" pathogens can be zoonotic (transmitted from animals

to humans), zooanthroponotic (transmitted from humans to animals), or transmitted between animal species. Arthropod vector borne diseases (i.e. Chagas Disease) and soil and tissue transmitted helminths (i.e. hookworms and roundworms), many of which are zoonotic, have been identified by the Pan American Health Organization as the first and second major groups of neglected tropical infectious diseases in the Latin American and Caribbean region, respectively (Dujardin et al. 2010).

Most wild and domestic animal species can serve as reservoir hosts for mulithost pathogens that negatively impact human and animal populations. These reservoir hosts are often infected with multiple pathogens (or increased parasite richness), and these multiple infections may influence a reservoir host's health and ability to be infected by other pathogens. The overall health condition of a host could impact its ability to fight off infection, and hosts with underlying poor body condition may be more susceptible to infectious disease, which in turn causes the host's body condition to further deteriorate, leading to a vicious cycle of negative health feedback (Beldomenico et al. 2009, Beldomenico and Begon 2010). The probability that a particular host will be a good reservoir for a pathogen is dependent on complex interactions between extrinsic factors (ecological conditions outside the host body, e.g. resource availability, habitat structure, climate) and intrinsic factors (internal mechanisms within the host body, e.g. genetics, sex, age, innate/adaptive immunity, infection with other pathogens) that can help shape the host's health (Wakelin 1975, 1978, Wakelin 1996, Christe et al. 2000, Bize et al. 2008).

The close relationship between humans and their pets, and the frequent interaction of these pets with sylvatic environments, particularly in rural tropical regions, allows for pet health to serve as an indicator for multihost pathogens that pose threats to human and/or wildlife populations (Deplazes et al. 2011, Jenkins et al. 2011, Kollataj et al. 2012). In particular, the domestic dog (*Canis familiaris*), the focus of our study, can play an important role as a reservoir host for many zoonotic multihost pathogens (e.g. *Trypanosoma cruzi*, Leishmania spp, rabies, cutaneous and visceral larval migrans) (Roberts and Janovy 2000, Dantas-Torres 2007). Given the close relationship humans have with dogs as companion animals, dogs may potentially increase the transmission risk of zoonotic pathogen such as *T. cruzi* to humans (Gurtler et al. 1996, Cohen and Gurtler 2001, Pineda et al. 2011).

The use of targeting animal populations for the detection and control of zoonotic diseases has a positive impact, and is often a more cost effective alternative, for interventions in human populations (Molyneux et al. 2011). Decreased human transmission of *Schistosoma japonicum* in China was achieved through limiting bovine-snail interaction (Wang et al. 2009), and human rabies can be prevented through mass vaccination of dog reservoirs (Cleaveland et al. 2006). Domestic animal health should be taken into consideration because of this interaction on both human and wildlife fronts. Therefore, investigating the specific role that the domestic dog health plays in transmission is key in infectious disease control.

Here, we studied domestic dog populations in rural communities of Panama. In this region, dogs may serve as reservoirs for Chagas disease, Leishmaniasis, helminths infection (both intestinal and visceral larval migrans), scabies, Leptosporosis, and toxoplasmosis (Etheredge et al. 2004, Cardinal et al. 2007, Labruna et al. 2009, Petersen et al. 2011, Teichmann et al. 2011). In this study, we investigate the zoonoses *Trypanosoma cruzi*, causative agent for Chagas disease, intestinal helminths (hookworms and roundworms), and Canine Distemper Virus, a pathogen that can be transmitted from domestic dogs to wildlife carnivores with potentially high morbidity and mortality effects (Munson et al. 2008).

This study seeks to elucidate 1) factors that influence domestic dog health in rural tropical communities, and 2) if dogs in poorer health may be more likely to serve as potential reservoirs for zoonotic and wildlife pathogens, and thus pose more of a risk to human and wildlife populations. The World Health Organization defines health as "physical, mental, and social well-being, not merely the absence of disease or infirmity" (Breslow 1972), and we employ this definition for our study by looking at physiological response and being in a state of normal nutritional condition by way of a body condition score.

Objectives and hypotheses

In the present study, we quantify body condition in dogs from three rural communities in the district of La Chorrera in Panama, and use this body condition as an

indicator for dog health to examine the associations between health and human poverty, pathogen richness, and physiological parameters by testing the following hypotheses.

Hypothesis 1, Parasite effect: *Dogs in lower body condition are more likely harbor more parasite types and have higher cytokine levels.*

To investigate this hypothesis, we examined the associations between parasite richness and body condition, presence/absence of zoonotic and wildlife parasites and body condition, and parasite richness and cytokine expression. A negative feedback cycle between dog health and infectious diseases can exist and exacerbate health and disease; less healthy animals are likely to be infected with pathogens, which in turn cause the animals to decrease even further in health (Craig et al. 2008, Beldomenico and Begon 2010). We expect dogs that have a lower body condition scores to have higher number of total parasites and therefore, higher possibility of being infected by zoonotic and wildlife parasites. The zoonoses of focus in this study are *Trypanosoma cruzi* (agent for Chagas disease) and potentially zoonotic helminths. Chagas disease is a significant cause of morbidity and mortality in Latin America, with approximately 10 million people infected and an additional 60 million people at risk (Tarleton et al. 2007). *Trypanosoma cruzi* is zoonotic (transmissible from animals to humans) and is transmitted from a variety of wild and domestic mammalian reservoir hosts to humans by the kissing bug vector (family *Reduviidae*). Soil transmitted helminths, particularly roundworms and hookworms cause developmental hindrances to physical and mental growth of children and can be zoonotic in nature (Dujardin et al. 2010). We use Canine Distemper Virus as a measure to evaluate relationships between dog health and their risk of transmission to wild animals. In

wildlife populations, domestic animal pathogens have been found to impact wildlife carnivore populations (Cleaveland and Dye 1995, Cleaveland et al. 2000, Fiorello et al. 2006). The extinction of the African Wild Dog (*Lycaon pictus*) was partly due to the introduction of rabies, Canine Distemper Virus (CDV), and parvovirus (Butler et al. 2004). Additionally, CDV can infect felids and has been cited to decrease lion populations in Africa (Munson et al. 2008).

Because of this increased possibility of acquiring multiple pathogens, we would expect dogs in in poor health to have increased immune responses to polyparasitism as compared to dogs in normal health. We may therefore expect to see a correlation of higher levels of cytokine expression in dogs with higher parasitism. Intracellular microparasites, such as *Mycobacterium,* have been shown to stimulate the Th1 arm of adaptive immunity, allowing the increased secretion of Th1 cytokines (TNF-B, IFN-g), and a Th2 immune response (cytokines IL-4, IL-5, IL-10, IL-13) dominates after host infection with macroparasites (Abbas et al. 2012). Therefore, the type of pathogens may potentially increase host susceptibility to infection with microparasites by interfering with host immunity (Ezenwa et al. 2010). The cytokines measured in the study were Interferon-γ (Th1 arm of adaptive response, corresponding to *T. cruzi* and CDV in this study) and Interleukin-10 (Th2 arm of adaptive response, corresponding to intestinal helminths in this study).

Hypothesis 2, Site effect: *Dog in lower body conditions are in communities of lower economic status.*

To examine this second hypothesis, we evaluate the association between body condition and site, parasite richness and site, zoonotic and wildlife diseases and site, and cytokine expression and site. Because dog health and human health are closely linked, we would expect dogs living in communities with higher levels of poverty to receive no or very low veterinary care (due to lack of owner resources to pay for care, or lack of transportation to the nearest town with a veterinarian) and may receive less food to eat in the households that are under economic duress (Chaves et al. 2008). Domestic animals can also be impacted by infectious diseases and poverty levels; dogs and cats in impoverished Chilean regions have been found to have a higher infection rate of zoonotic diseases than dogs in cats in less impoverished areas of Chile (Lopez et al. 2009, Schneider et al. 2011). For this hypothesis, we would expect dogs living in communities of lower economic status to have lower body condition scores and because of this lowered body condition score, have higher parasite richness and cytokine expression compared with those from communities of higher economic status.

Study design

The study was conducted in the Chagas disease endemic rural communities of Lagartera Grande (9° 6' 25N, 79° 54' 19W), Las Pavas (9° 6' 15N, 79° 53' 9W), and Los Hules (9° 3' 27N, 79° 59' 37W), all located west of the Panama Canal and approximately 40 km northwest of Panama City (Figure 2.1). Because of the circularity between heath and parasites, we choose to conduct a cross-sectional observational study on community-

volunteered domestic dogs for pathogen infection to test our hypotheses. The cross-sectional study allows us to take a "snapshot" health assessment of dogs at the time of study and does not imply cause and effect. The pathogens selected for this study were Canine Distemper Virus (CDV), *Trypanosoma cruzi* (causative agent for Chagas disease), *Dirofilaria immitis* (canine heartworm), and intestinal helminths (hookworm, roundworm, whipworm, and tapeworms). Rabies was excluded from this study as Panama has been free of canine rabies since 1972 and human rabies since 1973 (Belotto et al. 2005)**.** This community of pathogens was selected as we were interested in evaluating both directly transmitted and vector borne diseases that can infect humans, wildlife, and domestic animals populations.

Figure 2.1. Location of study sites in Panama.

Dog sampling

Health Assessment: Dogs were recruited by a local health official of the three regions; signs were placed around the communities the week prior to sampling days to inform community members that vaccination clinics were being held. Samples from 78 dogs were collected across three communities (27 from Lagartera Grande, 25 from Las Pavas, and 26 from Los Hules), on May 29, June 4, and June 11, 2011, respectively. Dogs were manually restrained and 3-5 mL of blood were collected from each dog from the cephalic vein. Whole blood was used for the Typanosoma Detect™ Rapid Test, IDEXX Heartworm Snap® Test, and blood smears. Remaining blood was stored in serum red top tubes and centrifuged at the lab to be separated and aliquoted for CBC for hematology analysis, ELISAs for cytokine detection, and DNA extraction for PCR. Ectoparasites were assessed for presence or absence and collected.

All dogs sampled were volunteered by owners, and volunteered dogs were given deworming treatment (subcutaneous Ivermectin injections), rabies vaccination (subcutaneous), and vitamins tablets after sampling was done.

Assessing Body condition: Body condition of each subject was assessed by sight and touch (spine and ribs). The body condition scored was based on the 9 point Body Condition Scale developed for dogs and cats (Baldwin et al. 2010). A score between 1-3 was considered too thin (emaciated, muscle mass loss, ribs, lumbar and pelvis easily visible and palpated), a score of 4-5 was considered ideal (ribs palpable but with some fat covering, abdominal tuck visible), and a score between 6-9 was considered too heavy.

Figure 2.2 shows a dog that was considered too thin with a body condition score of 1. Age of the dog was estimated based on dental wear as specified in Merck's Veterinary Manual (Siegmund and Merck & Co. 1955). The body condition score was then used as an indicator for health (Petersen et al. 2001). Sex of each animal was also recorded.

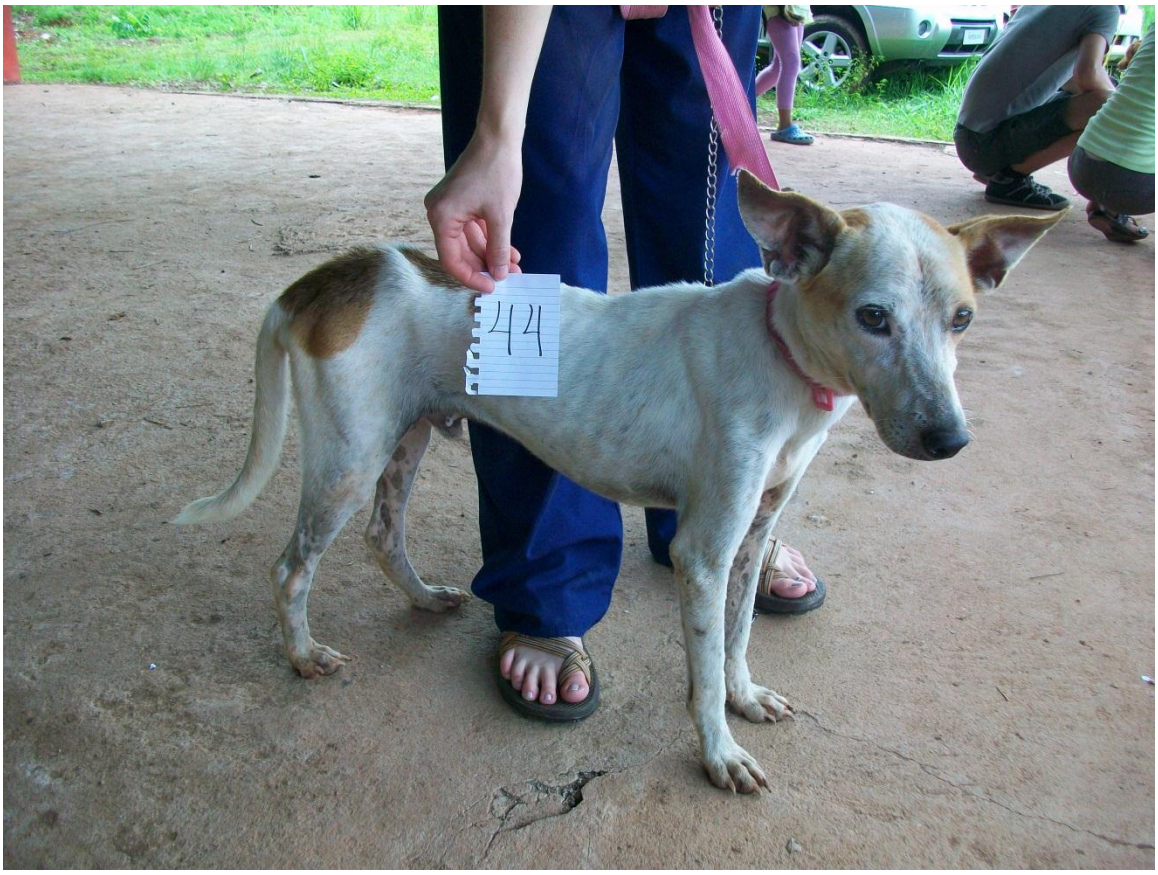

Figure 2.2. A dog from the community of Las Pavas with a body condition score of 1. Note the prominent ribs and pelvis bones, obvious loss of muscle mass, and no body fat.

Hematology: Blood samples from each dog were taken from the cephalic vein, centrifuged and sent out to a local veterinarian for a Complete Blood Count to assess physiological health. Blood panels (total white blood cell, red blood cell, hemoglobin,

mean hemoglobin concentration, mean cell volume) were analyzed using a CD-1700 Cell Dyn Analyzer. Hematocrit count was calculated from Red Blood Cell Count and Hemoglobin values. Differential White Blood Cells were determined by manually by slide examination. Reference ranges for canine panels were taken from Schalm's Veterinary Hematology (Weiss et al. 2010). Anemia was evaluated by red blood cell (RBC) count ($\times 10^6/\mu L$, hemoglobin (HGB, mg/deciliter), and hematocrit values (HCT, %). A HCT of greater than 30% was an indicator of chronic blood loss. In instances when the values conflicted, we weighed the hemoglobin greater than red blood cell or hematocrit. Normal red blood cell range was 5.5-8.5 $\times 10^6/\mu L$. Normal hemoglobin range was 12.0-18.0 g/dL, and any value below was considered Anemic. While reticulocyte count is the gold standard for determining anemia, the reticulocyte was unavailable for most samples, and we therefore relied on Mean Cell Volume (MCV) and Mean Cell Hemoglobin Concentration (MCHC) to determine whether anemia was regenerative or nonregenerative as an indicator for chronic blood loss. Normal white blood cell range referenced was 6,000-17,000/ μL. Values that fell outside the range were considered abnormal.

Cytokine assays: Canine specific enzyme-linked immunosorbent assay tests for Th1 cytokines (e.g. Interferon-γ) and Th2 (Interleukin-10) were performed on collected serum (Canine IL-10 and IFN-γ Quantikine™ ELISA Kits, R&D Systems, Minneapolis MN). Interferon-γ and Interleukin-10 cytokines were selected given the availability of canine specific ELISA kits. Prior to performing the ELISAs, serum being used for IL-10 and reagents had to be prepared. Samples were diluted 2-fold with Calibrator Diluent RD6-12

and mixed well. The Canine IL-10 Kit Control was reconstituted with 1 mL of distilled water. The wash buffer concentrate was diluted with 25mL added to 600 mL of distilled water. Then Canine IL-10 standards were created, each with a 2-fold dilution using 200 µL from the previous diluted standard and added to 200 µL Calibrator Diluent RD6-12. 50 µL of Assay Diluent RD1-14 was added to each well, followed by 50 µL of sample, standard, or control. The mixture was covered with an adhesive and incubated for 2 hours at room temperature. After incubation, the wells were aspirated and washed 5 times. 100 µL of diluted Canine IL-10 Conjugate was then added to each well and covered and incubated for an additional two hours. The wells were then aspirated and washed. Color Reagents A and B were mixed in equal volumes after the last wash and 100 µL was added to each well and incubated for 30 minutes protected from light. Finally, 100 µL of Stop Solution was added to each well and tapped to mix. The optical density was determined using a microplate reader set to 450 nm corrected at 570 nm. The procedure for IFN-γ is similar except the serum sample did not need additional preparation. The Canine IFN-γ Kit Control was reconstituted with 1 mL of distilled water. The wash buffer concentrate was diluted with 40 mL of the wash buffer concentrated added to 920 mL of distilled water. Then Canine IFN-γ standards were created, each with a 2-fold dilution using 200 µL from the previous diluted standard and added to 200 µL Calibrator Diluent RD6-12. 50 µL of Assay Diluent RD1-63 was added to each well, followed by 50 µL of sample, standard, or control. The mixture was covered with an adhesive and incubated for 2 hours at room temperature. After incubation, the wells were aspirated and washed 4 times. 100 µL of diluted Canine IFN-γ Biotin Conjugate was then added to each well and covered and incubated for one hour. The wells were then aspirated and

washed 4 times. 100 µL of Canine IFN-γ Streptavidin-HRP was then added to each well and covered and incubated for 30 minutes. The wells were then aspirated and washed 4 times. 100 µL of Substrate Solution was added to each well and incubated for 30 minutes protected from light. Finally, 100 µL of Stop Solution was added to each well and tapped to mix. The optical density was determined using a microplate reader set to 450 nm corrected at 570 nm.

Microparasite detection

T. cruzi detection: *T. cruzi* was detected by 4 methods. The first was a *T. cruzi* antibody test (Trypanosoma Detect™ InBios International Incorporated, Seattle, WA). The Trypanosoma Detect™ is a canine specific immunochromatographic dipstick derived from *T. cruzi* antigens designed for the determination of antibodies of *T. cruzi*. The sensitivity of Trypanosoma Detect™ is 100% and the specificity is 95%. The second method of *T. cruzi* detection was obtained through blood smears. The third method for detection was PCR using extracted DNA from the collected sample serum to search for trypanosome DNA fragments in host blood. The final method for *T. cruzi* detection was through blood culture for live trypanosomes.

PCR was conducted on DNA extracted from canine blood samples. The primers used were S35/S36 that amplify a 330-bp minicircle sequence and Tcz1/Tcz2 that amplify a 188-bp sequence of a 195-bp repetitive nuclear sequence. Each sample mixture contained 200 mM buffer, 2 mM dNTP, 25 mM $MgCl_2$, 5 U Taq polymerase, and 1 µL of DNA sample were used with 100 mM of each (forward and reverse) primer, yielding a 25 µL

solution per reaction sample. The samples were initially denatured at 94°C for 4 minutes, then underwent 35 cycles of 30 seconds at 55°C, 40 seconds at 72°C, and 1 minute at 94°C. The samples were then incubated for 1 minute at 55°C and 3 minutes at 72°C (Chiurillo et al. 2003). Amplification was then confirmed using a 2% agarose gel stained with ethidium bromide, and purified PCR product was send out for sequencing.

Canine Distemper Virus (CDV): CDV was detected at the University of Georgia Diagnostic Lab using collected serum with a serum neutralization assay.
Sera was placed in a 56°C water bath to inactivate complement before testing. The appropriate worksheet template for microtiter plates and record the identification of the sera to be tested was selected and 25 µL of serum were added to appropriate wells. 25 µL of base DMEM was then added to all wells in each column containing a serum sample or control using a micro-channel pipettor. Test Sera was then diluted in row H by transferring 25 µL of these diluted sera to row G using a 12 channel pipettor. Contents were missed will of the wells in row G by aspirating and dispensing several times before transferring 25 µL of these diluted sera to row F. This dilution process was repeated thru row A. the 2 fold dilution scheme will result in serum dilutions from 1:2 (row H) to 1:256 (row A). pipette tips were changed after each plate. One positive control (titer range 1:8-128) and one negative control (titer of <1:4) was left for each virus in each run. One column (8 wells) was left for the virus control and one column (8 wells) was left for the cell control. 25 µL of base DMEM was added to each well into the virus control column and 50 µL /well was added into the cell control column. Four 8-well columns were left for the virus back titration; and 25 µL of base DMEM was added into all wells of these

columns. Virus was diluted in base DMEM. 25 μL of appropriate virus dilution was added into wells except those of the cell control and the back titration columns. The addition of an equal amount of virus suspension to the serum dilutions effectively therefore doubled the dilutions (1:4 in row H thru 1:512 in row A). Microtiter plates containing the virus serum mixtures were incubated at 35.5 ± 2 degrees celcius/5±1% CO2 for 1 hour ± 5 minutes. During the incubation period, the appropriate cell line was prepared and diluted the cell suspension in complete DMEM and 10% FBS to obtain 125,000 cells/mL. 150 microliters of the cell suspension was then added to each well after incubation. The microtiter plates were then covered and incubated at conditions from step #9 for 3-4 days. Finally, the microtiter plates were examined using an inverted microscope at a magnification of 40-100X. Serum samples containing antibody neutralized the virus and with no CPE. Results were recorded as + (absence of CPE) or – (presence of CPE). The highest dilution showing complete neutralization of the virus is considered the endpoint titer of the antibody. A titer of at least 1:4 was considered positive for antibodies for CDV.

Macroparasite detection

Endoparasites-intestinal helminth detection: Fecal samples were obtained using fecal loops and placed in 1 mL eppendorf tubes with formalin for approximately 48 hours. Intestinal helminth infection was detected by fecal flotation analysis using a sugar solution with a specific gravity of 1.27. Helminths were identified by type – hookworm (*Ancylostoma or Uncinaria spp*), roundworm (*Ascaris spp or Toxocara spp*), tapeworm(*Dipylidium* spp) but not to the species level.

Ectoparasite detection: We combed the dog for fleas and ticks to evaluate infestation. For dogs infected with fleas, we attempted to catch them, and ticks were removed. Caught fleas and removed ticks were stored in in a 1 mL eppendorf tube in 10% ethanol.

Canine Heartworm Detection: IDEXX SNAP® Heartworm tests (Canine SNAP Heartworm Test, IDEXX Laboratories International, Westbrook ME) and blood culture was used to detect *D. immitis*. The SNAP® test has a sensitivity of 84 (78-89), specificity of 97 (84-100), and accuracy of 86 (81-90).

Total parasite richness and parasite types

The total number of pathogens was counted for each dog. Parasite type was broken down into two (non-mutually exclusive) groups: zoonotic (*T. cruzi*, hookworms, roundworms, tapeworms) or wildlife pathogens (CDV, *T. cruzi*, hookworms, roundworms, tapeworms). The variable "total parasites" was a total count of number of different types of parasites, irrespective of type or species (i.e. a dog that had tested positive for hookworms, roundworms, and CDV had a total parasite count of 3).

Census data

Census data obtained from the Panama government were used to compare economic status of the communities. While household income data was not available, data regarding household utilities was available. Table 2.1 shows the breakdown of households in each community with respect to floor material, availability of drinkable

water, and electricity. Lagartera Grande has the most houses with dirt floors, without drinkable water and electricity, and was considered the poorest community.

Site (n)	% houses with dirt floors (n)	% houses without potable water (n)	% houses without electricity (n)
LGA (71)	49.3% (35)	76.1% (54)	87.3% (62)
LPS (83)	42.2% (35)	19.3% (16)	60.2% (50)
LHS (98)	34.6% (34)	23.5% (23.5)	38.8% (38)

Table 2.1. Percentage of houses within each community of La Chorerra County, Panama, that have dirt floors, no potable water, and no electricity.

Statistical Analysis

Statistical analysis was conducted in R (The R Foundation for Statistical Software; http://www.r-project.org, version 2.13.1). We examined the association of body condition score and site, *T. cruzi* infection status, helminths infection status (for all helminths and individual helminths type, distemper status, and presence/absence of zoonoses, and wildlife parasites using a Kruskal-Wallis nonparametric test and Mann-Whitney U test when applicable. We also compared the association between site and parasite richness and presence/absence of zoonotic pathogens using a Chi-Squared analysis. Spearman correlation was used to examine the association for body condition and total number of parasites (parasite richness), Interleukin-10, and Interferon-γ cytokine expression. Finally, we compared the association of body condition score and

zoonotic parasites, wildlife parasites, and immune response (i.e. cytokine levels) across the three sites using generalized linear models.

Results

Health parameters: body condition, hematology, and parasite prevalence

Body condition

The overall mean body condition score for dogs across all three sites was 3.2, 95% CI [2.97, 3.44], with La gartera Grande (LGA) at 2.6, 95% CI [2.30, 2.89], Las Pavas (LPS) at 3.2, 95% CI [2.66,3.66], and Los Hules (LHS) at 3.9, 95% CI [3.60,4.17]. The total dog population consisted of 65% males (95% CI [53.98, 75.56]) and 35% females (95% CI [24.44, 46.32]), and the mean age was 30.2 months (95% CI [21.94, 38.47]. There was a significant difference in body condition score between sites (Kruskal-Wallis test, χ^2=22.02, df=2, p=1.7e-5).

Hematology

55.1%, 95% CI [46.02, 82.76] of all dogs across communities were anemic (67.7% in Lagartera Grande, 52.0% in Las Pavas, and 46.2% in Los Hules). All were classified as nonregenerative anemia due to MCV and MCHC within or below reference range of red blood cell (RBC) counts of less than $5.5\times 10^6/\mu L$ or hemoglobin (HGB) less than 12.0 g/dL. WBC was considered abnormal is the count was less than 6,000/ μL. A generalized linear model with poisson errors was used to compare the associations of the blood panel (white blood cell count, neutrophils, lymphocytes, monocytes, eosinophiles, and hemoglobin count) with body condition. There was no significant association between

body condition and blood panel; mean RBC was 5.35 ± 1.21, mean HGB count was 11.36 ± 2.85, and mean WBC was 16.45 ± 6.04.

Parasite prevalence

7 out of 78 dogs tested positive for *T. cruzi* (4 from Lagartera Grande, 1 from Las Pavas, 2 from Los Hules), a total of 9.0%. The PCR confirmed two dogs that were parasitemic for *T. cruzi*. *T. cruzi* prevalence in LGA was 14.8%, LPS was 4.0%, LHS was 7.7% (95% CIs [4.86, 34.61], [0.21, 22.32], and [1.34, 26.60], respectively). There was no significant difference in *T. cruzi* positive individuals across communities (Chi-squared test, $\chi^2 = 1.94$, df = 2, p = 0.38). The helminth infection in LGA was 81.5%, LPS was 88.0%, and LHS was 57.7% (95% CIs [61.25, 92.97], [67.66, 96.85], and [37.19, 76.03], respectively), and there was significant association between site and presence of helminth infection (Chi-squared test, χ^2=7.9357, df = 2, p = 0.019). Additionally, overall seroprevalence of distemper positive individuals was 61.5%, 95% CI [49.80, 72.13], with LGA at 81.5%, LPS at 60.0%, and LHS at 42.3% (95% CIs [61.25, 92.97], [38.89, 78.19], and [23.97, 62.81], respectively).

PARASITE EFFECT

Total parasites, zoonotic, and wildlife diseases and body condition

There was a significant negative association between body condition and total number of parasites (Spearman's correlation, ρ=-0.368, S= 108193.5, p= 0.000912)

There was no significant association between body condition and presence of zoonotic parasites (*T. cruzi*, hookworms, roundworms, tapeworms) (Mann-Whitney U test,

W=8.812.5, p=0.09589) There was significant association (Mann-Whitney, W=430.5, p=0.024) between body condition and the presence of parasites that can infect wildlife (CDV, *T. cruzi*, hookworms, roundworms, tapeworms), where lower body condition was associated with more wildlife parasites. There was a significant association between body condition and presence of distemper (Mann-Whitney test, W=928.5 p=0.0013), where dogs with distemper had lower body condition scores than dogs without distemper. Additionally in addition to a significant association between site and presence of distemper (Chi-squared test, χ^2=11.58, df=2, p=0.00031). There were, however, no significant association between body condition and *T. cruzi* presence (Mann-Whitney, W=302.5, p=0.33), hookworm egg shedding intensity (Kruskal-Wallis, χ^2=7.434, df=4, p=0.15), roundworm egg shedding intensity (Kruskal-Wallis, χ^2=3.70, df=4, p=0.45), and tapeworm egg shedding intensity (Kruskal-Wallis, χ^2=0.97, df=4, p=0.92).

Cytokines and parasite richness

There was a significant association between IFN-γ expression and parasite richness (Figure 2.3, Spearman's correlation, ρ=0.344, S= 51906.04, p= 0.0021), but there was not a significant association between IL-10 expression and parasite richness (Spearman's correlation, ρ=0.017, S= 77736.8, p= 0.88). There was, however, no significant association between IL-10 expression and presence of zoonotic pathogens (Mann-Whitney U-Test, W=675.5, p=0.89), IL-10 expression and presence of wildlife pathogens (Mann-Whitney U-Test, W=261, p=0.44), or IFN-γ expression and zoonotic parasites (Mann-Whitney U-Test, W=520.5, p=0.13), and IFN-γ expression and wildlife parasites (Mann-Whitney U-Test, W=218.5, p=0.15)

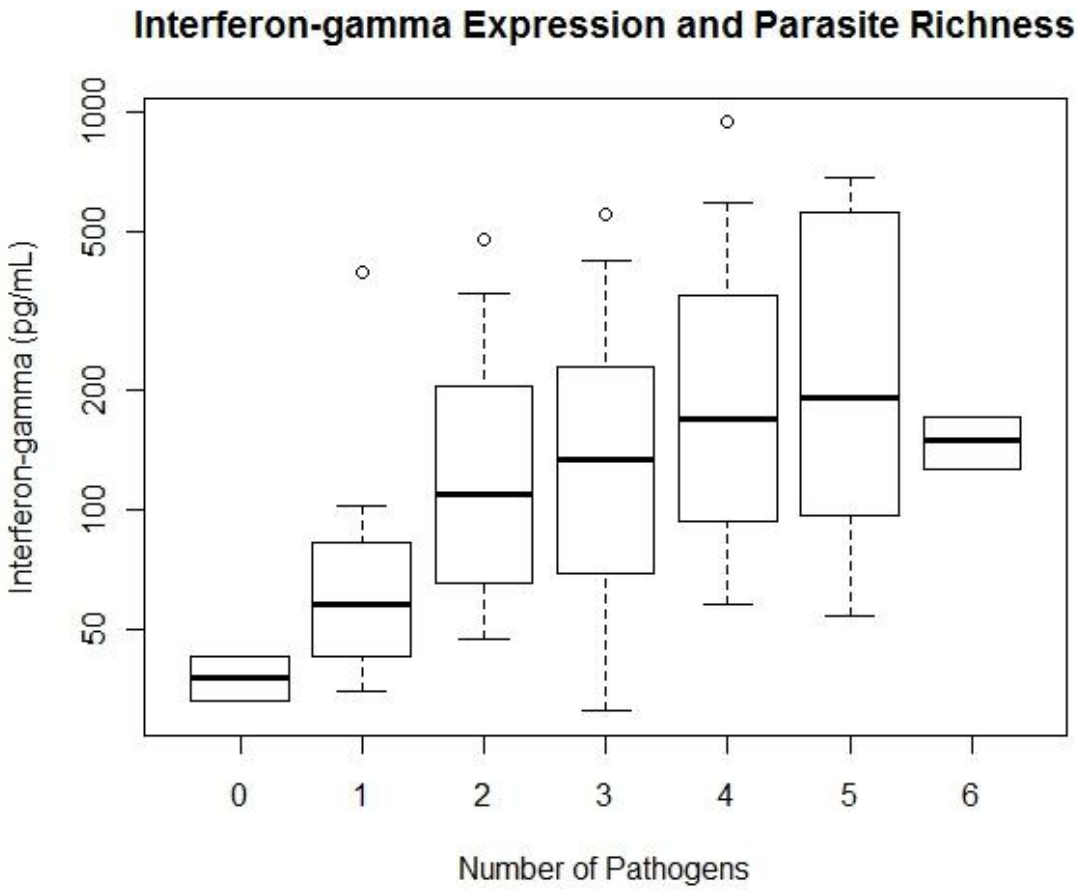

Figure 2.3. Significant association between IFN-γ expression and parasite richness (Spearman's correlation, ρ=0.344, S= 51906.04, p= 0.0021).

SITE EFFECT

Body condition and site

There was a significant association between body condition and site (Figure 2.4, Kruskal-Wallis, χ^2=22.023, df=2, p=1.65e-5), and a follow up multiple comparisons test showed a significant difference between Lagartera Grande and Los Hules. A significant difference was found between Lagartera Grande (LGA) and Las Pavas (LPS, p=0.017) and an even

greater difference was found between Lagartera Grande and Los Hules (LHS, p=0.00051). There was no significant difference between LPS and LHS.

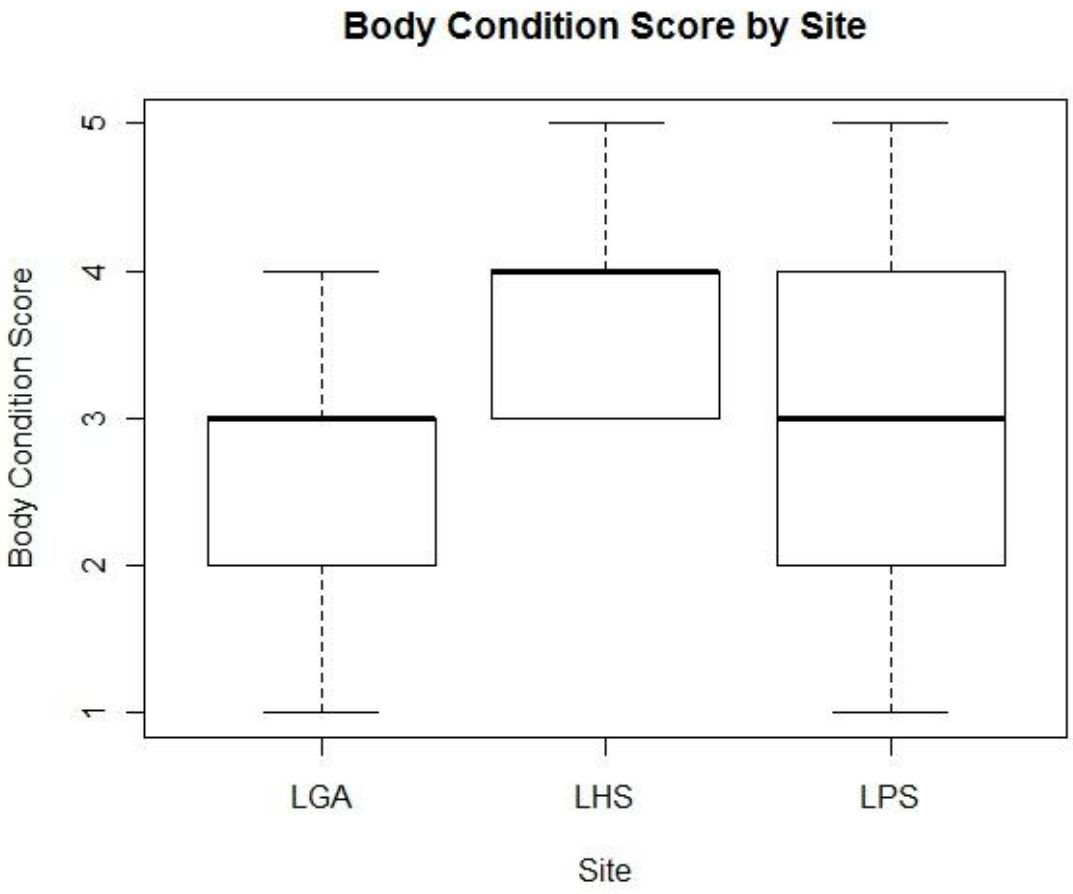

Figure 2.4. Body condition by site. A significant association was found between body condition and site (Kruskal-Wallis, χ^2=22.023, df=2, p=1.65e-5), (LGA: Lagartera Grande, LHS: Los Hules, LPS: Las Pavas).

Parasite richness, zoonotic, and wildlife diseases and site

While there was no association between site parasite richness (Chi-squared test, χ^2= 16.2, df=12, p=0.18), there was a significant association between site and the presence of

zoonotic parasites (*T. cruzi*, hookworms, roundworms, Chi-squared test, χ^2=12.00, df=2, p=0.0025), but not wildlife parasites (Chi-squared test, χ^2=6.16, df=4, p=0.19). However, we did find a significant association between site and presence of distemper virus (Chi-squared test, χ^2=11.58, df=2, p=0.0031). No dogs tested positive for heartworm.

Cytokine expression and site

There was statistical significance between IFN-γ expression and site (Kruskal-Wallis, χ^2=51.1, df=2, p=8.01e-12, Figure 2.5) but there was not a significant association between IL-10 expression and site (Kruskal-Wallis, χ^2=0.625, df=2, p=0.732)

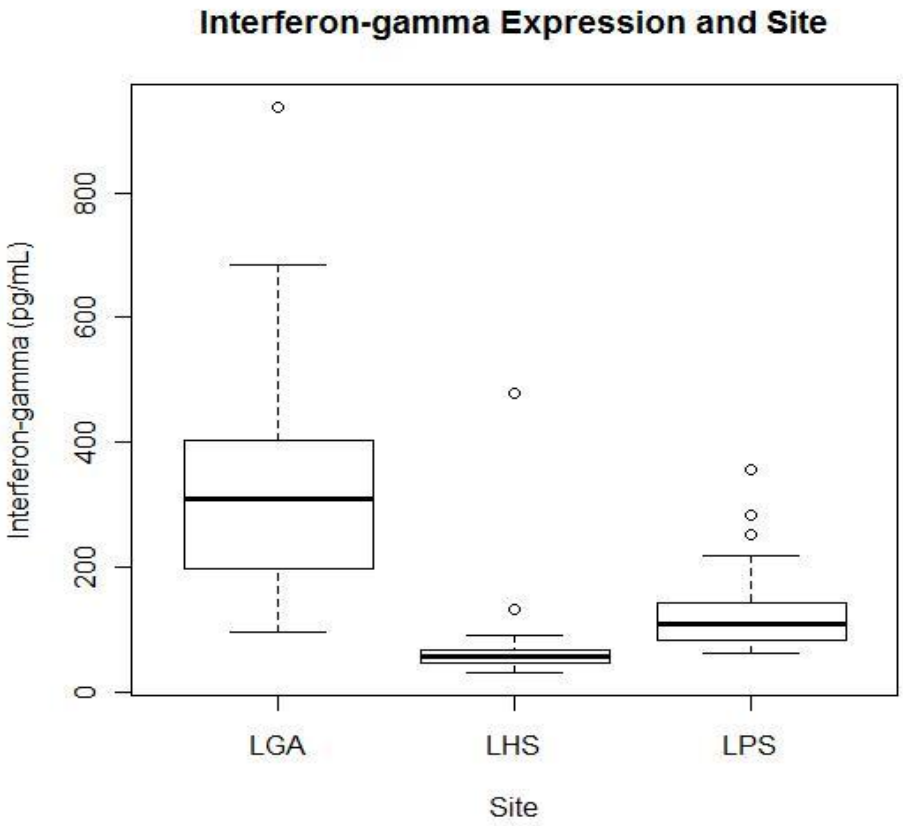

Figure 2.5. Preliminary analysis showed an association between IFN-γ and Lagartera Grande, Los Hules, and Las Pavas communities, and was confirmed with a Kruskal-Wallis (χ^2=51.1, df=2, p=8.01e-12).

Discussion

This study aimed to examine how human poverty, pathogen richness, and cytokine response contribute to domestic animal health. We found that 1) dogs with lower body conditions were found in more impoverished communities, 2) dogs with lower body conditions had higher parasite richness and also increased cytokine responses, and 3) dogs in the region of La Chorrera, Panama have the potential to harbor and transmit zoonotic diseases and wildlife diseases, in particular those in relatively poor

health. The complex interactions between body condition, parasite richness, and cytokine response could be contributing factors in influencing dog health. Body condition is an important aspect of health that can influence an animal's ability to fight off infection, and nutrition may have an impact on body condition. Constant feeding of mice with nematode infections, while prolonging nematode survival, has been shown to increase weight gain and allow for parasite clearance in the host (Tu et al. 2007). A balanced, diet provided to schoolchildren in Burkina Faso, Africa has also been shown to decrease the prevalence of intestinal helminths such as hookworms, roundworms, and tapeworms (Sanou et al. 2010). Because of the level of poverty in these communities, it is unlikely the dogs receive the proper nutrition in order to obtain a health body condition score and ultimately, fight infections. Disease-driven poverty traps are formed when income near poverty levels and infectious disease conditions interact, and can be released by improving the health condition of the population (Bonds et al. 2010). Because of the risk dogs pose for zoonotic and wildlife diseases, domestic animal health must be considered in control strategies for developing countries and countries of low economic status.

While body condition is an important variable in dog health, dog health could be influenced by other factors. Parasite richness can also play a role in influencing body condition, and cytokines expression could play a role in parasite richness, leading to a circularity of interacting factors. However, this study cannot assume directionality of these factors. Parasite richness may be influenced by body condition (and vice versa), cytokine response may be influenced by parasite richness and (vice versa), and body condition may be influenced by the economic poverty levels at the sites (and vice versa).

Therefore, complex, non-linear interactions between parasite richness, body condition, and poverty could be influencing dog health.

In the current study, dogs in communities of lower economic status were in poorer health than dogs found in communities with a relatively higher economic status. With dirt floors, lack of potable water, and lack of electricity as an indicator for poverty, Lagartera Grande was found to have the lowest body condition, highest prevalence of parasites, and (observationally) was the most isolated community sampled, approximately 10 km from a main road with bus access, and the main access road to Lagartera Grande is unpaved and frequently inaccessible by car during the rainy season. Isolated communities may not have access to the same resources such as food and medicine as communities closer to paved roads and buses, and owners may not be able to afford to take their pets to the veterinarian. Being located further away from the main road and major bus stops may also deter owners from taking their pets to the veterinarian either for routine checkups or problem visits and decrease likelihood of individuals seeking treatment for disease, either for themselves or family members. Additionally, there may be cultural differences in pet rearing and it is possible that most rural inhabitants, regardless of economic status, do not customarily take pets to the veterinarian for care. Dogs must be vaccinated for rabies in Panama by law, and other pathogen vaccinations are likely to be rare in poorer rural communities such as the ones studied.

Lagartera Grande, with its relatively high CDV seroprevalence, (81.5%, 95% CIs [61.25, 92.97]), may be a potential ‘source’ for the spread of distemper to nearby wild

carnivore populations. Lagatera Grande is located adjacent to protected forests of the Gigante Peninsula, part of Barro Colorado National Monument, and the probability for disease transmission may potentially be due to wildlife spillover (Kapil and Yeary 2011), and also from domestic dog spillover (Acosta-Jamett et al. 2011). The proximity of Lagartera Grande to protected forests and the distemper seroprevalence in the community may pose a risk to wild carnivores (such as coatis, kinkajou, otters, and tayra) where CDV has higher morbidity and mortality in other wildlife species (Appel and Summers 1995). It has been speculated that in this region peccaries may also be particularly susceptible to CDV, though this has yet to be studied and confirmed in the study region. Additionally, dogs can transmit generalist intestinal helminths by defecating in areas where wild mammals can come into contact with infective eggs. Dogs are often used for hunting and can even come into contact with wildlife through territorial conflicts (Fiorello et al. 2006).

Zoonoses are a concern due to the economic status of La Chorrera county and its vulnerability to neglected tropical diseases (Hotez et al. 2008). Because body condition was strongly correlated with site, and total number of parasites is associated with site, and we may infer that malnutrition may be contributing to these higher parasite loads. While we did not have a strong significant correlation between body condition, or site and *T. cruzi*, previous research has shown that malnutrition of dogs in Argentina lead to higher rates of canine *T. cruzi* infectivity to feeding vectors (Petersen et al. 2001). If sampling was continued, we may potentially see a similar trend given the current study had too small a sample size to make.

Because there was a significant negative association between body condition and cytokine expression, we may infer that dogs that are less healthy are more likely to have an increased cytokine inflammatory response. Lagartera Grande had the most significant levels of IFN-γ expression, which may be an indicator that the immune systems of the dogs are being challenged more so than the other sites. This may potentially be associated with a recent distemper outbreak in the community. Therefore, it is difficult to distinguish if the increased IFN-γ levels are correlated with the health of the dog or recent distemper outbreak. Additionally, there was a significant association between IFN-γ expression and parasite richness, and the dogs in Lagartera Grande with lower body condition, high IFN-γ expression and high parasite richness further emphasizes that there are many variables that contribute to dog health. Future directions on this portion of the study would be to complete a more comprehensive cytokine profile conducted to focus on cytokine response, in addition to a subsequent field season to compare distemper seroprevalence. The interaction between Th1 and Th2 cytokine expression tradeoff can also be evaluated within the context of multiple/coinfections, and this could be accomplished with additional sampling as well.

The primary limitation of our study was our sample size and sampling from 3 different communities only once, so there were no additional site replications of this study. The next step of this study would be to collect more samples to increase the sample size and power of the research by expanding to additional communities with different economic statuses and resampling the communities several times. Subsequent

field seasons would shed insight on transmission, immune function changes, physiological changes, and potentially seasonality (for example, the cytokine differences could have been due a recent distemper outbreak that only occurred in that location).

Despite limitations, the conclusions of the study that more rural, isolated, impoverished communities have domestic animals with higher pathogen richness and greater potential to spread diseases to humans and wildlife, are informative for poverty-related diseases and recommendations for prioritizing targeted communities for possible disease control strategies to prevent zoonotic and wildlife diseases originating from domestic animal populations.

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CHAPTER THREE

CONCLUSIONS

In addition to the limitations of the studies and future directions mentioned in the previous chapter, this project can be augmented with mathematical modeling. A vector-borne Ross-MacDonald mathematical model had been used to look at transmission of *Trypanosoma cruzi* (Figure 3.1) and then modified look at transmission dynamics of *T. cruzi* in a coinfected population (Figure 3.2). While our study had intended on using our field data to parameterize our models, our sample size was insufficient to implement in our models to examine transmission dynamics in the Panama communities. Additionally, a larger sample size would allow us to look more closely at the interaction of coinfection and transmission when a host is infected with multiple pathogens.

We used a compartmental vector-borne mathematical model developed for this study system, as modified from the Ross-MacDonald vector-borne model (Macdonald 1957). Figure 3.1 illustrates the S-I vector-borne model for this system. The Recovered compartment is changed to "D" to represent dead animals as once a host is infected with *T. cruzi*, the host is infected for life. This model shows transmission occurring as an infected vector (I_V) bites a susceptible dog (S_N) at bite rate "b", the dog acquiring *T. cruzi* with transmissibility "δ", and then a susceptible vector (S_V) biting tan infected dog (I_N) at the same bite rate "b" and the vector acquiring *T. cruzi* with transmissibility "α". Disease-

induced mortality is denoted as "γ". Also included in the model (not in figure) are the natural birth and death rates for both the vector and dogs. Because we had collected prevalence data for each community and we have vector abundance of reduviid bugs in the region (Gottdenker et al. 2011), data can then be put into the model to estimate remaining unknown parameters, particularly the transmission rate, by reconciling model and empirical disease dynamics. Regional variation in transmission rate can then be assessed with respect to body condition (Petersen et al. 2001). However, subsequent field seasons of resampling would be required to narrow down parameter estimates as it is difficult to exercise with the current smaller sample size.

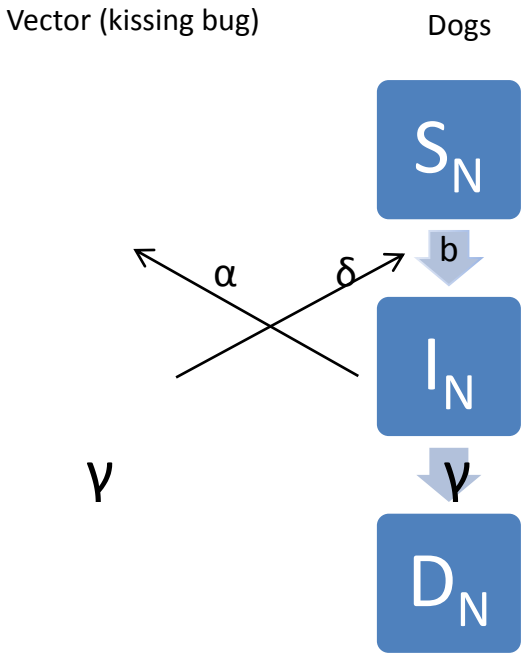

Figure 3.1. A Ross-McDonald Model applied to the *Trypanosoma cruzi* system.

The model was also extended to examine coinfection of *Trypanosoma cruzi* and intestinal helminths (Figure 3.2). The model was extended to include two classes of dogs,

dogs with worms (denoted with a “W” subscript) and dogs with no worms (denoted with an “N” subscript). Table 3.1 defines all the parameters for this model. From the model and the defined parameters, we constructed differential equations correlated with the compartments of this model in order to mimic true transmission (Table 3.3). Much of the parameters in this model would have to be obtained through xenodiagnoses and therefore would require more extensive field work and laboratory studies.

With the ability to create a model for both coinfection with intestinal helminths and *T. cruzi*, and also a model for *T. cruzi* transmission for populations with lower body conditions we can simulate conditions and examine transmission dynamics. Once we are able to examine these dynamics, we can potentially simulate different control interventions, like deworming a population, or implementing a nutritional regiment that results in dogs to have high body conditions.

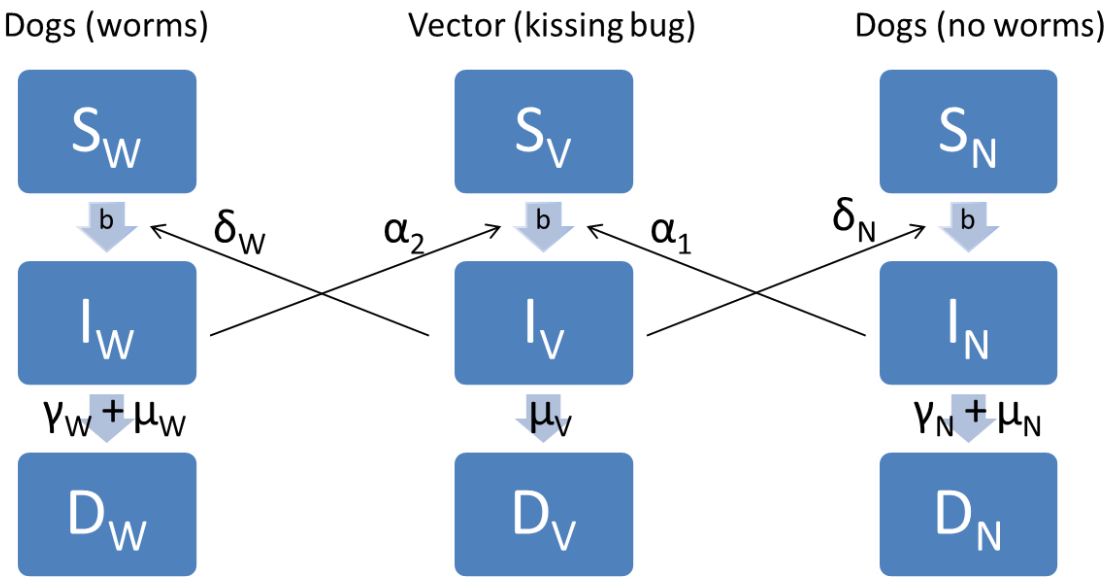

Figure 3.2. A modified Ross-McDonald model extended to incorporate *T. cruzi* transmission in an intestinal helminth coinfected population.

Parameter	Definition	Assumption and how to obtain
b	Rate of a vector biting a host	This parameter remains constant despite vector infection status, may have to be obtained through xenodiagnoses studies
α_1	Transmissibility of *T. cruzi* when an infected dog with no worms is bitten by a susceptible vector. Also called infectivity.	Infectivity, this parameter should impact disease transmission model.
α_2	Transmissibility of *T. cruzi* when an infected dog with worms is bitten by a susceptible vector. Also called infectivity.	Infectivity, this parameter should impact disease transmission model. According to reservoir competence definition, should be $> \alpha_2$.
δ_N	Transmissibility of *T. cruzi* when an infected vector bites a susceptible dog with no worms	Can to be determined through xenodiagnoses or varied, (Petersen et al. 2001).
δ_W	Transmissibility of *T. cruzi* when an infected vector bites a susceptible dog with worms	Can be determined through xenodiagnoses or varied, (Petersen et al. 2001).
$\sigma_{W,V,N}$	Natural birth rate of dogs with worms, vectors, and dogs with no worms	Can be varied in the model
$\mu_{W,V,N}$	Natural death rate of dogs with worms, vector, and dogs with no worms (respectively)	Can be varied in the model
$\gamma_{W,N}$	*T. cruzi* induced mortality of dogs with worms and dogs with no worms, respectively	Assumed to be very small for hosts due to time restraint of 100 days.

Table 3.1. The parameters used in the current models and assumptions made for those parameters.

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