Domestic dog health worsens with socio-economic deprivation of their home communities

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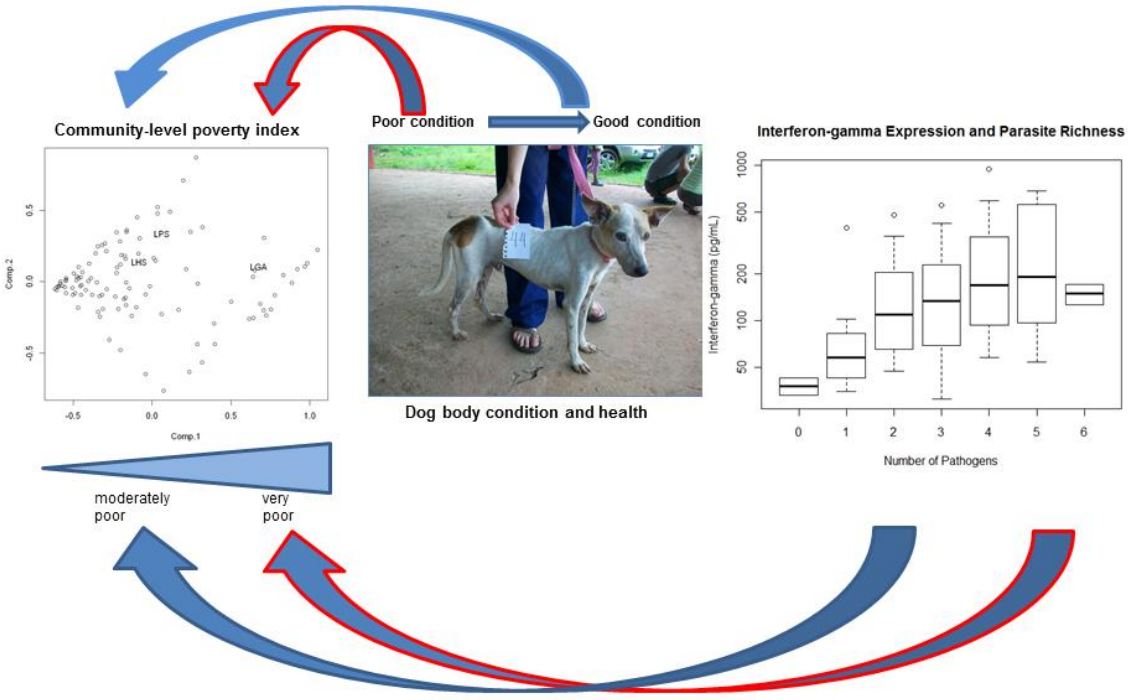
Evaluating relationships between poverty and dog health, we find that dogs in poor communities are sicker and more likely to be infected with zoonotic pathogens.



Domestic dog health worsens with socio-economic deprivation of their home communities

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Abstract

Dogs play an important role in infectious disease transmission as reservoir hosts of many zoonotic and wildlife pathogens. Nevertheless, unlike wildlife species involved in the life cycle of pathogens, whose health status might be a direct reflection of their fitness and competitive abilities, dog health condition could be sensitive to socio-economic factors impacting the well-being of their owners. Here, we compare several dog health indicators in three rural communities of Panama with different degrees of socio-economic deprivation. From a total of 78 individuals, we collected blood and fecal samples, and assessed their body condition. With the blood samples, we performed routine hematologic evaluation (complete blood counts) and measured cytokine levels (Interferon-γ and Interleukin-10) through enzyme-linked immunosorbent assays. With the fecal samples we diagnosed helminthiases. Dogs were also serologically tested for exposure to *Trypanosoma cruzi* and canine distemper virus, and molecular tests were done to assess *T. cruzi* infection status. We found significant differences between dog health measurements, pathogen prevalence, parasite richness, and economic status of the human communities where the dogs lived. We found dogs that were less healthy, more likely to be infected with zoonotic pathogens, and more likely to be seropositive to canine distemper virus in the communities with lower economic status. This study concludes that isolated communities of lower economic status in Panama may have less healthy dogs that could become major reservoirs in the transmission of diseases to humans and sympatric wildlife.

Keywords: domestic dog; zoonotic diseases; body condition; health assessment; poverty-disease; body condition; Panama; tropical; wildlife diseases; *Trypanosoma cruzi*; Canine distemper; helminthiasis

Introduction:

Because humans, domestic animals, and wildlife commonly share pathogens, targeting domestic animal populations for detection and control of zoonotic diseases (e.g. canine rabies vaccination) can minimize transmission to human populations and is often a more cost effective and feasible alternative to interventions in human populations, as animals are commonly reservoirs for zoonotic infections. Surveillance and control of domestic animal pathogens is also important to wildlife conservation and management efforts, because domestic animals can be reservoirs for pathogens that can negatively impact wildlife populations. For instance, domestic animal pathogens have been found to have a high transmission risk and negatively impact wildlife carnivore populations (Cleaveland and Dye 1995, Cleaveland et al. 2000, Fiorello et al. 2006, Butler et al. 2004, Zinsstag et al. 2011).

The probability that an animal will be a good reservoir for a pathogen is dependent on complex interactions between extrinsic factors (ecological conditions outside the host body, e.g. nutritional resource availability, habitat structure, vector presence, climate) and intrinsic factors (internal mechanisms within the host body, e.g. genetics, sex, age, innate/adaptive immunity, coinfections with other pathogens) (Wakelin 1975, 1978, Wakelin 1996, Christe et al. 2000, Bize et al. 2008, Molyneux et al. 2011). The overall nutritional condition of a host could impact its ability to fight off infection, and hosts with underlying poor 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 feedback on animal health (Beldomenico et al. 2009, Beldomenico and Begon 2010). This negative feedback cycle between animal health and infectious diseases can exacerbate disease, making less healthy animals more likely to be infected with pathogens (Craig et

al. 2008, Beldomenico and Begon 2010).

Similarly, human poverty and infectious disease can be related in a positive feedback cycle, because limited resources or social marginalization associated with poverty may lead to increased susceptibility to infectious disease due to a variety of ecological and nutritional factors (Factor et al. 2013, Bonds et al. 2010, Chaves et al. 2008, Karpati et al. 2002, Levins and Lopez 1999, Levins 1995). Although there are theoretical and empirical studies that explore relationships between poverty and disease in human populations (Plucinski et al. 2012, 2011, Chaves 2008), there is little attention given to the impact that human poverty may have on veterinary health, and how declines in animal health are related to human poverty.

Because dog and human health are closely linked, we would expect dogs living in high-poverty communities to receive little or no veterinary care (due to lack of owner resources to pay for care or lack of transportation to a veterinary clinic) and may receive less or poor quality food to eat in the households under economic duress. Domestic animals can also be impacted by infectious diseases and poverty levels. For instance, dogs and cats in impoverished Chilean regions have been found to have a higher infection rate of zoonotic diseases than dogs and cats in wealthier areas of Chile (Lopez et al. 2009, Schneider et al. 2011). In rural Panama and elsewhere, dogs are reservoirs for diseases that include trypanosomiasis, leishmaniasis, helminthiases (both intestinal and visceral larval migrans), scabies, Leptospirosis, and toxoplasmosis (Etheredge et al. 2004, Cardinal et al. 2007, Dantas-Torres 2007, Labruna et al. 2009, Deplazes et al. 2011, Jenkins et al. 2011, Petersen et al. 2011, Teichmann et al. 2011); however, little is understood about relationships between infectious diseases of domestic dogs, health status, and dog owner economic status.

The objectives of this study are 1) to evaluate dog health status in a rural area of Panama by measuring body condition, hematological and immunological parameters (Th1 and Th2 cytokine production), and infection with a diversity of macro and microparasite pathogens (especially those important to human and/or wildlife health in the studied area), 2) to evaluate relationships between

these canine health indicators, pathogen diversity, pathogen type, and socioeconomic deprivation at a community level.

First, we measure canine exposure to *Trypanosoma cruzi*, the causative agent for Chagas disease, intestinal helminths (hookworms and roundworms), and canine distemper. 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, Hotez et al. 2008). *T.cruzi* is transmitted from a variety of wild and domestic mammalian reservoir hosts, including dogs (Cohen & Gurtler 2001) to humans by the kissing bug vector (family *Reduviidae*). Soil transmitted helminths, particularly roundworms and hookworms, can be zoonotic, and can cause illness or hindrances to physical and mental growth in children (Dujardin et al. 2010). Canine distemper virus is a pathogen that can be transmitted from domestic dogs to wildlife carnivores with potentially high morbidity and mortality (Munson et al. 2008).

Second, we evaluate canine health indicators (body condition, hematology, and cytokine production). We expect dogs in lower body condition to have higher number pathogenic infections. Because of the many pathogens co-occurring in rural Panama (Pineda et al. 2011, Saldaña et al. 2013), we expect dogs in poor health to have hematological abnormalities (e.g. anemia, leukocytosis or leukopenia), and an increased immune response to polyparasitism. For example, intracellular microparasites, such as *Mycobacterium,* stimulate the Th1 arm of adaptive immunity, allowing increased secretion of Th1 cytokines (TNF-B, IFN-g). On the other hand, a Th2 immune response (cytokines IL-4, IL-5, IL-10, IL-13) may dominate after host infection with macroparasites (Abbas et al. 2012).The cytokines measured in the study are Interferon-γ (Th1 arm of adaptive response, expected to be high in the presence of *T. cruzi* and CDV infections) and Interleukin-10 (Th2 arm of adaptive response, expected to be high in the presence of parasitic worms).

METHODS

Study design:

The study was conducted in 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 1). Because of the cyclical feedback between heath and parasites, we conducted a cross-sectional observational study on community-volunteered domestic dogs for pathogen infection to test our hypotheses. The pathogens selected for this study were Canine Distemper Virus (CDV), *Trypanosoma cruzi* (causative agent for Chagas disease), canine heartworm (*Dirofilaria immitis*), 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 animal populations.

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. The dogs sampled from each community were estimated to be between 50-60% of the population of dogs in the community, based on visual counts and owner interviews. This proportion is powerful enough to make inferences about the prevalence of pathogens in each community, assuming a population size equal or less than 60 dogs per village, which given the lack of any prior information, requires a minimum sample size of 26 dogs to have prevalence estimates of 50% with a 15% precision (Kish, 1965).

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 Trypanosoma Detect™ Rapid Test, IDEXX

Hearworm 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 in 70% ethanol.

Body condition scoring: 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 between1-3 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 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.

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 hematological status. 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 and packed cell volume was evaluated by centrifugation of capillary tubes. Reference ranges for canine panels were taken from Schalm's Veterinary Hematology (Weiss et al. 2010). These reference values have wide range to take into account variations among dogs from many different locations, and were within the values for healthy dogs reported in Colombia (Bossa-Miranda et al. 2012). Anemia was characterized by decreases in one or more of the following: red blood cell (RBC) count ($\times 10^6/\mu L$; reference interval 5.5-8.5$\times$ $10^6/\mu L$), hemoglobin (HGB, mg/deciliter; reference interval 12.0-18.0 mg/dL), and packed cell volume (PCV, 37.0-55.0%). White blood cell counts were considered normal between the range of 5,500 and 19,500 leukocytes/ µL. 100 cell differential white blood cell counts were also performed on freshly prepared, Wright-Giemsa stained blood smears.

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) following manufacturer's recommendations. For the Interferon-γ, the manufacturer's reported sensitivity (based on 17 assays) was a mean minimum detectable dose of 25 pg/mL, with a range of 8-16 pg/mL, and no cross-reactivity was observed with recombinant canine IL-4, IL-6, IL-8, IL-10, GM-CSF, MCP-1, TNF-α, or VEGF. For IL-10, the manufacturer's reported sensitivity (based on 20 assays) was a mean minimum detectable dose of 2pg/mL with a range of 1-2.8 pg/mL and no cross reaction was observed with recombinant canine IFN-γ or IL-4.

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* (sensitivity 100%, specificity 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 blood sample to search for trypanosome DNA fragments in host blood. The final method for *T. cruzi* detection was through blood culture for live trypanosomes.

Canine Distemper Virus (CDV): CDV was detected at the University of Georgia Diagnostic Lab using collected serum with a serum neutralization assay. 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) (Roberts & Janovy 2000) 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. Fleas and removed ticks were stored in in a 1 mL eppendorf tube in 10% ethanol for further identification.

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 groups: zoonotic (*T. cruzi*, hookworms, roundworms, tapeworms) or potential wildlife pathogens (CDV, *T. cruzi,* hookworms, roundworms, tapeworms). Because dogs can be a reservoir of *T.* cruzi, (Gurtler et al. 2007), this pathogen is considered zoonotic. Regarding other pathogens considered zoonotic, canine hookworms can cause creeping eruption (cutaneous larval migrans) in humans and roundworms can cause visceral larval migrans in humans (Robertson and Thompson 2002, Robertson et al. 2000). Humans can accidentally acquire tapeworm infections from dogs by accidental ingestion of *Dipylidium caninum* fleas containing the infective stage of the parasite (Robertson and Thompson 2002). Canine distemper is a generalist virus of canids that can be transmitted from domestic dogs to wild carnivores, such as coati mundis, raccoons, (Kapil and Yeary 2011, Alexander et al. 2010). Canine hookworms and roundworms can be shared between domestic and many wild carnivore species (Wapenaar et al. 2013), and occasionally cause aberrant larval migrations in a variety of wildlife species, and tapeworm species may be shared or

transmitted from domestic dogs to wild carnivores (Viera et al. 2012). 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 and poverty quantification.

Census data were obtained from the publically available Panama government database at the National Institute of Statistics and Census (http://www.contraloria.gob.pa/inec/). Census data were used to compare economic status of the communities. Table 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 most economically disadvantaged community. To quantify poverty we developed a poverty index by performing a principal component analysis (PCA) on the three variables described in Table 1 and considering the 125 rural villages in La Chorrera county of Panamá Province, Republic of Panamá.

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, positive/negative of *T. cruzi*, presence/absence of helminths (for all helminths and individual helminths type), positive/negative distemper, presence/absence of zoonoses, and wildlife parasite using a Kruskal-Wallis nonparametric test and Mann-Whitney U test when applicable. We employed these non-parametric tests given the nature of our data, which was mostly presence/absence and scores, quantities best suited for non-parametric statistics. We also compared the association between site and parasite diversity and presence/absence of zoonotic pathogens using Contingency table analyses). 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 (Conover 1999).

Multiple correspondence analysis was also used to evaluate relationships between site and a variety of other categorical variables related to dog health and pathogen exposure. Multiple correspondence analysis allows for the visualization of associations between two or more categorical variables. The data set is decomposed and then the data is commonly projected in two dimensions representing two singular vectors with the largest values. Categorical variable centroids can be plotted and the proximity between different variables allows for the evaluation of the association between levels of categorical variables (Venables & Ripley 2002). We evaluated the following relationships: site, anemia, and distemper infection, and zoonotic pathogen presence. Finally, we compared the association of body condition score and zoonotic parasites, wildlife parasites, and immune response through cytokine data 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 was found to be 3.2, 95% CI [2.97, 3.44], with Lagartera 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 population consisted of 65% males (95% CI [53.98, 75.56]) and 35% females (95% CI [24.44, 46.32]), and the mean age at 30.2 months (95% CI [21.94, 38.47]. There was a significant difference in body condition score between each sites (Kruskal-Wallis test, χ^2=22.02, df=2, p=1.7e-5).

Hematology

Of all dogs across communities, 55.1%, 95% CI [46.02, 82.76] were anemic (67.7% in Lagartera Grande, 52.0% in Las Pavas, and 46.2% in Los Hules). All were classified as likely nonregenerative anemia due to MCV and MCHC within or below reference range, Red Blood Cell (RBC) countsof less than 5.5× 10^6/μL or HGB less than 12.0 g/dL. Of the 75 dogs we evaluated, 46.7% had a

leukocytosis, 13.3% were leukopenic, and 52% had normal total white blood cell counts. Thirty two percent of dogs had a neutrophilia, 29.3% of dogs had a lymphocytosis, and 33.3% had an eosinophilia. There was no significant difference of any blood values between sites or univariate relationships between body condition and white or red cell blood values.

Parasite prevalence

Seven dogs tested positive for antibodies to *T. cruzi* (4 from Lagartera Grande, 1 from Las Pavas, 2 from Los Hules). The PCR confirmed two dogs that were positive for *T. cruzi* on the antibody test. Trypanosomes were not detected on blood smears. *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-square test, $\chi^2 = 1.94$, df = 2, p = 0.38) but there was significant association between site and presence of helminth infection (Chi-square test, $\chi^2=7.9357$, 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. No dogs tested positive for heartworm on the antigen snap test, and an unidentified microfilaria was detected in the blood of one dog by direct microscopy.

PARASITE EFFECT

Parasite richness, infection with zoonotic and wildlife pathogens, and body condition

There was a statistically significant negative association between body condition and total number of different parasite types identified (Spearman's correlation, $\rho=-0.368$, S= 108193.5, $p<0.001$).

Across all dogs, there was no difference between body condition score and the presence or absence of zoonotic parasites (Mann-Whitney U test, W=8.812.5, p=0.096) There was a significant difference between (Mann-Whitney U test, W=430.5, p=0.024) body condition scores in animals and the presence of wildlife parasite infections. Animals that were positive for distemper had a

significantly lower body condition score than animals that were distemper negative (Mann-Whitney test, W=928.5 p=0.001), and there was significant association between site and presence of distemper (Chi-square test, χ^2=11.58, df =2, p=<0.0001). There were, however, no significant differences in body condition and *T. cruzi* presence (Mann-Whitney U test, W=302.5, p=0.33). There was no difference between body condition score and 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 3, Spearman's correlation, ρ=0.344, S= 51906.04, p= 0.002), 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 difference 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). Nor was there a significant difference between IFN-γ expression and zoonotic parasite presence (Mann-Whitney U-Test, W=520.5, p=0.13), and IFN-γ expression and wildlife parasite presence (Mann-Whitney U-Test, W=218.5, p=0.15)

Site Effect

Poverty indices

A poverty index was estimated based on principal components analysis (PCA) of the variables in Table 1 and considering all rural villages in La Chorrera county (n=125). The first PC, which accounted for 67% of the variability in the PCA, is a weighted average of the three variables

(Loadings on Table 2, where larger values indicate a more destitute community, thus rendering the 1st PC a reliable poverty index. The higher the index, the more impoverished the community. The poverty index for Lagartera Grande was 0.673, 0.062 for Las Pavas, and -0.082 for Los Hules. By performing the PCA we can also show that LGA is an extremely poor village in the context of this study, but also for La Chorrera county, while LHS is slightly better off than LPS, and both LHS and LPS are far from being as poor as LGA (Figure S1). When developing the index using a PCA we tried more variables, but dirt floors were strongly well correlated with income and other house materials rendering those additional variables not informative for the development of an index.

Body condition and site

There was a significant association between body condition and site (Figure 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 body condition in Lagartera Grande and Los Hules. A significant difference was found in body condition between Lagartera Grande (LGA) and Las Pavas (LPS, p=0.02) and between Lagartera Grande and Los Hules (LHS, p=0.001). GLM results predicted a significantly higher body condition dogs from in Los Hules ($p< 0.001$) compared to those in Las Pavas and Lagartera grande.

Parasite richness, zoonotic, and wildlife diseases and site

While there was a statistically insignificant association between site and parasite richness (Chi-square 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-square test, χ^2=12.00, df=2, p=0.0025), but no significant association site and parasites of concern to wildlife populations (Chi-square 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-square test, χ^2=11.58, df=2, p=0.0031).

Multiple correspondence analysis of the relationships between zoonotic pathogen presence, site, anemia, and distemper show (Figure 5) a relatively close association between dogs not carrying

zoonotic infections and residing in the wealthiest community (LHS), an association between canine distemper and site (LGA), with less wealthy communities (LGA and LPS) associated with dogs infected with zoonoses.

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 6) but there was not a significant association between IL-10 expression and site (Kruskal-Wallis, χ^2=0.625, df=2, p=0.732)

Discussion:

This study examines relationships between poverty, pathogen richness, and domestic animal health. The conclusions of the study are that 1) dogs with lower body condition and higher plasma interferon gamma levels were found in more impoverished communities, 2) dogs with lower body condition had higher parasite richness and also increased cytokine response, and 3) dogs in the region of La Chorrera, Panama have the potential to harbor and transmit diseases to humans and to wildlife, particularly those dogs in poor body condition.

In our study, polyparasitism was greater in animals with lower body condition. 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 allowed 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). Disease-driven poverty traps are formed when income near poverty levels and infectious disease conditions interact, and theoretically can be released by improving the health condition of the population (Bonds et al. 2010). Because body condition was strongly correlated with site and total number of parasites is associated with site, we may infer that malnutrition may be contributing to these higher

domestic dog parasite loads.

Overall, dogs in communities of poorer economic status were less healthy 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, highest proportion of anemic animals, 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 for their animals as communities closer to areas with better infrastructure, and owners may not be able to afford adequate food or veterinary care for their pets. Future studies should include surveys of domestic dog care among communities in relationship to socioeconomic marginalization. Additionally, there may be cultural differences in pet rearing and it is possible that the majority of rural inhabitants, regardless of economic status, do not customarily take pets to the veterinarian for routine 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.

Because there was an association between site and distemper infection, and Lagartera Grande had the highest seroprevalence of distemper (81.5%, 95% CIs [61.25, 92.97]), that community has a greater potential to spread distemper to wildlife carnivores. Lagartera 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 wildlife carnivores, 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 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 out 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 is a concern due to the economic status of La Chorrera county and neglected tropical diseases. Dogs in Las Pavas were less likely to be infected with zoonotic pathogens (Figure 5) than dogs in the communities that were less wealthy. While we did not found a strong significant correlation between body condition or site and *T. cruzi* infection, 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's sample size was too small to detect an association between body condition and *T. cruzi* infection.

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). 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.

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 or polyparasitism. Therefore, it is difficult to distinguish if the increased IFN-γ levels are correlated with the overall health of the dog or recent distemper outbreak. Further

directions on this portion of the study would be to complete a more comprehensive cytokine profile in dogs across the communities, in addition to further field sampling in these communities to compare levels of IFN-γ and distemper. The interaction between Th1 and Th2 cytokine expression tradeoff can also be looked within the context of multiple/coinfections, and this could be accomplished with additional sampling as well.

Although Lagartera Grande harbored the greatest proportion of anemic dogs, significant proportion of dogs (55%) across all communities were anemic with a non-regenerative anemia. Non-regenerative anemia can be due to chronic parasitism or other chronic infection (Weiss et al. 2010). Underlying causes for the WBC abnormalities seen in the dogs are uncertain, but eosinophilia is often attributable to ectoparasites or other macroparasites, such as helminths (Fabre et al. 2009). Leukocytosis and lymphocytosis may be due to epinephrine-mediated responses associated with blood collection or in some instances, inflammation.

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. Because of the risk dogs pose for zoonotic and wildlife diseases, domestic animal health must be considered together with information on owner economic status to develop disease control strategies, particularly in developing countries, countries of low economic status, or with large degrees of economic inequality.

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Table1. Percentage of houses within each community of La Chorerra County, Panama, that has dirt floors, no potable water, and no electricity. (Table adapted from INEC).

Site (n)	% Houses with dirt floors (n)	% Houses without potable water (n)	% Houses without electricity (n)
Lagartera Grande (71)	49.3% (35)	76.1% (54)	87.3% (62)
Las Pavas (83)	42.2% (35)	19.3% (16)	60.2% (50)
Los Hules (98)	34.6% (34)	23.5% (23.5)	38.8% (38)

Table 2. Principal components analysis (PCA) for the percent of houses with dirt floors, and lack of access to Potable water and electricity across the 125 rural towns of La Chorrera County, Panamá Province. Comp. 1 stands for the 1st principal component (PC), which accounted for 67% of the variability in the PCA, while Comp. 2 is the 2nd PC, which accounted for 23% of the variability in the PCA.

Loadings	Comp. 1	Comp. 2	Comp. 3
% Houses with dirt floors	0.324	0.458	0.828
% Houses without potable water	0.687	-0.715	0.127
% Houses without electricity	0.650	0.528	-0.547

Figure 1. Location of study sites in Panama.

Figure 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.

Figure 3. Boxplot of the association between parasite richness and IFN-γ expression (Spearman's correlation, ρ=0.344, S= 51906.04, p= 0.0021). Minimum and maximum IFN-γ levels are depicted by lines extending perpendicular to the vertical dotted line, small clear circles represent individual maximum outliers, the box represents upper and lower quartiles, and the median is represented by a bold black line within the box.

Figure 4. Boxplot of the relationship between site and body condition. A significant association was found between body condition and site (Kruskal-Wallis, χ^2=22.023, df=2, p=1.65e-5). Site abbreviations are as follows; LGA-Lagartera Grande, LPS-Las Pavas, LHS-Los Hules. The box represents upper and lower quartiles, and the median body condition value is represented by a bold black line. Maximum and minimum body condition scores for each site are delimited by the line extending perpendicular to the vertical dotted line extending from the box. For Los Hules, the minimum body condition score (3) is at the bottom of the quartile box.

Figure 5. Multiple correspondence analysis of associations between zoonotic pathogen presence, site, anemia, and distemper in domestic dogs). Site abbreviations are as follows; LGA-Lagartera Grande, LPS-Las Pavas, LHS-Los Hules. Anemia.Yes refers to anemic dogs, Anemia.no refers to non-anemic dogs, Zoonotic.N is the absence of zoonotic disease in dogs, zoonotic.y refers to dogs positive for zoonotic diseases. Distemper.YES and Distemper.NO refers to dogs positive and negative for antibodies to canine distemper virus.

Figure 6. Boxplot of the relationship between IFN-γ and Lagartera Grande (LGA), Los Hules (LHS), and Las Pavas (LPS) communities. Minimum and maximum IFN-γ levels are depicted by lines extending perpendicular to the vertical dotted line, small clear circles represent individual maximum outliers, the box represents upper and lower quartiles, and the median is represented by a bold black line within the box. Significant associations between site and IFN γ were detected with a

Kruskal-Wallis test (χ^2=51.1, df=2, p=8.01e-12).

Figure S1. Principal components analysis (PCA) for the percent of houses with dirt floors, and lack of access to Potable water and electricity across the 125 rural towns of La Chorrera County, Panamá Province, Republic of Panamá. LGA, LPS and LHS indicate the scores for Lagartera Grande, Las Pavas and Los Hules, respectively. Comp. 1 stands for the 1st principal component (PC), which accounted for 67% of the variability in the PCA, while Comp. 2 is the 2nd PC, which accounted for 23% of the variability in the PCA.

Figure 1

Figure 2

Figure 3

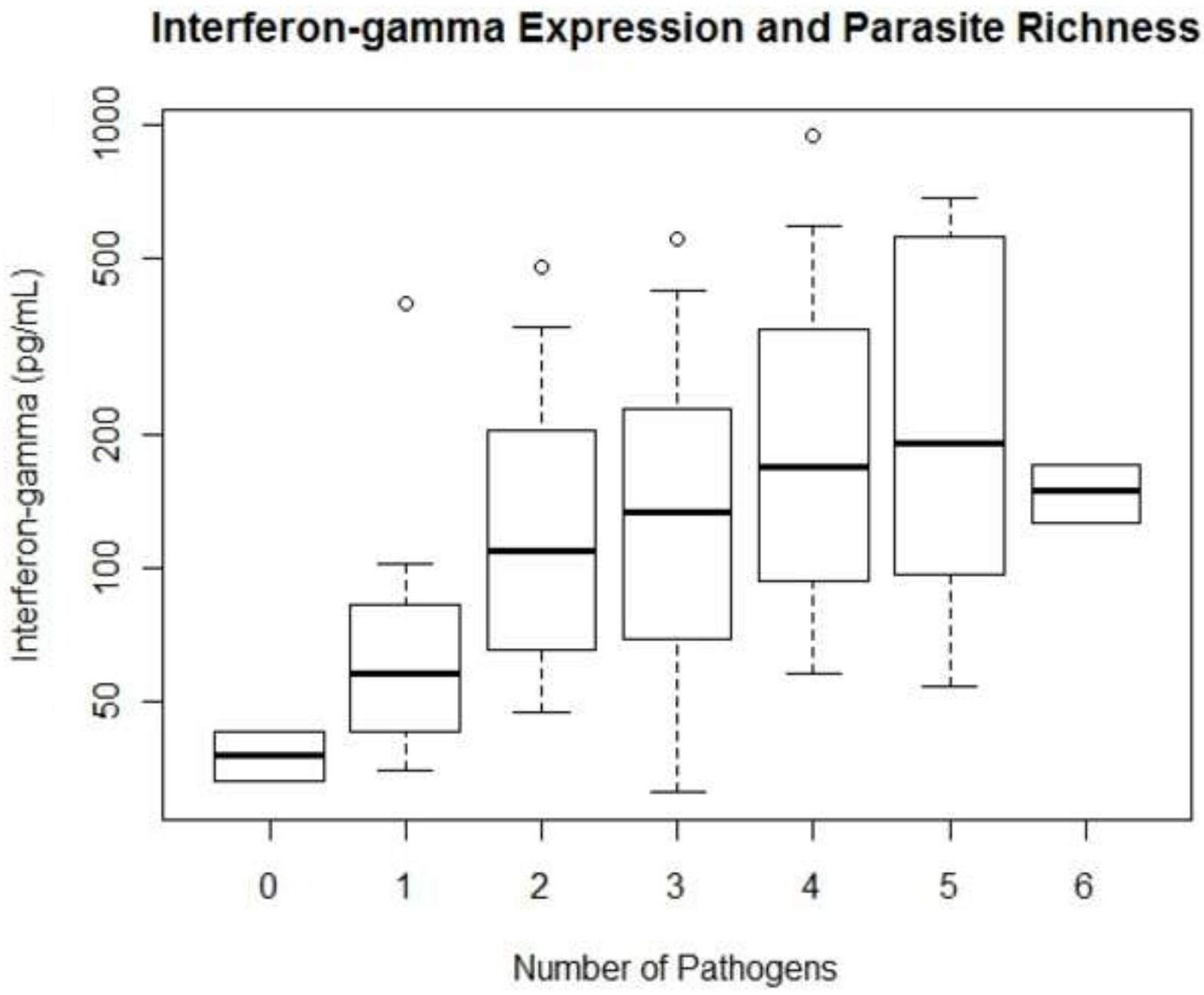
Interferon-gamma Expression and Parasite Richness
Interferon-gamma (pg/mL)
50
100
200
500
1000
0
1
2
3
4
5
6
Number of Pathogens

Figure 4

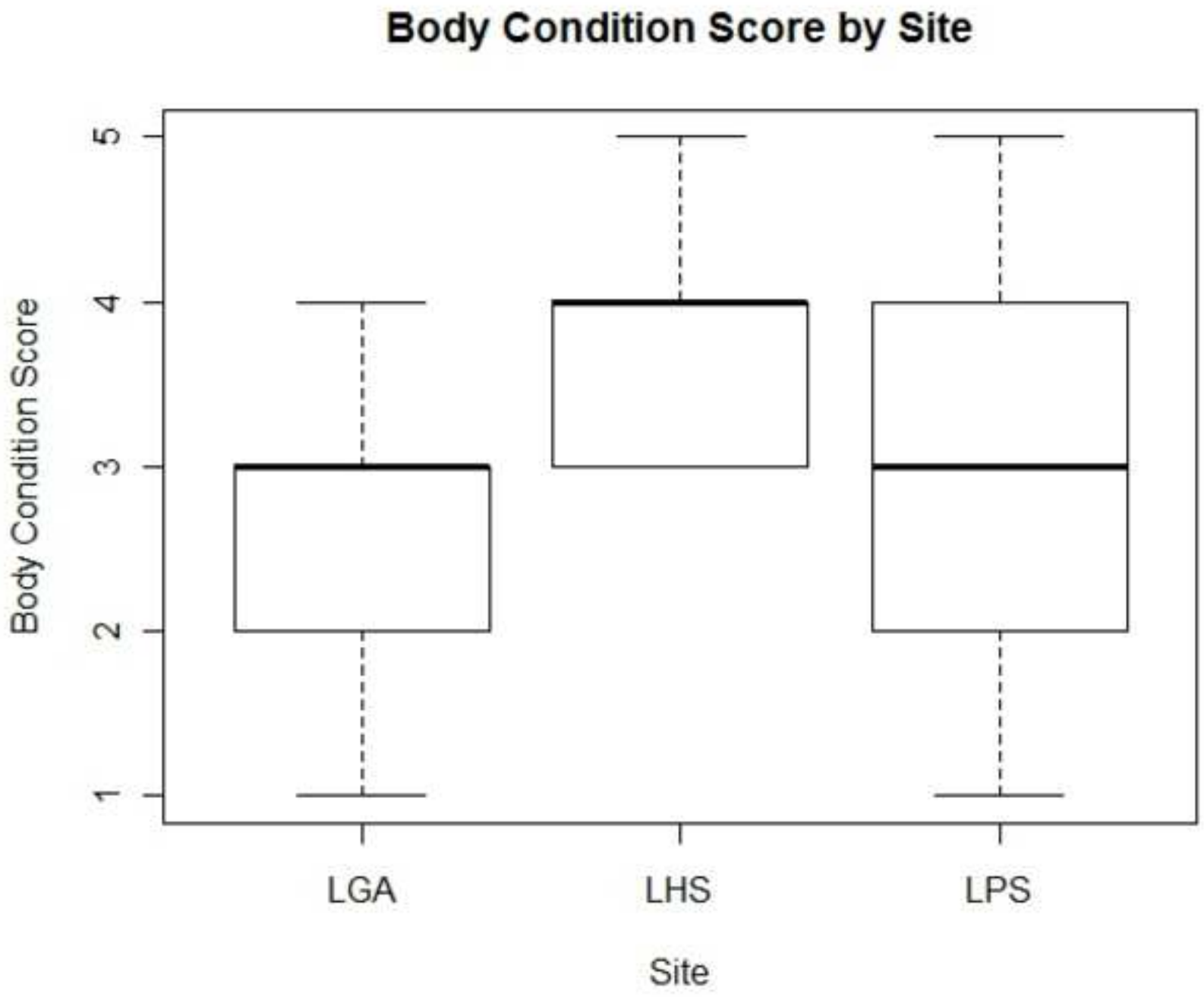
Body Condition Score by Site
Body Condition Score
1
2
3
4
5
LGA
LHS
LPS
Site

Figure 5

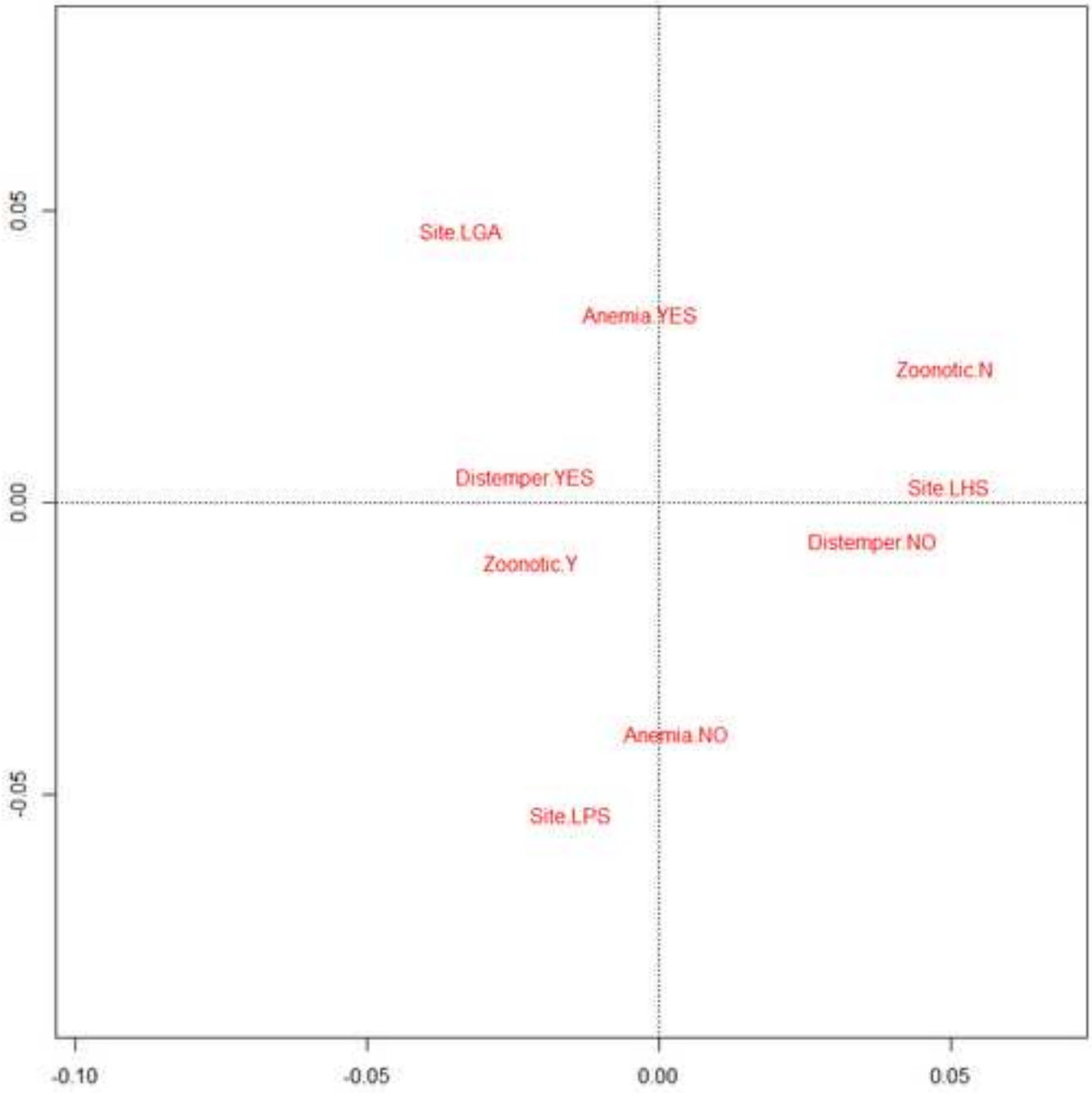
Site.LGA
Anemia.YES
Zoonotic.N
Distemper.YES
Site.LHS
Distemper.NO
Zoonotic.Y
Anemia.NO
Site.LPS
0.05
0.00
-0.05
-0.10
-0.05
0.00
0.05

Figure 6

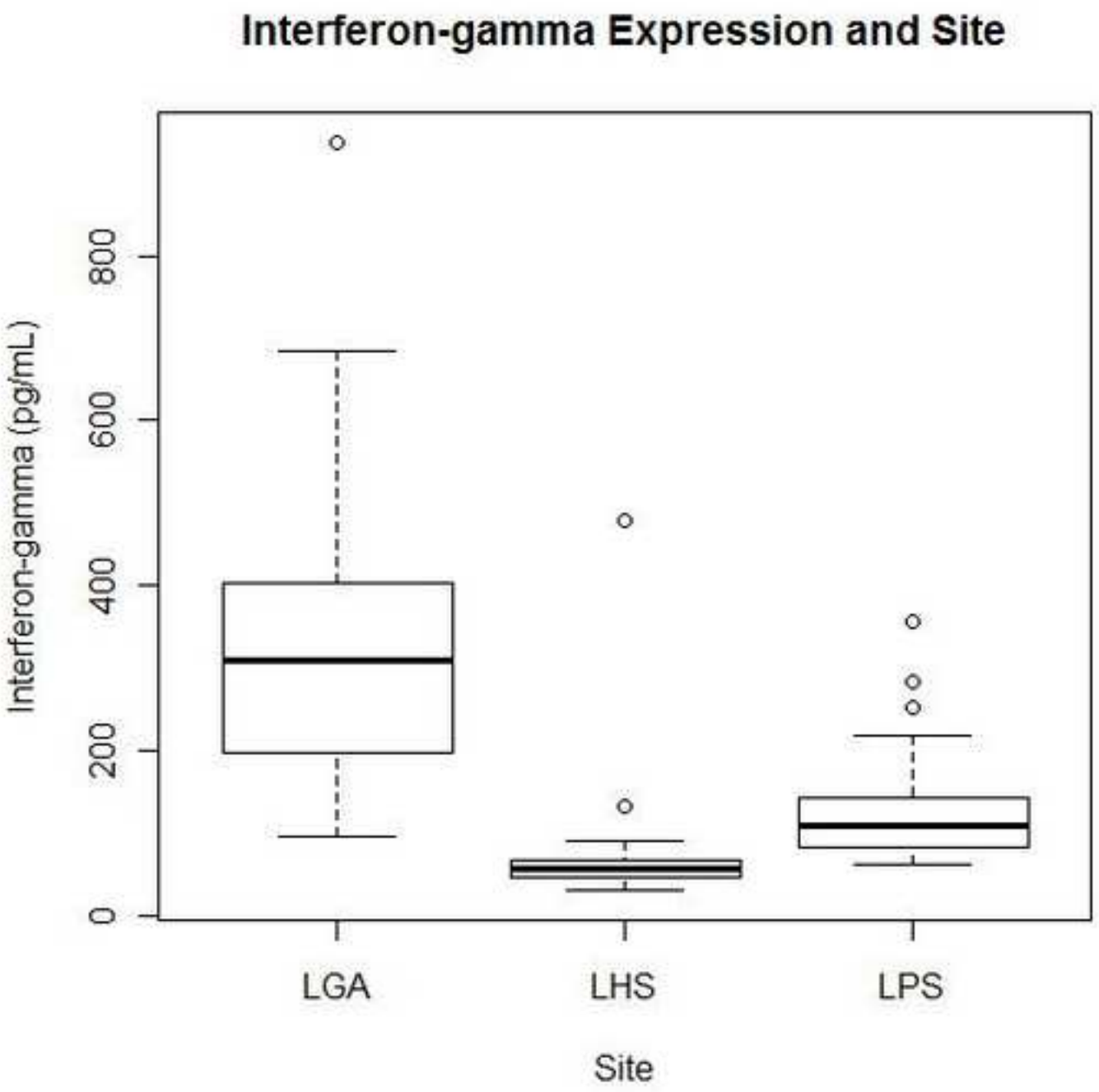
Interferon-gamma Expression and Site
Interferon-gamma (pg/mL)
0
200
400
600
800
LGA
LHS
LPS
Site

Figure S1