

Pathogen co-infection patterns within domestic dogs in rural environments

Djion A Holness¹, Amaka Nina Ananaba², Nicole L Gottdenker²
University of Connecticut¹, Storrs CT, University of Georgia, Athens GA²

Introduction

Pathogens can interact with each other within a host, which can influence patterns of co-infection in populations by complex interactions within the host's immune system; such as immunosuppression caused by infection with one pathogen which may influence susceptibility to infection with other pathogens.^{3, 4} Furthermore, behavioral and environmental factors, such as habitat-associated contacts with a vector, can influence patterns of host co-infection.^{1, 3}

Objective: To study relationships of intrinsic host factors and extrinsic (environmental) factors in relation to parasite/pathogen co-infections in domestic dogs from a rural landscape.

Study system: The pathogens in this study include a mosquito borne disease (heartworm, dirofilaria), kissing bug transmitted disease (Chagas disease caused by *Trypanosoma cruzi* (*T. cruzi*) infection, sandfly transmitted diseases (leishmania), and canine distemper (directly transmitted virus) within domestic dogs within individuals, households, communities, and across communities in rural Panama.^{2, 3}

Hypotheses

- Dogs in more isolated and and more forested communities will be more likely to be infected with a higher number of species, potentially due to lack of preventative care and complex habitats supporting a diverse array of pathogens.
- Vector-transmitted disease prevalence will be more associated with environmental factors (community or habitat type) than co-infection interactions
- Dogs in poor overall health (based on hematological analysis) are more likely to be co-infected with multiple pathogens.
- Animals who have been infected with/exposed to canine distemper (CDV) are more likely to be co-infected with multiple bloodborne pathogens, due to CDV-induced immunosuppression.

Background

- Data was collected from 275 domestic dogs from 6 communities that reside where there were distinct levels of deforestation (two replicates each of highly deforested, moderate deforestation, no deforestation), on each side of the Panama Canal.
- Intrinsic factors include dog nutritional status, sex, age, the type of pathogens co-occurring within a host, and host physiology/immune traits (e.g. red blood cell count, white blood cell count).
- Extrinsic factors include household type/dog care, community, and environmental (ecosystem, forest cover, altitude).


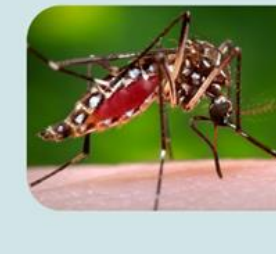






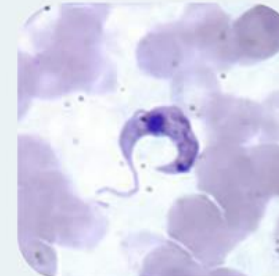

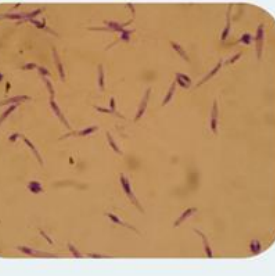
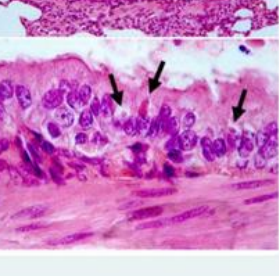
| | <i>Trypanosoma cruzi</i> | Dirofilaria | Leishmania | Canine Distemper Virus |
|-------------------|---|---|--|---|
| Vector | Kissing Bug | Mosquito | Sandfly | Direct transmission of virus |
| |  |  |  |  |
| Key Symptoms | Swollen lymph nodes, enlarged liver and spleen, dilated cardiomyopathy | Pulmonary artery blockage, coughing up blood, caval syndrome- cardiovascular collapse | Swollen lymph nodes, renal failure, nerve pain, intradermal nodes and ulcers, hyperkeratosis- thickening and depigmenting on muzzle and footpads | Eye redness, discharge from nose and eyes, immunosuppresses. |
| |  |  |  |  |
| Transmission mode | Kissing bug feces, consumption of an infected animal | Mosquito saliva from bite | Bite of infected sandfly | Airborne exposure, through placenta, contact with bodily fluids |
| Type of pathogen | Protozoa | Nematode | Protozoa | Virus |
| |  |  |  |  |

Table 1: Key information on each infection type studied.

Methods

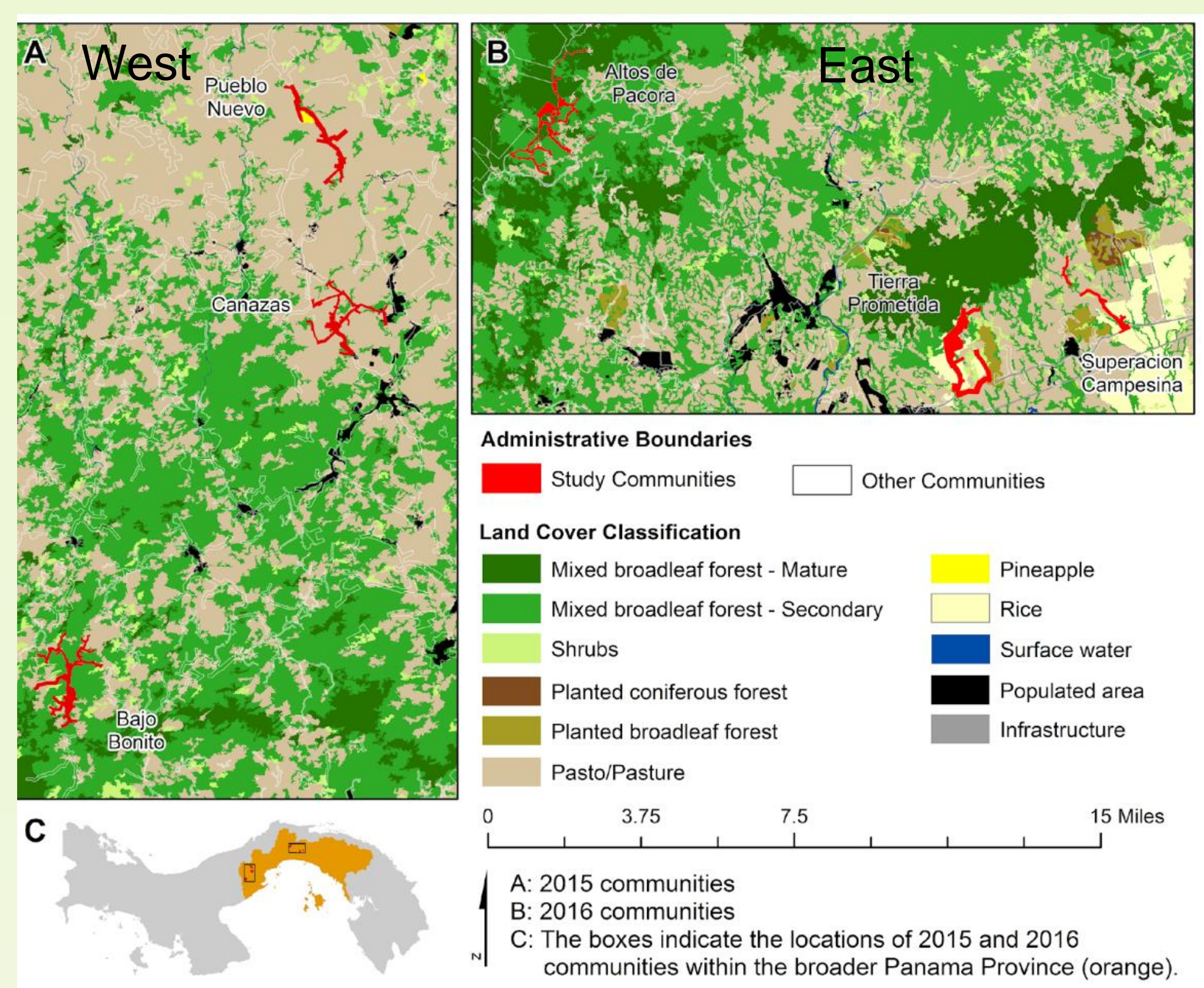


Figure 1: Map of studies sites with general vegetation data on surrounding area to the east and west of the Panama Canal. Bajo Bonito and Altos de Pacora (Forested), Cañazas and Tierra Prometida (Fragmented forests), Pueblo Nuevo and Superación Campesina (Deforested)




| Infection | Diagnostic Test Type |
|------------------------|--|
| <i>T. cruzi</i> | 4 tests performed, when an animal was positive on 2 or more of the following tests, or positive on PCR: immunochromatographic Rapid test dipstick assay (Inbios, Seattle, Washington), a Western Blot assay and an Immunofluorescent antibody test standardized for dog serum and using local <i>T. cruzi</i> strains from study sites in Panama, PCR for <i>T. cruzi</i> DNA in dog blood. |
| Dirofilaria | DiroCHECK® (San Diego, California) Heartworm ELISA, Tests for the presence of Dirofilaria antigen  |
| Leishmania | Leishmania serologic testing: Enzyme linked immunosorbent assay standardized for dogs and using local strains of Leishmania panamensis. |
| Canine Distemper Virus | Serum neutralization assay. Detects antibodies, by the absence of cytopathic effect (CPE). This assay cannot differentiate between positive antibodies from infection or from vaccination  Presence of CPE: Negative for CDV  Absence of CPE: Positive for CDV |

Table 2: Types of diagnostic tests for each infection.

- General body condition was evaluated for each dog from a standardized Purina Body condition scale for dogs. Blood samples were collected from dogs for hematologic and diagnostic assays
- T. cruzi*, leishmania, dirofilaria, and CDV exposure or infection were tested by a number of tests listed in Table 2 (above).

Results

Single Infection

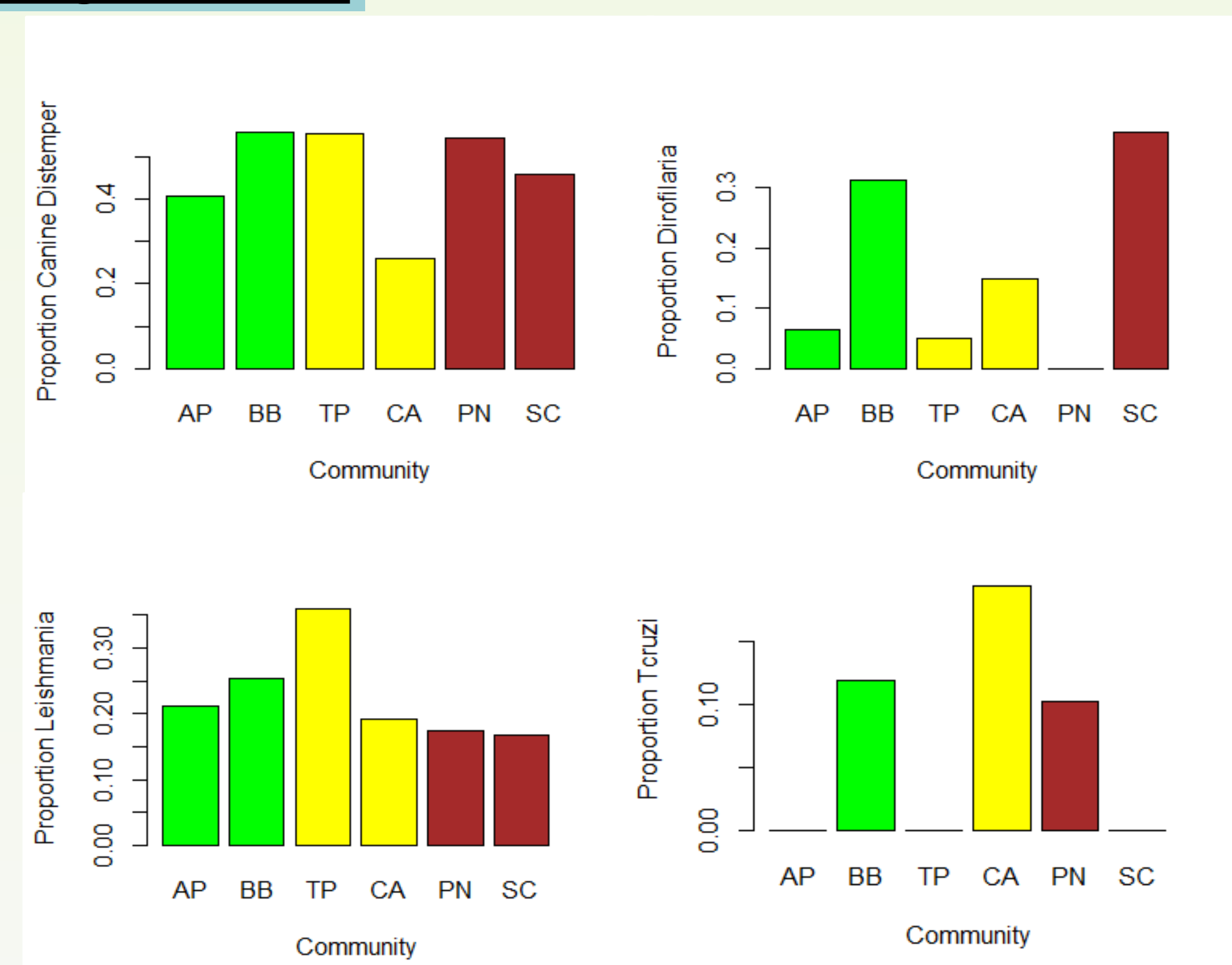


Figure 2: Proportion of infected individuals by community for each infection type. Rate of infection is heavily dependent on community. Green- forested, yellow- fragmented, red- deforested. The relationship between community and *T. cruzi* infection status is statistically significant (Fisher's Exact test, $p = 0.000830$). Dirofilaria infection status and habitat is marginally significant (Fisher's exact test, $p = 0.06605$). For the other two infection types there was no significant relationship between community and infection rate.

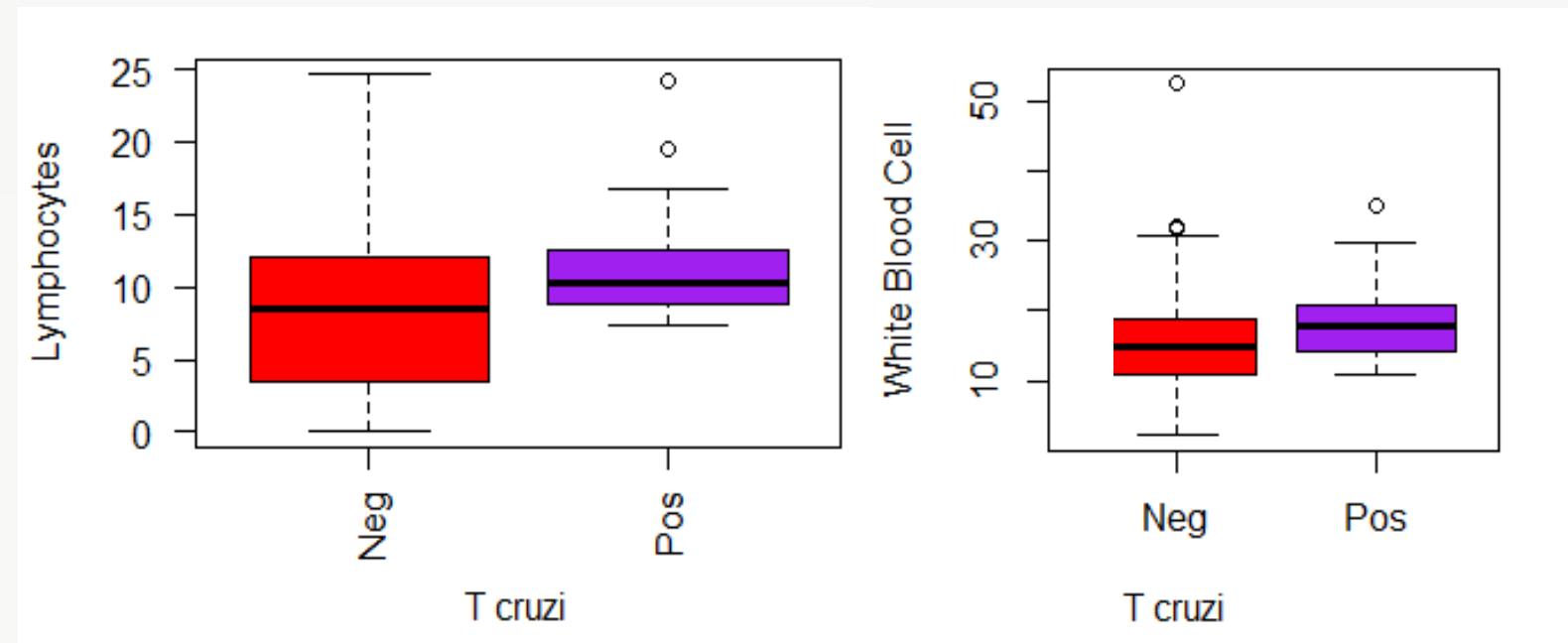


Figure 4: Lymphocyte and white blood cell count and *T. cruzi* presence.



Figure 3: Frequency (count) of infected individuals in each age group. (on left).

- There was no significant difference between infection and sex, although samples were male-biased.

There was a significant difference in *T. cruzi* infection status, total white blood cell (WBC), and lymphocyte (lym) cell count (10^3 cells/ μ l) count (Wilcox test, p -value 0.04602, and 0.01431 respectively), *T. cruzi* infection status and neutrophil count ($p = 0.04815$), and Leishmania with hematocrit ($p = 0.04456$). There was no significant relationship between dirofilaria and CDV infection data and hematology results.

Contact: djion.holness@uconn.edu

Results

Co- Infection

Species richness, community, and habitat type

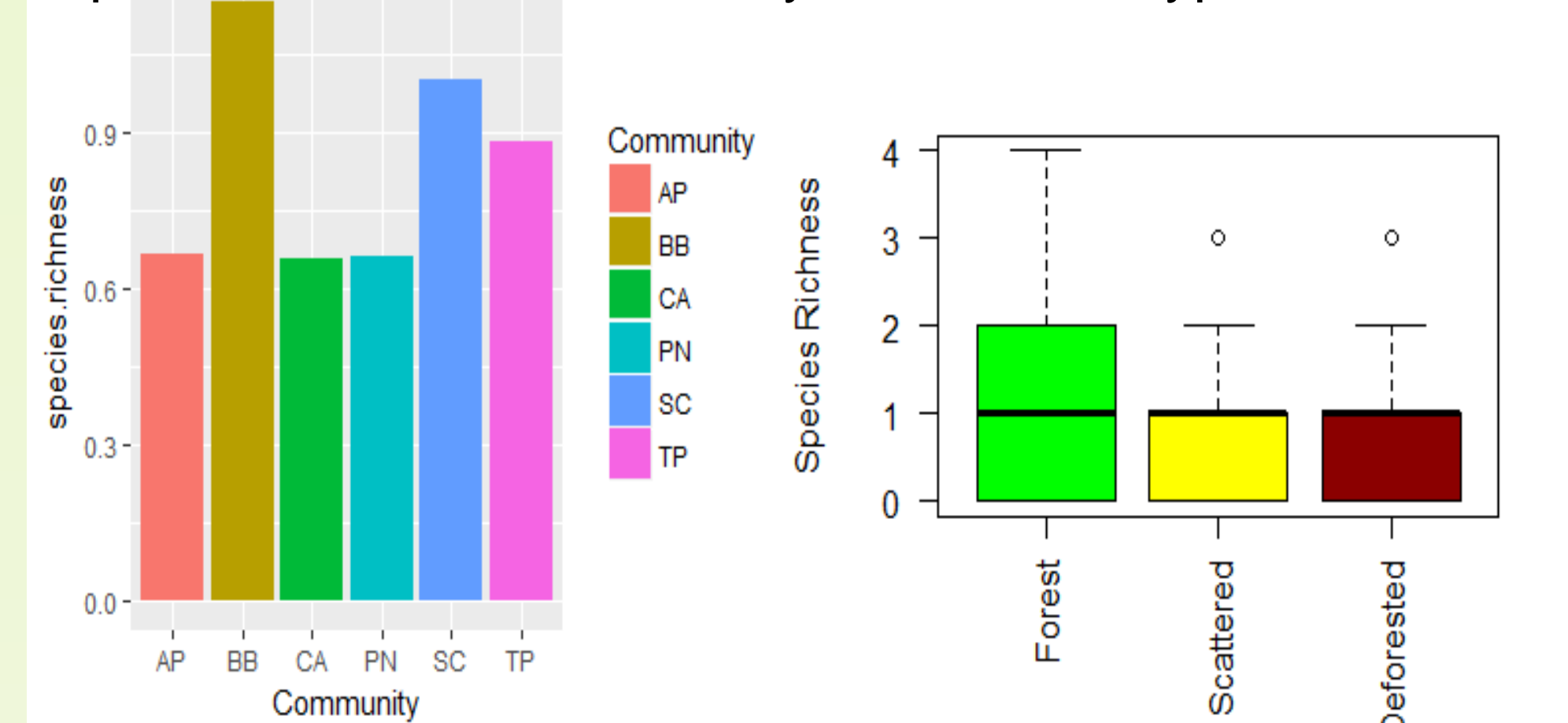


Figure 6: Pathogen species richness by community type (on left) and by habitat (on right). Using a generalized linear mixed model there was no significance difference between infection species richness across habitat types.

| sp1 | sp2 | Sp1_inc | Sp2_inc | Obs_cooccur | Prop_cooccur | Exp_cooccur | P_it | P_gt | Sp2_name | Sp2_name |
|-----|-----|---------|---------|-------------|--------------|-------------|---------|---------|-------------|-------------|
| 1 | 2 | 16 | 47 | 5 | 0.015 | 3.3 | 0.91234 | 0.21974 | T. cruzi | Leishmania |
| 1 | 3 | 16 | 37 | 4 | 0.012 | 2.6 | 0.90103 | 0.25499 | T. cruzi | Dirofilaria |
| 1 | 4 | 16 | 104 | 7 | 0.033 | 7.4 | 0.53072 | 0.67084 | T. cruzi | CDV |
| 2 | 3 | 47 | 37 | 8 | 0.034 | 7.7 | 0.64863 | 0.52255 | Leishmania | Dirofilaria |
| 2 | 4 | 47 | 104 | 19 | 0.096 | 21.6 | 0.24255 | 0.84823 | Leishmania | CDV |
| 3 | 4 | 37 | 104 | 21 | 0.075 | 17.0 | 0.94656 | 0.1052 | Dirofilaria | CDV |

Table 3: Co-occurrence test between each infection type. There is no significant association between expected and observed prevalence of different combinations of co-infecting pathogens

Conclusions

- Community identity appears to be an important factor in infection prevalence, as seen in Figure 2. This correlation may be accounted for by the varying natural habitats for vector-borne disease, i.e. palm tree presence, which may not be in every community relates to kissing bug and *T. cruzi* prevalence.
- There are no significant associations between community, habitat, and studied pathogen species richness.
- Based on the co-occurrence analysis of co-infection status, there seems to be no significant positive or negative associations between pathogen infections and co-infections. Other infection types of pathogens not evaluated in this study may influence patterns of pathogen co-occurrence. Tick-borne diseases such as *Ehrlichia spp* could potentially influence host susceptibility to infection than these infection types.
- T. cruzi* infection was associated with higher total white blood cell and total lymphocyte count.

References

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