

# Comparative Microbiome Analysis of Cohabiting Wildlife Species with Disease Vector Potential: Canada Geese vs. Domestic Flies

Adam Krantz, MS<sup>1</sup>; Annika Linde, DVM, PhD, MPH<sup>2</sup>; Janina Krumbek, PhD<sup>3</sup>; Gustavo A. Ramirez, PhD<sup>1,4</sup>; J. Santiago Aguilar, PhD<sup>1</sup>; Tonatiuh Melgarejo, DVM, MS, PhD<sup>1,2#</sup>  
<sup>1</sup>Western University of Health Sciences, CVM, Pomona, CA; <sup>2</sup>CePahtli Research Alliance, Claremont, CA; <sup>3</sup>MiDOG, LLC, Irvine, CA; <sup>4</sup>University of North Carolina, Chapel Hill, NC  
 #Corresponding Author: adam.krantz@westernu.edu; #Faculty Mentor (Summer 2019)

## Background & Rationale

Emerging/Re-emerging Infectious Disease (EID) surveillance is of increasing relevance in the face of anthropogenic forces such as accelerated human population increase, climate change, natural resource overuse, habitat and environmental destruction. Antimicrobial Resistance (AMR) to conventional therapeutics used to combat infections is similarly of growing concern in both human and veterinary medicine. Existing reports demonstrate increasing use of microbiome data to prognosticate about individual health outcomes as well as screen for selected agents of infection; e.g., flies have been used as a sentinel for populations of free ranging baboons in the detection of Treponemal agents in natural ecosystems. This pilot study focused on evaluating the potential role of a long-distance dispersal vector (Canada Goose) and short-range machinal vectors (Domestic Flies) in context of growing EID/AMR concerns, through use of whole insect and avian excrement microbiome analyses. We also aimed to elucidate any relationship between the microbiomes of cohabiting species in public parks that may serve as potential vectors for zoonotic disease agents.

## Hypothesis & Objectives

**Hypothesis:** Canada Geese and Domestic Flies, as cohabiting vector species in public spaces, share microbiome similarities that make flies useful as sentinels in a zoonotic disease surveillance context  
**Objectives:** 1) Collect excrement (feces/urine) from Canada Geese and (whole) domestic flies in a public park (Los Angeles area); 2) Extract and amplify microbial DNA from avian and insect samples; 3) Use Illumina MiSeq technology, and bioinformatics software, for metagenomic analyses (MiDOG); 4) Compare microbial abundance and diversity in domestic flies versus Canada geese excrements

## Design & Methods

A standard entomology net was used to catch flies at a public park in Los Angeles County with prior written permission from the City Manager. The flies were transferred to individual collection tubes, frozen using liquid nitrogen, and macerated in separate tubes prior to screening. Excrement samples from Canada Geese residing in the same public park were collected as soon as possible after excretion, without disturbing the birds. Nine (9) fly samples (including three samples from each of the families Muscidae, Sarcophagidae and Calliphoridae) along with nine (9) excrement samples from Canada Geese (*Branta canadensis*) were processed; plus an aggregate sample representing droppings from all geese. The Quick-DNA Fecal/Soil Microbe Miniprep Kit (Zymo Research, Irvine CA) and Illustra Ready-To-Go GenomiPhi V3 DNA Amplification Kit (GE Healthcare Life Sciences, Pittsburgh PA) were used for microbial DNA extraction and amplification, respectively. Microbiome data (geese excrements vs. whole flies) was generated for bacterial and fungal populations utilizing Illumina MiSeq technology (MiDOG, LLC, Irvine CA). Data was also analyzed for any evidence of AMR.

### 1. Collection of bird excrements



### 3. DNA Isolation & Amplification

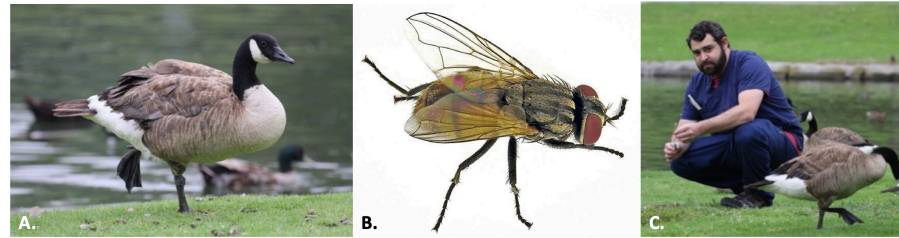


### 2. Collection of flies with net

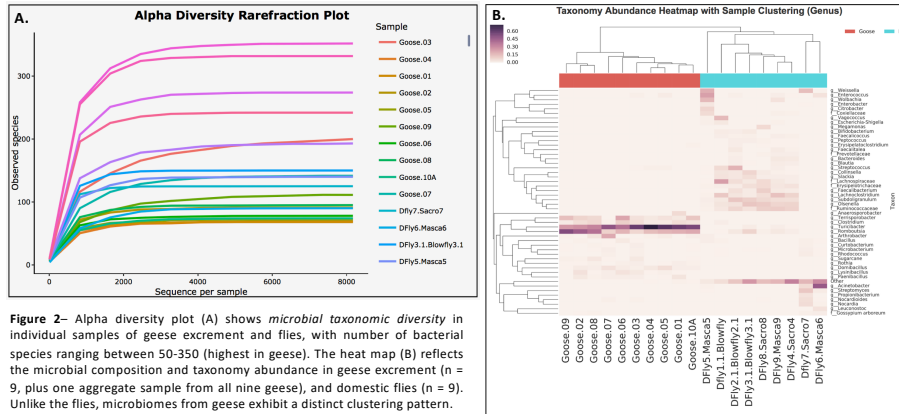


### 4. Metagenomics analyses (by MiDOG)

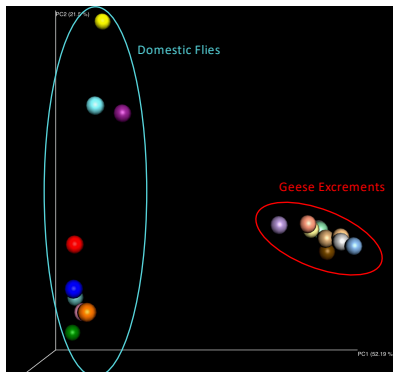
## Results



**Figure 1** – Public parks represent risk areas for pathogen transmission between birds (A), insects (B), and people (C). In this study we identified distinct differences in microbiome profiles of geese (feces/urine) vs. flies. The pilot data may have future implications in reference to EID/AMR surveillance efforts.



**Figure 2** – Alpha diversity plot (A) shows microbial taxonomic diversity in individual samples of geese excrement and flies, with number of bacterial species ranging between 50-350 (highest in geese). The heat map (B) reflects the microbial composition and taxonomy abundance in geese excrement (n = 9, plus one aggregate sample from all nine geese), and domestic flies (n = 9). Unlike the flies, microbiomes from geese exhibit a distinct clustering pattern.



**Figure 3** – Beta diversity plot (PCoA; Bray-Curtis dissimilarity) shows microbial structure diversity. Geese samples show clustering pattern

## Discussion & Conclusion

The study focused on the microbiome of cohabiting species in a public park system that presents risks for zoonotic pathogen transmission. Our data demonstrated extensive microbiome comparability amongst geese (droppings) using bioinformatic techniques (Fig.2.B, 3). Based on field observations (Fig.1), these similarities may be explained by geese flocking in constant close proximity, while feeding, resting, and excreting body waste on grassy areas that are relatively limited in size.

We found higher microbial taxonomic diversity in geese excrements (up to 350 species), as compared to ≤ 150 species in the flies (Fig.2.A). Also, the taxonomy abundance heatmap showed distinctly different composition of the microbial communities in geese vs. flies (Fig.2.B). Intestinal bacteria that were consistently present and highly abundant in geese included Turicibacter, Romboutsia, Terrisporobacter, and Clostridium (Fig.2.B), where species such as *Clostridium perfringens* or *Clostridium difficile* could present a zoonotic disease risk, and possible public health concern, especially for vulnerable human populations. Whereas the geese microbiomes showed a clear clustering pattern in microbial structure diversity, scattering was noted for the flies (Fig.3). As such, limited overlap was noted between the microbiomes in these vector species, which would suggest their interactions are infrequent. Still, some flies breed in bird debris and large volumes can also attract other pests that are associated with various human health hazards.

Our team noticed examples of microbial overlap that are of interest: MiDOG analyses highlighted that similar AMR profiles of *Enterococcus* (up to 350 species), as compared to ≤ 150 species in the flies (Fig.2.A). We were found in a fly (Fly9) and a goose (Goose3) sample against clinically important antimicrobials, including Azithromycin. *E. cecorum* (previously *Streptococcus cecorum*) is considered an EID of interest to human and veterinary medicine ('One Medicine'). This pathogen has been isolated from critically ill human patients (sepsis), and is deemed a potential threat to domestic animals (e.g., chicken).

Also, another fly sample (Fly5) contained a 6% relative abundance of Cxiellaceae spp. (where additional testing revealed 93% and 85% identity to *Rickettsiella grylli* and *Coxiella burnetii*, respectively). *C. burnetii* is the causative agent of Q Fever (human Q fever infection is a reportable disease in the U.S., and in livestock infections in California). These are relevant considerations in a One Health framework (Fig.4).

In conclusion, this pilot data suggest that flies might serve as sentinel species in context of EID/AMR surveillance. It also indicates different information is to be obtained from an avian versus an insect vector. Both may serve a valuable, yet different, sources of information. Larger sample sizes would be needed to define specific relationships between these two vector species living at this geographical location.

## Acknowledgements & References

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**Figure 4** - One Health is a concept that covers the triad of the abiotic-environment, biotic-environment and animals that live in both. It spans the disciplines of Public Health, Conservation Medicine, and Environmental Health to advance global health outcomes. The emphasis of this project is Public Health and Conservation Medicine.