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ARE DOGS ALTERING MICROBIAL COMMUNITIES AND CONTRIBUTING TO ANTIBIOTIC RESISTANCE IN URBAN PARK SOIL BACTERIA?

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INTRODUCTION

Dog ownership rates are increasing globally, and coupled with the trend towards increasing urbanization, more and more dogs are living in cities worldwide. Unlike wild animals that share our urban environments, the living and recreational spaces, movement and consumption patterns of dogs overlap to a large extent with those of humans. We share our homes, vehicles, sidewalks and parks with them, we buy their food from grocery stores, and we care for them as we would a family member. This also includes medical care when they are ill, and often times the prescription of antibiotics to cure infections. A Finnish study [1] found that ~22% of dogs admitted to a small-animal hospital were prescribed at least one course of antibiotics, while a researcher in the USA estimates that the administration rate there may be as high as 80% (A. Wayne, personal communication, 18 Dec 2018).

Animal agricultural is one of the largest consumers of antibiotics [2] and these compounds and resistant bacteria can be passed on to consumers [3] and then excreted in feces and urine. Low-level exposure to antibiotics has been shown to induce resistance [4] and a recent study [5] has shown that industrial dog foods of all types contain multidrug resistant bacteria.

Recent research [6] has shown that dog urine significantly impacts soils in spatially restricted areas of urban greenspaces. Since dogs are ingesting both antibiotics and resistant bacteria, these same areas are likely receiving both contaminates. Antibiotic resistance is a growing global concern [7], and as more people and their dogs move to urban areas, the potential for both the presence of resistant bacteria and exposure to them increases.

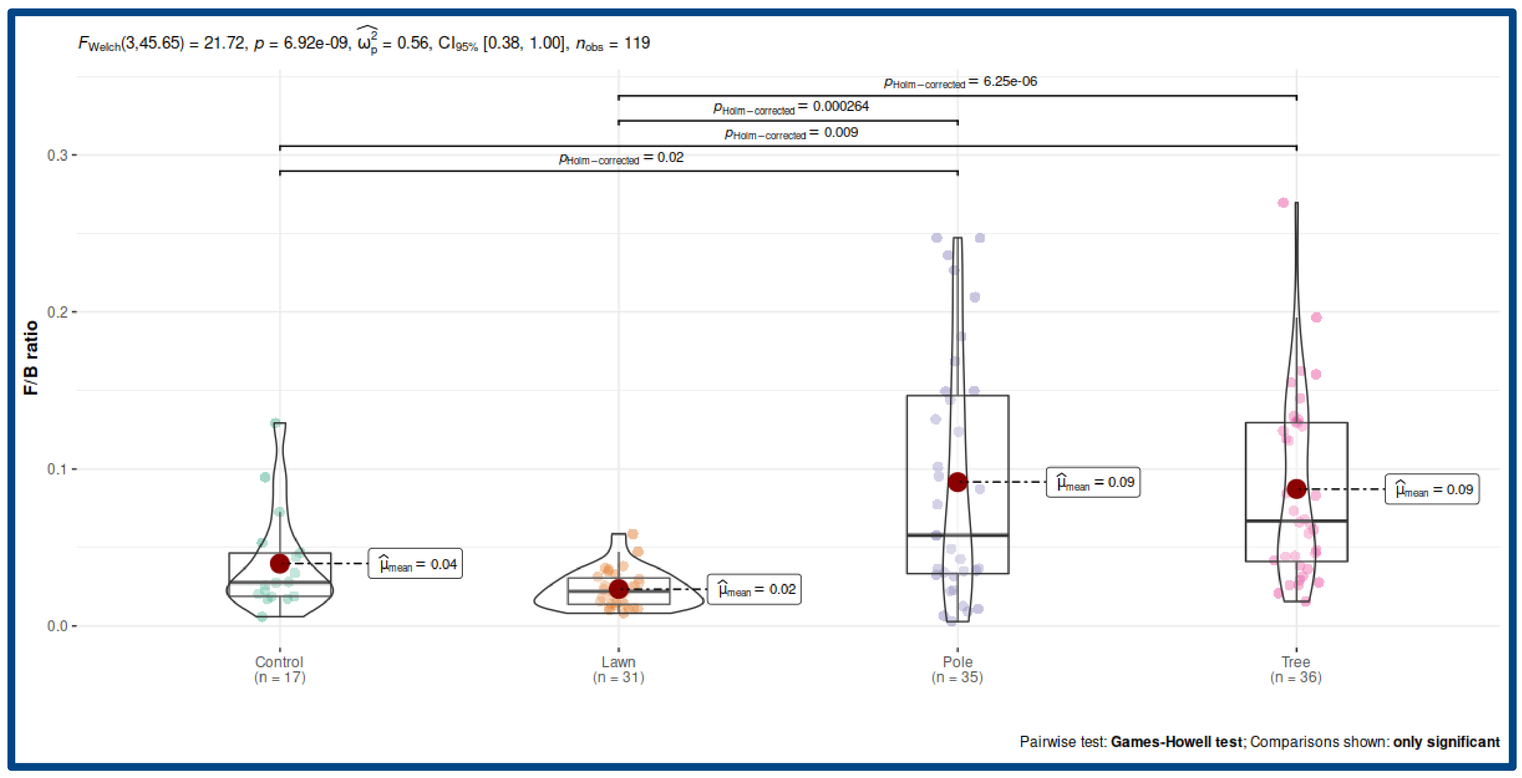

Figure 1. Fungal ITs to bacterial 16s ratios (F/B) of the four treatments in this study and their pair-wise comparisons.

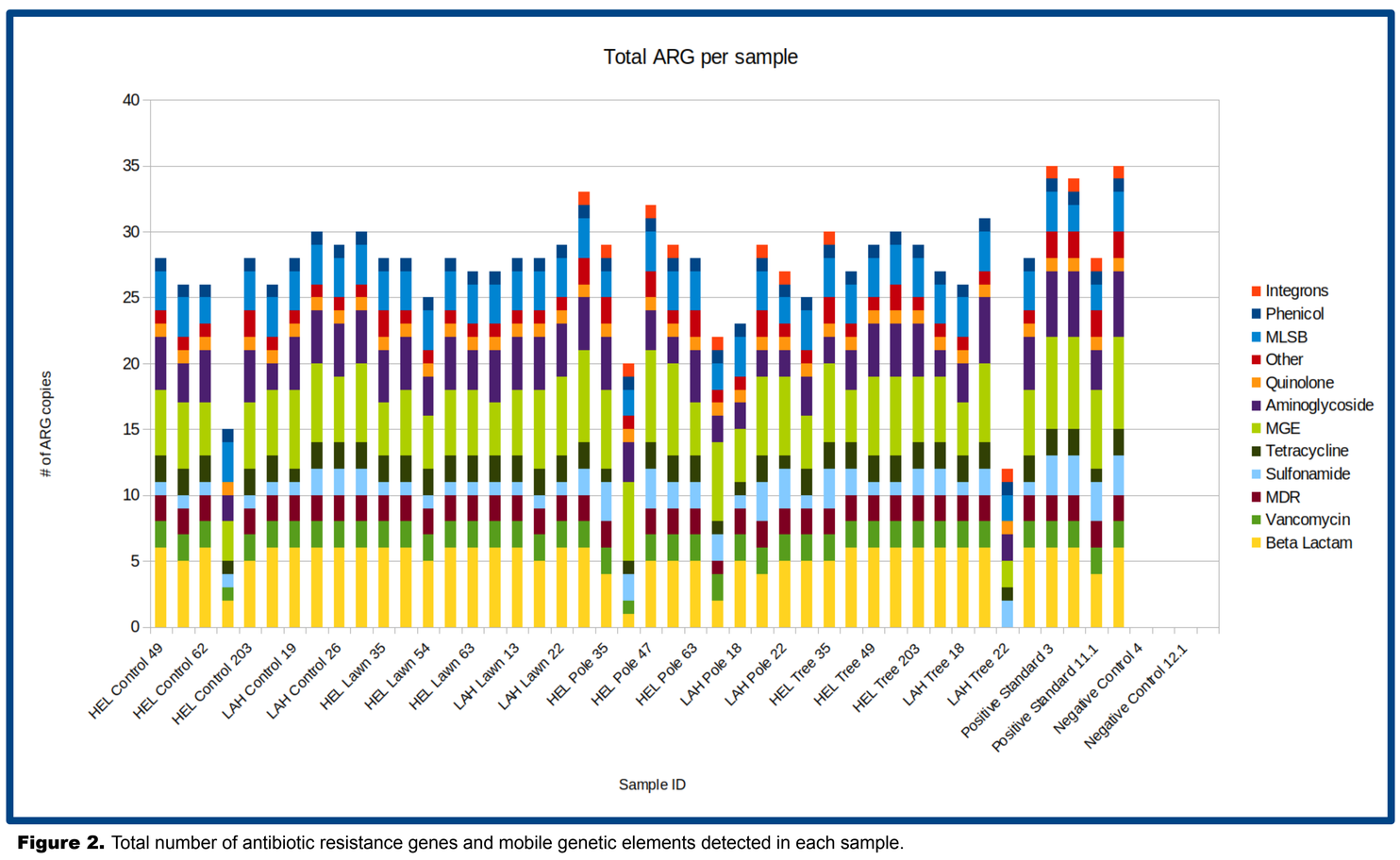

Figure 2. Total number of antibiotic resistance genes and mobile genetic elements detected in each sample.

METHODS

We included 32 areas of urban greenspace in Helsinki (n = 17) and Lahti (n = 15), and from these we collected soil samples from four treatments: Deciduous trees (Control, n = 17) and Lawn areas (Lawn, n = 31) at least 8 m away from pathways, and path-side deciduous trees (Tree, n = 36) lampposts (Pole, n = 35).

DNA was extracted from the samples using QIAGEN Power Soil and Fisher Scientific DNA extraction kits. We confirmed successful extractions by gel electrophoresis and used qPCR to check for the presence of PCR inhibitors and to determine the number of bacterial 16s and fungal ITS genes extracted. A subset of these samples were then submitted to ResistoMap Oy (Helsinki, Finland) for ARG sequencing.

Table 1. GLMM resluts testing the effect of treatment on ARGs. Only significant effects are shown.

Variable	Intercept (Control)	Lawn	Tree	Pole
Aminoglycoside	3.500	0.300	-0.200	**-0.800**
	(0.260)	(0.367)	(0.367)	**(0.367)**
	<0.001	0.414	0.586	**0.029**
Beta Lactam	5.400	0.400	-0.100	**-1.300**
	(0.409)	(0.579)	(0.579)	**(0.579)**
	<0.001	0.490	0.863	**0.025**
Integrons	0.000	0.100	0.200	**0.700**
	(0.107)	(0.152)	(0.152)	**(0.152)**
	1.000	0.510	0.187	**<0.001**
MLSB	2.886	0.132	0.010	**-0.282**
	(0.101)	(0.142)	(0.137)	**(0.141)**
	<0.001	0.353	0.944	**0.045**
Sulfonamide	1.292	-0.187	0.418	**0.907**
	(0.178)	(0.251)	(0.247)	**(0.249)**
	<0.001	0.455	0.090	**<0.001**

RESULTS & DISCUSSION

All statistical analyses were performed in R (v 4.1.1) [13]. Fungal ITS to bacterial 16S ratios (F/B) for each sample were calculated from the qPCR results (Fig 1). Treatment effects on the F/B ratio were analyzed using the ggbetweenstats function of the ggstatsplot package [8]

Using generalized linear mixed models (GLMM) [14] we tested the effect of *Treatment* (a factor with three levels; Control, Lawn, Tree, Pole) on the response variables (ARGs), with *siteID*, nested within C*ity* as a random term. Significant results from this analysis are given in Table 1.

The F/B ratios of path-side treatment were significantly different from those of the Lawn and Control trees. Interestingly, ARGs were found in all samples analyzed (Fig. 2). However, differences were observed between the path-side and control treatments both in terms of relative abundance and number of copies. Genes conveying sulfonamide and multidrug (MDR) resistance were the most numerous in both the control and treatment plots of both cities. Mobile genetic elements (MGE), important mechanisms for spreading ARG, were more common around the path-side treatments, and integrons were found only around Poles (80%) and Trees (20%). Sulfonamide resistance genes were significantly positively associated with Poles.

The impacts of dogs on on soil microbiology or the resistome is only now being looked at in detail. Our results indicate that dogs are significantly impacting soil microbial communities, and that the areas impacted by dogs have elevated levels of genes for Sulfonamide resistance and integrons. Further analyses will be needed to determine the mechanisms behind these changes.

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