INVESTIGATING THE DISEASE ECOLOGY OF URBAN FREE-RANGING CATS IN RELATION TO HABITAT TYPES AND SUPPLEMENTAL FEEDING

by

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(Under the Direction of Nicole Gottdenker)

ABSTRACT

Urbanization has led to the creation of novel ecosystems, exhibiting unique species compositions and ecological processes, and providing new opportunities and challenges to wildlife. In response to such environmental changes, urban wildlife managed to successfully adjust diverse aspects of their ecology. The main research question addressed in this dissertation is how the novel ecology of urban-dwelling animals affects host-parasite interactions by changing exposure and/or susceptibility of hosts to parasite infections. Studies were conducted in two different settings to address this question. First, I compared physiological body condition and prevalence of selected pathogens of feral cats from highly urban as well as rural areas to demonstrate how health status varies with respect to habitat types. Based on blood parameter analysis, I observed signs of higher body condition in urban feral cats versus rural cats. Additionally, comparison of pathogen prevalence between the two habitat type suggests a significant role of pathogen transmission mode and/or life-cycle in determining host-pathogen interactions regarding environmental conditions. Secondly, I focused on the disease

ecology of stray cat populations within an urban area. Specifically, I investigated the potential impact of human food provisioning on the population density and body condition of urban stray cats, and how its effect extends to pathogen prevalence and host immune function. Human food provisioning did not show an association with population density or body condition in urban stray cats. However, I found that the prevalence of a subset of pathogens and immune function parameters was significantly related with supplemental feeding. This pattern of observations suggests that transmission modes of pathogens and specific functions and/or energy requirements of different immune parameters may determine how host-pathogen interaction and host immune phenotype, respectively, changes in response to urban food provisioning.

Together, the findings of this study highlight the unique host-pathogen interactions in urban populations, and their heterogeneity within the city in response to human activities. Further, the observations from my research on feral and stray cats in relation to habitat types will have implications for the health and welfare management of not only free-ranging cats, but also various urban-dwelling wild and domestic animals.

INDEX WORDS: Urban-rural habitat, urban-adapted wildlife, domestic cat, supplemental feeding, population density, pathogen prevalence, immune phenotype

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DEDICATION

To my parents. For believing in me even when I showed you nothing.

For giving me the courage to start all over every time.

I am fortunate to have my family, and to be an aunt of my shiny nieces and nephews.

I thank my husband, for your love, patience and humor.

You make my good days better, and bad days tolerable.

It’s good to have you all by my side.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1. Introduction

With their ecological flexibility and the help of human activities, domestic cats are found throughout the globe, in a wide range of habitat types. Unlike their pet counterparts, free-ranging cats share many ecological characteristics with wild mesocarnivores, such as red foxes and raccoons, including the ability to successfully adapt to urbanized environments (Bateman and Fleming, 2012). Individuals and populations dwelling in urban habitats are known to differ in their physiological and/or ecological traits, such as breeding phenology, diet composition and home range size, compared to their conspecifics inhabiting rural or natural habitats (Lowry et al., 2013). In addition, novel biotic and abiotic characteristics of urban ecosystems, such as proximity to humans, microclimates, and vector species composition, may ultimately result in modified host-pathogen interactions in urban animal populations (Bradley and Altizer, 2007; Hamer et al., 2012; Watts et al., 2015).

Understanding the dynamics of host-pathogen interactions in wild populations is complex, as they are often a result of interactions among a myriad of factors related to hosts, vectors, pathogens, and environment. However, it can be further complicated in urban ecosystems, as current knowledge acquired from natural ecosystems may have limited implications for understanding urban ecological processes (Brearley et al., 2013). Hence, ample data from empirical studies are critical to understanding how the

susceptibility and/or exposure of hosts to pathogens vary with changing conditions in urban habitats.

The overarching goal of this dissertation research is to describe patterns of pathogen prevalence in urban-adapted host species in relation to urbanized habitats. I used free-ranging cats as a study species for two reasons; (1) free-ranging cats are an abundant and a significant component of modern urban ecosystems, and results on the disease ecology of urban free-ranging cats can be further applied to better understand host-pathogen interactions in other urban-adapted mesocarnivores; and (2) urban free-ranging cats are hosts of diverse pathogens, including many zoonotic pathogens and feline vector-borne diseases (FVBD) (Spada et al., 2014); therefore, understanding the dynamics of pathogens in free-ranging cats is related not only to their own health and welfare, but also to the health of wildlife, domestic animals, and humans, who can exchange pathogens with free-ranging cats (Gerhold and Jessup, 2013).

The word ‘free-ranging cat’ is loosely used to indicate domestic cats with various lifestyles, and therefore needs clarification for this study. In general, un-owned, free-ranging cats within or in the vicinity of human residential areas (e.g., cities or farms), using various forms of human resources are considered stray cats. In contrast, cats in rural and/or natural habitats are often defined as feral cats, and are considered to provide their own food through hunting and to find shelter in their natural habitat (Mendes-de-Almeida et al., 2006). In this dissertation, studies involving cats from both urban and rural habitats use the term feral (Chapters 2 and 3), whereas in chapters that only involve urban cats, the term stray is used (Chapters 4 and 5).

Chapters 2 and 3 of this dissertation present the results of exploring the association between the health status of feral cats and their habitat type by comparing various physiological body condition parameters and the prevalence of selected pathogens within urban and rural habitats. I hypothesized that feral cats in urban habitats would have higher body condition and that the prevalence of pathogens between the two habitat types would differ in relation to the life-cycle and transmission modes of each pathogen. Hence, my research objectives were to compare multiple blood parameters, biometric indices (body mass and body size), and infection status to selected feline pathogens in feral cats from contrasting habitat types (residential areas within a metropolitan city and rural farming areas). In general, urban-adapted wildlife, including feral cats, adapts well to both natural and urbanized habitat types (McKinney, 2002). However, this study will allow us to further understand the overall health status of feral cats across different habitats. In addition, results of pathogen prevalence may provide empirical evidence of whether pathogens with certain transmission modes and life-cycles are favored in an urbanized environment.

After presenting the research results on urban versus rural population in Chapters 2 and 3, in Chapters 4 and 5 of this dissertation, I focus on disease ecology in urban stray cats within the city. Among the diverse traits of urban habitats, abundant and constant food provisioning through various human acitivites has the potential to function as a powerful driver altering the ecology of urban-adapted animals, including individual behavior, population size, and home range (Oro et al., 2013; Shochat et al., 2006). It is suspected that accumulations of these ecological changes are followed by altered host-pathogen interactions (Becker et al., 2015). Although many urban-adapted wild animals

are known to utilize indirectly provisioned food sources, such as garbage bins, urban stray cats also benefit from additional food sources directly provided by cat caretakers. Cat caretakers are people who provide various resources for stray cats, mostly feeding them on a routine basis (Centonze and Levy, 2002), and are known to be common in many urbanized areas (Finkler et al., 2011; Kim et al., 2016). In Chapter 4 and Chapter 5, my objectives are to reveal the ecological associations between the supplemental feeding by cat caretakers and the ecology and health status of urban stray cats. In Chapter 4, I present data on the associations between supplemental feeding, population density of stray cats and the prevalence of multiple pathogens with different transmission modes. I hypothesized that supplemental feeding would increase the population density of stray cats in the neighborhood and that the prevalence of pathogens with density-dependent transmission would be higher in stray cat populations exposed to intensive cat caretaker activity. In Chapter 5, I further explore the potential association among supplemental feeding, body condition, and immune phenotype in urban stray cats. Maintaining immune components and immune responses are energetically-costly processes, and it is considered that nutritional resource availability is a critical factor shaping immune phenotype in individual animals (Becker et al., 2015; Ruiz et al., 2010). Therefore, I test whether use of supplemental food is associated with higher body condition, and additionally, in the composition of immune components and/or the ability of individuals to elicit immune responses.

2. Literature review

1) Disease ecology of urban adapted wildlife with emphasis on the impact of supplemental feeding

In response to ever-increasing urbanization, subsets of species with certain ecological traits, such as flexible diets and/or behavior, have successfully adapted in urbanized environments (Bateman and Fleming, 2012). Avian species and mesocarnivores, such as crows, feral cats, and red foxes, are the groups of animals most commonly studied as significant components of urban ecosystems (Gehrt et al., 2010; Kark et al., 2007; Sanz and Caula, 2014). These animals, commonly referred to as urban adapters, are known for their altered individual or population traits showing ecological divergence from their conspecifics inhabiting the original habitats (Bradley and Altizer, 2007; Brearley et al., 2013; Luniak, 2004). In this literature review, my goal is to address the disease ecology of urban dwelling animals, especially mesocarnivores, by classifying potentially involved mechanisms into two categories- one related to the intrinsic traits of urban-adapted host species/populations and the other related to characteristics of urban ecosystems-and to discuss how each variable can influence the exposure or susceptibility of host animals toward pathogens (Table 1-1, Fig. 1-1).

Host trait-based variables

The unique intrinsic traits of urban mesocarnivores, such as their diverse dietary range and flexible behavior, allow them to better adapt to novel urban environments. However, the resulting ecology of urban adapters can further influence the ways in which animals interact with pathogens by altering their exposure and/or susceptibility to

infection. I categorized the ecological mechanisms that link the ecology of urban adapters with altered exposure/susceptibility to pathogens as follows: (1) increased energy intake, (2) aggregation of individuals around clustered food sources, (3) reduced home range/higher home range overlap, (4) reduced dispersal rate, and (5) altered food sources and higher usage of anthropogenic subsidies.

***Increased energy intake*:** The availability of energy sources is one of the core determinants of wildlife ecology (Delany, 1982). Urban adapters are usually those that are successful in utilizing anthropogenic food sources, such as human refuse or recreational feeding. Previous empirical studies and reviews reported changes in population structure and/or demographic dynamics in urban mesocarnivores as a result of exploiting abundant energy sources (Bradley and Altizer, 2007; Bateman and Fleming, 2012). Such changes may include increased breeding success, survival rate, and body condition (Gehrt et al., 2010; McCleery et al., 2014). In the following, I present how this altered ecology can go beyond this to affect host-pathogen interactions in urban-dwelling animals (Table 1-1).

i) *Increased availability of energy for investment in immune phenotype*: Wild animals are constantly under pressure to strategically allocate energy among various energy-demanding physiological processes in ways that maximize their intrinsic fitness (Downs et al., 2014). As a result, immune response development is constrained by energy availability and by competition with other costly behaviors such as migration or reproduction (Sheldon and Verhulst, 1996). Increased energy intake in urban adapters may allow them to invest more in immune function, potentially *lowering susceptibility* to pathogen infections. In addition, urban-dwelling wildlife may also benefit by spending

less time and energy exploring and securing food sources (Becker et al., 2015). Some recent studies demonstrated higher immunocompetence in urban populations or in populations exposed to anthropogenic food provisioning (Audet et al., 2016; Wilcoxen et al., 2015). According to modeling and experimental studies, the association between resource availability and immune phenotype may have a significant influence on the outcome of pathogen infection in such host populations (Becker and Hall, 2014; Budischak et al., 2015), which warrants further studies of ecoimmunology in urban-adapted wildlife species.

ii) *The increased availability of young individuals*: Previous studies provide indirect evidence of higher juvenile proportion and/or higher juvenile survival rates within supplemented or urban populations as compared to unsupplemented or rural populations (Bino et al., 2010; red fox, McCleery, 2008; fox squirrel, Robb et al. 2008; avian species). Higher breeding and survival rates of juveniles may *increase the proportion of immunologically naïve, susceptible individuals* within the populations. Such shifted demographic structure may lower the probability of herd immunity preventing the pathogen outbreaks in host populations (Altizer et al., 2004; Lyles and Dobson, 1993; van Rensburg et al., 1987). Investigation of demographic structures and prevalence of endemic pathogens in supplemented and control populations may allow us to further clarify the impact of higher proportions of young and susceptible individuals in supplemented populations.

iii) *Lengthened infectious periods of individuals*: Higher body condition of individuals through rich energy intake has been suggested to lengthen the infectious period through processes such as higher survival rate or higher tolerance of infected

individuals (Becker et al., 2015). As a result, susceptible individuals sharing the habitat with infectious individuals shedding pathogens for a lengthened period of time may suffer from increased chances of pathogen exposure (Råberg et al., 1998; Vale et al., 2013). Such results have been observed in laboratory studies, but no such reports on wildlife were found during this literature review. Longitudinal studies investigating the association between the duration of pathogen shedding, body condition of infectious individuals and their resource availability would aid in clarifying the impact of supplemental feeding on elevating the dissemination rate of pathogens in urban areas.

Aggregation of animals: Anthropogenic food sources in urban habitats tend to be distributed in a clumped manner, attracting wild mesocarnivores to close proximity with each other at a frequency that may not occur in natural habitats (Harrison et al., 2011; Theimer et al., 2015; Wehtje and Gompper, 2011). The resulting aggregation of animals allows direct contact among intra- and interspecific individuals that can provide ideal conditions for pathogens transmitted through direct contact (e.g., feline leukemia virus, canine distemper virus, and fleas) (Becker et al., 2015). As such, previous empirical studies provide evidence of enhanced transmission of pathogens as a result of clumped food sources in various wildlife species (Cross et al., 2007; Lawson et al., 2012) including mesocarnivores such as raccoons (Wright and Gompper, 2005).

Reduced home range size/Increased habitat overlap: Use of human food can also modify the space-use of wild mesocarnivores (Salek et al. 2015), negating the need to explore a broader geographic area to acquire food sources (Adams, 2009; Davison et al., 2009; Prange et al., 2004). The reduction in home range size and/or increased home range overlap among neighboring populations/individuals has been reported from urban-

dwelling mesocarnivores or mesocarnivores that have access to anthropogenic food sources (Bino et al., 2010; Quinn and Whisson, 2005; Šálek et al., 2015). The resulting increased population density of urban host animals can increase the accumulation of parasite infective stage within the habitat area (Mbora and McPeek, 2009; Nunn et al., 2003), elevating the exposure risk of individuals to parasite infection. Such risks may be higher for parasites or pathogens that are resistant to external conditions and are environmentally transmitted to susceptible host individuals, such as various endoparasites or feline/canine parvovirus (Park, 2012).

Reduced dispersal rate: One of the ultimate drivers behind the evolution of animal dispersal is to avoid inbreeding and raise the genetic variability of a population (Gandon, 1999). However, previous studies show that availability of abundant food sources can cause reduced dispersal rates of animals, possibly leading to higher proportions of kin-related animals in the existing population (Grémillet et al., 2008; Monsarrat et al., 2013; Oro et al., 2013). The limited dispersal of individuals from urban populations was documented in previous studies on red foxes (Gosselink et al., 2010; Robinson and Marks, 2001). Reduced dispersal of urban populations may allow genetically related individuals to share a habitat, posing risks and causing detrimental effects on physiology of offspring through inbreeding. Among other negative impacts, inbreeding can lower overall genetic diversity of affected animals, including the genes related to immunity (MHC-major histocompatibility complex gene) which may lead to increased *susceptibility to pathogen infections* (Sommer, 2005).

Altered nutritional content: As a consequence of relying on food sources available in urban habitats, many mesocarnivores experience a shift in diet composition,

such as altered prey species and/or an increased proportion of food sources from human waste (e.g., garbage) (Bateman and Fleming, 2012; Widdows and Downs, 2015). Although human subsidies may provide sufficient energy intake for urban-adapters, alterations in the specific content of food sources can have further implications for the disease ecology of host populations. Two of the potential underlying mechanisms are discussed below.

i) *Reduced transmission of parasites with complex life-cycle*: Mesocarnivores in urban habitats are known to rely less on the predation of natural prey and instead utilize anthropogenic food refuse (Contesse et al., 2004; Grigione et al., 2011). This may have a negative impact on the development of parasites that require multiple host species to complete their life-cycle. For these parasites, their transmission to terminal hosts often occurs through the predation of intermediate host species. The transmission of *Toxoplasma gondii* to domestic/wild feline species, or *Echinococcus multilocularis* in domestic/wild canine species is a well-known example of such parasites (Hegglin et al., 2007; Lelu et al., 2010). Owing to the reduced predation of intermediate host species, urban mesocarnivores are likely to benefit from a *lowered risk of exposure* to such parasites. Previous empirical studies also support this possibility. For instance, urban raccoons showed a lower prevalence of raccoon roundworms (Page et al., 2008) and red foxes dwelling in cities had a lower prevalence of *E. multilocularis* or dixenous intestinal helminth infections compared to rural populations but this is not the case for monoxenous parasites (parasites in which development is restricted to a single host species) (Fischer et al., 2005; Reperant et al., 2007). Hence, despite the concern that the high population density of urban mesocarnivores would lead to higher a prevalence of

pathogens/parasites, such a pattern may not be universal, but may partly depend on the food composition of host individuals and the transmission mode of parasites involved.

ii) *Deficient consumption of critical nutrients:* With the reduced consumption of natural prey in urban mesocarnivores, it is possible that these animals suffer from insufficient supplies of critical nutrients. For instance, proteins and antioxidants are important for eliciting and/or maintaining immune responses in animals, and the lack of essential nutrients can be detrimental to the immune function of wild animals, which is tightly linked to their survival (Koski and Scott, 2001; Maggini et al., 2007). In many cases, anthropogenic food sources available in urban habitats are inadvertently disposed materials without consideration for the wildlife that consume them. As a consequence, wildlife relying on human food sources may suffer from an imbalanced diet and lowered immune function with *increased susceptibility to pathogen infections* (Knapp et al., 2013; Semeniuk et al., 2009).

Urban ecosystem-based variables

Urban ecosystems are innately novel to wildlife, composed of biotic and abiotic factors different from the original habitat (McKinney, 2002). In addition to changing the ecology of host animals living in them, urban environments can also present external factors interacting with urban-adapters that ultimately change the host-pathogen interactions. Among the many environmental variables of urban ecosystems, I discuss the following mechanisms: (1) spatial proximity to human residential areas, (2) altered species composition, and (3) altered microclimates.

***Spatial proximity to human residential areas*:** Urban mesocarnivores are spatially in close proximity to human and human-associated animals such as domestic and exotic pets (Riley et al., 2014). Considering that many urban adapted mesocarnivores include canine and feline species, or other omnivorous wildlife species that may share pathogens with dogs and/or cats, interaction between wildlife and domestic animals in urban habitats may pose epidemiologic threats to either or both animal groups (Bevins et al., 2012; Riley et al., 2014). Higher pathogen prevalence of urban wildlife populations has been reported in previous studies in association with an abundance of urban domestic animals (Lehrer et al., 2010; Suzán and Ceballos, 2005). In a study of gray foxes, higher prevalence of canine parvovirus was observed in correlation to the proximity of individuals to urban areas, potentially contracted from interacting with domestic dogs at the edge of urban developments (Riley et al., 2004). Similarly, Acosta-Jamett et al. (2011) reported a higher prevalence of canine distemper (CDV) in wild foxes in areas closer to human settlements. Based on the high prevalence of CDV in urban domestic dogs, the authors suggest that occurrence of CDV is a spillover from domestic dogs to wild foxes.

Altered species composition: Urbanization of natural habitats results in a shifted assemblage of wildlife species (McIntyre, 2014). The novel host species constituting urban habitats may have different compatibility (susceptibility to infection, feeding behavior of intermediate host species, etc.) as hosts to parasite infections compared to species that existed before the habitat development. Potential links between disease ecology and altered species composition have been highlighted by previous studies (Krasnov et al., 2004, 2006; LoGiudice et al., 2003), but rarely with respect to host

assemblages in urban settings. However, an example of such a link may occur by a process called ‘mesopredator release’. With the removal of large predators, mesocarnivores are known to flourish in urban areas (hence ‘mesopredator release’) (Prugh et al., 2009). The increase of various mesocarnivores may lead to novel competition or dominance structures among different species sharing an area (Ritchie and Johnson, 2009) with a potential impact on pathogen dynamics. For instance, in a theoretical study of Lyme disease, Levi et al. (2012) proposed that the existence of coyotes, a new top predator after the removal of gray wolves, may lower the population size of red foxes, which is a dominant predator species of small mammals. Since small mammals are an important host species involved in the life-cycle of Lyme disease, the authors suggest that the existence and abundance of coyotes may function as a predictor of the higher prevalence of Lyme disease in an area. Coyotes and red foxes are considered two of the most successful urban-adapters (Gehrt et al., 2010). Therefore, understanding how these two species interact may have a significant impact on the prevalence of pathogens where either or both species function as a host. Similarly, the urban disease ecology of *E. multilocularis* is suggested to depend on the community assemblage of various urban-adapted canine host species (Liccioli et al., 2015). Although many studies on the dynamics of *E. multilocularis* in urban habitats have been focused on red foxes, other canine urban species, such as raccoon dogs and coyotes, are also associated with the circulation of *E. multilocularis*. Each canine species may have different competency as a host. Therefore, the transmission of the parasite may be amplified or attenuated in different urban habitats reflecting the composition of the host canine species in the area (Liccioli et al., 2015).

Altered microclimate: Urban areas are characterized by an increased cover of artificial impervious surfaces, lack of shade from vegetation, and smog and effluent in the air from industry, which all lead to retention of heat (Baker et al., 2002; Saaroni et al., 2000). These charactersitics lead to a phenomenon known as the 'urban heat island (UHI) effect', which results in warmer temperatures and reduced seasonality. Among others, the UHI effect can have a significant impact on the dynamics of arthropod-borne diseases because arthropods tend to be sensitive to microclimates, and some vector species may do better than others in response to urbanization (Bradley and Altizer, 2007). For instance, *Rhipicephalus sanguineus*, the tick commonly called the 'brown dog tick', is known to flourish in urban habitats compared to other tick species, as its entire life cycle can be completed indoors, buffered from external environmental changes (Dantas-Torres, 2010). Previous studies reported higher *R. sanguineus* burden in urban domestic dogs, whereas dogs from rural areas were often infested with a higher diversity of ticks, with *R. sanguineus* sometimes being completely absent (Costa et al., 2013; Dantas-Torres, 2010; Labruna et al., 2000). Considering that *R. sanguineus* is a competent vector of pathogens that can cause disease in dogs (*Ehrlichia canis, Anaplasma platys*) (Dantas-Torres et al., 2012; Rymaszewska and Grenda, 2008), and humans (*Rickettsia rickettsia*), the infestation of *R. sanguineus* in urban adapted wildlife warrants further investigation. Fleas are another common group of ectoparasite found in mesocarnivores including domestic dogs and cats. In general, fleas are particularly sensitive to temperature and humidity, as these determine their survival, development, and reproduction (Krasnov et al., 2001; Silvermann et al., 1981). Prevalence of flea and flea-borne pathogens in relation to urban habitat is further discussed in the next subsection.

2) Meta-analysis of pathogen prevalence studies comparing urban versus rural inhabiting host or vector populations: Case study of two major feline pathogens, *Toxoplasma gondii* and flea/flea-borne pathogens

Domestic cats are an important host species of both *T. gondii* and flea/flea-borne pathogens. Owing to the complex life cycle of both pathogen groups, their ecology can be influenced by numerous environmental factors unique to habitat types, in addition to the ecology of its main host, such as free-ranging cats (Friggens and Beier, 2010). I conducted a meta-analysis of previous studies on the prevalence of *T. gondii* and flea or flea-borne pathogens in host or vectors species from urban versus rural habitats. Through meta-analysis, my objective was to seek a pattern in pathogen prevalence between different habitat types and the mechanism(s) behind the pattern observed.

***Toxoplasma gondii*:** Previous studies comparing the prevalence of *T. gondii* in various host species across urban and rural/natural habitat types were analyzed (Table 1-2). The studies are categorized depending on outcome; significantly higher prevalence in (1) urban or (2) rural areas, or (3) a non-significant difference of prevalence between the two habitat types (Table 1-2, Fig. 1-2). It is worth noting that many studies reporting a higher *T. gondii* prevalence in urban habitats had intermediate host species as a study subject (Ballash et al., 2015; Lehrer et al., 2010). Uniformly, these studies attributed the higher density of definitive host-domestic cats as the cause of a higher *T. gondii* prevalence in urban habitats than rural habitats. Similar patterns were reported in previous studies where the intermediate hosts of raccoon roundworms, *Peromyscus* spp., from urban habitats showed a higher prevalence of *Baylisascaris procyonis* compared to rural conspecifics (Kellner et al., 2012). However, studies of *T. gondii* prevalence in the

definitive host, mostly domestic cats, reported a higher prevalence in rural areas or non-significant differences between the two habitat types (Table 1-2, Fig. 1-2). Studies that reported a higher *T. gondii* prevalence in rural domestic cats suggested greater access to intermediate hosts or environmental oocyst contaminants as underlying mechanisms. However, it is worth pointing out that many of these studies were performed on owned cats (rather than stray/feral cats or wild felines). Whereas in farmland or rural areas owners allow their cats to roam freely, in highly urban habitats, owners tend to keep their cats indoors (Clancy et al., 2003; Lord, 2008). Urban cats that stay completely indoors are unlikely to be exposed to any kind of *T. gondii* infection source. Therefore, it is possible that comparing the results of domestic cat studies solely based on habitat type may is insufficient to explain the interaction between domestic cats and sources of *T. gondii* infection distributed in external urban environments in urban and rural areas. On the contrary, studies showing insignificant differences of *T. gondii* prevalence between urban and rural habitat types were performed on stray animals or wild hosts (Ballash et al., 2015; Spada et al., 2012). These findings suggest that the life-style of domestic cats, rather than their habitat type, may be a better explanation of the function of domestic cats as hosts of *T. gondii* in different habitat types.

Flea and flea-borne pathogens: In an attempt to better understand habitat-related flea-borne parasite patterns, I reviewed literatures on the prevalence of flea and/or flea-borne parasites between urban and rural habitats in cats, dogs, and wild animals (Table 1-3, Fig. 1-3). Among flea species, cat fleas (*Ctenocephalides felis*) and dog fleas (*Ctenocephalides canis)* have been more commonly studied in relation to urban habitats.

These fleas are known to prefer warm and relatively low humidity, which is a microclimate condition provided by many urban housings.

However, the pattern of prevalence between urban and rural habitats differed depending upon the host species studied. Studies reporting higher flea infestation or higher prevalence of a flea-borne bacterial pathogen (*Bartonella henselae*) in urban habitats were performed on domestic owned cats (Table 1-3). A suggested driver for this pattern was the larger home range of cats in rural areas (and a conversely higher population density in urban domestic cat populations) that may lead to the relatively sparse distribution of flea immature zones and reduced chances of cats coming into contact with these areas. However, in general, studies reporting a higher flea prevalence/infestation rate in rural habitats were more common in my research (Table 1-3). Potential causes were higher human control measures in urban areas, the role of wildlife as an additional host species in rural areas, and the negative impact of the urban microclimate on survival and reproduction of fleas. The most commonly studied flea-borne pathogen was *Rickettsia typhi*, which is the etiological agent of Murine typhus (Azad, 1990). Murine typhus has been previously considered a port or city disease, where humans and rodents share habitats in high population densities (Azad, 1990). However, I found that many recent articles show an insignificant difference of *R. typhi* prevalence between the two habitat types, or a higher prevalence in rural areas. The authors of these studies suggest that the geographical expansion of *R. typhi* infection may be due to human behavior that leads to the modification of environmental conditions (Lledó et al., 2001; Nogueras et al., 2006). Collectively, future studies should consider diverse factors,

such as human activity, host species composition, and host animal behavior, to unravel the disease ecology of fleas or flea-borne pathogens in relation to urban habitats.

In summary, the results of this meta-analysis in both pathogen groups show that the prevalence of pathogens between urban and rural habitats is inconsistent. The current review also illustrated a few important factors that warrant further considerations in future studies. Such findings include, first, the importance of acknowledging the role of the studied species within the life-cycle of the pathogens. For instance, the prevalence of pathogens in intermediate/accidental hosts is suspected to correlate with the abundance of definitive hosts and/or the probability of interaction between the two species. However, the prevalence of pathogens in definitive hosts may depend not only on its population abundance but also on various other environmental and anthropogenic factors. Secondly, the abundance of arthropod vector species may be more affected by human control measures, as highlighted by previous studies reporting lower infestation rates of fleas in urban-dwelling hosts. Therefore, in epidemiological studies of vector-borne pathogens in urban habitats, the impact of direct human activities should be considered a significant external factor.

Collectively, understanding the disease ecology of pathogens with complex life-cycles among different habitat types requires analysis of broader ecological factors involved. Importantly, the comparison of prevalence in host species that have different roles in the life-cycles of the pathogens (intermediate host, accidental host, or definitive host) may aid in verifying the potential need for different control measures depending on the target host species of interest in pathogen management.

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Table 1-1. Drivers and processes linking the ecology of urban wildlife with its interaction with pathogens

Approach	**Related ecological traits of urban wildlife/ ecosystem**	**Potential mechanisms behind urban host-pathogen interactions**		**Expected impact on;**	
				Host exposure to infection	**Host susceptibility to infection**
Host trait-based; focuses on ecological traits of urban adapted mesocarnivores	Traits related to successful exploitation/ adaptation of urban habitat • Behavioral flexibility • Wide dietary range (omnivorous) • Medium body size • Usage of anthropogenic resource • High fecundity	1. Increased energy intake -Increased breeding success -Increased survival rate -Increased body condition	Lengthened infectious period of infected individual	increase	
			Increased availability of young susceptible individuals	increase	increase
			Increased availability of energy for investment in immune components		decrease
		2. Aggregated distribution (centered around clumped food source)	Increased contact among individuals	increase	
		3. Reduced home-range size/higher home-range overlap	Increased accumulation of parasites in infective stage	increase	
		4. Reduced dispersal rate	Increased inbreeding		increase

		5. Altered dietary content; from natural prey to anthropogenic food source	Reduced transmission of parasites with complex life-cycle	decrease
			Deficient consumption of critical nutrient	increase
Urban ecosystem-context; focuses on ecological characteristic of urban ecosystem	Spatial proximity to human residential area	Increased interaction with domestic hosts that are taxonomically close to various mesocarnivores; potentially sharing pathogens		increase
	Altered microclimate	Shifted composition and abundance of arthropod-vector species		vary
	Altered species composition	Mesopredator release: Increased abundance of urban exploiting mesocarnivores		vary

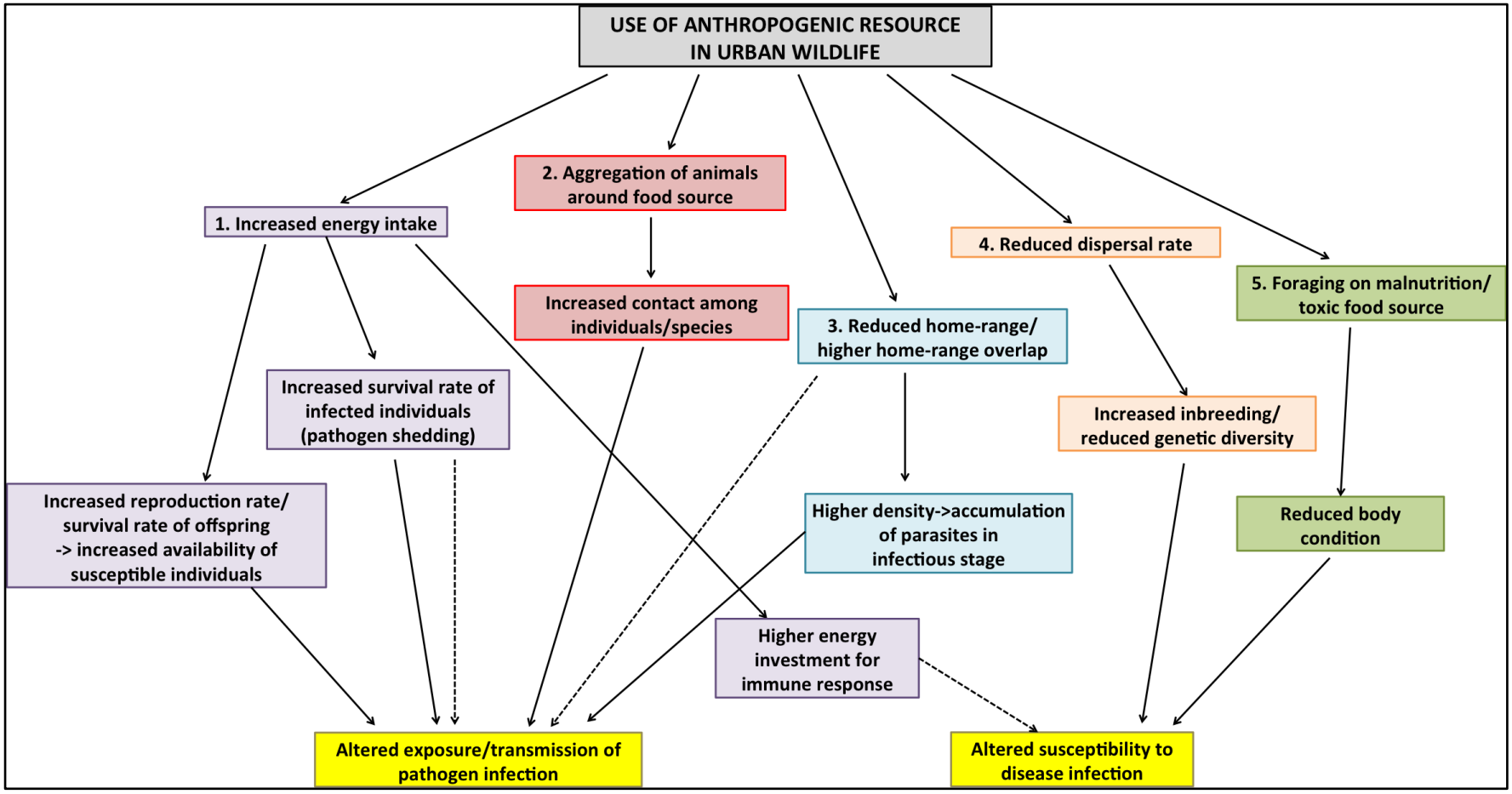

Figure. 1-1. Ecological processes observed in urban wildlife by utilizing anthropogenic resources, and its potential consequences on host-pathogen interactions (Number 1-5 in the figure corresponds to five host-trait based mechanisms in Table 1-1)

Table 1-2. Summary of relevant studies on the prevalence of *Toxoplasma gondii* between urban and rural habitats

Prevalence	**Urban vs. Rural (or suburban)**	**Host species**	**Function of studied host species**	**Factors discussed as potential mechanism behind the observed prevalences**	**Reference**
Toxoplasma gondii	Urban > rural	Woodchuck (*Marmota monax*)	Intermediate host	Greater densities of domestic and feral cats in urban areas	Lehrer et al. 2010
		White-tailed deer (*Odocoileus virginianus*)	Intermediate host	Higher household densities	Ballash et al. 2014
		Domestic goat (*Capra aegagrus hircus*)	Intermediate host	Higher cat and rodent densities	Bisson et al. 2000
	Urban < rural	Domestic cat (pet)	Definitive host	None	Opsteegh et al. 2012
		Domestic cat/ dog (pet)	Definitive host/ Intermediate host	1) Higher abundance of domestic and wild feline hosts in rural areas 2) Less soil exposure in urban areas	Ahmad et al. 2014
		Domestic cat (pet)	Definitive host	Different life-style; urban cats owners tend to keep cats indoors	Saevik et al. 2015
		Domestic cat (pet)	Definitive host	Higher access to local reservoirs of the parasite (prey animals and in the environment) in non-urban areas	Hornok et al. 2008
	Statistically insignificant differences	Domestic cat (outdoor cats and/or stray)	Definitive host	Higher direct transmission in urban habitats and combination of direct and trophic transmission in rural areas	Result from chapter3 /Ballash et al. 2014
		Domestic dog (stray)	Intermediate host	None	Yan et al. 2012
		Bobcats (*Lynx rufus*)	Definitive host	None	Riley et al. 2004
		Domestic cat (stray)	Definitive host	None	Spada et al. 2012

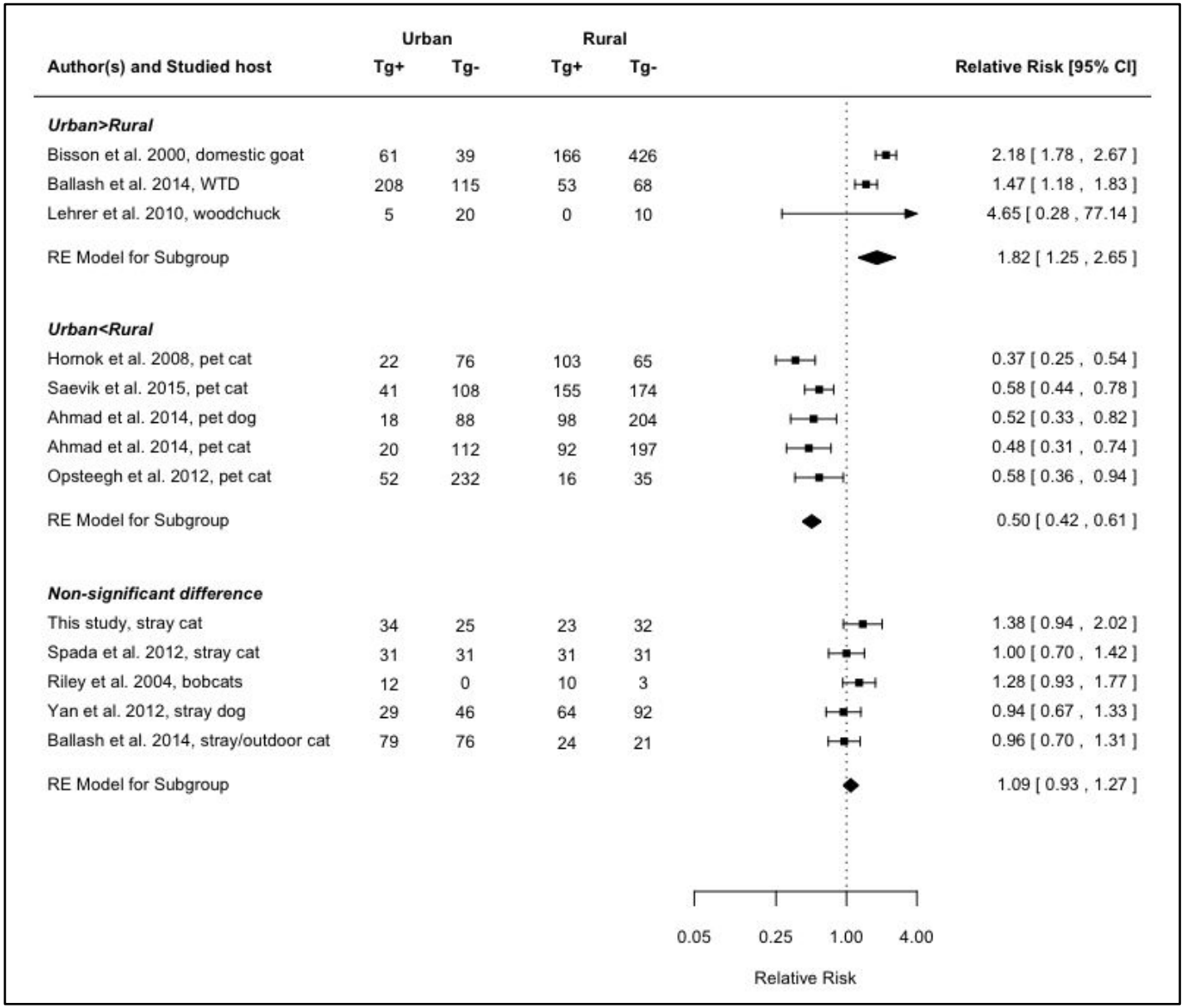
Urban
Rural
Author(s) and Studied host
Tg+
Tg-
Tg+
Tg-
Relative Risk [95% CI]
Urban>Rural
Bisson et al. 2000, domestic goat 61 39 166 426 2.18 [1.78 , 2.67]
Ballash et al. 2014, WTD 208 115 53 68 1.47 [1.18 , 1.83]
Lehrer et al. 2010, woodchuck 5 20 0 10 4.65 [0.28 , 77.14]
RE Model for Subgroup 1.82 [1.25 , 2.65]
Urban<Rural
Hornok et al. 2008, pet cat 22 76 103 65 0.37 [0.25 , 0.54]
Saevik et al. 2015, pet cat 41 108 155 174 0.58 [0.44 , 0.78]
Ahmad et al. 2014, pet dog 18 88 98 204 0.52 [0.33 , 0.82]
Ahmad et al. 2014, pet cat 20 112 92 197 0.48 [0.31 , 0.74]
Opsteegh et al. 2012, pet cat 52 232 16 35 0.58 [0.36 , 0.94]
RE Model for Subgroup 0.50 [0.42 , 0.61]
Non-significant difference
This study, stray cat 34 25 23 32 1.38 [0.94 , 2.02]
Spada et al. 2012, stray cat 31 31 31 31 1.00 [0.70 , 1.42]
Riley et al. 2004, bobcats 12 0 10 3 1.28 [0.93 , 1.77]
Yan et al. 2012, stray dog 29 46 64 92 0.94 [0.67 , 1.33]
Ballash et al. 2014, stray/outdoor cat 79 76 24 21 0.96 [0.70 , 1.31]
RE Model for Subgroup 1.09 [0.93 , 1.27]
0.05
0.25
1.00
4.00
Relative Risk

Figure 1-2. Summary of previous studies on *T. gondii* prevalence are shown grouped together according to their results (Result of prevalence; Urban>Rural or Urban<Rural or insignificant difference between two habitat types). Estimated relative risk and 95% confidence intervals of *T. gondii* prevalence outcomes are denoted through squares and horizontal lines. Higher relative risk above 1 indicate higher prevalence in urban habitats

Table 1-3. Summary of relevant studies on the prevalence of flea infestation and/or flea-borne pathogens between urban and rural habitats

Prevalence/infection intensity	Urban vs. Rural	Pathogen/Parasite	Host species	Factors discussed as potential mechanism behind the observed prevalence	Reference
Flea-borne pathogen/flea	Urban > rural	*Bartonella henselae*	Domestic cat	Higher aggregation of host species in urban habitats	Result from chapter 3.
		Flea (*Ctenocephalides felis*)	Domestic cat	Larger activity range of cats in rural area, 1) less concentration of flea immature development zones, and 2) less chances for cats to contact with these areas	Gracia et al. 2013
		Flea (*Ct. felis*)	Domestic dog	None	Alcaino et al. 2002
		Flea (*Ct. felis*)	Domestic cat	None	Wall et al. 1997
	Urban < rural	Flea (*Ct. canis*, *Ct. felis*)	Domestic dog/cat	Higher use of flea control in urban owners	Farkas et al. 2009/ Costa et al. 2013
		Flea (*Ct. canis*)	Domestic dog	1) More suitable habitats 2) Presence of wild carnivores which act as sources of infection	Gracia et al. 2008
		Flea (*Ct. canis*)	Domestic dog	None	Alcaino et al. 2002
		Rickettsia typhi	Human	Expansion to nonendemic areas (rural) through a peridomestic animal cycle involving e.g. cats, dogs, and opossums and their fleas	Lledo et al. 2001
		Rickettsia felis, Rickettsia typhi	Human	Human behavior modification and environmental changes which potentially led to expansion of *R. typhi* in to rural areas	Nogueras et al. 2006
		Yersinia pestis	Black-tailed	1) Impeded movement of hosts (prairie	McClure 2009

		(Plague)	prairie dog (*Cynomys ludovicianus*)	dog, flea) due to urban structures 2) Urban microclimate, reducing flea populations and/or plague transmission 3) Altered species composition and abundance in urbanized environment, possibly reducing host species influential in plague outbreaks, decreasing its transmission.	
	Statistically insignificant difference	*Rickettsia typhi*	Domestic dog	None	Lledo et al. 2003
		Rickettsia typhi	Human	None	Lledo et al. 2005
		Flea (*Ct. felis)*	Domestic dog	None	Gracia et al. 2008
		Flea (multiple species)	Domestic dog/cat	None	Beck et al. 2006

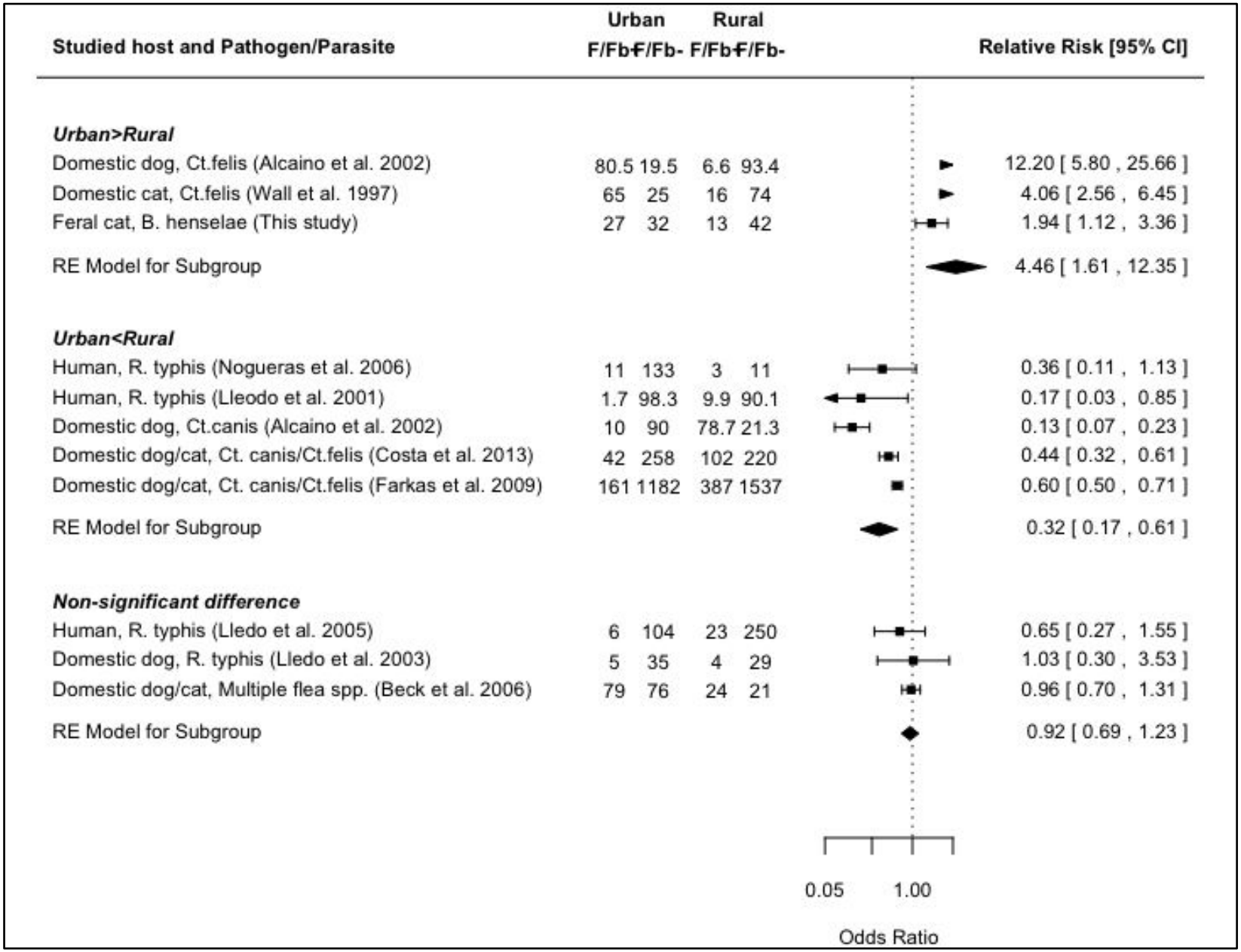
Studied host and Pathogen/Parasite
Urban F/Fb+ F/Fb-
Rural F/Fb+ F/Fb-
Relative Risk [95% CI]
Urban>Rural
Domestic dog, Ct.felis (Alcaino et al. 2002) 80.5 19.5 6.6 93.4 12.20 [5.80 , 25.66]
Domestic cat, Ct.felis (Wall et al. 1997) 65 25 16 74 4.06 [2.56 , 6.45]
Feral cat, B. henselae (This study) 27 32 13 42 1.94 [1.12 , 3.36]
RE Model for Subgroup 4.46 [1.61 , 12.35]
Urban<Rural
Human, R. typhis (Nogueras et al. 2006) 11 133 3 11 0.36 [0.11 , 1.13]
Human, R. typhis (Lledo et al. 2001) 1.7 98.3 9.9 90.1 0.17 [0.03 , 0.85]
Domestic dog, Ct.canis (Alcaino et al. 2002) 10 90 78.7 21.3 0.13 [0.07 , 0.23]
Domestic dog/cat, Ct. canis/Ct.felis (Costa et al. 2013) 42 258 102 220 0.44 [0.32 , 0.61]
Domestic dog/cat, Ct. canis/Ct.felis (Farkas et al. 2009) 161 1182 387 1537 0.60 [0.50 , 0.71]
RE Model for Subgroup 0.32 [0.17 , 0.61]
Non-significant difference
Human, R. typhis (Lledo et al. 2005) 6 104 23 250 0.65 [0.27 , 1.55]
Domestic dog, R. typhis (Lledo et al. 2003) 5 35 4 29 1.03 [0.30 , 3.53]
Domestic dog/cat, Multiple flea spp. (Beck et al. 2006) 79 76 24 21 0.96 [0.70 , 1.31]
RE Model for Subgroup 0.92 [0.69 , 1.23]
0.05
1.00
Odds Ratio

Figure 1-3. Summary of previous studies on flea and flea-boren pathogen infection are shown grouped together according to their results (Result of prevalence; Urban>Rural or Urban<Rural or insignificant difference between two habitat types). Estimated relative risk and 95% confidence intervals of Flea (F) and/or flea-borne pathogens (Fb) prevalence outcomes are denoted through squares and horizontal lines. Higher relative risk above 1 indicates higher prevalence in urban habitats

CHAPTER 2

EVALUATION OF BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS AND PREVALENCE OF SELECTED PATHOGENS IN FERAL CATS FROM URBAN AND RURAL HABITATS IN SOUTH KOREA[1]

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1 J. Hwang, N.L. Gottdenker, M.S. Min and M.S. Chun. *Journal of Feline Medicine and Surgery* 2015 May 27. pii: 1098612X15587572. [Epub ahead of print]

Abstract

Objective

In this study, we evaluated the potential association between the habitat types of feral cats and the prevalence of selected infectious pathogens and health status based on set of blood parameters.

Methods

We live-trapped 72 feral cats from two different habitat types: an urban area (N=48) and a rural agricultural area (N=24). We compared blood values and prevalence of FIV, FeLV and hemotropic *Mycoplasma* infection in feral cats from two contrasting habitats.

Results

Significant differences were observed in several blood values (HCT, RBC, BUN, Creatinine) depending on the habitat types and/or sex. Two individuals from the urban area were seropositive for FIV (3.0%), and 8 individuals (12.1%; 5 from urban and 3 from rural habitats) were positive for FeLV infection. However hemoplasma infection was more common. Based on molecular analysis, 38 cats (54.3%) were positive for hemoplasma with significantly higher infection rate in cats from rural habitats (70.8%) compared to urban cats (47.8%).

Conclusion and Relevance

Our study measured hematological and serum biochemical values, and prevalence of selected pathogens in feral cat populations from two different habitat types. A subset of important laboratory parameters from rural cats showed values under or above the corresponding reference ranges of healthy domestic cats, suggesting potential differences

in the health status of feral cats depending on the habitat type. Our findings provide information about the association between 1) blood values (hematological and serum biochemistry parameters) and 2) prevalence of selected pathogen infections with different habitat types that may provide important information to veterinarians who provide medical care for feral and/or stray cats and for overall cat welfare management.

Introduction

With their extreme adaptability and ecological flexibility, feral cats (*Felis catus*) are found in diverse habitat types, from deserted islands to the core of metropolitan areas (Denny et al., 2002). Feral cats in urbanized habitats typically have high population densities and are subject to increasing concerns regarding the potential role of feral cats in the transmission of zoonotic pathogens, the welfare of feral cats, and the conflicts with the public due to the wide range of human attitudes towards feral cats (Robertson, 2008). So far, there are a few studies evaluating the potential risk factors that may be related to pathogen infection and feral cats, such as habitat type (e.g. urban, agricultural) and/or life-style (pet, stray and feral) (Fromont et al., 2001; Little, 2005). However, many studies about pathogen infections in feral cats have focused on describing the prevalence of zoonotic diseases (Duarte et al., 2010; Lee et al., 2010). In this study, we compared blood values and prevalence of selected pathogens of feral cats inhabiting two different habitat types: an urban and a rural habitat. For the purposes of this study, feral cats were defined as unowned, untamed or unconfined cats (Levy and Crawford, 2004). Blood values composed of hematological and serum biochemistry parameters are considered to reflect the general health status of animals, including physiological condition and function of important organs, and are commonly applied to evaluate the trend of physiological status in natural feline populations, such as feral cats and the Iberian lynx (Beltrán et al., 1991; Macdonald et al., 1998; Wycislo et al., 2014). Although urbanized areas are generally not considered to be ideal habitats for wild mammals, feral cats in urban environments commonly use abundant food sources provided indirectly through human refuse or directly by cat caretakers (Finkler et al., 2011). In addition, feral cats

that rely on cat caretakers are known to show different ecology, such as smaller home range and breeding patterns (Schmidt et al., 2007), compared to their conspecifics living on farmlands or in rural areas where they are relatively independent from humans. We hypothesize that blood parameters of feral cats would differ depending on their habitats (urban versus rural) as a result of differences between the cats' environment and resources that would affect the physiological status of individual cats.

In addition to blood values, we examined the prevalence of selected feline pathogens, hemotropic *Mycoplasma*, feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) infections in rural and urban habitat types. Hemoplasmas and retroviruses are pathogens that have frequently been studied in domestic cats due to their potential to cause clinical signs that may be lethal to infected animals or negatively impact their health (Levy et al., 2006; Sykes et al., 2008; Willi et al., 2007). Hemotropic *Mycoplasma spp.* are bacteria that parasitize red blood cells that may cause anemia to varying degrees in cats (Messick, 2004), especially when co-infected with retroviruses (George et al., 2002). In cats, *Mycoplasma haemofelis,* Candidatus *M. hemominutum* and Candidatus *M. turicensis* have been reported to cause infection (Messick, 2004). FIV and FeLV are both retroviruses that have a significant impact on feline health (Hartmann, 2011). Although feline retroviruses have been widely studied, current knowledge about feline retroviruses is mostly from domestic cats rather than feral cats. However it should be noted that feral cats may physiologically and/or immunologically respond differently than domestic cats to retroviruses, due to different environmental factors such as diet and stress (Gibson et al., 2002; Plantinga et al., 2011). In pet cats, FIV can cause AIDS-like symptoms through immunosuppression and immunologic dysregulation, leading to

increased risk of opportunistic infections, onset of tumors, such as leukemia and lymphoma, and pathologic symptoms related to immune complex deposition disorders (Hartmann, 2011). FeLV is often more pathogenic than FIV, causing up to 80% mortality after infection (Levy, 2000). Clinical signs of FeLV are most commonly related to tumors, immunosuppression, hematologic disorders and immune-mediated diseases (Hartmann, 2011).

The aim of this study is to identify any differences in 1) hematological and serum biochemical values and 3) prevalence of infection for the selected pathogens, FIV, FeLV and hemoplasma, between feral cat (*Felis catus*) populations from two contrasting environments.

Material & Methods

Field site

We collected blood samples from feral cats in 7 different sites: 5 urbanized habitats and 2 rural areas. The urban sites were selected within Seoul (37°33′59.53″N 126°58′40.69″E), Korea, which is an intensively developed urban area, with population density of 17,000/km^2 (Kim and Baik, 2005). The Seoul landscape is highly fragmented and densely occupied with buildings and roads (Hong et al., 2003). Feral cats are mostly observed in residential areas within the city, where they engage in both positive and negative interactions with people, such as food provisioning and animal abuse incidents, respectively. Thus all urban trapping sites were selected among similar areas that represent densely packed multi-story residential buildings, one of the most typical housing structures in the city according to statistical data from the Seoul Metropolitan

Government population census (year 2013). Rural sites were selected among typical agricultural landscape composed of extensive rice paddies and sparsely dispersed houses. Two rural sites were selected among areas surrounding the Seoul, which were Yangpyeong county (37°46′15.262″N 127°42′10.875″E) and Gwangju city (37°42′83.21″N 127°33′48.157″E), located in distance of approximately 70 km from Seoul. The vegetation of the area is typically dominated by rice, except for shrubs between the rice paddies. The farming areas are surrounded by low hilly forest-covered landscapes. The population densities of the rural sites were approximately 136-189/km^2. In order to collect independent samples with respect to geographical locations, trapping sites from both urban and rural areas were sufficiently dispersed from each other (min.10km - max.18km) to avoid the possibility of trapping same cat (s) from different sites.

Live trapping and sample collection

Feral cats were live-trapped for a total of 480 trap nights in the summer of 2013 using Tomahawk traps (Tomahawk, Live Trap Company, WI). All traps were baited with wet cat food. In urban neighborhoods, traps were set in relatively hidden and confined places where feral cats were commonly observed. Such spots also reduced the chance of trapped cats being exposed to pedestrians before we checked the traps. In rural neighborhoods, most traps were placed within the farmland area with the permission of the farm owners. All traps were set between 6-8 pm in all areas, and sample collection started around 2-3 am the next morning in close proximity (less than 5 minute drive) to the trapping sites. Every trap with a cat was covered with a plastic panel to reduce the

visual stress of the trapped cats until sample collection. Trapped cats were anesthetized with a mixture of Zoletil® (zolazepam and tiletamine; 2 mg/kg) and Domitor® (medetomidine; 50 mg/kg) before sample collection. Physical examination was performed for each cat before samples collection, including measuring body weight, checking for teeth condition and ear-tipping (marking for neutering). Approximately 3-4 ml of blood were collected from the jugular vein. Half of the whole blood samples were collected in EDTA tube and the other half were collected in serum separation tubes containing clot activator. An aliquot of whole blood sample in EDTA tube was sent for hematology analysis within 1-2 days after collection, and rest of the blood samples were stored in -70°C for future DNA analysis. Serum separation tubes were maintained in an upright position until centrifugation. Centrifugation was performed within 3-4 hours after blood collection. Separated serum samples were stored in -70°**C** for serum biochemistry analysis and serology pathogen diagnostic assays.

Ethics statement

Ethical approval for the use of animals in this study was obtained from the IACUC of the University of Georgia (UGA IACUC: A2013 04-009-Y1-A0) and Seoul National University (SNUIBC-R131118-1). The fieldwork for this study was performed in areas where no specific permissions were required, and none of the field studies involved endangered or protected species.

Laboratory analyses

Both hematology and serum biochemistry analyses were performed at SNU Veterinary Medical Teaching Hospital (SNU VMTH) using automatic analyzers (Hematological analysis; Siemens ADVIA 2120i hematology system (Moritz et al., 2004), serum biochemistry analysis; Hitachi 7180 clinical analyzer). The hematology analytes included hematocrit, red blood cell count (RBC), white blood cell count (WBC), hemoglobin (HGB), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). Serum samples were tested for urea (BUN), creatinine (Crea), albumin, total protein (TP) and three major liver enzymes, alanine transaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP).

Serum samples were tested for identification of FIV antibodies and FeLV p27 antigen using commercialized serum diagnostic kits (SensPERT FELV Ag/FIV Ab kit; VetAll™). The manufacturer reported test sensitivities for FeLV antigen and FIV antibody assays as 98.5% and 99.7%, respectively, while the specificities for the assays were 97% and 99%, respectively (VetAll™, Seoul, Korea). All positive samples were re-tested with another commercialized ELISA kit from a different manufacturer (SNAP® FIV Antibody/FeLV Antigen Combo; IDEXX).

DNA was extracted from whole blood samples with the QIAamp DNA mini kit (Qiagen, Hilden, Germany). The extracted DNA was used to identify hemoplasma infection through a subsequent PCR assay. We amplified a partial segment of the 16S rRNA gene of *Mycoplasma* spp. using universal primer set HBT-F (ATACGGCCCATATTCCTACG) and HBT-R (TGCTCCACCACTTGTTCA) designed by Criado-Fornelio et al. (2003). The protocol for PCR was performed as previously

described (Criado-Fornelio et al., 2003). Every PCR run included a positive and negative control to detect bacterial contamination. Amplified products were separated by electrophoresis on a 1.2% agarose gel. PCR amplicons from the positive samples were purified and prepared for direct sequencing. The sequence homology of the sequenced 16S rRNA gene from the positive samples was examined using the National Center for Bio-technology Information (National Institute of Health) BLAST network service.

Statistical analyses

All statistical analyses were performed using statistical software R (http://cran.r-project.org). The significance association of sample numbers between habitat type and sex was analyzed using chi-square test. All continuous data, such as hematology and serum biochemistry parameters, were evaluated by the Shapiro-Wilk test before use in further tests. Mean and 95% confidence interval of continuous parameters (hematology and serum biochemistry parameters) were calculated with Bonferroni correction. Descriptive statistics are shown in tables. Sample sizes slightly varied according to analyses because of the missing data and/or specimens (whole blood or serum) of several individuals. The differences in hematology or serum biochemistry parameters between habitat types and sex groups were tested using two-way ANOVA. The difference in those parameters among sampling sites within each habitat type, 5 in urban and 2 in rural sites, was tested using the ANOVA and the post-hoc test (Tukey's HSD test) for pairwise comparison. Post hoc power analysis (1- beta error probability) was performed using G*Power to evaluate the ability of the analysis to detect significant association between blood parameters and/or pathogen infection and habitat types at an alpha error of 0.05

(Faul et al., 2009). The logistic regression model included two binary independent variables and their interaction (the habitat types, sexes and habitat type-sex interaction) to test its association with the three dependent variables (FIV, FeLV and hemoplasma infection). Odds ratio of two independent variables and its 95% confidence interval for hemoplasma infection was calculated. Lastly, a likelihood ratio test for the logistic regression model was performed comparing the tested model (sex, habitat, habitat x sex) and the model with intercept only.

Results

In total, biological samples were collected from 72 trapped feral cats. Among the 48 cats from the urban site, 16 individuals were female and 32 were male, while from the rural area, 12 cats were female and 12 were male. Although the samples from the urban area had more male cats, there was no significant association between sex and habitat type (chi-square test: p-value=0.20). However, it should be noted that power to detect the association between habitat and sex was only 0.29. Therefore careful interpretation is required when analyzing data combined by habitat type or sex (urban versus rural, male versus female) and the significance of interaction between the habitat type and sex.

Physical examination

Based on physical examinations, two female individuals showing round and swollen abdomen with large, well-developed nipples were determined as pregnant. Three male individuals weighing lower than 2.0kg and showing clean teeth, completely devoid of yellowing of tartar (Simking et al., 2010) were categorized as juveniles. In addition,

five male cats from urban habitat had ear-tipping, which is indication of neutering. The rest of the 67 animals were assumed to be intact.

Hematology

When hematologic parameters were tested for differences among sites (urban; 5 sites, rural; 2 sites) within each habitat type, none of the analytes showed a significant difference. Therefore, hematological parameters from different sites of the same habitat types were pooled for further analysis. There was a significant habitat type difference in HCT and RBC values, with urban cats having a higher mean value (Table 2-1). HCT, RBC and HGB values were also different between genders with male cats showing higher mean values. In all hematological values, habitat-sex interaction was not significant. The WBC values were not significantly associated with habitat type or sex (Table 2-1). However, in general, the WBC values of all the groups were high relative to the reference range provided by the SNU VMTH, with only the WBC values of the urban males falling within the reference range. There were three outlier samples with WBC value over 150, which were an urban female, a rural male and a rural female. Therefore, the impact of these three samples will need to be considered when interpreting the mean values of WBC in each group. Despite the limited sample size, statistical power (probability of rejecting false null hypothesis) of the tests comparing hematological parameters (RBC, HCT, HGB and WBC) between two habitat types was over 0.8 with the exception of WBC (0.59). Therefore, the lack of significance of the WBC result should be interpreted cautiously.

Serum biochemistry

Serum analytes, including BUN, creatinine, ALT, AST, total protein and albumin, were tested for significant differences among sites (urban; 5 sites, rural; 2 sites) within each habitat type. None of the analytes were significantly different among sites in urban or rural habitat types except for albumin and total protein (for both protein analytes, samples from one urban site showed a significantly higher value compared to other urban and rural sites. total protein=8.2 g/dl, albumin=3.5 g/dl). Therefore, serum parameters from different sites of same habitat types were pooled for further analysis. When the serum analytes were compared between the two habitat types, a significant difference was observed in the subset of parameters, with higher values of ALT in rural cats and higher values of BUN and creatinine in urban cats. Nevertheless, the values were within the reference range both provided by the SNU VMTH and by a previous study of New York free-ranging cats (Wycislo et al., 2014). In addition, sex was associated with TP and creatinine, with female cats having lower values (Table 2-2). A significant habitat-sex interaction was observed in AST values (Fig. 2-1). Power analysis of comparing serum parameters between the two-habitat types revealed statistical power of total protein and albumin analysis to be low (<0.8) which warrants cautious interpretation.

Identification of hemoplasma infection and its association with sex and habitat types

A total of 70 individuals (Urban = 46 and Rural = 24) were tested for hemotropic *Mycoplasma* infection by molecular diagnostics, and two samples were excluded due to failure of blood sample collection. Twenty-two (47.8%) out of 46 urban individuals and 16 (66.7%) out of 24 rural individuals were identified as hemotropic *Mycoplasma* PCR-

positive, giving 38/70 (54.3%) hemoplasma cases in the studied cats (Table 2-3, Fig. 2-2). Positive associations of *Mycoplasma* infection with rural habitat type (p-value=0.041, OR=7.00, 95% CI=1.22-58.56) and male (p-value=0.002, OR=14.00, 95% CI=3.13-101.49) were shown by the logistic regression analysis (likelihood ratio chi-square=18.83, d.f.=3, p-value=0.0003) (Table 2-4). The goodness-of-fit of the model assessed by Pseudo R^2 was 0.32. Post hoc power analysis showed that the power of detecting significant difference in hemoplasma infection between the two habitat types and two sexes were 0.32 and 0.92, respectively (alpha=0.05).

From the urban habitats, 5 out of 22 positive cats (22.7%) were *Mycoplasma haemofelis,* and the remaining 17 (77.3%) were *Mycoplasma haemominutum*. In the cats from the rural habitats, 1 cat (6.3%) was identified as *M. haemofelis* positive and the other 15 (93.7%) as *M. haemominutum positive.*

Identification of FIV/FeLV infection

Sixty-six samples were tested for FIV antibody and FeLV antigen using a serology assay kit. Two samples from the urban habitats showed positive exposure to FIV infection. Overall, occurrence of the FeLV antigen was 12.1% (8/66), and the proportion of FeLV infected cats was similar in both habitat types (Urban, 5/43, 11.9% and Rural, 3/23, 12.5%) (Table 2-3). When the occurrence of retrovirus infection was compared in the four groups (rural males, rural females, urban males and urban females), urban females had the highest infection rates (28.6%; n=14).

Discussion

In this study, we compared hematological and serum chemistry parameters and prevalence to selected pathogens of feral cats from urban and rural habitats. Subsets of important laboratory parameters, such as HCT, BUN and creatinine, of rural cats were under or above the corresponding reference ranges of healthy domestic cats, which suggests the potential influence of habitat type on the different health status of feral cats. In addition, the prevalence of hemoplasma infection was significantly higher in rural cats.

Among hematological parameters, HCT and RBC were significantly higher in male or urban cats compared to female or rural cats, respectively. In wild carnivores, low HCT values may indicate lower nutritional status, in some cases due to reduced prey availability and/or prey biomass (Dunbar et al., 1997). Similarly, rural cats may be under stronger nutritional stress than urban cats, due to resource limitations. However, testing additional variables, such as dehydration, toxin exposure and pathogen infection status (e.g. hookworm infection) would be required to further clarify the cause of the lower HCT values in rural cats. Meanwhile, values of HCT, RBC and HGB for rural females were consistently below the reference range. Two out of eleven (18%) rural female cats were pregnant and both cats showed hematology values lower than average, which may have caused the general decrease of values in rural female cats. However, it should be noted that RBC and HCT values of rural female cats were below reference range even when pregnant individuals were excluded from the dataset. The WBC values from the three groups, both sexes of cats from the rural areas and the urban females, were above the reference range. Although various mechanisms may cause an increase in the WBC,

higher WBC values are commonly considered to be associated with pathogen infection and/or inflammation (Aspinall and Aspinall, 2013).

Among biochemical analytes, serum BUN and creatinine values are known to reflect status of protein intake and muscle mass or kidney diseases in carnivorous mammals (Curi et al., 2014; Fuller et al., 1985; Kirkpatrick, 1975). Although the BUN and creatinine values of the studied cats were within the reference range, constantly lower BUN and creatinine values of the rural cats in both sexes could potentially suggest lower protein intake and/or muscle mass compared to cats from urban habitats.

In the case of liver enzymes, rural cats had higher levels of AST and ALT compared to urban cats, with the difference being larger in males for AST levels. Although it is possible that stress and tissue damage occurred during the trapping procedure, all cats were trapped through identical protocols and it is likely that other factors have caused the difference in liver enzyme levels between the two habitat types, such as chronic stress (e.g. malnutrition) and/or poor liver function (Fuller et al., 1985; Marco et al., 2000; Nájera et al., 2014). Alternatively, the liver enzyme levels from our result may be a characteristic feature of cats from each studied colony and that the observed ALT and AST values represents the normal range of healthy cats from each colony. The total protein and albumin for all the cats were within the reference range, which makes it unlikely for the cats to be suffering from serious dehydration.

A potentially important factor to consider when interpreting serum analytes is the individuality of each parameter. A recent study reported the high individuality of few serum analytes, including ALP, ALT, creatinine and globulin (Baral et al., 2014). For parameters with high individuality, population-based reference intervals are likely to

have limited significance, and when possible, repetitive sample collection of identical cats with time intervals should be encouraged for clear examination of the studied cat.

It should be also noted that the reference range used for the hematology and serology in this study was provided by the SNU Veterinary Medical Teaching Hospital (SNU VMTH) and thus is based on pet domestic cats rather than feral cats. There are not many reports about the reference range of stray/feral cat blood values. However, a recent biochemical survey of free-ranging cats in New York (Wycislo et al., 2014) reported a range of serum biochemistry values which was similar to the reference range used in our study, except for creatinine (0.6-1.4 mg/dl), which was lower than the range provided by the SNU VMTH (1.0-2.2 mg/dl). This could suggest that based on the creatinine value, stray/feral cats have lower muscle mass compared to pet domestic cats, or show a low-normal range for creatinine.

In our study, the hemoplasma infection rate was higher in cats from rural habitats and in male cats. Two hemoplasma species were identified; *M. haemominutum* was the most common species followed by *M. haemofelis*. The larger number of infected cats in rural areas could be related to the transmission mode of the hemoplasma species, which is suspected to be through fighting between male individuals and through blood-sucking arthropod vectors (Greene, 2011; Sykes, 2010; Sykes et al., 2008). It is possible that cats from rural habitats have daily contact with vegetation or soil, where ectoparasites live and quest for passing host animals (Lindström and Jaenson, 2003). Meanwhile, many urban feral cats are suspected to spend majority of their time using artificial structures, such as parking lots, empty buildings and basement areas (Calhoon and Haspel, 1989), limiting their chances to be exposed to ectoparasites (Sobrino et al., 2012). Further studies

simultaneously comparing occurrence of ectoparasites and vector-borne pathogens infection in relation to different habitat type are essential for revealing pattern between sex, habitat type and the occurrence of vectors and vector-borne diseases in feral cats.

Alternatively, a higher frequency of aggressive behaviors in rural males compared to urban male cats may be the cause for higher hemoplasma infection. In previous studies, male cats have been frequently identified to have a higher risk of hemoplasma infection compared to female cats (Bauer et al., 2011; Roura et al., 2010). Since fighting is likely to be one of the major transmission routes of hemoplasma infection in males, reduced aggressive behavior of neutered urban male cats (Scott et al., 2002) may have contributed to lower hemoplasma prevalence in the urban habitat. Out of five neutered male cats, all from urban habitats, four cats were negative for both saliva-borne pathogens, hemoplasma and FIV, and one neutered cat was co-infected with *Mycoplasma haemofelis* and *Mycoplasma haemominutum.*

The only two cats that were positive for FIV in this study were females from urban habitat, which made it difficult to observe the potential association between FIV infection and neutering or aggressiveness. For FeLV infection, the occurrence in our study was relatively high compared to previous reports on retrovirus prevalence in feral or stray cats which ranged from 0-12.2% (23-25, 45). The prevalence of FeLV from our study will have implications for the cat practitioners in Korea who treat both pet and stray/feral cats in their clinics. Nevertheless, considering that the prevalence of retroviruses is generally low when tested in a population level, studies with sufficient number of samples that fall into different categories of the tested variables would be required in order to explain the risk factors for retrovirus prevalence (Bande et al., 2012).

Lastly, it should be noted that insufficient sample size might have caused the lack of statistical significance in some of the analyses, such as comparison of serum biochemical parameters by sex, as evidenced by power lower than 0.8. Thus, number of samples from each sex or habitat type should be considered when interpreting the result in relation to the tested factors (habitat type, sex and pathogen infection).

Conclusions

Although there is controversy regarding the role of feral cats in an ecosystem (Longcore et al., 2009), there is no doubt that they are ecologically interacting with the various components of their environment, including wildlife, domestic animals and humans, with the potential for pathogen transmission. Individual behaviors and/or population structure of domestic cat with different life-style (e.g. pet, feral, shelter) varies in several aspects such as population density, allogrooming and nurturing behavior among individuals, all of which may influence pathogen transmission (Liberg et al., 2000). Therefore, the prevalence of disease and possible interactions between pathogens and cats should be monitored separately in relation to different environmental settings and habitat types. Our study reveals possible habitat-related differences in feral cat health status, such as blood parameters and infection with selected pathogens. Findings from this study can provide important information for domestic cat practitioners and those who are involved in managing feral cat populations (e.g. Trap-Neuter-Return). In addition, pathogen infection status of feral cats in rural habitats may aid conservation efforts of wildlife species that can share pathogens with feral cats, such as leopard cats (*Prionailurus bengalensis euptilurus*) in Korea.

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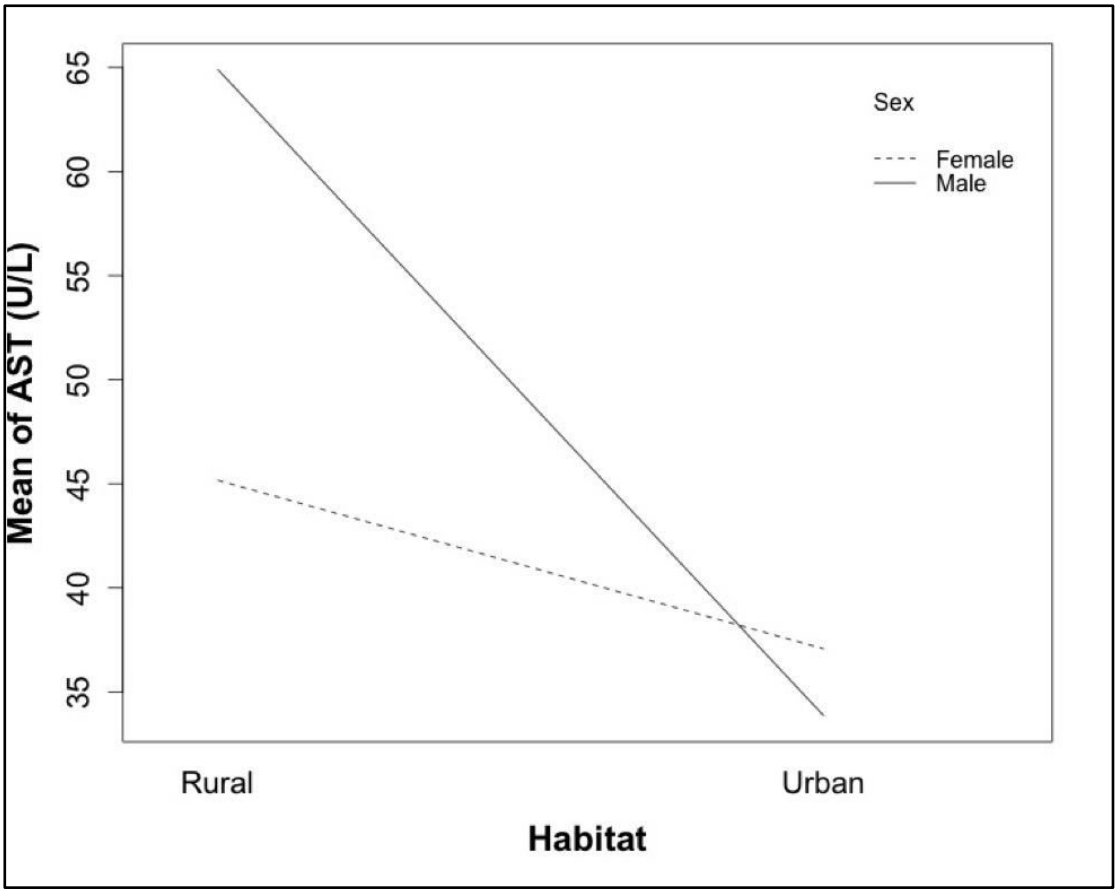

Figure 2-1. Interaction plot of AST showing significant interaction between habitat type and sex

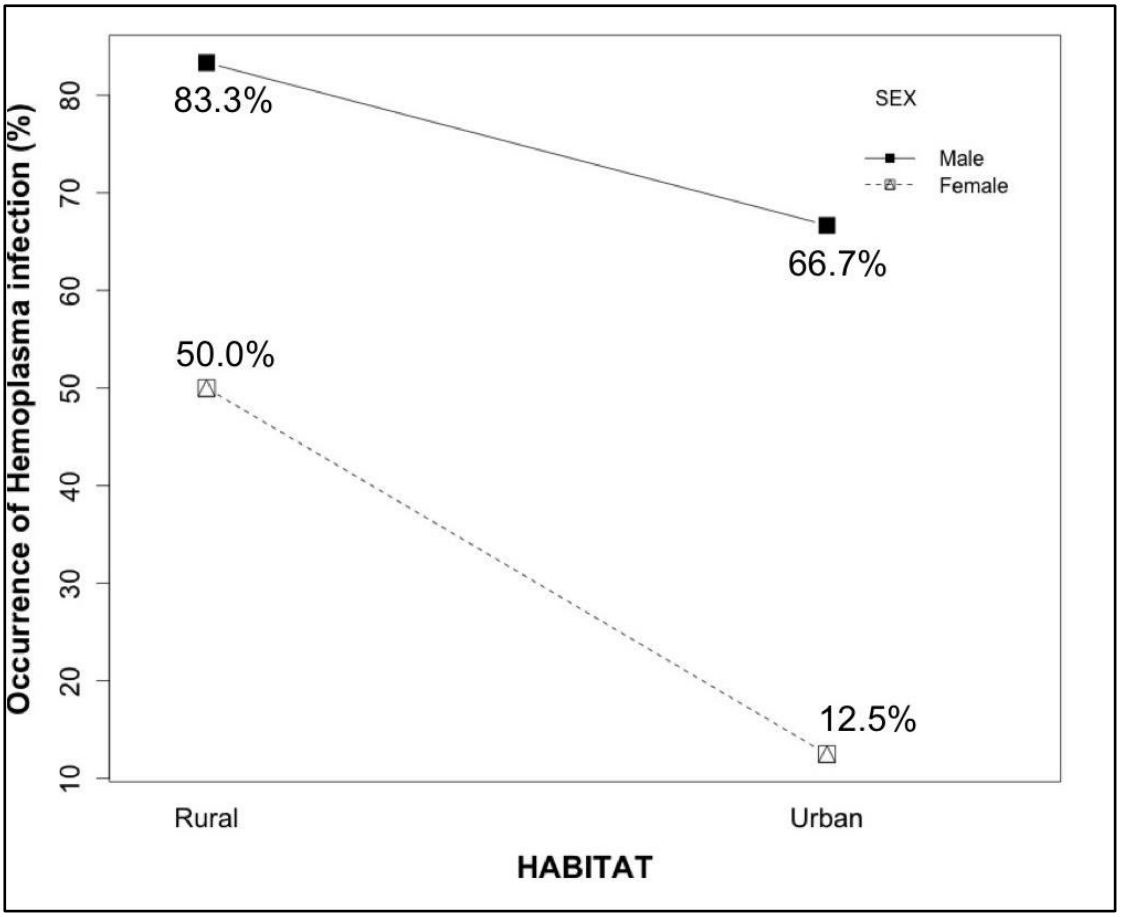

Figure 2-2. Occurrence of hemoplasma infection in feral cats by habitat type and sex

Table 2-1. Hematological values of studied feral cats by habitat and sex, including results of 2-way analyses of variance.

(Mean, 95% Bonferroni confidence interval)

	Reference range	Urban		Rural		P values		
		Male (n=31)	Female (n=15)	Male (n=9)	Female (n=12)	Habitat	Sex	Interaction; Habitat and Sex
HCT (%)	27.7-46.8	32.2 (29.3-35.1)	27.6 (23.5-31.8)	27.0 (21.7-32.4)	22.4 (17.8-27.1)	0.003	0.009	0.99
RBC ($x10^6$/L)	6.0-10.1	7.3 (6.65-7.94)	6.0 (5.07-6.92)	5.8 (4.63-7.01)	4.9 (3.90-5.96)	0.001	0.005	0.59
HGB (g/dL)	8.1-14.2	11.2 (10.0-12.4)	9.9 (8.2-11.6)	11.5 (9.3-13.7)	8.1 (6.2-10.0)	0.28	0.001	0.14
MCV (fL)	41.3-52.6	44.3 (42.8-45.9)	46.2 (44.0-48.3)	46.6 (43.9-49.4)	45.5 (43.1-48.0)	0.35	0.70	0.10
WBC* ($x 10^3$/uL)	6.3-19.6	17.3 (-0.74-35.3)	40.0 (14.1-65.9)	45.2 (11.7-78.6)	39.4 (10.4-68.3)	0.053	0.60	0.17

Table 2-2. Serum biochemistry values of studied feral cats by habitat and sex, including results of 2-way analyses of variance. (Mean, 95% Bonferroni confidence interval)

	Reference range	Urban		Rural		P value		
		Male (n=28)	Female (n=13)	Male (n=10)	Female (n=12)	Habitat	Sex	Interaction; Habitat and Sex
AST (U/L)	12-40	33.8 (25.7-41.9)	37.4 (25.5-49.2)	64.9 (51.4-78.4)	45.2 (32.8-57.7)	<0.001	0.078	0.012
ALT (U/L)	28-100	42.5 (33.5-51.4)	39.8 (26.6-52.9)	58.2 (43.2-73.2)	59.2 (45.5-72.8)	0.001	0.86	0.72
Albumin (g/dl)	2.4-4.1	3.2 (3.01-3.36)	3.2 (2.97-3.49)	3.4 (3.10-3.69)	3.2 (2.97-3.50)	0.29	0.58	0.30
TP (g/dl)	5.8-8.5	7.1 (6.81-7.48)	6.9 (6.40-7.38)	7.1 (6.59-7.70)	6.5 (6.0-7.02)	0.31	0.021	0.31
BUN (mg/dl)	15-34	25.1 (22.2-25.2)	21.1 (16.9-21.2)	19.8 (15.0-20.0)	18.1 (13.8-18.3)	0.012	0.079	0.47
Creatinine (mg/dl)	1.0-2.2	1.3 (1.15-1.39)	1.1 (0.97-1.31)	1.1 (0.91-1.29)	1.0 (0.78-1.14)	0.009	0.042	0.94

Table 2-3. Occurrence of hemoplasma and retroviruses infection in feral cats from the two habitat types

	Urban		**Rural**		**Total**
	Female	**Male**	**Female**	**Male**	
Hemotropic *Mycoplasma*					
Sample size (n)	16	30	12	12	70
Infected animals	2/16 (12.5%)	20/30 (66.7%)	6/12 (50%)	10/12 (83.3%)	38/70 (54.3%)
FIV and FeLV					
Sample size (n)	14	29	12	11	66
Infected animals; FIV	2/14 (14.3%)	0/29 (0%)	0/12 (0%)	0/11 (0%)	2/66 (3.0%)
Infected animals; FeLV	4/14 (28.6%)	1/29 (3.4%)	1/12 (8.3%)	2/11 (18.1%)	8/66 (12.1%)

Table 2-4. Association between hemoplasma infection and external factors in the sampled feral cats (n=70).

Significant associations are shown in bold

Variable	**Categories**	**Estimate**	**SE**	**p-value**	**Odds ratio (OR)**	**OR C.I. (95%)**
Habitat	Urban	-	-	**0.041**	1.00	
	Rural	1.95	0.95		7.00	1.22-58.56
Sex	Female	-	-	**0.002**	1.00	
	Male	2.64	0.85		14.00	3.13-101.49
Interaction; Habitat and Sex	-	-1.03	1.29	0.42	-	

CHAPTER 3

PREVALENCE OF PATHOGENS WITH COMPLEX LIFE CYCLES IN CONTRASTING HABITAT TYPES; STUDY ON URBAN AND RURAL FERAL CATS[1]

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Abstract

Background: Pathogens transmitted through intermediate hosts or vector species can be particularly sensitive to changes in environmental conditions due to their complex life cycles and the diversity of biotic and abiotic factors involved in their transmission. In this study, we examine the prevalence of three infectious pathogens with different transmission modes (*Bartonella henselae* and hemoplasma; vector-borne transmission, *Toxoplasma gondii*; trophic or direct transmission) in feral cats (*Felis catus*) from two unique habitat types, urban and rural. We collected feral cat samples from contrasting habitat types (total N=117): an urban area (N=63) and a rural agricultural area (N=54). We determined the infection status of three pathogens in blood samples of feral cats through molecular and serological diagnostic methods.

Results: Overall prevalence of hemoplasma, *T. gondii* and *B. henselae* was 47.9%, 50% and 35.7%, respectively. Between-habitat type differences in prevalence were insignificant in hemoplasma and *T. gondii* infection, whereas occurrence of *B. henselae* antibodies was significantly higher in urban cats.

Conclusions: From this study, we discuss how pathogens with distinct transmission modes may show different infection patterns between the two habitat types. We suggest further efforts to identify underlying mechanisms explaining the potential association among transmission modes, habitat types, and host-pathogen interactions.

Key words: Feral cat, *Toxoplasma gondii*, *Bartonella henselae*, hemoplasma, PCR, IFA

Introduction

The life-history of vector-borne and/or trophically transmitted pathogens involve complex associations with a wide range of environmental factors including abiotic/biotic components, and the ecology of both vector and host species, making them particularly vulnerable to environmental changes (LaDeau et al., 2015; Lambin et al., 2010). Therefore attempts to better understand the transmission of pathogens with complex life cycles and/or pathogen prevalence in different host populations should consider potential shifts of host-pathogen interactions with respect to different landscape conditions (Gilot-Fromont et al., 2012; Reisen, 2009). Globally, domestic cats are distributed in wide range of habitat types, from sites of intense urbanization to protected natural areas (Denny et al., 2002). As a result, free-ranging domestic cats (*Felis catus*), including pet cats with outdoor access, and feral cats, are exposed to diverse environmental conditions that may cause variation in their interactions with pathogens (Ostfeld et al., 2005). Domestic cats are also hosts for a wide range of pathogens with complex life-cycles, including *Toxoplasma gondii,* and Feline vector borne diseases (FVBD) such as *Bartonella* spp., *Anaplasma/Ehrlichia* spp., and *Rickettsia* spp. (Maia et al., 2014; Spada et al., 2012), making them excellent model systems to evaluate impacts of habitat types on the transmission of vector-borne and/or trophically transmitted pathogens.

Here, we attempt to identify the potential association between habitat type (urban and rural) and feral cat infection with three pathogens that have different transmission modes; *Toxoplasma gondii, Bartonella henselae* and hemoplasma. *T. gondii* is a zoonotic protozoan that is a significant threat to human, domestic animal, and wildlife health. Only felids, such as domestic cats, serve as definitive hosts, but almost all warm-blooded

animals can be infected as an intermediate host (Dubey and Jones, 2008). Domestic cats can be infected with *T. gondii* trophically through predation of an infected intermediate host, such as rodents, or through environmental transmission-contact with oocysts in fecal matter or contaminated abiotic materials such as soil and vegetation (Afonso et al., 2013; Gilot-Fromont et al., 2012). *B. henselae* is a bacterial pathogen known as the etiological agent of Cat Scratch Disease in humans (Breitschwerdt et al., 2010). *B. henselae* infection is usually asymptomatic in feline hosts and is flea-borne (*Ctenocephalides felis*), primarily transmitted through flea feces (Chomel et al., 2009). *Mycoplasma haemofelis* and '*Candidatus* Mycoplasma haemominutum' are important bacterial pathogens in feline species that may cause mild to severe anemia, especially when cats are co-infected with FIV and/or FeLV (Luria et al., 2004; Macieira et al., 2008). Hemoplasma in domestic cats can be transmitted through either direct contact with saliva or blood of infected individuals (e.g. biting, tick bites) (Sykes, 2010; Willi et al., 2007a).

In this study, we hypothesize that the prevalence of vector-borne pathogens (*B. henselae*, hemoplasma) would be higher in rural habitats due to frequent exposure of feral cats to potential vector species, such as ticks and fleas. Previous studies on arthropod-borne pathogens and/or arthropod infestation rates in various host species showed a higher prevalence and/or infestation rate in rural host populations (Costa et al., 2013; Labruna et al., 2007; Rizzoli et al., 2014), although certain vector species were better adapted to urbanized environments (Dantas-Torres, 2010; Shimada et al., 2003). We also expect a higher prevalence of primarily trophically transmitted parasites (*T. gondii*) in feral cats from rural populations due to higher predation rates of rural feral cats on

intermediate host. Similar to other urban-adapted mesocarnivores, urban feral cats rely heavily on anthropogenic food sources (Finkler et al., 2011a; Gilot-Fromont et al., 2012), potentially reducing intermediate host predation rates. Since transmission of *T. gondii* primarily occur through prey-predator system (Gilot-Fromont et al., 2012), we predict a lower prevalence of *T. gondii* in urban cat populations.

Materials and Methods

Study area

We collected samples from feral cats (N=117) in five different districts within a metropolitan area (Seoul, Korea) and four rural areas. Seoul (37°33′59.53″N 126°58′40.69″E) is an intensively developed city, with a human population density of 17,000/km^2 (Kim and Baik, 2005). All five urban trapping sites were selected among residential areas densely packed with multi-story residential buildings (Fig. 3-1), one of the most typical housing structures in the city according to statistical data from the Seoul Metropolitan Government. Rural sites were selected among areas with typical agricultural landscapes composed of extensive rice paddies and sparsely dispersed houses (Fig. 3-1). Four rural sites were sampled from different regions, Yangpyeong county (37°46′15.262″N 127°42′10.875″E), Gwangju city (37°42′83.21″N 127°33′48.157″E), Cheorwon county (38.2092°N, 127.2175°E) and Chuncheon city (37.8667°N, 127.7333°E). The vegetation of these areas is typically dominated by paddy fields, except for shrubs between the rice paddies. The farming areas are surrounded by low hilly forest-covered landscapes. The human population densities of rural sites are approximately 136-189/km^2. In order to collect independent samples with respect to

geographical locations, trapping sites were sufficiently dispersed from each other (min.10km - max.18km) to prevent potential overlapping and/or geographic clustering of samples in both urban and rural sites.

Live trapping and sample collection

Feral cats were live-trapped for a total of approximately 550 trap nights in the summer of 2013 and 2015 using ten Tomahawk traps (Tomahawk, Live Trap Company, WI). All traps were baited with wet cat food, set between 6-8 pm and retrieved around 3-4 am the next morning. The sample collection was performed in close proximity (less than a 5 minute drive) to trapping sites. Trapped cats were anesthetized with a mixture of Zoletil® (zolazepam and tiletamine; 2 mg/kg) and Domitor® (medetomidine; 50 mcg/kg) before sample collection. Approximately 3-4 ml of blood were collected from the jugular vein or saphenous vein. Whole blood samples were collected in an EDTA tube and serum separation tube. Samples were stored in a 4°C refrigerator in the field, and in a -70°C freezer in laboratory until DNA extraction and serum analysis.

A total of 107 cats were trapped, 53 cats from urban habitats and 54 cats from rural areas. In addition, 10 additional samples from a district within Seoul were collected through a Trap-Neuter-Return center, giving a total sum of 117 samples available for analysis. Male cats comprised 59.8% (70/117) and female cats approximately 40.2% (47/117) of the sampled cats. The ratio of male to female cats was not significantly different between the two habitat types (chi-square test; P=0.39).

Laboratory analyses

DNA was extracted from whole-blood samples with the QIAamp DNA mini kit (Qiagen). The extracted DNA was used to identify hemoplasma infection through a subsequent PCR assay. We amplified a partial segment of the 16S rRNA gene of hemoplasma species using the universal primer set HBT-F (5'-ATACGGCCCATATTCCTACG-3') and HBT-R (5'-TGCTCCACCACTTGTTCA-3') designed by Criado-Fornelio et al. 2003. The protocol for PCR was performed as previously described (Criado-Fornelio et al., 2003). Every PCR run included a positive control (DNA from cat blood sample identified as positive for '*Ca.* M. haemominutum' through PCR and sequencing) and negative control (sterilized water). Amplified products were separated by electrophoresis on a 1.2% agarose gel. PCR amplicons from the positive samples were purified and prepared for direct sequencing to identify the nature of amplified product. The sequence homology of the sequenced 16S rRNA gene from the positive samples was examined using the program BLAST (National Center for Biotechnology Information; available at: http://www.ncbi.nlm.nih.gov). Serological assays were performed for *T. gondii* and *B. henselae*, using commercialized ELISA (SensPERT Feline Toxo IgG Ab test kit; VetAll, Seoul, Korea) and IFA (*Bartonella henselae* IFA feline IgG antibody kit; Fuller laboratories, Fullerton, CA, USA) kits, respectively. Tests for both pathogens were performed based on the protocol provided by the manufacturers.

Statistical analyses

Statistical analyses for the association between the prevalence of tested pathogens, habitat type, and sex were performed with a chi-square test. Phi-coefficient (φ) was calculated to show the effect size of the association between the variables. The odds ratio (OR) and the 95% CI for pathogen infection was also calculated for each pathogen. The significant occurrence of each pathogen co-infection combination (*T. gondii* and hemoplasma, hemoplasma and *B. henselae*, *B. henselae* and *T. gondii*, all three pathogens), and the association between the habitat type and occurrence of each co-infection combination was also analyzed using chi-square test. All statistical analyses were performed using the statistical software R (R Development Core Team, 2005).

Results

Among 117 cats, 56 cats (47.9%) were PCR positive for hemoplasma infection. From urban habitats, 26 cats were positive (26/63, 41.3%) whereas 30 cats were positive from rural areas (30/54, 55.6%) (Table 3-1). Through sequencing of PCR amplicons, we identified 4 cases of co-infection with '*Ca.* M. haemominutum' and *M. haemofelis*, 2 cases of "Candidatus *M. turicensis*", 10 cases of *M. haemofelis* and 40 cases of '*Ca.* M. haemominutum' infection among 56 hemoplasma positives (Table S1). Serological detection of IgG for *T. gondii* and *B. henselae* antibodies was performed in 112 of the 117 cats. Fifty-six cats (50%) and 40 cats (35.7%) were positive for *T. gondii* and *B. henselae*, respectively. Among 56 cats positive for *T. gondii* antibodies, 34 cats were from urban habitats (34/59, 57.6%) and 22 cats from rural habitats (22/53, 41.5%). Among 40 cats positive for *B. henselae* antibodies, 27 cats were from urban habitat

(27/59, 45.8%) whereas 13 cats were from rural habitats (13/53, 24.5%) (Table 3-1). Prevalence for *T. gondii* infection showed a higher percentage of positive individuals in urban areas compared to rural populations but this difference was not significant (chi-square test P = 0.13). However, the prevalence of infection with *B. henselae* between the habitat type was significantly different (chi-square test P = 0.03) (Table 3-2, Fig. 3-2). Between the two sexes, a significant difference in infection was observed only in hemoplasma prevalence (hemoplasma; male=46/70, female 10/47, chi-square test P = 0.00) (Table 3-2). Positivity to more than one tested pathogen, co-infection, was found in 46 individuals. Among four combinations of co-infection among three pathogens, significant association was found between hemoplasma and *B. henselae* infection (chi-square test P=0.03). None of the four co-infection combinations showed significant association with its occurrence and habitat type.

Discussion

This study evaluated the exposure of feral cats from urban and rural areas to three pathogens with different transmission modes; *T. gondii* (trophic and environmental), *B. henselae* (flea-borne) and hemoplasma (through blood and/or saliva of infectious host individual, and potentially arthropod-borne). Results of *T. gondii* seroprevalence showed higher, but statistically non-significant, exposure rates in urban populations compared to rural cats. In previous studies, parasites relying on trophic transmission, such as raccoon roundworms, had lower prevalence in urban habitats (Hegglin et al., 2007; Page et al., 2008), presumably due to lower intermediate host prey availability and predation. However, unlike parasites that are transferred to the definitive host solely by predation on

intermediate hosts, *T. gondii* can be also transmitted to the definitive hosts by direct contact of susceptible host individuals via environmental contamination with *T. gondii* oocysts. Under the assumption that intensity of cat predation on wild intermediate hosts decreases along rural to urban gradients (Deplazes et al., 2004; Lepczyk et al., 2004), it is possible that our study results were due to high exposure of feral cats in urban habitats to *T. gondii* oocysts through environment contamination, which compensated for reduced trophic transmission of *T. gondii*. The behavior of feral cats, defecating in soil and burying fecal matter, may present potential risks in urban playgrounds and/or parks, exposing the public, especially pets and children, to oocysts (Du et al., 2012; dos Santos et al., 2010). Distribution and intensity of *T. gondii* oocyst contamination in urban habitat warrants further investigation.

Contrary to our initial prediction, seroprevalence of *B. henselae* was significantly higher in urban feral cats. In many cases, prevalence of vector-borne pathogens or infestation of vector species has been reported as higher in rural-dwelling host populations (Costa et al., 2013; Labruna et al., 2007). However, variation of such patterns may exist, depending on the ecology of the vector species involved in the transmission. For instance, Krasnov et al. 2002 reported that flea prevalence and infestation rate was higher in hosts with high population density (Krasnov et al., 2002). Urban feral cats are known to live at high population densities frequently sharing food sources, which may explain the higher prevalence of *B. henselae* observed in urban feral cats in this study (Mirmovitch, 1995; Natoli, 1985; Tennent and Downs, 2008). However the association between host population density and flea prevalence and infestation was mostly studied in rodents, whose burrowing behavior has lot of influence on host-flea interactions.

Hence, additional studies are required to verify the potential impact of feral cat population density on prevalence of fleas and flea-borne pathogens. Alternatively, larger activity range of rural cats may reduce concentration of immature flea development zones and lessen the chances of cats getting contact with these areas (Gracia et al. 2013). The buffered microclimate of urban buildings can also provide ideal condition for fleas. Although dry and high temperature of urban area may have negative affect on the survival of fleas (Krasnov et al., 2006), it is also possible that indoor habitats, such as abandoned buildings, may provide environment where flea population can grow throughout the year (Krämer and Mencke, 2001). As free-ranging dogs are rare in Seoul, it is less likely for dogs to contribute in the epidemiology of flea or flea-borne pathogens in urban feral cats. Although several studies tested the potential transmission of *B. henselae* through ticks, evidence suggests that tick-borne *Bartonella* transmission is unlikely (Cotte´ et al., 2008; Telford and Wormser, 2010). Nevertheless, according to the effect size (Phi coefficient 0.22), the strength of association between habitat type and *B. henselae* prevalence is considered to be relatively weak (Babu et al., 2014). Further study seeking for infestation rate of flea and prevalence of additional flea-borne pathogens will be required to clarify the impact of habitat type on flea epidemiology.

Hemoplasma is another bacterial pathogen infecting the erythrocytes of hosts. In this study we did not observe significant habitat-related differences in hemoplasma prevalence. However significant sexual difference was observed, with males showing higher prevalence in both habitat types (urban males: 60%, rural males: 74.2%). This pattern is similar to previous studies and is potentially explained by bite-related transmission in male cats (Duarte et al., 2015; Luria et al., 2004; Willi et al., 2006).

Meanwhile, the prevalence in females between habitat types was also significantly different, with females from rural areas showing approximately 4-times greater prevalence (urban females: 8.7 %, rural females: 32 %). Considering that feline hemoplasma may also be transmitted through blood-sucking arthropod vectors in addition to saliva (Fyumagwa et al., 2008; Sykes, 2010; Taroura et al., 2005), the pattern of hemoplasma prevalence between urban and rural female cats may be the combined result of two transmission modes (vector-borne and saliva through biting). We detected *M. turicensis* sequences in two cat samples. Previous studies showed that although the occurrence of *M. turicensis* is less frequent compared to *M. haemofelis* or '*Ca*. M. haemominutum' (although geographical differences may exist), it is widely distributed around the globe (Willi et al., 2011). To best of our knowledge, this is the first report of *M. turicensis* in domestic cats in Korea, which calls for further studies regarding its pathogenicity and interaction with host and other hemoplasma species.

Among four co-infection combinations, occurrence of hemoplasma and *B. henselae* co-infection was significantly frequent compare to other combinations (P=0.03). Although prevalence of both pathogens have been frequently studied in domestic cat populations, patterns of co-infection and/or its implications have been rarely discussed, especially in stray or feral cats (Barrs et al., 2010; Juvet et al., 2010; Spada et al., 2014a). One of the potential mechanisms behind observed co-infections may be the role of fleas in transmitting hemoplasma in addition to *B. henselae*. Transmission of hemoplasma through fleas has been suggested in previous studies (Woods et al., 2005). Although it is not likely to be the only transmission route of hemoplasma, its significance may vary depending on environmental conditions (Sykes, 2010).

Conclusions

In our study, we observed difference of pathogens prevalence from feral cats in urban versus rural habitats. Based on our results, we suggest that the association between habitat type and host-parasite interactions may vary in relation to the pathogen's transmission strategy and the factors involved in transmission process. Further investigations on infestation of ectoparasites, dietary content of feral cats and environmental contamination of parasites between two habitat types In order to clarify the mechanisms underlying the observed pattern in this study. Feral cats are hosts of a number of zoonotic pathogens, including *T. gondii* and *B. henselae*, many of them with complex life histories and diverse transmission modes. Previous studies on the prevalence of zoonotic pathogens in feral cats have been mostly focused in urban habitats. However, considering increasing human activities in diverse habitat types other than urbanized areas (e.g. sururban development of rural areas, agricultural land use change), it is imperative that we understand how interactions between feral cats and their pathogens vary across different environmental conditions and transmission modes.

Ethics statement

Ethical approval for the use of animals in this study was obtained from the IACUC of the University of Georgia (UGA IACUC: A2013 04-009-Y1-A0) and Seoul National University (SNUIBC-R131118-1). The fieldwork for this study was performed in areas where no specific permissions were required, and none of the field studies involved endangered or protected species.

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Table 3-1. Prevalence of pathogen infections in feral cats from this study

Pathogens	Sex	Urban	Rural	Total
		No. of positive/tested, % of positives (95% C.I.)		
Hemoplasma	**Female**	2/23 8.7% (2.4, 26.8)	8/24 33.3% (18, 53.3)	10/47 21.3% (12.0, 34.9)
	Male	24/40 60% (44.5, 73.7)	22/30 73.3% (55.6, 85.8)	46/70 65.7% (54.0, 75.8)
	Total	26/63 41.3% (30.0, 53.6)	30/54 55.6% (42.4, 68.0)	
Toxoplasma gondii	**Female**	12/21 57.1% (36.4, 75.6)	10/24 41.7% (24.5, 61.2)	22/45 48.9% (35.0, 63.0)
	Male	22/38 57.9% (42, 72.2)	12/29 41.4% (25.5, 59.3)	34/67 50.7% (39.1, 62.3)
	Total	34/59 57.6% (44.9, 69.4)	22/53 41.5% (29.3, 54.9)	
Bartonella henselae	**Female**	8/21 38.1% (20.7, 59.3)	5/24 20.8% (9.2, 40.5)	13/45 28.9% (17.7, 43.4)
	Male	19/38 50% (34.8, 65.2)	8/29 27.6% (14.7, 45.7)	27/67 40.3% (29.4, 52.3)
	Total	**27/59** 45.8% (33.7, 58.3)	**13/53** 24.5% (14.9, 37.6)	

Table 3-2. Association between pathogen infection and external factors in the samples feral cats

Risk factor		**Hemoplasma**			***Toxoplasma gondii***			***Bartonella henselae***		
		Chi-square P	Phi (φ) coefficient	Odds ratio (95% C.I.)	Chi-square P	Phi (φ) coefficient	Odds ratio (95% C.I.)	Chi-square P	Phi (φ) coefficient	Odds ratio (95% C.I.)
Habitat type	**Urban**	0.192	0.14	0.56 (0.26-1.19)	0.13	0.16	1.91 (0.90-4.06)	0.03	0.22	2.60 (1.16-5.83)
	Rural			1			1			1
Sex	**Male**	0.00	0.44	7.15 (3.00-17.06)	1.0	0.02	1.08 (0.51-2.29)	0.30	0.12	1.66 (0.74-3.73)
	Female			1			1			1

Figure 3-1. Typical landscape of trapping sites in urban (left) and rural (right) areas

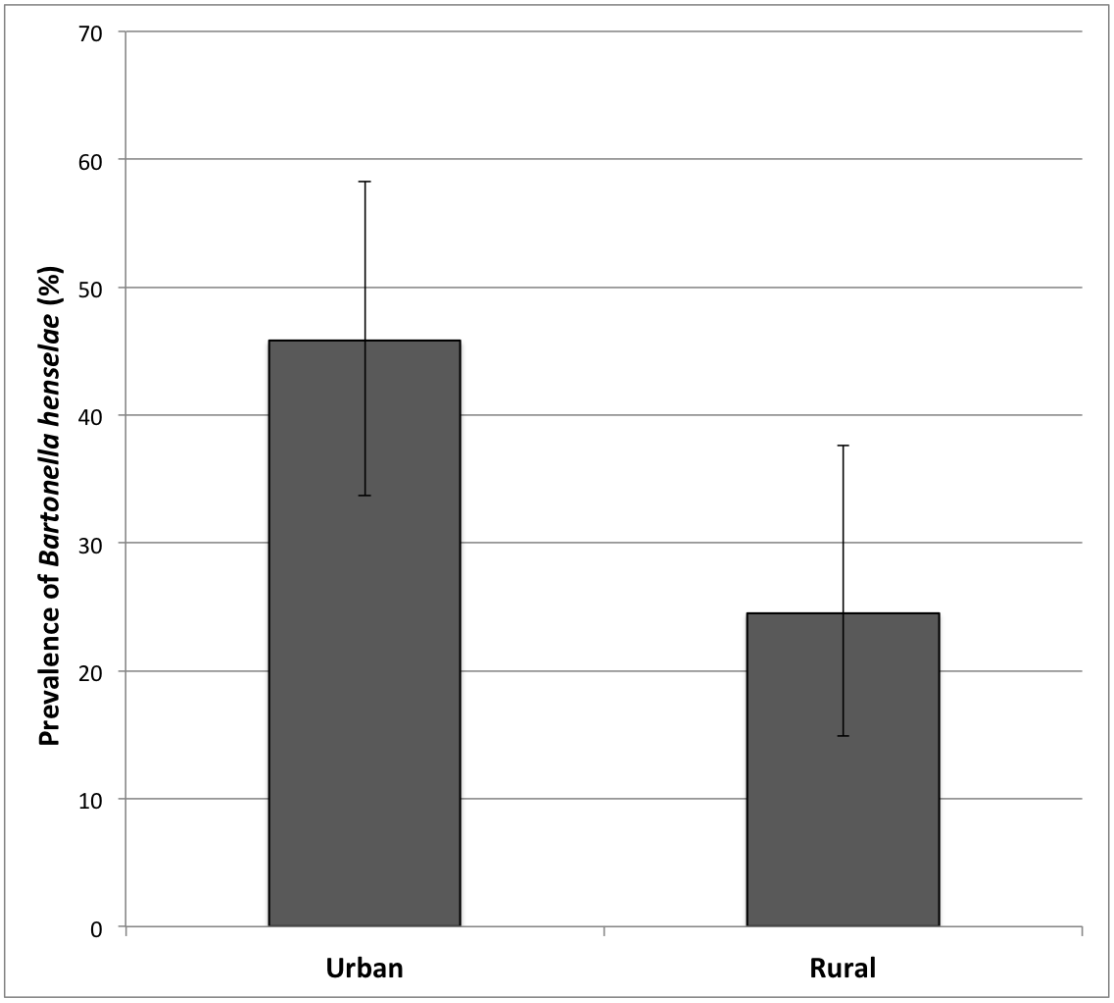

Figure 3-2. Occurrence of *B. henselae* infection in feral cats by habitat type

CHAPTER 4

DISENTANGLING THE LINK BETWEEN FOOD PROVISIONING, POPULATION DENSITY AND PREVALENCE OF PATHOGENS WITH DIFFERENT TRANSMISSION MODES IN URBAN STRAY CATS[1]

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Abstract

Supplemental feeding in free-ranging animals can alter host-pathogen interactions through mechanisms such as increased host population density and/or aggregated host distribution. In this study, we investigated the potential link between pathogen prevalence and host population density in urban stray cats, which are subject to varying intensities of cat caretaker activity involving supplemental feeding. We selected 6 districts as our study sites in Seoul, Korea, 3 with high and 3 low intensity areas of cat caretaker activity (CCA). Population density of stray cats was estimated in residential areas of each district. Blood samples of stray cats (N=302) were collected by local animal hospitals participating in a trap-neuter-release (TNR) program and tested for 8 different pathogens (feline immunodeficiency virus, feline leukemia virus, feline panleukopenia virus, feline calicivirus, feline herpesvirus-1, *Bartonella henselae*, hemoplasma, *Toxoplasma gondii*). We analyzed the prevalence of tested pathogens in relation to stray cat population density and intensity of CCA. There was no significant association between stray cat population density and CCA. Neither *B. henselae* nor feline hemoplasma infection was associated with CCA or host population density. However, prevalence of feline leukemia virus was significantly higher in areas with high CCA ($P=0.03$), whereas that of feline parvovirus was higher in areas with low CCA ($P<0.001$). We suggest that supplemental feeding may influence the prevalence of feline leukemia virus and feline parvovirus in urban stray cats by reducing foraging behavior and/or increasing aggregation. Results from our study may have further implications for other urban-adapted wildlife using anthropogenic food sources.

Key words: feline pathogens, urban stray cat, cat caretaker activity, supplemental feeding, urban ecology, host population density

Introduction

Anthropogenic food provisioning of free-ranging animals is a common practice and may be direct (e.g. conservation management) or indirect (e.g. urban trash cans, pet food) (Oro et al., 2013). In wild animals, food provisioning can shift individual phenotypic traits (e.g. body size, immunocompetence) and/or population parameters (e.g. survival rate, breeding rate, population density) (Fedriani et al., 2001; Jessop et al., 2012; Robb et al., 2008; Rodriguez-Hidalgo et al., 2010). Altered demographic processes of animals due to anthropogenic food sources can lead to increased local population size and/or density often followed by higher prevalence, intensity, or abundance of pathogen/parasite infections (Navarro-Gonzalez et al. 2013; Forbes et al. 2015). Larger population size/density can facilitate pathogen invasion through two potential mechanisms: host population size maintained above the threshold required for pathogen invasion, and/or by increased contact rates between infected and susceptible individuals (Becker et al., 2015; Becker and Hall, 2014). To date, few studies have jointly evaluated linkages between supplemental feeding, host population density, and prevalence of pathogens (Brennan et al., 2014; Miller et al., 2003; Vicente et al., 2007).

Here, we simultaneously examine the link between food provisioning, host population density, and pathogen prevalence while also asking how these relationships vary with transmission modes. Specifically, we estimate population densities of urban stray cat that are under influences of varying intensity of human food provisioning activities. Stray cats are abundant in many urban ecosystem, actively using anthropogenic food sources through diverse routes, including deliberate food provisioning by cat caretakers (Centonze and Levy, 2002; Finkler et al., 2011b). We also investigate the

prevalence of 8 pathogens: feline calicivirus (FCV), feline herpesvirus-1 (FHV-1), feline leukemia virus (FeLV), feline panleukopenia virus (FPV; feline parvovirus), *Toxoplasma gondii*, feline immunodeficiency virus (FIV), feline hemoplasma, and *Bartonella henselae*. We test multiple pathogens to account for variation in response of pathogen with different transmission modes with respect to host population density (Greer et al., 2008). Transmission modes of each pathogen are suggested to fall somewhere along the continuum of two extreme transmission modes- density-dependent and frequency-dependent (Begon et al., 2002; Fenton et al., 2002; Greer et al., 2008). If supplemental feeding increases host population density, we hypothesize that it should increase the prevalence of pathogens transmitted through direct contact (density-dependent transmission; FCV, FHV-1, FeLV) (Lutz et al., 2009; Radford et al., 2009; Thiry et al., 2009), compared to sexually-transmitted, vector-borne, or trophically transmitted pathogens (frequency-dependent transmission; *T. gondii*, FIV, hemoplasma, *B. henselae*) (Afonso et al., 2013; Chomel et al., 2009; Courchamp et al., 2000; Willi et al., 2007a), which is predicted to show relatively dull or no response towards increased host population density. In addition, feline panleukopenia virus (FPV; feline parvovirus) and *T. gondii* are transmitted by exposure to contaminated materials (e.g. feces, soil) in the environment (Tenter et al., 2000; Truyen et al., 2009). We predict that prevalence of two environmentally transmitted pathogens will also show a positive association with the population density of stray cats (Ballash et al., 2015), as accumulation of parasites of infectious stages will increase in areas with high host density.

Methods and Materials

Study site

The study was performed in Seoul, the capital city of Korea. Seoul is one of the largest megacities in the world with a population density of approximately 17, 000 people per km^2 (Kim and Baik, 2005). Stray cats in the city are most commonly observed in the residential areas including apartment complexes and intensive housing areas. Seoul is divided into 25 administrative local districts, and 6 districts were selected as study sites based on the intensity of "cat caretaker activity" (hereafter CCA). A synthetic index summarizing the intensity of CCA was composed of results from a nation-wide survey of cat-caretakers performed in 2013 (Kim et al. 2016). Variables used for the index were: 1) number of respondents per district; 2) number of respondents taking care of > 10 cats; 3) number of respondents who have been working as a cat caretaker for > 5 year; and 4) score from the subjective perception of each respondent about the intensity of CCA in their residing district. Additionally, the survey used demographic information provided by the Seoul local government, including: 5) matriculation rates of university; 6) property tax (index of wealth); and 7) computer ownership, as indices reflecting socioeconomic status (SES) of each district (Finkler et al., 2011b). Here, a principal component analysis for 7 above factors was performed. The first principal component (PC1) explained 54% of the variance in the data and the districts were ranked based on PC1, used as index of CCA. From this rank, we selected 6 districts; 3 districts from the top 5 (high CCA), and 3 from the bottom 5 districts (low CCA) (Fig. 4-1). The 6 studied districts are as follows; GN (Gangnam district), SC (Seocho district), MP (Mapo district) for high CCA areas and

GC (Geumchon district), DDM (Dongdaemoon district) and SD (Seongdong district) as low CCA areas (Fig. 4-2).

Estimating population density

Survey methods: We performed population density surveys in 12 sites from 6 districts (2 sites per district as replicates) during April-October of 2014 and 2015. In each site, a strip transect of approximately 2km, covering 0.13-0.20km^2 was surveyed. The survey routes were selected from high-density residential areas within each district. Apartment complexes were excluded from the survey routes to prevent sampling bias, since different apartment complex are known to have specific stray cat management policies. Surveys were conducted between 1700 and 2000 hours, and usually completed within 60-70 min. These times were selected based on the nocturnal characteristic of cat activity (Izawa 1983; Horn et al. 2011). For each site, 5 consecutive surveys (all within 2 weeks) were performed. Population density estimation was performed by direct observation of individual cats identified via morphological characteristics (Finkler et al. 2011c). We employed a capture–recapture sampling technique for the surveys, encompassing an initial 'capture' survey during which each observed cat was individually identified and recorded, and 4 consecutive 'recapture' surveys, in which we identified cats as re-sighted or newly observed. Each new cat was recorded during the survey in which it was first observed (any time in between the first and the fifth day). For every cat observed, GPS point, time of observation and morphological characteristics were recorded. Data on the cats' neutering status were identified and recorded by the ear tip cut made during the neutering procedure. Cats were also divided into 2 age categories according to their physical characteristics: kittens, up to the age of 6 months; and adults,

older than 6 months (Gunther et al., 2011). Kittens were not included in the density analysis.

Population density data analysis: Population density was estimated from 5 consecutive surveys at each site, with the null model M_0 and heterogeneity model M_h (using Jackknife estimator) in the software program MARK (White and Garrott, 1990), using CAPTURE (Otis et al., 1978). Model M0 represents status where there is no variation in capture probability associated with individuals or capture occasions. Model Mh allows heterogeneity in capture probability for each individual. However this heterogeneity does not assume capture differences among sampling occasions or capture history of individuals (Karanth and Nichols, 1998). A closed population model was used based on the assumption of limited immigration/emigration, birth and death during the 2-week survey period (Gross et al., 2011). We only included heterogeneity model in our analysis. Since each 'trap' was based on visual identification rather than physical trapping, we considered behavioral response (e.g. "trap-shy") before and after the 'trap' an unlikely source of variation in capture probability. The total number of individuals observed at each site was divided by the size of the survey area to obtain estimates of cat density (per km^2) and 95% confidence intervals. Survey areas were determined in ArcView 9.0 (ESRI, Redlands, CA) by creating a polygon with a 50-meter buffer zone around each survey route. Population density estimated from 2 sites within each district was averaged to permit analysis of each district's association with the prevalence of tested pathogens.

Biological sample collection

Biological samples were collected from the 6 study districts from August of 2014 till April of 2015 (GN, SC, MP (high CCA) and GC, DDM and SD (low CCA)). There were animal hospitals from each district that were contracted to perform Trap-Neuter-Release (TNR) of stray cats. We built a network with the TNR performing animal hospitals from the 6 districts and requested to collect 50 adult cat blood samples per district during their neutering procedures. All cats determined to be yearlings or younger were released without being neutered. Half of each blood sample (approx. 1.5-2ml) was placed into a tube containing the anticoagulant ethylene diamine tetra-acetic acid (EDTA; Becton Dickinson, Rutherford, New Jersey, USA), and the other half was placed into a serum separator tube. Blood samples collected for serum separation were permitted to clot, then centrifuged for 10 min at 1200 X G. Next, serum was removed from the clot using a sterile pipet and transferred to a sterile 1.5ml Eppendorf tube. All serum and whole blood samples were stored at -80 until analyses were conducted.

Laboratory analysis

PCR was used for identification of feline hemoplasma in the whole blood samples. Frozen whole blood samples were thawed at room temperature and genomic DNA was extracted using the QIAamp DNA mini kit (Qiagen). The extracted DNA was used as a template for the subsequent PCR assay. We amplified a partial segment of the 16S rRNA gene of mycoplasma species (including hemoplasma, which is the hemotropic mycoplasma) using the universal primer set HBT-F (ATACGGCCCATATTCCTACG) and HBT-R (TGCTCCACCACTTGTTCA) designed by Criado-Fornelio et al. 2003. The

PCR protocol was performed as previously described (Criado-Fornelio et al., 2003). Every PCR run included a positive and negative control to detect bacterial contamination. Amplified products were separated by electrophoresis on a 1.2% agarose gel. PCR amplicons from the positive samples were purified and prepared for direct sequencing. The sequence homology of the sequenced 16S rRNA gene from the positive samples was examined using BLAST software (National Center for Biotechnology Information; available at: http://www.ncbi.nlm.nih.gov).

Serological assays were performed to determine the prevalence of *T. gondii, B, henselae*, FIV, FeLV and FCV, FHV-1 and FPV in the serum samples. Since different serological tests were not performed on the same day, aliquots of serum samples were stored in several tubes, so that each tube was thawed for a single analysis only, in attempt to prevent repeated thawing and freezing of samples. Commercial ELISA test kits were used to detect 1) antibodies for FIV and FeLV p27 antigen detection (SensPERT FeLV Ag/FIV Ab kit; VetAll™) and 2) antibodies to *T. gondii* (Rapid diagnostic test (RDT) developed by Chong et al. 2011 (Chong et al., 2011), using recombinant SAG1 antigen.). Tests to detect *B. henselae* antibodies were performed using the indirect immunofluorescence (IFA) slides test (*Bartonella henselae* IFA feline IgG antibody kit; Fuller laboratories). A titer of > 1:64 IgG immunoglobulins was considered positive for exposure to *B. henselae*. Lastly, modified in-house ELISA kits (ImmunoComb, Feline VacciCheck, Biogal Galed Laboratories) were used for testing FCV, FHV-1 and FPV antibodies. The ImmunoComb® test is based on solid phase “dot”-ELISA technology consisting in a comb-shaped plastic card. On each 12 teeth of the plastic card there are 4 spots as follows: FCV antigen is the lowest spot; FHV antigen is next to the lowest; FPV

antigen is the second from the top; and the uppermost spot is the positive reference. A titer of positive index used to test for antibodies to each pathogen in ImmunoComb was as follows: FPV antibodies at 1:80 in hemagglutination-inhibition (H.I.) reaction; FHV-1 antibodies at 1:16; and FCV antibodies equal to 1:32 in virus neutralization reactions, as provided by the manufacturer. Tests for all antigens and antibodies were performed based on the protocols provided by the manufacturers.

Statistical analysis

All statistical analyses were performed using statistical software R (http://cran.r-project.org). Chi-square tests were performed to ensure that there was no sexual bias in sample sizes among the 6 districts; no significant difference of sample sizes was observed between the two sexes (χ^2 =7.09, P=0.21). The normality of population density from the 12 survey sites was confirmed through Shapiro-Wilk tests. Generalized linear mixed-models (GLMMs) were used to test associations between population density and CCA, with district set as a random effect. We also tested for the presence of associations between the prevalence of each pathogen and sex, population density, and CCA using GLM with logic function. Moran's I was calculated using coordinates and population density estimates of twelve survey sites to test whether estimated population density values were clustered in space (positive spatial autocorrelation).

Results

Cat caretaker activity (CCA) and population density of stray cats

Density estimations for each site are shown in Table 4-1 and Fig. 4-3. There was no significant association between population density and CCA (estimate: -21.18, SE: 14, P=0.13), although trends of higher population density in low CCA districts (GC, SD) and lower population density in high CCA districts (GN, MP) were observed. In addition, density estimates from 2 survey sites (one from district DDM, the other from district SC) did not coincide with the pattern observed in the other 4 districts. A survey site from district DDM (low CCA) had the lowest population density among all sites, whereas a site from district SC (high CCA) showed a higher range of population density. There was no evidence of spatial autocorrelation of population density (Moran's I value, P=0.26).

Pathogen prevalence

The prevalence of 8 pathogens from 6 districts is summarized in Table 4-2. The overall prevalence based on the antibodies against each pathogen was as follows: FCV, 94.3% (282/299); FHV-1, 97.9% (293/299); *Toxoplasma gondii*, 7.7% (23/298); FIV, 3.6% (11/302); FPV, 79.2% (236/298); *B. henselae*, 34.8% (105/302). Prevalence of FeLV antigens was 23.2% (70/302) and feline hemoplasma was 32.5% (98/302). FeLV prevalence was highest in GN (40%) and SC (36%) (both high CCA districts), whereas FPV prevalence was highest in DDM (94%) and SD (90%), followed by GC (80%) (3 low CCA districts).

Association between supplemental feeding, population density and pathogen prevalence

Prevalence of FeLV and FPV showed a significant association with CCA (FeLV, P = 0.03; FPV, P = 0.00) (Fig. 4-4, Table 4-3). However, the pattern of association between prevalence and CCA differed for FPV and FeLV: FPV prevalence was higher in low CCA districts, whereas FeLV showed higher prevalence in high CCA districts (Table 4-3). No significant association was observed between population density and the prevalence of 4 pathogens (*B. henselae,* P = 0.41; hemoplasma, P = 0.73; FPV, P = 0.63; FeLV, P = 0.38). In addition, the prevalence of *B. henselae* and feline hemoplasma was not associated with the intensity of CCA (*B. henselae*, P = 0.48; hemoplasma, P = 0.15). Significant sex differences were also observed in FeLV and hemoplasma, with a higher prevalence of feline hemoplasma in males (P = 0.00), and a higher prevalence of FeLV in females (P = 0.01) (Table 4-3).

Discussion

Stray cat population density and cat caretaking; food provisioning and neutering

The population density of stray cats was not significantly linked to CCA (cat caretaker activity) and the general pattern of population densities from 6 districts disagreed with our initial expectation with respect to CCA. Two districts with lower CCA (SD and GC) showed the highest population densities, whereas 2 districts with high CCA (GN and MP) showed lower population densities (Table 1). This pattern may be explained by heterogeneous trap-neuter-release (TNR) efforts among districts. In addition to TNR performed by local districts, which began in 2008, active cat caretakers also contribute to neutering of urban stray cats via aggressive campaigning and education.

Two high CCA districts (GN and SC), were among the top districts with regards to the number of cat caretakers who had experience in performing TNR (Kim et al. 2016). These 2 districts also have the highest income in Seoul, a socioeconomic factor that has previously been linked to either the proportion of neutered stray cats or the level of neutering by caretakers (Finkler et al., 2011b, 2011c). Thus, although stray cats from the districts with higher CCA may be exposed to more supplemental feeding, it is likely that a higher proportion of cats in these areas are also neutered, resulting in smaller or similar population sizes compared to districts with lower CCA.

Neutering may modify infectious disease transmission through changing individual behavioral traits, and/or demographic population structure. Neutering can reduce aggressive behavior and/or increase amicable behavior in both sexes (Finkler et al., 2011a; Finkler and Terkel, 2010). These behavioral shifts can increase or decrease spread of pathogens transmitted through either aggressive or affiliative interaction, such as FIV and FeLV, respectively. Neutering can also influence the demographic structure of cat populations, through processes such as increased survival rates of neutered individuals (Gunther et al., 2011; Lee et al., 2014), lowered immigration or emigration rates (Gunther et al., 2011; Mendes-de-Almeida et al., 2006) and/or lowered birth rates (Levy et al., 2003; Mendes-de-Almeida et al., 2006). Such changes can lower the availability of susceptible host individuals (e.g. newborns, immigrants) in the area, while increasing the proportion of adult individuals who may have recovered from prior infection and are immunized, subsequently leading to heightened herd immunity in the area (Becker et al., 2015). With expanding adaptation of TNR as a stray cat population

management strategy, additional efforts are warranted to predict the epidemiological consequences of neutering in urban stray cat populations.

Prevalence of FCV, FHV-1, FIV and T. gondii

Out of 8 pathogens tested, 4 of them (FIV, FCV, FHV-1 and *T. gondii*) showed either consistently high or low prevalence in all districts. Feline calicivirus and FHV-1 are 2 of the main pathogens causing feline respiratory tract disease (Radford et al., 2009; Thiry et al., 2009). Both pathogens are highly contagious and tend to show high prevalence in larger populations (Bannasch and Foley, 2005; Radford et al., 2009; Thiry et al., 2009). Compared to previous seroprevalence reports on pet or shelter cats (FCV, 36.6-92.4%; FHV-1, 11-73.3%) (DiGangi et al., 2012; Lappin et al., 2002; Lickey et al., 2005), results from our study show higher prevalence for both FCV (95%) and FHV-1 (97.3%). On the other hand, Yamaguchi et al. (1996) reported high seroprevalence values for both FCV and FHV-1 (FCV, 100%; FHV-1, 100%) in a study of free-ranging farm cats, many of which where known to share supplemental food and latrine sites. Considering that FCV and FHV-1 are pathogens with high morbidity and low mortality, we suggest that mechanisms caused by an abundant food supply in free-ranging cats, such as the aggregation of individuals in close proximity and/or higher survival rates of individuals after infection (Bitew et al., 2011; DiGangi et al., 2012; Lembo et al., 2011), may have further contributed to the high seroprevalence of FCV or FHV-1 observed in our study and that reported by Yamaguchi et al. (1996) (Yamaguchi et al., 1996). However additional empirical studies are required to verify the potential impact of supplemental food on FCV and/or FHV-1 prevalence in free-ranging cats.

A low prevalence of FIV, similar to this study (total prevalence: 3.6%; 95% C.I. 0.018-0.049) has been reported in previous population-level studies on free-ranging or household cats (Lee et al., 2002; Levy et al., 2006; Ravi et al., 2010). Pontier et al. (2009) suggested that urban stray cats maintain a promiscuous mating system due to the high local density of both male and female cats (unlike its rural counterparts, wherein a polygynous mating system is maintained), which may lead to fewer aggressive interactions between males in breeding seasons and the lower prevalence of FIV in urban male cats (Courchamp et al., 2000; Pontier et al., 2009; Ravi et al., 2010). The additional impact of neutering/spaying on the behavioral modification of urban males may also contribute in the low prevalence of FIV, as previously mentioned.

Although we expected the prevalence of *T. gondii* to show some association with population density, the overall number of positive individuals was small (Table 4-2). However, our result was similar to other studies that investigated seroprevalence of *T. gondii* in stray cats residing in highly urbanized habitats, which ranged from 5.61-14.3% (Lee et al., 2011; Oi et al., 2015; Wang et al., 2012). With respect to CCA, the lack of consistent supplemental food in low CCA districts may force stray cats to prey on urban wild rodents, which are potential intermediate hosts of *T. gondii* (Mercier et al., 2013; Reperant et al., 2009), leading to higher *T. gondii* prevalence in low CCA areas. However, there are no data on the predation behavior of urban stray cats or their dietary content, or on the prevalence of *T. gondii* in urban rodents in the study area. Further study with higher sample size is required to explain the potential association between *T. gondii* prevalence and CCA intensity, especially in relation to dietary components and foraging behavior.

Prevalence of B. henselae*, feline hemoplasma, FeLV and FPV and its association with food provisioning and stray cat population density*

Patterns of *B. henselae* and feline hemoplasma prevalence were not significantly associated to CCA or population density. The prevalence of *B. henselae* and feline hemoplasma from 6 districts ranged from 28.4-42% and 26-48%, respectively, which fall within the ranges reported by previous studies from domestic cats (serological assays of *B. henselae*: 33.6% to 54% (Fabbi et al., 2004; Luria et al., 2004; Marston et al., 1999); molecular assays of feline hemoplasma: 20.5% to 33.1% (Duarte et al., 2015; Luria et al., 2004; Spada et al., 2014a)). In cats, *B. henselae* is mainly transmitted through infected fleas or through direct contact with infected blood, whereas feline hemoplasma is suspected to use various routes for transmission, including arthropod vectors and saliva (i.e., biting). In comparison to pathogens that largely rely on close contact between host individuals for transmission, the spread of vector-borne and sexually transmitted diseases are generally considered less affected by host population density, although the specific dependence on host population density may vary (Antonovics et al., 1995; Ryder et al., 2007; Thrall et al., 1993). We suggest that the relatively independent nature of *B. henselae* and hemoplasma transmission modes with regards to host population density or aggregation of individuals may explain the results presented here.

We found that FeLV prevalence was significantly higher in districts with high CCA (with GN and SC showing the highest FeLV prevalence), but unrelated to population density of stray cats. In comparison to previous studies on FeLV (2.3-23% (Alves et al., 2011; Levy et al., 2006; Stojanovic and Foley, 2011)), prevalence from our study is relatively high (12-42%), especially in 2 of our studied districts (GN, SC).

Transmission of FeLV usually occurs through proximate contacts, such as food sharing, licking and grooming (Barratt, 1997; Rojko and Olsen, 1984). With this in mind, we suggest that a potential cause for higher FeLV prevalence in the 2 districts with high CCA is the increased aggregation of cats around food provisioned by the cat caretakers. Using data from FeLV infection in stray cats as parameters, Becker and Hall 2014 built a simulation model predicting the impact of supplemental feeding on prevalence of FeLV, through altered demography, contact behavior and immune response. This study demonstrated that increased aggregation and population density of supplemented cats led to higher FeLV prevalence, although such impact varied depending on the influence of provisioned food on the immune response of cats.

The prevalence of FPV from our study, ranging from 59-94%, is similar to results from previous studies that performed on feral or pet cats with no history of vaccination (45%-76.3%; (Blanco et al., 2009; Levy et al., 2008; Pavlova et al., 2015)). In contrast to FeLV, FPV prevalence was highest in 2 districts with lowest CCA (GC, SD), but not significantly associated to host population density. Feline parvovirus (FPV), the etiologic agent of feline panleukopenia is extremely resistant to environmental conditions, allowing environmental transmission via contact with biotic or abiotic materials contaminated with fluids, fomites or feces of an infected animal (Ikeda et al., 2002; Truyen et al., 2009). Lack of food provisioning by caretakers in low CCA districts may have led stray cats in the area to maintain a larger activity range, with more intensive foraging activity compared to conspecifics in high CCA districts with a relatively abundant and stable food supplement (Barratt, 1997; Tennent and Downs, 2008). This in turn may lead to a higher exposure rate of host individuals to pathogens transmitted via

contact with environmental contaminants (Lepczyk et al., 2015). Similar association has been discussed in other systems, such as prevalence of *T. gondii* in diverse wildlife (Fredebaugh et al. 2011) or increased infection to *Strongyloides* in wild primates (Parr et al. 2013).

Lastly, we observed a lack of positive association between cat population density and prevalence of pathogens whose transmission is density dependent (FeLV and FPV). Most pathogens have an asymptotic relationship between contact rate and host density (Fenton et al., 2002; McCallum et al., 2001). Therefore, when the population size of hosts expands to a certain point (asymptote), the contact rate among individuals for transmission of directly transmitted pathogens becomes saturated and does not serve as a function of host population density (Altizer et al., 2003; Antonovics et al., 1995; McCallum et al., 2001). Similarly, we believe that the lack of association between pathogen prevalence and cat population density reported here was due to the high population size of stray cats across the city, allowing all studied districts to maintain a maximized contact rate among individuals, and leading to a lack of significant variation in the prevalence of the studied pathogens. This observation holds true unless the contact rate among individuals is manipulated through external forces such as human food provisioning.

One limitation of this study was the lack of information about the vaccination history of the cats used for sampling. However, we argue that the number of vaccinated cats among our samples would have been negligible. Stray cats that were born as a stray cat and never adopted are unlikely to have a vaccination history. Regarding the possibility of a cat being once adopted, vaccinated and abandoned, the majority of stray

cats in Seoul are classified as Korean shorthair breeds (91.3%) (Hwang, 2015), which are mostly adopted through local animal rescue centers, volunteer cat caretakers or animal rights NGO groups, which strictly require vaccination and neutering prior to adoption. Since our samples were only from cats newly neutered by the TNR animal hospitals, abandoned cats would not have been used for our sampling.

Our study shows that the activities of cat caretakers can influence stray cat populations through diverse routes, in addition to food provisioning. This is important because different human activities can have conflicting influences on host ecology, further complicating our ability to predict complex host-pathogen interaction in urban animals. Our results also suggest that the relationships between prevalence and supplemental feeding can further vary depending on the unique transmission mode and/or life-history of each pathogen (Becker et al. 2015). Thus incorporating information about the ecology of each pathogen of interest would be crucial when elucidating the link between human activities and host/pathogen ecology, in order to provide insights on prioritizing epidemiological focuses (e.g. pathogen, geographical area, etc.) for improved management capabilities of urban ecosystems.

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Table 4-1. Estimated densities and 95% confidence intervals of cats among observed cats in each of the survey sites from six districts (CCA indicated in parenthesis; high-H, low-L)

District (CCA)	Site ID	Area size (km^2)	Estimated cat density and 95% CI (cats per km^2)
GN (H)	GP	0.142	148 (134-239)
	IW	0.167	174 (144-258)
SC (H)	YJ	0.173	249 (191-387)
	BB	0.186	172 (135-280)
MP (H)	SSa	0.151	186 (159-285)
	DG	0.182	188 (160-265)
DDM (L)	JN	0.132	206 (168-327)
	JA	0.175	138 (127-212)
GC (L)	SH	0.156	231 (193-334)
	DS	0.190	226 (174-352)
SD (L)	MJ	0.178	254 (214-344)
	SS	0.206	292 (224-432)

Table 4-2. Occurrence of pathogens (prevalence (%), 95% confidence interval) in each district (CCA indicated in parentheses; H-high, L-low)

Districts		**FIV**	**Feline Hemoplasma**	***B. henselae***	***T. gondii***	**FPV**	**FeLV**	**FCV**	**FHV-1**
GN (H)	Positive/ Total tested	0/50	13/50	21/50	3/50	29/49	21/50	50/50	50/50
	Prevalence % (95% C.I.)	0 % (0.00-0.05)	26% (0.15-0.39)	42% (0.29-0.56)	6.0% (0.02-0.15)	59% (0.45-0.72)	42% (0.28-0.56)	100% (0.95-1.00)	100% (0.95-1.00)
SC (H)	Positive/ Total tested	3/50	19/50	15/50	3/50	38/50	18/50	46/50	47/50
	Prevalence (95% C.I.)	6.0% (0.02-0.15)	38% (0.26-0.52)	30% (0.19-0.44)	6.0% (0.02-0.15)	76% (0.63-0.86)	36% (0.24-0.50)	92% (0.82-0.97)	94% (0.85-0.98)
MP (H)	Positive/ Total tested	3/50	24/50	21/50	3/48	37/49	6/50	48/49	47/49
	Prevalence (95% C.I.)	6.0%, (0.02-0.15)	48% 0.35-0.62)	42% (0.29-0.56)	6.3% (0.02-0.16)	75.5% (0.64-0.87)	12% (0.03-0.21)	98% (0.91-1.00)	96% (0.88-0.99)
DDM (L)	Positive/ Total tested	2/50	14/50	16/50	2/50	47/50	10/50	50/50	50/50
	Prevalence (95% C.I.)	4.0% (0.01-0.12)	28% (0.17-0.41)	32% (0.20-0.46)	4.0% (0.01-0.12)	94% (0.85-0.98)	20% (0.11-0.33)	100% (0.95-1.00)	100% (0.95-1.00)
GC (L)	Positive/ Total tested	2/50	14/50	17/50	6/50	40/50	7/50	46/50	50/50

	Prevalence (95% C.I.)	4.0% (0.01-0.12)	28% (0.17-0.41)	34% (0.22-0.48)	12% (0.05-0.23)	80% (0.67-0.89)	14% (0.07-0.26)	92% (0.82-0.97)	100% (0.95-1.00)
SD (L)	Positive/ Total tested	1/52	14/52	15/52	6/50	45/50	8/52	43/50	50/50
	Prevalence (95% C.I.)	1.9% (0.00-0.09)	26.9% (0.16-0.5)	28.8% (0.18-0.42)	12% (0.05-0.23)	90% (0.80-0.96)	15.4% (0.08-0.27)	86% (0.75-0.94)	100% (0.95-1.00)

Table 4-3. Generlized linear model analysis of the prevalence of four tested pathogens (feline hemoplasma, *B. henselae*, FPV, FELV) by sex and CCA (cat caretaker activity)

Pathogens	Variable	Categories	Odds ratio	95% CI	Parameter	SE	P value
Hemoplasma	CCA	High	1		-0.42	0.30	0.15
		Low	0.65	0.36-1.17			
B. henselae		High	1		-0.20	0.28	0.48
		Low	0.82	0.47-1.42			
FPV		High	1		1.01	0.36	0.00*
		Low	2.76	1.29-5.74			
FeLV		High	1		-0.72	0.33	0.03*
		Low	0.48	0.24-0.92			
Hemoplasma	Sex	Female	1		0.75	0.25	0.00*
		Male	2.12	1.30-3.50			
B. henselae		Female	1		-0.15	0.24	0.54
		Male	0.86	0.53-1.38			
FPV		Female	1		-0.39	0.30	0.18
		Male	0.67	0.37-1.20			
FeLV		Female	1		-0.74	0.87	0.01 *
		Male	0.48	0.27-0.83			

* P values below 0.05

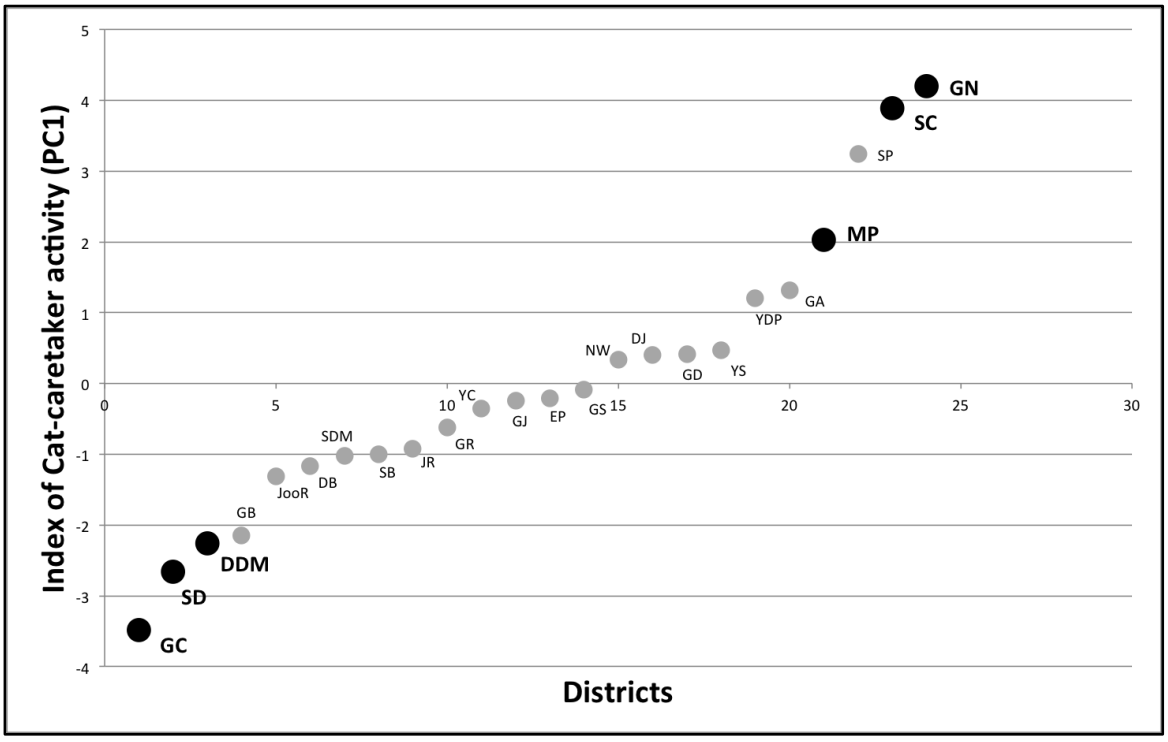

Figure 4-1. Seoul districts ranked based on PCA result of CCA (PC1). The six districts selected for the study are marked as filled circles (on the upper right side-high CCA districts and on the lower left side-lower CCA districts).

Figure 4-2. Map of Seoul highlighting 12 sites from 6 districts in which cat density surveys were carried out: (a) 2 survey sites from MP (high CCA district); (b) 2 survey sites from GC (low CCA district); (C) 4 survey sites from GN and SC (both high CCA districts); (d) 4 survey sites from SD and DDM (both low CCA districts)

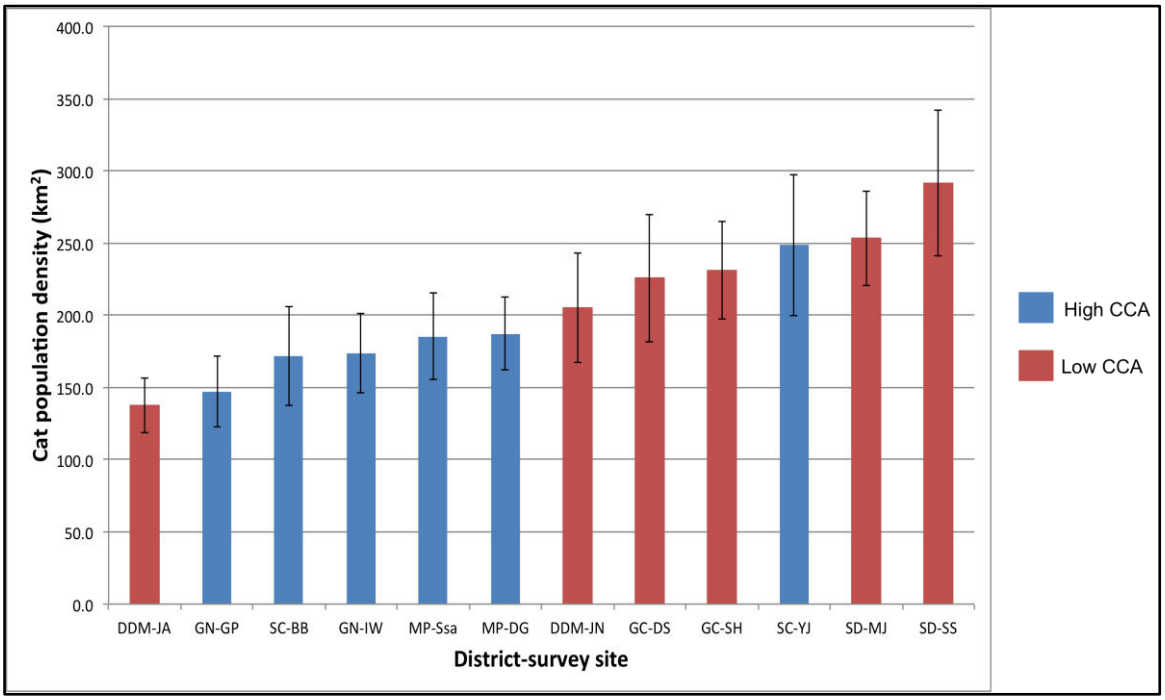

Figure 4-3. Cat population density estimates and standard errors from 12 survey sites.

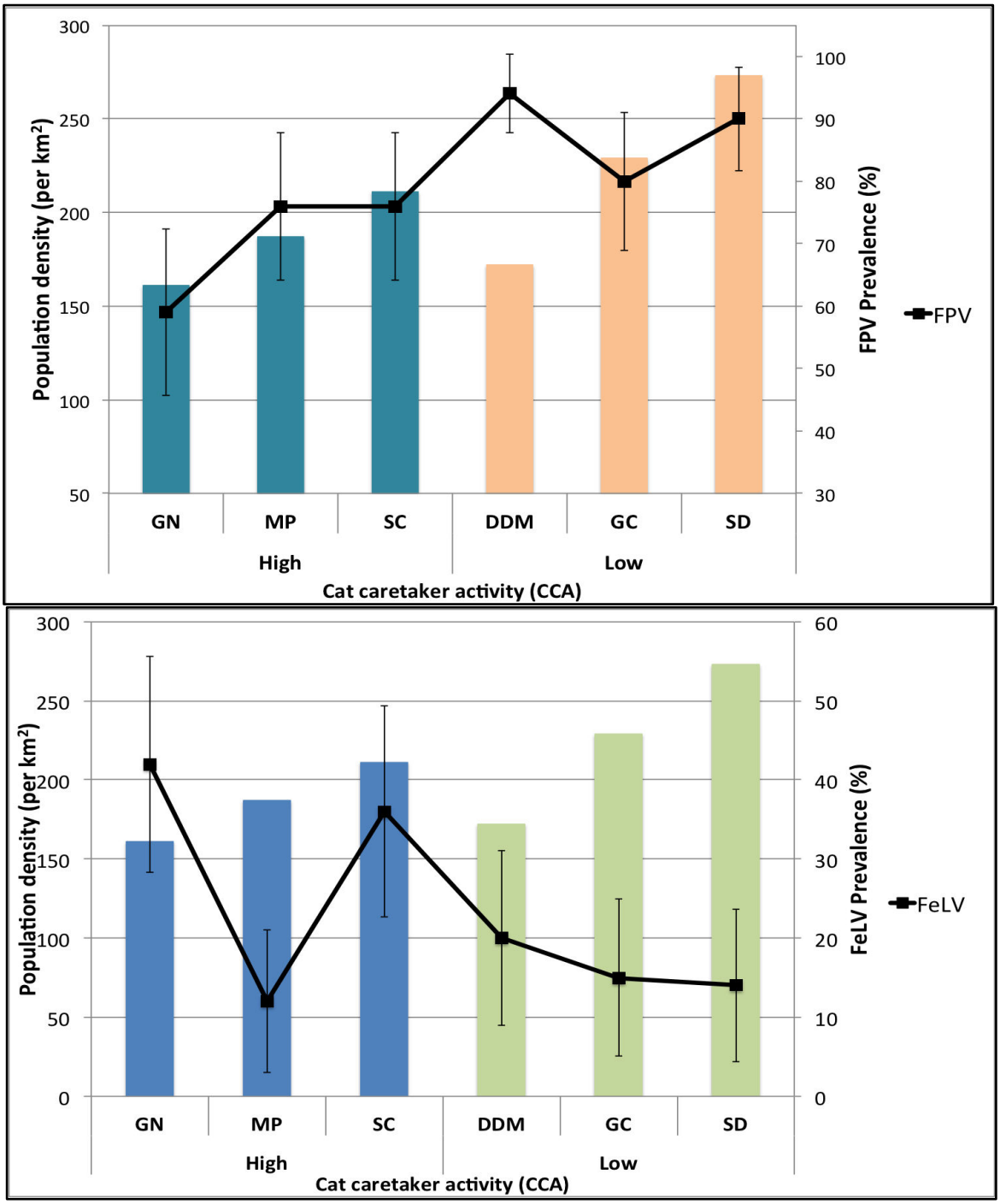

Figure 4-4. Associations between estimated cat population density and prevalence of a) FeLV and b) FPV by districts and CCA (errors bars: 95% confidence interval of pathogen prevalence)

CHAPTER 5

ANTHROPOGENIC FOOD PROVISIONING AND IMMUNE PHENOTYPE: ASSOCIATION AMONG SUPPLEMENTAL FOOD, BODY CONDITION, AND IMMUNOLOGICAL PARAMETERS IN AN URBAN HABITAT[1]

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Abstract

1. Direct or indirect supplemental feeding of free-ranging animals is widely performed worldwide, with significant impacts on ecological aspects of animals, such as increasing population density or shifting demographic processes. Another potential impact of improved body condition through higher energy intake is altered immunocompetence.

2. Since immune system maintenance is energetically costly, immune responses and other energy-demanding physiological processes are subject to trade-offs in individual animals. Although increased availability of food sources through supplemental feeding is suspected to increase the overall immunocompetence of animals, empirical data verifying the association between supplemental feeding and different immune parameters are lacking. Understanding the potential influence of supplemental feeding on immune phenotypes is critical, as it may further affect the host-pathogen dynamics in free-ranging animals.

3. Using urban stray cats as a study model, we tested for associations among intensity of supplemental feeding through cat caretaker activity (CCA); body condition through body mass and set of blood parameters; and immune phenotype through bacterial killing assay (BKA), immunoglobulin G (IgG) concentration, and leukocyte counts.

4. Significantly higher bacterial killing ability was observed in cats from high CCA districts, whereas significantly higher IgG concentration and eosinophil counts were observed in cats from low CCA districts. Other leukocyte counts and body condition index showed no significant association with CCA.

5. In our study, we observed varying patterns of different immune components in relation to intensity of supplemental feeding, potentially due to different function and energy

costs of each immune response and component. In addition, our data suggests that supplemental feeding influences immune phenotype, not only by means of provisioning a surplus energy source, but also through routes such as provisioning of critical nutrition and reduction of exposure rates to parasite infections through behavioral alterations.

Key words: urban habitat, stray cat, supplemental feeding, immune components, cat caretaker, bacterial killing assay, IgG concentration

Introduction

Host immunity plays a critical role in regulating parasite infections (Downs et al., 2014; Hawley and Altizer, 2011). Immune response activation is energetically costly and may affect various life-history traits (Downs et al., 2014; Downs and Stewart, 2014; Martin et al., 2003) through energy trade-offs and resource allocation (Ardia et al., 2011; French et al., 2009). Reduction of immune responses in exchange for other energy demanding processes, such as nestling growth, molting, or reproduction (Ardia, 2005; Landete-Castillejos et al., 2002; Moreno-Rueda, 2010; Nordling et al., 1998) can further affect host-parasite interactions by increasing the susceptibility of animals to parasitic infections (French et al., 2010; Lifjeld et al., 2002; Nordling et al., 1998; Sheldon and Verhulst, 1996). The occurrence of such trade-offs between immune responses and other physiological processes is determined by multiple factors, such as availability of food sources (Downs et al., 2014; French and Moore, 2008; Martin et al., 2007). For instance, wild female tree lizards (*Urosaurus ornatus*) had lowered wound-healing rates in gravid reproductive stages with higher energy demands, but female individuals with ad libitum access to food did not show such a trade-off, reflecting the significance of resource

availability for immune function of individual animals (French et al., 2009; French and Moore, 2008). The positive association between higher resource quality or resource availability and higher immune responses has been observed in both experimental and observational studies (Martin et al., 2008, 2007; Wilcoxen et al., 2015), emphasizing the role of sufficient energy sources for maintaining immune function in wild animals.

In this context, supplemental feeding of wildlife may also have an effect on the immune responses of free-ranging animals (Becker et al., 2015; Brzek and Konarzewski, 2007). Human food subsidies can provide additional energy sources for wildlife that may minimize the trade-offs between immune responses and other energetically costly physiological processes (Downs et al., 2014; Oro et al., 2013). Although the positive effect of supplemental feeding on body condition has been addressed (Auman et al., 2008; Cypher and Frost, 1999; Jessop et al., 2012; Otali and Gilchrist, 2004), few studies explore interactions among supplemental feeding, body condition and immune responses (Neve et al., 2007; Wilcoxen et al., 2015). Nevertheless, epidemiological models have shown that how immune defense responds to supplemental feeding is an important predictor in whether resource shifts increase or decrease infectious disease spread (Becker & Hall 2014). Such findings motivate further examining of associations among food resources, host condition, and immune function in urbanized wildlife exposed to human food provisioning.

The objective of this study is to investigate potential associations among supplemental feeding, body condition, and immune phenotype in stray cats using multiple immune parameters. Stray cats in urban habitats are exposed to different intensities of cat caretaker activity (hereafter CCA), which represents provisioning of

supplemental food, and are excellent models for this study. We hypothesize that cats from districts with high CCA will show higher body condition, and that host immunocompetence will increase with improved body condition.

Materials & Methods

Study site

This study was performed in Seoul, Korea, which is one of largest megacities in the world with a population density of approximately 17,000/km^2 (Kim and Baik, 2005). Stray cats in the city are most commonly observed in residential areas including apartment complexes and intensive housing areas. Among 25 administrative districts of Seoul, six districts were selected as study sites based on the results of a nation-wide survey of cat-caretakers performed at the end of 2013 (Kim et al., 2016). All districts within the city were ranked based on the intensity of CCA. Indices from the survey used for ranking were; (1) number of respondents per district, (2) number of respondents who are taking care of more than 10 cats, (3) number of respondents who have been working as cat caretaker for more than five years, and (4) score from subjective perception of each respondent about intensity of cat caretaking activity in hist/her residing district. Additional demographic factors, including (5) matriculation rates, (6) property tax (index of wealth), and (7) computer ownership, were used as indices reflecting the socioeconomic status (SES) of residents, which is known to be positively associated with the intensity of CCA (Finkler et al., 2011b). We performed a principal component analysis for these seven indices of CCA. The first principal component (PC1) explained 54% of the variance in the data, and was used to rank the districts as a single index of

CCA. From this rank, three districts from the top range (high CCA) and three districts at the bottom (low CCA) were selected for this study.

Sample collection

Selected animal hospitals from each district within Seoul have annual contracts with their local district government to perform Trap-Neuter-Release (TNR) of stray cats. From the six focal districts, we collected blood samples during TNR procedures. Information about sex and body mass of each cat was also provided. All individuals previously neutered or determined by the veterinarians as under-aged (below 1.5kg), were released without neutering or sampling. Whole blood samples stored in EDTA tubes were used for the complete hematology blood test and for making blood smear for differential counts of white blood cells (WBC). Serum collected from whole blood samples and aliquots was stored in a -70°C freezer to preserve sample integrity.

Analysis of body condition estimates

The following five commonly used biological parameters were used as indices of body condition in this study: body mass (MASS; measured in kg), hematocrit (HCT), serum albumin (ALB), serum creatinine (CREA), and blood urea nitrogen (BUN) from serum (Gilot-Fromont et al., 2012; Milner et al., 2003). HCT is an indicator of red blood cells mass and thus the aerobic capacity of blood. HCT has been commonly used to assess body condition, and its association with survival, reproduction, and pathogen infection status of host animals has been observed in previous studies (Bókony et al., 2012; Budischak et al., 2012). ALB, CREA, and BUN are also used as indicators of body

condition, providing information on protein supply and metabolism (Milner et al., 2003; Säkkinen et al., 2001). For instance, serum albumin and creatinine concentration detect long-term protein under-nutrition, whereas BUN is used as an indicator of short-term protein status (Caldeira et al., 2007; Robert and Schwanz, 2013). Both the hematology blood test and serum biochemistry analyses were performed at Seoul National University Veterinary Medical Teaching Hospital (SNU VMTH) using automatic analyzers (hematological analysis: Siemens ADVIA 2120i hematology system; 25 serum biochemistry analysis: Hitachi 7180 clinical analyzer). The result of tested blood parameters were interpreted based on the reference range of healthy domestic cats provided by SNU VMTH (Table S2).

Analysis of immune parameters

To capture the complexity of vertebrate immune defenses, we measured seven immune parameters encompassing innate and adaptive defenses. Among WBC differential counts (neutrophils, monocytes, lymphocytes, and eosinophils), neutrophil and monocytes were used as indicators of innate immunity whereas lymphocytes and eosinophils may reflect both innate and adaptive immune function aspects. The neutrophil to lymphocyte ratio (N:L ratio) was also calculated as an index of stress in each individual, as lymphocyte decrease is often observed in response to increased stress hormones, corticosteroids (Davis et al., 2008). We also measured two humoral immune parameters, the bacterial killing assay (BKA) and total immunoglobulin G (IgG) concentration as indicators of innate and adaptive immune defenses, respectively (Lee, 2006; Schneeberger et al., 2014). Specifically, BKA against *E.coli* and *S.aureus*

characterizes functionally relevant actions of complement proteins and natural antibodies (French et al., 2010). IgG concentration, among others, reflects the cumulative contact of host individuals with pathogens and mirrors the on-going chronic pathogen infection (Schneeberger et al., 2014).

A leukocyte differential count was performed via examination of air-dried, whole blood films on a microscope slide stained with Diff-Quick stain (Medion Diagnostics, Dudingen, Switzerland.). Under the microscope, a differential count of 100 leukocytes was performed, classifying WBC as neutrophils, lymphocytes, eosinophils, or monocytes based on cellular morphology. The N:L ratio was calculated from this differential count.

Total IgG in serum samples was quantified using a commercialized sandwich ELISA kit (Abnova IgG (Cat) ELISA-Abnova, Taipei City, Taiwan), following protocols provided by the manufacturer. Absorbance of the ELISA kit was quantified by reading the optical density (OD) of the reaction plate using a microplate reader (Biotek, Winooski, VT, U.S.A.) at wavelength 450nm. We measured bacterial killing activity (hereafter BKA) following the method of French and Lee (2012) with slight modifications. To perform the BKA, commercially standardized pellets of *E. coli* (ATCC No. 8739) and *S. aureus* (ATCC No. 6538) (KCCM, Seoul, Korea) were used. Each pathogen pellet was dissolved in 50 ml of sterile 1 M phosphate-buffered saline (PBS) and used as original stock; it was then diluted step-wise from 10^1 to 10^5 in order to compare and select the dilution factor to be used for assay. Log phase growth was determined by incubating five different diluent stocks (10^1 to 10^5) in 37 °C simultaneously with four replicates for each diluent and four negative controls in a 96-well plate. The absorbance of each diluent was measured before the incubation and after

2, 4, 6, 12, and 24 hours to identify the time-point at which the absorbance saturated using a spectrophotometer at wavelength 600nm. For both pathogens, the diluent of 10^3 showed a clear saturation point at 6 hours and at 12 hours for *E. coli* and *S. aureus*, respectively. For the assay of samples, a 1:8 dilution of each serum samples was mixed with media and diluted pathogen stock, and incubated for 6 and 12 hours in 37°C for *E. coli* and *S. aureus*, respectively. Positive controls containing only media and bacterial solution, and negative controls containing only media, were also included in all tested 96-well plates. The absorbance was read before the incubation (background absorbance) and after the incubation. The bactericidal ability was calculated by dividing the mean absorbance for each sample (all samples ran in duplicate) by mean absorbance for the positive controls, subtracted from one, which provided the BKA of each sample.

Statistical analysis

We collected 186 samples for the study, 83 from high and 103 from low CCA districts, respectively. Among them, one individual from a low CCA district showed extremely high BUN (52.9 mg/dL) and CREA (2.59 mg/dL) values, indicative of chronic renal failure, and this influential outlier was removed from all analysis. All statistical analyses were performed using the statistical software R (http://cran.r-project.org). The sex ratio of samples between high and low CCA districts was analyzed through chi-square tests. We tested for associations among body conditions, immune phenotype, and CCA. First, we performed principal component analysis (PCA), on the five body condition parameters (body mass, HCT, ALB, BUN and CREA) (Gilot-Fromont et al., 2012). The first component of PCA (hereafter PC1) captured 25.6% of the total inertia,

showing positive correlation with all tested body condition parameters (Fig. 5-1). Thus, PC1 from PCA of body condition estimates was used as a body condition index for further analysis. In contrast, immune parameters were not combined as a single index for two reasons; (1) immunologically, tested immune parameters had potential not to behave in same direction due to different functions and/or energy cost, and (2) statistically when the parameters were analyzed using PCA, a subset of immune parameters correlated in opposite direction with regards to PC1 or PC2, and could not be represented by a single index. Next, to test for evidence of association of body condition index and immune parameters with CCA and sex, we performed a linear mixed model (LMM). Separate models were built for each response variable (body condition index, N:L ratio, and each immune parameter), and all models were set with CCA, sex, and their interaction as fixed variables and individual district identity as a random variable. Transformation was applied to response variables when required to fulfill model normality assumptions of LMM. Square root, logarithmic, and square power transformation were applied to values of BKA-*E.coli*, N:L ratio and IgG concentration, respectively. Owing to the skewed distribution of the count of each WBC (neutrophil, monocyte, lymphocyte, and eosinophil), the association between CCA and sex with neutrophil and lymphocyte was analyzed through GLMM, fitted using the penalized quasi-likelihood approach, whereas eosinophils and monocytes were analyzed by negative binomial GLMM. Lastly, we looked for associations of body condition index and N:L ratio with seven immune phenotype estimates, BKA of *E. coli* and *S. aureus*, IgG concentration and components of WBC counts. A general linear model (GLM) was built with body condition index or N:L ratio as the response variable and all the immune parameters as independent variables.

Wald chi-square tests were used to determine the effect size of estimated parameters in (G)LMM.

Results

We analyzed 185 individuals, 83 and 102 from high and low CCA districts, respectively. The PC1 body condition index did not show a clear association with CCA (P = 0.54) nor did the CCA and sex interaction (P = 0.30). In analyzing each immune phenotype estimate, only neutrophil count showed a significant and negative association with body condition index (Wald χ^2 = 6.50, P = 0.01). Body condition index was significantly higher in males (Wald χ^2 = 8.50, P < 0.01), but sex ratio did not differ between high and low CCA sites (chi-square test; P = 0.76).

Each immune parameter was separately analyzed to investigate its association with CCA and sex. IgG concentration was significantly higher in low CCA districts (Wald χ^2 = 20.00, P < 0.01) (Fig. 5-2, Table 5-1). Conversely, BKA of *E. coli* and *S.aureus* (BKA *E.coli*; Wald χ^2 = 16.50, P < 0.01, BKA *S.aureus*; Wald χ^2 = 31.83, P < 0.01) was significantly higher in cats from high CCA districts (Fig. 5-3, Table 5-1). None of the WBC components showed significant differences between high and low CCA populations except for eosinophils; where cats from low CCA districts showed significantly higher eosinophil cell counts (Wald χ^2 =3.67, P = 0.05) (Fig 5-4). BKA for *S.aureus* was significantly higher in females (Wald χ^2 = 9.22, P < 0.01), whereas other immune parameters showed no significant associations with sex. The N:L ratio did not vary with CCA or sex (CCA; P = 0.84, Sex; P = 0.18).

Discussion

The goal of this study was to examine how human food provisioning is associated with the body condition and immune phenotype of stray cats. Interestingly, overall CCA (cat caretaker activity) did not show a significant association with body condition indices. However, our results suggest that different aspects of immunity can vary in the directionality of their association with supplemental feeding. Stray cats from high intensity CCA sites had significantly higher innate defenses (BKA) but significantly lower adaptive and anti-parasite defenses (IgG concentration and eosinophil counts, respectively) as compared to cats from low CCA areas. Our data suggest that usage of supplemental food sources influences immune phenotype of animals through routes unrelated to overall body condition.

Constitutive innate immune responses, such as BKA, are sensitive to energy availability (Lee et al. 2006). Previous studies reported direct trade-off between innate immune response and other energy-demanding physiological processes, and signs of trade-off being relieved by food supplementation (Brzek & Konarzewski 2007; French & Moore 2008). Overall, animals in better condition are suggested to maintain higher constitutive innate immune function (Lee et al. 2006). In this study, BKA for both pathogens were higher in high CCA districts and lower in low CCA districts. This may be explained by different availability of stable food source in each area. For instance, food provisioning by cat caretakers can allow cats to spend less time and energy foraging and/or competing to secure food sources, leaving more energy for other physiological processes including innate immunity (Lane *et al.* 2011; Becker *et al.* 2015). Higher body mass of cats in high CCA districts may also reflect their higher energy availability

compare to cats in low CCA areas. Alternatively, higher BKA may have been related to the nutritional quality of the food source available. Stray cats in the low CCA area, like other urban wildlife, will commonly rely on indirectly provisioned human food sources, such as garbage. These food sources are unlikely to contain nutritional elements critical for maintaining essential immune components, such as proteins or antioxidants (Koski & Scott 2001; Marcos, Nova & Montero 2003; Maggini *et al.* 2007). In comparison, the cats in the high CCA districts, supplemented with commercial cat food, may have had the advantage of maintaining relatively balanced nutritional status, which allowed them to elicit stronger bacteria killing ability, a protein demanding process (Venesky *et al.* 2012).

However, the abundance of energy availability due to supplemental feeding had limited implications on explaining our observation of IgG concentration as we observed higher IgG in low CCA areas compared to high CCA areas. IgG is predominantly involved in secondary immune response. Because the assay in this study measured concentration of cumulated IgG rather than ephemeral antibody response, the result of IgG concentration may be reflecting the history of pathogen infection, which may be affected by characteristics of infections, such as the duration and/or frequency (Brock et al. 2013; Listi et al. 2006). Similarly, previous studies translated increased IgG concentration as sign of chronic infection or accumulation of repeated pathogen exposure (Brock et al. 2013; Schneeberger et al. 2014). Although extreme malnutrition may hinder production of IgG (Glick, Day & Thompson 1981; Frouin et al. 2013), this is less likely to apply in our study, as overall cats showed similarly moderate body condition regardless of the CCA intensity. Hence, our observation of higher IgG levels in low CCA areas may be better explained by the difference in pathogen infection history of cats in

the high and low CCA areas. For instance, supplemental feeding in wildlife is known to shift the behavior of animals in ways that can affect the exposure and transmission of pathogens in host animals. For instance, smaller home range areas and/or less time spent foraging (Gilchrist and Otali, 2002; Lemel et al., 2003; Schoepf et al., 2015) are often reported in animals with accessibility to abundant supplemental food. Such alteration of foraging behavior and/or habitat use can potentially lower the exposure of animals to environmentally transmitted pathogens (Fredebaugh et al. 2011; Parr et al., 2013). Similarly, stray cats in low CCA districts are expected to use larger home range and/or spend more time seeking food sources, giving them more spatiotemporal chances to be exposed to infectious stages of pathogens or parasites in the environment. An epidemiological analysis of stray cats used in this study revealed a higher prevalence of feline parvovirus antibodies in cats from low CCA districts (unpublished data). Feline parvovirus is very stable in the environment and can be transmitted through contact with external objects, such as soil or vegetation, contaminated with the virus from fomites of infectious individuals (Petersen et al., 2008). Feline parvovirus may represent one of the pathogens transmitted through environmental contact to which cats in low CCA districts were more frequently exposed. Hence, higher IgG in low CCA districts may be reflecting higher exposure of cats in these areas to pathogen infections.

Similarly, eosinophil count, which is an indicator of parasite (e.g. helminth, ectoparasite) infections among others, was also significantly higher in low CCA districts (Klion and Nutman, 2004). Stray cats are hosts of a suite of parasites, such as hookworms, fleas and *Toxocara* sp., many of them transmitted through environmental contact such as vegetation, soil and fecal matter (Borji et al., 2011; Waap et al., 2014).

Thus, higher eosinophil count in low CCA districts may be due to higher helminth worm burden of cats in the areas, potentially acquired through environmental transmission, similar to feline parvovirus. Taken together, lack of supplemental food in lower CCA districts may have contributed to active foraging and/or intensive land-use by stray cats, facilitating repeated contact with infectious stages of parasites distributed in the habitat followed by higher IgG concentration and/or eosinophil count. Further efforts investigating direct links among supplemental feeding, home range size and prevalence of environmentally transmitted pathogens will be required to verify the role of altered foraging behavior and land-use as mechanisms behind the link between supplemental feeding and observed immune phenotype.

The lower BKA observed in the low CCA sites in contrast to the higher IgG may be the result of parasite infection, commonly followed by body condition debilitation (McDade et al., 2016; Palacios et al., 2012). Indeed, concentration of IgG has been previously reported to show a negative relationship with body condition of animals, potentially reflecting on-going infection and/or cumulative exposure to pathogen infection (Brock et al., 2013). Lowered body condition can lead to reduced energy available for individuals to invest in other energetically expensive physiological processes, such as innate immune responses (Brock et al., 2013; Schneeberger et al., 2014). Future studies seeking evidence of trade-offs among immune components in relation to parasite infection histories and resource availability may benefit from considering various forms of infection, such as its duration, repetitivenvess, and the novelty of parasites that may alter immune phenotypes in host animals (Budischak et al. 2015).

Based on observation of the overall hematological panel independently from body condition index, thirteen individuals, five from high CCA and eight from low CCA districts, were showing concurrent signs of inflammation (leukocytosis with neutrophilia), anemia (low HCT and RBC), and protein-deficiency (low CREA or BUN or ALB) (data not shown). Median value of BKA (for both *E.coli* and *S.aureus*) was lower and IgG concentration was higher in thirteen individuals, than that of the entire samples. Although the body condition index did not show a statistically significant association with tested immune parameters, the data suggest that the overall health status of an animal reflected in the entire hematological panels yet provides meaningful information on the capacity of individuals to elicit immune responses. Our result warrants further efforts to reveal the associations between the health status reflected on hematological observations and the ability to elicit various immune responses.

Our study demonstrated different immune parameters showing association with supplemental feeding in varying directions. This may be due, in part, to (1) multiple mechanisms behind the interaction of human food provisioning and immune phenotype, including surplus energy source, stress, or parasite exposure; and (2) difference in the response and behavior of each immune component toward energy availability or parasite infection. This study was limited by lack of information regarding the prevalence of environmentally transmitted pathogens. In particular, collecting information on prevalence/infestation rates of helminths is suggested, as it commonly causes chronic and/or repetitive infection in free-ranging animals with potentially strong influences on shaping immune phenotypes (Klion and Nutman, 2004; Koski and Scott, 2001). Supplemental feeding and human subsidies are directly or indirectly provisioned to free-

ranging animals worldwide (Oro et al., 2013; Robb et al., 2008). The variation of immune function in response to supplemental feeding can have an immense influence on host-pathogen interaction, altering the outcome of pathogen invasion in host populations (Becker and Hall, 2014). Results from this study suggest multiple potential routes that human provisioning can affect immune phenotype of individual, highlighting the diverse influence of supplemental feeding on physiology of free-ranging animals. Our study also underscores the importance of taking into account the different behavior of immune components in response to external factors such as energy availability and/or pathogen infection, in future ecoimmunological studies.

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Table 5-1. Result of GLMM association analysis of immune parameters by CCA and sex

Pathogens	Variable	Estimate	SE	LCI	UCI	t, z value	P value
BKA-*E.coli*	**CCA-high**	0.08	0.02	0.03	1.33	4.00	0.00*
BKA-*S.aureus*		0.16	0.03	0.09	0.22	5.61	0.00*
IgG		-0.52	0.12	-0.81	-0.26	-4.48	0.00*
Eosinophil		-0.41	0.22	-0.88	-0.09	-1.92	0.05*
BKA-*E.coli*	**Sex-female**	0.03	0.02	0.00	0.06	1.78	0.08
BKA-*S.aureus*		0.06	0.02	0.02	0.10	3.13	0.00*
IgG		-0.19	0.12	-0.42	0.04	-1.66	0.09
Eosinophil		-0.00	0.14	-0.28	0.28	-0.01	0.84

* P values below 0.00

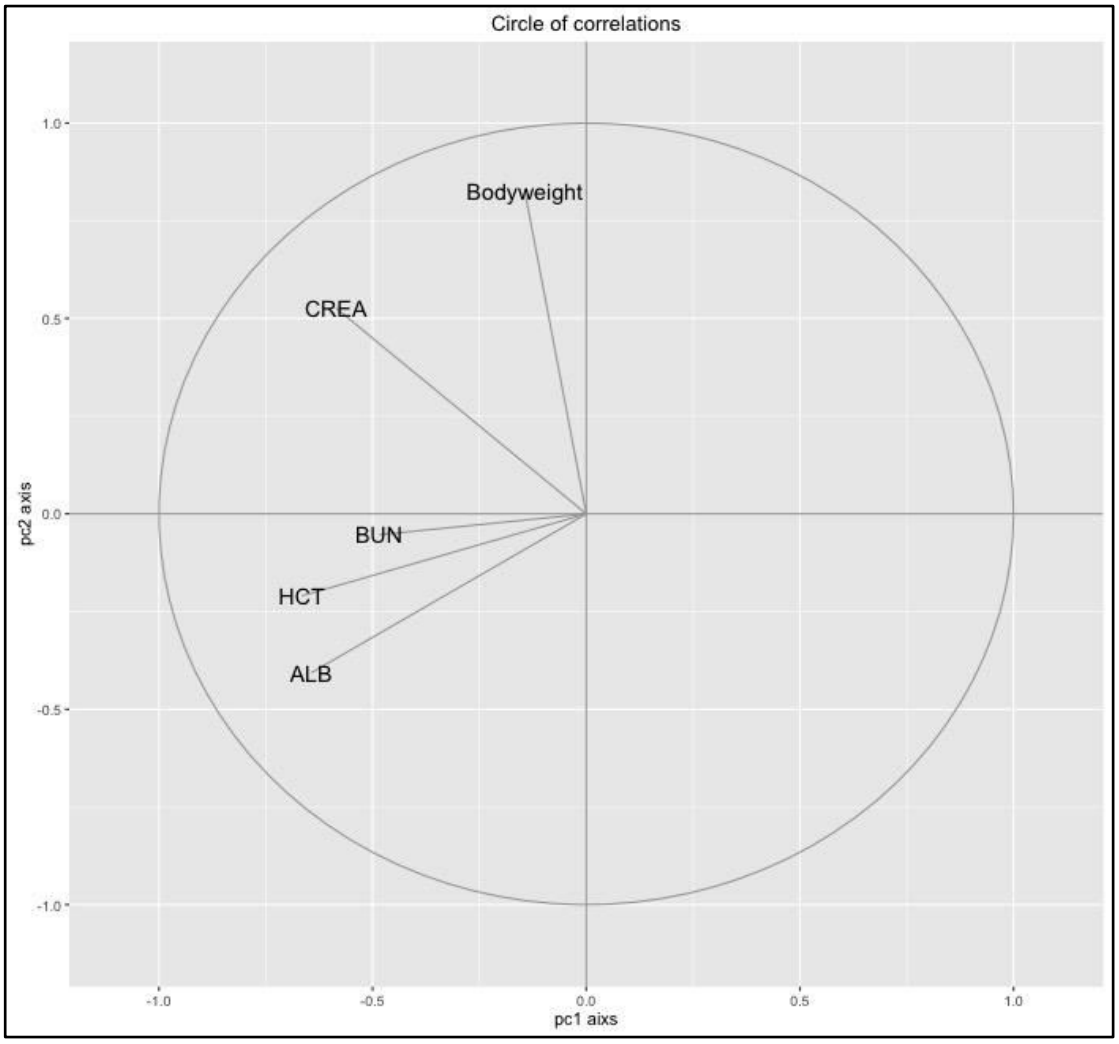

Figure 5-1. Correlation circle showing the projection of body condition parameters on principal components. See text for definition of variables.

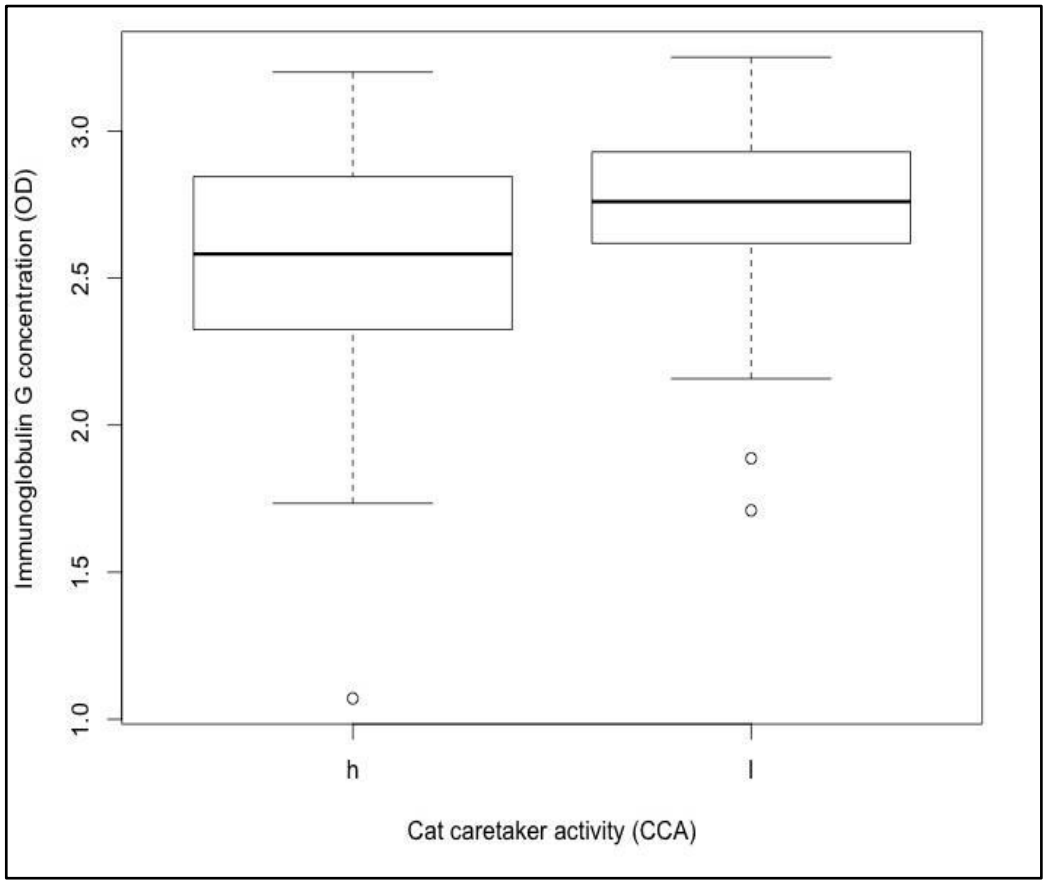

Figure 5-2. Box plot of IgG concentration by CCA (h=High, l=Low)

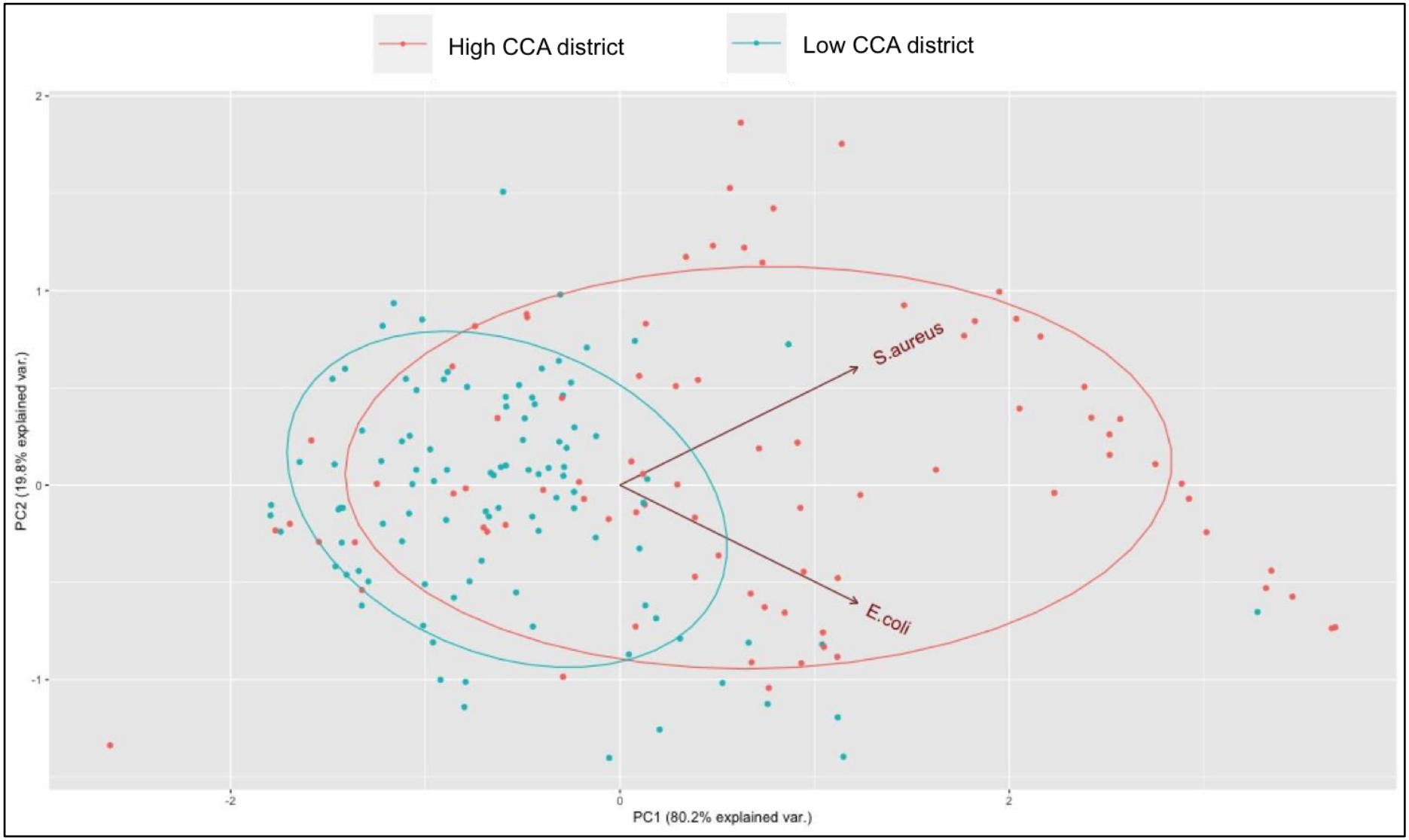
High CCA district
Low CCA district
S.aureus
E.coli
PC2 (19.8% explained var.)
PC1 (80.2% explained var.)
2
1
0
-1
-2
0
2

Figure 5-3. BKA results between two CCA districts. Projections of BKA value of all studied individuals on the first two principal components of PCA simultaneously analyzing BKA for *E.coli* and *S.aureus* (displayed in the PC1-PC2 plane)

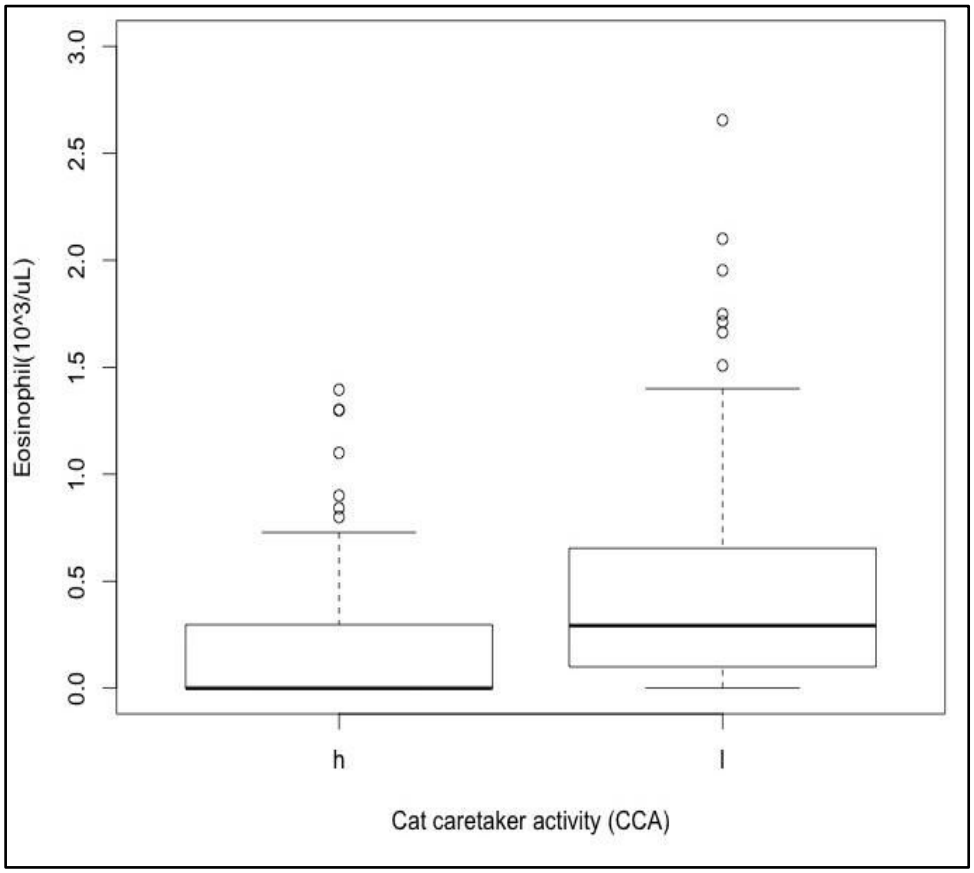

Figure 5-4. Box plot of Eosinophil counts by CCA (h=High, l=Low)

CHAPTER 6

CONCLUSION AND FUTURE DIRECTIONS

The goal of this dissertation was to improve understanding of the disease ecology of urban-adapted animals in relation to habitat types and human behaviors, and to seek associated mechanisms and consequences. I used free-ranging domestic cats to answer my research questions, as they are one of most ubiquitous mesocarnivore species found in modern society, interacting with various human activities.

In the first half of my research (Chapters 2 and 3), I conducted a comparative study on health parameters and pathogen prevalence in feral cat populations from urban versus rural habitat types. Specifically, Chapter 2 found that stray cats from urban habitats had higher body condition levels compares to their conspecifics from rural habitats, suggesting the potential influence of habitat type on the health status of feral cats. The results of this study have implications on management of the welfare and health status of feral cats in various habitat types, along with domestic animals and humans that are sharing the habitats. Future studies investigating immune function of feral cats in different habitat types may further allow us to answer how the different health stautses observed in this study are linked to resistance of feral cats to pathogen infections.

Chapter 3 demonstrated that the means through which host-pathogen interactions respond to different habitat types might be determined by the transmission mode of each pathogen. Among three pathogens tested, only a pathogen (*B. henselae*) transmitted

through fleas showed significantly higher prevalence in urban cats. Although number of previous studies reported higher prevalence and/or infestation rate of fleas in rural habitat compare to urban area, contrasting results have been also observed as reviewed in the literature review subsection of this thesis. As suggested in the literatures with similar results from this study, further attempts at testing the associations of host population density, home range size and urban microclimate may allow further clarifications of the dynamics of the flea and flea-boren pathogens in urban habitat. Another interesting finding from this chapter was the similar prevalence of *T. gondii* between the two habitat types. Based on findings from a previous modeling study (Lelu et al. 2010), I suggested the ability of *T. gondii* to switch its dominant transmission mode between the two habitat types as the potential mechanism for similar prevalence in urban and rural habitats. However, further study on comparing diet content, especially the frequency of consuming intermediate host species in feral cats between the two habitat types, may provide further understanding on the dominant transmission mode of *T. gondii* (direct or trophic) in response to habitat types.

In the second half of my study, the main question shifted to looking at the disease ecology of stray cats within Seoul, Korea. Chapters 4 and 5 attempt to verify how human food provisioning is associated with not only health status or population density of stray cats but the history of pathogen infections and capacity of cats to immunologically interact with infections. Chapter 4 investigated the potential links among the supplemental feeding, population density, and prevalence of multiple pathogens in urban stray cats. The results stated in Chapter 4 demonstrate the potential influence of supplemental feeding on pathogen prevalence, but in a manner that relates to the

transmission mode of each pathogen. Specifically, pathogens with transmission modes that are considered 'frequency-dependent', such as vector-borne transmission, did not show a significant relationship with supplemental feeding. Meanwhile, higher supplemental feeding had a positive relationship with the prevalence of pathogens transmitted through direct contact (higher prevalence of feline leukemia virus in high supplemental feeding), whereas the prevalence of pathogen infection acquired through environmental transmission showed a negative relationship (higher seroprevalence of feline parvovirus in low supplemental feeding). Findings from this study suggest that altered behavior in response to supplemental feeding, such as higher aggregation and/or small home range, may have had a significant impact on the exposure rate of host animals to different pathogens, resulting in the observed patterns of this study. Hence, further study may benefit from quantifying the aggregation rate of animals in response to different intensities of food provisioning and how it affects the prevalence of pathogens transmitted through direct contact between infectious and susceptible individuals.

Lastly, Chapter 5 was an attempt to investigate the impact of supplemental feeding on body condition and immune phenotype in stray cats. In disagreement with my initial prediction, body condition measured by selected blood parameters was not associated with the intensity of supplemental feeding. However, two tested immune components (BKA and IgG) reflecting the function of different immune arms, showed significant, yet opposite, association with availability of supplemental feeding. This result is interesting, because it suggests that availability of additional food sources may have had an influence in shaping immune phenotypes through routes other than supplying an extra energy source. Based on the observed pattern of immune functions from this study,

which was higher IgG concentration and eosinophil in low food provisioned stray cats, a higher frequency of cat exposure to parasite infections, especially helminthes, may be one hidden driver shaping the immune phenotype. I propose that relatively lower availability of supplemental feeding (in low CCA districts) may have resulted cats in the area to use larger home range and/or perform intensive foraging behavior. This may have increased the exposure of cats to parasites within the inhabiting area compared to cats that benefit from abundant and stable anthropogenic food sources. An additional observation on BKA, which showed an opposite response from IgG and eosinophil counts, might be considered a result of energy trade-off among different immune arms. Simultaneous investigation of food availability, immune phenotype, and prevalence/infestation of parasites in stray cats or other urban-adapted wildlife may further clarify the mechanisms suggested.

There is a rich literature explaining the patterns and potential mechanisms of urban wildlife ecology. This dissertation research was an attempt to expand our understanding about how the well-known ecology of urban wildlife is interacting with pathogens in this novel environment. Higher body condition and higher population density of urban animals have been two of most commonly discussed mechanisms that have been suspected to have significant impacts on urban disease ecology. However, the results of this study suggest that host-pathogen interaction in urban habitats can be more complex, requiring consideration of wider range of drivers and processes involved. In particular, further studies focusing on the impact of different transmission modes of pathogens and altered host behavior in respect to urbanization and use of human resources may aid in unraveling disease ecology in urban wildlife.

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APPENDIX

Table S1. PCR results for cat hemoplasma positives from this study

PCR result	Number of infected cats
Hemoplasma positive	56
"*Candidatus* Mycoplasma haemominutum"	40
Mycoplasma haemofelis	10
"*Candidatus* Mycoplasma turicensis"	2
Copositive; CMhm+Mhf	4

*CMhm: "*Ca.* M. haemominutum"; Mhf: *M. haemofelis*

Table S2. Hematological parameter values and body mass by CCA and sex

	Reference range	High CCA		Low CCA	
		Male (n=41)	**Female (n=42)**	**Male (n=47)**	**Female (n=55)**
Body mass (kg)	-	4.54 (4.19-4.90)	3.96 (3.69-4.23)	4.16 (3.85-4.47)	3.39 (3.20-3.58)
HCT (%)	27.7-46.8	30.48 (27.75-33.30)	28.74 (27.04-30.44)	30.36 (28.84-31.88)	28.85 (27.60-30.10)
ALB (g/dL)	8.1-14.2	2.92 (2.81-3.02)	2.74 (2.57-2.91)	2.88 (2.80-2.98)	2.94 (2.88-2.99)
BUN (mg/dL)	41.3-52.6	19.34 (18.15-20.52)	18.59 (17.43-19.75)	17.97 (16.94-18.99)	17.23 (16.13-18.32)
CREA (mg/dL)	6.3-19.6	1.04 (0.99-1.10)	0.97 (0.92-1.03)	1.07 (1.00-1.13)	1.03 (0.97-1.08)