A Walk in the Park: Zoonotic Risks Associated with Dogs that Frequent Dog Parks in Southern Ontario

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ABSTRACT

A Walk in the Park: Zoonotic Risks Associated with Dogs that Frequent Dog Parks in Southern Ontario

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A cross-sectional study investigated the shedding of zoonotic organisms (Campylobacter, Giardia, and Salmonella) and antimicrobial resistant generic E. coli in dogs that visited dog parks in southern Ontario. Logistic regression models were constructed to identify risk factors. Factors for the shedding of Campylobacter spp. included consumption of a commercial dry diet, exposure to compost, and age. Factors for the shedding of C. upsaliensis included outdoor water access and age. A risk factor for ampicillin resistance was attending a dog day care. For resistance to at least one antimicrobial, factors included attending a dog day care, breed size, consumption of a commercial dry diet and consumption of a homemade cooked diet. For multiclass resistance, exposure to compost, breed size, and consumption of a commercial dry diet were identified. Park was not significant in any model. Dogs that visit dog parks shed organisms that may pose a human health risk.
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Chapter 1:

Introduction, literature review, study rationale, and objectives

Introduction

Companion animals are of great significance in today’s society; more than 32% of Canadian households have a dog, with an estimated 6 million dogs in Canada (Perrin, 2009). In a survey conducted by the American Animal Hospital Association (2004), 94% of pet owners considered their pet to have human-like personality traits, 93% were likely to risk their lives for their pets, 40% would bring a dog as their only companion on a deserted island, and 58% visit their pet’s veterinarian more often than their own physician. Considering the large number of people sharing their homes with dogs and the close relationship that many owners share with their dogs, the public health impacts of dogs as companion animals is important to consider, particularly with the various One Health initiatives which aim to integrate human, animal and environmental health (e.g., Wurapa et al., 2011; Blaha, 2012; Leung, Middleton, and Morrison, 2012).

This thesis focuses on zoonotic risks related to dogs that visit dog parks, and therefore the following literature review aims to describe issues relevant to both the positive and negative aspects of owning dogs and visiting dog parks through the following topics:

1. Social, physical and emotional benefits of dog ownership;
2. Zoonoses in dogs with a focus on Salmonella, Giardia, and Campylobacter;
3. Antimicrobial resistant organisms in dogs;
4. Dog parks as a unique environment; and
5. Methods used to study dogs in a community.
Literature review

Benefits of dog ownership

Dogs have been living with humans for more than 14,000 years (Case, 2008) and provide many benefits to their owners. The term “zooeyia” has recently been coined to describe the positive impact animals have on human health (Hodgson and Darling, 2011). There are several physical, social and psychological benefits that have been associated with the companionship of a dog.

Evidence suggests that dog owners are more physically active than non-owners, which has been attributed to walking dogs (Cutt et al., 2007; Hodgson and Darling, 2011). The ownership of a dog has been associated with decreased likelihood of high blood pressure, obesity, and cardiovascular disease (McConnell et al., 2011). These effects may be due to the increased physical activity as a result of owning a dog; dogs provide positive motivation for physical activity, such as regular walking, even in detrimental weather (Temple, Rhodes, and Wharf Higgins, 2011). Dogs have also been shown to be a positive factor in recovery from heart attacks (Wells, 2007; McConnell et al., 2011), which may be a result of the companionship and social support a dog can provide. In addition, dogs can be used to detect and predict certain physical ailments including cancer, seizures, and hypoglycemia (Wells, 2007; Knight and Herzong, 2009). Dogs are also used as service animals to provide eyes, ears and companionship to the blind, deaf, autistic, physically disabled, or residents of nursing homes and prisons (Wells, 2007).
Pets can provide social support to their owners, as well as reduce feelings of isolation and loneliness (Wells, 2007; Wood et al., 2007; Hodgson and Darling, 2011). Wood et al., (2007) reported that 84% of dog owners talked to other pet owners while walking their dog, which facilitated relationships between neighbours. The community can also benefit from the presence of a dog; pet owners are more likely to be civically engaged compared to non-pet owners (Wood et al., 2007), and dogs in the neighbourhood provide a sense of safety in the community and increase the likelihood of neighbours meeting and talking to each other (Wood et al., 2007).

In addition to increasing social connections with other humans, dogs themselves can act as a companion, especially for the elderly and those with barriers to regular social interactions. Dogs can create a sense of belonging, reduce levels of anxiety, loneliness and depression, enhance self-esteem and increase feelings of independence and competence (Wells, 2007; Hodgson and Darling, 2011; McConnell et al., 2011). Furthermore, thinking about one’s pet has been shown to reduce feelings of negativity after social rejection similar to thinking of a best friend (McConnell et al., 2011).

The many benefits of a relationship with a dog may be part of the reason they are such common pets and explain the close relationship that many people share with their dogs. It is important, however, to be aware of all aspects of owning and caring for a dog, including the potential health risks.
Zoonoses in dogs

Although dogs do provide many health benefits, they can also pose a health risk to humans through bites and zoonoses. Zoonoses are diseases that are naturally transmitted between humans and animals (Plaut, Zimmerman, and Goldstein, 1996). Infection can occur through direct contact with skin, saliva, urine or feces, or indirect contact from contaminated food or water (Plaut, Zimmerman, and Goldstein, 1996). Pets may also put humans at greater risk of infection from tick-transmitted diseases, such as Lyme disease and Rocky Mountain spotted fever, as a result of ticks on pets being introduced into the home environment (Plaut, Zimmerman, and Goldstein, 1996). Individuals that are immune-compromised due to illness, as well as the very young and very old, are in even greater danger of infection with zoonotic diseases (Plaut, Zimmerman, and Goldstein, 1996). In particular, bacterial zoonoses such as Salmonella, Giardia, and Campylobacter can be carried and shed asymptotically by dogs, and may cause disease in humans (Grøndalen, Sævik, and Sørum, 2008).

a. Salmonella

Salmonella is a gram negative, motile, non-spore-forming, facultative anaerobic organism, which can be found in many species of mammals, birds, and reptiles (Marks et al., 2011). In humans and dogs, the common sources of exposure to Salmonella include contaminated food and direct fecal-oral ingestion (Grøndalen, Sævik, and Sørum, 2008). Salmonella is associated primarily with acute signs that present 3-5 days after exposure, with the severity of fever, vomiting, abdominal pain and diarrhea in both humans and dogs dependent on the individual (Marks et al., 2011). Infection can also spread throughout the body leading to severe and sometimes fatal illness (Marks and Kather, 2003). Salmonella is often shed...
asymptomatically by dogs, and can be shed up to six weeks after infection (Cantor et al., 1997; Bagcigil et al., 2007; Grøndalen, Sævik, and Sørum, 2008; Marks et al., 2011). Previous studies have reported the prevalence of *Salmonella* in healthy dogs ranging from 1-4% (Hackett and Lappin, 2003; Marks et al., 2011), and over 30% for hospitalized dogs (Marks et al., 2011).

In a study by Leonard et al. (2011a), a high prevalence of *Salmonella* was detected (23%) among healthy dogs. A prevalence this high in healthy dogs was thought to be due to the large number of dogs in the study consuming a raw diet, which has been identified as a risk factor for *Salmonella* carriage in a number of studies (Cantor et al., 1997; Finley et al., 2007; Lefebvre et al., 2008; Leonard et al., 2011a). In addition, Leonard et al. (2011a) collected several fecal samples from each dog, resulting in a period prevalence, rather than a point prevalence, which may have affected the probability of a dog testing positive.

The relatively common asymptomatic shedding of *Salmonella*, and the requirement to remove dog feces from public areas, including dog parks, may expose humans to *Salmonella*, without being aware of the risk. In addition, if feces are not removed from dog parks, the environment, such as lakes and rivers, as well as wild animals, including small mammals (e.g., squirrels, raccoons, and mice) and birds, may be exposed to this pathogen.

b. *Giardia*

*Giardia* is a protozoan transmitted fecal-oraly that colonizes the intestinal tracts of humans, and other animals, and can cause diarrhea, nausea and vomiting, which can be self-limiting or chronic (Thompson, Palmer, and O`Handley, 2008; Ballweber et al., 2010). Most infections are sub-clinical, however, making detection and control difficult (Thompson, Palmer, and O`Handley, 2008). Cysts are shed in feces of infected individuals and are immediately
infectious (Thompson, Palmer, and O’Handley, 2008). Cysts remain infectious and can survive and accumulate in cool, damp environments, as well as in water for several months (Thompson, Palmer, and O’Handley, 2008).

Previous studies have reported *Giardia* in 5-9% of healthy dogs (Jacobs, Forrester, and Yang, 2001; Hackett and Lappin, 2003; Mircean, Györke, and Cozma, 2012; Wang et al., 2012). While a relatively small proportion of the canine population may be infected, a substantial proportion of dogs and humans may be at risk of infection due to the long infectious period and survivability of *Giardia* in the environment.

*Giardia* is separated into seven genetically different assemblages referred to as A-G. Humans are only infected with assemblages A and B, and dogs primarily with C and D. However, dogs have also been reported to be infected with assemblages A and B (Xiao and Fayer, 2008; Thompson, Palmer, and O’Handley, 2008; Ballweber et al., 2010; McDowall et al., 2011). In a recent study, McDowall et al. (2011) reported that in canine fecal samples in Ontario submitted for *Giardia* testing only 1% of the samples contained the potentially zoonotic assemblage B, and none of them contained assemblage A. The majority of samples were assemblage C and D (31% and 68%, respectfully), which are not considered zoonotic. Similarly, in Colorado, only assemblages C and D were detected in fecal samples from dogs owned by veterinary students and staff members at a veterinarian hospital (Wang et al., 2012). These findings suggest that the risk of *Giardia* from dogs to humans may be low.

Although dog parks have not been extensively studied in relation to zoonoses in dogs, Wang et al. (2012) reported that dogs who visited dog parks in Colorado had a higher probability of shedding *Giardia* compared to dogs that did not visit dog parks. However, only assemblages
C and D were detected in these samples. The study by Wang et al. (2008) is among the first that has investigated zoonoses in dogs that have visited dog parks.

c. **Campylobacter**

*Campylobacter* is a gram negative, anaerobic organism and is responsible for campylobacteriosis, which has been identified as the most common cause of bacterial enteritis in people in Canada (Government of Canada, 2009). Campylobacteriosis is characterized by acute diarrhea, vomiting, fever and intense abdominal pain, lasting 3-15 days (Nachmkin, Szymanski, and Blaser, 2008). Following infection, there is also a risk of chronic conditions such as colitis, nephritis, reactive arthritis, and Guillain-Barré syndrome (Bourke, Chan, and Sherman, 1998; Nachmkin, Szymanski, and Blaser, 2008). Dogs may experience gastro-intestinal signs similar to humans, such as diarrhea, but can also be infected sub-clinically and shed *Campylobacter* spp. without showing signs (Grøndalen, Sævik, and Sørum, 2008). Routes of exposure to *Campylobacter* include fecal-oral, ingestion of contaminated food or water and consumption of raw meat, particularly poultry. Cats and dogs have also been identified as a potential source of exposure of *Campylobacter* spp. to humans (Government of Canada, 2007).

The most commonly identified species of *Campylobacter* infecting humans in Canada is *Campylobacter jejuni*, followed distantly by *C. coli* and *C. lari* (Government of Canada, 2009). Although the catalase negative species *Campylobacter upsaliensis* is not recovered as often from humans, it is possible that available data under-estimate the prevalence of this species. Public health laboratories focus on thermotolerant strains and may not attempt to speciate catalase negative species. In addition, standard laboratory methods used to isolate catalase positive *Campylobacter* spp. can hinder the recovery of catalase negative strains, particularly *C.*
upsaliensis (Labarca et al., 2002; Lastovica and Le Roux, 2003); the use of a broth that contains cephalothin, which C. upsaliensis has been shown to be susceptible to, as well as a shorter incubation time, have been noted as possible factors that might result in under-detection of this species (Lastovica and Le Roux, 2003).

In contrast, C. upsaliensis is frequently recovered from clinically normal companion animals, with dogs and cats believed to be the reservoir species for this organism (Workman, Mathison, and Lavoie, 2005). Campylobacter upsaliensis was first isolated from canine feces in Uppsala, Sweden in 1983 (Bourke, Chan, and Sherman, 1998). Shortly after the identification of C. upsaliensis in dogs, it was detected in human samples, using appropriate techniques and has been implicated as a human pathogen (Bourke, Chan, and Sherman, 1998). Campylobacter upsaliensis has since been associated with chronic and recurring diarrhea, weight loss, bacteremia in immune-compromised patients, abortion, hemolytic-uremic syndrome, and Guillain-Barré syndrome in humans (Bourke, Chan, and Sherman, 1998).

Previous canine prevalence studies on Campylobacter spp. have been completed mostly in Europe, in household or shelter dogs. In these studies, the prevalence of Campylobacter spp. in the feces of domestic dogs has ranged from 22-87%, with 19-99% being C. upsaliensis (e.g., Sandberg et al., 2002; Wieland et al., 2005; Westgarth et al., 2009; Parsons et al., 2010; Leonard et al., 2011b.) The shedding of this pathogen by dogs can pose a risk for human health (Bourke, Chan, and Sherman, 1998; Government of Canada, 2007).

Previously identified risk factors associated with the shedding of Campylobacter spp. in dogs include the following: age of the dog, with young animals more likely to shed (Hald et al., 2004; Wieland et al., 2005; Acke et al., 2006; Westgarth et al., 2009; Parsons et al., 2010; Leonard et al., 2011b.)
Leonard et al., 2011b); diet, with homemade cooked foods increasing the probability of shedding (Leonard et al., 2011b); participation in group activities (Leonard et al., 2011b); children in the home (Leonard et al., 2011b); and season, with shedding more frequent in the summer (Sandberg et al., 2002). Treatment with antibiotics has been identified as a sparing factor (Leonard et al., 2011b). Previously identified risk factors specifically for the shedding of *C. upsaliensis* include consumption of a homemade diet (Leonard et al., 2011b), humans with access to cats from outside the home (Leonard et al., 2011b), the dog having suffered diarrhea 14 days prior to sampling (Wieland et al., 2005), living with another dog (Westgarth et al., 2009; Parsons et al., 2010), size of dog, with small dogs most likely to shed this organism (Westgarth et al., 2009), sharing a household with fish (Westgarth et al., 2009), and being fed human “tidbits” (Westgarth et al., 2009).

The prevalence and risk factors for the shedding of *Salmonella*, *Giardia*, and *Campylobacter* in dogs are important to consider, as dogs may provide a reservoir for these zoonotic pathogens, which can result in severe health problems for both humans and dogs. Healthy, active dogs, such as those that visit dog parks have not been extensively studied and may represent a population different from those that present at veterinary clinics, animal hospitals or animal shelters, which are more frequently studied.
Antimicrobial resistant organisms in dogs

Antimicrobial resistance is an international human and animal health problem, and with the use of antimicrobials in companion animals, this population presents a potential reservoir for resistant bacteria (Guardabassi, Schwarz, and Lloyd, 2004; Lloyd, 2007; Umber and Bender, 2009). Antimicrobial resistance can result in a change of virulence in an organism, or a decreased response to treatment (Travers and Barza, 2002). Due to antimicrobial resistance, longer treatment times and hospitalizations, increased days off work and more severe human infections are possible (Travers and Barza, 2002). The transmission pathway of antimicrobial resistance genes between humans and their pets is not clear, and is difficult to determine, but Stenske et al. (2009) have reported that within a household, dogs and their owners are more likely to share common antimicrobial patterns than those in different households. Similarly, Harada et al. (2012) reported that dogs and their owners may share fecal antimicrobial resistant E. coli within the household. These results suggest that there is some cross-species transmission of antimicrobial resistant organisms and the presence of resistant organisms in dogs may pose a risk to humans.

Antimicrobial resistance can occur in both pathogenic and commensal organisms. Commensal organisms, including generic E. coli, may represent a large group of bacteria that can contribute to the spread of antimicrobial resistance genes (McEwen and Fedorka-Cray, 2002; O’Brien, 2002; Marshall, Ochieng, and Levy, 2009). The large numbers of bacteria present in the gastro-intestinal system, combined with the relatively small space, provides ideal conditions for gene transfer between commensal and potentially pathogenic bacteria (O’Brien, 2002; Marshall, Ochieng, and Levy, 2009). Generic E. coli can also be used as an indicator bacterium to
determine which resistance genes may be present in an individual or population (Caprioli et al., 2000).

The development of antimicrobial resistant genes may occur by several different mechanisms that vary based on the organism and the class of antimicrobial agent involved (Boerlin and Reid-Smith, 2008; Umber and Bender, 2009). Antimicrobial resistance can be either intrinsic or acquired. Intrinsic or “natural” resistance mechanisms are those that are based on the natural characteristics of the organism, such as the lack of a cell wall or transport mechanism that allows the antimicrobial to be effective (Umber and Bender, 2009). Acquired resistance mechanisms include mutation of DNA, transfer of chromosomal DNA, and the transfer of extra-chromosomal DNA, located on plasmids or transposons through transduction, conjugation and transformation (Boerlin and Reid-Smith, 2008; Umber and Bender, 2009).

Antimicrobial drug use and resistant organisms are not commonly monitored in companion animals in North America. The lack of reporting antimicrobial use and resistance patterns makes it difficult to estimate how much resistance is present in companion animals and whether there should be changes in how antimicrobials are used to prevent increased resistance. There are guidelines on the prudent use of antimicrobials in veterinary medicine; however these guidelines may not affect how veterinarians choose treatments or the occurrence of antimicrobial resistance (Prescott et al., 2002). Practitioners may choose not to follow these guidelines, as some veterinarians do not like restrictions on their rights to prescribe, and may think prudent use of antimicrobials in companion animals is not worthwhile (Prescott et al., 2002). It is possible to determine the effect of these guidelines on antimicrobial resistance in Scandinavian countries, as there is a national antimicrobial use and resistance surveillance program that includes
information on antimicrobial use in companion animals (Guardabassi, Schwarz, and Lloyd, 2004). These surveillance programs can provide baseline data so antimicrobial usage and resistance patterns can be monitored over time.

In previous studies of antimicrobial resistance in generic *E. coli* in dogs, up to 76% of isolates have shown resistance to at least one antimicrobial, including antimicrobials such as amoxicillin, streptomycin, ampicillin, sulfamethoxazole, tetracycline, and chloramphenicol (Normand et al., 2000; Pedersen et al., 2007; Murphy et al., 2010; Shaheen et al., 2010; Harada et al., 2011; Leonard et al., 2012). In healthy dogs, estimates of the prevalence of antimicrobial resistant *E. coli* isolates have ranged from 0-60%, with most studies falling within a range of 10-15% (De Graef et al., 2004; Costa et al., 2008; Murphy et al., 2009; Stenske et al., 2009; Murphy et al., 2010; Leonard et al., 2012). The prevalence of antimicrobial resistance in *E. coli* varies based on what type of dog is sampled (e.g., healthy, admitted to veterinary hospital, living in breeding kennel, etc.), the organism isolated (*E. coli*, *Salmonella*, *Enterococcus*, *Campylobacter*), and the country where the study takes place. Many studies examine dogs that are in animal hospitals, which can bias the results upwards, as these animals may represent only the worst cases where other treatments have failed, the development of resistance due to the use of antimicrobial for treatment, or be reflective of resistance genes present in environmental bacteria which could complicate infections. In addition, breeding kennels have been shown to have a higher rate of antimicrobial resistance than individually owned household dogs, possibly due to the close contact and higher density of animals (De Graef et al., 2004). Different countries may use antimicrobials differently, which may alter the prevalence of antimicrobial resistant isolates in dogs as well as the distribution of resistance among different antimicrobial classes (Caprioli et al., 2000).
Bacterial isolates from dogs show resistance to antimicrobials typically used in companion animal medicine and of human medical importance. In Denmark, where there is a national surveillance database for antimicrobial use, penicillins, cephalosporins, aminoglycosides (streptomycin), and sulphonamides/trimethoprim combinations are common antimicrobials used in companion animals (Guardabassi, Schwarz, and Lloyd, 2004). Similarly, *E. coli* isolates from dogs in Scandinavian countries commonly show resistance to these antimicrobials (Guardabassi, Schwarz, and Lloyd, 2004; Pedersen et al., 2007).

Multidrug and multiclass resistance, which can limit the effectiveness of several antimicrobials and reduce available treatment options, has been reported in generic *E. coli* isolates from dogs. This is concerning for both veterinary and human health due to the possible transmission of resistance genes between humans and dogs. In a United States study of multidrug resistance in dogs and cats recruited at veterinary clinics, almost 20% of collected isolates showed resistance to all seven antimicrobials that were tested, and 57% were resistant to multiple antimicrobials (Shaheen et al., 2010). These results show that multidrug resistance in companion animals is common and should be considered when monitoring antimicrobial resistance patterns in a population.

The risk factors for the carriage of antimicrobial resistant generic *E. coli* have not been thoroughly investigated, particularly in healthy household dogs and dogs that visit dog parks. Most studies focus on dogs at veterinary clinics or veterinary hospitals, which may over-estimate the prevalence of resistance that is actually present in the general population of dogs. Dogs in breeding kennels have also been reported to shed higher levels of antimicrobial resistant isolates (25%), compared to household dogs (12%) (De Graef et al., 2004; Harada et al., 2011). There is
a need to determine the prevalence of antimicrobial resistance in pet dogs that share a home with humans and who might visit people with immune-compromised systems. Studying dogs that visit dog parks may provide an indication of antimicrobial resistance in a typical population of pet dogs.

**Dog parks as a unique environment**

Dog parks provide a designated off-leash area, which provides a safe, controlled environment allowing dogs to play, socialize and exercise with other dogs, and provides people an opportunity to exercise and interact with other dog owners with similar interests (Lee, Shepley, and Huang, 2009; McCormack et al., 2011). These parks can also provide a designated green space in an otherwise urban environment, which encourages physical activity, enjoyment of nature, social interaction, and escape from everyday stressors for owners (McCormack et al., 2010).

In addition to the many benefits of dog parks, there are however, some negative aspects. With a large number of dogs socializing off-leash, there is a possibility of aggressive behaviour among the dogs. However, inter-dog aggression in parks may not be a significant risk. Shyan, Fortune, and King (2003) reported that less than 7% of dogs showed aggression toward other dogs less than 0.5% of the time, and no dogs inflicted wounds. It is also possible that there is a “self-policing” effect happening in the parks, where owners of aggressive dogs make an effort to keep their dogs away from other dogs to prevent confrontations or are asked not to return to the park. This could create a population bias, which minimizes the number of aggressive interactions and stressed animals. While there is a risk of aggression at dog parks, this risk appears to be relatively low.
Another negative aspect that is associated with dog parks is the introduction of bacteria to the park by owners who fail to remove their dog’s feces from the ground. Fecal bacteria can pose a health risk to dogs, their owners, other users of the park, and wildlife that may live in or near the park. With a group of dogs it may be difficult for owners to identify their own pet’s feces and some may be left on the park grounds. Furthermore, some owners may not remove their dog’s feces even if they can identify them, because they see feces as “natural” and “biodegradable” (Webley and Siviter, 2000; Wells, 2006). In addition to being an annoyance to other park users, dog feces left in a park can contribute to microbial populations in the park and surrounding areas, increasing the potential spread of pathogens and/or antimicrobial resistant organisms. These organisms can also contaminate the wildlife living in and around the park and bodies of water in the park, which can potentially spread these organisms over a larger area, affecting not only the users of the parks, but also other people and animals in the community.

Dogs that visit dog parks may represent a unique study population that has not been previously investigated in Canada. The interaction between dogs and humans, as well as the presence of wildlife and sometimes water at the parks presents a set of risk factors for various pathogens and health related outcomes that are distinctive in this population. In addition, health status does not directly determine inclusion in this population, as may be the case when dogs that visit clinics are studied. The dogs that attend dog parks are generally healthy, social, active dogs that may better represent the general population of dogs in a community.
Methods used to study dogs in the community

Observational studies are frequently used in epidemiology, particularly for large population studies as they provide an ethical, non-invasive way to collect data. In particular, cross-sectional studies provide a straightforward, effective way to collect a considerable amount of information about a population and generate hypotheses (Grimes and Schulz, 2002a; Dohoo, Martin, and Stryhn, 2009). A concern in using cross-sectional studies is that data on prevalence rather than incidence are collected. When interpreting the risk factors identified in cross-sectional studies, it is important to remember that they are associated with having the outcome, rather than acquiring the outcome. This may lead to the identification of factors that are associated with duration, rather than the acquisition of an outcome. This drawback needs to be considered, as causal inferences are not well supported using this study design (Grimes and Schulz, 2002a; Dohoo, Martin, and Stryhn, 2009).

In addition, with cross-sectional studies, the study population is often obtained purposively, which can create a selection bias, and limit the external validity of the study (Grimes and Schulz, 2002b; Dohoo, Martin, and Stryhn, 2009). A variety of risk factors can be explored in a cross-sectional study, which can be beneficial in generating hypotheses and identifying potential factors that are associated with the outcome. A drawback of this study design is that due to so many factors being examined, the analysis may be unfocused and there is a strong possibility of false associations by chance (i.e., type I errors) (Dohoo, Martin, and Stryhn, 2009).

While there are some limitations to using cross-sectional studies, it is a suitable method for studying a population of dogs that frequent dog parks. A cohort study, in which a population
with known exposures is followed through time until an outcome, is not feasible for this population, as it would not allow exploratory investigation of several potential risk factors, and it would require the following of participants for a long time until the outcome occurs, if it ever does. Similarly, a case-control study, in which participants with the outcome of interest are selected and previous exposures determined, would also not be suitable for this population. A case-control study would not be practical for this population as it would require follow-up after initial fecal sample testing which would require a large time commitment from participants.

The use of a survey to collect data has several strengths and weaknesses. It provides an effective, inexpensive method to collect data from a population with minimum time commitment from participants. At a dog park, the use of an in-person intercept survey ensures that anyone entering the park has an equal opportunity to participate in the study, and increases awareness about the study to users of dog parks. It also provides an opportunity for participants to ask questions if they do not understand any items on the survey, or are interested in more information about the study. By having a person administering the survey, the response rate, and the reliability and validity of the data may also be increased (Franklin and Walker, 2003). The in-person intercept survey method is an efficient way to obtain a suitable sample size to allow statistical analysis in a short amount of time, as compared to mail or phone surveys. It also ensures specific inclusion criteria, such as the use of a dog park, are met prior to completing the survey.

A limitation of the intercept survey is that people may feel that they are obliged to participate because there is no way to by-pass the people administering the survey. Also, with the use of people recording the answers participants provide, there is the possibility of recording
errors (Franklin and Walker, 2003). The use of multiple interviewers administering the survey could also have an effect on the quality and consistency of the responses. For example, the way an interviewer interprets a question may affect the way participants respond to the question, resulting in interviewer bias (Franklin and Walker, 2003). There is also the possibility that participants will respond in the way they think is socially responsible, especially with sensitive questions, which can bias the results.

To address the limitations of administering a cross-sectional survey, the study objectives should be specific and the study design should support the objectives (Grimes and Schulz, 2002). Interviewers should be trained and have thorough knowledge of the study and survey, not pressure potential participants to take part in the study, and be able to answer any questions they may have (Franklin and Walker, 2003). In addition, participants should be given assurances that all information collected is confidential (Franklin and Walker, 2003).

There have been few published studies that have investigated pathogens in canine feces in parks (Wright, 1982; Rinaldi et al., 2006; Wang et al., 2012). None of these studies collected feces from owners at a dog park, but rather from the ground at a park or collected by the owners at their home. Wright (1982) collected fecal samples from the ground in a public park in England. The history of the dog was not collected, and the length of time samples were on the ground could not be determined. This study provided an estimate of C. jejuni in canine feces, but could not investigate any pet-management risk factors and included many biases due to the method of fecal collection.

Similar to Wright (1982), canine fecal samples were collected from public areas in southern Italy and tested for parasites (Rinaldi et al. 2006). Again, these samples were not
collected directly from owners, but off the ground, so no pet-related risk factors could be examined. In addition, the samples were not all collected at parks, as some were collected from the street. The objectives of these two studies (Wright, 1982; Rinaldi et al., 2006) were to determine canine contamination in the environment, not to describe dogs that attend dog parks, so the ability to extrapolate the findings to this population is limited and should be done cautiously.

In contrast, Wang et al. (2012) collected canine feces and characteristics of dogs that were owned by veterinary medical students and staff at a veterinary hospital. Wang et al. (2012) examined the differences in parasite carriage between dogs that visited dog parks and those that did not. The samples were not collected at a park, but rather voluntarily submitted by participating owners. Information about pet-related risk factors was collected, but the study was not targeted specifically at dogs that visit dog parks.

**Study rationale and objectives**

The carriage of zoonotic pathogens and antimicrobial resistant bacteria in dogs may be described by various pet demographic and management related factors. The pet-related risk factors for the carriage of these organisms has been explored in relation to shelter dogs, household dogs, and dogs recruited at veterinary clinics and hospitals, but dogs that visit dog parks have not been well represented in the literature. Dog parks are an important environment to study due to their popularity and the opportunity for contact between humans, dogs, and wildlife. In order to address the need to focus on public health issues related to dogs that visit dog parks, the objectives for this study were as follows:
1. Characterize and describe the population of dogs that visit dog parks (Chapters 2, 3);
2. Determine the prevalence of zoonotic bacteria, particularly *Salmonella*, *Giardia*, and *Campylobacter* in dogs that visit dog parks (Chapter 2);
3. Assess associations between the prevalence of *Campylobacter* in dogs and various demographic and pet-management factors (Chapter 2);
4. Investigate the presence of antimicrobial resistance in commensal bacteria in dogs that visit dog parks (Chapter 3);
5. Assess associations between the prevalence of antimicrobial resistance in dogs and various demographic and pet-management factors (Chapter 3); and
6. Assess the clustering of shedding status of zoonoses and antimicrobial resistance by park that dogs visit (Chapters 2, 3).
References


Chapter 2:

A cross-sectional study examining the shedding of *Campylobacter* spp. and other zoonotic enteric pathogens in dogs that frequent dog parks in southern Ontario, and risk factors for shedding of *Campylobacter* spp.

(Procter et al. formatted for submission to Zoonoses and Public Health)

Abstract

There are an estimated 6.4 million pet dogs in Canadian households with the potential to transmit zoonotic pathogens to humans. Dogs have been identified as carriers of *Salmonella*, *Giardia*, and *Campylobacter* spp., particularly *C. upsaliensis*, but little is known about the prevalence and risk factors for these pathogens in pet dogs that visit dog parks. This study examined the prevalence of *Salmonella*, *Giardia*, and *Campylobacter* spp. in the feces of dogs visiting dog parks in southern Ontario, as well as risk factors for shedding *Campylobacter* spp. and *Campylobacter upsaliensis*. From May to August 2009, canine fecal samples were collected at ten dog parks in the cities of Guelph and Kitchener-Waterloo, Ontario, Canada. Owners were asked to complete a questionnaire related to pet characteristics and management factors including age, diet, exposure to livestock, and medical history of the dog, including treatment with antibiotics. Fecal samples were collected from 251 dogs and 189 questionnaires were completed. *Salmonella*, *Giardia*, and *Campylobacter* spp. were present in 1.2%, 6.4%, and 43.0% of fecal samples, respectively. Of the *Campylobacter* spp. detected, 86.1% were *C. upsaliensis*, 13% were *C. jejuni* and 0.9% *C. coli*. Statistically significant sparing factors associated with the shedding of *Campylobacter* spp. included the feeding of a commercial dry
diet and the dog’s exposure to compost. Age of dog had a quadratic effect, with young dogs and senior dogs having an increased probability of shedding *Campylobacter* spp. compared to adult dogs. The only statistically significant risk factor for shedding *C. upsaliensis* was outdoor water access, including lakes and ditches, while increasing age of the dog was a sparing factor. Based on the findings of this study, feeding commercial dry food, being exposed to compost and reducing outdoor water access may reduce the probability of shedding *Campylobacter* spp. by dogs that frequent dog parks.

**Introduction**

Approximately 32% of Canadian households have at least one dog, with an estimated population of more than 6 million pet dogs in Canada (Perrin, 2009). Sharing a home with an animal can be rewarding in many ways, but it can also pose a health risk, especially to the very young and old and those who are immune compromised; domestic dogs have been identified as potential reservoirs of zoonotic enteric pathogens, such as *Salmonella, Giardia,* and *Campylobacter* (Grøndalen, Sævik, and Sørum, 2008).

*Salmonella* and *Giardia* are associated with clinical signs including diarrhea and vomiting in both humans and dogs, but many infections in dogs are subclinical (Cantor et al., 1997; Bagcigil et al., 2007; Grøndalen, Sævik, and Sørum, 2008; Ballweber et al., 2010). *Giardia* is genetically separated into seven assemblages known as A-G. Humans are only infected with assemblages A and B, and dogs primarily with C and D; however, dogs have also been reported to be infected with assemblages A and B and therefore pose a zoonotic risk (Xiao and Fayer, 2008; Ballweber et al., 2010). Due to possible sub-clinical infections in dogs while
shedding, and the requirement to remove dog feces from public areas, humans may be exposed to *Salmonella* and *Giardia* without being aware of the risk.

Campylobacteriosis has been identified as the most common cause of bacterial enteritis in people in Canada (Government of Canada, 2009). Campylobacteriosis is characterized by acute diarrhea, vomiting, fever and intense abdominal pain, lasting for 3-15 days (Nachmkin, Szymanski, and Blaser, 2008). Following infection, there is also a risk of chronic conditions such as colitis, nephritis, reactive arthritis, and Guillain-Barré syndrome (Bourke et al., 1998; Nachmkin, Szymanski, and Blaser, 2008). Dogs may experience gastro-intestinal signs similar to humans, such as diarrhea, but can also shed *Campylobacter* spp. without showing any signs (Grøndalen, Sævik, and Sørum, 2008). Routes of exposure to *Campylobacter* include fecal-oral, ingestion of contaminated food or water and consumption of raw meat, particularly poultry. Cats and dogs have also been identified as a potential source of exposure of *Campylobacter* spp. to humans (Government of Canada, 2007).

The most commonly identified species of *Campylobacter* infecting humans in Canada is *Campylobacter jejuni*, followed distantly by *C. coli* and *C. lari* (Government of Canada, 2009). The catalase-negative species *Campylobacter upsaliensis* is not recovered as often as *C. jejuni* from humans, however it is possible that available data under-estimate the prevalence of this species. Public health laboratories focus on thermotolerant strains, which may miss *C. upsaliensis*, and may not attempt to speciate catalase-negative species. In addition, standard laboratory methods used to isolate *Campylobacter* spp. can hinder the recovery of catalase-negative strains, particularly *C. upsaliensis* (Labarca et al., 2002; Lastovica and Le Roux, 2003); the use of a broth that contains cephalothin, to which *C. upsaliensis* has been shown to be
susceptible, as well as a shorter incubation time, have been noted as possible factors that might result in under-detection of this species (Lastovica and Le Roux, 2003).

*Campylobacter upsaliensis* is frequently recovered from clinically normal companion animals, with dogs and cats believed to be the reservoir species for this organism (Workman et al., 2005). In previous studies, the prevalence of *Campylobacter* spp. in the feces of domestic dogs has ranged from 22-87%, with 19-99% of those being *C. upsaliensis* (e.g., Sandberg et al., 2002; Wieland et al., 2005; Westgarth et al., 2009; Parsons et al., 2010; Leonard et al., 2011a.) This is a concern as the shedding of this pathogen by dogs may pose a risk for human health (Bourke et al., 1998; Government of Canada, 2007).

Dogs that attend dog parks may represent a different population from those previously studied as they may have off-leash contact with other dogs or their feces, humans other than their owners, and possibly wildlife, depending on the type of park visited. Furthermore, infected dogs that visit public parks have more opportunities to expose other dogs, as well as humans, to zoonotic bacteria that are shed in their feces.

As part of a larger study examining the prevalence of, and risk factors for, gastrointestinal organisms in dogs, this study focused on determining the prevalence of shedding of zoonotic organisms including *Salmonella, Giardia*, and particularly *Campylobacter* spp. and *C. upsaliensis*. The study also investigated the possibility of the clustering of shedding of *Campylobacter* spp. by park and date of visit. Pet characteristics and management factors that may impact the likelihood of shedding of *Campylobacter* spp. in dogs, such as age, diet, and medical history were also investigated.
Methods

Collection of Fecal Samples

Thirteen off-leash dog parks in the Guelph and Kitchener-Waterloo, Ontario, Canada area were identified using internet searches and personal correspondence. Parks were visited at least three times over the collection period for two to three hours each time either in the morning or afternoon between May and August 2009. Parks where few or no participants were recruited, usually due to limited activity, were not visited again. Dog owners were approached and asked to participate in the study and provide the researcher, one of two interviewers, with feces collected from their dog while in the park. Each submitted fecal sample was given a unique identification number for each dog, and placed into a specimen container for testing of *Salmonella* and *Giardia* and a CultureSwab™ Cary-Blair Agar swab (BD, Franklin Lakes, New Jersey) was immediately inserted into the fecal sample for *Campylobacter* recovery. The sample was stored in a cooler until submitted to the Canadian Research Institute for Food Safety (CRIFS) and Animal Health Laboratory, University of Guelph (AHL) for processing. This study was approved by the University of Guelph’s Research Ethics Board.

Questionnaire

A questionnaire adapted from one previously used by Leonard et al. (2011a) was administered to owners at the time of sample submission. Questions were asked relating to pet management factors concerning the dog’s main diet, presence of other animals in the home, the dog’s previous illnesses or treatments, veterinary care, and human illness in the home over the previous 30 days. Data on breed, age, sex and neuter status were collected for all dogs with a
submitted fecal sample. The complete questionnaire is available upon request from the authors (Appendix I).

**Laboratory Analysis**

*Salmonella* culture was performed as previously reported by Leonard et al. (2011b) using an enrichment followed by selective plating method and further characterized for specific serotypes at the Laboratory for Foodborne Zoonoses, Guelph. Fecal samples were tested for *Giardia* antigen using a SNAP test (IDEXX Laboratories, Westbrook, Maine). Positive samples were typed by PCR as described previously (McDowall et al., 2011).

*Campylobacter* spp. testing was carried out using the method reported in Leonard et al. (2011a). In brief, direct plating onto modified cefoperazone charcoal deoxyylate agar (mCCDA) and a Bolton broth enrichment using a dilutions procedure was used. Both the plates and the broth were incubated at 42°C for 48 hours in microaerophilic conditions. Suspect colonies were tested for oxygen tolerance, growth at 25°C, gram stain, and dark field microscopy. Typical *Campylobacter* spp. colonies were confirmed using catalase, oxidase, oxygen tolerance and microscopy tests. Hippurate and indoxyl acetate hydrolysis tests, and cephalothin susceptibility tests, were performed to speciate suspected *Campylobacter* into *C. jejuni*, *C. coli* and *C. lari*. All *Campylobacter* isolates were frozen in glycerol at -70°C to allow for future molecular typing. Catalase-negative *Campylobacter* spp. were speciated using a series of polymerase chain reaction (PCR) assays targeting the 16S rRNA encoding genes, as previously described (Leonard et al., 2011a). Catalase-positive cultures were identified using PCR methods previously described (Denis et al., 1999). A second PCR method was used to identify *C. lari* if the PCR methods described above were negative for both *C. jejuni* and *C. coli* (Linton et al., 1996).
Finally, catalase-negative isolates were identified using a previously described PCR method for *C. upsaliensis* and *C. helveticus* (Linton et al., 1996). The primers and targets used for *Campylobacter* spp. identification have been described in previous publications (Linton et al., 1996; Denis et al., 1999).

**Statistical Analysis**

Statistical analyses were performed using Stata Intercooled 10.1 (StataCorp LP, College Station, Texas). Descriptive statistics, such as prevalence, frequency and mean, were performed for each variable examined and pathogen tested. The association between continuous variables was evaluated for colinearity using Spearman rank correlations using a cut-point of 0.7, with the most biologically meaningful variable selected for further analysis. Linearity was assessed between the log odds of *Campylobacter* spp. or *C. upsaliensis* and continuous variables using locally weighted regression (lowess) curves. If not linear, a quadratic term was added; if the addition of the quadratic term was not significant, the variable was categorized. To identify potential risk factors for shedding *Campylobacter* spp. or *C. upsaliensis*, univariable logistic regression was initially performed on all variables where there were at least five responses in each category for that variable. *Campylobacter* spp. and *C. upsaliensis* were considered as separate outcomes.

Multivariable models for each outcome were created using the variables identified as significant at $\alpha \leq 0.20$ in univariable logistic regression. Each variable was tested for statistical significance ($\alpha \leq 0.05$) in the model using manual backward selection with likelihood ratio tests. Confounders were identified as non-intervening variables that changed coefficients of significant variables by $\geq 20\%$, and were forced into the model. Once the model was complete, all possible
pair-wise interactions between the remaining variables in the model were assessed by likelihood ratio tests.

When the multivariable models, including interactions if appropriate, were generated, quadrature techniques were used to create models using either the park visited or a combination of park and date of visit as a random effect to determine if there was any evidence of clustering in the data. Akaike’s information criteria (AIC) and Bayesian information criteria (BIC) were used to assess whether the random effects model or regular logistic model fit the data best.

If the multi-level model fit the data better, best linear unbiased predictors (BLUPs) were evaluated to assess model fit. If the logistic regression model fit the data better, Pearson residuals were examined visually to assess the fit of the model. In addition, a Hosmer-Lemeshow goodness-of-fit test was performed. A $\alpha$-value $\leq 0.05$ for the goodness-of-fit test indicated the model did not fit the data (Dohoo, Martin, and Stryhn, 2009).

**Results**

*Collection of Samples*

Of the 13 dog parks identified and visited from May to August, 2009, ten resulted in the recruitment of study participants. Between 1-90 samples were collected from each park; more than 25 samples were collected from each of five parks, with less than 10 samples from each of the remaining five parks. Fecal samples were obtained from a total of 251 dogs; 189 surveys were completed, each representing an individual dog.
Laboratory Analysis

The prevalence of *Campylobacter* spp. was 43% and catalase negative *C. upsaliensis* was 37% (Table 2.1). The prevalence of both *Salmonella* (1%) and *Giardia* (6%) were low, and thus these pathogens were not considered as outcomes for risk factor analysis, but were considered as potential risk factors in the *Campylobacter* models. *Salmonella* serotypes identified were Enteritidis (n=1), Mbandaka (n=1), and Thompson (n=1). *Giardia* was identified in 16 samples using the SNAP test (IDEXX Laboratories, Westbrook, Maine); eight were able to be typed using the small subunit ribosomal RNA PCR method, three of those were assemblage C, and five assemblage D. Using the β-gardin PCR, only two of the 16 samples were able to be typed, both of which were assemblage D.

Statistical Analysis

Descriptive statistics for individual variables and univariable associations with presence of *Campylobacter* spp. and *C. upsaliensis* that were significant at α<0.20 and considered for inclusion in the multivariable models are shown in Table 2.2. The results of the univariable analyses were similar for *Campylobacter* spp. and *Campylobacter upsaliensis*.

A high correlation was found between age and time with the same diet. Time with diet was therefore excluded as a risk factor from the models, as age has more biological meaning and had fewer missing values. A high correlation was also present between the age of the dog and amount of time owned; age was included for evaluation in the multivariable model as age was a more plausible risk factor for shedding *Campylobacter* spp. in dogs.
The relation between age of the dog and the log odds of *Campylobacter* spp. and *C. upsaliensis* showed a curvilinear relationship based on the lowess curve so a quadratic term for age was included in the multivariable model. The quadratic term for age was not significant when the outcome was *C. upsaliensis*. Therefore, age was categorized into young (dogs <1 year old), and adult (dogs > 1 year old) dogs.

In the final *Campylobacter* spp. multivariable model, consumption of a commercial dry diet, and exposure to compost were negatively associated with *Campylobacter* spp. shedding (Table 2.3). Age was modelled with a quadratic term, with young dogs having a high likelihood of shedding *Campylobacter* spp., with the likelihood of shedding declining with age, but increasing for dogs in their senior years (Figure 2.1). Significant interactions were not found between age and consumption of a commercial dry diet (p=0.77), age and exposure to compost (p=0.16), nor consumption of a commercial dry diet and exposure to compost (p=0.99). In the multivariable model for shedding of *C. upsaliensis*, positive associations were found between dogs being young and having access to outdoor water, such as lakes and ditches for swimming or drinking (Table 2.4). No significant interaction was found between age (young vs. old) and outdoor water access (p=0.20). There was no evidence of clustering by park or visit (park and date of visit) in either the Campylobacter spp. or the *C. upsaliensis* models, as assessed by evaluating the variance component associated with the random effect and the results of a likelihood ratio test. In addition, information criteria (AIC and BIC) indicated that there was no substantial improvement in model fit with the inclusion of either random effect (Table 2.5).

Pearson residuals from both multivariable models were examined for outliers and influential covariate patterns. There were a few observations with large residuals, which were
examined for errors in data entry; since there were no errors, all observations were retained in the models. The Hosmer-Lemeshow tests performed to assess the fit of the *Campylobacter* spp. and *C. upsaliensis* models were not significant (p=0.19 and p=0.20, respectively), indicating that the models fit the data.

**Discussion**

In this study we investigated the prevalence of selected zoonotic pathogens (*Salmonella*, *Giardia*, and *Campylobacter*), and pet-related risk factors for *Campylobacter* spp. and *C. upsaliensis* shedding in dogs that visited dog parks in southern Ontario. Previous studies have reported *Salmonella* prevalence in healthy dogs ranging from 1-4% (Cantor et al., 1997; Hackett and Lappin, 2003), consistent with the prevalence observed in this study (1%).

The prevalence for *Giardia* (6%) found in this study is consistent with other studies that have reported a prevalence of 5-9% (Jacobs, Forrester, and Yang, 2001; Hackett and Lappin, 2003; Mircean et al., 2012; Wang et al., 2012). Interestingly, Wang et al. (2012) found that dogs that visited dog parks in Colorado had a higher probability of shedding *Giardia* compared to dogs that did not visit dog parks. In the current study, the majority of the assemblages were type D, with a small number of type C. Some of the samples could not be typed, although the results were similar to McDowall et al. (2011) in southern Ontario, where 99% of the typeable samples were not zoonotic. As humans are typically only infected with assemblages A or B (Ballweber et al., 2010), the zoonotic risk of *Giardia* from the dogs in this study appeared to be very low, although a limited number of samples were typed.
Previous canine prevalence studies on *Campylobacter* have been completed mostly in Europe, on household or shelter dogs. In this study, 43% of fecal samples tested positive for *Campylobacter* spp. This is comparable to similar studies of household dogs, which have reported prevalences ranging from 22%-75% (e.g., Sandberg et al., 2002; Wieland et al., 2005; Westgarth et al., 2009; Parsons et al., 2010; Leonard et al, 2011a). Leonard et al. (2011a) found a prevalence of *Campylobacter* spp. of 22% (95% CI: 17 -27%) in the same study area as the current study. The difference in prevalence may be due to the type of animals examined. Leonard et al. (2011a) investigated dogs that visited one of several veterinary clinics over a full year, which were reported to be more likely to be kept on a leash and less likely to visit dog parks compared to dogs from this study, and may therefore differ from dogs that visit dog parks during the summer months.

All samples in this study were collected during the summer months (May to August). Wright (1982) found that canine fecal samples collected off the ground in a public park in the United Kingdom during the summer months, particularly June and July, comprised 75% of the *Campylobacter* spp. detected in the feces collected over the twelve months of the study. More recently, Sandberg et al. (2002) reported increased probability of dogs at small animal clinics in Norway testing positive for *Campylobacter* spp. when they were tested between May and September, compared to the rest of the year. This could be due to increased time spent outside potentially exposing them to other dogs and wildlife. As a result of this seasonality effect, the prevalence of *Campylobacter* spp. detected in this study provides only an estimate of prevalence of *Campylobacter* spp. in this population for the summer months. Interestingly, Leonard et al. (2011a) studied a population of dogs in the same study area that visited clinics over a full year, but did not find a seasonal effect. However, this could have been due to the lack of outdoor
exposure that these dogs were allowed, reducing external sources for *Campylobacter* colonization. A future study should investigate the prevalence of *Campylobacter* spp. in dogs that go to dog parks over an entire year to get a clearer picture of the potential risk of *Campylobacter* spp. exposure to humans at different times of the year and the variability of risk factors across time.

A number of the pet-related demographic and management factors associated with the shedding of *Campylobacter* spp. found in this study were consistent with previous studies, such as age (Hald et al., 2004; Wieland et al., 2005; Acke et al., 2006; Westgarth et al., 2009; Parsons et al., 2010; Leonard et al., 2011a) and diet (Leonard et al., 2011a). Studies have shown that younger animals are more likely to shed *Campylobacter* spp. (Hald et al., 2004; Wieland et al., 2005; Acke et al., 2006; Westgarth et al., 2009; Parsons et al., 2010; Leonard et al., 2011a), as they may be more susceptible to colonization due to a naïve immune system or the stress of being introduced to a new home. Age modelled as a quadratic term, highlighting the risk of shedding *Campylobacter* spp. in senior dogs, is consistent with the reported decline of immune function as dogs reach their senior years (Kil et al., 2010).

A high correlation between age and time of dog ownership was observed, both of which were significant in univariable analysis, preventing the further exploration of the effect of length of time a dog had been in its current home on shedding *Campylobacter* spp. The time an animal had been on its current diet was also related to the age of the dog, which may affect gastrointestinal microbial populations. While the time an animal had been on its current diet could not be examined in this study, Stavisky et al. (2011) reported that a change of diet was
associated with an increased risk of diarrhea, suggesting that a new diet may disrupt the balance of the gastrointestinal system.

The consumption of a commercial dry diet was found to have an inverse association with the risk of *Campylobacter* spp. shedding. This may be the result of the dogs not consuming a homemade or raw diet, which has been found to increase both the probability of *Campylobacter* spp. shedding, as well as the probability of diarrhea (Finley et al., 2008; Leonard et al., 2011a; Stavisky et al., 2011). It could also be a reflection of the production processes in place for commercial dry diets which would eliminate the presence of any *Campylobacter* spp. if there were to be any in the raw ingredients of these pet food products.

This study identified access to outdoor water sources (e.g., lakes, rivers, ditches and puddles) as a risk factor for shedding *C. upsaliensis*. Evidence of *Campylobacter* spp. in river water and waterfowl in southern Ontario (Van Dyke et al., 2010), suggests that water could be a source of *Campylobacter* spp. exposure for both humans and animals. However, the *Campylobacter* spp. identified in the water in the Van Dyke (2010) study were not speciated. It is possible these water sources could become contaminated from waterfowl, as well as other wild animals such as squirrels and raccoons, or pets, including dogs that come into contact with this water (Van Dyke et al., 2010; Lee et al., 2011; Mandrell et al., 2011). There was water access at six of the ten parks where fecal samples were collected; however, the non-significance of the random effects suggests little evidence of clustering at the parks. In addition, the questionnaire did not differentiate as to where the dogs had access to water.

The decreased probability of shedding *Campylobacter* spp. associated with exposure to compost has not previously been reported. It is unclear why this relationship exists, and may be a
result of compost acting as a proxy variable for a factor that was not examined in this study. Type of housing, such as apartment or farm, as well as education and socio-economic status may be variables not examined that may influence the probability of owners having compost. Exposure to compost may expose dogs to other bacterial organisms which may act as probiotics and prevent the colonization of the gastro-intestinal tract with pathogens like *Campylobacter* (Cutting, 2011). It was not specified in the questionnaire if the dog actually consumed compost, or simply had access to it; therefore, while it is possible that compost has a protective effect against shedding of *Campylobacter* spp. in dogs, this relationship requires further study.

The differences between the risk factors identified for the two outcomes may indicate an underlying difference between the epidemiology of generic *Campylobacter* spp. and *C. upsaliensis*, as has been suggested by the different risk factor models that have been developed (Leonard et al., 2011a, Wieland et al., 2005). It is difficult to determine if there was a difference with these data, however. There were few *C. jejuni* and *C. coli* isolates recovered, thus a separate model could not be constructed for these species. In addition, subtypes were mixed in the *Campylobacter* spp. model, whereas the outcome was much more specific in the *C. upsaliensis* model.

This cross-sectional study provides a general description of the canine population that visits dog parks in the Guelph and tri-cities (Kitchener-Waterloo, Cambridge) in southern Ontario. The prevalence and identified risk factors for *Campylobacter* spp. and *C. upsaliensis* shedding were similar to other studies from North America and Europe (Sandberg et al., 2002; Hald et al., 2004; Wieland et al., 2005; Acke et al., 2006; Westgarth et al., 2009; Parsons et al., 2010; Leonard et al., 2011a). Exposure to compost as a sparing factor, as well as access to
outdoor water sources were newly identified factors contributing to the probability of shedding *Campylobacter* spp. in domestic dogs. It was important to characterize this population to determine if there were different risk factors that are unique to this population, as well as to determine the risk to humans from this population of dogs.

The high prevalence of *Campylobacter* spp., particularly *C. upsaliensis*, which was found in this population of dogs, indicates that this population is a potential source of exposure to humans. Consequently, more research and surveillance on catalase-negative species in humans is required to determine the burden of *C. upsaliensis* in the Canadian population. Further research into the risk factors and sources of exposure are necessary to fully understand the epidemiology of *Campylobacter* spp. as well as to develop management and control programs that can be used to reduce the risk of transmission of zoonotic pathogens between dogs and humans.
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Xiao, L, and R Fayer 2008: Molecular characterisation of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission. *Int. J. Parasitol.* 38, 1239-1255
Table 2.1. Prevalence of organisms present in 251 dog fecal samples collected from dog parks in southern Ontario in the summer of 2009, and the results of examining the association between *Giardia* and *Salmonella* and the presence of *Campylobacter* spp. or *Campylobacter upsaliensis* in dog fecal samples.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Number of positive samples</th>
<th>% of total (95% CI)</th>
<th>Campylobacter spp. p-value (LR-Test)</th>
<th>OR (95% CI)</th>
<th>C. upsaliensis p-value (LR-Test)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catalase Positive (C. jejuni, C. coli)</td>
<td>108</td>
<td>43.03 (36.86-49.20)</td>
<td>5.98 (3.02-8.93)</td>
<td></td>
<td>37.05 (31.04-43.07)</td>
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</tr>
<tr>
<td>Catalase Negative (C. upsaliensis)</td>
<td>15 (14,1)</td>
<td>93</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Giardia</em></td>
<td>16</td>
<td>6.37 (3.33-9.42)</td>
<td>0.11</td>
<td>2.33 (0.82-6.62)</td>
<td>0.57</td>
<td>1.35 (0.48-3.75)</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>3</td>
<td>1.20 (0-2.55)</td>
<td>0.26</td>
<td>0.34 (0-3.20)</td>
<td>0.30</td>
<td>0.44 (0-4.11)</td>
</tr>
</tbody>
</table>

*CI: confidence interval; LR-Test: likelihood ratio test; OR: odds ratio*
Table 2.2. Survey responses of all variables and univariable logistic regression analysis for the presence of *Campylobacter* spp. or *C. upsaliensis* in dog fecal samples collected from dog parks in southern Ontario in the summer of 2009.

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Overall p (LR-Test)</th>
<th>OR (95% CI)</th>
<th>Overall p (LR-Test)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Park</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>38</td>
<td>0.2960 Ref</td>
<td></td>
<td>0.2573 Ref</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>--</td>
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<tr>
<td>C</td>
<td>2</td>
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<td></td>
</tr>
<tr>
<td>D</td>
<td>0</td>
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<td></td>
<td>--</td>
<td></td>
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<tr>
<td>E</td>
<td>90</td>
<td>1.08 (0.50-2.32)</td>
<td>1.02 (0.47-2.22)</td>
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<td>F</td>
<td>41</td>
<td>0.79 (0.32-1.94)</td>
<td>0.88 (0.36-2.20)</td>
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<td>G</td>
<td>0</td>
<td>--</td>
<td></td>
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</tr>
<tr>
<td>H</td>
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<tr>
<td>I</td>
<td>39</td>
<td>1.05 (0.43-2.60)</td>
<td>0.77 (0.30-1.94)</td>
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<td>J</td>
<td>29</td>
<td>0.47 (0.17-1.33)</td>
<td>0.49 (0.17-1.42)</td>
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<td>K</td>
<td>8</td>
<td>3.71 (0.66-20.76)</td>
<td>4.60 (0.82-25.88)</td>
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<tr>
<td>L</td>
<td>0</td>
<td>--</td>
<td></td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>2</td>
<td>1.23 (0.07-21.24)</td>
<td>1.53 (0.09-26.43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Park location:</td>
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<tr>
<td>Guelph</td>
<td>125</td>
<td>0.4776 Ref</td>
<td>0.5451 Ref</td>
<td>0.85 (0.51-1.43)</td>
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</tr>
<tr>
<td>Outside Guelph (K-W, Cambridge)</td>
<td>126</td>
<td>Ref</td>
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<td>Month of collection:</td>
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<tr>
<td>May</td>
<td>54</td>
<td>0.1029 Ref</td>
<td>0.2993 Ref</td>
<td></td>
<td></td>
</tr>
<tr>
<td>June</td>
<td>53</td>
<td>0.71 (0.32-1.52)</td>
<td>0.74 (0.33-1.64)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>July</td>
<td>68</td>
<td>0.52 (0.24-1.08)</td>
<td>0.70 (0.33-1.49)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>August</td>
<td>76</td>
<td>1.14 (0.56-2.28)</td>
<td>1.27 (0.63-2.59)</td>
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</tr>
<tr>
<td>Breed:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large Mix^a</td>
<td>76</td>
<td>0.4356 Ref</td>
<td>0.1228 Ref</td>
<td></td>
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</tr>
<tr>
<td>Large Pure</td>
<td>116</td>
<td>0.83 (0.46-1.48)</td>
<td>0.64 (0.35-1.17)</td>
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<tr>
<td>Small Mix</td>
<td>28</td>
<td>0.55 (0.22-1.38)</td>
<td>0.55 (0.21-1.40)</td>
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<tr>
<td>Small Pure</td>
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<td>1.47 (0.36-3.39)</td>
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<tr>
<td>Gender of Owner: Female</td>
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<td>0.8771 Ref</td>
<td>0.8914 Ref</td>
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<tr>
<td>Male</td>
<td>85</td>
<td>0.96 (0.57-1.63)</td>
<td>0.96 (0.56-1.66)</td>
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<tr>
<td>Owner Age:</td>
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<tr>
<td>19&lt;30</td>
<td>50</td>
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<td>0.3458 Ref</td>
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<tr>
<td>30&lt;60</td>
<td>160</td>
<td>0.68 (0.36-1.29)</td>
<td>0.74 (0.39-1.42)</td>
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<tr>
<td>&gt;60</td>
<td>38</td>
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<td>0.52 (0.21-1.27)</td>
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<td>Gender of Dog: Female</td>
<td>105</td>
<td>0.4556 Ref</td>
<td>0.5581 Ref</td>
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<tr>
<td>Male</td>
<td>144</td>
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<td>0.86 (0.51-1.44)</td>
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<tr>
<td>Variable</td>
<td>N</td>
<td>Overall p (LR-Test)</td>
<td>OR (95% CI)</td>
<td>Overall p (LR-Test)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>-----</td>
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<tr>
<td><strong>Campylobacter spp.</strong></td>
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<tr>
<td>Age of Dog: Mean (SE)</td>
<td>3.98 (0.23)</td>
<td>0.0003</td>
<td>0.87 (0.80-0.94)</td>
<td>0.0001</td>
<td>0.84 (0.77-0.92)</td>
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<td>Range</td>
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<tr>
<td>Neutered: Yes</td>
<td>197</td>
<td>0.0229</td>
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<td>0.1156</td>
<td>0.59 (0.31-1.13)</td>
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<td>No</td>
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<tr>
<td>Years dog owned: Mean (SE)</td>
<td>3.19 (0.23)</td>
<td>0.0027</td>
<td>0.86 (0.78-0.96)</td>
<td>0.0003</td>
<td>0.82 (0.73-0.93)</td>
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<td>Range</td>
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<td>Breeder</td>
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<td>Ref</td>
<td>0.1937</td>
<td>Ref</td>
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<td>Humane Society</td>
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<td>0.64 (0.16-2.53)</td>
<td>1.86 (0.96-3.59)</td>
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<td>0.50 (0.13-1.94)</td>
<td>1.81 (0.95-3.44)</td>
<td>0.59 (0.31-1.13)</td>
<td>1.13 (0.56-2.28)</td>
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<tr>
<td>Other</td>
<td>69</td>
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<tr>
<td>Number of dogs in house: Mean (SE)</td>
<td>1.33 (0.04)</td>
<td>0.6490</td>
<td>0.90 (0.56-1.44)</td>
<td>0.9928</td>
<td>1.00 (0.62-1.62)</td>
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<td>Median</td>
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<tr>
<td>Range</td>
<td>1-5</td>
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<tr>
<td>Number of other animals in house: Mean (SE)</td>
<td>0.75 (0.08)</td>
<td>0.4374</td>
<td>1.11 (0.85-1.44)</td>
<td>0.2331</td>
<td>1.18 (0.90-1.53)</td>
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<td>Median</td>
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<td>Range</td>
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<td>Visited veterinarian in the past year: Yes</td>
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<td>Dewormed in past 6 months: Yes</td>
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<td>No (Ref)</td>
<td>57</td>
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<tr>
<td>Treated with anything in past month: Yes</td>
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<td>1.02 (0.45-2.31)</td>
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<td>Treated with other meds/supplements: Yes</td>
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<td>Dog diarrhea in past month: Yes</td>
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<td>1.04 (0.50-2.19)</td>
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<td>Dog vomiting in past month: Yes</td>
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<tr>
<td>Dog gastrointestinal upset in past month (diarrhea and/or vomiting): Yes</td>
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<td>OR (95% CI)</td>
<td>Overall p (LR-Test)</td>
<td>OR (95% CI)</td>
</tr>
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<td>---------------------</td>
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</tr>
<tr>
<td>Human diarrhea in past month: Yes</td>
<td>13</td>
<td>0.1787</td>
<td>2.18 (0.69-6.94)</td>
<td>0.2073</td>
<td>2.07 (0.67-6.44)</td>
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<td>175</td>
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<tr>
<td>Human vomiting in past month: Yes</td>
<td>9</td>
<td>0.1537</td>
<td>2.71 (0.66-11.18)</td>
<td>0.0667</td>
<td>3.59 (0.87-14.86)</td>
</tr>
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<td>179</td>
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<td>Human gastro-intestinal upset in past month (diarrhea and/or vomiting): Yes</td>
<td>14</td>
<td>0.1060</td>
<td>2.49 (0.80-7.74)</td>
<td>0.1163</td>
<td>2.41 (0.80-7.26)</td>
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<td>174</td>
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<tr>
<td>Hunt and catch or eat any prey: Yes</td>
<td>25</td>
<td>0.3486</td>
<td>1.50 (0.64-3.48)</td>
<td>0.4335</td>
<td>1.41 (0.60-3.30)</td>
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<td></td>
<td>162</td>
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<td></td>
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<tr>
<td>Hunt and catch or eat rodents: Yes</td>
<td>16</td>
<td>0.2630</td>
<td>1.80 (0.64-5.06)</td>
<td>0.2454</td>
<td>1.85 (0.66-5.17)</td>
</tr>
<tr>
<td></td>
<td>168</td>
<td></td>
<td></td>
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<tr>
<td>Hunt and catch or eat birds: Yes</td>
<td>9</td>
<td>0.5459</td>
<td>0.65 (0.16-2.69)</td>
<td>0.3468</td>
<td>0.48 (0.10-2.40)</td>
</tr>
<tr>
<td></td>
<td>175</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attend dog day care: Yes</td>
<td>11</td>
<td>0.1642</td>
<td>2.40 (0.68-8.51)</td>
<td>0.0655</td>
<td>3.19 (0.90-11.33)</td>
</tr>
<tr>
<td></td>
<td>178</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participation in obedience/agility/flyball: Yes</td>
<td>31</td>
<td>0.1612</td>
<td>1.74 (0.80-3.77)</td>
<td>0.0701</td>
<td>2.05 (0.94-4.47)</td>
</tr>
<tr>
<td></td>
<td>158</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participation in therapy/education programs: Yes</td>
<td>8</td>
<td>0.7298</td>
<td>0.77 (0.18-3.34)</td>
<td>0.4587</td>
<td>0.55 (0.11-2.82)</td>
</tr>
<tr>
<td></td>
<td>181</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kennelled/hospitalized in last 6 months: Yes</td>
<td>33</td>
<td>0.9022</td>
<td>0.95 (0.45-2.04)</td>
<td>0.3724</td>
<td>0.70 (0.31-1.56)</td>
</tr>
<tr>
<td></td>
<td>156</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Access to livestock: Yes</td>
<td>17</td>
<td>0.4070</td>
<td>1.53 (0.56-4.14)</td>
<td>0.3760</td>
<td>1.58 (0.58-4.30)</td>
</tr>
<tr>
<td></td>
<td>172</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Access to outdoor water source (lakes, ditches, etc.): Yes</td>
<td>128</td>
<td>0.0498</td>
<td>1.88 (0.99-3.56)</td>
<td>0.0156</td>
<td>2.26 (1.14-4.47)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Access to lakes, rivers, creeks: Yes</td>
<td>117</td>
<td>0.0689</td>
<td>1.75 (0.95-3.21)</td>
<td>0.0432</td>
<td>1.90 (1.01-3.59)</td>
</tr>
<tr>
<td></td>
<td>71</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Access to ditches, puddles: Yes</td>
<td>92</td>
<td>0.3980</td>
<td>1.28 (0.72-2.29)</td>
<td>0.1518</td>
<td>1.54 (0.85-2.80)</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Access to toilets: Yes</td>
<td>36</td>
<td>0.2194</td>
<td>1.58 (0.76-3.27)</td>
<td>0.3236</td>
<td>1.45 (0.70-3.03)</td>
</tr>
<tr>
<td></td>
<td>152</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposure to garbage: Yes</td>
<td>26</td>
<td>0.9866</td>
<td>0.99 (0.43-2.30)</td>
<td>0.7564</td>
<td>1.15 (0.49-2.70)</td>
</tr>
<tr>
<td></td>
<td>153</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposure to plates/bowls: Yes</td>
<td>71</td>
<td>0.7917</td>
<td>1.09 (0.59-1.99)</td>
<td>0.8448</td>
<td>1.06 (0.57-1.99)</td>
</tr>
<tr>
<td></td>
<td>108</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variable</td>
<td>N</td>
<td>Campylobacter spp.</td>
<td></td>
<td>C. upsaliensis</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>-------</td>
<td>--------------------</td>
<td>---</td>
<td>----------------</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>Overall p (LR-Test)</td>
<td>OR (95% CI)</td>
<td>Overall p (LR-Test)</td>
<td>OR (95% CI)</td>
<td></td>
</tr>
<tr>
<td>Exposure to compost: Yes</td>
<td>0.1158</td>
<td>0.47 (0.17-1.25)</td>
<td>0.3667</td>
<td>0.64 (0.24-1.73)</td>
<td></td>
</tr>
<tr>
<td>No (Ref)</td>
<td>0.9686</td>
<td>1.02 (0.41-2.56)</td>
<td>0.8124</td>
<td>1.12 (0.44-2.87)</td>
<td></td>
</tr>
<tr>
<td>Exposure to dead animals: Yes</td>
<td>0.0267</td>
<td>5.12 (1.03-25.40)</td>
<td>0.5820</td>
<td>1.47 (0.38-5.67)</td>
<td></td>
</tr>
<tr>
<td>No (Ref)</td>
<td>0.4713</td>
<td>1.41 (0.56-3.58)</td>
<td>0.9404</td>
<td>0.96 (0.36-2.55)</td>
<td></td>
</tr>
<tr>
<td>Exposure to animal feces (not litter): Yes</td>
<td>0.0178</td>
<td>0.12 (0.01-1.01)</td>
<td>0.0597</td>
<td>0.22 (0.04-1.18)</td>
<td></td>
</tr>
<tr>
<td>No (Ref)</td>
<td>0.6933</td>
<td>1.17 (0.54-2.56)</td>
<td>0.9634</td>
<td>0.98 (0.44-2.20)</td>
<td></td>
</tr>
<tr>
<td>Homemade cooked diet: Yes</td>
<td>0.0733</td>
<td>2.07 (0.93-4.62)</td>
<td>0.3497</td>
<td>1.47 (0.66-3.27)</td>
<td></td>
</tr>
<tr>
<td>No (Ref)</td>
<td>0.0011</td>
<td>0.34 (0.18-0.66)</td>
<td>0.0046</td>
<td>0.40 (0.21-0.75)</td>
<td></td>
</tr>
<tr>
<td>Time with diet: &lt;1 year (Ref)</td>
<td>0.6959</td>
<td>1.15 (0.57-2.34)</td>
<td>0.5645</td>
<td>1.24 (0.60-2.54)</td>
<td></td>
</tr>
<tr>
<td>&gt;1 year</td>
<td>0.2759</td>
<td>1.72 (0.65-4.57)</td>
<td>0.0933</td>
<td>2.31 (0.87-6.17)</td>
<td></td>
</tr>
<tr>
<td>Pig ears: Yes</td>
<td>0.0171</td>
<td>2.32 (1.15-4.67)</td>
<td>0.0512</td>
<td>2.00 (1.00-4.01)</td>
<td></td>
</tr>
<tr>
<td>No (Ref)</td>
<td>0.2163</td>
<td>1.60 (0.76-3.37)</td>
<td>0.3492</td>
<td>1.44 (0.68-3.05)</td>
<td></td>
</tr>
<tr>
<td>Raw bones: Yes</td>
<td>0.9746</td>
<td>0.99 (0.55-1.79)</td>
<td>0.6235</td>
<td>1.16 (0.64-2.13)</td>
<td></td>
</tr>
<tr>
<td>No (Ref)</td>
<td>0.1769</td>
<td>1.87 (0.75-4.69)</td>
<td>0.2864</td>
<td>1.65 (0.66-4.11)</td>
<td></td>
</tr>
</tbody>
</table>

*Dog breeds were categorized into large mix, large pure, small mix or small pure following the American Kennel Club guidelines (Available at: [http://www.akc.org/breeds/complete_breed_list.cfm](http://www.akc.org/breeds/complete_breed_list.cfm) (accessed on 4 July 2011)).

*CI: confidence interval; LR-Test: likelihood ratio test; OR: odds ratio; Ref: referent category
Table 2.3. Final multivariable logistic regression model for the shedding of *Campylobacter* spp. among dogs that visited dog parks in southern Ontario in May-August 2009.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>p-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial dry diet(a)</td>
<td>0.08</td>
<td>0.03</td>
<td>0.01-0.79</td>
</tr>
<tr>
<td>Compost exposure(b)</td>
<td>0.30</td>
<td>0.03</td>
<td>0.10-0.88</td>
</tr>
<tr>
<td>Dog age (years)</td>
<td>0.56</td>
<td>&lt;0.01</td>
<td>0.40-0.78</td>
</tr>
<tr>
<td>Dog age squared</td>
<td>1.04</td>
<td>&lt;0.01</td>
<td>1.01-1.06</td>
</tr>
</tbody>
</table>

\(a\) versus other diets
\(b\) versus no exposure to compost

*CI: confidence interval; OR: odds ratio

Table 2.4. Final multivariable logistic regression model for the shedding of *Campylobacter upsaliensis* among dogs that visited dog parks in southern Ontario in May-August 2009.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>p-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young dog (&lt;1 year)</td>
<td>2.76</td>
<td>&lt;0.01</td>
<td>1.36-5.62</td>
</tr>
<tr>
<td>Outdoor water access(a)</td>
<td>2.30</td>
<td>0.02</td>
<td>1.15-4.62</td>
</tr>
</tbody>
</table>

\(a\) versus no access to outdoor water

*CI: confidence interval; OR: odds ratio

Table 2.5. Comparison of final regular and multi-level logistic models concerning risk factors for the shedding of *Campylobacter* spp. and *C. upsaliensis* using information criteria.

<table>
<thead>
<tr>
<th>Random effect</th>
<th><em>Campylobacter</em> spp. model</th>
<th><em>C. upsaliensis</em> model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AIC</td>
<td>BIC</td>
</tr>
<tr>
<td>No random effect</td>
<td>229.05</td>
<td>244.99</td>
</tr>
<tr>
<td>Park</td>
<td>231.05</td>
<td>250.18</td>
</tr>
<tr>
<td>Visit (park and date)</td>
<td>229.36</td>
<td>248.45</td>
</tr>
</tbody>
</table>

*AIC: Akaike’s Information Criteria; BIC: Bayesian Information Criteria
Figure 2.1. Predicted probability of shedding *Campylobacter* spp. for dogs between 0 and 15 years of age based on a multivariable logistic regression model for the shedding of *Campylobacter* spp. in dogs that visited dog parks in southern Ontario.

*The variables for diet and compost exposure were fixed on commercial dry diet and no exposure to compost.*
Chapter 3:

A cross-sectional study examining prevalence and risk factors for antimicrobial resistant generic *E. coli* in domestic dogs that frequent dog parks in southern Ontario, Canada

(Procter et al. formatted for submission to Zoonoses and Public Health)

Abstract

Antimicrobial resistance can create a health threat by limiting treatment options, increasing the risk of hospitalization and severity of infection. Companion animals can shed antimicrobial-resistant bacteria which can expose other dogs and humans to antimicrobial-resistant genes. This study examined the prevalence and risk factors for the shedding of antimicrobial-resistant generic *Escherichia coli* in the feces of dogs that visited dog parks in southern Ontario. From May to August 2009, canine fecal samples were collected at ten dog parks in the cities of Guelph and Kitchener-Waterloo, Ontario, Canada. Owners were asked to complete a questionnaire related to pet characteristics and management factors including age, diet, exposure to livestock, and medical history of the dog, including recent treatment with antibiotics. Fecal samples were collected from 251 dogs and 189 surveys were completed. Generic *E. coli* was isolated from 237 of the fecal samples, and up to 3 isolates per sample were tested for antimicrobial susceptibility. Eighty-nine percent of isolates were pan-susceptible; 82.3% of dogs shed isolates that were pan-susceptible. Multiclass resistance was detected in 7.2% of the isolates from 10.1% of the dogs. Based on multi-level multivariable logistic regression, a risk factor for the shedding of generic *E. coli* resistant to ampicillin was attending dog day care. Risk factors for the shedding of *E. coli* resistant to at least one antimicrobial included attending dog day care and being a large mixed breed dog; consumption of commercial
dry and home cooked diets were protective factors. In a multi-level multivariable model for the shedding of multiclass-resistant *E. coli*, exposure to compost and being a large mixed breed dog were risk factors, while consumption of a commercial dry diet was a sparing factor. Pet dogs are a potential reservoir of antimicrobial-resistant generic *E. coli*, which may pose a health risk to humans.

**Introduction**

Antimicrobial resistance is an international public health problem, and with the use of antimicrobials in companion animals, this population is a potential reservoir for antimicrobial-resistant bacteria (Boerlin and Reid-Smith, 2008). With the increased prevalence of antimicrobial resistant bacteria, treatment options can be limited, treatment time may be increased, and infection may be more severe (Umber and Bender, 2009). Consequently, increased cost of healthcare and higher mortality rates are associated with antimicrobial-resistant infections (Neidell et al., 2012). As a result, there is the potential of a serious health risk given that approximately 32% of Canadian households have at least one dog, with an estimated population of 6 million pet dogs in Canada (Perrin, 2009).

The role of companion animals in the transmission of antimicrobial resistance genes and antimicrobial resistant organisms are poorly understood, and difficult to define. National monitoring programs on antimicrobial consumption or resistance do not include companion animals, nor is there routine testing for the presence of resistant organisms in companion animals (Caprioli et al., 2000; Guardabassi, Schwarz, and Lloyd, 2004). Fecal shedding of antimicrobial resistant bacteria in dogs may contribute to the transmission of resistance genes, and the introduction of antimicrobial resistant organisms into environments shared with humans.
Furthermore, canine feces represent a potential reservoir for the human acquisition of commensal bacteria, such as *E. coli*, that can act as a major source of antimicrobial resistance genes (Caprioli et al., 2000). Resistance in these reservoir bacteria are also thought to be a greater threat to health than direct selection pressure on pathogens themselves, due to the vast number of commensal bacteria and the flexibility of genes to move from *E. coli* to other organisms (Boerlin and Reid-Smith, 2008). The close relationship between humans and their pets also provides opportunities for the transmission of bacteria, either directly or indirectly through contact and the environment (Guardabassi, Schwarz, and Lloyd, 2004; Sannes, Kuskowski, and Johnson, 2004). The exchange of resistance genes can occur with low bacterial numbers, which further heightens the risk of transmission of antimicrobial resistance between humans and their pets (Guardabassi, Schwarz, and Lloyd, 2004).

Dog owners are required to remove the feces of their dog(s) from park grounds, potentially becoming exposed to resistant bacteria. Dogs may also be exposed to resistant bacteria from other dogs, wildlife, and the environment in the parks. Few studies have been conducted on antimicrobial resistance in dogs and thus, risk factors for the carriage of antimicrobial resistant bacteria are not well documented. The objectives of this study were to determine the prevalence of antimicrobial resistance in generic *E. coli*, as well as to investigate risk factors for the carriage of resistant generic *E. coli* in dogs that visit dog parks in southern Ontario.
Methods

Collection of Fecal Samples

Thirteen off-leash dog parks in the Guelph and Kitchener-Waterloo area were selected for this study. The parks were identified using internet searches and personal correspondence. Parks were visited at least three times for two to three hours each time either in the morning or afternoon between May and August 2009 by two individuals. Parks where few or no participants were recruited, usually due to limited activity, were not visited again. Dog owners were approached and asked to participate in the study and provide the researcher with canine fecal samples voided by their dog while in the park. As owners were leaving the park they submitted the fecal samples, which were given a unique identification number for each dog, then placed into a specimen container for future isolation and antimicrobial susceptibility testing of *E. coli*. The container with the sample was stored in a cooler until submitted to the Canadian Research Institute for Food Safety (CRIFS), University of Guelph, for *E. coli* isolation. This study was approved by the University of Guelph’s Research Ethics Board.

Questionnaire

A questionnaire adapted from one previously used by Leonard et al. (2012) was administered to owners at the time of sample submission. Questions were asked relating to pet management factors concerning the dog’s main diet, presence of other animals in the home, the dog’s previous illnesses or treatments, veterinary care, and human illness in the home over the previous 30 days. Data on breed, age, sex and neuter status were collected for all dogs. The risk factors investigated are shown in Table 3.1; the complete questionnaire is available upon request from the authors (Appendix I)
Laboratory Analysis

All canine fecal samples were cultured for generic *E. coli* at the Canadian Research Institutes for Food Safety (CRIFS) Laboratory, University of Guelph, as previously described (Leonard et al., 2012). In brief, pre-enriched buffer peptone water was combined with *E. coli* broth and incubated for 18-24 h, followed by plating and incubation on Eosin Methylene Blue agar (Difco, Becton Dikinson); purification followed on MacConkey agar (Difco, Becton Dikinson). Three *E. coli* isolates from each dog were tested for antimicrobial susceptibility at the Laboratory for Foodborne Zoonoses, Public Health Canada, using the Sensititre™ automated broth microdilution system (Trek Diagnostic Systems Ltd.) using methods which have been reported by the Government of Canada (2011). The National Antimicrobial Resistance Monitoring System (NARMS) susceptibility panel CMV1AGNF was used, using methods described by the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) (Government of Canada, 2011; FDA, 2010). Resistance breakpoints were those derived from the Clinical and Laboratory Standards Institute (CLSI) (Government of Canada, 2011; FDA, 2010), and a lower MIC breakpoint for ceftriaxone, based on the updated CLSI Informational Supplement M100-S20 (Clinical and Laboratory Standards Institute, 2010). A list of the antimicrobials and their MIC breakpoints used for *E. coli* susceptibility testing can be found in Table 3.2. Antimicrobials were classified based on their use in veterinary medicine and importance to human medicine, as defined by the Government of Canada (2005), with category I representing antimicrobials of very high importance in human medicine and essential to the treatment of serious bacterial infections with no alternatives for resistant infections, including amoxicillin/clavulanic acid, ceftiofur, ceftriaxone, ciprofloxacin and trimethoprim/sulfamethoxazole. Category II included antimicrobials of high importance in
human medicine, such as amikacin, ampicillin, cefoxitin, gentamicin, kanamycin, nalidixic acid, and streptomycin, used to treat a variety of infections and act as alternatives in the event of resistance to category III antimicrobials (Government of Canada, 2005). Antimicrobials that are of medium importance in human medicine and are used as first-line drugs with alternatives for resistance generally available fall into category III, including chloramphenicol, sulfisoxazole, and tetracycline (Government of Canada, 2005). Antimicrobials were also grouped by class of antimicrobial, including aminoglycosides, beta-lactams, folate pathway inhibitors, phenicols, quinolones, and tetracylines.

Statistical Analysis

Isolates were considered ‘susceptible’ or ‘resistant’ based on the MIC breakpoints; isolates with intermediate susceptibility were classified as ‘susceptible’. Prevalence of resistance, as well as 95% exact binomial confidence intervals for resistance to each antimicrobial, was calculated at the isolate and dog level. A one-tailed 97.5% confidence interval was calculated for zero values. Prevalence and confidence intervals were also determined for each antimicrobial class and category of importance to human health. The prevalence of antimicrobial resistance patterns were also described (combinations of resistance to two or more antimicrobials). After reviewing the prevalence and frequency of resistance to each antimicrobial, class, and category, as well as multiclass resistance, outcomes for statistical modelling were chosen. Outcomes that affected between 10-90% of dogs were chosen for statistical model building; a cut point of 10% was chosen to ensure an adequate effective sample size for statistics.

To assess co-linearity between continuous variables, Spearman rank correlations with a cut-off point of 0.70 were assessed with the most biologically meaningful variable selected for
further analysis. Linearity was assessed between the log odds of the outcomes and continuous variables using locally weighted regression (lowess) curves. If not linear or suitably modelled with the addition of a quadratic term ($\alpha<0.05$), the variable was categorized. Where the breed of each dog was provided, it was assigned to one of large mix, large pure, small mix, or small pure categories (American Kennel Club, 2011)

Multi-level logistic regression models were built to identify univariable risk factors for the chosen outcomes, at the isolate level, controlling for dog and park visited using random effects to control for clustering, as multiple isolates were tested per dogs and multiple dogs were tested per park. Using a cut-off point of $\alpha \leq 0.20$ in univariable analysis, risk factors were selected for multivariable models. The multivariable models were constructed using forward step-wise selection. Both univariable and multivariable analyses were conducted using dog and park as random effects, using predictive quasi-likelihood with the first order derivative of the Taylor series expansion (PQL1) methods and the use of re-weighted iterative generalized least squares (RIGLS). The variance estimates from these models were used to determine the percentage of variance in resistant isolates at the dog level, and the proportion at the park level using the latent variable technique (Dohoo, Martin, and Stryhn, 2009). A Shapiro-Wilks test was used to determine if the residuals were normally distributed. Model residuals, were evaluated visually to identify outliers, and assess the normality of best linear unbiased predictors (BLUPs) (Dohoo, Martin, and Stryhn, 2009).

All statistical analyses were performed using Stata 11.2MP (Stata Corp., College Station, Texas, USA), except for the random effects models involving quasi-likelihood methods which
were performed using MLwiN 2.25 (London, UK) through Stata 11.2MP using the command “runmlwin” (Rasbash et al., 2009; Leckie and Charlton, 2011).

Results

Collection of Samples

Participants were recruited from ten of the 13 dog parks visited between May and August 2009. A range of 1-90 samples were collected from each of the ten parks, with five of the parks providing more than 25 samples each, and less than 10 samples from each of the other five parks. Fecal samples were obtained from a total of 251 dogs and 189 surveys were completed concerning the management of individual dogs. From these fecal samples, 709 generic E. coli isolates were obtained from 237 dogs, and were further tested for antimicrobial susceptibility.

Descriptive statistics

Of the isolates, 11% were resistant to at least one antimicrobial, with 8% resistant to 2-5 antimicrobials and 1% resistant to 5 or more antimicrobials. The most frequent antimicrobial that isolates were resistant to was ampicillin (8%), followed by sulfisoxazole (5%) and tetracycline (5%). There was no resistance found to ciprofloxacin, amikacin, or nalidixic acid (Table 3.2). Overall, 18% of dogs had at least one isolate that was resistant to at least one antimicrobial, and 12%, 9%, and 8% of dogs carried resistant isolates to ampicillin, sulfisoxazole, and tetracycline, respectively. Of note, 3% of isolates from 4% of the dogs were resistant to ceftriaxone, which is often used to treat enteric diseases.

The most frequent antimicrobial class that isolates were resistant to was the beta-lactams (8%) followed by folate pathway inhibitors (5%) and tetracyclines (5%) (Table 3.3). No
resistance was found to quinolones. Multiclass resistance was present in 7% of the isolates. Resistance to beta-lactams, folate pathway inhibitors, and tetracyclines were also the most frequent antimicrobial classes present at the dog level (13%, 9%, and 8%, respectively). Multiclass resistant isolates were present in *E. coli* from 10% of the dogs.

Based on importance to human medicine, 4% of isolates were resistant to category I antimicrobials, 10% to category II antimicrobials, and 8% to category III antimicrobials. At the dog level, 8% carried isolates resistant to category I antimicrobials, 14% to category II, and 13% to category III antimicrobials (Table 3.4).

**Resistance Patterns**

Frequent antimicrobial resistance patterns among the *E. coli* isolates from this population of dogs were AMP-TCY (n=28), AMC-AMP (n=24), SOX-STR (n=24), AMP-SOX (n=23), AMP-STR (n=22), and AMP-FOX (n=20). The same patterns were also the most frequent at the dog level. Most antimicrobial resistance patterns found in these isolates included 2-4 antimicrobials, however, there were a large number of patterns that contained 5 antimicrobials as well (Table 3.5). Four patterns had 9 or more antimicrobials and the largest pattern included ten antimicrobials (AMC-AMP-FOX-TIO-CRO-CHL-GEN-SOX-TCY-STR), but this pattern was present in only one isolate.

**Multivariable models**

The following three outcomes were chosen to investigate risk factors: resistance to ampicillin, resistance to at least one antimicrobial, and multiclass resistance. The only risk factor significantly associated with ampicillin resistance in dogs that visited dog parks was attending a dog day care (Table 3.6). Factors positively associated with antimicrobial resistance to at least
one of the antimicrobials tested included dogs attending a day care, and breed size, with large mixed breed dogs more likely to be resistant to at least one antimicrobial. Factors negatively associated with antimicrobial resistance to at least one antimicrobial included consumption of a commercial dry diet and consumption of a homemade cooked diet compared to other diets (Table 3.6). Risk factors associated with multiclass antimicrobial resistance in dogs that visited dog parks included exposure to compost and breed size with large mixed breed dogs more likely to shed multiclass resistant isolates. Consumption of a commercial dry diet was a sparing factor in this model (Table 3.6).

The variance due to park as a random effect was small in all of the models (i.e., $<10^{-4}$), so park as a random effect was not included in the multivariable models. Dog was retained as a random effect in the models to account for within-dog variance. Intraclass-correlation coefficients at the animal level were 50%, 49%, and 51% for the models ampicillin resistance, resistance to at least one antimicrobial, and multiclass resistance, respectively (Table 3.6). Residuals were not normal as indicated with a Shapiro-Wilks test, and there was some variation from the normal line on a q-plot of the BLUPs. However, there were no extreme outliers when examining the standardized, leverage, and deletion residuals.

**Discussion**

This study examined the prevalence of antimicrobial resistance in *E. coli* recovered from pet dogs that visited dog parks in southern Ontario over the summer of 2009. Approximately 11% of isolates and 18% of dogs carried resistance to at least one antimicrobial, with the majority of isolates being pan-susceptible. The prevalence of resistance was highest to ampicillin, tetracycline, and sulfisoxazole in both isolates and dogs. Resistance to these
antimicrobials reflect common antimicrobials that are used in companion animals (Guardabassi, Schwarz, and Lloyd, 2004).

Isolates were found that were resistant to antimicrobials of very high importance in human medicine, such as amoxicillin/clavulanic acid, ceftiofur, ceftriaxone, and trimethoprim/sulfamethoxazole. Resistance to category I antimicrobials was found in 3% of isolates from 8% of dogs. Also of concern, multiclass resistance was present in 7% of the isolates and 10% of the isolates at the dog level. Consequently, pet dogs may be an important reservoir for antimicrobial resistant bacteria, as demonstrated in this and other studies (Pedersen et al., 2007; Murphy et al., 2009; Murphy et al., 2010; Leonard et al., 2012).

The prevalence of antimicrobial resistant *E. coli* observed in this study is comparable to previous studies of dogs in Canada which have reported the prevalence of antimicrobial resistance in *E. coli* isolates to range from 0-20% (Murphy et al., 2009; Murphy et al., 2010; Leonard et al., 2012). There have also been similar studies of antimicrobial resistance in dogs conducted in Europe, the United States, and Japan, with varying prevalences of antimicrobial resistance, ranging from 0-76% (Normand et al., 2000; De Graef et al., 2004; Costa et al., 2008; Shaheen et al., 2010; Harada et al., 2011). The variability in the reported prevalence of antimicrobial resistance among these studies may be due to difference in study location since different countries may have different consumption patterns of antimicrobials among companion animals, and there may be different levels of antimicrobial resistance in the community (Guardabassi, Schwarz, and Lloyd, 2004). In addition, dogs recruited at a veterinary clinic may have a different prevalence of resistance and resistance patterns compared to dogs recruited at animal shelters, which may also be different than dogs recruited at dog parks, or healthy dogs,
due to population density, health status, or recent treatment history. The use of antimicrobials in these different populations, as well as the contact between dogs potentially resulting in transfer of resistance genes and/or bacteria, can also influence the reported prevalence of resistance and resistance patterns.

Pet management risk factors for the shedding of antimicrobial resistant generic *E. coli* in dogs have not been extensively studied in the past. In this study, attending a dog day care program was associated with the carriage of ampicillin resistant *E. coli*, as well as resistance to at least one antimicrobial. At a day care, dogs may be in contact with a number of other dogs, in close contact and potentially sharing antimicrobial resistant bacteria. The spread of antimicrobial resistant bacteria in canine day care centres has not been previously studied, but there have been some studies at dog breeding kennels (De Graef et al., 2004; Harada et al., 2011). Harada, et al. (2011) reported that dogs within the same breeding kennel showed greater similarity in resistance patterns compared to dogs from different breeding kennels. Additionally, De Graef et al. (2004) reported that multidrug resistance was more frequent in breeding kennel dogs than individually owned dogs. These findings support the notion that close contact between dogs, whether living together or staying together for a large proportion of the day may increase the likelihood of sharing antimicrobial bacteria and genes.

Consumption of commercial dry food was found to be a sparing factor for the presence of resistance to at least one of the tested antimicrobials, as well as multiclass resistance. In addition, homemade cooked diets were found to be a sparing factor for the presence of isolates resistant to at least one of the tested antimicrobials. In a previous study that examined commercial raw food diets (Finley et al., 2008), antimicrobial resistant *Salmonella* were found in various products,
some of which were resistant to multiple classes of antimicrobials. This reduced risk found in this study could be due to the elimination of *E. coli* from commercial and cooked diets through production processes, particularly where heat is applied to eliminate bacteria from the final product. In the multivariable analysis, consumption of a homemade cooked diet was a significant protective factor for the presence of *E. coli* with resistance to at least one antimicrobial, but not when considering multiclass resistance. This finding may be a result of the small sample size used and specificity of the outcomes; further research regarding the association between multiclass antimicrobial resistance in dogs and their diets should be conducted to further understand this relationship.

Category of dog, based on purity of breed and size, was found to be associated with the likelihood of the carriage of antimicrobial resistant *E. coli* strains resistant to at least one antimicrobial, as well as multiple antimicrobial classes. Large dogs of a mixed breed were found to be significantly more likely to shed antimicrobial resistant *E. coli* than large pure and small mixed and pure dogs. This relationship has not been reported previously. It is possible that the lifestyle of large mixed breed dogs may be substantively different than smaller dogs or pure bred dogs, and this difference could result in an increased probability of shedding antimicrobial resistant bacteria. In addition, approximately 20% of large mixed breed dogs were acquired from the humane society; it has been reported that animals living together have a higher prevalence of antimicrobial resistance than individually housed dogs (De Graef et al., 2004).

Exposure to compost was found to be associated with the occurrence of multiclass resistant *E. coli* in dogs. It was not specified in the survey if the dogs consumed compost or simply had access to it. It was also not specified if the compost was indoors or outdoors. It is
possible that the compost included animal by-products that contained antimicrobial resistant bacteria or residues. Low levels of antimicrobial resistance have been found in residential garden soil, as well as manure that may be mixed with compost (Esiobu, Armenta, and Ike, 2002). There could also be contamination of the compost with antimicrobial resistance from the feces of small wild animals (Allen et al., 2011). Interestingly, in a study of the same population, compost was found to be a protective factor against the shedding of *Campylobacter* spp. (Chapter 2). Perhaps the population of microbes present in the compost provides a competitive environment that selects for resistance to antimicrobials, but prevents the growth of *Campylobacter* spp.

Interestingly, the presence of antimicrobial resistant isolates in an animal was not clustered by the park where the dogs were recruited. The negligible park level variance component suggests little variation in the status of animals was explained at the park level. This finding may be due to the relatively close geographic proximity of the parks to each other, as the parks were all located within the Wellington and Waterloo counties. There is also the possibility that dogs visit more than one park, or that there is no environmental exposure at the parks. It is possible that wildlife at the park, including mice, squirrels, and racoons, contribute to antimicrobial resistance in the environment (Allen, et al., 2011), however the low significance of park as a random effect suggests that non-park factors are more important in explaining the variation in the shedding of antimicrobial resistant generic *E. coli*.

The results of this study support commensal bacteria populations as a potential reservoir for antimicrobial resistance in dogs. It is unclear, however, if the resistance developed in dogs, or if it was transferred from humans or other environmental or food sources. Evidence suggests that sharing of antimicrobial resistant genes and bacteria within households is possible, whether
directly or through the environment (Stenske et al., 2009). Dogs may also acquire resistant *E. coli* strains from humans (Sannes, Kuskowski, and Johnson, 2004). In this study, however, none of the human health factors were significant in the models. Further research should be conducted to examine the similarities between antimicrobial resistant bacteria in dogs and humans, as well as the transfer of these bacterial populations between dogs and humans to more fully understand this relationship.

The design of this study does not provide an indication of the source of the antimicrobial resistance genes and resistant bacteria, nor the duration the dogs are colonized or shedding antimicrobial resistant bacteria. In addition, a lower MIC of ceftriaxone was used in this study as compared to previous studies, based on current CLSI standards. This is important to consider when comparing results of this study with results of earlier antimicrobial resistance studies in dogs. Furthermore, there were many outcomes measured and a long list of variables which increased the probability of type I errors.

The study of prevalence and risk factors for the carriage of antimicrobial resistant *E. coli* in dogs that visit dog parks in southern Ontario provides a way to characterize a companion animal population distinct from a veterinary clinic or hospital, and could be used as a baseline to consider the trends of antimicrobial resistance in this population. In addition, the consumption patterns of antimicrobials in companion animals should be monitored along with the prevalence of antimicrobial resistant bacteria. In this study, both dog and human health variables, such as experiencing a gastro-intestinal upset in the previous month and a dog’s treatment with antimicrobials in the past month were not significant in the models. Perhaps the selection of antimicrobial resistant isolates requires more than one month, and the time period in which data
are obtained could be extended in future studies. This effect may also be a function of low power due to relatively homogenous responses, which limited the ability to evaluate these variables. One key to understanding the role of companion animals on the public health impacts from antimicrobial resistant infections may be the monitoring of antimicrobial resistant bacteria in companion animals. The occurrence of antimicrobial resistant bacteria in dogs, along with the close relationship that most owners share with their dogs may make these animals a potential health risk to owners and other users of dog parks.
References


Clinical and Laboratory Standards Institute 2010: *Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement M100-S20*. CLSI, Wayne, PA, USA.


Government of Canada 2005: Proposed categorization of antimicrobial drugs in current thinking on risk management measures to address antimicrobial resistance associated with the use of antimicrobial agents in food-producing animals. Ottawa, ON: Health Canada, Veterinary Drugs Directorate.


Table 3.1. List of pet-related management variables evaluated for an association with antimicrobial resistant generic *E. coli* shedding in dogs that visited dog parks in the Guelph and Kitchener-Waterloo area in May-August 2009.

<table>
<thead>
<tr>
<th>Owner demographic information</th>
<th>Diet information (Y/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Gender</td>
<td>• Store-bought/commercial processed dry food</td>
</tr>
<tr>
<td>• Age</td>
<td>• Store-bought/commercial processed canned food</td>
</tr>
<tr>
<td>• City</td>
<td>• Homemade cooked</td>
</tr>
<tr>
<td>• Province</td>
<td>• Homemade raw</td>
</tr>
<tr>
<td>• Type of home</td>
<td>• Commercial cooked</td>
</tr>
<tr>
<td>o Urban</td>
<td>• Commercial raw</td>
</tr>
<tr>
<td>o Suburban</td>
<td>• Combination of diets</td>
</tr>
<tr>
<td>o Small town rural</td>
<td>• Other diet (open)</td>
</tr>
<tr>
<td>o Non-farm rural</td>
<td>• Treats provided to dog (Y/N)</td>
</tr>
<tr>
<td>o Farm</td>
<td>o Dried pig’s ears</td>
</tr>
<tr>
<td>Dog demographic information</td>
<td>o Raw bones</td>
</tr>
<tr>
<td>• Name</td>
<td>o Cooked bones</td>
</tr>
<tr>
<td>• Breed</td>
<td>o Store-bought bones</td>
</tr>
<tr>
<td>• Age (years)</td>
<td>o Rawhide chews</td>
</tr>
<tr>
<td>• Gender (M/F)</td>
<td>o Other (open)</td>
</tr>
<tr>
<td>• Spayed or neutered (Y/N)</td>
<td>• Raw meat or animal products (Y/N)</td>
</tr>
<tr>
<td>• Time dog has been owned (years)</td>
<td>o How often</td>
</tr>
<tr>
<td>• Number of dogs in household</td>
<td>• Daily</td>
</tr>
<tr>
<td>• Number of other animals in house</td>
<td>• Weekly</td>
</tr>
<tr>
<td>• Source of dog</td>
<td>• Monthly</td>
</tr>
<tr>
<td>o Pet Store</td>
<td>• Rarely</td>
</tr>
<tr>
<td>o Breeder</td>
<td>• Time dog has been on current diet</td>
</tr>
<tr>
<td>o Humane Society</td>
<td>o Days</td>
</tr>
<tr>
<td>o Other</td>
<td>o Weeks</td>
</tr>
<tr>
<td>Dog medical history (Y/N)</td>
<td>o Months</td>
</tr>
<tr>
<td>• Seen veterinarian in past year</td>
<td>o Years</td>
</tr>
<tr>
<td>• Dewormed in past 6 months</td>
<td>• Other animal exposures (Y/N)</td>
</tr>
<tr>
<td>• Given antimicrobials in the past month</td>
<td>• Access to farms with livestock</td>
</tr>
<tr>
<td>• Given probiotics/active bacterial culture in past month</td>
<td>• Hunt and catch or eat prey</td>
</tr>
<tr>
<td>• Given other medications/supplements in past month</td>
<td>• Off-leash with other dogs</td>
</tr>
<tr>
<td>• Diagnosed with <em>Salmonella, Campylobacter, Giardia</em> or <em>Clostridium difficile</em> infection in the past 6 months</td>
<td>• Hunting activities</td>
</tr>
<tr>
<td>• Diarrhea in past month</td>
<td>• Dog day care</td>
</tr>
<tr>
<td>• Vomiting in past month</td>
<td>• Obedience/agility/flyball</td>
</tr>
<tr>
<td>Water exposure (Y/N)</td>
<td>• Therapy/education programs</td>
</tr>
<tr>
<td>• Access to lakes, rivers, creeks in past 6 months</td>
<td>• Kennel in past 6 months</td>
</tr>
<tr>
<td>• Access to ditches, puddles in past 6 months</td>
<td>• Hospitalized in past 6 months</td>
</tr>
<tr>
<td>• Drank out of toilet in past 6 months</td>
<td>• Access to garbage in past 2 weeks</td>
</tr>
<tr>
<td>• Other water exposures (open)</td>
<td>• Access to table scraps in past 2 weeks</td>
</tr>
<tr>
<td>Human health (anyone in household) (Y/N)</td>
<td>• Access to compost in past 2 weeks</td>
</tr>
<tr>
<td>• Diarrhea in past 30 days</td>
<td>• Access to cat litter in past 2 weeks</td>
</tr>
<tr>
<td>• Vomiting in past 30 days</td>
<td>• Access to dead animals in past 2 weeks</td>
</tr>
<tr>
<td></td>
<td>• Access to animal feces (excluding cat litter) in past 2 weeks</td>
</tr>
</tbody>
</table>
Table 3.2. Breakpoints for defining resistance and the prevalence of antimicrobial resistant generic E. coli isolates to antimicrobials classified based on human medicine importance from pet dogs that visited dog parks in Guelph and Kitchener-Waterloo in the summer of 2009.

<table>
<thead>
<tr>
<th>Category&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Antibiotic</th>
<th>Resistance breakpoint (ug/mL)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Proportion of isolates resistant (n=709)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Proportion of dogs resistant (n=237)&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Amoxicillin/clavulanic acid (AMC)</td>
<td>≥ 32</td>
<td>3.4% (2.2-5.0)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.1% (2.6-8.7)</td>
</tr>
<tr>
<td>I</td>
<td>Ceftiofur (TIO)</td>
<td>≥ 8</td>
<td>2.5% (1.5-4.0)</td>
<td>3.8% (1.8-7.1)</td>
</tr>
<tr>
<td>I</td>
<td>Ceftriaxone (CRO)</td>
<td>≥ 4</td>
<td>2.5% (1.5-4.0)</td>
<td>3.8% (1.8-7.1)</td>
</tr>
<tr>
<td>I</td>
<td>Ciprofloxacin (CIP)</td>
<td>≥ 4</td>
<td>0.0% (0.0-0.5)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.0% (0.0-1.5)</td>
</tr>
<tr>
<td>I</td>
<td>Trimethoprim/sulfamethoxazole (SXT)</td>
<td>≥ 4</td>
<td>1.6% (0.8-2.8)</td>
<td>4.2% (2.0-7.6)</td>
</tr>
<tr>
<td>II</td>
<td>Amikacin (AMK)</td>
<td>≥ 64</td>
<td>0.0% (0.0-0.5)</td>
<td>0.0% (0.0-1.5)</td>
</tr>
<tr>
<td>II</td>
<td>Ampicillin (AMP)</td>
<td>≥ 32</td>
<td>7.9% (6.0-10.1)</td>
<td>12.2% (8.4-17.1)</td>
</tr>
<tr>
<td>II</td>
<td>Cefoxitin (FOX)</td>
<td>≥ 32</td>
<td>3.0% (1.8-4.5)</td>
<td>5.1% (2.6-8.7)</td>
</tr>
<tr>
<td>II</td>
<td>Gentamicin (GEN)</td>
<td>≥ 16</td>
<td>0.1% (0.0-0.8)</td>
<td>0.4% (0.01-2.3)</td>
</tr>
<tr>
<td>II</td>
<td>Kanamycin (KAN)</td>
<td>≥ 64</td>
<td>0.7% (0.2-1.6)</td>
<td>1.7% (0.5-4.3)</td>
</tr>
<tr>
<td>II</td>
<td>Nalidixic acid (NAL)</td>
<td>≥ 32</td>
<td>0.0% (0.0-0.5)</td>
<td>0.0% (0.0-1.5)</td>
</tr>
<tr>
<td>II</td>
<td>Streptomycin (STR)</td>
<td>≥ 64</td>
<td>4.4% (3.0-6.1)</td>
<td>7.2% (4.2-11.2)</td>
</tr>
<tr>
<td>III</td>
<td>Chloramphenicol (CHL)</td>
<td>≥ 32</td>
<td>1.4% (0.7-2.6)</td>
<td>1.7% (0.5-4.3)</td>
</tr>
<tr>
<td>III</td>
<td>Sulfisoxazole (SOX)</td>
<td>≥ 512</td>
<td>5.2% (3.7-7.1)</td>
<td>8.9% (5.6-13.2)</td>
</tr>
<tr>
<td>III</td>
<td>Tetracycline (TCY)</td>
<td>≥ 16</td>
<td>5.1% (3.6-7.0)</td>
<td>8.4% (5.2-12.7)</td>
</tr>
<tr>
<td>NA</td>
<td>Pan-susceptible</td>
<td></td>
<td>89.0% (86.5-91.2)</td>
<td>82.3% (76.8-86.9)</td>
</tr>
</tbody>
</table>

NA, not applicable.

<sup>a</sup>Category I- antimicrobials of very high importance in human medicine, essential to the treatment of serious bacterial infections, no alternatives for resistant infections; Category II- antimicrobials of high importance in human medicine, used to treat a variety of infections, alternatives for resistance to category III antimicrobials; Category III- antimicrobials of medium importance in human medicine, used as first-line drugs, alternatives for resistance are generally available (Government of Canada, 2005).

<sup>b</sup>CLSI. Informational Supplement M100-S20.; breakpoints were based on the distribution of minimal inhibitory concentrations and were harmonized with those of the National Antimicrobial Resistance Monitoring System.

<sup>c</sup>Columns total may be greater than the number of isolates or 100% because multiresistant isolates were recovered from several dogs.

<sup>d</sup>95% confidence intervals (CIs)

<sup>e</sup>One-sided, 97.5% CIs were calculated for 0 values.
Table 3.3. Proportion of antimicrobial resistant generic *E. coli* at the isolate and dog level by antimicrobial class from pet dogs that visited dog parks in Guelph and Kitchener-Waterloo in the summer of 2009.

<table>
<thead>
<tr>
<th>Class</th>
<th>Isolates (n=709)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Dogs (n=237)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoglycosides</td>
<td>4.8% (3.3-6.6)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.0% (4.9-12.2)</td>
</tr>
<tr>
<td>Beta-lactams</td>
<td>8.0% (6.1-10.3)</td>
<td>12.7% (8.7-17.6)</td>
</tr>
<tr>
<td>Folate pathway inhibitors</td>
<td>5.2% (3.7-7.1)</td>
<td>8.9% (5.6-13.2)</td>
</tr>
<tr>
<td>Phenicols</td>
<td>1.4% (0.7-2.6)</td>
<td>1.7% (0.5-4.3)</td>
</tr>
<tr>
<td>Quinolones</td>
<td>0.0% (0.0-0.5)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.0% (0.0-1.5)</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>5.1% (3.6-7.0)</td>
<td>8.9% (5.6-13.2)</td>
</tr>
<tr>
<td>Multiclass resistance&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7.2% (5.4-9.3)</td>
<td>10.1% (6.6-14.7)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Aminoglycosides- amikacin, gentamicin, kanamycin, and streptomycin; Beta-lactams- ampicillin, amoxicillin-clavulanic acid, ceftriaxone, cefoxitin, and ceftiofur; Folate-pathway inhibitors- sulfisoxazole and sulfamethoxazole-trimethoprim; Phenicols- chloramphenicol; Quinolones- ciprofloxacin and nalidixic acid; Tetracyclines- tetracycline.

<sup>b</sup>Column total may be greater than the number of isolates or 100% because multiresistant isolates were recovered from several dogs.

<sup>c</sup>95% CIs

<sup>d</sup>One-sided, 97.5% CIs were calculated for 0 values.

<sup>e</sup>Resistant to two or more classes of antimicrobials.
Table 3.4. Distribution of antimicrobial resistant generic *E. coli* isolates and carrier dogs by category of antimicrobial importance in human medicine from pet dogs that visited dog parks in Guelph and Kitchener-Waterloo in the summer of 2009.

<table>
<thead>
<tr>
<th>Category(^a)</th>
<th>Isolates (n=709)(^b)</th>
<th>Dogs (n=237)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3.4% (2.2-5.0)(^c)</td>
<td>8.0% (4.9-12.2)</td>
</tr>
<tr>
<td>II</td>
<td>9.9% (7.8-12.3)</td>
<td>14.8% (10.5-19.9)</td>
</tr>
<tr>
<td>III</td>
<td>7.8% (5.9-10.0)</td>
<td>12.7% (8.7-17.6)</td>
</tr>
</tbody>
</table>

\(^a\)Category I- antimicrobials of very high importance in human medicine, essential to the treatment of serious bacterial infections, no alternatives for resistant infections; Category II- antimicrobials of high importance in human medicine, used to treat a variety of infections, alternatives for resistance to category III antimicrobials; Category III- antimicrobials of medium importance in human medicine, used as first-line drugs, alternatives for resistance are generally available (Government of Canada, 2005).

\(^b\)Column total may be greater than the number of isolates or 100% because multiresistant isolates were recovered from several dogs.

\(^c\)95% CIs

Table 3.5. Distribution of the number of antimicrobials within antimicrobial resistance patterns among generic *E. coli* isolated from pet dogs that visited dog parks in Guelph and Kitchener-Waterloo in the summer of 2009.

<table>
<thead>
<tr>
<th>Number of antimicrobials in resistance pattern</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patterns</td>
<td>59</td>
<td>71</td>
<td>60</td>
<td>33</td>
<td>15</td>
<td>6</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>
Table 3.6. Multi-level multivariable risk factor models for the carriage of antimicrobial resistant generic *E. coli* in dogs that visit dog parks in southern Ontario, May-August 2009.

<table>
<thead>
<tr>
<th>Outcome:</th>
<th>Ampicillin Resistance</th>
<th>Resistance to ≥ 1 antimicrobial</th>
<th>Multiclass resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>OR  p-value  95% CI</td>
<td>OR  p-value  95% CI</td>
<td>OR  p-value  95% CI</td>
</tr>
<tr>
<td>Attend dog day care</td>
<td>6.64 0.02 1.31-33.66</td>
<td>5.92 0.04 1.06-32.95</td>
<td>-</td>
</tr>
<tr>
<td>Home cooked diet*</td>
<td>- - -</td>
<td>0.05 0.01 0.005-0.50</td>
<td>-</td>
</tr>
<tr>
<td>Commercial dry diet*</td>
<td>- - -</td>
<td>0.02 &lt;0.01 0.002-0.21</td>
<td>0.04 0.01 0.004-0.43</td>
</tr>
<tr>
<td>Breed size</td>
<td>- - -</td>
<td>&lt;0.01b</td>
<td>&lt;0.01b</td>
</tr>
<tr>
<td>Large mix</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Large pure</td>
<td>0.16 0.05-0.47</td>
<td>0.03 0.004-0.20</td>
<td></td>
</tr>
<tr>
<td>Small mix</td>
<td>0.35 0.08-1.60</td>
<td>0.34 0.06-1.91</td>
<td></td>
</tr>
<tr>
<td>Small pure</td>
<td>0.11 0.02-0.86</td>
<td>0.08 0.01-0.96</td>
<td></td>
</tr>
<tr>
<td>Compost</td>
<td>- - -</td>
<td>5.48 0.04 1.12-26.72</td>
<td></td>
</tr>
<tr>
<td>Animal-level variancec</td>
<td>3.23 (SE: 1.04); ICC: 50%</td>
<td>3.19 (SE: 0.92); ICC: 49%</td>
<td>3.43 (SE: 1.23); ICC: 51%</td>
</tr>
</tbody>
</table>

*Compared to all other diets
b Overall p-value for variable
cSE: Standard error; ICC: Intraclass correlation coefficient
*CI: confidence intercal; OR: odds ratio; Ref: referent category
Chapter 4:

Summary discussion and conclusions

The objective of this study was to examine the potential public health impacts of dogs that visit dog parks. Specifically, to describe the population of dogs that attend dog parks, determine the prevalence of selected zoonotic pathogens and antimicrobial resistant generic E. coli in these dogs, assess significant associations between pet-related management risk factors for the carriage of selected zoonotic pathogens and antimicrobial resistant E. coli, and determine if carriage of antimicrobial resistant isolates and selected zoonotic pathogens clustered by park visited. These objectives were met through the use of a cross-sectional study that included a survey component to identify potential pet-related factors. The development of multi-level multivariable models using laboratory results from submitted canine fecal samples and the completed surveys, allowed for the identification of statistically significant associations within these models. The degree of clustering by park for these pathogens and presence of antimicrobial resistant bacteria were also assessed.

To my knowledge, this thesis is the first report in the Canadian literature that examines zoonotic pathogens and antimicrobial resistant bacteria in dogs recruited from dog parks. The results indicate that the prevalence of Salmonella, Giardia, and Campylobacter in dogs that visit dog parks is similar to those recruited at veterinary clinics or from volunteer households (Jacobs, Forrester and Yang, 2001; Hackett and Lappin, 2003; Wieland et al., 2005; Westgarth et al., 2009; Parsons et al., 2010; Leonard et al., 2011; Marks et al., 2011; Mircean, Györke, and Cozma, 2012; Wang et al, 2012). The similarities between this study and studies where dogs
were recruited at veterinary clinics may be because most (96%) of the dogs that were recruited in this study had attended a veterinary clinic in the past year.

The pet-related factors associated with the carriage of *Campylobacter* spp. and *C. upsaliensis* were similar to previously identified factors including age and diet (Hald et al., 2004; Wieland et al., 2005; Acke et al., 2006; Westgarth et al., 2009; Parsons et al., 2010; Leonard et al., 2011). However, there were also some newly identified factors, including access to compost, which was protective, and access to outdoor water sources, such as lakes, rivers, and ditches which was a risk factor. The identification of these pet-management factors prompts the need for further research into these factors, to confirm consistency and attempt to uncover the mechanisms responsible for the observed effects.

The prevalence of antimicrobial resistant *E. coli* isolates was similar to what has been previously reported in healthy dogs (De Graef et al., 2004; Costa et al., 2008; Murphy et al., 2009; Stenske et al., 2009; Murphy et al., 2010; Leonard et al., 2012). Risk factors for the carriage of antimicrobial resistant bacteria are not well studied in healthy pet dogs. In this study, attending a dog day care, diet, exposure to compost and purity and size of breed were found to have an association with the presence of antimicrobial resistant isolates. Consumption of commercial dry and homemade cooked diets were found to be protective, whereas dogs that attended dog day care, had exposure to compost and large dogs of mixed breed had an increased probability of shedding antimicrobial resistant generic *E. coli* isolates.

Clustering of *Campylobacter* shedding status and shedding of antimicrobial resistant generic *E. coli* by park was not significant in any of the constructed models. This suggests the
status of dogs in terms of shedding *Campylobacter* or antimicrobial resistant generic *E. coli* is independent of the park where they were recruited.

**Strengths and limitations**

The cross-sectional study design is limited in that only prevalence data are collected. The true association between the identified risk factors and either acquiring or maintaining the outcome is difficult to determine, as prevalence is a function of both incidence and duration. It is possible that the identified factors are a consequence of the outcome, rather than a determinant. The modelling of prevalence data is not as much an issue when determining if the dogs are shedding the organisms of interest. This study was concerned with the presence of zoonotic pathogens and antimicrobial resistance, rather than the development or acquisition of these organisms. In addition, the identification of risk factors associated with shedding the organisms, which if identified and altered may help in the reduction of the shedding, and therefore the risk to humans. Also, most of the factors examined are constant and generally would not change over time, such as breed and diet. For any factors that may change over time, a time frame was provided on the questionnaire. Although efforts were made, the results may not help in the development of strategies to prevent the acquisition of these organisms. Furthermore, the issue of reverse causality should be considered in the context of antimicrobial resistance, as it is unclear if resistance originated in the individual dog due to exposure to a risk factor, or was acquired from a shared environment with other dogs, humans, or animals.

The cross-sectional design is useful for a preliminary study on this population of dogs since owners do not have to commit a large amount of time to the study, and it ensures that most dogs that visit the studied dog parks over the sampling period have a chance to be part of the
study. It also allows a large number of variables to be tested and can help generate hypotheses. In addition, this study provided baseline data for future studies and surveillance into changes in the prevalence of these zoonotic pathogens and antimicrobial resistant bacteria in this population of dogs.

The use of a short, in-person questionnaire to collect information about pet demographic and management factors has both limitations and strengths. The short, one-page questionnaire used in this study allowed participants to complete it quickly and did not limit participation in the study. By having a short questionnaire, the number of potential risk factors that could be assessed was limited. Having a person administering the questionnaire may help increase participation simply by being present, as compared to the use of on-line or mail surveys (Franklin and Walker, 2003). The presence of a person can also help explain or clarify any unclear questions about the survey and/or the study. In addition, by providing the option of submitting a fecal sample without completing the questionnaire participation was increased, and helped to provide a clearer picture of the true prevalence of zoonotic organisms and the shedding of antimicrobial resistant organisms in dogs.

While there are benefits of having a person present, there are also some negative effects that should be considered. Firstly, a person is required to visit all the parks, irrespective of weather, to collect samples which can lead to considerable costs and time commitment to complete the study. Also, the presence of someone “watching” while participants complete the questionnaire may result in un-truthful responses toward socially acceptable answers, especially for sensitive questions (Franklin and Walker, 2003). For example, a participant may respond that they take their pet to the veterinarian regularly, or may not report a previous gastrointestinal illness. Confidentiality was assured in this study to minimize this effect.
Despite having a relatively large sample size for a small animal study, the power of this study was low for several exposures and pathogens of interest. For instance, use of probiotics (n=3) and consumption of commercial raw diet (n=1) had too few responses to include as variables in the models. A final consideration is the number of variables that were examined in this study. In this study, more than 50 items were investigated, which creates an increased probability of a type I error. This is a common issue with exploratory studies and the causal nature of specific variables should be considered in terms of consistency between past and future studies.

**Future research**

As this is the first study that has investigated dogs at dog parks, it is necessary to further explore this population to validate the results from this study and build evidence for the identified pet-related factors in this population. Future work should focus on describing the people who use these parks as well as the population of dogs that visit dog parks in different regions within provinces (urban vs. Rural), different provinces within Canada and different countries. This study did not collect extensive data on the potential exposures and risk factors due to the short questionnaire used, so a future study should aim to examine factors that were significant in this and other studies and perhaps some additional factors that have not yet been reported in the literature. For instance, data concerning owner socio-economic status, frequency of visits to the park, number of parks regularly visited and concurrent health status of owners were not collected in this study and may provide further insight into the relationship between humans and their dogs.
In a future study, water in dog parks could be tested for various pathogens, including *Campylobacter* spp. to determine if exposure at the parks is a risk. The survey did not specify where dogs had access to water, so it is not clear if it was water at the parks, or another source. Similarly, another factor that should be further studied is a dog’s exposure to compost. It was not specified if the dog had eaten compost or simply had access to it. Furthermore, the content of the compost was unknown and future studies could address this issue. It is possible that there was a can or bucket of compost material in the house or yard that the dog may be able to reach, or there may have been a compost pile in the yard that the dog could roll in. Additionally, the compost may be a mix of food scraps or may contain animal waste or manure. Compost samples could be collected to determine if these different types of compost present distinct exposures that could potentially affect the carriage of zoonotic pathogens and antimicrobial resistant organisms.

Further research into the transmission of antimicrobial resistant bacteria and genes between humans and dogs should be undertaken. By looking at the genetic similarities of antimicrobial resistant organisms in dogs and humans, the amount of sharing of antimicrobial resistance genes between the two species can be assessed.

**Surveillance**

Due to the large proportion of dogs shedding *Campylobacter upsaliensis* and antimicrobial resistant *E. coli*, surveillance systems should be developed to monitor the health burden of these organisms in dogs, and their effect on humans. In Canada, there are currently surveillance systems for gastro-intestinal pathogens (C-EnterNet) and antimicrobial resistance (Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS)) in humans. These systems, supported by the Public Health Agency of Canada, provide human-centric data.
While the CIPARS surveillance system also monitors antimicrobial use and resistance among agricultural animals, neither surveillance program collects data on companion animals and the health burden they may pose for Canadians.

**Conclusion**

This study demonstrated that although there are similarities between these results and results from studies focusing on dogs recruited at veterinary clinics or households, dogs that visit dog parks may have unique exposures that may affect their shedding of zoonotic pathogens and antimicrobial resistant bacteria. With more than 32% of Canadian homes shared with dogs (Perrin, 2009), and the increasing popularity of dog parks (Wang et al, 2012), this population of dogs should be considered when attempting to determine the overall health of a dog population, as well as its potential public health risk to humans. Dogs may play a large role in the lives of humans and can make our lives better in many ways, but when sharing a home with another species, there are also health risks that require awareness.
References


Appendix I:

Questionnaire used to obtain dog and owner information at dog parks in southern Ontario
(May-August 2009)
Salmonella, Campylobacter and Giardia in Dogs
Household ID #: ____________________ Dog ID # ________________

Dog Park _____________________________
Date: __________ Interviewed by: ________

Dog/Owner Assessment Questionnaire (Dog Parks)

Thank you very much for agreeing to participate in this study. The interview will last 10-15 minutes. Please feel free to stop me at any time. I would like to remind you that your responses will be very important to this study and will be kept confidential. Please let me know if there are any questions you do not wish to answer.

Owner Information
Sex: ______ Approximate Age: ______
City: ______________ Province: __________ Postal Code __________
Is your home: [ ] Urban [ ] Suburban [ ] Small town rural [ ] Non farm rural [ ] Farm
Type of farm ____________________________

Dog Information
Dog’s Name: ____________________________
Breed: __________________ Age: ______ Sex: ______ Neutered: ______
How long have you owned your dog? ____________ Number of dogs in household ________
How many other animals are in your home? __________________________________________
Where did you get your dog?
[ ] Pet Store [ ] Breeder [ ] Humane Society
[ ] Other __________________

Exposures, Diet, Medical History
1.1 Does your dog have access to farms with livestock?
[ ] Yes [ ] No [ ] Don’t Know
What type(s)________________________________________

1.2 Does your dog hunt and catch or eat prey?
[ ] Yes [ ] No [ ] Don’t Know
[ ] Small Rodents [ ] Birds [ ] Other____________________

1.3 Has your dog seen a veterinarian in the past year?
[ ] Yes [ ] No [ ] Don’t Know

1.4 Has your dog been dewormed in the past 6 months?
[ ] Yes [ ] No [ ] Don’t Know
If yes, which product____________________________________
Is it a year-round heartworm product? _________

1.5 Has your dog been treated with any of the following in the past month?
[ ] Antibiotics [ ] Probiotics/active bacterial culture [ ] Other medications/supplements
[ ] Don’t Know
If yes, what____________________________________________

1.6 Has your dog been diagnosed with Salmonella, Campylobacter, Giardia (Beaver Fever) or Clostridium difficile in the last 6 months?
[ ] Yes [ ] No [ ] Don’t Know
If yes, which one____________________________
Salmonella, Campylobacter and Giardia in Dogs
Household ID #: ________________ Dog ID #: ________________
Dog Park_____________________
Date: __________ Interviewed by: ______

1.7 Has your dog had any of the following gastro-intestinal upset signs during the last month?
Diarrhea (3 or more loose stools in 24 hours) [ ] Yes [ ] No [ ] Don’t Know
Vomiting (not regurgitation) [ ] Yes [ ] No [ ] Don’t Know

1.8 Has your dog had access to any of the following water sources, either for drinking or swimming, in the last 6 months?
[ ] Lakes, rivers, creeks [ ] Water in ditches, puddles [ ] Toilet
[ ] Other – please specify__________________________________________
[ ] No [ ] Don’t Know

1.9 What diet are you currently feeding your dog? (check all that apply)
[ ] store bought/commercial processed dry food
[ ] store bought/commercial processed canned food
[ ] homemade cooked
[ ] homemade raw
[ ] commercial cooked
[ ] commercial raw
[ ] combination
[ ] other (specify) ____________________________

1.10 How long have you been feeding the current diet to your dog?
___days ___weeks ___months ___years

1.11 Have you provided your dog with any of the following treats?:
[ ] Dried pig’s ears [ ] Raw Bones [ ] Cooked Bones [ ] Store bought bones
[ ] Rawhide chews [ ] Other pet treats, please specify______________________

1.12 Does your dog receive any raw meats or other animal products?
[ ] Yes [ ] No [ ] Don’t Know
How often [ ] daily [ ] weekly [ ] monthly [ ] rarely [ ] never
Specify what:__________________________________________

1.13 Does your dog participate in any of the following activities?
[ ] Off-leash with other dogs [ ] Hunting [ ] Dog day care
[ ] Obedience/agility/flyball [ ] Therapy/education programs
[ ] Kennelled/hospitalized in the last 6 months
[ ] other (specify) ____________________________

1.14 In the last 2 weeks has your dog eaten/had access to any of the following?
[ ] Garbage [ ] Licking plates/bowls [ ] Compost [ ] Cat litter
[ ] Dead Animals [ ] Animal Feces (excluding cat litter)
[ ] No [ ] Don’t Know

1.15 Has anyone in your family had any of the following gastro-intestinal upset signs during the preceding 30 days?
Diarrhea (3 or more loose stools in 24 hours) [ ] Yes [ ] No [ ] Don’t Know
Vomiting [ ] Yes [ ] No [ ] Don’t Know

Do you wish to be contacted with the results of this study? [ ] Yes [ ] No
Salmonella, Campylobacter and Giardia in Dogs
Household ID #: ___________________ Dog ID #: ______________
Dog Park: _______________________
Date: ___________ Interviewed by: ______

Contact Information

Name: ____________________________
Address: __________________________
City: ____________________ Province: __________ Postal Code: __________
Phone number: _________________ Email: ________________________________