
by

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In this study we investigated the prevalence of selected zoonotic pathogens (Salmonella and Campylobacter) and antimicrobial resistant bacteria (Salmonella spp. and generic Escherichia coli) in client-owned dogs in Southwestern Ontario. The pet-related risk factors for shedding Salmonella spp., Campylobacter spp., and antimicrobial resistant Salmonella spp. and generic Escherichia coli in the recruited pet dogs were also investigated. Pet-related data were collected through owner-administered questionnaires, and bacterial prevalence and antimicrobial resistance (AMR) data were obtained through repeated canine fecal samples. These data were evaluated using single-level and multilevel logistic regression models. The first cross-sectional study was conducted from October 2005 through May 2006, and involved 138 dogs from 84 households. Twenty-three percent of dogs had at least one fecal sample positive for Salmonella, and 25% of households had at least one dog shedding Salmonella. Statistically significant risk factors included contact with livestock, receiving a probiotic in the previous month, feeding a raw food diet or a homemade cooked diet, and having more than one dog in the household. Antimicrobial susceptibility testing was performed on 515 Salmonella and E. coli isolates recovered
from 136 dogs from 83 households. The majority of bacterial isolates (80%) were pan-susceptible and 11% were resistant to two or more antimicrobial classes. Multilevel logistic regression models with random intercepts for household and dog identified bacterial species, being fed a homemade diet, and being fed a raw diet as statistically significant risk factors for AMR in this population of dogs. The second cross-sectional study took place from July 2008 through May 2009; 240 client-owned pet dogs were recruited. The prevalence of *Campylobacter* spp. carriage in this population of pet dogs was 22%, with 19% positive for *C. upsaliensis*, and 3% positive for *C. jejuni*. Significant risk factors from multivariable logistic regression models were being fed a homemade cooked diet, dog age, and treatment with antibiotics in the previous month. Knowledge of the epidemiology of these zoonotic pathogens in pet dogs and the role of pet-related management factors is critical for controlling *Salmonella, Campylobacter* and AMR in pet dogs and for developing evidence-based pet ownership guidelines.
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STATEMENT OF WORK

All authors were involved in the study design for the research presented in this thesis. Dr. Scott McEwen also contributed to the study design of the *Salmonella/E. coli* study. Drs. David Pearl and J. Scott Weese were involved with writing the initial proposal and acquiring Ontario Veterinary College Pet Trust funding for *Campylobacter* sample processing for this project. Dr. Pearl also acquired funding from the Canada Foundation for Innovation and the Ontario Research Fund for the infrastructure for statistical analyses. The projects presented in this thesis were also funded in part by the Public Health Agency of Canada.

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INTRODUCTION

The domestic dog has been a companion to humans for tens of thousands of years (Parker, et al., 2004; Druzhkova, et al., 2013); throughout those years, their role in our lives has changed from one of guardian and hunting companion, to farm hand and most recently, pet and family member. It is estimated that there are over 6 million dogs in Canada, with at least 30-40% of households owning one or more dogs (Perrin, 2009; Stull, et al., 2012; Canadian Animal Health Institute, 2013). Beyond family pets, modern dogs are also used as service animals and therapy dogs, exposing new and potentially vulnerable populations of people to zoonotic pathogens (Robinson, 2000; Lefebvre, et al., 2006; Lefebvre, et al., 2008a; Mani and Maguire, 2009). The close relationships that most people share with their pet dogs and the increased exposure of vulnerable populations to dogs requires research into the potential zoonotic risks associated with dog contact.

Dogs can carry numerous zoonotic pathogens (i.e., infections that can spread from animals to humans), including several species of bacteria, parasites, viruses, and fungi. Some of the zoonotic diseases of concern include salmonellosis, campylobacteriosis, giardiasis, rabies, vector-borne zoonoses, including Leishmaniasis, zoonotic helminths, and zoonotic dermatoses such as ringworm.
(Greene, 2012; Ballweber, et al., 2010; Chomel and Sun, 2011; Deplazes, et al., 2011; Weese, 2011; Stull, et al., 2012). Bacterial antimicrobial resistance is an increasingly serious concern in both human and animal, which given the close relationship we share with our pets, is yet another zoonotic infectious disease risk that requires thorough study, understanding, and consideration (Guardabassi, et al., 2004; Clarke, 2006; Weese, 2008; Umber and Bender, 2009).

However, despite the multiple potential zoonotic disease risks, there are many potential benefits to having dogs in our lives. Those benefits include companionship, physical activity, protection, improved mental health and stress relief, animal-assisted therapies, and increased independence for those with disabilities (Edney, 1995; Jennings, 1997; Hodgson and Darling, 2011b; Beetz, et al., 2012a; Beetz, et al., 2012b). The key to balancing the benefits and risks of dog ownership is comprehensive consideration of pet-related management factors, such as diet, environmental exposures, proper veterinary care, and owner-related factors, such as immune competency, age and health status. In addition, since dogs share the same environments, foods, and many of the possible infectious disease exposures as people, they are a potentially rich source of information for public health surveillance, including surveillance related to enteric bacteria, emerging infectious diseases, antimicrobial resistance and environmental contaminants (Kile, et al., 2005; Gubernot, et al., 2008; Maciejewski, et al., 2008; Moore and Lund, 2009; Schmidt, 2009; Reif, 2011).
In this thesis, the prevalence and related risk factors of several enteric zoonotic bacteria of public health importance in two populations of pet dogs in Ontario, Canada are investigated. The study focused on *Salmonella*, *Campylobacter*, the occurrence of antimicrobial resistance, and the pet-related management factors that may be associated with increased or decreased risk of shedding these bacteria pet dogs. Multilevel statistical modeling was used to examine the potential associations and effects of these risk factors at the individual dog and household level. Given the close relationship that most owners have with their dogs, understanding the role that pet dogs may play in human zoonotic infections is crucial for the development of evidence-based guidelines for safe dog ownership, and to protect the public through responsible pet management. The following literature review is intended to provide a brief introduction to the following areas:

1) Key companion animal zoonoses, with a focus on pet dogs and enteric bacteria;

2) *Salmonella* and *Campylobacter* infections in humans and their public health significance;

3) The epidemiology of *Salmonella* and *Campylobacter* in dogs and their potential role in human infections;

4) The public health implications of antimicrobial resistance in humans and dogs, with a focus on *Salmonella* and generic *Escherichia coli*; and

5) The use of multilevel models in companion animal research.
LITERATURE REVIEW

Public Health Implications of Zoonotic Pathogens in Dogs

Zoonotic diseases, or zoonoses, are infectious diseases that can spread between animals and humans (Stehr-Green and Schantz, 1987; Macpherson, et al., 2000; Acha and Szyfres, 2001c; Weese and Fulford, 2011a). Given the large population of pet dogs in Canada, the high percentage of households owning a dog (Perrin, 2009; Stull, et al., 2012; Canadian Animal Health Institute, 2013), and the fact that many dogs are considered family members, concerns about the transmission of zoonotic infections between dogs and humans have been raised. However, dogs also provide numerous physical, social and psychological benefits through their relationships with humans, including improved physical and mental health, protection, and increased independence (Lefebvre, et al., 2006; Lefebvre, et al., 2008a; Hodgson and Darling, 2011b; Silveira, et al., 2011; Beetz, et al., 2012b).

Dog-related zoonoses are an important public health issue, as dogs are capable of carrying many types of pathogens, such as bacteria, viruses, parasites, and fungi, including several common enteric and non-enteric infectious diseases. Domestic dogs have long been recognized as potential sources of several zoonotic enteric pathogens (Bisseru, 1967; Beran and Steele, 1994; Acha and Szyfres, 2001a), and oftentimes, dogs are subclinical carriers of these pathogens, making the extent of their true risk to human health difficult to measure (Hackett and Lappin, 2003; Weese and Fulford,
In companion animal practice, veterinarians tend to be concerned with only a
handful of zoonotic diseases, most importantly rabies, parasitic worms (roundworm,
hookworm, etc.), dermatophytosis (ringworm), and some bacterial pathogens
(*Leptospira, Brucella*, etc.) (Glickman, et al., 2006; Mani and Maguire, 2009; Weese
and Fulford, 2011b; Weese, et al., 2011). Rabies in humans is very rare in Canada,
with only 5 human cases in Canada since 1985 (Government of Canada, 2008b;
Government of Canada, 2012c); and it is uncommon in domestic animals, with only
16 infected dogs, and 2 each of infected cats, cattle and horses reported in 2012
(Government of Canada, 2012c) due to routine rabies vaccination of domestic animals
and control of pet populations. However, even though rabies is rare, due to the lethal
nature of the disease, and continually reported cases in wildlife, it is an important
public health concern in Canada.

Enteric parasites (e.g. *Toxocara, Giardia*) are another example of important
zoonoses emphasized in veterinary training and in practice, most often from the
perspective of animal health, even though these organisms can cause infections in
humans. Again, the occurrences of human parasitic infections related to companion
animal exposure (visceral, ocular and cutaneous larva migrans, giardiasis) in Canada
are extremely rare (Weese, et al., 2011; Lee, et al., 2010). However, parasitic worms
like *Toxocara* spp. are commonly found in companion animals, especially puppies and
kittens, in North America (Lee, et al., 2010; Weese, et al., 2011; Bolivar-Mejia, et al.,
2014), and preventative treatment is widely advocated (Hotez, 2008; CDC, 2013).

In recent years, the trend of feeding raw and homemade diets, and treats made from dehydrated animal parts (e.g., pig ears, rawhide) to pet dogs has increased in popularity (Lenz, et al., 2009; Schlesinger and Joffe, 2011). These feeding practices bring about new public health risks, including increased risk of exposure to enteric pathogens like *Salmonella, Campylobacter* and verotoxogenic *E. coli* (LeJeune and Hancock, 2001; Weese, et al., 2005; Finley, et al., 2006; Finley, et al., 2007; Lefebvre, et al., 2008b), and outbreaks of salmonellosis have been linked to exposure to rawhide and pig ear treats (Health Canada, 2000; Clark, et al., 2001; CDC, 2006). As well, given the popularity of these diets and treats, contamination of the household environment, especially the kitchen, may lead to exposure of especially vulnerable populations, including young children, the elderly and the immunocompromised (Kusumaningrum, et al., 2004; Redmond, et al., 2004). Proper hand and household hygiene, particularly for children, are of paramount importance in these situations and require guidance and recommendations for clients from small animal veterinarians, including discouraging feeding these diets in many circumstances (Robinson and Pugh, 2002; Lefebvre, et al., 2008b; Mani and Maguire, 2009; Stull, et al., 2012).

A new and concerning public health risk in companion animal medicine that is often underappreciated is antimicrobial resistance (AMR) (Van Immerseel, et al., 2004; Finley, et al., 2008a; Weese, 2008; Jackson, et al., 2009; Umber and Bender, 2009; Gibson, et al., 2011b; Ewers, et al., 2012). Given the closeness of the
relationship that most people share with their pets and the fact that many of the same antimicrobials are used in human and veterinary medicine, the potential exchange of resistant bacteria between dogs and their owners could potentially be quite large. Records of antimicrobial use and surveillance of AMR infections in pets are often insufficient, making it very difficult to determine the true role that pets play in the dissemination, carriage and transmission of antimicrobial resistant organisms to humans (Guardabassi, et al., 2004; Weese, 2008; Moore and Lund, 2009; Umber and Bender, 2009; Boothe, et al., 2012; Day, et al., 2012).

Veterinarians increasingly need to be aware of the public health risks for specific segments of the population that are of increased concern in relation to pet-related zoonoses; these include the very young (under 5 years old), the elderly, the immunocompromised, those with chronic diseases, and pregnant women (Lefebvre, et al., 2008a; Hodgson and Darling, 2011a; Hodgson and Darling, 2011b). Dogs are a popular animal used in hospital-based visitation programs, animal assisted therapy and as service animals, potentially exposing some of the most vulnerable populations to zoonotic pathogens, some of which might be resistant to antimicrobials used for treating human infections. Increased precautions and improved hygiene are of particular importance for these programs to lower the risk of transmitting any zoonotic infectious agents to potentially immunocompromised patients. However, given the many benefits derived from these animals, a balance needs to be achieved between the risks and advantages of exposure to pet, service, and therapy animals. Companion
animals, such as dogs, can also serve as valuable sentinels for infectious diseases, like Lyme disease, and emerging diseases not previously observed in the clinical setting (Day, et al., 2012; Smith and Whitfield, 2012).

The estimated burden of human illness from companion animal zoonoses is difficult to determine due to the lack of surveillance in companion animals (in part because many of the diseases are not nationally notifiable and there is often underreporting), the fact that often only serious human cases receive medical attention and diagnosis, and the actual source of these infections is difficult to determine because they have multiple potential origins, including animals (Stehr-Green and Schantz, 1987; Government of Canada, 2013b). Utilizing available zoonotic disease data in the late 1980s, Stehr-Green and Schantz estimated that the economic cost of pet-related zoonoses could surpass $300 million USD each year; this estimate includes enteric pathogens, non-enteric viruses and other pathogens (Stehr-Green and Schantz, 1987). More recently, Hale et al (2012) estimated that animal contact accounted for 14% of enteric illness in the United States, but the proportion specifically attributable to pet contact was not determined. More detailed estimates of the extent of companion animal zoonoses are not readily available, and due to the large amount of underreporting and difficulty in source attribution, any available data are likely to underestimate the burden of pet dog zoonoses. In recent years, calls have been made to increase companion animal surveillance (Day, et al., 2012; Smith and Whitfield, 2012), which would help to provide estimates of the real burden of companion animal
zoonoses and their associated economic costs.

Public health risks of *Salmonella* in humans

Human salmonellosis in North America is relatively common and *Salmonella* is one of the most frequently reported gastrointestinal pathogens from national surveillance programs, with approximately 5000 - 7000 cases of salmonellosis reported each year in Canada (Government of Canada, 2012d; Government of Canada, 2013a). However, *Salmonella* cases often go unreported and the true incidence of salmonellosis in Canada may actually be closer to hundreds of thousands of cases each year (Majowicz, et al., 2005; Thomas, et al., 2006b; Thomas, et al., 2013). *Salmonella* infection in humans can have a broad assortment of clinical signs and sequelae, ranging from asymptomatic carriage and self-resolving diarrhea, to chronic carrier states and severe clinical disease, including reactive arthritis and death (Keusch, 2001; Buxton, et al., 2002). Salmonellosis in otherwise healthy adults usually results in a self-limiting infection that does not require antimicrobial treatment, but children, the elderly, pregnant women and immunocompromised individuals frequently require treatment and infection in these individuals can be very severe (Keusch, 2001). *Salmonella* can be spread to humans through contaminated food, water, or contact with infected animals and their environments; however it is suspected that the majority of human cases are likely foodborne (Ravel, et al., 2010; Dumoulin, et al., 2012; Nesbitt, et al., 2012). The majority of *Salmonella* cases are
sporadic, often go unreported, and many of the sources of infection are not identified. Consequently, the proportion of cases that could be due to contact with dogs or contaminated dog food products is unclear (Finley, et al., 2006; Domingues, et al., 2012b; Ziehm, et al., 2012). Feldman and Riley (1984) estimated that about 1% of human salmonellosis cases were associated with contact with companion animals, and recent estimates from the Centers for Disease Control and Prevention in the United States (US CDC) indicate that animal contact, including contact with pets, may account for 6% of reported salmonellosis cases in humans (Hale, et al., 2012).

Among the 5000-7000 *Salmonella* cases reported annually to the National Enteric Surveillance Program of Canada (NESP), the three most common *Salmonella* serotypes are *S. enterica* subsp. *enterica* serotype Enteritidis, *S. Typhimurium* and *S. Heidelberg* (Government of Canada, 2012d). *Salmonella* Enteritidis is often associated with outbreaks, poultry products and travel (Nesbitt, et al., 2012). *Salmonella Typhimurium* is commonly isolated from poultry products, and less commonly from beef, pork, water and contaminated produce (Lapidot and Yaron, 2009; Liu, et al., 2009; Semenov, et al., 2009; Government of Canada, 2010); and *S. Heidelberg* is also commonly found in chicken, eggs and other poultry products (Andrysiak, et al., 2008; Government of Canada, 2008a).

The economic burden of gastrointestinal infections, including salmonellosis in humans, can be quite large, with some estimates suggesting a cost of about $120-$150 per capita per year, resulting in millions of dollars per year in hospital expenses and
losses in productivity (Majowicz, et al., 2006; Thomas, et al., 2006a; Henson, et al., 2008; Ruzante, et al., 2010). Voetsch et al. (2004) estimated there were approximately 1.4 million non-typhoidal *Salmonella* infections in the United States per year, resulting in 168,000 physician office visits per year (Voetsch, et al., 2004). In Canada, there are approximately 19-26 cases per 100,000 residents per year, with approximately 13% of these requiring hospitalization, with higher percentages of hospitalization in the very young, the elderly and the immunocompromised (Keegan, et al., 2009; Ruzante, et al., 2010).

Several outbreaks of human salmonellosis that have been reported in the last 10-15 years have been traced back to exposure to contaminated pet food and pet treat products (Health Canada, 2000; Clark, et al., 2001; CDC, 2006; CDC, 2008b; CDC, 2008c; Behravesh, et al., 2010; CDC, 2012; Hale, et al., 2012). Additionally, pet dogs have been directly linked with a handful of cases of human salmonellosis (Morse, et al., 1976; Sato, et al., 2000; Cherry, et al., 2004; Wright, et al., 2005), and contact with dogs was identified as a significant risk factor for human infection with *Salmonella* Enteriditis in the province of Ontario (Varga, et al., 2012).

**Epidemiology of Salmonella in dogs**

The prevalence of *Salmonella* isolation from clinically healthy dogs is normally low, and has been estimated to be between 0 - 4%, although it can be closer to 6% in stray dogs and over 30% in hospitalized dogs (Cantor, et al., 1997; Cave, et
al., 2002; Hackett and Lappin, 2003; Marks and Kather, 2003; Sokolow, et al., 2005; Bagcigil, et al., 2007; Tsai, et al., 2007; Greene, 2012). Even higher prevalences have been documented among certain populations of dogs, such as sled dogs and greyhounds, where raw meat and animal products make up a large proportion of their diet (Chengappa, et al., 1993; Stone, et al., 1993; Cantor, et al., 1997; Morley, et al., 2006). Dogs are often subclinical carriers of *Salmonella* (McDonough and Simpson, 1996; Sanchez, et al., 2002; Bagcigil, et al., 2007; Rabinowitz and Conti, 2010; Greene, 2012) so it is uncertain how commonly the pathogen is shed by pet dogs. However, puppies, immunocompromised dogs and stressed animals may develop clinical signs of salmonellosis, including diarrhea and septicemia (Rabinowitz and Conti, 2010; Greene, 2012). The most prevalent serotypes of *Salmonella* found in dogs globally are *Salmonella Typhimurium* and *Salmonella Enteritidis* (Carter and Quinn, 2000; Greene, 2012). Dogs frequently shed *Salmonella* intermittently (Bagcigil, et al., 2007; Finley, et al., 2007; Weese and Fulford, 2011a; Greene, 2012), making recovery of the pathogen difficult.

A number of pet-related management factors, such as diet fed, pet treats, and animal exposure, are important when considering *Salmonella* carriage in pet dogs. Raw diets (i.e., those composed of raw meat, bones and produce) are becoming increasingly popular in North American pet dog populations and recently have been associated with the carriage of *Salmonella* in dogs (Joffe and Schlesinger, 2002; Finley, et al., 2006; Finley, et al., 2007; Lefebvre, et al., 2008b; Lenz, et al., 2009;
Schlesinger and Joffe, 2011); several studies have investigated the contamination of these diets and their ingredients (Chengappa, et al., 1993; Lister, 1997; Freeman and Michel, 2001; LeJeune and Hancock, 2001; Joffe and Schlesinger, 2002; Weese, et al., 2005; CDC, 2006; Morley, et al., 2006; Strohmeyer, et al., 2006). Raw meat and raw animal products, most frequently chicken and turkey, and occasionally beef, pork and eggs, are contaminated with *Salmonella* (Government of Canada, 2007b; Government of Canada, 2008a), and consequently, commercial and/or homemade raw diets made with these products are a potential source of *Salmonella*. As well, with respect to homemade diets and commercial raw diets, improper food handling and preparation by dog owners may potentially increase the spread of *Salmonella* in food preparation areas and lead to contamination of raw and cooked foods fed to pet dogs (Parry, et al., 2002; Kusumaningrum, et al., 2004; Parry, et al., 2005; Luber, 2009; Redmond and Griffith, 2009). *Salmonella* can be recovered from pet treats and rawhide treats fed to pet dogs (Peart, 1999; Willis, 2001; Pitout, et al., 2003; White, et al., 2003; Wong, et al., 2007; Adley, et al., 2011; Kukanich, 2011; Freeman, et al., 2013). Livestock exposure (e.g., cattle, pigs, horses, and poultry) or contact with their feces or contaminated environments may lead to increased *Salmonella* exposure in pet dogs, as these animals have been shown to commonly carry *Salmonella* (LeJeune and Hancock, 2001; Liebana, et al., 2002). Previous research has also found that having multiple dogs in a household may lead to *Salmonella* spread between dogs, and outbreaks of salmonellosis in dogs have been linked to group housing and dog-to-dog
transmission (Cherry, et al., 2004; Morley, et al., 2006; Schotte, et al., 2007).

**Public health risks of Campylobacter in humans**

Campylobacteriosis is one of the most common bacterial causes of enteric disease in humans worldwide, as well as in Canada, with approximately 9,000-10,000 laboratory-confirmed cases each year in Canada, representing about one third of all reported enteric illnesses (Government of Canada, 2007c; Government of Canada, 2012b; Government of Canada, 2013a). As with Salmonella, many Campylobacter cases go unreported; the true incidence of campylobacteriosis in Canada may actually be closer to hundreds of thousands of cases each year, with approximately 13 to 37 cases in the community for each case that is reported (Majowicz, et al., 2005; Thomas, et al., 2006b; Thomas, et al., 2013). Many reported cases of campylobacteriosis are food or travel related, and the most commonly recovered species of Campylobacter from humans is *C. jejuni*, followed distantly by *C. coli, C. upsaliensis* and *C. fetus* (Lee, et al., 2004; Government of Canada, 2007a; Government of Canada, 2007b; Government of Canada, 2007c; Karenlampi, et al., 2007; Inglis, et al., 2011; Bullman, et al., 2012; Government of Canada, 2012b; Government of Canada, 2012d; Government of Canada, 2013a). Similar to *Salmonella* infections, companion animals can be a potential source of infection, but since the majority of Campylobacter cases are sporadic, often go unreported, and many of the sources of infection are not identified (Thomas, et al., 2006b), the number of infections that are pet-related is
likely underestimated (Weese, 2011; Weese and Fulford, 2011a).

Campylobacteriosis is a gastrointestinal infection that is usually mild to severe in humans, causing nausea, vomiting and watery diarrhea, but long-term and potentially life-threatening sequelae such as Guillain-Barré syndrome, reactive arthritis, and post-infection irritable bowel syndrome can occur (Blaser, 2001; Altekruse and Tollefson, 2003; CDC, 2008a; Thabane and Marshall, 2009). In a recent study using laboratory confirmed cases reported in Canada, over two thirds of patients reported moderate to severe symptoms, occurring on average for more than a week (Deckert, et al., 2013). In another recent Canadian study, approximately 5% of reported campylobacteriosis cases were hospitalized, with hospitalization occurring more frequently in the very young and elderly (Ruzante, et al., 2010).

While the majority of *Campylobacter* cases in Canada are sporadic and believed to be foodborne, several studies in the 1980s from other countries began examining the role of companion animals as potential sources of human campylobacteriosis (Blaser, et al., 1980; Fox, et al., 1983; Hopkins, et al., 1984). Many international studies in the past 20 years have identified that having a household pet, especially a puppy or kitten, or a pet with diarrhea, are significant risk factors for *Campylobacter* infection in humans (Goossens, et al., 1991; Kapperud, et al., 1992; Adak, et al., 1995; Hald and Madsen, 1997; Tenkate and Stafford, 2001; Neimann, et al., 2003; Damborg, et al., 2004; Friedman, et al., 2004; Carrique-Mas, et al., 2005; Fullerton, et al., 2007; Damborg, et al., 2008; Stafford, et al., 2008; Buettner, et al.,
It is estimated that contact with pet dogs and cats may account for approximately 5% of campylobacteriosis cases in humans internationally (Altekruse and Tollefson, 2003; Domingues, et al., 2012a), and recent estimates from the US CDC state that animal contact in general, including contact with pets, may account for an average of 17% of reported campylobacteriosis in humans (Hale, et al., 2012). Dogs have also been suspected as the direct source of infection in several human cases of campylobacteriosis (Fox, et al., 1983; Goossens, et al., 1991; Jimenez, et al., 1999; Wolfs, et al., 2001; Damborg, et al., 2004).

**Epidemiology of Campylobacter in Dogs**

Dogs are often healthy carriers of *Campylobacter*, and the bacteria has been recovered from approximately 3-75% of clinically healthy pet dogs (Hald and Madsen, 1997; Baker, et al., 1999; Sandberg, et al., 2002; Hald, et al., 2004; Wieland, et al., 2005; Tsai, et al., 2007; Damborg, et al., 2008; Acke, et al., 2009b; Westgarth, et al., 2009; Chaban, et al., 2010; Parsons, et al., 2010), and up to 80% of stray animals (Fernandez and Martin, 1991; Acke, et al., 2006). *Campylobacter* can cause both clinical and non-clinical disease in dogs, with the most severe infections occurring in young and immunocompromised dogs, often associated with a *C. jejuni* infection (Fox, et al., 1983; Fox, 2012; Weese and Fulford, 2011a). Treatment of clinical campylobacteriosis with antimicrobials is controversial in animals, as it is in
humans, with the recommendation for antimicrobial therapy to be reserved for moderate and severe cases, or those animals with underlying health issues (Marks, et al., 2011; Weese, 2011; Weese and Fulford, 2011a; Greene, 2012).

The most frequently recovered species of *Campylobacter* in dogs is *C. upsaliensis*, followed by *C. jejuni*, *C. coli*, and *C. lari* (Hald, et al., 2004; Modolo and Giuffrida, 2004; Rossi, et al., 2008; Acke, et al., 2009a; Acke, et al., 2009b; Koene, et al., 2009; Westgarth, et al., 2009; Chaban, et al., 2010; Parsons, et al., 2010; Carbonero, et al., 2012; Parsons, et al., 2012; Gras, et al., 2013). However, the predominant *Campylobacter* species recovered from dogs often depends on the culture and isolation methods used because *C. upsaliensis* is much more sensitive to antimicrobials and environmental conditions than other species of *Campylobacter* (Bourke, et al., 1998; Lastovica and le Roux, 2000; Lastovica and Skirrow, 2000; Labarca, et al., 2002; Lastovica and Le Roux, 2003; Acke, et al., 2009a).

Some of the common risk factors and predictors identified for *Campylobacter* carriage in dogs include young age (< 1 year old), the presence of diarrhea, season of the year (autumn, spring and summer), and high density housing, such as kennels and shelters (Torre and Tello, 1993; Baker, et al., 1999; Sandberg, et al., 2002; Hald, et al., 2004; Modolo and Giuffrida, 2004; Wieland, et al., 2005; Acke, et al., 2006; Acke, et al., 2009b; Westgarth, et al., 2009; Chaban, et al., 2010; Parsons, et al., 2010). As a dog matures, the occurrence of *Campylobacter* carriage has generally been found to decrease (Fox, et al., 1983; Hald, et al., 2004).
**Campylobacter upsaliensis as a human pathogen**

Dogs and cats are believed to be the only reservoir hosts for *C. upsaliensis* (Bourke, et al., 1998). However, *C. upsaliensis* has recently been recovered from chickens at slaughter in Africa and Asia, and minced beef in Ireland (Lynch, et al., 2011; Garin, et al., 2012). *Campylobacter upsaliensis* is increasingly being recognized as a human pathogen of importance (Patton, et al., 1989; Labarca, et al., 2002; Lastovica and Le Roux, 2003; Bullman, et al., 2012; Couturier, et al., 2012), yet due to very limited information on sources of *C. upsaliensis* other than dogs and cats, it is difficult to determine their contribution to the burden of *C. upsaliensis* in human infection (Lynch, et al., 2011; Garin, et al., 2012). As a result of current laboratory methods used for the detection of *C. jejuni* and *C. coli* (i.e., catalase positive *Campylobacter* spp.), numerous cases of *C. upsaliensis* may go undetected, and it is possible that *C. upsaliensis* is more widespread in food sources and is a more frequent cause of human campylobacteriosis than is currently recognized. *Campylobacter* spp. isolation methods for human feces and food samples often involve media that contain cefaperazone, nalidixic acid and cephalothin at levels that prevent the growth of *C. upsaliensis* (Bourke, et al., 1998). Interestingly, Lastovica and Le Roux (2003) found that almost 25% of human campylobacteriosis cases in South Africa were due to *C. upsaliensis*, through use of a filtration method (Lastovica and Le Roux, 2003), and studies from the United States and Belgium suggest that *C. upsaliensis* is the second most commonly isolated *Campylobacter* spp. in humans, after *C. jejuni* (Goossens, et
al., 1991; Labarca, et al., 2002). *Campylobacter upsaliensis* may also be more common in Canadian human infections (Taylor, et al., 1989; Bourke, et al., 1998) and further research into the role of *C. upsaliensis* in human gastrointestinal disease, the potential sources of *C. upsaliensis* in human infection, and the impact on surveillance from changing laboratory methods for the recovery of *C. upsaliensis* is warranted.

**Public health implications of antimicrobial resistant bacteria in companion animals**

Antimicrobial resistance (AMR) in bacteria in animals is a concern globally, and companion animals are a potential reservoir for resistant bacteria (Guardabassi, et al., 2004; Boerlin and Reid-Smith, 2008; Acar and Moulin, 2012). Due to a lack of information about antimicrobial resistant bacteria in pets, such as cats and dogs, the true role of companion animals in the spread of resistant bacteria to humans is poorly understood (Normand, et al., 2000; Guardabassi, et al., 2004; Wright, et al., 2005; Lloyd, 2007; Weese, 2008). Antimicrobial resistant bacteria in companion animals is difficult to study, due to a lack of surveillance and routine testing (Lloyd, 2007; Murphy, et al., 2009). As early as 1978, researchers in Australia found that antimicrobial resistance among bacteria in human and pet populations was similar, and suggested that resistant organisms could emerge in one population and act as a source of infection for the other (Davies and Stewart, 1978). Recent studies have begun to shed more light on the occurrence of antimicrobial resistant bacteria in pets.
(Authier, et al., 2006; Ogeer-Gyles, et al., 2006; Costa, et al., 2008; Lefebvre, et al., 2008b; Acke, et al., 2009c; Murphy, et al., 2009). It has also been suggested that pets can and often do share resistant bacteria with their owners, and that they may act as a reservoir for human infection (Prescott, 2008). In a study by Johnson et al. (2006), transmission of multidrug-resistant E. coli and Enterococcus faecalis from a dog to its owner through a bite wound was reported. Furthermore, pet owners and their dogs in the same household were found to carry similar urinary E. coli clones, suggesting possible within-household transmission of E. coli between pet owners and dogs (Damborg, et al., 2009). As well, an outbreak of antimicrobial resistant Salmonella occurred at three veterinary clinics in the United States in 1999 and 2000, with infections occurring in animals, pet owners and clinic staff (Wright, et al., 2005).

Because of the close relationship most people have with their pets and the frequent contact between dogs and humans, antimicrobial resistance in zoonotic pathogens of companion animals is of great importance and a significant public health issue (Sato, et al., 2000; Acha and Szyfres, 2001a; Acha and Szyfres, 2001b; Cherry, et al., 2004; Guardabassi, et al., 2004; Clarke, 2006; Boerlin and Reid-Smith, 2008; Jackson, et al., 2009). The close contact is also of particular concern to owners and veterinary staff that may be immunocompromised, as well as those patients who come in contact with dogs as part of therapy visitation programs (Lefebvre, et al., 2006; Lefebvre, et al., 2008a; Lefebvre, et al., 2008b).

It is estimated that 70-90% of antimicrobials sold in many developed countries
are used in animals, with the majority of this amount being used in food animal production, but the proportion used in companion animals is generally unknown (Food and Drug Administration, 2011; Government of Canada, 2011; Food and Drug Administration, 2012). However, the Danish AMR surveillance system (DANMAP) has recently estimated that approximately 2.5% of all antimicrobials sold in Denmark are intended for use in companion animals (DANMAP, 2011). Not only are antimicrobials commonly used in human medicine also used in companion animals, but antimicrobials used in animals which might not be used in human medicine may belong to the same classes of antimicrobials used to treat human infection. This relationship between antimicrobials used in animals and humans is a concern due to the correlation of resistance that can be seen to different drugs within the same class. Of public health concern are findings from recent studies which have determined that close contact between humans and pets can lead to the exchange of pathogenic bacteria, including those carrying AMR genes (Guardabassi, et al., 2004; Clarke, 2006; Johnson, et al., 2006; Skurnik, et al., 2006).

**AMR in dogs focusing on *Salmonella* spp. and generic *E. coli***

Non-human animals, primarily livestock, are considered the main reservoir for *Salmonella* spp. causing infection in people, with the majority of human cases believed to be foodborne (Finley, et al., 2006; DuPont, 2009; Government of Canada, 2012a; Nesbitt, et al., 2012; Polpakdee, et al., 2012). However, *Salmonella* isolates
have also been recovered from other sources such as veterinary hospitals and shelter environments (Murphy, et al., 2010; Steneroden, et al., 2011). Until recently, very few studies have investigated the role of pet dogs as a potential source of antimicrobial resistant bacteria in humans. The majority of studies have focused on food animals and farm environments, since food animals are believed to be the major source of resistant strains of non-typhoidal *Salmonella* (van den Bogaard and Stobberingh, 1999; van den Bogaard and Stobberingh, 2000). Antimicrobial resistant *Salmonella* isolates, including several multidrug-resistant (MDR) isolates and isolates resistant to cephalosporins and fluoroquinolones have, however, been found in dogs and other companion animals (Guardabassi, et al., 2004; Lloyd, 2007; Umber and Bender, 2009).

Antimicrobial resistance in commensal and pathogenic *E. coli* has also been found to be prevalent in dogs in recent Canadian and European studies (De Graef, et al., 2004; Rantala, et al., 2004; Pedersen, et al., 2007; Murphy, et al., 2009; NORM/NORM-VET, 2009), with several studies reporting resistance to at least one antimicrobial in canine *E. coli* isolates from Canada, the United States, the United Kingdom and South Korea (Authier, et al., 2006; Ogeer-Gyles, et al., 2006; Ball, et al., 2008; Nam, et al., 2010; Shaheen, et al., 2010; Wedley, et al., 2011). However, it should be noted that the majority of these studies and reports investigated clinical samples of *E. coli* rather than fecal samples from healthy dogs (Prescott, et al., 2002; Weese, 2008), and therefore may not be representative of the healthy canine
Recent studies have identified previous treatment with antimicrobials and recent hospitalization as risk factors for the carriage of antimicrobial resistant *Salmonella* and *E. coli* in pet dogs (Rantala, et al., 2004; Aarestrup, 2006; Mentula, et al., 2006; Ogeer-Gyles, et al., 2006; Damborg, et al., 2008; Gronvold, et al., 2010; Gibson, et al., 2011a; Gibson, et al., 2011b). Another potential source of antimicrobial resistant bacteria in pet dogs is the raw animal products fed to pets or raw diets made with these products (Finley, et al., 2008a; Finley, et al., 2008b); resistant *Salmonella* spp. and *E. coli* are commonly recovered from raw meat and other animal products in Canada, including chicken, and less commonly beef, pork and turkey (Government of Canada, 2010; Government of Canada, 2011). In 2008, *E. coli* with resistance to 1 or more antimicrobial was detected in 20-30% of retail beef samples, 40-50% of retail pork samples and 60-80% of retail chicken samples from across Canada; *Salmonella* spp. with resistance to at least one antimicrobial were recovered from 40-50% of retail chicken meat samples and almost 70% of retail pork samples collected in the same timeframe (Government of Canada, 2011). Antimicrobial resistant *Salmonella* and *E. coli* have also been recovered directly from commercially-prepared raw diets and rawhide pet treats available in Canada and the United States (Strohmeyer, et al., 2006; Finley, et al., 2008a; Finley, et al., 2008b), and raw meat consumption has been found to be significantly associated with multidrug resistant *E. coli* carriage in therapy dogs (Lefebvre, et al., 2008b). Finally, type of housing has been found to be another risk
factor for resistant bacteria carriage by dogs, where kenneled dogs were more likely than dogs from private homes to carry resistant *E. coli* (De Graef, et al., 2004; Harada, et al., 2011).

Due to antimicrobial use in veterinary and human medicine, commensal bacteria in animals and humans, particularly enteric bacteria, are constantly subjected to antimicrobial pressure (Skurnik, et al., 2006). *Escherichia coli*, a ubiquitous commensal bacterium, is considered a major potential source of antimicrobial resistance genes (Weese, 2008; Marshall, et al., 2009). Recently, the prevalence of resistance to at least one antimicrobial in *E. coli* isolates from healthy pet animals has been estimated to be between 10% and 30% from surveillance and scientific studies in Europe and Canada (Skurnik, et al., 2006; Murphy, et al., 2009; NORM/NORM-VET 2008, 2009). Because *E. coli* is easily and inexpensively recovered in most animals and is a reservoir of antimicrobial resistance genes, generic *E. coli* is frequently used as an indicator organism for AMR surveillance (Varga, et al., 2008; Weese, 2008; Marshall, et al., 2009). Unfortunately, in some cases, *E. coli* was found to be generally poor at predicting the AMR patterns of pathogenic bacteria, such as *Salmonella*, recovered from the same animal (Varga, et al., 2008). However, generic *E. coli* can still be used to monitor potential emerging resistance issues which might be transferred to pathogenic bacteria.
Multilevel Modeling in Companion Animal Research

Multilevel modeling is a statistical tool used to account for the fact that individual animals in common groups, such as chickens in a flock, puppies in a litter or cows in a herd, often share common characteristics and exposures (Diez Roux and Aiello, 2005; Dohoo, et al., 2009). Clustering of disease status is also typical in most infectious diseases and must be accounted for in the analysis if multiple individuals are taken from a common litter, home or herd (Dohoo, et al., 2003; Diez Roux and Aiello, 2005). Multilevel models allow researchers to account for the lack of independence of clustered individuals and can also account for repeated measurements from the same animal or groups over time (Dohoo, et al., 2009; Rothman, et al., 2012). Random effects are included in the model to account for the impact of a common group or cluster in estimating the size of the effect and standard errors for a particular exposure. The clustering that often occurs in these common groups must be taken into account in order to obtain valid estimates of the effects of interest in the study, otherwise the size of standard errors may be incorrect and is most often underestimated (Bingenheimer and Raudenbush, 2004; Dohoo, et al., 2009).

Multilevel modeling also allows for the concurrent examination of individual and group level risk factors. In addition, for random intercept models, researchers can estimate the variance partition coefficients (VPCs) and determine the level at which most of the variance is explained. These VPCs can be used to target interventions at the level where they will have the most impact (Bonten, et al., 1998; Dohoo, et al.,
2001; Dohoo, et al., 2009).

Whereas multilevel models can provide more accurate estimates of standard errors and model coefficients by accounting for clustering by group, they do come with drawbacks; multilevel models are more complex than traditional models (i.e., less parsimonious), and often require larger databases for models to converge (De Leeuw and Kreft, 1986; de Leeuw and Kreft, 1995). As well, variance estimates, sample size calculations and power calculations become more complicated for multilevel models (Snijders and Bosker, 1993; Snijders and Bosker, 1994).

Oftentimes in veterinary research, there is a tendency to treat companion animals, like dogs and cats, as individual units rather than accounting for clustering. Even when the number of individuals per cluster is small (i.e. <5), the impact of clustering can be large depending on the size of the intra-class correlation coefficient (Bingenheimer and Raudenbush, 2004; Clarke, 2008). Consequently, multilevel models are sometimes appropriate and essential for companion animal studies. Some examples of multilevel models used in recent companion animal studies include a repeated measures longitudinal study on dogs in a canine model for obesity (Kabir, et al., 2011), and repeated measures modeling of multiple teeth per dog in a dental study (Ramirez-Echave, et al., 2011). Multilevel modeling has also been successfully used to examine computed tomography diagnosis of shoulder lesions in dogs, comparing various diagnostic results in both shoulders in study dogs (Maddox, et al., 2013).

Krontveit et al. (2010) used multilevel models with random effects for litter to study
risk factors for hip dysplasia in large breed dogs in Norway (Krontveit, et al., 2010), while Wisener et al. (2010) also used multilevel random effects models to examine individual and community-level variables as risk factors for calcium oxalate compared to struvite uroliths in dogs (Wisener, et al., 2010).

**STUDY RATIONALE**

Due to a lack of detailed investigations examining pet-related management factors and their association with *Salmonella, Campylobacter* and antimicrobial resistant bacteria carriage in pet dogs in Canada, investigations are required to establish the prevalence of these bacteria, identify the risk factors associated with bacterial carriage, and to determine which management practices may potentially put pet owners at increased risk and which practices may reduce the risk of acquiring infections from their pets. In order to address these needs, the objectives for this study were as follows:

1. Determine the prevalence of zoonotic enteric bacteria, particularly *Salmonella* and *Campylobacter* in client-owned pet dogs (Chapters 2 & 3);

2. Assess associations between the prevalence of *Salmonella* and *Campylobacter* in pet dogs and various demographic and pet-management factors (Chapters 2 & 3);
3. Determine the prevalence of antimicrobial resistant *Salmonella* and *E. coli* and common resistance patterns in client-owned pet dogs (Chapter 4);

4. Determine the ability of commensal *E. coli* to predict antimicrobial resistance in *Salmonella* bacteria within the same dog (Chapter 4);

5. Assess associations between the prevalence of antimicrobial resistant *Salmonella* and *E. coli* in pet dogs and various demographic and pet-management factors (Chapter 5);

6. Assess the proportion of variance in shedding status of *Campylobacter*, and of antimicrobial resistance in *Salmonella* and *E. coli* at the pet and household levels (Chapters 2, 4 & 5).
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CHAPTER TWO


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ABSTRACT

The purpose of this study was to determine pet-related management factors that may be associated with the presence of *Salmonella* spp. in feces of pet dogs from volunteer households. From October 2005 until May 2006, 138 dogs from 84 households in Ontario were recruited to participate in a cross-sectional study. Five consecutive daily fecal samples were collected from each dog and enrichment culture for *Salmonella* spp. was performed. A higher than expected number of the dogs (23.2%; 32/138) had at least one fecal sample positive for *Salmonella*, and 25% (21/84) of the households had at least one dog shedding *Salmonella*. Twelve serotypes of *Salmonella enterica* subsp. *enterica* were identified, with the predominant serotypes being Typhimurium (33.3%; 13/39), Kentucky (15.4%; 6/39), Brandenburg (15.4%; 6/39) and Heidelberg (12.8%; 5/39). Univariable logistic regression models were created with a random effect for household to account for clustering. Statistically significant risk factors for a dog testing positive included having contact with livestock, receiving a probiotic in the previous 30 days, feeding a commercial or homemade raw food diet, feeding raw meat and eggs, feeding a homemade cooked diet, and having more than one dog in the
household. In two-variable models that controlled for feeding raw food, the non-
dietary variables were no longer statistically significant. These results highlight the
potential public health risk of including raw animal products in canine diets.

*Impacts*

- Consuming a commercial or homemade raw diet, a homemade cooked diet, or
  raw meat and eggs, increases a pet dog’s risk of carrying *Salmonella*.
- Testing multiple consecutive whole fecal samples greatly improves *Salmonella*
  recovery in dogs.
- Having multiple dogs in a household, using probiotics and contact with
  livestock are important potential risk factors that need to be investigated
  further.

Keywords: *Salmonella*; dogs; raw food; zoonoses; public health

**INTRODUCTION**

Incidents of human salmonellosis in North America are relatively common,
and each year, there are approximately 6000–12 000 cases of salmonellosis reported
in Canada (Lee and Middleton, 2003). However, it has been suggested that the true
incidence of salmonellosis in Canada is greatly under-reported, and may actually be
closer to hundreds of thousands of cases each year (Majowicz et al., 2005; Thomas et
al., 2006). *Salmonella* infection in humans can have a broad array of clinical signs and
sequelae, ranging from asymptomatic carriage to severe clinical disease (Keusch, 2001). It is suspected that the majority of these cases are likely foodborne. However, an unknown proportion of the remaining cases could be due to contact with dogs or contaminated dog food products (Finley et al., 2006).

Several outbreaks of salmonellosis in humans related to exposure to contaminated pet food and pet treat products have been reported in the last 10 years (Clark et al., 2001; Centers for Disease Control and Prevention, 2006, 2008a, b). As well, pet dogs have been directly implicated in several cases of salmonellosis in humans (Sato et al., 2000; Cherry et al., 2004). The prevalence of Salmonella isolation from clinically healthy dogs has been estimated to be between 1–4% and over 30% in hospitalized dogs (Hackett and Lappin, 2003; Marks and Kather, 2003; Tsai et al., 2007), but even higher prevalences have been recorded among certain populations of non-hospitalized dogs, such as sled dogs, where raw animal products make up a large proportion of their diet (Cantor et al., 1997). Dogs are often subclinical carriers of Salmonella (McDonough and Simpson, 1996; Greene, 2006; Bagcigil et al., 2007), so it is unknown how commonly the pathogen is shed by pet dogs and the role they play in the spread of this bacterium to humans. The most prevalent serotypes of Salmonella found in dogs globally are Salmonella enterica subsp. enterica serotype Typhimurium and Salmonella enterica subsp. enterica serotype Enteritidis (Carter and Quinn, 2000).

It is estimated that there are approximately 6 million dogs in Canada, with
32% of households owning at least one dog (Perrin, 2009). In North America, the nature of the human-animal bond results in many dogs being considered family members rather than simply ‘pets’. It is this close relationship that causes concern with respect to the transmission of zoonotic infections from dogs to humans. The occurrence of Salmonella in pet dogs and the risk factors and pet management factors that may increase or decrease that carriage must be investigated thoroughly to develop guidelines for safe pet ownership, particularly for the young, elderly and immunocompromised.

Raw diets, those composed of raw meat, bones and produce, are becoming increasingly popular in the pet dog population and in recent years have been implicated in the carriage of Salmonella in dogs (Joffe and Schlesinger, 2002; Finley et al., 2007), with several studies investigating the contamination of the ingredients often used in these diets (Freeman and Michel, 2001; Weese et al., 2005; Strohmeyer et al., 2006; Finley et al., 2008). This suggests that some health management practices of dogs may have a potential role in increasing the risk of pet dogs carrying Salmonella. Further investigations are required to identify these factors, and determine which management decisions are most easily changed by pet owners. The purpose of this study was to determine which pet-related management factors potentially increase or decrease the risk of carriage of Salmonella in a population of pet dogs from volunteer households.
MATERIALS AND METHODS

Recruitment

Between October 2005 and May 2006, a convenience sample of households in Ontario was recruited to participate in a study to investigate the presence of *Salmonella*, *Clostridium difficile* and generic *Escherichia coli* (for the purpose of researching antimicrobial resistance) in household dogs. This study was part of a larger study conducted by the Public Health Agency of Canada and the University of Guelph investigating household environmental contamination with *Salmonella*, *Clostridium difficile* and generic *Escherichia coli*. The household study examined the environmental contamination of several areas in the home, as well as investigating the carriage of the above pathogens in pet dogs. Additional analyses of the results for the household portion of this study are in progress. Participants were recruited through advertising in brochures, e-mail, and listserves that targeted the University of Guelph, the Ontario Veterinary College, pet therapy organizations in Southwestern Ontario, local veterinary conferences and meetings, and veterinary clinics in Ontario that agreed to display brochures related to the project. In total, 97 households were recruited for the household contamination study. Eighty-four dog-owning households with a total of 138 dogs were recruited. All owners of dogs that were found to be shedding any of the pathogens being investigated were contacted by phone and mail and counseled on the zoonotic risks of these pathogens and proper fecal handling and hand hygiene. The study was approved by the University of Guelph Research Ethics
sample collection and questionnaire

Study homes were visited by trained technicians, who collected environmental samples, administered the questionnaire (Appendix A) and provided a fecal collection kit for the primary care givers of each dog to collect and return a single fecal sample per day for five consecutive days. The fecal kit contained an instruction guide, tongue depressors, disposable gloves, biohazard bags, five 100-ml sterile specimen containers, and pre-addressed, postage-paid cushioned envelopes for mailing the samples. The instruction guide provided guidelines for safe fecal sample handling, as well as information about the pathogens being tested and answers to frequently asked questions about these pathogens. If fecal samples were not received at the university within 2 weeks, the owners were contacted by phone or email, as a reminder to submit the samples. The questionnaire was administered by the technician to the primary care takers of the dog in each household. The questionnaire included questions concerning the following: the dog’s main diet and whether additional animal products were added to the diet; the presence and type of other pets in the home; humans living in the household; the dog’s activities; whether the pet had experienced vomiting or diarrhea in the previous month; veterinary care, including deworming; any contact with livestock; and the use of a probiotic (e.g. Lactobacillus) in the previous month.

Breed, age, sex and neuter status were collected from 129 (93.5%) of the 138 dogs.
**Microbiological analysis**

All samples were received via express post at the University of Guelph. Upon arrival, 10 g of fresh feces was combined with a 0.85% saline solution and homogenized. Samples were pre-enriched using a 1:10 ratio in buffered peptone water (BPW) for 24 h at 37°C. Two methods were used in parallel for increased sensitivity. In the first method, 0.1 ml of the BPW mixture was inoculated into modified semi-solid Rappaport-Vassiliadis (MSRV) agar and incubated at 42°C for 24–72 h. A loopful of positive MSRV inoculum was plated on MacConkey agar and incubated at 37°C for 18–24 h. Next, two presumptive non-lactose fermenting colonies were inoculated on full tryptic soy agar (TSA) and incubated at 37°C for 18–24 h. In the second method, once the BPW solution was incubated, 1 ml of BPW was inoculated into 10 ml of Rappaport-Vassiliadis (RV) broth and incubated at 42°C for 24 h. Once incubated, 1 ml of RV mixture was added to 10 ml of tetrathionate (TT) broth and incubated under the same conditions. Next, one loopful of TT broth was inoculated onto each of full xylose lysine tergitol 4 (XLT4), brilliant green sulphur, and bismuth sulphate agars and incubated at 37°C for 18–24 h. Two typical colonies from each agar plate were sub-cultured onto MacConkey agar and incubated at 37°C for 18–24 hours. Non-lactose fermenting colonies were plated onto TSA. Biochemical testing for both methods was conducted using Christensen’s urea, triple sugar iron and *Salmonella* O antiserum Poly A-I & Vi agglutination test. *Salmonella* positive isolates were submitted to the Office Internationale des Epizooties Reference Laboratory for
Salmonellosis [Laboratory for Foodborne Zoonoses (LFZ), Public Health Agency of Canada, Guelph] for serotyping and phagetyping, and the Canadian Integrated Program for Antimicrobial Resistance Surveillance (LFZ, Guelph) for antimicrobial susceptibility testing. A dog was considered positive for *Salmonella* if one or more of their fecal samples tested positive by either method. All five fecal samples were received from 134 (97.1%) of 138 dogs, and four fecal samples were received from four dogs.

**Statistical analysis**

The data were entered and analyzed in Intercooled Stata© 10 for Windows (College Station, TX, USA) and MLwiN© 2.02 (Centre for Multilevel Modeling, London, UK). Univariable logistic regression models with a random intercept for household to account for clustering were built using MLwiN© 2.02. The random intercept models were created using re-weighted iterative generalized least squares with predictive quasi-likelihood and the first order derivative of the Taylor series expansion for linearization (Dohoo et al., 2003; Goldstein, 2003). Using the latent variable technique, intraclass correlation coefficients (ICCs) for dogs in the same household were estimated for each model and an intercept-only model (Dohoo et al., 2003). Models with two explanatory variables, combining potential confounders with the significant variables from the univariable models in a pairwise fashion, were used to test the impact of potential confounders. The potential confounders included: breed
(mixed, small, medium and large); age category (young, adult and senior); sex (male and female); neuter status (intact and neutered); raw feeding (feeding a raw diet or adding raw food to the diet); and exposure to livestock. A variable was determined to be a confounder if the odds ratio of the independent variable changed by more than 20% when the potentially confounding variable was added to the model (Dohoo et al., 2003; Thrusfield, 2005). All tests were two-tailed with a statistical significance level of 5%.

In addition, sub-analyses on variables available for the dogs fed raw diets were performed using conditional estimates in exact univariable logistic models. Data were available for 23 dogs from 14 households, and variables relating to the length of time the raw diet had been fed were examined. Due to the small sample sizes in these subcategories, exact models were built in Intercooled Stata© 10 for Windows. Random effects could not be included in these models to control for clustering by household because of the very small effective sample sizes. Sensitivity analyses were also calculated in Intercooled Stata© 10 for Windows, comparing the sensitivity of using 1, 2, 3 or 4 consecutive fecal samples versus five samples for detecting dogs shedding *Salmonella*. The collection of five samples was used as the gold standard based on the temporal shedding patterns of *Salmonella* in dogs from a previous study (Finley et al., 2007). The microbiological laboratory tests were treated as 100% specific for these purposes.
RESULTS

Approximately 23% [32/138; 95% confidence interval (CI) = 16.4–31.1%] of the dogs enrolled in this study had at least one fecal sample positive for Salmonella. One quarter (25%; 21/84; 95% CI = 16.2–35.6%) of the participating homes had at least one dog with a sample positive for Salmonella. Among those dogs shedding Salmonella (n = 32), the median number of positive daily samples was 1 and varied from 1 to 5, with 59.4% (19/32; 95% CI = 40.7–76.3%) of the dogs positive on only 1 day (Fig. 2.1). Only four (12.5%; 95% CI = 3.5–28.9%) of the 32 dogs shedding Salmonella had a history of diarrhea in the previous month.

In total, 80 variables relating to the dogs’ health, diet and common exposures were examined in univariable models (Table 2.1), and 12 were found to be significant at the 5% level (Table 2.2). The distribution of significant risk factors (P ≤ 0.05) and the risk for carriage of Salmonella in the dogs that were and were not exposed to those risk factors can be found in Table 2.2. Many of the risk factors that were positively associated with Salmonella carriage in univariable logistic models, with a random effect for home, were related to feeding raw or home-cooked foods of animal origin (Table 2.2). Of the 32 dogs positive for Salmonella, 14 (43.8%; 95% CI = 26.4–62.3%) were fed a raw diet or given raw animal products, and 10 (31.3%; 95% CI = 16.1–50.0%) were fed home-cooked food. Statistically significant risk factors unrelated to foods of animal origin included having contact with livestock, being given a probiotic in the past 30 days and having more than one dog in the household.
The only risk factor that appeared to have a sparing effect on the carriage of *Salmonella* was being fed a rawhide treat in the week before the fecal sample was tested (Table 2.2). Variables regarding each dog’s age, breed, health, veterinary care (deworming and antibiotics), and the occurrence of diarrhea or vomiting in the previous month, were not found to be significantly associated with *Salmonella* carriage.

When breed, age category, sex, neuter status and exposure to livestock were individually examined for their potential confounding effect on the significant univariable associations, only breed, age category, sex and neuter status had appreciable effects on the independent variables. Being fed raw eggs in the past week became insignificant when breed, age category, sex and neuter status were added to the model and being fed rawhide chews in the past week became insignificant when breed was added to the model (Table 2.3). The odds ratios of several variables did change by more than 20% with the inclusion of each examined confounding variable, but the direction of the relationship remained unchanged (Table 2.3). When the raw feeding variable was included as a potential confounder, all of the non-dietary significant variables became insignificant (Table 2.3). The raw feeding variable itself remained significant in all of the models, except as a covariate in the probiotic use model. The ICCs with one or two fixed effects ranged from 29.8% to 34.8% (Table 2.2). The intercept only model had an ICC of 32.0%. The ICCs indicate the degree to which dogs within the same household are similar in *Salmonella* status (i.e.,
clustering), and 30–40% was considered a moderate degree of clustering by household.

Among raw diet fed dogs, being fed a raw diet for more than 2 years (OR = 17.08; 95% CI = 1.27–229.15) was the only variable found to be statistically significantly associated with *Salmonella* carriage.

A total of 39 *Salmonella* isolates were recovered from the fecal samples collected and all isolates belonged to *Salmonella enterica* subsp. *enterica*. Twelve serotypes of *Salmonella* were identified in this study. The most common serotypes identified were (n = 39): Typhimurium 33.3% (13/39), Kentucky 15.4% (6/39), Brandenburg 15.4% (6/39) and Heidelberg 12.8% (5/39). Serotypes Thompson, Tennessee, I:Rough-O:r:-, I:Rough-O:z29:-, Infantis, Indiana, Ouakam and I:8,20:i:- were all isolated from one or two dogs and more than one serotype was isolated from seven dogs. None of the isolate combinations appeared more than once, but *Salmonella* Heidelberg appeared in four of the mixed infections.

The five consecutive fecal samples offered an opportunity to look at the sensitivity of multiple samples for finding *Salmonella*. Assuming that the microbiological laboratory tests were 100% specific, and testing five fecal samples was the best method or ‘gold standard’ for finding *Salmonella* (Finley et al., 2007), the sensitivities of sampling for 1, 2, 3 or 4 days were calculated. The sensitivities of the multiple samples were 35.5%, 64.5%, 83.9% and 90.3% for 1, 2, 3 and 4 days of consecutive sampling, respectively.
DISCUSSION

This study is the first of its kind in Canada, and has greatly improved our knowledge of *Salmonella* in household pet dogs. The occurrence of *Salmonella* (23%) in these dogs was higher than expected and many of the pet-related management factors positively associated with the carriage of *Salmonella* related to raw food feeding. The other factors investigated in this study, for example, exposure to livestock, probiotic use, having multiple dogs in the home and the use of rawhide treats, are also important in pet-related management, and must be studied further. This study also demonstrated the correlation of *Salmonella* status among dogs in the same household, and how important multiple sample testing is for the recovery of *Salmonella* in individual dogs. We recommend that a minimum of three consecutive full fecal samples be tested in clinically healthy dogs.

Similar to previous studies, in univariable analyses, we found that access to livestock, having multiple dogs in a household, feeding raw meat and feeding a raw diet were significant risk factors for carriage of *Salmonella* in dogs (LeJeune and Hancock, 2001; Joffe and Schlesinger, 2002; Cherry et al., 2004; Morley et al., 2006; Finley et al., 2007; Schotte et al., 2007). Livestock, like cattle, pigs, horses, and poultry, have been shown to commonly carry *Salmonella* and dogs having contact with these animals may acquire this bacterium from exposure to their feces or contaminated environments (LeJeune and Hancock, 2001; Liebana et al., 2002). Having multiple dogs in a household may also represent a potential source of
contamination, and outbreaks of salmonellosis in dogs have been linked to group housing and dog-to-dog transmission (Cherry et al., 2004; Morley et al., 2006; Schotte et al., 2007). As the dogs are likely to share similar sources of *Salmonella* (in their environment, food, dishes and handling), *Salmonella* could be present in many dogs and increase the potential spread and shedding of *Salmonella*. Raw meat and meat products are frequently contaminated with *Salmonella*, and consequently, commercial and homemade raw diets made with these products are a potential source of *Salmonella* (Government of Canada, 2007a, 2008). As well, improper food handling and preparation by dog owners may increase the spread of *Salmonella* and contamination of raw and cooked foods fed to pet dogs (Luber, 2009; Redmond and Griffith, 2009). Questions regarding hand hygiene were asked in the questionnaire, however, none of these variables were found to be statistically significant.

Contrary to expectation, in univariable analyses, rawhide chews were found to have a sparing effect for the carriage of *Salmonella*, and probiotics were found to increase the odds of *Salmonella* carriage in dogs in our study. Previously, rawhide chews have been associated with outbreaks of *Salmonella* in humans (Health Canada, 2000; Willis, 2001; Wong et al., 2007). In our study, the only dogs given rawhide chews were those fed commercial, processed diets; none of the raw fed dogs were given these products. As the raw diet fed dogs represent such a large portion of the *Salmonella* positive dogs, this may account for the sparing effect. In addition, recent research suggests that contamination of rawhide and pig ear treats is decreasing, due
to changes in the industry after the previously mentioned *Salmonella* outbreaks (Finley et al., 2006; Chiewchan et al., 2007); therefore, this could be one reason for the negative association between rawhide chews and *Salmonella*. The results concerning probiotics used in the previous 30 days are also unexpected, considering the expected function of probiotics. Probiotics, including various species of *Lactobacillus*, have been noted for their potential positive health effects, as a treatment for diarrhea, and as a means to control various canine intestinal infections (Rinkinen et al., 2003; Vahjen and Manner, 2003; McCoy and Gilliland, 2007). However, research involving probiotic use in dogs is lacking and contradictory. McCoy and Gilliland (2007) found that certain canine *Lactobacillus* bacteria can inhibit the growth of *Salmonella* in vitro, but Rinkinen et al. (2003) demonstrated unchanged *Salmonella* adhesion with the use of certain probiotics in vitro. In the only in vivo study, Vahjen and Manner (2003) showed an increase in *Salmonella* shedding in dogs with the use of certain probiotics. It should be noted that it is unknown why the owners of the dogs in our study were feeding the probiotics and which types and dosage of probiotics were given. As well, of the 36 dogs that were given a probiotic, 21 of them were also fed a raw diet, and this may account for the positive association due to the large number of raw fed dogs that were found to be shedding *Salmonella*.

In the two-variable models, all of the non-raw diet variables that were statistically significant in our univariable analyses became non-significant when we controlled for the potential confounding effect of being fed a raw diet. However, the
direction of the odds ratios remained positive for these non-rain diet variables, and the 
magnitude of these measures of association was still relatively large. Future studies 
should not ignore the influence of access to livestock, having multiple dogs in a 
household, or being given a probiotic in the last 30 days on *Salmonella* shedding in 
dogs. The lack of statistical significance may simply be a function of the small sample 
size of this and other canine studies. Still, these results highlight the importance of 
raw diets in understanding the shedding of *Salmonella* in dogs.

The large ICCs for *Salmonella* carriage among dogs within the same 
households were expected. The ICCs represent the degree to which dogs within the 
same household are similar in *Salmonella* status. Dogs in the same household would 
likely have had similar diets, the same environment and the same possible sources of 
exposure. Clustering of disease status within a herd or household is typical in most 
infectious diseases and must be accounted for in the analysis if multiple subjects are 
taken from a common home or herd (Dohoo et al., 2003; Diez Roux and Aiello, 
2005). Subsequent researchers should be cautious, and analytically control for 
clustering, if multiple dogs per household are to be included in their studies.

The four most common serotypes identified in this study are similar to those 
commonly found in humans, food animals and pets in Canada (Government of 
Canada, 2007b, 2008). *Salmonella* Typhimurium is commonly found in pork, beef, 
veal, and less commonly in chicken, as well as in water, contaminated produce, and a 
variety of animals (Guardabassi et al., 2004; Swanson et al., 2007; Lapidot and Yaron,
Salmonella Brandenburg is found in pork and chicken, and has also been recovered from humans and numerous animals (Clark et al., 2004). Both S. Kentucky and S. Heidelberg are found in chicken, and S. Kentucky has also been found in dairy cattle and surface water (Government of Canada, 2008). Unfortunately, in this study, the exact components of the raw diets fed to the participating dogs were not determined, so connections between the serotypes found and raw animal products and diet fed cannot be assumed. It is very important, however, to recognize that the majority of the serotypes isolated in this study are also capable of infecting humans.

The prevalence of Salmonella found in this study was approximately 20% higher than previously reported for household dogs, where the prevalence of Salmonella in clinically healthy dogs was found to range from 1% to 4% (Cantor et al., 1997; Hackett and Lappin, 2003). This may be due to the differences in feeding practices and geographical variations, but may also be due to the increased sensitivity of our testing program or the fact that this was not a random sample of dogs, and a larger than normal proportion of dogs were fed raw diets or raw foods of animal origin. Dogs fed a raw food diet appeared to be over-represented in the study population, and anecdotal evidence from veterinary practitioners suggests that the prevalence of raw feeding is <5%, as it is believed that only 8% of owners feed human food, whether cooked or raw, to their dog (APPMA, 2004). Dogs fed raw food diets have been shown to shed Salmonella (Joffe and Schlesinger, 2002; Finley et al., 2009; Liu et al., 2009; Semenov et al., 2009).
and the ingredients used in their diets are commonly contaminated with this bacterium (Chengappa et al., 1993; LeJeune and Hancock, 2001; Weese et al., 2005; Strohmeyer et al., 2006; Finley et al., 2008). Also, unlike other studies, we sampled dogs for five consecutive days, and used whole fecal specimens instead of fecal or rectal swabs. This greatly increased the sensitivity of our testing procedure, and illustrates the value of testing multiple full fecal samples for recovering *Salmonella* in dogs. As well, the temporal pattern of *Salmonella* shedding by day in this study is consistent with the intermittent pattern of shedding found in other canine studies (Greene, 2006; Baggigil et al., 2007; Finley et al., 2007). The variability in the shedding patterns of these dogs emphasizes the importance of testing multiple fecal samples to find *Salmonella*, and we would recommend that a minimum of three consecutive full fecal samples be tested in clinically healthy dogs.

The study’s design needs to be considered when interpreting our study’s results. Dogs were recruited as a convenience sample, through several non-random routes, and this process may have created several distinct groups of dogs that are not representative of the typical canine population in Ontario. In addition, if dogs were related through common social groups (e.g., therapy or agility programs), there may be some unrecorded clustering that was not accounted for in our analyses. As well, volunteer bias, a form of selection bias, could be an issue, given the process for recruitment in this study and many studies of this nature. Selection bias would only have occurred if the recruitment process affected both the disease status and the
exposure, and not either alone (Dohoo et al., 2003; Rothman et al., 2008). It is possible that raw feeding dog owners participating in the study were only willing to do so if they believed their dog was healthy, due to the controversy over raw feeding. However, an over-representation of healthy raw-fed dogs in our study, assuming *Salmonella* status and gastrointestinal disease were highly associated, would have resulted in smaller odds ratios (bias toward the null). Consequently, we are more confident in the strong association found between raw feeding and *Salmonella* carriage. Lastly, in view of the fact that this study was cross-sectional in nature, we cannot determine which factors cause *Salmonella* carriage and which factors prolong carriage as prevalence is a function of incidence and duration (Dohoo et al., 2003; Rothman et al., 2008). However, controlling management factors related to incidence or duration would be useful for protecting public health.

Finally, given that only 32 dogs were found to be shedding *Salmonella*, caution should be taken when interpreting the non-significant results in this study. Potential risk factors for carriage of *Salmonella* may have been missed due to the large effect and/or small amount of variation that is often needed to observe statistical significance in small studies (Dohoo et al., 2003). The small effective sample size also restricted the analysis to univariable and two-variable models, and interactions could not be investigated. Several multivariable models were attempted, but were deemed too unstable due to the small effective sample size when subcategories were examined.
The results of this study have important implications in the potential role of dogs in human Salmonella infections. Given the close relationship that most owners have with their dogs, further studies into the health management factors that may increase or decrease a dog’s risk of carrying Salmonella must be completed. Studies concerning the amount of Salmonella shed by dogs, the risk shedding dogs pose to their owners, and other potential household and environmental sources of exposure are needed. The information collected from this study and future studies like this are crucial for the development of evidence-based guidelines for safe dog ownership and to protect the public through responsible pet management.

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Fig. 2.1. Distribution of the duration of shedding of *Salmonella* by day, among the 32 dogs that tested positive
Table 2.1. Risk factor variables investigated for their association with *Salmonella* spp. carriage in pet dogs (n = 138) from volunteer households in Ontario, 2005–2006

<table>
<thead>
<tr>
<th>General Diet Information</th>
<th>Raw Diet Information</th>
<th>Cooked Diet Information</th>
<th>Activities &amp; Pet Information</th>
<th>Dog Health Information</th>
<th>Household Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Fed raw beef in past week</td>
<td>• How long raw diet fed</td>
<td>• How long cooked diet fed</td>
<td>• More than one dog</td>
<td>• Given a probiotic in past month</td>
<td>• Number of children in home</td>
</tr>
<tr>
<td>• Fed raw chicken in past week</td>
<td>- &lt;1 month</td>
<td>- &lt;1 month</td>
<td>• Cats in home</td>
<td>• Diarrhea in past month</td>
<td>• Any infants in home</td>
</tr>
<tr>
<td>• Fed raw pork in past week</td>
<td>- 1-6 months</td>
<td>- 1-6 months</td>
<td>• Other pets in home</td>
<td>• Vomiting in past month</td>
<td>• Any persons &gt;65 years old. in home</td>
</tr>
<tr>
<td>• Fed raw eggs in past week</td>
<td>- 6-12 months</td>
<td>- 6-12 months</td>
<td>• Allowed to run freely in park</td>
<td>• Age category</td>
<td>• Any immunocompromised individuals in home</td>
</tr>
<tr>
<td>• Fed dried pig’s ears in past week</td>
<td>- 12-24 months</td>
<td>- 12-24 months</td>
<td>• Contact with livestock</td>
<td>• Breed size</td>
<td></td>
</tr>
<tr>
<td>• Fed bones in past week</td>
<td>- &gt;2 years</td>
<td>- &gt;2 years</td>
<td>• Contact with other cats</td>
<td>• Sex</td>
<td></td>
</tr>
<tr>
<td>• Fed rawhide chews in past week</td>
<td>• Raw meats used in last month</td>
<td>• Raw meats used in cooked diet in last month</td>
<td></td>
<td>• Neuter status</td>
<td></td>
</tr>
<tr>
<td>• Fed table scraps in past week</td>
<td>- Chicken</td>
<td>- Chicken</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Fed other pet treats in past week</td>
<td>- Turkey</td>
<td>- Turkey</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Main type of diet fed</td>
<td>- Fish</td>
<td>- Fish</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Commercial dry/canned</td>
<td>- Beef</td>
<td>- Beef</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Commercial raw diet</td>
<td>- Lamb</td>
<td>- Lamb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Homemade raw diet</td>
<td>- Pork</td>
<td>- Pork</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Homemade cooked diet</td>
<td>- Ostrich/Emu</td>
<td>- Ostrich/Emu</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Commercial home-cooked diet</td>
<td>- Venison</td>
<td>- Venison</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Other</td>
<td>- Other</td>
<td>- Other</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Anything added to commercial food</td>
<td>• Is raw diet mixed with dry food</td>
<td>• Is cooked diet mixed with dry food</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Raw feeding (anything raw fed or added to diet)</td>
<td>• After handling raw food for raw diet:</td>
<td>• After handling raw food for cooked diet:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Homemade (anything homemade fed or added to diet)</td>
<td>- Always wash hands</td>
<td>- Always wash hands</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Fed a raw diet for more than 6 months</td>
<td>- Always rinse hands</td>
<td>- Always rinse hands</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Fed a cooked diet for more than 6 months</td>
<td>- Usually wash/rinse hands</td>
<td>- Usually wash/rinse hands</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Sometimes wash/rinse hands</td>
<td>- Sometimes wash/rinse hands</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Rarely wash/rinse hands</td>
<td>- Rarely wash/rinse hands</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2.2. The risk of *Salmonella* spp. carriage among dogs from Southern Ontario (2005–2006), exposed and unexposed to statistically significant* variables identified with a mixed† univariable logistic regression model (n = 138)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exp.</th>
<th>Unexp.</th>
<th>% Exp.</th>
<th>Risk Exp. (%)a</th>
<th>Risk Unexp. (%)b</th>
<th>Odds Ratio</th>
<th>95% C.I.²</th>
<th>ICCd</th>
</tr>
</thead>
<tbody>
<tr>
<td>anything homemade fed or added to diet</td>
<td>39</td>
<td>99</td>
<td>28.3</td>
<td>48.7</td>
<td>13.1</td>
<td>6.52</td>
<td>2.17 – 19.64</td>
<td>29.8</td>
</tr>
<tr>
<td>anything raw fed or added to diet (raw feeding)</td>
<td>28</td>
<td>110</td>
<td>20.3</td>
<td>50.0</td>
<td>16.4</td>
<td>5.44</td>
<td>1.64 – 18.10</td>
<td>31.1</td>
</tr>
<tr>
<td>dog fed raw beef, chicken, pork or eggs in last week</td>
<td>27</td>
<td>111</td>
<td>19.6</td>
<td>51.9</td>
<td>16.2</td>
<td>6.11</td>
<td>1.80 – 20.77</td>
<td>31.2</td>
</tr>
<tr>
<td>fed raw chicken in past week</td>
<td>18</td>
<td>120</td>
<td>13.0</td>
<td>61.1</td>
<td>17.5</td>
<td>8.69</td>
<td>2.11 – 35.74</td>
<td>31.7</td>
</tr>
<tr>
<td>fed raw eggs in past week</td>
<td>7</td>
<td>131</td>
<td>5.1</td>
<td>71.4</td>
<td>20.6</td>
<td>8.69</td>
<td>1.00 – 75.17</td>
<td>30.0</td>
</tr>
<tr>
<td>fed commercial raw food diet</td>
<td>7</td>
<td>131</td>
<td>5.1</td>
<td>71.4</td>
<td>20.6</td>
<td>8.69</td>
<td>1.00 – 75.17</td>
<td>30.0</td>
</tr>
<tr>
<td>fed homemade raw food diet</td>
<td>22</td>
<td>116</td>
<td>15.9</td>
<td>59.1</td>
<td>16.4</td>
<td>8.59</td>
<td>2.27 – 32.54</td>
<td>30.7</td>
</tr>
<tr>
<td>fed homemade cooked diet</td>
<td>22</td>
<td>116</td>
<td>15.9</td>
<td>45.5</td>
<td>19.0</td>
<td>3.75</td>
<td>1.03 – 13.64</td>
<td>31.8</td>
</tr>
<tr>
<td>fed rawhide chews in past week</td>
<td>39</td>
<td>99</td>
<td>28.3</td>
<td>7.7</td>
<td>29.3</td>
<td>0.19</td>
<td>0.04 – 0.84</td>
<td>32.6</td>
</tr>
<tr>
<td>has been given a probiotic in the last 30 days</td>
<td>36</td>
<td>102</td>
<td>26.1</td>
<td>44.4</td>
<td>15.7</td>
<td>5.24</td>
<td>1.65 – 16.69</td>
<td>33.5</td>
</tr>
<tr>
<td>more than one dog in the household</td>
<td>99</td>
<td>39</td>
<td>71.7</td>
<td>29.3</td>
<td>7.7</td>
<td>4.91</td>
<td>1.19 – 20.26</td>
<td>34.8</td>
</tr>
<tr>
<td>has contact with livestock</td>
<td>18</td>
<td>120</td>
<td>13.0</td>
<td>50.0</td>
<td>19.2</td>
<td>4.25</td>
<td>1.08 – 16.70</td>
<td>31.1</td>
</tr>
</tbody>
</table>

*P≤0.05; †Includes a random intercept for household of dog; ºRisk of *Salmonella* carriage in the exposed dogs; ‼Risk of *Salmonella* carriage in the unexposed dogs; ²95% confidence interval of the Odds Ratio; ³Intra-class correlations were calculated in MLwiN using a latent variable technique.
Table 2.3. Unadjusted and adjusted odds ratios (ORs) for *Salmonella* carriage that changed by >20% with the inclusion of breed size, age, sex, neuter status or raw feeding as potential confounders to the significant variables from the initial univariable analyses

<table>
<thead>
<tr>
<th>Confounders (bold) and Independent Variables</th>
<th>Unadjusted OR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>95% C.I.&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Adjusted OR&lt;sup&gt;b&lt;/sup&gt;</th>
<th>95% C.I.&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Breed Size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed raw eggs in past week</td>
<td>8.69</td>
<td>1.00 – 75.17</td>
<td>4.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.28 – 63.92</td>
</tr>
<tr>
<td>Fed rawhide chews in past week</td>
<td>0.19</td>
<td>0.04 – 0.84</td>
<td>0.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.05 – 1.09</td>
</tr>
<tr>
<td>Fed commercial raw diet</td>
<td>8.69</td>
<td>1.00 – 75.17</td>
<td>10.43</td>
<td>1.05 – 103.31</td>
</tr>
<tr>
<td>Fed homemade cooked diet</td>
<td>3.75</td>
<td>1.03 – 13.64</td>
<td>5.52</td>
<td>1.32 – 23.05</td>
</tr>
<tr>
<td>Has been given a probiotic in past 30 days</td>
<td>5.24</td>
<td>1.65 – 16.69</td>
<td>4.04</td>
<td>1.08 – 15.16</td>
</tr>
<tr>
<td><strong>II. Age (categories)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anything raw fed or added to diet (raw feeding)</td>
<td>5.44</td>
<td>1.64 – 18.10</td>
<td>4.18</td>
<td>1.13 – 15.50</td>
</tr>
<tr>
<td>Dog fed raw beef, chicken, pork or eggs in last week</td>
<td>6.11</td>
<td>1.80 – 20.77</td>
<td>4.74</td>
<td>1.24 – 18.02</td>
</tr>
<tr>
<td>Fed raw chicken in past week</td>
<td>8.69</td>
<td>2.11 – 35.74</td>
<td>6.85</td>
<td>1.36 – 34.51</td>
</tr>
<tr>
<td>Fed raw eggs in past week</td>
<td>8.69</td>
<td>1.00 – 75.17</td>
<td>4.61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.37 – 58.11</td>
</tr>
</tbody>
</table>
### III. Sex

<table>
<thead>
<tr>
<th>Activity</th>
<th>Mean</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
<th>Standard Deviation</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Has been given a probiotic in past 30 days</td>
<td>5.24</td>
<td>1.65 – 16.69</td>
<td>4.16</td>
<td>1.20 – 14.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anything raw fed or added to diet (raw feeding)</td>
<td>5.44</td>
<td>1.64 – 18.10</td>
<td>4.14</td>
<td>1.12 – 15.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog fed raw beef, chicken, pork or eggs in last week</td>
<td>6.11</td>
<td>1.80 – 20.77</td>
<td>4.73</td>
<td>1.24 – 17.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed raw chicken in past week</td>
<td>8.69</td>
<td>2.11 – 35.74</td>
<td>6.70</td>
<td>1.35 – 33.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed raw eggs in past week</td>
<td>8.69</td>
<td>1.00 – 75.17</td>
<td>4.41</td>
<td>0.35 – 54.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed homemade raw food diet</td>
<td>8.59</td>
<td>2.27 – 32.54</td>
<td>6.82</td>
<td>1.51 – 30.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Has been given a probiotic in past 30 days</td>
<td>5.24</td>
<td>1.65 – 16.69</td>
<td>4.15</td>
<td>1.21 – 14.25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### IV. Neuter Status

<table>
<thead>
<tr>
<th>Activity</th>
<th>Mean</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
<th>Standard Deviation</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Has been given a probiotic in past 30 days</td>
<td>5.24</td>
<td>1.65 – 16.69</td>
<td>4.16</td>
<td>1.20 – 14.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anything homemade fed or added to diet (homemade)</td>
<td>6.52</td>
<td>2.17 – 19.64</td>
<td>5.13</td>
<td>1.58 – 16.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anything raw fed or added to diet (raw feeding)</td>
<td>5.44</td>
<td>1.64 – 18.10</td>
<td>4.29</td>
<td>1.15 – 16.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog fed raw beef, chicken, pork or eggs in last week</td>
<td>6.11</td>
<td>1.80 – 20.77</td>
<td>4.84</td>
<td>1.26 – 18.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed raw eggs in past week</td>
<td>8.69</td>
<td>1.00 – 75.17</td>
<td>4.33</td>
<td>0.33 – 56.88</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
From univariable logistic regression model with a random intercept for household, for 129 dogs where the breed, sex, neuter status and age were provided; The adjusted ORs and 95% confidence intervals were obtained using two variable logistic regression models, including the independent variable and the potential confounder, and a random intercept for household; Associations became non-significant (p>0.05) with the inclusion of the confounding variable.

<table>
<thead>
<tr>
<th>Behavior</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed homemade raw food diet</td>
<td>8.59</td>
<td>2.27 – 32.54</td>
<td></td>
<td>1.53 – 30.23</td>
</tr>
<tr>
<td>Has been given a probiotic in past 30 days</td>
<td>5.24</td>
<td>1.65 – 16.69</td>
<td></td>
<td>1.13 – 13.71</td>
</tr>
</tbody>
</table>

V. Raw Feeding

<table>
<thead>
<tr>
<th>Behavior</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed rawhide chews in past week</td>
<td>0.19</td>
<td>0.04 – 0.84</td>
<td>0.29</td>
<td>0.06 – 1.36</td>
</tr>
<tr>
<td>Has been given a probiotic in past 30 days</td>
<td>5.24</td>
<td>1.65 – 16.69</td>
<td>3.06</td>
<td>0.75 – 12.40</td>
</tr>
<tr>
<td>More than one dog in the household</td>
<td>4.91</td>
<td>1.19 – 20.26</td>
<td>3.74</td>
<td>0.89 – 15.70</td>
</tr>
<tr>
<td>Has contact with livestock</td>
<td>4.25</td>
<td>1.08 – 16.70</td>
<td>2.93</td>
<td>0.68 – 12.54</td>
</tr>
</tbody>
</table>
CHAPTER THREE

Factors related to *Campylobacter* spp. carriage in client-owned dogs visiting veterinary clinics in a region of Ontario, Canada

(As published in Epidemiology and Infection, 2011, 139, 1531–1541)

SUMMARY

From July 2008 until May 2009, 240 client-owned pet dogs from seven veterinary clinics in the Region of Waterloo, Ontario, Canada participated in a study to determine pet-related management factors that may be associated with the presence of *Campylobacter* spp. in dogs. The prevalence of *Campylobacter* spp. carriage in our study population of pet dogs was 22%, with 19% of the dogs positive for *C. upsaliensis*, and 3% positive for *C. jejuni*. A significant risk factor from multivariable logistic regression models for both *Campylobacter* spp. and *C. upsaliensis* carriage was having homemade cooked food as the dog’s diet or added to its diet, and a significant sparing factor for both models was treatment with antibiotics in the previous month. Increasing age of the dog decreased the odds of *Campylobacter* spp. and *C. upsaliensis* carriage. Based on the high prevalence of *Campylobacter*, and specifically *C. upsaliensis*, further research concerning pet dogs as a risk factor for campylobacteriosis in humans is warranted.

Key words: *Campylobacter*, *C. upsaliensis*, dogs, public health, zoonoses.
INTRODUCTION

Campylobacteriosis is the most common cause of bacterial enteritis in people in Canada, with approximately 9500 laboratory confirmed cases each year [1]. The most commonly recovered species is *Campylobacter jejuni*, followed distantly by *Campylobacter coli* and *C. lari* [1, 2]. *Campylobacter* usually causes mild to severe gastrointestinal infection in humans, including nausea, vomiting and watery diarrhoea, but potentially life-threatening sequelae can occur (e.g. Guillain-Barré syndrome) [3, 4]. The majority of human cases are sporadic and believed to be foodborne; however, since the early 1980’s, several studies have investigated the role of companion animals as potential sources of human infections (e.g. Blaser, et al. and Fox, et al. [5, 6]). Many studies have identified having a household pet, especially a puppy, or a dog with diarrhoea, as a risk factor for *Campylobacter* infection in people [7-12]. Dogs have also been suspected as the source of transmission in several cases of campylobacteriosis [13-15].

Domestic dogs have long been recognized as potential sources of zoonotic enteric pathogens like *Salmonella, Campylobacter* and *Giardia* [16, 17]. The prevalence of *Campylobacter* carriage in clinically healthy pet dogs has been estimated to be between 15-58% [18-24], but can be as high as 87% in stray animals [25]. *Campylobacter* can cause both clinical and non-clinical infections in dogs, with the most severe sequelae occurring in young and immunocompromized dogs; often associated with *C. jejuni* infection [6, 26]. The most commonly isolated species of *Campylobacter* in dogs has varied among studies due to microbiological methods, but
in recent work, *C. upsaliensis* has been the most frequently recovered species in dogs [21, 22, 24, 27].

It is estimated that there are approximately six million dogs in Canada, with 32% of households owning at least one dog [28]. In North America, a strong human-animal bond means that dogs are often considered family members rather than simply “pets”. It is this close relationship that causes concern with respect to the potential transmission of zoonotic infections from dogs to humans. Several studies have identified young age, the presence of diarrhoea, season, and high density housing, like kennels and shelters, as significant risk factors for the carriage of *Campylobacter* in dogs [20-24, 29]. Due to a lack of detailed investigations examining pet-related management factors and their association with *Campylobacter* carriage in dogs in North America, investigations are required to identify these factors, and determine which management practices may potentially put pet owners at increased risk of infection from their pets. The purpose of this study was to determine which pet-related management factors, including type of diet fed to the dog, the dog’s exposure/access to other pets and livestock, the dog’s involvement in group activities (e.g. obedience), and veterinary treatments, are associated with the carriage of *Campylobacter*. In addition, human-related factors, including the presence of children in the home, household members’ exposure/access to other animals and livestock, whether household members have visited or worked in a hospital, and any household members experiencing vomiting or diarrhoea in the previous week, have also been examined.

This study will be used to explore the epidemiology of carriage of *Campylobacter* and specific *Campylobacter* species in a population of client-owned pet dogs from the
Region of Waterloo, Ontario, Canada.

METHODS

Recruitment

Between July 2008 and May 2009, dogs visiting seven veterinary clinics in the Region of Waterloo, Ontario were recruited to participate in a study to investigate the occurrence of *Campylobacter*, *Salmonella*, *Giardia*, and antimicrobial resistance in generic *Escherichia coli* in client-owned pet dogs. This manuscript contains the *Campylobacter* results only. Veterinarians from all 44 veterinary clinics in the Region of Waterloo, Ontario were sent letters inviting them to participate in the study, with nine clinics responding and seven clinics agreeing to participate. Once a clinic agreed to take part, the primary author (EL) visited each of the clinics every 7-14 days for ten months to recruit client-owned pet dogs for the study. Any dog visiting the clinic was eligible to participate, including those with signs of gastrointestinal disease and those being treated with antimicrobials; however, only one dog per household was included in the study and dogs were only eligible to participate in the study once. Dog owners visiting the veterinary clinics were asked by their veterinarian or the primary author to participate in the study. Those who agreed to participate then spoke with the primary author, the owner questionnaire was administered (Appendix B), and the owner was provided with a fecal collection kit to collect and return a single fecal swab per day for two consecutive days. The study was approved by the University of Guelph Research Ethics Board.
Samples

The fecal kit provided to the dog owners contained an instruction guide, tongue depressors, disposable gloves, biohazard bags, sterile specimen containers, two sterile Cary-Blair agar swabs (CultureSwab™ Cary-Blair Agar; Becton Dickson and Company; Sparks, Maryland, USA), and pre-addressed, postage-paid cushioned envelopes for mailing the samples. Fecal swabs were used for *Campylobacter* isolation because the samples had to travel in the mail, and due to the fastidiousness of *Campylobacter*, it was felt the agar in the Cary-Blair swabs would provide better recovery. In a small trial completed by our laboratory using fecal samples spiked with *Campylobacter*, the fecal swabs remained positive after being mailed, whereas the full fecal samples did not (results unpublished). The fecal swab was plunged into the freshly passed feces collected in the sterile specimen containers, and then placed in the Cary-Blair agar tube. Proper use of the Cary-Blair swabs was demonstrated for each owner at the time of recruitment. The two Cary-Blair swabs were to be tested for *Campylobacter* spp. only.

Microbiological Analysis

All samples were received via express post at the University of Guelph. Upon arrival, information pertaining to fecal swabs was documented and swabs were immediately sent to the Laboratory Services Division (LSD), University of Guelph for *Campylobacter* isolation. The fecal swab was streaked directly on to modified cefoperazone charcoal deoxycholate agar (mCCDA) plates (*Campylobacter* selective
blood-free agar [CM0739] and CCDA selective supplement [SR0155], Oxoid, Nepean, ON, Canada) and the swab was then inserted into 5mL of Bolton broth (Oxoid, Nepean, ON, Canada). A 1mL aliquot of the inoculated Bolton broth was then added to 9mL of new Bolton broth for further enrichment. The plates and broth were incubated for 48 hours at 42°C in a micro-aerophilic atmosphere, based on standard Campylobacter spp. isolation methods at LSD. The mCCDA plate with the direct streak was then read and both Bolton broth dilutions were plated onto mCCDA and incubated for another 72 hours. Controls were used at every stage of the procedure. All mCCDA plates were observed for Campylobacter based on the presence of grey colonies. If present, colonies were re-streaked for purity, and tested for oxygen tolerance and growth at 25°C. Additionally, dark-field microscopy, catalase and oxidase tests, and antibiotic sensitivity tests for cephalothin and nalidixic acid, were conducted on all suspect colonies to confirm the presence of Campylobacter. All Campylobacter isolates were frozen in glycerol at -70°C to allow for future molecular typing. A dog was considered positive for Campylobacter if at least one swab tested positive.

**PCR Species Identification**

A series of polymerase chain reaction (PCR) assays were performed targeting the 16S rRNA encoding genes to determine the species of Campylobacter. A loopful of the glycerol frozen broth containing the isolate was inoculated onto Columbia agar and incubated with CampyGen™ (Oxoid, Nepean, ON, Canada), an atmosphere
generation system, at 37°C for two days. This culture was then sub-cultured on another Columbia agar plate and incubated with CampyGen™ at 37°C for one day. Once the isolate had been purified, the DNA was extracted using InstaGene™ (Bio-Rad Laboratories, Hercules, CA, USA) and the remaining culture was stored on Cryostor™ beads (Oxoid, Nepean, ON, Canada) at -70°C. If the culture was catalase positive from previous biochemical testing done at LSD, PCR methods previously described were used to identify the isolate [30]. If the catalase positive culture was negative for both *C. jejuni* and *C. coli* based on the above PCR methods, a second PCR method was used to identify *C. lari* [31]. Finally, if the culture was catalase negative, a previously described PCR method for *C. upsaliensis* and *C. helveticus* was used to identify the isolate [31]. The primers and targets used for *Campylobacter* spp. identification can be found in Linton *et al.* (1996) and Denis *et al.* (1999) [30, 31].

*Questionnaire*

Each questionnaire was administered by the primary author to the primary caregiver of the recruited dog during their visit at the veterinary clinic. The questionnaire included questions concerning the following: the dog’s main diet and whether additional animal products were added to the diet; the presence of other pets in the home; the dog’s activities; the occurrence of vomiting and diarrhoea in the previous month; veterinary care, including deworming; any contact with livestock; and the use of antibiotics in the previous month. Breed, age, sex and neuter status were also collected from all dogs. The variables investigated in this study can be
found in Table 3.1 and the questionnaire can be found in Appendix B.

**Statistical Analysis**

Data from the questionnaires were entered into EpiData® 3.1 (The EpiData Association, Odense, DK) and analysed in Intercooled Stata/MP® 11.0 for Windows (College Station, Texas, USA). All tests were two-tailed with a statistical significance level of 5%. Univariable logistic regression models were used to screen all variables from the questionnaire for an association with *Campylobacter* spp. carriage and for each species of *Campylobacter* isolated if sufficient data were available (e.g., *C. upsaliensis*, *C. jejuni*). Significant continuous variables were evaluated for linearity with the log odds of the outcome using lowess curves and categorical linear trends (lntrend plots) [32]. Pair-wise correlations between significant variables from the univariable analysis (p≤ 0.20) were examined using the Spearman correlation test. Variables with correlation values greater than 0.7 were investigated and the variable that was more biologically plausible, or had the least number of missing values, was included in the model [32].

Multivariable models were constructed for dogs that were positive for *Campylobacter* spp., and for individual *Campylobacter* species where data were sufficient. The main effects models were created with the significant variables from the univariable analysis (p≤ 0.20). A manual backwards step-wise procedure was used to construct the multivariable model. Likelihood ratio tests were used to assess the significance of each model as variables were removed. Confounding was evaluated by
examining the effect of the removed variables on the coefficients of the remaining variables. A variable was determined to be a confounder if the log odds of the other independent variables changed by ≥ 20% [32, 33]. The potential confounding effects and interactions of breed (mixed, pure small, pure medium, pure large); age (in years); sex (male, female); and neuter status (intact, neutered) were examined regardless of statistical significance due to the suspected impact of these demographic characteristics on management-related risk factors. Interaction terms were examined for all remaining variables in the final model. To assess clustering, clinic was modeled using two approaches. In the first, clinic was modeled as a random effect. Clinic was also modeled as a fixed effect because of concerns that due to the limited number of clinics, the random effects would not be properly estimated. The significance of clinic both as a random effect and fixed effect was assessed based on a likelihood ratio (LR) test. If the clinic variables were not significant and did not confound any measures of association, the simpler multivariable models were reported. For the multilevel models, the normality of best linear unbiased predictors (BLUPs) were assessed with normal quantile plots to determine model fit [32]. For standard logistic regression, residuals and Hosmer-Lemeshow goodness-of-fit tests for the final models were assessed. A p-value ≤ 0.05 from the goodness-of-fit test indicated that the model did not fit the data [32].
RESULTS

In total, 240 client-owned pet dogs were recruited for the study. From the seven participating veterinary clinics, 492 pet owners were approached to participate in the study, 279 dogs were recruited for the study, and complete samples were received for 240 dogs. Both fecal swabs were received from 98% (234/240; 95% C.I. = 94.64-99.08) of the dogs, and only one swab was received from 2.5% (6/240; 95% C.I. = 0.92-5.36) of the dogs. The median number of dogs recruited from the clinics was 35, with a minimum of three and maximum of 80. Questionnaires were completed for all dogs recruited for the study. Among participating dogs, 53% (127/240; 95% confidence interval (C.I.) = 46.39-59.37) were female and 17% (40/240; 95% C.I. = 12.18-21.99) were less than one year of age, with the average age of all participating dogs being 4.9 years (95% C.I. = 4.39-5.39). Demographic information of the participating dogs can be found in Table 3.2.

Approximately twenty-two percent (52/240; 95% C.I. = 16.63-27.42) of the dogs enrolled in this study had at least one fecal swab positive for Campylobacter. The predominate species of Campylobacter recovered was C. upsaliensis, which was shed by 19% (46/240; 95% C.I. = 14.39-24.73) of the dogs in the study. Of the Campylobacter positive dogs, 89% (46/52; 95% C.I. = 76.56-95.65) carried C. upsaliensis, and 14% (7/52; 95% C.I. = 5.59-25.79) carried C. jejuni. One dog had both C. upsaliensis and C. jejuni and no other species of Campylobacter were recovered.

In total, 81 variables relating to the dogs’ health, diet, and common exposures
were examined in univariable models (Table 3.1). The variables found to be significant at the 20% level in the *Campylobacter* spp. and *C. upsaliensis* univariable logistic regression models can be found in Table 3.3. There were no statistically significant associations found in any of the models between *Campylobacter* carriage and vomiting or diarrhoea, or *Campylobacter* carriage and season. As well, clinic was not found to be significant as a random effect or fixed effect in any multilevel logistic regression models, with p-values greater than 0.99 and variance less than 0.001, and insignificant LR tests. Age (in years) was statistically significant and was found to be linearly associated with carrier status on the lowess and lintrend plots, and was kept as a continuous variable in all of the models. Univariable and multivariable logistic regression models were not examined for *C. jejuni* carriage because only 7 dogs were found to be carrying *C. jejuni* creating a very small effective sample size.

From the multivariable model for *Campylobacter* spp. carriage, not being treated with antibiotics in the previous month, not having children in the home, and having homemade cooked food as the dog’s diet or added to the dog’s diet increased the odds of carriage. The odds of *Campylobacter* spp. carriage decreased by 0.8 as the age of the dog increased (Table 3.4).

In the multivariable model for *C. upsaliensis* carriage, not being treated with antibiotics in the previous month, a household member having contact with a cat that was not their own in the previous week, and having homemade cooked food as the dog’s diet or added to the dog’s diet increased the odds of carriage. The odds of *C. upsaliensis* carriage decreased by 0.8 as the age of the dog increased (Table 3.4).
In the multivariable models for *Campylobacter* spp. and *C. upsaliensis* carriage, interactions between the significant variables were not found to be statistically significant (p>0.05). Residuals from both final multivariable models were examined for outliers and influential covariate patterns. There were several observations with large residuals, however, the data were examined and found to be correct, and therefore all observations were kept in the final models. The final models for *Campylobacter* spp. and *C. upsaliensis* were not significant at the 5% level with the Hosmer-Lemeshow goodness-of-fit tests (p=0.64 for both models), indicating that the models fit the data.

**DISCUSSION**

This study offers a detailed investigation of pet-related risk factors for *Campylobacter* carriage in client-owned pet dogs in North America. Previous risk factor research has been completed mostly in Europe and Australia. The occurrences of *Campylobacter*, *C. upsaliensis*, and *C. jejuni* found in this study were consistent with the estimated prevalences previously reported for household dogs [21-24]. A number of the investigated pet-related management factors (i.e. age and antibiotic use) associated with the carriage of *Campylobacter*, were consistent with those found in prior studies [20-22, 24]. The potential role of adding cooked human food to a pet dog’s diet as a risk factor for *C. upsaliensis* carriage needs to be investigated further, as a similar association has also been demonstrated in a recent study [22]. Our study also demonstrated that *C. upsaliensis* was much more common in this population of
pet dogs than *C. jejuni*, which is in agreement with several preceding studies [21, 22, 24, 27]. This study highlights the fact that pet dogs may be an important source of *Campylobacter*, especially *C. upsaliensis*, and exposure to dogs must be considered in human cases of campylobacteriosis. As well, *Campylobacter* from positive dogs should be speciated in order to determine the risk for human infection and any species-specific control methods that may be necessary.

This study has the following limitations that need to be considered to avoid over-interpreting our results: the subjects were not recruited randomly; the response rates by clinic and client were poor; and the exploratory nature of the study resulted in many variables being examined. Without a random sample, the reader should be cautious about extrapolating the prevalence in our study population to the Region of Waterloo or Ontario. However, similar prevalences of *Campylobacter* carriage in pet dogs have been found in several recent studies [21-24]. In terms of poor response level, non-response is a form of selection bias that could have altered the size and direction of the odds ratios estimated from our models. However, for this selection bias to occur, non-participation (non-response) by dog owners or veterinary clinics needs to relate to both the examined pet-related risk factors and *Campylobacter* carriage [32]. Considering that few animals were showing clinical signs, and no association was found between diarrhoea or vomiting and *Campylobacter* carriage, it is unlikely that owner willingness to participate was related to both the outcome and the exposures of interest. As well, no association was found between clinic and *Campylobacter* carriage, therefore it is unlikely that clinic willingness to participate was related to both the outcome and the exposures of interest. Finally, like many
exploratory studies, a large number of variables were examined, so the possibility of type I errors should be noted. Where we have identified novel risk factors for *Campylobacter* carriage, we suggest these variables be examined in future studies. Also, in view of the fact that this study was cross-sectional in nature, we cannot determine which factors cause *Campylobacter* carriage and which factors prolong carriage since prevalence is a function of incidence and duration [32, 34]. However, controlling management factors related to prevalence itself would be useful for protecting public health.

Feeding homemade cooked food was found to increase the odds of *Campylobacter* spp. and *C. upsaliensis* carriage in dogs in our study. Previously, *C. jejuni* contaminated food has been associated with infection with *Campylobacter* in humans and animals [35, 36]. To date, dogs and cats have been assumed to be the only reservoir for *C. upsaliensis* [37]. However, a study by Westgarth and colleagues (2009) [22] also found an association between feeding leftover human food and *C. upsaliensis* carriage in community dogs. In our study, only one participating dog was fed a raw food diet; therefore, the association with feeding human food may be due to poor food handling practices rather than direct exposure from raw food. Nonetheless, these findings of an association between feeding homemade cooked food and leftovers, and the presence of *C. upsaliensis* in canine faeces, may warrant the inclusion of *C. upsaliensis* in food safety surveillance programs in the future. Microbiological testing of the foods fed to the dogs in this study was not done, so a direct connection cannot be made. However, sample size needs to be taken into account with this association in our study, as only 11/240 dogs were fed homemade
cooked food, either as their main diet or added to their diet.

Similar to previous studies, a significant difference in *Campylobacter* and *C. upsaliensis* carriage was observed based on age [20-22, 24, 38], and with every year increase in age, the odds of *Campylobacter* and *C. upsaliensis* carriage decreased by 0.8 (Table 3.4). This is likely due to the inexperienced immune systems of the younger dogs; as dogs mature the occurrence of *Campylobacter* carriage decreases [6, 29].

Lack of exposure to antibiotics in the month prior to sample testing was found to increase the odds of *Campylobacter* carriage in our study. A similar finding has been discussed in previous studies, but, unlike in our study, the association was not found to be statistically significant [22, 24]. The association between lack of antibiotic use and an increase in the risk of *Campylobacter* carriage is potentially logical given the antibacterial function of most antibiotics; however, treatment with antibiotics is controversial and only recommended in severely ill animals [26]. Consequently, the use of antibiotics to prevent the carriage of *Campylobacter* in clinically healthy dogs is not normally recommended.

Interestingly, two previously unreported findings, not having children living in the home and a household member having contact with a cat that was not their own, were associated with an increase in the odds of carriage of *Campylobacter* spp. and *C. upsaliensis* in pet dogs, respectively. In previous studies, having a dog or puppy was associated with an increase in the risk of *Campylobacter* carriage in children [8, 10], however current research has not looked at the association in the opposite direction.
For *C. upsaliensis* carriage and cat contact, it is possible that these owners were experiencing a greater deal of contact with other animals and may have been acting as a vector of *Campylobacter* for their pets. Cats have been found to carry *Campylobacter*, including *C. upsaliensis* [39] and have been identified as a significant risk factor for *C. jejuni* infection [40]. It is also possible that these variables are acting as proxies for other statistical associations, or could simply be due to chance because of the large number of variables investigated. Nonetheless, these associations should be investigated in future studies.

Finally, given that only 52 dogs were found to be shedding *Campylobacter* spp., caution should be taken when interpreting the non-significant results in this study. Potential risk factors for carriage of *Campylobacter* spp. may have been missed due to the large effect and/or small amount of variation that is often needed to observe statistical significance in small studies [32]. Weaker associations could have been disguised by the small sample size.

This study identified several novel risk factors for *Campylobacter* spp. carriage in pet dogs, including lack of antibiotic exposure, not having children in the home, exposure to cats and other pets, and including homemade cooked food in the dog’s diet, that require further investigation. These results may warrant a change in the current surveillance of *Campylobacter* species in food sources, specifically in the case of *C. upsaliensis*. Recent changes in laboratory methods for processing canine fecal samples have given rise to an increased prevalence of *C. upsaliensis* in dogs [41, 42]. It is possible that *C. upsaliensis* is more common in food sources and in human
cases of campylobacteriosis than is currently appreciated, since it may be missed as a result of using laboratory methods designed to detect *C. jejuni* and *C. coli* (i.e., catalase positive *Campylobacter* spp.). Current laboratory methods used for isolation of *Campylobacter* spp. from human feces and food samples often involve the use of agar plates and broth suspensions that contain cefaperazone, nalidixic acid, and cephalothin at levels that prevent the growth of *C. upsaliensis* [37]. Using a previously described filtration method, Lastovica and Le Roux [43] found that almost 25% of campylobacteriosis cases in humans in South Africa were due to *C. upsaliensis*. A study from the United States has suggested that *C. upsaliensis* is the second most commonly isolated *Campylobacter* spp. in humans, after *C. jejuni* [44]. A Belgian study also found that *C. upsaliensis* was recovered more often than *C. coli* in humans, indicating that *C. upsaliensis* may be of greater importance than previously thought [45]. *Campylobacter upsaliensis* is certainly capable of causing disease in humans and may be more common than believed in Canadian human infections [37, 46]. Further research into the prevalence of *C. upsaliensis* in human gastrointestinal disease and the potential sources of *C. upsaliensis* is warranted. The information collected from this study and future studies like it, is crucial for the development of evidence-based guidelines for safe dog ownership and to protect the public through responsible pet management.
ACKNOWLEDGEMENTS

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DECLARATION OF INTEREST

None
REFERENCES


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Table 3.1: List of pet-related management variables evaluated for an association with *Campylobacter* spp. carriage in client-owned pet dogs in the Region of Waterloo, Ontario 2008-2009 (n=240)

<table>
<thead>
<tr>
<th>Demographic and Dog Information</th>
<th>Other Pet Information (Y/N)</th>
<th>Dog Health Information (Y/N)</th>
<th>Diet Information (Y/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Age (in years)</td>
<td>• More than one dog</td>
<td>• Dewormed in past 6 months</td>
<td>• Store bought/commercial processed food (dry or can)</td>
</tr>
<tr>
<td>• Breed size&lt;sup&gt;a&lt;/sup&gt;</td>
<td>• Cats in home</td>
<td>• Diagnosed with enteric illness in past 6 months&lt;sup&gt;d&lt;/sup&gt;</td>
<td>• Homemade cooked</td>
</tr>
<tr>
<td>• Sex (M/F)</td>
<td>• Other pets in home</td>
<td>• Given a probiotic in past month</td>
<td>• Homemade raw</td>
</tr>
<tr>
<td>• Intact (Y/N)</td>
<td>• Access to livestock&lt;sup&gt;c&lt;/sup&gt;</td>
<td>• Given antibiotics in past month</td>
<td>• Commercial cooked</td>
</tr>
<tr>
<td>• Source of dog</td>
<td>• Access to garbage in past 2 weeks</td>
<td>• Given any other medications or supplements in past month</td>
<td>• Commercial raw</td>
</tr>
<tr>
<td>- Pet store</td>
<td>• Licked plates/bowls in past 2 weeks</td>
<td>• Diarrhea in past month</td>
<td>• Combination of diets</td>
</tr>
<tr>
<td>- Breeder</td>
<td>• Access to compost in past 2 weeks</td>
<td>• Vomiting in past month</td>
<td>• Food added to diet (daily or weekly)</td>
</tr>
<tr>
<td>- Humane Society</td>
<td>• Access to cat litter in past 2 weeks</td>
<td>Water Exposure (Y/N)</td>
<td>(Y/N)</td>
</tr>
<tr>
<td>- Other</td>
<td>• Access to dead animals in past 2 weeks</td>
<td>• Given tap water</td>
<td>- Table scraps</td>
</tr>
<tr>
<td>• Reason for vet visit (open)</td>
<td>• Access to animal feces in past 2 weeks</td>
<td>• Given well water</td>
<td>- Raw beef</td>
</tr>
<tr>
<td>• Type of home</td>
<td>• Access to lakes/rivers/creeks in past 6 months</td>
<td>• Given bottled water</td>
<td>- Cooked beef</td>
</tr>
<tr>
<td>- Urban</td>
<td>• Drinks out of toilet</td>
<td>• Drinks out of toilet</td>
<td>- Raw chicken</td>
</tr>
<tr>
<td>- Suburban</td>
<td>• Access to ditches or puddles in last 6 months</td>
<td>• Access to lakes/rivers/creeks in past 6 months</td>
<td>- Cooked chicken</td>
</tr>
<tr>
<td>- Small town rural</td>
<td>• Household member with vomiting in past month</td>
<td>• Household member with diarrhea in past month</td>
<td>- Raw turkey</td>
</tr>
<tr>
<td>- Non-farm rural</td>
<td>• Household living working/visited been in hospital in past month</td>
<td>• Household member worked/visited/been in hospital in past month</td>
<td>- Cooked pork</td>
</tr>
<tr>
<td>- Farm</td>
<td>• Household member treated with antibiotics in past month</td>
<td>• Household member visited a petting zoo in past month</td>
<td>- Cooked eggs</td>
</tr>
<tr>
<td>• Season (determined from date of enrollment)</td>
<td>• Household member had contact with other cats in past month</td>
<td>• Household member had contact with other birds in past month</td>
<td>- Fish</td>
</tr>
<tr>
<td>• Veterinary Clinic (A-G)</td>
<td>• Household member had contact with other cats in past month</td>
<td>• Household member visited a petting zoo in past month</td>
<td>• Treats given more than once a month (Y/N)</td>
</tr>
<tr>
<td>Activities &amp; Pet Information (Y/N)</td>
<td>• Household member treated with antibiotics in past month</td>
<td>• Household member had contact with livestock in past month</td>
<td>- Dried pig’s ears</td>
</tr>
<tr>
<td>• Involved in hunting</td>
<td>• Household member had contact with other cats in past month</td>
<td>• Household member visited a petting zoo in past month</td>
<td>- Raw bones</td>
</tr>
<tr>
<td>• Has contact with other dogs</td>
<td>• Household member had contact with other birds in past month</td>
<td>• Household member visited a petting zoo in past month</td>
<td>- Cooked bones</td>
</tr>
<tr>
<td>• Visits dog park</td>
<td>• Household member visited a petting zoo in past month</td>
<td>• Household member had contact with livestock in past month</td>
<td>- Store bought bones</td>
</tr>
<tr>
<td>• Goes to dog day care</td>
<td>• Household member had contact with other birds in past month</td>
<td>• Household member had contact with livestock in past month</td>
<td>- Rawhide chews</td>
</tr>
<tr>
<td>• Group activity&lt;sup&gt;b&lt;/sup&gt;</td>
<td>• Household member visited a petting zoo in past month</td>
<td>• Household member had contact with livestock in past month</td>
<td>• Homemade cooked food fed or added to diet (collapsed variable)</td>
</tr>
<tr>
<td>• Involved in therapy program</td>
<td>• Household member visited a petting zoo in past month</td>
<td>• Household member had contact with livestock in past month</td>
<td></td>
</tr>
<tr>
<td>• Vet clinic stay in last 6 months</td>
<td>• Household member had contact with other birds in past month</td>
<td>• Household member visited a petting zoo in past month</td>
<td></td>
</tr>
<tr>
<td>• Kennel stay in last 6 months</td>
<td>• Household member visited a petting zoo in past month</td>
<td>• Household member had contact with livestock in past month</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Small, medium, large/giant breed or mixed breed; <sup>b</sup>Participation in activities like obedience, flyball, agility; <sup>c</sup>Livestock includes cattle, sheep, goats, pigs, or horses; <sup>d</sup>Diagnosed with *Salmonella*, *Campylobacter*, *Giardia* or *C. difficile*. 

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Table 3.2: Demographic information of the client-owned pet dogs sampled in this study from the Region of Waterloo, Ontario 2008-2009 (n=240)

<table>
<thead>
<tr>
<th>Clinic (clinic type)</th>
<th>Number of Dogs n=240 (%)</th>
<th>Campylobacter spp. Positive/Negative</th>
<th>C. upsaliensis Positive/Negative</th>
<th>C. jejuni Positive/Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (small animal, urban)</td>
<td>60 (25.0%)</td>
<td>10/50</td>
<td>8/52</td>
<td>2/58</td>
</tr>
<tr>
<td>B (small animal, suburban)</td>
<td>80 (33.3%)</td>
<td>17/63</td>
<td>16/64</td>
<td>1/79</td>
</tr>
<tr>
<td>C (mixed practice, rural)</td>
<td>8 (3.3%)</td>
<td>5/3</td>
<td>5/3(^a)</td>
<td>1/7(^a)</td>
</tr>
<tr>
<td>D (small animal, urban)</td>
<td>3 (1.3%)</td>
<td>0/3</td>
<td>0/3</td>
<td></td>
</tr>
<tr>
<td>E (small animal, suburban)</td>
<td>47 (19.6%)</td>
<td>13/34</td>
<td>11/36</td>
<td>2/45</td>
</tr>
<tr>
<td>F (small animal, suburban)</td>
<td>35 (14.6%)</td>
<td>6/29</td>
<td>5/30</td>
<td>1/34</td>
</tr>
<tr>
<td>G (small animal &amp; exotics, urban)</td>
<td>7 (2.9%)</td>
<td>1/6</td>
<td>1/6</td>
<td>0/7</td>
</tr>
</tbody>
</table>

Dog Demographics

<table>
<thead>
<tr>
<th>Sex</th>
<th>Male</th>
<th>Female</th>
<th>C. upsaliensis Positive/Negative</th>
<th>C. jejuni Positive/Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>113 (47.1%)</td>
<td>25/88</td>
<td>22/91(^a)</td>
<td>4/99(^a)</td>
</tr>
<tr>
<td>Female</td>
<td>127 (52.9%)</td>
<td>27/100</td>
<td>24/103</td>
<td>3/124</td>
</tr>
</tbody>
</table>

| Intact                  | Yes        | 50 (20.8%) | 16/34                           | 15/35\(^a\)                | 2/48\(^a\) |
| No                      | 190 (79.2%)| 36/154     | 31/159                          | 5/185                       |

| Breed Size               | Pure Small (<25 lbs) | 68 (28.3%) | 15/53                           | 14/54                       | 1/67       |
| Pure Medium (25-60 lbs)  | 27 (11.3%)          | 7/20       | 6/21\(^a\)                      | 2/25\(^a\)                 |
| Pure Large/Giant (>60 lbs)| 69 (28.8%)        | 17/52      | 15/54                           | 2/67                        |
| Mixed (any size)         | 76 (31.7%)         | 13/63      | 11/65                           | 2/74                        |

<p>| Source of dog            | Pet Store | 16 (6.7%)  | 1/15                            | 1/15                        | 0/15       |
| Breeder                  | 122 (50.8%)| 24/98      | 21/101                          | 3/119                       |
| Humane Society           | 20 (8.3%)  | 2/18       | 2/18                            | 0/20                        |
| Other(^b)              | 82 (34.2%) | 25/57      | 22/60(^a)                     | 4/78(^a)                 |</p>
<table>
<thead>
<tr>
<th>Type of home</th>
<th>55 (22.9%)</th>
<th>11/44</th>
<th>8/47</th>
<th>3/52</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suburban</td>
<td>165 (68.8%)</td>
<td>36/129</td>
<td>33/132</td>
<td>3/162</td>
</tr>
<tr>
<td>Rural</td>
<td>20 (8.3%)</td>
<td>5/15</td>
<td>5/15a</td>
<td>1/19a</td>
</tr>
<tr>
<td>Season when recruited</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>43 (17.9%)</td>
<td>6/37</td>
<td>6/37</td>
<td>0/43</td>
</tr>
<tr>
<td>Summer</td>
<td>76 (31.7%)</td>
<td>18/58</td>
<td>16/60a</td>
<td>3/73a</td>
</tr>
<tr>
<td>Fall</td>
<td>71 (29.6%)</td>
<td>21/50</td>
<td>18/53</td>
<td>3/68</td>
</tr>
<tr>
<td>Winter</td>
<td>50 (20.8%)</td>
<td>7/43</td>
<td>6/44</td>
<td>1/49</td>
</tr>
<tr>
<td>Age in years</td>
<td>Mean (minimum, maximum)</td>
<td>4.9 (0.14, 17)</td>
<td>2.6 (0.14, 9.6)/5.5 (0.14, 17)</td>
<td>2.8 (0.14, 9.6)/5.4 (0.14, 17)</td>
</tr>
</tbody>
</table>

a Total is greater than total number positive because one dog had both *C. upsaliensis* and *jejuni*; b Included farms, friends, rescue groups, etc.
Table 3.3: Descriptive statistics and significant associations (p≤ 0.20) from univariable logistic regression analysis of pet-related management factors and *Campylobacter* spp. and *C. upsaliensis* carriage in client-owned pet dogs, recruited through veterinary clinics in the Region of Waterloo, Ontario, 2008-2009 (n=240)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exp.</th>
<th>Unexp.</th>
<th>O.R.</th>
<th>P-value</th>
<th>95% C.I.</th>
<th>O.R.</th>
<th>P-value</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (in years)</td>
<td>n/a</td>
<td>n/a</td>
<td>0.8</td>
<td>0.000</td>
<td>0.7-0.9</td>
<td>0.8</td>
<td>0.000</td>
<td>0.7-0.9</td>
</tr>
<tr>
<td>Intact</td>
<td>50</td>
<td>190</td>
<td>2.0</td>
<td>0.049</td>
<td>1.0-4.0</td>
<td>2.2</td>
<td>0.031</td>
<td>1.1-4.5</td>
</tr>
<tr>
<td>Participates in a group activity&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16</td>
<td>224</td>
<td>4.1</td>
<td>0.008</td>
<td>1.5-11.5</td>
<td>2.8</td>
<td>0.063</td>
<td>1.0-8.0</td>
</tr>
<tr>
<td>No antibiotics in previous month</td>
<td>180</td>
<td>60</td>
<td>5.1</td>
<td>0.003</td>
<td>1.8-14.8</td>
<td>6.0</td>
<td>0.004</td>
<td>1.8-20.0</td>
</tr>
<tr>
<td>Homemade cooked food fed or added to diet</td>
<td>11</td>
<td>229</td>
<td>4.8</td>
<td>0.013</td>
<td>1.4-16.3</td>
<td>5.7</td>
<td>0.006</td>
<td>1.7-19.5</td>
</tr>
<tr>
<td>Fed table scraps</td>
<td>71</td>
<td>169</td>
<td>0.4</td>
<td>0.014</td>
<td>0.2-0.8</td>
<td>0.4</td>
<td>0.021</td>
<td>0.2-0.9</td>
</tr>
<tr>
<td>Catches or hunts prey animals&lt;sup&gt;f&lt;/sup&gt;</td>
<td>26</td>
<td>214</td>
<td>5.1</td>
<td>0.095</td>
<td>0.9-5.1</td>
<td>2.5</td>
<td>0.039</td>
<td>1.1-6.1</td>
</tr>
<tr>
<td>Drinks out of ditches and puddles</td>
<td>103</td>
<td>137</td>
<td>3.3</td>
<td>0.074</td>
<td>1.0-3.3</td>
<td>2.0</td>
<td>0.040</td>
<td>1.0-3.8</td>
</tr>
<tr>
<td>Contact with other dogs</td>
<td>190</td>
<td>50</td>
<td>4.5</td>
<td>0.144</td>
<td>0.8-4.5</td>
<td>2.0</td>
<td>0.154</td>
<td>0.8-4.9</td>
</tr>
<tr>
<td>Kennel stay in past 6 months</td>
<td>42</td>
<td>198</td>
<td>1.8</td>
<td>0.111</td>
<td>0.9-3.8</td>
<td>1.9</td>
<td>0.092</td>
<td>0.9-4.1</td>
</tr>
<tr>
<td>More than one dog in the house</td>
<td>61</td>
<td>179</td>
<td>0.6</td>
<td>0.133</td>
<td>0.3-1.2</td>
<td>0.6</td>
<td>0.133</td>
<td>0.3-1.3</td>
</tr>
<tr>
<td>No children in the household&lt;sup&gt;g&lt;/sup&gt;</td>
<td>161</td>
<td>79</td>
<td>1.9</td>
<td>0.091</td>
<td>0.9-3.8</td>
<td>1.7</td>
<td>0.152</td>
<td>0.8-3.6</td>
</tr>
<tr>
<td>Household member works in/has visited a hospital in the past week</td>
<td>51</td>
<td>189</td>
<td>0.5</td>
<td>0.126</td>
<td>0.2-1.2</td>
<td>0.4</td>
<td>0.063</td>
<td>0.2-1.1</td>
</tr>
<tr>
<td>------------------------------------------------------------------</td>
<td>----</td>
<td>-----</td>
<td>-----</td>
<td>--------</td>
<td>---------</td>
<td>-----</td>
<td>--------</td>
<td>---------</td>
</tr>
<tr>
<td>Household member had contact with other cats in past week $^h$</td>
<td>57</td>
<td>183</td>
<td>1.8</td>
<td>0.089</td>
<td>0.9-3.5</td>
<td>2.0</td>
<td>0.053</td>
<td>1.0-4.0</td>
</tr>
<tr>
<td>Household member had contact with livestock in past week $^i$</td>
<td>13</td>
<td>227</td>
<td>2.4</td>
<td>0.141</td>
<td>0.8-7.7</td>
<td>2.8</td>
<td>0.080</td>
<td>0.9-9.1</td>
</tr>
<tr>
<td>Dog has had access to dead animals in past 2 weeks</td>
<td>11</td>
<td>229</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>2.6</td>
<td>0.151</td>
</tr>
<tr>
<td>Dog drinks out of toilet</td>
<td>28</td>
<td>212</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1.8</td>
<td>0.184</td>
</tr>
<tr>
<td>Household member has had vomiting or diarrhoea in past week</td>
<td>35</td>
<td>205</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.4</td>
<td>0.143</td>
</tr>
</tbody>
</table>

$^a$Exposed dogs (i.e. those that were positive for the risk factor); $^b$Unexposed dogs (i.e. those that were negative for the risk factor); $^c$Odds Ratio calculated in Stata/MP 11.0; $^d$95% confidence interval of the Odds Ratio calculated in Stata/MP 11.0; $^e$Included obedience, flyball and agility classes; $^f$Included birds, small rodents and other small prey; $^g$Children <18 years old that live in the home on a regular basis; $^h$Someone who lives in the home had contact with a cat that was not their own in the previous 7 days; $^i$Someone who lives in the home had contact with livestock (cattle, sheep, goats, pigs, or horses) in the previous 7 days; Dashes signify that the variable was not significant at the 20% level in that univariable model.
Table 3.4: Significant risk factors (p≤ 0.05) from multivariable logistic regression analysis of pet-related management factors and *Campylobacter* spp. and *C. upsaliensis* carriage for client-owned pet dogs recruited through veterinary clinics in the Region of Waterloo, Ontario, 2008-2009 (n=240).

| Variable | *Campylobacter* spp. model | | | | | | *Campylobacter upsaliensis* model | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Odds Ratio | P-value | 95% C.I. | Odds Ratio | P-value | 95% C.I. |
| Age | 0.8 | 0.000 | 0.7 – 0.9 | 0.8 | 0.000 | 0.7 – 0.9 |
| Participates in a group activity | | | | | | | | | |
| No (referent) | | | | | | | | | |
| Yes | 4.0 | 0.027 | 1.2-13.8 | -- | -- | -- |
| Children in the home | | | | | | | | | |
| Yes (referent) | | | | | | | | | |
| No | 2.5 | 0.029 | 1.1 – 5.6 | -- | -- | -- |
| Antibiotics in previous month | | | | | | | | | |
| Yes (referent) | | | | | | | | | |
| No | 8.7 | 0.001 | 2.4 – 31.7 | 10.7 | 0.003 | 2.3 – 50.0 |
| Homemade cooked food fed or added to diet | | | | | | | | | |
| No (referent) | | | | | | | | | |
| Yes | 17.8 | 0.001 | 3.4 – 93.2 | 25.3 | 0.000 | 4.3 – 148.0 |
| Household member had contact with other cats in the previous week | | | | | | | | | |
| No (referent) | | | | | | | | | |
| Yes | -- | -- | -- | 2.3 | 0.040 | 1.0 – 5.2 |

*95% confidence interval of the Odds Ratio; bParticipation in activities like obedience, flyball, agility; cChildren <18 years old that live in the home on a regular basis; dSomeone who lived in the home had contact with a cat that was not their own in the previous 7 days; Dashes signify that the variable was not significant at the 5% level in that multivariable model.*
CHAPTER FOUR

Comparison of antimicrobial resistance patterns of *Salmonella* spp. and *Escherichia coli* recovered from pet dogs from volunteer households in Ontario (2005-2006)


SYNOPSIS

*Objective*: To compare the antimicrobial resistance (AMR) patterns of *Salmonella* spp. and *Escherichia coli* in the feces of pet dogs from volunteer households in Southwestern Ontario, Canada.

*Methods*: From October 2005-May 2006, 138 dogs from 84 Ontario households were recruited to participate in a cross-sectional study. Five consecutive daily fecal samples were collected from each dog and cultured for *Salmonella* spp. and *E. coli*. A panel of 15 antimicrobials from 7 antimicrobial classes was used for susceptibility testing.

*Results*: *E. coli* and *Salmonella* spp. were recovered from 96.4% and 23.2% of dogs, respectively. In total, 515 bacterial isolates from 136 dogs from 83 households were sent for antimicrobial susceptibility testing with 80.4% of isolates being pan-susceptible. The most common resistance pattern was to amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur and ceftriaxone (AMC-AMP-FOX-TIO-CRO), present in 13.3% of *Salmonella* isolates and 1.3% of *E. coli* isolates. Fifty-eight of the isolates were resistant to two or more drug classes, with 70.7% and 29.3% being *E. coli* and *Salmonella*, respectively. Based on multilevel logistic regression, the odds of
resistance were greater in *E. coli* than *Salmonella* (odds ratio (OR) = 3.2; 95% confidence interval (C.I.) = 1.22-8.43). Agreement in resistance between *E. coli* and *Salmonella* isolates from the same dog was low (prevalence-adjusted, bias-adjusted kappa (PABAK) = 0.38; 95% C.I. = 0.30-0.46).

**Conclusions:** Pet dogs are a potential household source of antimicrobial resistant *Salmonella* spp. and *E. coli*. However, extrapolating the epidemiology of AMR in pathogens, like *Salmonella*, from *E. coli* should be done with caution.

**Keywords:** canine, AMR, clustering, zoonoses, public health

**INTRODUCTION**

Antimicrobial resistance (AMR) is an international health issue, and as animals are a potential microbial reservoir for resistant bacteria, antimicrobial resistance in animals is a growing concern.¹ The true role of companion animals, particularly dogs and cats, in the spread of resistant bacteria to humans is poorly understood.² Additionally, companion animals are a difficult reservoir of antimicrobial resistance to study, due to a lack of surveillance and routine testing. However, recent studies have begun to shed some light on the occurrence of antimicrobial resistance in pets.³-⁵ Antimicrobial resistance in zoonotic pathogens from companion animals, particularly *Salmonella* spp. and *Campylobacter* spp., is of great importance as their close relationship and frequent contact with humans may make these animals a source of resistant bacteria and an important public health issue.¹ ⁶ Recent studies have concluded that close contact between humans and pets can lead to the exchange of pathogenic bacteria, including those carrying AMR.⁷ ⁸
Non-human animals are considered the main microbial reservoir for *Salmonella* spp. that infect people and the majority of human cases are believed to be foodborne. \(^9,10\) Until recently, few studies have investigated the role of pet dogs as a potential source of antimicrobial-resistant *Salmonella* in humans. Antimicrobial resistant strains have been found in canine *Salmonella* isolates, including several multidrug-resistant (MDR) isolates and isolates resistant to cephalosporins and fluoroquinolones.\(^6,11\)

Commensal bacteria in animals and humans, especially enteric bacteria, are continuously subjected to many antimicrobial pressures due to veterinary and human medicine.\(^7\) Commensal bacteria, like *E. coli*, are considered a major source of antimicrobial resistance genes, with selection for antimicrobial resistance usually occurring in non-pathogenic bacterial species.\(^2,12\) Recent surveillance and scientific studies in Europe and Canada have estimated the prevalence of resistance to at least one antimicrobial in *E. coli* isolates from healthy pet animals to be between 10% and 30%.\(^5,7,13\)

It is estimated that there are approximately 6 million dogs in Canada, with 32% of households owning at least one dog\(^14\) and the close contact most people have with their pets causes concern with respect to the potential transmission of zoonotic AMR bacteria from dogs to humans. The objective of this study was to determine the prevalence of and compare common antimicrobial resistance patterns in *E. coli* and *Salmonella* spp. isolates recovered from a population of healthy pet dogs from volunteer households in Ontario, Canada.
MATERIALS AND METHODS

Recruitment

Between October 2005 and May 2006, a convenience sample of households in Ontario were recruited to participate in a study to investigate the presence of *Salmonella*, *Clostridium difficile* and *E. coli* (for the purpose of researching antimicrobial resistance) in household dogs, as previously described. Briefly, a total of 138 dogs from 84 households were recruited for the study through advertising in brochures, e-mail, and listserves that targeted the University of Guelph, the Ontario Veterinary College, pet therapy organizations in Southwestern Ontario, local veterinary conferences and meetings, and veterinary clinics in Ontario that agreed to display brochures related to the project. The only inclusion criterion was the presence of one or more dogs in the household. There were no exclusion criteria. The study was approved by the University of Guelph Research Ethics Board and owner consent was obtained for all participating dogs.

Samples and Questionnaire

As previously described, study homes were visited by trained technicians, who for this study administered a questionnaire, and provided a fecal collection kit to the primary care givers of each dog to collect and return a single fecal sample per day for five consecutive days. Descriptions of the fecal kit, instruction guide, and questionnaire can be found elsewhere.
Salmonella Isolation

All samples were received via express post at the University of Guelph. A complete description of the isolation of *Salmonella* from the canine fecal samples can be found in Leonard, *et al.* (2010). In brief, 10g of fresh feces were combined with a 0.85% saline solution, homogenized and then pre-enriched using buffered peptone water (BPW) and two parallel isolation methods were then used. In the first method, the BPW mixture was inoculated into modified semi-solid Rappaport-Vassiliadis (MSRV) agar, plated on MacConkey agar and two presumptive non-lactose fermenting colonies were inoculated on full tryptic soy agar (TSA). In the second method, the BPW solution was inoculated into Rappaport-Vassiliadis (RV) broth, then added to tetrathionate (TT) broth, then inoculated onto each of full xylose lysine tergitol 4 (XLT4), brilliant green sulfa (BGS), and bismuth sulfate (BS) agars and finally two typical colonies were sub-cultured onto MacConkey agar and non-lactose fermenting colonies were plated onto TSA. Biochemical testing for isolates recovered using either method was conducted using Christensen's urea, triple sugar iron, and *Salmonella* O antiserum Poly A-I & Vi agglutination test. Up to three *Salmonella* positive isolates per day per dog were submitted to the Office Internationale des Epizooties (OIE) Reference Laboratory for Salmonellosis (Laboratory for Foodborne Zoonoses (LFZ), Public Health Agency of Canada, Guelph) for serotyping and phagetyping, and the Canadian Integrated Program for Antimicrobial Resistance Surveillance (LFZ, Guelph) for antimicrobial susceptibility testing.
E. coli Isolation

All fecal samples from the participating dogs were cultured for *E. coli* at the Canadian Research Institute for Food Safety laboratory, University of Guelph. Fifty millilitres of the BPW mixture from the *Salmonella* isolation were combined with 50 mL of double strength *E. coli* (EC) broth (Difco, Becton Dickinson) and incubated at 42°C for 18-24h. A loopful of EC broth was then plated on Eosin Methylene Blue (Difco, Becton Dickinson) agar and incubated at 37°C for 18-24h. Presumptive *E. coli* colonies were transferred to MacConkey agar (Difco, Becton Dickinson) for purification and incubated at 37°C for 18-24h. Isolated *E. coli* colonies were transferred onto Luria-Bertani agar (Difco, Becton Dickinson) and incubated at 37°C for 18-24h. Confirmation testing of *E. coli* was conducted using Kovac’s indole spot reagent (PML Microbiologicals, Mississauga, Ontario, Canada) and Simmon’s citrate agar (Difco, Becton Dickinson). No further subtyping was performed on the *E. coli* isolates. Three *E. coli* isolates per dog were taken from the first *E. coli* positive fecal sample and forwarded to the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) at the Laboratory for Foodborne Zoonoses (LFZ), Public Health Agency of Canada, Guelph, Ontario for antimicrobial susceptibility testing.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing for the *Salmonella* and *E. coli* isolates was conducted using the Sensititre™ automated broth microdilution system (Trek™ Diagnostic Systems Ltd.). The National Antimicrobial Resistance Monitoring System
(NARMS) susceptibility panel CMV1AGNF was used for the *E. coli* and *Salmonella* isolates, using methods described by CIPARS. A list of the antimicrobials and their minimum inhibitory concentration (MIC) breakpoints used for *E. coli* and *Salmonella* susceptibility testing in this project can be found in Table 4.1. The breakpoints for resistance are those used by CIPARS and NARMS, which were derived from the Clinical Laboratory Standards Institute (CLSI) where available, and a lowered MIC breakpoint for ceftriaxone was used in this study, based on the CLSI Informational supplement M100-S20. Antimicrobials were also classified based on their importance to human medicine.

**Statistical Analysis**

The data were entered and analyzed in Intercooled Stata/MP® 11.1 for Windows (College Station, Texas, USA). All tests were two-tailed with a statistical significance level of 5%. Isolates were considered “susceptible” or “resistant” based on the MIC breakpoints found in Table 4.1 and isolates with intermediate susceptibility based on MIC values to the tested antimicrobials were considered “susceptible” for this project.

Using Intercooled Stata/MP® 11.1, the prevalence of resistance for each antimicrobial was calculated at the isolate, dog and household level. Ninety-five percent confidence intervals were calculated for binomial proportions for descriptive purposes, using the Clopper-Pearson exact method, not accounting for clustering. As well, the prevalence of resistance to each antimicrobial was calculated by bacterial species (*E. coli* vs. *Salmonella*) and *Salmonella* serovar. Finally, the prevalences of
antimicrobial resistance (AMR) patterns were also analyzed for each antimicrobial.

Multilevel multivariable logistic regression models for resistance (i.e., resistant to any antimicrobial) were developed with latent variable techniques and quadrature methods.\textsuperscript{21,22} The multilevel models contained random effects for dog and household to account for clustering, as multiple isolates were tested per dog and multiple dogs were tested per household. A fixed effect for bacterial species, \textit{Salmonella} spp. vs. \textit{E. coli}, was also included in the models. These models were used to determine variance estimates for isolates in the same dog and dogs in the same household.\textsuperscript{21,22} They were also used to estimate prevalence of resistance for \textit{Salmonella} and \textit{E. coli} at the isolate level after accounting for clustering at the dog and household level using the \texttt{<predict>} command in Stata. Separate \textit{Salmonella} and \textit{E. coli} models were also created with random effects for dog and household. The variance estimates from these models were used to determine what percentage of variance in resistant isolates was at the dog level, and what percentage was at the household level. Improvement to model fit was assessed using Akaike information criterion (AIC) values as the random effects were added to the model.\textsuperscript{21}

To determine the ability of \textit{E. coli} to predict resistance in \textit{Salmonella} spp., McNemar’s $\chi^2$, the kappa statistic, and PABAK were used to evaluate the agreement in resistance between the \textit{E. coli} and \textit{Salmonella} spp. isolates within the same dog.\textsuperscript{23} If a statistically significant McNemar’s $\chi^2$ was found, PABAK was calculated. PABAK is an adjusted kappa used to correct for imbalances due to high (>80%) or low (<20%) prevalences, and significant differences in the proportion positive among tests, that can result in biased or unstable kappa values.\textsuperscript{23} A kappa or PABAK value
less than 0.4 was considered poor to fair agreement, based on the kappa degree of agreement scale developed by Landis and Koch.\textsuperscript{21,24} However, it should be noted that kappa and PABAK scales are for intra-observer agreement and we would expect the values to be higher for objective tests.\textsuperscript{25}

**RESULTS**

Descriptive Statistics

In total, 515 bacterial isolates were tested for antimicrobial susceptibility, from 136 dogs and 83 households, no bacteria were recovered from 2 dogs in one household. Approximately 23% (32/138; 95% C.I. =16.4-31.1%) of the dogs enrolled in this study had at least one fecal sample positive for *Salmonella*, providing 120 *Salmonella* isolates. The median number of *Salmonella* isolates recovered from positive dogs was two (range 1-12). *E. coli* was recovered from 96% (133/138; 95% C.I. =91.75-98.81%) of the dogs, which provided 395 *E. coli* isolates for antimicrobial susceptibility testing. The majority of *Salmonella* isolates (80%; 96/120), and *E. coli* isolates (81%; 318/395), were susceptible to all antimicrobials tested (pan-susceptible). At the dog and household level, only pan-susceptible isolates were recovered from 72% (98/136) of the dogs and 65% (54/83) of the households. Of the *Salmonella* isolates, 14% (17/120) were multiclass resistant (resistance to two or more classes of antimicrobial) and of the *E. coli* isolates, 10% (41/395) were multiclass resistant. The distribution of all resistant isolates, divided by antimicrobial, antimicrobial category, bacterial species, dog and household, can be found in Table 4.2 and Figure 4.1.
Resistance Patterns

Among the resistant *Salmonella* isolates, 79% (19/24) were serovar Heidelberg, 12.5% (3/24) were serovar Kentucky and 8.3% (2/24) were serovar Indiana. The most common *Salmonella* AMR pattern found in this population of dogs was resistance to amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur and ceftriaxone, (AMC-AMP-FOX-TIO-CRO), and was only found in *S*. Heidelberg. All of the serovar AMR patterns broken down by isolate, dog and household can be found in Table 4.3.

Common AMR patterns among the resistant *E. coli* isolates, broken down by isolate, dog and household, can be found in Table 4.4. The most common *E. coli* antimicrobial resistance found in isolates from this population of dogs was resistance to ampicillin, followed by ampicillin and tetracycline (AMP-TET) resistance.

Although only involving a few homes, common patterns of resistance were found in dogs within the same household, as well as between *Salmonella* and *E. coli* within and between dogs. A comparison of all the similar AMR patterns can be found in Table 4.5.

Multilevel Models

The multilevel random effects in the logistic regression models allowed for the comparison of variance within and between the different households and dogs. As the random effects for household and dog were added into the model with a fixed effect for bacterial species, the AIC improved from 513.81 to 384.72 over the original simple model. From the multilevel model for resistance, approximately 53% and 31%
of the variance was accounted for at the household and individual dog levels, respectively; the percentage of total variance specifies where most of the variance in the outcome is occurring. Of the households recruited for this study, 54% (45/83) had one dog, 35% (22/83) had two dogs and 12% (10/83) had 3 or more dogs. From this multilevel model, it was also determined that the odds of resistance were 3.2 times greater in *E. coli* than *Salmonella* (p=0.02; 95% C.I.=1.22-8.43). From the model for *E. coli* resistance only, the variance due to clustering was fairly evenly distributed between the household level (variance = 17.38; 49%) and the individual dog level (variance = 14.56; 41%). From the model for *Salmonella* resistance only, the majority of variance was at the household level (16.20; 83%), with less than 1% (0.05) of the variation at the individual dog level.

Using the predict command in Stata/MP® 11.1 with the multilevel model with a fixed effect for bacterial species, the predicted prevalence of resistance in *E. coli* isolates was 1.6% (95% C.I.=0.29-8.47) and the predicted prevalence for resistance in *Salmonella* isolates was 0.5% (95% C.I.=0.06-3.64).

**Agreement in Resistance between E. coli and Salmonella**

Using only dogs that were positive for both *E. coli* and *Salmonella* spp. (n=29), the agreement in resistance in general, resistance to ampicillin and resistance to tetracycline between the *E. coli* and *Salmonella* isolates was evaluated with McNemar’s $\chi^2$, kappa statistics, and PABAK (Table 4.6). All three agreement statistics were non-significant or low for each resistance category, except for the statistics for tetracycline resistance. For tetracycline resistance, there was a
statistically significant McNemar’s $\chi^2 (7.00; p=0.01)$ meaning the kappa statistic was biased and not reliable, but the PABAK statistic for tetracycline resistance indicated moderate agreement (0.52; 95% C.I.= 0.43-0.60).

**DISCUSSION**

In this study, the occurrence of antimicrobial resistance (AMR) in *E. coli* and *Salmonella* spp. recovered from pet dogs in Ontario and their phenotypic resistance patterns were examined. The prevalence of resistance to at least one antimicrobial in enteric bacteria isolated from this population of pet dogs was approximately 20%, with the majority of dog and household isolates being pan-susceptible. Specifically, 20% of *Salmonella* spp. isolates were resistant to at least one antimicrobial, and the prevalence of resistance was highest to ampicillin, amoxicillin/clavulanic acid, and the cephalosporins. Similarly, approximately 20% of *E. coli* isolates were resistant to at least one antimicrobial, and the prevalence of resistance was highest to ampicillin, tetracycline, and sulfisoxazole. In both *E. coli* and *Salmonella*, isolates were found that were resistant to antimicrobials of very high importance in human medicine, such as amoxicillin/clavulanic acid (n=32), the cephalosporins (cefoxitin, ceftiofur, ceftriaxone; n=30) and ciprofloxacin (n=10). Also of concern, multiclass resistance was present in over 10% of the tested isolates (58/515; 95% C.I.=8.66-14.32). As demonstrated in this study and others like it, pet dogs may be an important reservoir of antimicrobial resistant bacteria. The human-animal bond and close contact that most owners share with their pets make AMR bacteria carried by dogs an important public health risk to owners and veterinary staff, particularly those who are
immunocompromised.

Few studies have looked at AMR phenotypes in *Salmonella* isolated from pet dogs. Previous AMR studies of canine *Salmonella* have found higher amounts of resistance to ampicillin than that reported here, and also commonly found resistance to tetracycline and streptomycin.\(^{26,27}\) A similar level of ceftiofur resistance was found in *Salmonella* recovered from dogs in the United States.\(^{28}\) However, it should be noted that the majority of *Salmonella* serovars isolated from the pet dogs in our study were susceptible to all of the 15 antimicrobials tested (Table 4.3). *Salmonella* Heidelberg exhibited the highest prevalence of resistance in comparison to the other serovars recovered and was most commonly resistant to amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur and ceftriaxone (AMC-AMP-FOX-TIO-CRO). The cephalosporin resistance phenotype (cefoxitin, ceftiofur, ceftriaxone) appears to be common in *S*. Heidelberg, and has been reported in previous studies.\(^{28,29}\) It is possible that the *S*. Heidelberg isolates in our study may have carried plasmids conferring beta-lactam resistance, however genetic typing for the samples in our study has not yet been performed. Multiclass resistance was demonstrated in the salmonellae isolated in our study (14%) and has been reported in dogs and other companion animals.\(^2\)

The occurrence of resistance to at least one antimicrobial and common resistance patterns in the *E. coli* isolates from the group of pet dogs observed here are comparable to the prevalence of resistance observed in dogs in a recent Canadian study, and several European studies.\(^{5,30-32}\) Higher prevalences of resistance to at least one antimicrobial in canine *E. coli* isolates from Canada, the United States and South Korea\(^{3,4,33,34}\) may be due to the use of clinical samples rather than samples from
healthy dogs,\textsuperscript{2,35} and may also be due to geographical differences, differences in antimicrobial use and differences in the population of dogs studied. Relative to food producing animals in Canada, pan-susceptibility appears to be higher in pet dogs.\textsuperscript{16}

After controlling for clustering with random effects for dog and household, the predicted prevalence of resistance for \textit{Salmonella} and \textit{E. coli} isolates from the multilevel model were noticeably smaller than the true prevalence in our study. This discrepancy commonly occurs with predictive models using large multiple random effects, and has been reported in previous studies.\textsuperscript{21,36} The odds of resistance and predicted prevalence of resistance were also higher in \textit{E. coli} than in \textit{Salmonella} spp., suggesting that \textit{E. coli} may be more susceptible to antimicrobial selection pressure than \textit{Salmonella}. This is one of the main reasons for using \textit{E. coli} for AMR surveillance.\textsuperscript{2,12,36} As well, \textit{E. coli} is easily and inexpensively recovered in most animals, supporting its potential use for AMR monitoring. However, in our study, \textit{E. coli} was generally poor at predicting the AMR patterns of \textit{Salmonella} recovered from the same dog. This finding has also been concluded in a previous study involving porcine \textit{Salmonella} and \textit{E. coli},\textsuperscript{36} and caution should therefore be taken when extrapolating AMR epidemiology from \textit{E. coli} to pathogens like \textit{Salmonella} at the individual dog and household level.

Although we did not find very good agreement between \textit{Salmonella} and \textit{E. coli} isolates within the same dog, there were occasions when there were similar phenotypic resistance patterns within dogs and within households (Table 4.5). It is logical that dogs within the same household would have similar exposures and regular contact, making the potential spread of AMR bacteria possible. To date, molecular
genetic typing has not been completed on the isolates in our study, so confirmed linkages between isolates from different dogs within the same household cannot be made. However, this theory is supported by previous studies, where kenneled dogs were more likely than dogs from private homes to have AMR E. coli, and E. coli exhibiting similar resistance patterns and genes were found in humans and dogs within the same household. In addition, the high variance components at the household level found in our study are evidence of clustering, supporting the possible spread of resistant bacteria among dogs within the same household and/or common sources of infection within households. The differences in variance components amongst the Salmonella and E. coli isolates in our study may reflect differences in clonal diversity between the two bacterial populations.

Our study’s design needs to be considered when interpreting the results. This was a cross-sectional study and dogs were recruited as a convenience sample, and through several non-random routes. This process may have created several distinct groups of dogs that are not representative of the typical canine population in Ontario. In addition, if dogs were related through common social groups (e.g. therapy or agility programs), there may be some unrecorded clustering that was not accounted for in our analyses. Also, sample size needs to be taken into account, given that only 32 dogs were found to be shedding Salmonella, and only 138 dogs were recruited for the study in total. However, multiple isolates were examined per dog and per household, giving a total of 515 isolates, which allowed for the evaluation of within dog and within household variation. Finally, this study, based on current CLSI standards, used a lower MIC for ceftriaxone than previous studies. This must be taken into
consideration when comparing the results of this study with those of earlier AMR studies in dogs.

Antimicrobial resistance in this population of pet dogs was found to be at levels similar to those found in several previous studies, and pet dogs should be considered as an important potential source of resistant bacteria. The similarities in the AMR patterns of dogs within the same household could be an indication of the potential spread of AMR bacteria between dogs or could be due to common exposure sources of bacteria, like raw meats. *Salmonella* has been reported to have spread from dogs to humans,\(^{39,40}\) so the potential spread of antimicrobial resistant *Salmonella* from dogs to humans is an additional concern. Given the close relationship that most owners have with their dogs, the spread of AMR bacteria between dogs and owners must be considered, especially for those who are immunocompromised. Further studies evaluating the genetic similarity of AMR bacteria isolated from dogs and humans and pet-related risk factors for carriage of these bacteria in dogs, are needed to fully determine the public health impact of AMR in pet dogs.

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2011, presentation #32).

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**TRANSPARENCY DECLARATIONS**

None to declare.
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1 Boerlin P, Reid-Smith RJ. Antimicrobial resistance: its emergence and transmission. 


9 DuPont HL. Antimicrobial Resistance of Shigella spp., Typhoid Salmonella and


24 Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977; 33: 159-174.


27 Tsai HJ, Huang HC, Lin CM, *et al*. Salmonellae and campylobacters in household


35 Prescott JF, Hanna WJ, Reid-Smith R, *et al.* Antimicrobial drug use and resistance


Table 4.1. Resistance breakpoints for antimicrobial resistance testing of *Salmonella* spp. and generic *E. coli* isolates from pet dogs from volunteer households in Ontario, 2005-06 (*n*=136 dogs, 515 isolates)

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Resistant (mg/L)</th>
<th>Class</th>
<th>Category&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin (AMK)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>≥64</td>
<td>aminoglycosides</td>
<td>II</td>
</tr>
<tr>
<td>Amoxicillin/clavulanic acid (AMC)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>≥32</td>
<td>Penicillin/β-lactam inhibitor combination</td>
<td>I</td>
</tr>
<tr>
<td>Ampicillin (AMP)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>≥32</td>
<td>penicillins</td>
<td>II</td>
</tr>
<tr>
<td>Cefoxitin (FOX)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>≥32</td>
<td>second-generation cephalosporins</td>
<td>II</td>
</tr>
<tr>
<td>Ceftiofur (TIO)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>≥8</td>
<td>third-generation cephalosporins</td>
<td>I</td>
</tr>
<tr>
<td>Ceftriaxone (CRO)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>≥4</td>
<td>third-generation cephalosporins</td>
<td>I</td>
</tr>
<tr>
<td>Chloramphenicol (CHL)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>≥32</td>
<td>phenicols</td>
<td>III</td>
</tr>
<tr>
<td>Ciprofloxacin (CIP)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>≥4</td>
<td>fluoroquinolones</td>
<td>I</td>
</tr>
<tr>
<td>Gentamicin (GEN)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>≥16</td>
<td>aminoglycosides</td>
<td>II</td>
</tr>
<tr>
<td>Kanamycin (KAN)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>≥64</td>
<td>aminoglycosides</td>
<td>II</td>
</tr>
<tr>
<td>Nalidixic Acid (NAL)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>≥32</td>
<td>quinolones</td>
<td>II</td>
</tr>
<tr>
<td>Streptomycin (STR)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>≥64</td>
<td>aminoglycosides</td>
<td>II</td>
</tr>
<tr>
<td>Sulfisoxazole (SOX)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>≥512</td>
<td>sulphonamides</td>
<td>III</td>
</tr>
<tr>
<td>Tetracycline (TET)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>≥16</td>
<td>tetracyclines</td>
<td>III</td>
</tr>
<tr>
<td>Category</td>
<td>Antimicrobials</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>-----------------------------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Category I</td>
<td>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 (Health Canada, Veterinary Drugs Directorate, 2005).</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ \text{Trimethoprim/sulfamethoxazole (SXT)}^b \geq 4 \text{ sulphonamide combination} \quad \text{II} \]

\(^a\text{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 (Health Canada, Veterinary Drugs Directorate, 2005).} \]

\(^b\text{CLSI. Informational Supplement M100-S20.} \]

Table 4.2: Distribution of all resistant isolates by antibiotic, broken down by species of bacteria, number of dogs and number of households, from pet dogs from volunteer households in Ontario, 2005-2006

<table>
<thead>
<tr>
<th>Category*</th>
<th>Antibiotic</th>
<th>Number of isolates</th>
<th>Number of dogs</th>
<th>Number of households</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Amoxicillin/Clavulanic Acid (AMC)</td>
<td>3.8% (2.1-6.2)c</td>
<td>6.8% (3.1-12.5)</td>
<td>8.5% (3.5-16.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.2% (8.5-21.7)</td>
<td>12.5% (3.5-29.0)</td>
<td>14.3% (3.1-36.3)</td>
</tr>
<tr>
<td>I</td>
<td>Ceftiofur (TIO)</td>
<td>3.3% (1.8-5.6)</td>
<td>4.5% (1.7-9.6)</td>
<td>6.1% (2.0-13.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.2% (8.5-21.7)</td>
<td>12.5% (3.5-29.0)</td>
<td>14.3% (3.1-36.3)</td>
</tr>
<tr>
<td>I</td>
<td>Ceftriaxone (CRO)</td>
<td>3.3% (1.8-5.6)</td>
<td>4.5% (1.7-9.6)</td>
<td>6.1% (2.0-13.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.2% (8.5-21.7)</td>
<td>12.5% (3.5-29.0)</td>
<td>14.3% (3.1-36.3)</td>
</tr>
<tr>
<td>I</td>
<td>Ciprofloxacin (CIP)</td>
<td>2.5% (1.2-4.6)</td>
<td>3.0% (0.8-7.5)</td>
<td>3.7% (0.8-10.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 (0.0-0.03)</td>
<td>0 (0.0-0.1)</td>
<td>0 (0.0-0.2)</td>
</tr>
<tr>
<td>I</td>
<td>Trimethoprim/Sulphamethoxazole (SXT)</td>
<td>4.8% (2.9-7.4)</td>
<td>9.0% (4.8-15.2)</td>
<td>12.2% (6.0-21.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 (0.0-0.03)</td>
<td>0 (0.0-0.1)</td>
<td>0 (0.0-0.2)</td>
</tr>
<tr>
<td>II</td>
<td>Amikacin (AMK)</td>
<td>0 (0.0-0.01)b</td>
<td>0 (0.0-0.03)</td>
<td>0 (0.0-0.04)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 (0.0-0.03)</td>
<td>0 (0.0-0.1)</td>
<td>0 (0.0-0.2)</td>
</tr>
<tr>
<td>II</td>
<td>Ampicillin (AMP)</td>
<td>12.7%</td>
<td>18.8%</td>
<td>25.6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16.7%</td>
<td>15.6%</td>
<td>19.1%</td>
</tr>
<tr>
<td>II</td>
<td>Cefoxitin (FOX)</td>
<td>(9.5-16.3)</td>
<td>(10.5-24.6)</td>
<td>(12.6-26.5)</td>
</tr>
<tr>
<td>------</td>
<td>----------------</td>
<td>------------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td></td>
<td>3.3%</td>
<td>(1.8-5.6)</td>
<td>(8.5-21.7)</td>
<td>(1.7-9.6)</td>
</tr>
<tr>
<td>II</td>
<td>Gentamicin (GEN)</td>
<td>0.3%</td>
<td>0</td>
<td>0.8%</td>
</tr>
<tr>
<td></td>
<td>(0.0-1.4)</td>
<td>(0.0-0.03)</td>
<td>(0.0-4.1)</td>
<td>(0.0-0.1)</td>
</tr>
<tr>
<td>II</td>
<td>Kanamycin (KAN)</td>
<td>0.3%</td>
<td>0</td>
<td>0.8%</td>
</tr>
<tr>
<td></td>
<td>(0.0-1.4)</td>
<td>(0.0-0.03)</td>
<td>(0.0-4.1)</td>
<td>(0.0-0.1)</td>
</tr>
<tr>
<td>II</td>
<td>Nalidixic Acid (NAL)</td>
<td>4.8%</td>
<td>6.0%</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(2.9-7.4)</td>
<td>(2.6-11.5)</td>
<td>(0.0-0.1)</td>
<td>(0.0-0.2)</td>
</tr>
<tr>
<td>II</td>
<td>Streptomycin (STR)</td>
<td>4.3%</td>
<td>4.2%</td>
<td>7.5%</td>
</tr>
<tr>
<td></td>
<td>(2.5-6.8)</td>
<td>(1.4-9.5)</td>
<td>(3.7-13.4)</td>
<td>(0.8-20.8)</td>
</tr>
<tr>
<td>III</td>
<td>Chloramphenicol (CHL)</td>
<td>2.5%</td>
<td>3.0%</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(1.2-4.6)</td>
<td>(0.8-7.5)</td>
<td>(0.0-0.1)</td>
<td>(0.8-10.3)</td>
</tr>
<tr>
<td>III</td>
<td>Sulfisoxazole (SOX)</td>
<td>6.6%</td>
<td>11.3%</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(4.4-9.5)</td>
<td>(6.5-17.9)</td>
<td>(0.0-0.1)</td>
<td>(7.8-24.2)</td>
</tr>
<tr>
<td>III</td>
<td>Tetracycline (TET)</td>
<td>9.6%</td>
<td>2.5%</td>
<td>15.8%</td>
</tr>
<tr>
<td></td>
<td>(6.9-13.0)</td>
<td>(0.5-7.1)</td>
<td>(10.1-23.1)</td>
<td>(0.1-16.2)</td>
</tr>
<tr>
<td>----------------</td>
<td>------------</td>
<td>-----------</td>
<td>-------------</td>
<td>------------</td>
</tr>
<tr>
<td>n/a</td>
<td>Multiclass Resistant&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.4%</td>
<td>14.2%</td>
<td>16.5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(7.6-13.8)</td>
<td>(8.5-21.7)</td>
<td>(10.7-24.0)</td>
</tr>
<tr>
<td>n/a</td>
<td>Pan-susceptible</td>
<td>80.5%</td>
<td>80.0%</td>
<td>73.7%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(76.3-84.3)</td>
<td>(71.7-86.8)</td>
<td>(65.4-80.9)</td>
</tr>
</tbody>
</table>

*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.<sup>19</sup>

<sup>a</sup> Columns may total greater than the total number of isolates or 100% because multiple resistant E. coli and Salmonella isolates were recovered from several dogs; <sup>b</sup> One-sided, 97.5% confidence interval were calculated in Stata/MP 11.1 for 0 values; <sup>c</sup> 95% confidence intervals calculated in Stata/MP 11.1; <sup>c</sup> Resistant to two or more classes of antimicrobial.
Table 4.3: Antimicrobial resistance (AMR) patterns of *Salmonella* spp. isolated from pet dogs from volunteer households in Ontario, 2005-2006

<table>
<thead>
<tr>
<th><em>Salmonella</em> serovar</th>
<th>AMR Patterns</th>
<th>Number of Isolates n=120</th>
<th>Number of dogs n=32*</th>
<th>Number of households n=21*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brandenburg</td>
<td>pan-susceptible</td>
<td>15 (12.5%)</td>
<td>6 (18.8%)</td>
<td>6 (28.6%)</td>
</tr>
<tr>
<td>Heidelberg</td>
<td>AMC-AMP-FOX-TIO-CRO</td>
<td>16 (13.3%)</td>
<td>3 (9.4%)</td>
<td>2 (9.5%)</td>
</tr>
<tr>
<td>Heidelberg</td>
<td>AMP</td>
<td>3 (2.5%)</td>
<td>2 (6.3%)</td>
<td>2 (9.5%)</td>
</tr>
<tr>
<td>Heidelberg</td>
<td>pan-susceptible</td>
<td>10 (8.3%)</td>
<td>3 (9.4%)</td>
<td>2 (9.5%)</td>
</tr>
<tr>
<td>Infantis</td>
<td>pan-susceptible</td>
<td>3 (2.5%)</td>
<td>1 (3.1%)</td>
<td>1 (4.8%)</td>
</tr>
<tr>
<td>Indiana</td>
<td>STR</td>
<td>2 (1.7%)</td>
<td>1 (3.1%)</td>
<td>1 (4.8%)</td>
</tr>
<tr>
<td>I:Rough-O:r:-</td>
<td>pan-susceptible</td>
<td>1 (0.8%)</td>
<td>1 (3.1%)</td>
<td>1 (4.8%)</td>
</tr>
<tr>
<td>I:Rough-O:z29:-</td>
<td>pan-susceptible</td>
<td>2 (1.7%)</td>
<td>1 (3.1%)</td>
<td>1 (4.8%)</td>
</tr>
<tr>
<td>I:8,20:i:-</td>
<td>pan-susceptible</td>
<td>1 (0.8%)</td>
<td>1 (3.1%)</td>
<td>1 (4.8%)</td>
</tr>
<tr>
<td>Kentucky</td>
<td>STR-TET</td>
<td>2 (1.7%)</td>
<td>1 (3.1%)</td>
<td>1 (4.8%)</td>
</tr>
<tr>
<td>Kentucky</td>
<td>AMC-AMP-FOX-TIO-CRO-STR-TET</td>
<td>1 (0.8%)</td>
<td>1 (3.1%)</td>
<td>1 (4.8%)</td>
</tr>
<tr>
<td>Kentucky</td>
<td>pan-susceptible</td>
<td>19 (15.8%)</td>
<td>5 (15.6%)</td>
<td>4 (19.1%)</td>
</tr>
<tr>
<td>Ouakam</td>
<td>pan-susceptible</td>
<td>3 (2.5%)</td>
<td>1 (3.1%)</td>
<td>1 (4.8%)</td>
</tr>
<tr>
<td></td>
<td>pan-susceptible</td>
<td>7 (5.8%)</td>
<td>2 (6.3%)</td>
<td>1 (4.8%)</td>
</tr>
<tr>
<td>----------------</td>
<td>-----------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>Tennessee</td>
<td>pan-susceptible</td>
<td>7 (5.8%)</td>
<td>2 (6.3%)</td>
<td>1 (4.8%)</td>
</tr>
<tr>
<td>Thompson</td>
<td>pan-susceptible</td>
<td>9 (7.5%)</td>
<td>2 (6.3%)</td>
<td>1 (4.8%)</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>pan-susceptible</td>
<td>26 (21.7%)</td>
<td>12 (37.5%)</td>
<td>7 (33.3%)</td>
</tr>
</tbody>
</table>

Abbreviations: AMC = amoxicillin/clavulanic acid; AMP = ampicillin; FOX = cefoxitin; TIO = ceftiofur; CRO = ceftriaxone; STR = streptomycin; TET = tetracycline; *columns may total greater than “n” because several dogs had multiple serovars of *Salmonella*. 
Table 4.4: Antimicrobial resistance (AMR) patterns of generic *E. coli* isolated from pet dogs from volunteer households in Ontario, 2005-2006

<table>
<thead>
<tr>
<th><em>E. coli</em> AMR Patterns</th>
<th>Number of Isolates</th>
<th>Number of dogs</th>
<th>Number of households</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMC-AMP-CIP-KAN-NAL-STR-TET</td>
<td>1 (0.25%)</td>
<td>1 (0.8%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>AMC-AMP-FOX-TIO-CRO</td>
<td>5 (1.3%)</td>
<td>3 (2.3%)</td>
<td>3 (3.7%)</td>
</tr>
<tr>
<td>AMC-AMP-FOX-TIO-CRO-GEN-STR-SOX-TET-SXT</td>
<td>1 (0.25%)</td>
<td>1 (0.8%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>AMC-AMP-FOX-TIO-CRO-STR</td>
<td>6 (1.5%)</td>
<td>2 (1.5%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>AMC-AMP-FOX-TIO-CRO-TET</td>
<td>1 (0.25%)</td>
<td>1 (0.8%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>AMC-AMP-SOX-SXT</td>
<td>1 (0.25%)</td>
<td>1 (0.8%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>AMP</td>
<td>11 (2.8%)</td>
<td>5 (3.8%)</td>
<td>5 (6.1%)</td>
</tr>
<tr>
<td>AMP-CHL-CIP-NAL-SOX-TET-SXT</td>
<td>6 (1.5%)</td>
<td>2 (1.5%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>AMP-NAL-SOX-TET-SXT</td>
<td>2 (0.5%)</td>
<td>1 (0.8%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>AMP-SOX-SXT</td>
<td>2 (0.5%)</td>
<td>2 (1.5%)</td>
<td>2 (2.4%)</td>
</tr>
<tr>
<td>AMP-SOX-TCY-SXT</td>
<td>1 (0.25%)</td>
<td>1 (0.8%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>AMP-STR-SOX-SXT</td>
<td>1 (0.25%)</td>
<td>1 (0.8%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>AMP-STR-SOX-TET-SXT</td>
<td>2 (0.5%)</td>
<td>1 (0.8%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>Resistance Pattern</td>
<td>AMP-STR-TET</td>
<td>AMP-TET</td>
<td>CHL-NAL</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td></td>
<td>2 (0.5%)</td>
<td>1 (0.8%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>AMP-STR-TET</td>
<td>8 (2.0%)</td>
<td>5 (3.8%)</td>
<td>5 (6.1%)</td>
</tr>
<tr>
<td>AMP-TR-TET</td>
<td>1 (0.25%)</td>
<td>1 (0.8%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>CHL-SOX-TET</td>
<td>3 (0.8%)</td>
<td>1 (0.8%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>CIP-NAL</td>
<td>3 (0.8%)</td>
<td>1 (0.8%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>NAL</td>
<td>6 (1.5%)</td>
<td>3 (2.3%)</td>
<td>2 (2.4%)</td>
</tr>
<tr>
<td>SOX-SXT</td>
<td>2 (0.5%)</td>
<td>2 (1.5%)</td>
<td>2 (2.4%)</td>
</tr>
<tr>
<td>SOX-TET</td>
<td>1 (0.25%)</td>
<td>1 (0.8%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>SOX-TET-SXT</td>
<td>1 (0.25%)</td>
<td>1 (0.8%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>STR</td>
<td>1 (0.25%)</td>
<td>1 (0.8%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>STR-SOX-TET</td>
<td>3 (0.8%)</td>
<td>2 (1.5%)</td>
<td>2 (2.4%)</td>
</tr>
<tr>
<td>TET</td>
<td>6 (1.5%)</td>
<td>5 (3.8%)</td>
<td>5 (6.1%)</td>
</tr>
<tr>
<td>Pan-susceptible</td>
<td>318 (80.5%)</td>
<td>120 (90.2%)</td>
<td>70 (85.4%)</td>
</tr>
</tbody>
</table>

Abbreviations: AMC = amoxicillin/clavulanic acid; AMP = ampicillin; CHL = chloramphenicol; CIP = ciprofloxacin; FOX = cefoxitin; CRO = ceftriaxone; GEN = gentamicin; KAN = kanamycin; NAL = nalidixic acid; SOX = sulfadoxazole; STR = streptomycin; SXT = trimethoprim/sulphamethoxazole; TET = tetracycline; TIO = ceftiofur; * columns may total greater than “n” because several dogs had more than one *E. coli* resistance pattern.
Table 4.5: All isolates of generic *E. coli* and *Salmonella* spp. showing similar resistance to at least one antimicrobial within the same dog and/or within the same household, from pet dogs from volunteer households in Ontario, 2005-2006 (n=133 dogs, 82 households, 395 isolates)

<table>
<thead>
<tr>
<th>Household ID</th>
<th>Dog ID</th>
<th>Bacterial Species</th>
<th>Serovar</th>
<th>AMR patterns</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>09</td>
<td><em>E. coli</em> (3 isolates)</td>
<td></td>
<td>NAL</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td><em>E. coli</em> (1 isolate)</td>
<td></td>
<td>NAL</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td><em>E. coli</em> (1 isolate)</td>
<td></td>
<td>CHL-NAL</td>
</tr>
<tr>
<td>11</td>
<td>12</td>
<td><em>E. coli</em> (1 isolate)</td>
<td></td>
<td>AMP</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td><em>E. coli</em> (2 isolates)</td>
<td></td>
<td>AMP</td>
</tr>
<tr>
<td>13</td>
<td>19</td>
<td><em>E. coli</em> (3 isolates)</td>
<td></td>
<td>CHL-SOX-TET</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td><em>E. coli</em> (2 isolates)</td>
<td></td>
<td>AMP-TET</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td><em>E. coli</em> (1 isolate)</td>
<td></td>
<td>AMP-STR-SOX-SXT</td>
</tr>
<tr>
<td>19</td>
<td>68</td>
<td><em>Salmonella</em> (5 isolates)</td>
<td>Heidelberg</td>
<td>AMC-AMP-FOX-TIO-CRO</td>
</tr>
<tr>
<td></td>
<td>68</td>
<td><em>E. coli</em> (2 isolates)</td>
<td></td>
<td>AMP-TET</td>
</tr>
<tr>
<td></td>
<td>69</td>
<td><em>Salmonella</em> (8 isolates)</td>
<td>Heidelberg</td>
<td>AMC-AMP-FOX-TIO-CRO</td>
</tr>
<tr>
<td>26</td>
<td>48</td>
<td><em>E. coli</em> (1 isolate)</td>
<td>Heidelberg</td>
<td>AMC-AMP-FOX-TIO-CRO</td>
</tr>
<tr>
<td></td>
<td>49</td>
<td><em>Salmonella</em> (2 isolates)</td>
<td>Heidelberg</td>
<td>AMP</td>
</tr>
<tr>
<td></td>
<td>49</td>
<td><em>Salmonella</em> (3 isolates)</td>
<td>Heidelberg</td>
<td>AMC-AMP-FOX-TIO-CRO</td>
</tr>
<tr>
<td>29</td>
<td>70</td>
<td><em>E. coli</em> (3 isolates)</td>
<td></td>
<td>AMC-AMP-FOX-TIO-CRO-STR</td>
</tr>
<tr>
<td></td>
<td>73</td>
<td><em>E. coli</em> (3 isolates)</td>
<td></td>
<td>AMC-AMP-FOX-TIO-CRO-STR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>35</td>
<td>50</td>
<td><em>E. coli</em> (3 isolates)</td>
<td></td>
<td>AMP-CHL-CIP-NAL-SOX-TET-SXT</td>
</tr>
<tr>
<td>51</td>
<td></td>
<td><em>E. coli</em> (3 isolates)</td>
<td></td>
<td>AMP-CHL-CIP-NAL-SOX-TET-SXT</td>
</tr>
<tr>
<td>46</td>
<td>116</td>
<td><em>Salmonella</em> (2 isolates)</td>
<td>Indiana</td>
<td>STR</td>
</tr>
<tr>
<td>116</td>
<td></td>
<td><em>E. coli</em> (1 isolate)</td>
<td></td>
<td>AMP-TET</td>
</tr>
<tr>
<td>116</td>
<td></td>
<td><em>E. coli</em> (1 isolate)</td>
<td></td>
<td>SOX-TET-SXT</td>
</tr>
<tr>
<td>118</td>
<td></td>
<td><em>Salmonella</em> (2 isolates)</td>
<td>Kentucky</td>
<td>STR-TET</td>
</tr>
<tr>
<td>118</td>
<td></td>
<td><em>Salmonella</em> (1 isolate)</td>
<td>Kentucky</td>
<td>AMC-AMP-FOX-TIO-CRO-STR-TET TET</td>
</tr>
<tr>
<td>118</td>
<td></td>
<td><em>E. coli</em> (1 isolate)</td>
<td></td>
<td>AMP-SOX-SXT</td>
</tr>
<tr>
<td>118</td>
<td></td>
<td><em>E. coli</em> (1 isolate)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AMC = amoxicillin/clavulanic acid; AMP = ampicillin; CHL = chloramphenicol; CIP = ciprofloxacin; FOX = cefoxitin; CRO = ceftriaxone; NAL = nalidixic acid; SOX = sulfizoxazole; STR = streptomycin; SXT = trimethoprim/sulphamethoxazole; TET = tetracycline; TIO = ceftiofur
Table 4.6. McNemar’s $\chi^2$, kappa statistics and PABAK statistics used to assess the level of agreement between generic *E. coli* and *Salmonella* spp. for general antimicrobial resistance, ampicillin resistance and tetracycline resistance in pet dogs from volunteer households in Ontario, 2005-06 ($n=29$ dogs, 19 households, 199 isolates$^a$)

<table>
<thead>
<tr>
<th>Model</th>
<th>McNemar’s $\chi^2$</th>
<th>McNemar’s $\chi^2$ $P$ value</th>
<th>Kappa$^b$ statistic</th>
<th>Kappa $P$ value</th>
<th>PABAK$^b$ statistic</th>
<th>PABAK 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimicrobial resistance</td>
<td>1.00</td>
<td>0.32</td>
<td>0.20</td>
<td>0.13</td>
<td>0.38</td>
<td>0.30-0.46</td>
</tr>
<tr>
<td>Ampicillin resistance</td>
<td>1.60</td>
<td>0.21</td>
<td>0.17</td>
<td>0.32</td>
<td>0.31</td>
<td>0.23-0.39</td>
</tr>
<tr>
<td>Tetracycline resistance</td>
<td>7.00</td>
<td>0.01</td>
<td>0.17</td>
<td>0.05</td>
<td>0.52</td>
<td>0.43-0.60</td>
</tr>
</tbody>
</table>

$^a$Only 29 dogs were used for these tests because dogs had to be positive for both *Salmonella* spp. and generic *E. coli*.

$^b$For kappa and PABAK, 0.0-0.4=poor to fair agreement, 0.4-0.8=moderate to substantial agreement and $>0.8$=almost perfect agreement, calculated in Stata/MP 11.1.
Figure 4.1. Percentage of resistant isolates of *Salmonella* spp. and generic *E. coli* isolates from pet dogs from volunteer households in Ontario, 2005-06.

Error bars represent the 95% CIs (n=136 dogs, 515 isolates). **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 (Health Canada, Veterinary Drugs Directorate, 2005).**
CHAPTER FIVE

Risk factors for antimicrobial-resistant *Salmonella* spp. and *Escherichia coli* carriage in pet dogs from volunteer households in Ontario, Canada (2005-2006)

(As prepared for submission to the American Journal of Veterinary Research)

ABSTRACT

*Objective:* To determine which pet-related management factors are associated with the carriage of antimicrobial resistant *Salmonella* and *E. coli* in a population of pet dogs.

*Sample:* 138 dogs from 84 households in Ontario, Canada. *Procedure:* From October 2005 until May 2006, dogs and households in Ontario, Canada were recruited to participate in a cross-sectional study. Fecal samples were cultured for *Salmonella* and *E. coli*, providing 515 bacterial isolates for antimicrobial susceptibility testing. Multilevel logistic regression models with random effects for household and dog were created to identify pet-related management factors associated with antimicrobial resistance (AMR). *Results:* Bacterial species, the dog being fed a homemade diet or having homemade food added to dog’s diet, the dog being fed a raw diet or having anything raw added to a dog’s diet, the dog being fed a homemade raw food diet, and the dog being fed raw chicken in the past week were statistically significant risk factors for AMR in this population of dogs. *Conclusions and Clinical Relevance:* This study identified several important pet-related risk factors for the carriage of antimicrobial resistant *Salmonella* and *E. coli* in pet dogs. *Impact for Human Medicine:* The information collected from this study is crucial for the development of
evidence-based guidelines for safe dog ownership and to protect the public through responsible pet management, especially for those pet owners who are immunocompromised.

INTRODUCTION

Antimicrobial resistance (AMR) in bacteria in animals is a health concern globally, with the true role of companion animals in the spread of resistant bacteria to humans, particularly dogs and cats, being poorly understood.\textsuperscript{1-3} Recent studies have begun to shed light on the occurrence of antimicrobial resistant bacteria in pets;\textsuperscript{4-7} however, studies investigating pet-related risk factors for carriage are lacking. Antimicrobial resistance in zoonotic pathogens in companion animals, particularly \textit{Salmonella} spp. and \textit{Campylobacter} spp., is of great importance as pets have been shown to be statistically significant risk factors for infections by these pathogens in humans.\textsuperscript{8-11} Their close relationship and frequent contact with humans may make these animals a potential source of resistant bacteria and an important public health issue.\textsuperscript{1, 12, 13} Johnson \textit{et al.} \textsuperscript{14} reported the transmission of multidrug-resistant \textit{Escherichia coli} and \textit{Enterococcus faecalis} from a dog to its owner through a penetrating bite wound. Similarly, \textit{E. coli} exhibiting similar resistance patterns and resistance genes were found both in dogs and cats sharing a household and in humans and dogs sharing another household.\textsuperscript{15}

Dog ownership is common in Canada, and it has recently been estimated that there are over 6 million pet dogs living in approximately 32\% of Canadian
households, and a recent survey in the region of Waterloo, Ontario found that 43% of survey participants owned a dog. In North America, dogs are often considered family members rather than merely “pets”, most often due to an increasingly strong human-animal bond. Because of this close relationship, concerns have been raised about the potential transmission of zoonotic antimicrobial resistant bacteria between dogs and humans. Recent studies have identified previous treatment of dogs with antibiotics as a risk factor for the carriage of antimicrobial resistant Salmonella and E. coli in pet dogs. Hospitalization of dogs has also been reported as a significant risk factor for the shedding of antimicrobial resistant generic E. coli. Furthermore, Rantala et al. discovered a potential association between age of the dog and resistance to tetracycline in E. coli.

To date there appear to be limited published studies that have looked at pet-related management factors and their association with antimicrobial resistant Salmonella spp. and generic E. coli carriage in pet dogs in Canada. The purpose of this study was therefore to determine which pet-related management factors, including type of diet fed (e.g., raw, cooked or processed), exposure/access to other pets and livestock, veterinary treatments, and pet-related demographics (age, breed and sex) are associated with the carriage of antimicrobial resistant Salmonella and E. coli in a population of pet dogs from volunteer households in Ontario, Canada.
MATERIALS AND METHODS

Recruitment

Between October 2005 and May 2006, a convenience sample of households in Ontario was recruited to participate in a study that investigated the presence of *Salmonella*, *Clostridium difficile* and *E. coli* (for the purpose of researching antimicrobial resistance) in household dogs, as previously described. Briefly, a total of 138 dogs from 84 households were recruited through advertising in brochures, e-mail, and listserves that targeted the University of Guelph, the Ontario Veterinary College, pet therapy organizations in Southwestern Ontario, local veterinary conferences and meetings, and veterinary clinics in Ontario that agreed to display brochures related to the project. The study was approved by the University of Guelph’s Research Ethics Board and owner consent was obtained for all participating dogs.

Samples and questionnaire

As previously described, study homes were visited by trained technicians who collected environmental samples, administered the questionnaire, and provided a fecal collection kit for the primary care givers of each household dog to collect and return a single fecal sample per day for five consecutive days. The questionnaire collected information on the following topics: the dog’s main diet and whether additional animal products were added to the diet; the presence and type of other pets in the
home; people living in the household; the dog’s activities; veterinary care, including deworming; any contact with livestock; and the use of a probiotic in the previous month. Breed, age, sex and neuter status were collected from 129 (93.5%) of the 138 dogs. The questionnaire can be found in Appendix A.

Salmonella isolation

All samples were shipped on a daily basis by the dog owners in prepared kits and were received via express post at the Department of Population Medicine, University of Guelph. Samples were not submitted in transport media or with temperature controls. A complete description of the isolation of Salmonella from the fecal samples can be found elsewhere. In brief, 10g of fresh feces were combined with a 0.85% saline solution, homogenized and then pre-enriched using buffered peptone water (BPW). Two parallel isolation methods were then used. In the first, the BPW mixture was inoculated into modified semi-solid Rappaport-Vassiliadis agar, plated on MacConkey agar and two presumptive non-lactose fermenting colonies were inoculated on full tryptic soy agar (TSA). In the second method, the BPW solution was inoculated into Rappaport-Vassiliadis broth, then added to tetrathionate broth, and inoculated onto each of full xylose lysine tergitol 4, brilliant green sulfa and bismuth sulfate agars. Two typical colonies from each agar plate were sub-cultured onto MacConkey agar and then non-lactose fermenting colonies were plated onto TSA. Biochemical testing for both methods was conducted using Christensen's urea, triple sugar iron, and Salmonella O antiserum Poly A-I & Vi agglutination tests.
Up to three *Salmonella* positive isolates per positive day per dog were submitted to the Office Internationale des Epizooties Reference Laboratory for Salmonellosis (Laboratory for Foodborne Zoonoses (LFZ), Public Health Agency of Canada, Guelph, ON) for serotyping and phagetyping, and the LFZ susceptibility testing laboratory (Public Health Agency of Canada, Guelph, ON) for antimicrobial susceptibility testing.

**Escherichia coli isolation**

All fecal samples from the participating dogs were cultured for *E. coli* at the Canadian Research Institute for Food Safety laboratory, University of Guelph. A complete description of the isolation of *E. coli* from the fecal samples can be found elsewhere.23 In brief, a loopful of fecal slurry from the *Salmonella* isolation procedure was plated onto MacConkey agar; presumptive *E. coli* colonies were transferred to a secondary purification stage MacConkey agar and then transferred onto tryptic soy agar. Confirmation testing for *E. coli* was conducted using Kovac’s indole spot reagent and Simmon’s citrate agar. Three *E. coli* isolates per dog were taken from the first fecal sample positive for *E. coli* and forwarded to LFZ for antimicrobial susceptibility testing.
Antimicrobial susceptibility testing

Antimicrobial susceptibility testing for the *Salmonella* and *E. coli* isolates was conducted using the Sensititre™ automated broth microdilution system.\(^a\) The National Antimicrobial Resistance Monitoring System (NARMS) susceptibility panel CMV1AGNF was used for *E. coli* and *Salmonella*, using methods described by the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS).\(^{24,25}\) A list of the antimicrobials and their minimum inhibitory concentration (MIC) breakpoints used for *E. coli* and *Salmonella* susceptibility testing in this project can be found in Table 5.1. The breakpoints for resistance are those used by CIPARS and NARMS, which were derived from the Clinical Laboratory Standards Institute (CLSI), where available.\(^{24,25}\) A lowered breakpoint for ceftriaxone was used in this study, and is adapted from the CLSI Informational supplement M100-S20.\(^{26}\) Antimicrobials were also classified based on their importance to human medicine, due to the fact that many of the chemical classes of antimicrobial drugs used in animals are also used in humans and some of these antimicrobials are essential for treatment of serious life-threatening human infections.\(^{27}\)

Statistical analysis

The data were entered and analysed in Intercooled Stata/MP\(^{11.1}\) for Windows.\(^b\) All tests were two-tailed with a statistical significance level of 5%.

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\(^a\) Trek™ Diagnostic Systems Ltd., Ohio, USA

\(^b\) StataCorp. 2009. Stata Statistical Software: Release 11. College Station, TX: StataCorp LP
Isolates were considered “susceptible” or “resistant” based on the MIC breakpoints found in Table 5.1; isolates with intermediate susceptibility to the tested antimicrobials were considered “susceptible” for this project.

Multilevel logistic regression models were used to screen each variable in the questionnaire one at a time for association with the following outcomes: “resistant” (i.e., resistant to any antimicrobial), “multiclass resistant” (i.e., resistant to ≥ 2 antimicrobial classes), and specific antimicrobial resistances. Multilevel models were created using the <runmlwin> macro within Intercooled Stata/MP® 11.1,28,29 which runs MLwiN© 2.1© through Stata. All models contained random intercepts for dog and household to account for clustering, as multiple isolates were tested per dog and multiple dogs were tested per household. The random intercept models were created using re-weighted iterative generalized least squares with predictive quasi-likelihood or marginal quasi-likelihood and the first order derivative of the Taylor series expansion for linearization.30,31 Only antimicrobials and resistance categories for which resistant isolates of *E. coli* and/or *Salmonella* were found in greater than 25 dogs were examined using multilevel logistic regression. Variables significant at ≥20% were examined for collinearity using Pearson phi correlation coefficients before any further multivariable models were built.

If model convergence could be achieved, models with two explanatory variables, combining potential confounders with the significant variables from the univariable models in a pairwise fashion, were used in order to test the impact of

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potential biological confounders. The potential confounders included: breed size (mixed, small, medium, large); age category (young <1yr, adult 1-7yrs, senior >7yrs); sex (male, female); and neuter status (intact, neutered). A variable was considered a confounder if it was a non-intervening variable and its removal from the model resulted in >20% change in the coefficient of another variable.\textsuperscript{30,32} The normality of best linear unbiased predictors was assessed with normal quantile plots to determine model fit for all multilevel models.\textsuperscript{30} Due to the limited effective sample size of the dataset and model instability, full multivariable models are not presented in this manuscript. Variance estimates from each model were used to estimate the percent variance at each level in the model (i.e., isolate, dog, and household), using the latent variable technique.\textsuperscript{30}

RESULTS

In total, 138 dogs from 84 households were recruited for the study, providing 515 bacterial isolates that were tested for AMR. Approximately 23% (32/138; 95% confidence interval (C.I.) =16.4-31.1\%) of the dogs enrolled in this study had at least one fecal sample positive for \textit{Salmonella}, providing 120 \textit{Salmonella} isolates. \textit{Escherichia coli} was recovered from approximately 96\% (133/138; 95\% C.I. =91.75-98.81\%) of the dogs, providing 395 \textit{E. coli} isolates for antimicrobial susceptibility testing. Of the \textit{Salmonella} isolates, 20\% (24/120; 95\% C.I. =13.26-28.28\%) were resistant to at least one antimicrobial (“resistant”) and 14\% (17/120; 95\% C.I. =8.48-21.71\%) were multiclass resistant (“multiclass”). Of the \textit{E. coli} isolates, 20\% (77/395;
95% C.I. = 15.70-23.75) were resistant to at least one antimicrobial and 10% (41/395; 95% C.I. = 7.55-13.82) were multiclass resistant. Of the individual antimicrobials, ampicillin resistance was the most common, with 13.6% (70/515; 95% C.I. = 10.75-16.86) of isolates being resistant to this antimicrobial.

Univariable analyses

In total, 48 pet-related variables including type of diet fed (e.g., raw, cooked or processed), exposure/access to other pets and livestock, veterinary treatments, and pet-related demographics, were examined in univariable multilevel logistic models for associations with the following outcomes: “resistant”, “multiclass”, and “ampicillin” (Table 5.2). Breed, age, sex and neuter status were collected from 129 (93.5%) of the 138 dogs. Dog identification number and household identification number were used as random intercepts in all of the multilevel logistic regression models. All variables found to be significant at the 20% level and where model convergence could be achieved in the “resistant”, “multiclass”, or “ampicillin” univariable multilevel logistic regression models, can be found in Tables 5.3-5.5.

From the “resistant” univariable multilevel logistic regression models, bacterial species (E. coli vs. Salmonella), being fed a homemade diet or having homemade food added to dog’s diet, being fed a raw diet or having any raw animal product added to a dog’s diet, being fed a homemade raw food diet, and being fed raw chicken in the past week were found to be statistically significant risk factors for resistance to at least one antimicrobial (Table 5.3). Being vaccinated annually and
being treated with a heartworm preventive in the past six months were found to be significant sparing factors for resistance to at least one antimicrobial (Table 5.3). Many of the significant variables from the resistance models were highly correlated (rho = 0.68-0.99) and related to raw feeding.

From the “multiclass” univariable multilevel logistic regression models, being given a herbal product was the only risk factor significant at a level of 5% (Table 5.4). From the ampicillin resistance univariable multilevel logistic regression models, being given a herbal product was again the only statistically significant risk factor (Table 5.5).

Confounder analyses for resistance

When breed size, age category, sex, and neuter status were individually examined for their potential confounding effect on the significant univariable associations, all had appreciable effects on the majority of independent variables (Table 5.6). The greatest confounding effects were seen for species of bacteria (E. coli vs. Salmonella), being fed rawhide treats in the past week, and being allowed to run free in a dog park. Dogs fed rawhide treats in the past week, and dogs allowed to run free in a dog park became significant with the inclusion of each of the examined confounders; however, the direction of the associations stayed the same (Table 5.6). Two variable models, and consequently the impact of potentially confounding and extraneous variables, could not be assessed for both multiclass and ampicillin resistance due to small effective sample sizes.
Residuals from the multilevel models that included confounding variables were examined for outliers and influential covariate patterns. There were a small number of observations with large residuals, which were examined for mistakes in data entry and for the impact of their removal from the models; due to a lack of errors in data entry, all observations were retained in the models. The best linear unbiased predictors for each of the “resistant” multilevel models investigated appeared to be normally distributed.

**Variance estimation**

The multilevel random effects in the “resistant”, “multiclass” and “ampicillin” univariable models allowed for comparison of variance within and between the different households and dogs. Approximately 28-35% and 14-20% of the variance in the resistant, multiclass and ampicillin models was explained at the household level and the dog level, respectively (Tables 5.3-5.5).

**DISCUSSION**

In this study, potential pet-related risk factors for carriage of antimicrobial resistant *Salmonella* spp. and/or *E. coli* by pet dogs from volunteer households in Ontario were examined. This is one of the few Canadian studies examining the potential risk factors for AMR in *Salmonella* and *E. coli* in pet dogs. The prevalence of AMR in *Salmonella* and/or *E. coli* isolates was approximately 20% and the
prevalence of dogs carrying an AMR isolate was approximately 28%. Multilevel logistic regression models identified several important pet-related management factors that were associated with an increased risk of resistance in Salmonella or E. coli in this population of pet dogs. Many of the associations were related to raw food feeding, which highlights another potential public health risk of adding raw animal products to pet dog diets. Specifically, bacterial species (E. coli vs. Salmonella), being fed a homemade diet or having homemade food added to dog’s diet, being fed a raw diet or having anything raw added to a dog’s diet, being fed a homemade raw food diet, and being fed raw chicken in the past week were all found to be significant risk factors for carriage of antimicrobial resistant Salmonella spp. or E. coli. Using variance estimates for dog and household, from the multilevel models for “resistant” it was found that approximately 50% of the variance was explained at the household and dog levels, with the residual variance at the isolate level.

From univariable and two variable models for antimicrobial resistance in general (“resistant”), bacterial species (E. coli vs. Salmonella) and several raw feeding pet-related management factors were found to remain significant even after controlling for confounding variables individually (breed size, age category, sex, and neuter status). Antimicrobial resistant Salmonella spp. and E. coli are commonly recovered from raw animal products in Canada, particularly chicken and turkey. Consequently, raw animal products fed to pets or raw diets made with these products could be a potential source of AMR Salmonella spp. and E. coli. In 2011, CIPARS recovered E. coli with resistance to at least one antimicrobial from approximately 75% of retail chicken meat samples from across Canada; Salmonella
spp. with resistance to at least one antimicrobial was recovered from approximately 56% of retail chicken meat samples collected in the same timeframe using the same retail samples. Ampicillin resistance in *E. coli* was very common in Canadian retail meat samples, with approximately 47% of *E. coli* recovered from chicken meat samples showing decreased susceptibility to ampicillin. Ampicillin resistance was also common among *Salmonella* spp. isolates recovered from retail meat samples, with approximately 41% of retail chicken meat isolates resistant to this drug, depending on the province where the meat was purchased. The exact ingredients of the raw and homemade diets fed to the pet dogs in our study were not identified, so a clear connection with the meat source cannot be made. However, antimicrobial resistant *Salmonella* and *E. coli* have been recovered from commercially prepared raw diets available in Canada and the United States, and Lefebvre et al. (2008) found raw meat consumption to be significantly associated with multidrug resistant *E. coli* carriage in therapy dogs.

The use of herbal products for dogs was identified through univariable analyses to be a statistically significant risk factor for multiclass and ampicillin resistance (Tables 5.4 and 5.5). In this study, 45 dog owners indicated that they were giving their dog a “herbal product”. This question was intended to identify naturopathic products used by dog owners, and of these, information on the herbal product used was available for 20 dogs; glucosamine was the most common product recorded. This association has not been reported in previous studies in pet dogs, and may be acting as a proxy for other pet-related factors, but due to large number of variables investigated, it may also be a type I error. In addition, complete information
regarding the herbal products given to the dogs in this study was not available, making a conclusion based on this association difficult. Multidrug-resistant *Salmonella* spp. and *E. coli* have been recovered from dogs in other studies, but an association with the medications or herbal products the dogs were given was not reported.²⁻³⁻³⁷⁻³⁹

Being vaccinated annually and being treated with a heartworm preventive in the previous six months were found to be sparing factors in the “resistant” models. This connection has not been reported in previous studies and these variables could be acting as proxies for consistent veterinary care. As well, the variables could be acting as a proxy for feeding processed dry or canned diets since owners that follow these particular preventive health measures may be more likely to purchase these diets. Consequently, these pets may be less likely to be fed raw diets or raw animal products, reducing their risk of carrying *Salmonella*.³⁵

When controlling for confounders (breed size, age category, sex and neuter status) in the two variable multilevel models for “resistant”, a dog being fed a rawhide pet treat in the past week and a dog being allowed to run free in a dog park, became statistically significant, assuming the examined confounders were not intervening variables. These associations have not been reported in earlier studies. However, the authors of this study previously reported that being fed a rawhide treat was associated with a decreased risk of *Salmonella* carriage, but this association became insignificant when breed was introduced into the model.²² Antimicrobial resistant *Salmonella* have been recovered from rawhide treats,³⁵ and it is possible they could act as a source of resistant *Salmonella* in dogs. The association with a dog being allowed to run free in a
dog park makes sense biologically, since such dogs would be exposed to many other dogs and other sources of bacteria.

The point estimate for species of bacteria (E. coli vs. Salmonella) increased when controlling for confounders, with E. coli isolates being over seven times as likely to be antimicrobial resistant than Salmonella spp. isolates. Generic E. coli is commonly used in AMR studies because it readily acquires and maintains resistance genes from other bacteria.\textsuperscript{40-42} However, E. coli has not been shown to be a reliable predictor of resistance in Salmonella spp. at the animal or sample level.\textsuperscript{23,40}

The high amount of variation explained at the dog and household level for the various AMR outcomes was expected. Clustering of infection status within a herd or household is typical in most infectious diseases and must be accounted for in the analysis if multiple subjects are taken from a common household or herd.\textsuperscript{30,43} Our results indicate that interventions targeted at the household level, reflecting the general pet-related management of the dog (e.g. diet, veterinary care), could be effective in limiting resistant bacteria in pet dogs.

The design of the study described here needs to be considered when interpreting the results. Furthermore, caution must be taken when extrapolating our findings to other dog and pet populations. Dogs were not recruited through a truly random route, which may have created a distinct group of dogs that are not representative of the typical canine population in Ontario. In addition, if dogs were related through common social groups (e.g., therapy or agility programmes), there may be some unrecorded clustering that was not accounted for in our analyses. As we
noted previously, *Salmonella* carriage was abnormally high in this population of dogs, and may have been due to the large number of dogs fed a raw diet, but may also have been due to the fact that more than one method of isolation was used with three selective media, improving test sensitivity and recovery of *Salmonella*. However, given that potential clustering by dog and household was accounted for in our analyses, and a large number of pet-related factors were examined, the results are still important for the development of safe pet ownership guidelines, and to help inform the development of AMR surveillance for small animals. Selection bias would have affected the odds ratios estimated if the recruitment process affected both the infection status and the exposure, but not either alone. This bias has been discussed extensively in a previous paper, and would only have been an issue if the owners’ decisions to participate were based on the bacterial status of their dogs and the antimicrobial susceptibility of the bacteria isolated from their dogs. While recruitment could have been affected by exposure, it is unlikely that owner participation was affected by bacterial carriage. Finally, in view of the fact that this study was cross-sectional in nature, we cannot determine which factors cause AMR *Salmonella* spp. and *E. coli* carriage, and which factors prolong carriage, since prevalence is a function of incidence and duration. However, controlling management factors related to prevalence would be useful for protecting public health, if not necessarily suitable for understanding the causal relationship between exposure and pathogen/AMR carriage.

A major limitation of this study is that previous antimicrobial use was not questioned through the survey, since the original project was not specifically designed for this purpose. Antimicrobials are an important risk factor for the development of
AMR in bacteria and have been identified as a risk factor in several canine studies.\textsuperscript{18-21,37} However, it should be noted that these studies often examined \textit{E. coli} from clinical infections and dogs admitted to veterinary hospitals, which may not be comparable to \textit{E. coli} isolated from clinically healthy dogs.\textsuperscript{2,45}

Finally, given the small sample size in this study, caution should be taken when interpreting the non-significant results. Potential risk factors for carriage of antimicrobial resistant \textit{Salmonella} and \textit{E. coli} may have been missed due to the large effect and/or small amount of variation that is needed to observe statistical significance in small studies.\textsuperscript{30} Weaker associations may not have been detected, especially for the models examining multiclass and ampicillin resistance. As well, we were restricted in our ability to control for confounding variables. However, it should be noted that the sample size in this study is comparable to, or larger than, many previous pet dog studies, which ranged from less than 10 dogs to almost 200 dogs.\textsuperscript{7,18,20,21,46}

In summary, this study identified several important risk factors for carriage of antimicrobial resistant \textit{Salmonella} and \textit{E. coli} in pet dogs. As such, it can be concluded that pet dogs are a potential source of antimicrobial resistant \textit{Salmonella} and \textit{E. coli}. These results may warrant a change in the current surveillance of antimicrobial resistance and supports the recommendation that companion animals be included in national, provincial and local AMR surveillance programs.\textsuperscript{47} As well, the associations with raw food diets and raw animal products highlight the potential health risk of adding raw or poorly prepared animal products to pet dog diets. The
protective associations related to vaccination and other veterinary care require further investigation. Given the close relationship that most owners have with their dogs, further studies into the pet-related health management factors that may increase or decrease a dog’s risk of antimicrobial resistant bacteria carriage should be completed. The information collected from such studies is crucial for the development of evidence-based guidelines for safe dog ownership and to protect the public through responsible pet management. Such information is particularly important for those pet owners who are immunocompromised.

ACKNOWLEDGEMENTS

The work presented in this article was performed as part of a PhD thesis for the primary author (E. K. Leonard). Antimicrobial susceptibility testing was completed by the Canadian Integrated Program for Antimicrobial Resistance Surveillance at the Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, ON, Canada. Sample collection and testing for this study were supported by the Public Health Agency of Canada. The infrastructure for statistical analyses completed by E. K. Leonard was supported through a grant to D. L. Pearl from the Canada Foundation for Innovation and the Ontario Research Fund. E. K. Leonard was supported through the Blake Graham Fellowship from the Ontario Veterinary College.
REFERENCES


http://dx.doi.org/10.3201/eid1812.120664.
Table 5.1: Resistance breakpoints for antimicrobial susceptibility testing of *Salmonella* spp. and generic *E.coli* isolates from pet dogs from volunteer households in Ontario, 2005-2006

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Resistant*†‡ (μg/ml)</th>
<th>Class</th>
<th>Category§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin*</td>
<td>≥ 64</td>
<td>Aminoglycosides</td>
<td>II</td>
</tr>
<tr>
<td>Amoxicillin/Clavulanic Acid*</td>
<td>≥ 32</td>
<td>penicillin-β-lactamase inhibitor combinations</td>
<td>I</td>
</tr>
<tr>
<td>Ampicillin*</td>
<td>≥ 32</td>
<td>Penicillins</td>
<td>II</td>
</tr>
<tr>
<td>Cefoxitin*</td>
<td>≥ 32</td>
<td>2nd generation cephalosporins</td>
<td>II</td>
</tr>
<tr>
<td>Ceftiofur*</td>
<td>≥ 8</td>
<td>3rd generation cephalosporins</td>
<td>I</td>
</tr>
<tr>
<td>Ceftriaxone†</td>
<td>≥ 4</td>
<td>3rd generation cephalosporins</td>
<td>I</td>
</tr>
<tr>
<td>Chloramphenicol*</td>
<td>≥ 32</td>
<td>Chloramphenicols</td>
<td>III</td>
</tr>
<tr>
<td>Ciprofloxacin*</td>
<td>≥ 4</td>
<td>Fluoroquinolones</td>
<td>I</td>
</tr>
<tr>
<td>Gentamicin*</td>
<td>≥ 16</td>
<td>Aminoglycosides</td>
<td>II</td>
</tr>
<tr>
<td>Kanamycin*</td>
<td>≥ 64</td>
<td>Aminoglycosides</td>
<td>II</td>
</tr>
<tr>
<td>Nalidixic Acid*</td>
<td>≥ 32</td>
<td>Quinolones</td>
<td>II</td>
</tr>
<tr>
<td>Streptomycin†</td>
<td>≥ 64</td>
<td>Aminoglycosides</td>
<td>II</td>
</tr>
<tr>
<td>Sulfisoxazole*</td>
<td>≥ 512</td>
<td>Sulphonamides</td>
<td>III</td>
</tr>
<tr>
<td></td>
<td>Tetracycline *</td>
<td>≥ 16</td>
<td>Tetracyclines</td>
</tr>
<tr>
<td>-------------------------</td>
<td>----------------</td>
<td>------</td>
<td>---------------</td>
</tr>
<tr>
<td>Trimethoprim/Sulphamethoxazole *</td>
<td>≥ 4</td>
<td></td>
<td>Sulfonamide combinations</td>
</tr>
</tbody>
</table>

*CLSI. M100-S16 Table 2A. M7-A6-MIC Testing section.
†CLSI. Informational Supplement. M100-S20.

§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\(^27\).
Table 5.2: Variables investigated for their association with antimicrobial resistance in *Salmonella* spp. and *E. coli* from pet dogs from volunteer households in Ontario, 2005-2006.*

<table>
<thead>
<tr>
<th>General Diet Information</th>
<th>Dog Health Information and Demographics</th>
<th>Activities &amp; Pet Information</th>
<th>Household Information</th>
<th>Species of bacteria recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Fed raw beef in past week</td>
<td>• Given a probiotic in past month</td>
<td>• More than one dog (yes/no)</td>
<td>• Number of children (&lt;18y.o.) in home</td>
<td>• <em>E. coli or Salmonella</em></td>
</tr>
<tr>
<td>• Fed raw chicken in past week</td>
<td>• Given an herbal product in the past month</td>
<td>• Cats in home (yes/no)</td>
<td>• Are there any infants (&lt;1y.o.) in home (yes/no)</td>
<td></td>
</tr>
<tr>
<td>• Fed raw pork in past week</td>
<td>• Diarrhea in past month</td>
<td>• Other pets in home (yes/no)</td>
<td>• Are there any persons &gt;65 years old in home (yes/no)</td>
<td></td>
</tr>
<tr>
<td>• Fed raw eggs in past week</td>
<td>• Vomiting in past month</td>
<td>• Allowed to run freely in park (yes/no)</td>
<td>• Are there any immunocompromised individuals in home (yes/no)</td>
<td></td>
</tr>
<tr>
<td>• Fed dried pig’s ears in past week</td>
<td>• Age (in years)</td>
<td>• Confined to fenced yard (yes/no)</td>
<td>• Owner’s occupation</td>
<td></td>
</tr>
<tr>
<td>• Fed bones in past week</td>
<td>• Age category</td>
<td>• Contact with livestock (yes/no– cattle, sheep, goats, pigs, or horses)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Fed rawhide chews in past week</td>
<td>- Puppy (&lt;1 yr)</td>
<td>• Contact with other cats (yes/no)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Fed cooked table scraps in past week</td>
<td>- Adult (1-7 yrs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Fed other pet treats in past week</td>
<td>- Senior (&gt;7 yrs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Main type of diet fed:</td>
<td>• Breed size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Commercial dry/canned</td>
<td>- Small pure breed (&lt;25 lbs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Commercial raw diet</td>
<td>- Medium pure breed (25-60 lbs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Homemade raw diet</td>
<td>- Large/giant pure breed (&gt;60 lbs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Homemade cooked diet</td>
<td>- Mixed breed (all sizes)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Commercial home-cooked diet</td>
<td>• Sex (male or female)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Other</td>
<td>• Neuter status (intact or neutered/spayed)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Anything added to commercial dry/canned food</td>
<td>• Is the dog vaccinated (yes/no)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Given heartworm preventive (yes/no)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Retriever breed (yes/no)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Where was the dog’s diet purchased</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Grocery store</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Pet store</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Prepared at home</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Modified from Leonard *et al* 2011*22
Table 5.3: Univariable associations between pet-related factors and the risk of antimicrobial resistance in *Salmonella* spp. or *E. coli* isolates, dogs, and households, for pet dogs from volunteer households in Ontario, 2005-2006 (n=515 isolates, 136 dogs, 83 households).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>P-value</th>
<th>95% C.I.</th>
<th>HouseID Var (S.E.)</th>
<th>DogID Var (S.E.)</th>
<th>% House Var</th>
<th>% Dog Var</th>
<th>Exposed Isolates/Dogs/Houses</th>
<th>Unexposed Isolates/Dogs/Houses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species (<em>E. coli</em> vs <em>Salmonella</em>)</td>
<td>2.34</td>
<td>0.036</td>
<td>1.06-5.19</td>
<td>2.39 (0.94)</td>
<td>1.15 (0.70)</td>
<td>35.05</td>
<td>16.77</td>
<td>395/133/82</td>
<td>120/32/21</td>
</tr>
<tr>
<td>Homemade diet fed or homemade food added to diet</td>
<td>3.50</td>
<td>0.024</td>
<td>1.18-10.37</td>
<td>2.13 (0.92)</td>
<td>1.26 (0.72)</td>
<td>31.95</td>
<td>18.80</td>
<td>204/39/19</td>
<td>311/97/64</td>
</tr>
<tr>
<td>Raw diet fed or anything raw added to diet</td>
<td>3.28</td>
<td>0.049</td>
<td>1.00-10.74</td>
<td>2.22 (0.93)</td>
<td>1.24 (0.72)</td>
<td>32.90</td>
<td>18.35</td>
<td>160/28/13</td>
<td>355/108/70</td>
</tr>
<tr>
<td>Dog fed raw beef, chicken, pork or eggs in past week</td>
<td>3.11</td>
<td>0.065</td>
<td>0.93-10.39</td>
<td>2.22 (0.93)</td>
<td>1.23 (0.72)</td>
<td>32.92</td>
<td>18.28</td>
<td>157/27/12</td>
<td>358/109/71</td>
</tr>
<tr>
<td>Fed homemade raw food diet</td>
<td>4.43</td>
<td>0.022</td>
<td>1.24-15.91</td>
<td>2.16 (0.92)</td>
<td>1.24 (0.72)</td>
<td>32.27</td>
<td>18.57</td>
<td>140/22/10</td>
<td>375/114/73</td>
</tr>
<tr>
<td>Fed raw chicken in the past week</td>
<td>5.17</td>
<td>0.015</td>
<td>1.38-19.32</td>
<td>2.01 (0.91)</td>
<td>1.32 (0.74)</td>
<td>30.43</td>
<td>19.88</td>
<td>120/18/9</td>
<td>395/118/74</td>
</tr>
<tr>
<td>Diarrhea in previous 30 days</td>
<td>0.38</td>
<td>0.169</td>
<td>0.10-1.50</td>
<td>2.25 (0.92)</td>
<td>1.19 (0.71)</td>
<td>33.46</td>
<td>17.69</td>
<td>98/28/18</td>
<td>417/108/65</td>
</tr>
<tr>
<td>Owner works in healthcare or veterinary related field</td>
<td>0.33</td>
<td>0.065</td>
<td>0.10-1.07</td>
<td>2.06 (0.90)</td>
<td>1.25 (0.72)</td>
<td>31.20</td>
<td>18.94</td>
<td>146/41/27</td>
<td>369/95/56</td>
</tr>
<tr>
<td>Vaccinated annually</td>
<td>0.30</td>
<td>0.031</td>
<td>0.10-0.90</td>
<td>2.19 (0.92)</td>
<td>1.22 (0.71)</td>
<td>32.69</td>
<td>18.26</td>
<td>331/99/65</td>
<td>184/37/18</td>
</tr>
<tr>
<td>Treated with heartworm preventive in the past 6 months</td>
<td>0.31</td>
<td>0.036</td>
<td>0.10-0.93</td>
<td>2.21 (0.93)</td>
<td>1.24 (0.72)</td>
<td>32.82</td>
<td>18.38</td>
<td>365/99/62</td>
<td>150/37/21</td>
</tr>
<tr>
<td>Fed commercial dry/canned food diet</td>
<td>0.31</td>
<td>0.155</td>
<td>0.06-1.56</td>
<td>2.26 (0.93)</td>
<td>1.21 (0.71)</td>
<td>33.40</td>
<td>17.93</td>
<td>104/17/8</td>
<td>411/119/75</td>
</tr>
<tr>
<td>Fed homemade cooked food diet</td>
<td>2.47</td>
<td>0.177</td>
<td>0.66-9.16</td>
<td>2.25 (0.92)</td>
<td>1.20 (0.71)</td>
<td>33.36</td>
<td>17.81</td>
<td>94/22/12</td>
<td>421/114/71</td>
</tr>
<tr>
<td>Diet bought in grocery store</td>
<td>2.34</td>
<td>0.149</td>
<td>0.74-7.43</td>
<td>2.07 (0.92)</td>
<td>1.24 (0.71)</td>
<td>31.37</td>
<td>18.73</td>
<td>124/29/19</td>
<td>391/107/64</td>
</tr>
</tbody>
</table>
Each variable of interest (p ≤ 0.20) was examined one at a time in a multilevel model with random effects for dog and household; †95% confidence interval (95% C.I.) of the Odds Ratio, variance (Var) and standard error (S.E.) for the household and dog level variance calculated with multilevel logistic regression using MLwiN within Stata/MP 11.2.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate (S.E.)</th>
<th>95% C.I.</th>
<th>Var (S.E.)</th>
<th>S.E. (S.E.)</th>
<th>Household (S.E.)</th>
<th>Dog (S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed rawhide in the past week</td>
<td>2.51 (0.101)</td>
<td>0.84-7.53</td>
<td>2.31 (0.92)</td>
<td>1.12 (0.69)</td>
<td>34.32 (6.73)</td>
<td>117/38/25</td>
</tr>
<tr>
<td>Allowed to run free in dog park</td>
<td>2.36 (0.132)</td>
<td>0.77-7.21</td>
<td>2.27 (0.92)</td>
<td>1.16 (0.70)</td>
<td>33.80 (6.73)</td>
<td>17.29</td>
</tr>
<tr>
<td>Given herbal products</td>
<td>2.86 (0.054)</td>
<td>0.98-8.33</td>
<td>2.18 (0.93)</td>
<td>1.24 (0.73)</td>
<td>32.46 (6.73)</td>
<td>18.46</td>
</tr>
</tbody>
</table>
Table 5.4: Univariable associations between pet-related factors and the risk of multiclass resistance in *Salmonella* spp. or *E. coli* isolates, dogs, and households*, for pet dogs from volunteer households in Ontario, 2005-2006 (n=515 isolates, 136 dogs, 83 households).

<table>
<thead>
<tr>
<th>Variable</th>
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<th>P-value</th>
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<th>HouseID Var (S.E.)</th>
<th>DogID Var (S.E.)</th>
<th>% House Var</th>
<th>% Dog Var</th>
<th>Exposed Isolates/Dogs/Houses</th>
<th>Unexposed Isolates/Dogs/Houses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed raw eggs in the past week</td>
<td>4.26</td>
<td>0.135</td>
<td>0.64-28.59</td>
<td>1.82 (0.95)</td>
<td>0.96 (0.78)</td>
<td>29.95</td>
<td>15.82</td>
<td>54/7/4</td>
<td>461/129/79</td>
</tr>
<tr>
<td>Owner works in healthcare or veterinary related field</td>
<td>0.32</td>
<td>0.087</td>
<td>0.09-1.18</td>
<td>1.67 (0.92)</td>
<td>0.98 (0.79)</td>
<td>28.13</td>
<td>16.47</td>
<td>146/41/27</td>
<td>369/95/56</td>
</tr>
<tr>
<td>Vaccinated annually</td>
<td>0.42</td>
<td>0.135</td>
<td>0.13-1.31</td>
<td>1.91 (0.97)</td>
<td>0.94 (0.77)</td>
<td>31.13</td>
<td>15.29</td>
<td>331/99/65</td>
<td>184/37/18</td>
</tr>
<tr>
<td>Fed rawhide in the past week</td>
<td>2.18</td>
<td>0.171</td>
<td>0.71-6.67</td>
<td>1.80 (0.94)</td>
<td>0.92 (0.77)</td>
<td>29.96</td>
<td>15.27</td>
<td>117/38/25</td>
<td>398/98/58</td>
</tr>
<tr>
<td>Given herbal products</td>
<td>3.37</td>
<td>0.029</td>
<td>1.13-10.02</td>
<td>1.70 (0.94)</td>
<td>1.04 (0.80)</td>
<td>28.13</td>
<td>17.29</td>
<td>191/45/24</td>
<td>318/91/59</td>
</tr>
<tr>
<td>Infants (&lt;1yr) living in the home</td>
<td>5.55</td>
<td>0.195</td>
<td>0.41-74.40</td>
<td>1.70 (0.93)</td>
<td>1.00 (0.80)</td>
<td>28.37</td>
<td>16.73</td>
<td>32/3/2</td>
<td>483/133/81</td>
</tr>
</tbody>
</table>

*Each variable of interest (p≤ 0.20) was examined one at a time in a multilevel model with random effects for dog and household; †95% confidence interval (95% C.I.) of the Odds Ratio, variance (Var) and standard error (S.E.) for the household and dog level variance calculated with multilevel logistic regression using MLwiN within Stata/MP 11.2.*
Table 5.5: Univariable associations between pet-related factors and the risk of ampicillin resistance in *Salmonella* spp. or *E. coli* isolates, dogs, and households*, for pet dogs from volunteer households in Ontario, 2005-2006 (n=515 isolates, 136 dogs, 83 households).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>P-value</th>
<th>95% C.I.†</th>
<th>HouseID Var (S.E.)†</th>
<th>DogID Var (S.E.)†</th>
<th>% House Var</th>
<th>% Dog Var</th>
<th>Exposed Isolates/Dogs/Houses</th>
<th>Unexposed Isolates/Dogs/Houses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homemade diet fed or homemade food added to diet</td>
<td>2.98</td>
<td>0.061</td>
<td>0.95-9.39</td>
<td>2.26 (0.99)</td>
<td>0.90 (0.70)</td>
<td>35.02</td>
<td>13.90</td>
<td>204/39/19</td>
<td>311/97/64</td>
</tr>
<tr>
<td>Fed homemade raw food diet</td>
<td>2.85</td>
<td>0.124</td>
<td>0.75-10.83</td>
<td>2.28 (0.99)</td>
<td>0.89 (0.70)</td>
<td>35.28</td>
<td>13.77</td>
<td>140/22/10</td>
<td>375/114/73</td>
</tr>
<tr>
<td>Fed commercial dry/canned food diet</td>
<td>0.33</td>
<td>0.183</td>
<td>0.06-1.70</td>
<td>2.13 (0.96)</td>
<td>0.91 (0.72)</td>
<td>33.62</td>
<td>14.43</td>
<td>104/17/8</td>
<td>411/119/75</td>
</tr>
<tr>
<td>Fed homemade cooked food diet</td>
<td>2.71</td>
<td>0.138</td>
<td>0.73-10.10</td>
<td>2.20 (0.98)</td>
<td>0.90 (0.71)</td>
<td>34.46</td>
<td>14.10</td>
<td>94/22/12</td>
<td>421/114/71</td>
</tr>
<tr>
<td>Fed raw chicken in the past week</td>
<td>3.06</td>
<td>0.114</td>
<td>0.76-12.22</td>
<td>2.20 (0.98)</td>
<td>0.92 (0.72)</td>
<td>34.29</td>
<td>14.42</td>
<td>120/18/9</td>
<td>395/118/74</td>
</tr>
<tr>
<td>Fed raw eggs in the past week</td>
<td>4.08</td>
<td>0.164</td>
<td>0.56-29.49</td>
<td>2.13 (0.96)</td>
<td>0.92 (0.72)</td>
<td>33.60</td>
<td>14.50</td>
<td>54/7/4</td>
<td>461/129/79</td>
</tr>
<tr>
<td>Diet bought in grocery store</td>
<td>2.37</td>
<td>0.152</td>
<td>0.73-7.74</td>
<td>1.99 (0.93)</td>
<td>0.94 (0.73)</td>
<td>31.92</td>
<td>15.16</td>
<td>124/29/19</td>
<td>391/107/64</td>
</tr>
<tr>
<td>Diet prepared at home</td>
<td>2.94</td>
<td>0.141</td>
<td>0.70-12.41</td>
<td>2.13 (0.96)</td>
<td>0.91 (0.73)</td>
<td>33.65</td>
<td>14.37</td>
<td>104/17/8</td>
<td>411/119/75</td>
</tr>
<tr>
<td>Diarrhea in previous 30 days</td>
<td>0.27</td>
<td>0.112</td>
<td>0.05-1.36</td>
<td>2.15 (0.96)</td>
<td>0.89 (0.71)</td>
<td>33.98</td>
<td>14.06</td>
<td>98/28/18</td>
<td>417/108/65</td>
</tr>
<tr>
<td>Vaccinated annually</td>
<td>0.42</td>
<td>0.148</td>
<td>0.13-1.36</td>
<td>2.24 (0.98)</td>
<td>0.91 (0.71)</td>
<td>34.76</td>
<td>14.13</td>
<td>331/99/65</td>
<td>184/37/18</td>
</tr>
<tr>
<td>Confined to a fenced yard</td>
<td>0.46</td>
<td>0.164</td>
<td>0.15-1.38</td>
<td>1.93 (0.92)</td>
<td>0.93 (0.73)</td>
<td>31.39</td>
<td>15.17</td>
<td>334/84/53</td>
<td>181/52/30</td>
</tr>
<tr>
<td>Given herbal products</td>
<td>3.49</td>
<td>0.025</td>
<td>1.17-10.42</td>
<td>1.96 (1.04)</td>
<td>1.04 (1.04)</td>
<td>31.18</td>
<td>16.49</td>
<td>191/45/24</td>
<td>318/91/59</td>
</tr>
<tr>
<td></td>
<td>Odds Ratio</td>
<td>95% CI (95% C.I.)</td>
<td>Variance (Var)</td>
<td>Standard Error (S.E.)</td>
<td>Household</td>
<td>Dog</td>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>------------</td>
<td>-------------------</td>
<td>----------------</td>
<td>-----------------------</td>
<td>-----------</td>
<td>-----</td>
<td>-------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infants (&lt;1yr) living in the home</td>
<td>6.08</td>
<td>0.41-89.76</td>
<td>1.98</td>
<td>0.97</td>
<td>31.78</td>
<td>15.51</td>
<td>32/32</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.96)</td>
<td>(0.76)</td>
<td>(0.94)</td>
<td>(0.74)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other pets in the home (other than cats and dogs)</td>
<td>2.84</td>
<td>0.93-8.65</td>
<td>2.18</td>
<td>0.86</td>
<td>34.45</td>
<td>13.54</td>
<td>150/42/25</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.96)</td>
<td>(0.70)</td>
<td>(0.96)</td>
<td>(0.70)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Each variable of interest (p≤ 0.20) was examined one at a time in a multilevel model with random effects for dog and household; †95% confidence interval (95% C.I.) of the Odds Ratio, variance (Var) and standard error (S.E.) for the household and dog level variance calculated with multilevel logistic regression using MLwiN through Stata/MP 11.2.*
Table 5.6: Unadjusted and adjusted odds ratios (OR) for the risk of antimicrobial resistance in *Salmonella* spp. or *E. coli* isolates, that changed by greater than 20% with the inclusion of breed size(I), age(II), sex(III), or neuter status(IV) as potential confounders to the significant variables from the initial multilevel analyses* (n=457 isolates, 127 dogs, 78 households†).

<table>
<thead>
<tr>
<th>Confounders (bold) and Independent Variables</th>
<th>Unadjusted OR*</th>
<th>P-value*</th>
<th>95% C.I.†</th>
<th>Adjusted ORc</th>
<th>P-value‡</th>
<th>95% C.I.‡</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Breed Size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial species (<em>E. coli</em> vs. <em>Salmonella</em>)</td>
<td>2.34</td>
<td>0.036</td>
<td>1.06-5.19</td>
<td>7.96</td>
<td>0.001§</td>
<td>2.39-26.51</td>
</tr>
<tr>
<td>Owner works in healthcare or veterinary related field</td>
<td>0.33</td>
<td>0.065</td>
<td>0.10-1.07</td>
<td>0.45</td>
<td>0.206</td>
<td>0.13-1.55</td>
</tr>
<tr>
<td>Treated with heartworm preventive in the past 6 months</td>
<td>0.31</td>
<td>0.036</td>
<td>0.10-0.93</td>
<td>0.23</td>
<td>0.014§</td>
<td>0.07-0.74</td>
</tr>
<tr>
<td>Fed commercial dry/canned food diet</td>
<td>0.31</td>
<td>0.155</td>
<td>0.06-1.56</td>
<td>0.51</td>
<td>0.532</td>
<td>0.06-4.20</td>
</tr>
<tr>
<td>Fed homemade cooked food diet</td>
<td>2.47</td>
<td>0.177</td>
<td>0.66-9.16</td>
<td>3.42</td>
<td>0.076</td>
<td>0.88-13.35</td>
</tr>
<tr>
<td>Diet bought in grocery store</td>
<td>2.34</td>
<td>0.149</td>
<td>0.74-7.46</td>
<td>2.62</td>
<td>0.134</td>
<td>0.74-9.21</td>
</tr>
<tr>
<td>Fed rawhide in the past week</td>
<td>2.51</td>
<td>0.101</td>
<td>0.84-7.53</td>
<td>3.90</td>
<td>0.022§</td>
<td>1.22-12.50</td>
</tr>
<tr>
<td>Allowed to run free in dog park</td>
<td>2.36</td>
<td>0.132</td>
<td>0.77-7.21</td>
<td>4.22</td>
<td>0.027§</td>
<td>1.18-15.11</td>
</tr>
<tr>
<td><strong>II. Age (categories)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial species (<em>E. coli</em> vs. <em>Salmonella</em>)</td>
<td>2.34</td>
<td>0.036</td>
<td>1.06-5.19</td>
<td>7.58</td>
<td>0.001§</td>
<td>2.28-25.19</td>
</tr>
<tr>
<td>Activity</td>
<td>Odds Ratio</td>
<td>95% CI</td>
<td>P Value</td>
<td>OR 95% CI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>------------</td>
<td>-----------</td>
<td>---------</td>
<td>------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homemade diet fed or homemade food added to diet</td>
<td>3.50</td>
<td>1.18-10.37</td>
<td>4.51</td>
<td>0.014§</td>
<td>1.36-14.91</td>
<td></td>
</tr>
<tr>
<td>Diarrhea in previous 30 days</td>
<td>0.38</td>
<td>0.10-1.50</td>
<td>0.27</td>
<td>0.092</td>
<td>0.06-1.24</td>
<td></td>
</tr>
<tr>
<td>Fed commercial dry/canned food diet</td>
<td>0.31</td>
<td>0.06-1.56</td>
<td>0.47</td>
<td>0.483</td>
<td>0.06-3.84</td>
<td></td>
</tr>
<tr>
<td>Fed homemade cooked food diet</td>
<td>2.47</td>
<td>0.66-9.16</td>
<td>3.78</td>
<td>0.053</td>
<td>0.98-14.59</td>
<td></td>
</tr>
<tr>
<td>Fed rawhide in the past week</td>
<td>2.51</td>
<td>0.84-7.53</td>
<td>3.89</td>
<td>0.022§</td>
<td>1.21-12.48</td>
<td></td>
</tr>
<tr>
<td>Allowed to run free in dog park</td>
<td>2.36</td>
<td>0.77-7.21</td>
<td>3.79</td>
<td>0.038§</td>
<td>1.08-13.32</td>
<td></td>
</tr>
<tr>
<td>III. Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial species (E. coli vs. Salmonella)</td>
<td>2.34</td>
<td>1.06-5.19</td>
<td>7.92</td>
<td>0.001§</td>
<td>2.40-26.17</td>
<td></td>
</tr>
<tr>
<td>Owner works in healthcare or veterinary related field</td>
<td>0.33</td>
<td>0.10-1.07</td>
<td>0.42</td>
<td>0.148</td>
<td>0.13-1.36</td>
<td></td>
</tr>
<tr>
<td>Vaccinated annually</td>
<td>0.30</td>
<td>0.10-0.90</td>
<td>0.33</td>
<td>0.054</td>
<td>0.10-1.02</td>
<td></td>
</tr>
<tr>
<td>Treated with heartworm preventive in the past 6 months</td>
<td>0.31</td>
<td>0.10-0.93</td>
<td>0.22</td>
<td>0.009§</td>
<td>0.07-0.68</td>
<td></td>
</tr>
<tr>
<td>Fed commercial dry/canned food diet</td>
<td>0.31</td>
<td>0.06-1.56</td>
<td>0.48</td>
<td>0.483</td>
<td>0.06-3.67</td>
<td></td>
</tr>
<tr>
<td>Fed homemade cooked food diet</td>
<td>2.47</td>
<td>0.66-9.16</td>
<td>3.11</td>
<td>0.083</td>
<td>0.86-11.20</td>
<td></td>
</tr>
<tr>
<td>Fed rawhide in the past week</td>
<td>2.51</td>
<td>0.84-7.53</td>
<td>3.94</td>
<td>0.016§</td>
<td>1.29-12.00</td>
<td></td>
</tr>
<tr>
<td>Allowed to run free in dog park</td>
<td>2.36</td>
<td>0.77-7.21</td>
<td>3.41</td>
<td>0.048§</td>
<td>1.01-11.51</td>
<td></td>
</tr>
</tbody>
</table>
## IV. Neuter Status

<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>SE</th>
<th>95% CI</th>
<th>p</th>
<th>Adjusted p</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial species (E. coli vs. Salmonella)</td>
<td>2.34</td>
<td>0.036</td>
<td>1.06-5.19</td>
<td>7.63</td>
<td>0.001$</td>
<td>2.32-25.08</td>
</tr>
<tr>
<td>Fed commercial dry/canned food diet</td>
<td>0.31</td>
<td>0.155</td>
<td>0.06-1.56</td>
<td>0.48</td>
<td>0.479</td>
<td>0.06-3.64</td>
</tr>
<tr>
<td>Fed homemade cooked food diet</td>
<td>2.47</td>
<td>0.177</td>
<td>0.66-9.16</td>
<td>3.32</td>
<td>0.065</td>
<td>0.93-11.88</td>
</tr>
<tr>
<td>Diet bought in grocery store</td>
<td>2.34</td>
<td>0.149</td>
<td>0.74-7.46</td>
<td>2.49</td>
<td>0.133</td>
<td>0.76-8.20</td>
</tr>
<tr>
<td>Fed rawhide in the past week</td>
<td>2.51</td>
<td>0.101</td>
<td>0.84-7.53</td>
<td>3.74</td>
<td>0.021$</td>
<td>1.23-11.44</td>
</tr>
<tr>
<td>Allowed to run free in dog park</td>
<td>2.36</td>
<td>0.132</td>
<td>0.77-7.21</td>
<td>3.68</td>
<td>0.034$</td>
<td>1.10-12.27</td>
</tr>
</tbody>
</table>

*From multilevel logistic regression model with a random intercept for household and dog; †For 457 isolates, 127 dogs, 78 households where the breed, sex, neuter status and age were provided; ‡The adjusted ORs and 95% confidence intervals (C.I.) were obtained using two variable multilevel logistic regression models from MLwiN through Stata, including the independent variable (p≤ 0.05-0.20) and the potential confounder, and random intercepts for household and dog; §The ORs stayed significant or became significant after controlling for the potential confounder.
CHAPTER 6

CONCLUSIONS

SUMMARY

The objectives of the studies in this thesis were to determine the occurrence and factors associated with fecal shedding of several enteric zoonotic bacteria of public health importance in two populations of pet dogs in Ontario, Canada. The studies focused on *Salmonella* and *Campylobacter*, the occurrence of antimicrobial resistance in *Salmonella* and generic *Escherichia coli*, and assessing pet-related management factors associated with increasing or decreasing the risk of shedding these zoonotic bacteria in pet dogs. These objectives were met through the use of two cross-sectional studies that included pet owner questionnaires and the collection of canine fecal samples for microbiological analysis. A number of regression and multilevel regression modeling methodologies were used to investigate the potential associations of these risk factors at the individual dog and household level using laboratory results and antimicrobial susceptibility testing from submitted canine fecal samples and information collected from questionnaires.

The first study of this thesis (Chapter 2) examined the occurrence of *Salmonella* spp. and pet-related management factors associated with *Salmonella* carriage in pet dogs and highlights the potential role that dogs could play in human *Salmonella* infections. The occurrence of *Salmonella* in this population of dogs was higher than expected at 23%; the prevalence of *Salmonella* in clinically healthy dogs has previously been found to range from 1% to 4% (Cantor, et al., 1997; Hackett and
Lappin, 2003). As previously discussed, this may be due to the multiple fecal samples being collected for each dog (i.e., period prevalence over 5 days), the enhanced isolation methods and the fact that a large proportion (20%) of dogs in this study population were fed raw foods; many of the pet-related factors found to be associated with the carriage of *Salmonella* in the analysis related to raw food feeding. Some of the other factors investigated in this study, for instance exposure to livestock, probiotic use, having multiple dogs in the home, and the use of rawhide treats, are other important and potentially modifiable pet-related factors, and should be studied further in order to reduce the risk of *Salmonella* carriage in pet dogs. Multiple fecal sample testing was also essential for the recovery of *Salmonella* in individual dogs based on the sporadic shedding seen in this group of dogs and in previous studies (Bagcigil, et al., 2007; Greene, 2012). The high correlation of *Salmonella* status among dogs in the same household in this study demonstrated the importance of *Salmonella* spread between dogs and this should be considered with respect to *Salmonella* carriage and spread in multi-dog households. Several of the most common serotypes of *Salmonella* identified in dogs in this study are similar to those commonly found in humans in Canada (Government of Canada, 2012a; Government of Canada, 2012b), and further emphasizes the potential risk of *Salmonella* spread from pet dogs to people. In light of the close relationship most owners have with their pet dogs, further studies into the pet-related management factors that may increase or decrease a dog’s risk of carrying *Salmonella* should be completed. Specifically, studies regarding potential household and environmental sources of *Salmonella* exposure in pet dogs, the amount of *Salmonella* shed by dogs, and the risk *Salmonella*-shedding dogs pose
to their owners are needed.

The *Campylobacter* study in Chapter 3 provided a detailed examination of the occurrence of *Campylobacter* in pet dogs in Southwestern Ontario and identified risk factors for *Campylobacter* spp. carriage in that population of dogs. The prevalence of *Campylobacter* spp., *C. upsaliensis*, and *C. jejuni* found in this population of dogs was consistent with the prevalences previously reported for household dogs in Canada and the United Kingdom, with *C. upsaliensis* being much more common than other species of *Campylobacter* (Acke, et al., 2009; Westgarth, et al., 2009; Chaban, et al., 2010; Parsons, et al., 2010). Some of the key pet-related risk factors identified in this study, including lack of antimicrobial exposure, not having children in the home, exposure to cats and other pets, and including homemade cooked food in the dog’s diet, have not previously been identified as risk factors for *Campylobacter* carriage in dogs and require further investigation. Given the associations discovered in this study between a dog’s diet and *Campylobacter* carriage, a change in the current surveillance of *Campylobacter* species in food sources, specifically in the case of *C. upsaliensis*, may be warranted. Current routine laboratory methods in Canada and abroad are designed to detect *C. jejuni* and *C. coli* (i.e., catalase positive *Campylobacter* spp.) and use cefaperazone, nalidixic acid, and cephalothin at levels that inhibit the growth of *C. upsaliensis* (Bourke, et al., 1998). While potentially of limited clinical relevance in dogs, *C. upsaliensis* is capable of causing disease in humans and may be more common in food sources and Canadian human infections than was previously thought (Taylor, et al., 1989; Bourke, et al., 1998). Further research into the prevalence of *C. upsaliensis* in human gastrointestinal disease, the role that pet dogs may play in
human infections, and potential sources of C. upsaliensis is warranted. As this study demonstrates, pet dogs are an important potential source of Campylobacter spp., especially C. upsaliensis, and exposure to dogs should be investigated in human cases of campylobacteriosis.

In chapters 4 and 5 of this thesis, we examined antimicrobial resistance in Salmonella and E. coli recovered from pet dogs in Ontario; the prevalence of antimicrobial resistance (AMR) in E. coli and Salmonella spp. and phenotypic resistance patterns were explored in chapter 4, and pet-related factors associated with antimicrobial resistance in E. coli and Salmonella spp. were evaluated in chapter 5. The prevalence of resistance to at least one antimicrobial in this population of pet dogs was 28%, indicating that the majority of enteric bacteria isolated from the recruited dogs and households were pan-susceptible. However, pet dogs should still be considered an important potential source of resistant bacteria as multiclass resistance and resistance to antimicrobials of very high importance in human medicine were found in this population of dogs. Salmonella has been reported to have spread from dogs to humans (Sato, et al., 2000; Cherry, et al., 2004), so the potential spread of antimicrobial resistant Salmonella, E. coli or AMR genetic determinants from dogs to humans is a public health concern. The similarities in the AMR patterns of dogs within the same household also highlighted the potential spread of AMR bacteria between dogs and potentially common source transmission of resistant bacteria, presenting another risk for AMR bacteria transmission to humans. In chapter 4, we also investigated the ability of generic E. coli isolates to predict resistance in Salmonella recovered from the same dog; generic E. coli was a poor predictor of
resistance in *Salmonella* recovered from this population of dogs, at the individual dog level. A similar conclusion was found in a study involving *Salmonella* and *E. coli* in pigs (Varga, et al., 2008). *Escherichia coli* is often used to monitor AMR because it is easily and inexpensively recovered in most animals, but caution should be taken when extrapolating AMR epidemiology from *E. coli* to pathogens like *Salmonella* at the individual animal level.

In chapter 5, we identified several important risk factors for carriage of antimicrobial resistant *Salmonella* and/or *E. coli* in pet dogs. Risk factors for the carriage of antimicrobial resistant bacteria are not well studied in healthy pet dogs and several important pet-related management factors, including multiple risk factors associated with raw food feeding, were found to have an association with the presence of antimicrobial resistant bacteria in this population of dogs. As well, the associations with raw food diets and raw animal products, also found to be risk factors for *Salmonella* carriage in general (chapter 2), highlight the potential health risk of adding raw or poorly prepared animal products to pet dog diets. The multilevel modelling results (chapter 5) indicate that AMR status in this population of dogs clustered strongly by household, suggesting that public health interventions targeted at the household level, reflecting general pet-related management factors, could be effective in controlling AMR in pet dogs. The current surveillance of antimicrobial resistance in companion animals is lacking and needs improvement, and the inclusion of pets in national, provincial and local AMR surveillance programs is necessary given the close relationship between dogs and humans. Future research comparing the similarities of AMR bacteria isolated from dogs and humans in the same household and further
analysis of the pet-related risk factors for carriage of these bacteria in dogs are needed to fully determine the public health impact of pet dogs in the spread of AMR pathogens and genetic resistance determinants.

ADVANTAGES & LIMITATIONS OF STUDY DESIGN

All of the research presented in this thesis was exploratory in nature and completed through cross-sectional studies. As a result, the study design needs to be considered both positively and negatively when interpreting the results. The cross-sectional design of the studies is valuable for examining a large number of variables to generate hypotheses and develop concepts for future research. As well, these studies provide baseline data for comparison in future Canadian and international studies on the prevalence of these important zoonotic pathogens and antimicrobial resistant bacteria in pet dogs. The reasonably short study period, multiple, consecutive fecal samples per dog, and comprehensive fecal collection kits provided to the owners allowed for enrollment of a broad range of dogs, providing a reasonably representative study group. The total sample size of individual dogs in these studies was relatively small, but was large when compared to previous small animal studies (Bagcigil, et al., 2007; Acke, et al., 2009; Chaban, et al., 2010). In each of our studies, multiple fecal samples were collected per dog and both whole fecal samples and fecal swabs were used to increase the probability of recovering pathogens in the studies, increasing the sensitivity of the testing completed. As well, for the Salmonella and E. coli analyses, multiple isolates were examined for AMR for each dog and household, giving a larger
total of bacterial isolates, which allowed for complex statistical modeling, permitting more comprehensive and accurate analysis. The relatively high recovery rate of pathogens in the presented research illustrates the value of testing multiple, full fecal samples for recovering zoonotic pathogens and examining AMR in dogs, in comparison to single samples or swabs.

The research in this thesis investigated a large number of pet-related variables through a questionnaire administered to dog owners. Due to the large effect and/or small amount of variation that is often needed to observe statistical significance in small studies, potential risk factors for carriage of the investigated pathogens may have been missed (Dohoo, et al., 2009). As well, because so many variables were investigated, there is an increased possibility of type I errors, which should not be ignored. It is also possible that some of the variables that were associated with pathogen carriage were acting as proxies for other statistical associations. However, the large number of risk factors investigated allowed for a more comprehensive examination of pet-related management factors in this population of dogs, and helped to create new hypotheses for future research.

Another potential limitation for this research was that dogs were not recruited in a random manner, although truly random sampling of such a population is virtually impossible. Dogs and their owners were recruited through several routes as a convenience sample, which may have created a study population that is not representative of the typical canine population in southern Ontario, affecting external validity. Nonetheless, considering the popularity of dogs as pets in this region, and the zoonotic potential of the pathogens investigated, the results are still important for
public health.

Several types of bias need to be taken into consideration when interpreting the results, given the recruitment process in these studies. Selection bias could have occurred because of the previously mentioned methods of recruitment, though this bias would have only occurred if the recruitment process influenced both the infection status and exposures in the dogs, but not either alone (Dohoo, et al., 2009). Consequently, selection bias was unlikely given that the dogs’ owners were not likely to have known the shedding status of their pets.

Another potential bias encountered in this research involves information bias, due to interviewer or response bias. As the owners in these studies were given face-to-face interviews, their answers may have been influenced by the interviewer or by social desirability to provide acceptable answers, especially in relation to raw food feeding. However, if owners claimed falsely that they did not feed raw diets, this would have biased the results to the null, and our studies would have actually underestimated the associations (Rothman, et al., 2008; Dohoo, et al., 2009). On the other hand, having the interviews conducted in person ensured that the questionnaires were fully completed and allowed for clarification of any questions the dog owners may have had.

Finally, because these studies were cross-sectional, the prevalence of exposures and outcomes were measured, and not incidence, so it is difficult to determine which factors cause pathogen carriage and which factors prolong carriage as prevalence is a function of incidence and duration (Rothman, et al., 2008; Dohoo, et al., 2009). However, controlling pet-related management factors related to
incidence and/or duration would be beneficial for public health and preventing the spread of pet-related zoonoses, if not necessarily appropriate for understanding the causal relationship between the exposures investigated and pathogen carriage.

**IMPLICATIONS FOR SURVEILLANCE**

From the results presented in this thesis, it can be concluded that pet dogs are an important potential source of *Salmonella*, *Campylobacter* and antimicrobial resistant *Salmonella* and *E. coli*. These results may necessitate a change in the current surveillance of enteric pathogens and antimicrobial resistance and supports the inclusion of pets in national, provincial and local enteric bacteria and AMR surveillance programs. Companion animals such as dogs can be seen as sentinels for human disease and antimicrobial resistance trends, especially because many of the same antimicrobials are used in human medicine and companion animal medicine, and pets can also be potential reservoirs/sources of resistant bacteria. Companion animal surveillance could give an improved perspective for human AMR trends and disease because of the close relationship shared with pets and shared environments and exposures.

**FUTURE RESEARCH AND CONCLUSIONS**

The research and results presented in this thesis are among the first in Canada to the author’s knowledge to extensively examine pet-related management factors for carriage of several important zoonotic pathogens and AMR bacteria, and identified several pet-related factors that require further investigation. In order to validate the
results and determine further pet-related management and environmental factors that could be changed to decrease the risks of dog ownership, particularly those related to the role of food, whether cooked or raw, in influencing the shedding of *Salmonella*, *Campylobacter* and antimicrobial resistant *Salmonella* and *E. coli* in pet dogs, larger study populations of dogs should be recruited when possible. Furthermore, more diverse populations of dogs should be included, in order to improve the external validity of the results and the applicability of any pet-related recommendations. Research should also be repeated in other provinces and areas to determine regional differences and similarities, thereby increasing the utility of future pet ownership guidelines. As well, the true role of *C. upsaliensis* in human illness, and its potential sources, including food, needs to be determined given the large percentage of healthy dogs that appear to be shedding this zoonotic bacterium and the fact that it is not being tested for in most food and human fecal samples. In regards to antimicrobial resistance, more detailed research investigating the role of antimicrobial use and AMR development in healthy dogs needs to be completed, as well as comparative testing of dogs and humans in the same households, in order to determine common risk factors and sources of exposure. For the majority of healthy pet owners, dogs are not likely to be a great risk for human illness with the investigated bacteria; however, special consideration needs to be taken with vulnerable pet owners, including the very young, the elderly and immunocompromised individuals (Lefebvre et al. 2008). Given the results of this thesis concerning pathogen carriage and age of the dog, particular consideration must be given regarding the age of pet dogs in contact with vulnerable populations. More importantly, because of the greatly increased risk for shedding
pathogens like *Salmonella*, raw meat and other raw animal products should not be fed to dogs within households of or in contact with vulnerable people. The evidence collected from these studies and similar future research is of public health importance and will improve the development and application of evidence-based guidelines for safe dog ownership and enhanced companion animal surveillance.
REFERENCES


APPENDIX A

Questionnaire used to obtain dog and household information for pet dogs in Ontario (October 2005 to May 2006)
Environmental Home Assessment

Thank you very much for agreeing to participate in this study. The interview will last 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.

Household ID #:  
Dog Name: _______ Animal ID#: _______  
Dog Name: _______ Animal ID#: _______  
Dog Name: _______ Animal ID#: _______  
Dog Name: _______ Animal ID#: _______  
Dog Name: _______ Animal ID#: _______  
Other Animals: _______ Animal ID#: _______  
Other Animals: _______ Animal ID#: _______  
Other Animals: _______ Animal ID#: _______  

Demographic Information
Date: _______  
Interviewed by: ________________________  
Name: ____________________________________  
Address: _________________________________  
City: ___________ Province: ______ Postal Code _______  
Phone number: _________________________  
Gender: M/F male/female Age: ___  

Section 1: Pet Information
I am going to ask you about pets you might have at home and food products that you might buy for their feeding.

1.1 Do you have any of the following pets in your home:
   - Dogs [ ]Y [ ]N [ ]DK
   - Cats [ ]Y [ ]N [ ]DK
   - Hamsters [ ]Y [ ]N [ ]DK
   - Reptiles [ ]Y [ ]N [ ]DK
   - Fish [ ]Y [ ]N [ ]DK
   - Other: ____________________________________

1.2 How many dogs do you have in your home? _______  

1.3 Where do you buy your dog’s food?
   - [ ] grocery, discount store
   - [ ] pet store
   - [ ] order from internet
   - [ ] prepared at home
   - [ ] other (specify) _________________________

1.4 Brand of pet food fed during last week ________________

1.5 In the past week, did you feed your dog any of the following items:
   - Raw beef [ ]Yes [ ]No
<table>
<thead>
<tr>
<th>Item</th>
<th>Yes</th>
<th>No</th>
<th>DK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw chicken</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw pork</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw eggs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dried pig’s ears</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rawhide chews</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cooked table scraps</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other pet treats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specify: __________________________</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1.6 How frequently do you feed your dog:
- [ ] less than once a day
- [ ] once a day
- [ ] twice a day
- [ ] all day

1.7 Who feeds the dog:
- [ ] Yourself
- [ ] Spouse/partner
- [ ] Other ____________________

1.8 Which of the following apply to your dog:
- [ ] can run freely in a park or other location where other dogs may have visited
- [ ] is confined to a fenced yard
- [ ] is always on a leash
- [ ] has contact with livestock (cattle, sheep, goats, pigs, or horses)
- [ ] has contact with cats

1.9 Do you treat your dog with medication to prevent heartworm (ie. Interceptor, Heartgard, Sentinel, Revolution)?
- [ ] Yes
- [ ] No
- [ ] DK

1.10 Do you vaccinate your dog annually, in addition to rabies vaccination?
- [ ] Yes
- [ ] No
- [ ] DK

Specify: ____________________

1.11 Have you administered an herbal product to your dog during the preceding 30 days?
- [ ] Yes
- [ ] No
- [ ] DK

Specify: ____________________

1.12 Have you given your dog a probiotic (*Lactobacillus*, active bacterial culture...) during the preceding 30 days?
- [ ] Yes
- [ ] No
- [ ] DK

1.13 Has your dog had any of the following gastro-intestinal upset signs during the preceding 30 days?
- Diarrhea [ ] Yes [ ] No [ ] DK
- Vomiting [ ] Yes [ ] No [ ] DK

1.14 Which of the following types of food do you use for feeding your dog:
- Commercial dry/canned dog food [ ] Yes [ ] No [ ] DK *(section 4)*
- Commercial raw food diets [ ] Yes [ ] No [ ] DK *(section 2)*
Section 2: Raw Food Diet Information
I will now ask you about the raw food diets that you buy to feed your dogs.

2.1 How long have you been feeding raw food diets to your dog(s)?
[ ] <1 month
[ ] 1-6 months
[ ] 6-12 months
[ ] 12-24 months
[ ] >2 years

2.2 What is the brand name of the raw food diets?
____________________________________________

2.3 If raw food diets are prepared at home, where do you obtain your recipes?
____________________________________________

2.4 What raw meats have you used in the last 30 days?
[ ] Chicken
[ ] Turkey
[ ] Fish
[ ] Beef
[ ] Other:_____________

2.5 Where do you purchase raw food?
[ ] Grocery store
[ ] Butcher
[ ] Directly from the farm
[ ] Pet store
[ ] Veterinary clinic
[ ] Other: __________

2.6 How long after mixing/opening do you keep food before discarding it? _______

2.7 Do you mix the raw food diet with any commercial dry food?
[ ] Yes [ ] No [ ] DK

2.8 Do you add anything else to the raw food diet? (vitamins, calcium, etc.)
[ ] Yes [ ] No [ ] DK

If yes, specify: ________________________________

2.9 If you feed frozen raw meat, how do you thaw it?
[ ] At room temperature
[ ] In a refrigerator
[ ] In a microwave or other oven that is turned on
[ ] In water
[ ] Don't know / Not sure
[ ] Don't thaw

2.10 For thawing the dog's frozen meat, do you:
[ ] Keep it in its original packaging
[ ] Move the meat to another plate
[ ] Place it on a cutting board
[ ] Place it on another container

2.11 How do you determine when a raw food sample is no longer edible?
[ ] How long you've had it
[ ] Expiry date on product
[ ] Appearance
[ ] Odour
[ ] Other:________

2.12 Why did you start feeding raw food?
[ ] More natural diet
[ ] Concerned about additives/preservatives in processed food
[ ] Healthier diet
[ ] Cost
[ ] To treat ongoing health problems
[ ] Other:_______________________

2.13 What health benefits do you feel occur as a result of feeding a raw diet?
___________________________________________________
___________________________________________________
___________________________________________________

2.14 Where did/do you obtain information about feeding raw diets? (check all that apply)
[ ] Friends
[ ] Other dog owners
[ ] Pet store employees
[ ] Your Veterinarian
[ ] Other veterinarians
[ ] The internet
[ ] Books
[ ] Other:_________

2.15 What do you do with the food bowl after feeding?
[ ] Immediately remove and clean it
[ ] Leave it until food is consumed
[ ] Leave it until the next meal

2.16 How often do you clean the food bowl?
[ ] daily
[ ] 3-6 times/wk
[ ] 1-2 times/wk
[ ] less than once a week

2.17 How do you clean the food bowl?
[ ] Cold water rinse
[ ] Hot water rinse
[ ] Rinse and scrub: no soap
[ ] Rinse and scrub with soap (Hot water or Cold water)
[ ] Dishwasher clean
[ ] Soak in disinfectant
    which disinfectant: _____________
[ ] Other: _______________________

2.18 After handling raw food, do you:
[ ] Always thoroughly wash your hands (soap and water)
[ ] Always rinse your hands
[ ] Usually wash/rinse your hands
[ ] Sometimes wash/rinse your hands
[ ] Rarely wash/rinse your hands

2.19 Which of the following statements do you agree with most?
[ ] Dogs fed raw diets are healthier than dogs fed processed diets
[ ] Dogs fed processed diets are healthier than dogs fed raw diets
[ ] Dogs fed homecooked diets are healthier than dogs fed processed diets
[ ] There is no health difference between dogs fed raw diets or processed diets

2.20 What health concerns regarding feeding raw diets are you aware of:

________________________________________________________________________
________________________________________________________________________

2.21 Has your veterinarian discussed possible risks associated with raw diets?
[ ] Yes [ ] No [ ] DK
    Further Comments: __________________________

2.22 Do you believe that safety concerns regarding the feeding of raw diets are:
[ ] Accurately portrayed
[ ] Exaggerated
[ ] Downplayed
[ ] Unaware of any safety concerns

**Section 3: Home Cooked Food Diet Information**
I will now ask you about the home cooked food diets that you buy and make to feed your dogs.

3.1 How long have you been feeding home cooked food diets to your dog(s)?
[ ] <1 month
[ ] 1-6 months
[ ] 6-12 months
[ ] 12-24 months
[ ] >2 years

3.2 If commercial brand used, what is the brand name of the home cooked food diets?

________________________________________________________________________

3.3 If home cooked food diets are prepared at home, where do you obtain your recipes?

________________________________________________________________________

3.4 What raw meats have you used in the last 30 days for home cooked recipes?
[ ] Chicken [ ] Lamb
[ ] Turkey [ ] Pork
[ ] Fish [ ] Ostrich/emu
[ ] Beef [ ] Venison
[ ] Other: ______________

3.5 Where do you purchase ingredients for home cooked food?
[ ] Grocery store
[ ] Butcher
[ ] Directly from the farm
[ ] Pet store
[ ] Veterinary clinic
[ ] Other: ______________

3.6 How long after mixing/opening do you keep food before discarding it? ______

3.7 Do you mix the home cooked food diet with any commercial dry food?
[ ] Yes [ ] No [ ] DK

3.8 Do you add anything else to the home cooked food diet? (vitamins, calcium, etc.)
[ ] Yes [ ] No [ ] DK

If yes, specify: __________________________________________________________

3.9 If you feed frozen meat, how do you thaw it?
[ ] At room temperature
[ ] In a refrigerator
[ ] In a microwave or other oven that is turned on
[ ] In water
[ ] Don't know / Not sure
[ ] Don't thaw

3.10 For thawing the dog’s frozen meat, do you:
[ ] Keep it in its original packaging
[ ] Move the meat to another plate
[ ] Place it on a cutting board
[ ] Place it on another container

3.11 How do you determine when a home cooked food sample is no longer edible?
[ ] How long you’ve had it
[ ] Expiry date on product
[ ] Appearance
[ ] Odour
[ ] Other: ______________

3.12 Why did you start feeding home cooked food?
[ ] More natural diet
[ ] Concerned about additives/preservatives in processed food
[ ] Healthier diet
[ ] Cost
[ ] To treat ongoing health problems
[ ] Other: ____________________
3.13 What health benefits do you feel occur as a result of feeding a home cooked diet?
________________________________________________________________________
________________________________________________________________________

3.14 Where did/do you obtain information about feeding home cooked diets? (check all that apply)
[ ] Friends
[ ] Other dog owners
[ ] Pet store employees
[ ] Your Veterinarian
[ ] Other veterinarians
[ ] The internet
[ ] Books
[ ] Other: ____________________

3.15 What do you do with the food bowl after feeding?
[ ] Immediately remove and clean it
[ ] Leave it until food is consumed
[ ] Leave it until the next meal

3.16 How often do you clean the food bowl?
[ ] daily
[ ] 3-6 times/wk
[ ] 1-2 times/wk
[ ] less than once a week

3.17 How do you clean the food bowl?
[ ] Cold water rinse
[ ] Hot water rinse
[ ] Rinse and scrub: no soap
[ ] Rinse and scrub with soap (Hot water or Cold water)
[ ] Dishwasher clean
[ ] Soak in disinfectant
  which disinfectant: ___________
[ ] Other:________________________

3.18 After handling raw food, do you:
[ ] Always thoroughly wash your hands (soap and water)
[ ] Always rinse your hands
[ ] Usually wash/rinse your hands
[ ] Sometimes wash/rinse your hands
[ ] Rarely wash/rinse your hands

3.19 Which of the following statements do you agree with most?
[ ] Dogs fed raw diets are healthier than dogs fed processed diets
[ ] Dogs fed processed diets are healthier than dogs fed raw diets
[ ] Dogs fed home cooked diets are healthier than dogs fed processed diets
[ ] There is no health difference between dogs fed raw diets or processed diets

3.20 What health concerns if any regarding feeding home cooked diets are you aware of:
_____________________________________________________________________
_____________________________________________________________________

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3.21 Has your veterinarian discussed possible risks associated with home cooked diets?
[ ] Yes    [ ] No    [ ] DK

3.22 Do you believe that safety concerns regarding the feeding of home cooked diets are:
[ ] Accurately portrayed
[ ] Exaggerated
[ ] Downplayed
[ ] Unaware of any safety concerns

**Section 4: Commercial Dog Food Information**

4.1 Which of the following statements do you agree with most?
[ ] Dogs fed raw diets are healthier than dogs fed processed diets
[ ] Dogs fed processed diets are healthier than dogs fed raw diets
[ ] There is no health difference between dogs fed raw diets or processed diets
[ ] Dogs fed homecooked diets are healthier than dogs fed processed diets

3.2 What health concerns regarding feeding raw diets are you aware of?
__________________________________________________________________________
__________________________________________________________________________

4.3 Do you believe that safety concerns regarding the feeding of raw diets are:
[ ] Accurately portrayed
[ ] Exaggerated
[ ] Downplayed
[ ] Unaware of any safety concerns

4.4 Do you add anything to the food? (such as eggs, table scraps, etc.)
[ ] Yes    [ ] No    [ ] DK

4.5 What do you do with the food bowl after feeding?
[ ] Immediately remove and clean it
[ ] Leave it until food is consumed
[ ] Leave it until the next meal

4.6 How often do you clean the food bowl?
[ ] daily
[ ] 3-6 times/wk
[ ] 1-2 times/wk
[ ] less than once a week

4.7 How do you clean the food bowl?
[ ] Cold water rinse
[ ] Hot water rinse
[ ] Rinse and scrub: no soap
[ ] Rinse and scrub with soap (Hot water or Cold water)
[ ] Dishwasher clean
[ ] Soak in disinfectant
  which disinfectant:________
[ ] Other:___________________
Section 5: Household Information
I am now going to ask you some questions about your household.

5.1 How many children live in your household? Please state their ages.
   
   # of Children:_____
   age 1: _____   age 2: _____   age 3:_____

5.2 Are any of the following groups of people present in your household?
   [ ] Infants (<1 yr of age)
   [ ] Persons over 65 years of age
   [ ] Immunocompromised individuals (someone whose immune system is decreased through disease or treatment)

5.3 Are any of the following groups in regular (at least weekly) contact with your dog?
   [ ] Infants (<1 yr of age)
   [ ] Persons over 65 years of age
   [ ] Immunocompromised individuals (through disease or treatment)

5.4 Have you purchased any product for yourself or your family from a health food store, or health food section of a grocery store, during the preceding 30 days?
   [ ] Yes   [ ] No   [ ]DK

5.5 Have you purchased any organic products (fruit, vegetables, etc) for yourself or your family during the preceding 30 days?
   [ ] Yes   [ ] No   [ ]DK

5.6 In general, how is frozen meat thawed or defrosted in your household?
   [ ] At room temperature
   [ ] In a refrigerator
   [ ] In a microwave or other oven that is turned on
   [ ] In water
   [ ] Don't know / Not sure
   [ ] Don't thaw

5.7 For thawing your meat, do you:
   [ ] Keep it in its original packaging
   [ ] Move the meat to another plate
   [ ] Place it on a cutting board
   [ ] Place it on another container

Section 6: Travel Information
Now I am going to ask you about any travel you might have done in the last 30 days.

6.1 Did you travel anywhere in the province in the last 30 days?
   [ ]Yes   [ ]No   [ ]DK
   If yes, please specify destination and date traveled ____________

6.2 Did you travel anywhere outside the province, but within Canada, in the last 30 days?
   [ ]Yes   [ ]No   [ ]DK
   If yes, please specify destination and date traveled _______________

6.3 Did you travel anywhere outside Canada in the last 30 days?
[ ] Yes  [ ] No  [ ] DK
If yes, please specify destination and date traveled ______________________

Section 7: Occupational Information

7.1 What is your main occupation?

____________________________________________________________________

Comments:
APPENDIX B

Questionnaire used to obtain dog and household information for pet dogs in the Region of Waterloo, Ontario (July 2008 to May 2009)
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.

Household ID #: ____________
Dog’s Name :________________            Animal ID#:_____
Breed:________________                 Age:_______         Sex:________________
How long have you owned this dog?____________________________________________
Where did you get this dog?
   [ ] Pet Store       [ ] Breeder       [ ] Humane Society
   [ ] Other_____________________
Why is this dog visiting your veterinarian today?
___________________________________________________________________

Demographic Information
Date: __________
Interviewed by: _________________________________
Name: __________________
Address: ______________________________________
City:_____________________  Province:_____________     Postal Code___________
Phone number: __________________     Email: _______________________________________
Is your home: [ ] Urban       [ ] Suburban       [ ] Small town rural     [ ] Non farm rural     [ ] Farm
   Type of farm____________________________

Section 1: Activities Information
The following questions relate to activities that your dog is currently involved in or has been involved in during the past year.

1.1 Is your dog involved in hunting activities?:
   [ ] Yes          [ ] No               [ ] Don’t Know
   If yes, what type of hunting______________________________

1.2 Has your dog had contact with other dogs?:
   [ ] Yes          [ ] No               [ ] Don’t Know

1.3 Does your dog participate/visit in any of the following activities?
   [ ] Dog park
   [ ] Dog day care
   [ ] Obedience/Agility/Flyball/Other classes

1.4 Is your dog involved in therapy/education programs?
   [ ] Yes          [ ] No
   If yes, please specify______________________________________________

1.5 Has your dog been boarded or hospitalized in the last 6 months?
   [ ] Vet clinic         [ ] Kennel       [ ] No
   If yes, when ______days_______months_______years

* Questionnaire was pretested with 3 veterinary clinicians and 5 pet owners.
Section 2: Other Animal Information
The following questions refer to exposure that your dog has had with other animals

2.1 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

2.2 How many dogs are in your home (excluding the dog in the survey)?
__________ Ages:________________________________________

2.3 How many cats are in your home?
__________ Ages:________________________________________

2.4 How many other animals are in your home?
__________ What kinds:______________________________________

2.5 Does your dog have access to farms with livestock?
[ ] Yes [ ] No [ ] Don’t Know
What type(s)_________________________________________

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

Section 3: Medical History
The following questions refer to your dog’s medical history. Questions will be verified by checking your dog’s medical records.

3.1 Has your dog been dewormed in the past 6 months?
[ ] Yes [ ] No [ ] Don’t Know
If yes, which product_____________________________________________

3.2 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__________________________

3.3 Do you vaccinate your dog for Giardia, in addition to rabies vaccination?
[ ] Yes [ ] No [ ] Don’t Know
If yes, when was the last dose given_________________________

3.4 Have you given your dog a probiotic (Lactobacillus, active bacterial culture...) during the preceding
month?
[ ] Yes [ ] No [ ] Don’t Know
If yes, what kind/how and why_____________________________________________

3.5 Has your dog been treated with antibiotics in the past month?
[ ] Yes [ ] No [ ] Don’t Know
What antibiotic________________________________________

3.6 Is your dog receiving any other medications or supplements currently?
[ ] Yes [ ] No [ ] Don’t Know
If yes, what______________________________

3.7 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

Section 4: Water Exposure
The following questions refer to water sources that your dog has had exposure or access to

4.1 What is the primary source of water for your dog?
   [ ] Municipal tap water
   [ ] Well tap water
   Has the water undergone treatment (excluding water softener)?
     [ ] Yes [ ] No [ ] Don’t Know
     If yes, what?______________________________
   [ ] Bottled water
   [ ] Other – please specify______________________________

4.2 Does your dog drink out of the toilet?
   [ ] Yes [ ] No [ ] Don’t Know

4.3 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
   [ ] Other – please specify______________________________
   [ ] No [ ] Don’t Know

Section 5: Diet Information
The following questions refer to the primary diet you are currently feeding your dog.

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

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

5.3 How often do you feed your dog any of the following items in their diet or as a treat?
   Table scraps [ ] daily [ ] weekly [ ] monthly [ ] rarely [ ] never
   Raw beef [ ] daily [ ] weekly [ ] monthly [ ] rarely [ ] never
   Cooked beef [ ] daily [ ] weekly [ ] monthly [ ] rarely [ ] never
   Raw chicken [ ] daily [ ] weekly [ ] monthly [ ] rarely [ ] never
   Cooked chicken [ ] daily [ ] weekly [ ] monthly [ ] rarely [ ] never
   Raw turkey [ ] daily [ ] weekly [ ] monthly [ ] rarely [ ] never
   Cooked turkey [ ] daily [ ] weekly [ ] monthly [ ] rarely [ ] never
   Raw pork [ ] daily [ ] weekly [ ] monthly [ ] rarely [ ] never
   Cooked pork [ ] daily [ ] weekly [ ] monthly [ ] rarely [ ] never
   Raw eggs [ ] daily [ ] weekly [ ] monthly [ ] rarely [ ] never
   Cooked eggs [ ] daily [ ] weekly [ ] monthly [ ] rarely [ ] never
   Fish [ ] daily [ ] weekly [ ] monthly [ ] rarely [ ] never
   Other [ ] daily [ ] weekly [ ] monthly [ ] rarely [ ] never
   Specify:___________________________________
5.4 Have you provided your dog with any of the following treats?:

- Dried pig’s ears
  - [ ] daily
  - [ ] weekly
  - [ ] monthly
  - [ ] rarely
  - [ ] never

- Raw Bones
  - [ ] daily
  - [ ] weekly
  - [ ] monthly
  - [ ] rarely
  - [ ] never

- Cooked Bones
  - [ ] daily
  - [ ] weekly
  - [ ] monthly
  - [ ] rarely
  - [ ] never

- Store bought bones
  - [ ] daily
  - [ ] weekly
  - [ ] monthly
  - [ ] rarely
  - [ ] never

- Rawhide chews
  - [ ] daily
  - [ ] weekly
  - [ ] monthly
  - [ ] rarely
  - [ ] never

- Other pet treats
  - [ ] daily
  - [ ] weekly
  - [ ] monthly
  - [ ] rarely
  - [ ] never

Specify: _______________________________________________________

If not given daily, weekly or monthly, when was the last treat given_____________________

**Section 6: Exposure to Humans**

The following questions relate to people currently living in your home

6.1 How many children live in your household? Please state their ages.

____________________________________________________________________________________

6.2 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

6.3 Have any human members in your family worked in/ visited/ or been in a hospital in the past 30 days?

  - [ ] Yes
  - [ ] No
  - [ ] Don’t Know
  - [ ] Don’t want to answer

If yes, in what capacity______________________________________________

6.4 Has anyone in your family been treated with antibiotics in the past month (oral or injectable)?

  - [ ] Yes
  - [ ] No
  - [ ] Don’t Know

6.5 In the past seven days has anyone in the household been in contact with any of the following animals?

  - [ ] cats
  - [ ] birds
  - [ ] petting zoos
  - [ ] livestock (cattle, sheep, goats, pigs or horses)
  - [ ] Other: ____________________________________________