To the Graduate Council:

I am submitting herewith a dissertation written by Katherine Anne Stenske entitled "Comparison of fecal *Escherichia coli* from dogs and their owners." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Comparative and Experimental Medicine.

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Joseph Bartges, Major Professor

We have read this dissertation
And recommend its acceptance:

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David Bemis

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Ann Draughon

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Karla Matteson

Accepted for the Council:

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Carolyn R. Hodges, Vice Provost
and Dean of the Graduate School

Comparison of fecal *Escherichia coli* from dogs and their owners

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Abstract

Contact between human beings and dogs may allow sharing of antimicrobial resistant and virulent bacteria. Objectives of this study were to determine the prevalence of cross-species sharing of fecal *E. coli* based on pulse field gel electrophoresis (PFGE) profile similarity, to compare antimicrobial susceptibility patterns and virulence factor patterns between dog-owner pairs, and to analyze the epidemiology of cross-species sharing using a questionnaire.

A cross-sectional study comparing fecal *E. coli* isolates from dogs and their owners was conducted. A questionnaire and fecal sample was collected from 61 dog-owner pairs and 30 controls. Three *E. coli* colonies were isolated from each participant and confirmed biochemically. Antimicrobial susceptibility of each isolate was determined via disc diffusion for 17 antimicrobial agents routinely monitored by the National Antimicrobial Resistance Monitoring System. PFGE profiles were used to establish relatedness among bacterial isolates. A multiplex PCR was developed to determine presence or absence of 4 urovirulence factor genes: *cnf*, *hlyD*, *sfa*, and *papGIII*. The questionnaire asked about medical history, antimicrobial therapy, hygiene, and relationship with dog.

A wide array of PFGE profiles was observed in *E. coli* isolates from all participants. Within-household sharing occurred with 9.8% prevalence, and across-household sharing occurred with 0.26% prevalence. No specific behaviors were associated with increased clonal sharing between dog and owner.

No differences were found in susceptibility results or virulence factor patterns between dog-owner pairs. Control isolates were more resistant than canine isolates, and human beings carried more multiple-drug resistant *E. coli* than dogs. Isolates from owners who did not wash their hands after petting their dogs had increased resistance to ampicillin. An association was found in women between history of UTI and presence of each virulence factor in their dog's fecal *E. coli*. Antimicrobial resistance was associated with reduction of virulence factors.

Within-household sharing of *E. coli* occurred more commonly than across-household sharing, but both direct contact and environmental reservoirs may be important routes for cross-species sharing of bacteria and genes for resistance and virulence. Cross-species bacterial sharing is a potential public health concern, and good hygiene is recommended.

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List of Abbreviations

aer = aerobactin
AM = ampicillin
AmC = amoxicillin-clavulanic acid
AN = amikacin
ARF = acute renal failure
ATCC = American Type Culture Collection
BHI = brain heart infusion
bp = base pair
C = chloramphenicol
CDC = Center for Disease Control
CF = cephalothin
CIP = ciprofloxacin
CLSI = Clinical and Laboratory Standards Institute
cnf = cytotoxic necrotizing factor
CPD = cefpodoxime
CRGV = cutaneous and renal glomerular vasculopathy
CRO = ceftriaxone
DNA = deoxyribonucleic acid
EAggEC = enteroaggregative *E. coli*
EC = *Escherichia coli*
E. coli = *Escherichia coli*
EDTA = ethylenediamine tetra acetate
EHEC = enterohemorrhagic *E. coli*
EIEC = enteroinvasive *E. coli*
EMB = eosin methylene blue
EPEC = enteropathogenic *E. coli*
ETEC = enterotoxigenic *E. coli*
ExPEC = extraintestinal pathogenic *E. coli*
F = forward
FDA = Food and Drug Administration
FOX = cefoxitin
Gal-Gal = Galactose(α1-4)Galactoseβ
GI = gastrointestinal
GM = gentamicin
hlyD = hemolysin
HUS = hemolytic uremic syndrome
ICU = intensive care unit
ID# = identification number
IPM = imipenem
K = kanamycin
LB = Luria-Bertani broth

LEE = locus for enterocyte effacement
LPS = lipopolysaccharide
MDR = multiple drug resistance
MRSA = methicillin-resistant *Staphylococcus aureus*
MUG = 4-methylumbelliferyl-β-D-glucuronide
NA = nalidixic acid
NARMS = National Antimicrobial Resistance Monitoring System
NCCLS = National Committee for Clinical Laboratory Standards
NXL = ceftiofur
PAI = pathogenicity island
Pap = pyelonephritis associated pili
papGIII = pyelonephritis associated pili G allele III
PBS = phosphate buffered saline
PCR = polymerase chain reaction
PFGE = pulse field gel electrophoresis
R = reverse
RAPD = random amplified polymorphic DNA
S = streptomycin
S. aureus = *Staphylococcus aureus*
sfa = S fimbriae adhesin
stx2 = shigatoxin 2
Te = tetracycline
TMS = trimethoprim-sulfamethoxazole
tRNA = transfer ribonucleic acid
TSI = triple sugar iron
UPEC = uropathogenic *E. coli*
USDA = United States Department of Agriculture
UTCVM = University of Tennessee, College of Veterinary Medicine
UTI = urinary tract infection

Nomenclature

C = Celsius
cm = centimeter
h = hour
g = gram
G = x gravity
L = liter
ml = milliliter
mm = millimeter
M = molar
mM = micromolar
ng = nanogram
nm = nanometer
rpm = revolutions per minute
s = second
μg = micrograms
μl = microliter
V = voltage

Literature Review

Introduction

Escherichia coli (*E. coli*) is a gram-negative, non-spore-forming, motile, coccobacillus in the Family, *Enterobacteriaceae*. Strains of this species are highly versatile bacteria that can respond and adapt to environmental changes in temperature, pH, chemicals, oxygen and nutrient availability. This versatility allows them to survive as benign commensal flora in various body sites of mammals such as the gastrointestinal (GI) and distal urogenital tracts, and to cause opportunistic infections when host defenses are compromised. Some strains of *E. coli* are naturally pathogenic, such as *E. coli* O157:H7, while other strains are normally nonpathogenic; however, new pathogenic strains can arise quickly through acquisition of virulence genes. A better understanding of the epidemiology, genetics, and response of *E. coli* to antimicrobial agents will help advance strategies to control transmission and treatment of these infections while reducing the economic impact of *E. coli* and improving overall public health.

Classification Schemes of *E. coli*

Several classification schemes are used to identify and characterize strains of *E. coli* because of their diversity. These are based on colony morphology, biochemical reactions, antimicrobial susceptibility, antigenic variation, production of

virulence factors, and deoxyribonucleic acid (DNA) fingerprint profiles. Serologic testing is used to classify strains based on outer surface antigens, including somatic antigens (O-antigens) that are part of the lipopolysaccharide (LPS), capsular antigens (K-antigens), flagellar antigens (H-antigens), and fimbrial antigens (F-antigens). Serogroups are determined based on the O-antigen, and serotypes are determined by the full compliment of O, K, F, and H-antigens, resulting in nomenclature such as *E. coli* O157:H7. Classification schemes based on newer molecular techniques are emerging, including use of polymerase chain reaction (PCR) to determine presence of virulence factors (virotyping), and use of DNA fingerprinting techniques (genotyping).

Role of *E. coli* in the Normal Gastrointestinal and Urogenital Flora

The *Enterobacteriaceae* family was so named because its members are primarily found in the enteric tract of mammals. In addition to *E. coli*, other prominent *Enterobacteriaceae* include *Proteus, Klebsiella, Enterobacter, Salmonella, and Shigella*. The mammalian GI tract is sterile during fetal development, and the fetus is originally exposed to bacteria during passage through the birth canal. Bacteria are ingested from the local environment and travel through the GI tract, competing with other bacteria and ultimately colonizing in their ideal niche. Initially, as the flora evolves it is very similar to the mother's GI flora, due to close physical contact and nursing; however, with maturation and independence, the adult GI flora

becomes an individualized complex ecosystem based on environment, health, stress level, nutrition, and exposure to antimicrobial drugs and probiotics [1]. As a result of these contributory factors, there can be marked variation in the bacterial microflora of the intestinal compartments and feces even among individuals living in the same environment [2, 3].

The oral cavity is inhabited by a mixture of aerobic and anaerobic bacteria. The esophagus is not colonized by bacteria but is contaminated by saliva that contains a mixture of aerobic and anaerobic bacteria. The stomach and intestines are inhabited by a combination of anaerobes, coliforms, gram-positive bacteria, and spirochetes, with the total number of bacteria increasing progressively and peaking in the large intestine [4].

The GI flora remains fairly stable over prolonged periods, experiencing gradual shifts in bacterial species and predominant strains. Rapid changes can occur with administration of antimicrobial therapy or infection with virulent pathogens [3]. Administration of nutraceuticals such as prebiotics and probiotics will alter the GI flora. Prebiotics are undigested foods that alter replication of specific enteric bacteria. Soluble fibers such as fructooligosaccharides are prebiotics that reach the colon undigested, are fermented by anaerobic bacteria, and may decrease growth of *E. coli* and *Clostridium*. Probiotics are live microorganisms, such as *Lactobacilli, Bifidobacteria*, and *Enterococcus faecium* that incorporate into the GI flora, inhibit pathogenic bacteria, and stimulate immune defense mechanisms [3].

The normal GI flora is a symbiotic partner with the host mammal and is responsible for several physiologic and metabolic activities. A major role is to compete with potential pathogens for binding sites, stimulate immunoglobulin A (IgA) production, and produce antibacterial compounds to suppress activity of potential pathogens. The normal GI flora also plays a role in regulating GI transit time, carbohydrate fermentation, bile acid metabolism, short-chain fatty acid production, and folic acid synthesis [1, 3, 5].

In addition to the GI flora, *E. coli* are part of the normal flora in the distal urogenital tract, including the distal urethra, prepuce, and vagina [6]. Although *E. coli* may be a source of ascending infection, they are also protective, competing to inhibit adherence, growth, and ascension of potential pathogens up the urethra into the urinary bladder, prostate, and uterus [6]. As in the GI tract, the normal distal urogenital flora contains a mixture of bacteria, and *E. coli* comprise only a small fraction. *Lactobacilli* are more commonly recognized bacteria in the urogenital defense system. When outside pressures such as antimicrobial treatment alter *Lactobacillus* numbers, risk of ascending urinary tract infections (UTI) and opportunistic vaginal yeast infections is increased. Therapeutically replacing *Lactobacilli* via live cultures in yogurt or probiotics may restore the normal urogenital flora and assist in management of opportunistic and pathogenic ascending infections.

Acquisition of Virulence

Although some *E. coli* live in harmony with and protect the mammalian host, other strains of *E. coli* cause significant disease. This distinction is determined by bacterial and host factors. Bacterial factors can increase virulence and pathogenicity, while decreased host immune defenses allow opportunistic infections to occur. Certain strains of *E. coli* acquire genes that encode virulence factors and increase the bacterium's ability to adhere to host cells, invade, produce toxins, utilize host nutrients, and avoid the host's immune system. By definition, virulence is the relative ability of an organism to cause disease in a particular host, and virulence genes are major factors involved in determining if a bacterial strain is benign or pathogenic [7]. Host factors such as immunosuppression, anatomical abnormalities, and previous antimicrobial therapy are also important in *E. coli* epidemiology.

Increased virulence can develop from chromosomal mutations in individual bacteria or acquisition of virulence genes through horizontal gene transfer. Mutations occur spontaneously within the genome, and the effect of the mutation depends on which gene is mutated and the type of mutation. Certain mutations improve survival, allowing propagation into a clone within the host environment. If chromosomal mutations promote increased virulence or antimicrobial resistance, the mutated clone can overcome the host's normal flora and immune defenses and cause clinical disease. Nonsense, deletion, and inversion mutations can cause fatal defects in protein products, leading to premature bacterial death.

Horizontal gene transfer is a more common, rapid, and efficient method of acquiring virulence genes. This is the spread of DNA from one bacterium to another, occurring via three main routes: transformation, transduction, and conjugation. With transformation, transduction, and conjugation, new DNA is brought into a recipient bacterium, and if it has similar base pair (bp) segments as the recipient's chromosomal DNA, homologous recombination can occur to integrate the new DNA into the recipient's chromosome.

Transformation is the direct uptake of naked DNA from a nearby ruptured bacterium. Transduction is the transfer of DNA between bacteria via bacteriophages. Phages have the ability to integrate into the chromosome, via lysogeny, and during times of DNA stress, including ultraviolet light damage or use of an antimicrobial drug inhibiting DNA replication, the phage can excise from the host chromosome. Occasionally when this occurs, a small portion of bacterial host DNA is packaged into phage nucleocapsids when virus particles are assembled and released from infected host cells. These new virions with chimeric genomes can bind and inject their nucleic acid into new bacterial hosts, and recombination can then occur with the recipient's chromosome.

Conjugation is the most common and efficient route of horizontal gene transfer, a form of direct mating between bacteria that results in transfer of plasmids from a donor to a recipient bacterium. Plasmids are pieces of DNA independent from a bacterium's chromosome that often carry genes for virulence and antimicrobial resistance. In short, during conjugation, a donor bacterium with a self-transmissible

or mobilizable plasmid extends a pilus to attract a recipient bacterium that lacks the same type of plasmid. Following attachment of donor to recipient, the pair comes into very close contact, a hollow pore is formed between the two, and a copy of the plasmid is transferred from donor to recipient. Homologous recombination then occurs between the plasmid and the recipient's chromosome to produce offspring unlike either parent bacterium. When a donor bacterium with a unique plasmid is first introduced to a new population, conjugation occurs rapidly, providing plasmids and their genes for virulence or antimicrobial resistance to the majority of naïve recipients. The pilus that attracts recipients also attracts bacteriophages, so tight regulation stops conjugation after the initial burst to prevent destruction of the donor by phage lysis.

Transposons and integrons are DNA segments that can jump between strands of DNA. This action is classified as nonhomologous recombination, because its occurrence is determined by transposases and integrases that recognize specific sites in recipient DNA. Transposons carry genes for virulence factors and antimicrobial resistance and can be carried on plasmids or phages and transfer DNA after conjugation or transduction. This most often occurs within the same species of bacteria, but can also occur between different genera and species. Integrons are a type of transposon that encodes integrase, an enzyme helping them serve as recipients for antimicrobial resistance genes. The functional integrity of genetic elements acquired by nonhomologous recombination may be readily lost, leaving behind

unique insertion sequences that are sometimes used for species identification or genomic clusters of genes encoding antimicrobial resistance or virulence traits.

Pathogenicity islands (PAI) are condensed clusters of genes encoding virulence factors able to be acquired through horizontal gene transfer. PAI sequences are large (>30 kilobase) blocks of DNA located near tRNA genes and have a guanine-cytosine (GC) content that differs from the rest of the genome [8]. They contain transposases and integrases to allow insertion into recipient DNA as transposons, but PAI gene clusters can also transfer via plasmids or phages [9]. Due to frequent recombination and genetic rearrangement, PAIs have constant modifications in exact composition of virulence factors [9]. PAIs and virulence genes they contain play an important role in the uropathogenesis of *E. coli*. One study using PCR showed that 40% of commensal fecal *E. coli* isolates but 93% of uropathogenic *E. coli* (UPEC) carried PAIs [10]. Another study identified PAIs in 22% of fecal strains from healthy subjects, but in 79% of isolates from patients with acute pyelonephritis and 82% of isolates from patients with cystitis (82%) [8].

Pathogenic *E. coli* and their Virulence Factors

Disease from *E. coli* can come from either opportunistic or pathogenic *E. coli.* Opportunistic infection and disease occur when there is a change in the local environment or temporary break in immune defenses. Many bacterial strains, irrespective of their possession or lack of virulence traits, can cause opportunistic

infection and disease. Common risk factors include immunosuppressive, chemotherapeutic, and antimicrobial therapies, natural diseases such as GI ulceration, and iatrogenic damage such as that caused by an indwelling urinary catheter. Alternatively, pathogenic *E. coli* can cause infection and illness without concurrent disease or immunosuppression, because they have virulence factors that can overcome the host's intact natural defenses.

Four major categories of virulence factors in *E. coli* are: adhesins, toxins, iron-acquisition systems, and the outer capsule. Adhesins are critical proteins that mediate adherence of pathogenic bacteria to their target host cells. Toxins either locally destroy host target cells or enter the bloodstream and cause systemic damage, and stimulate the immune system by triggering inflammatory cascades. Iron-acquisition systems help bacteria compete with host cells for critical iron supplies. Finally, the capsule is an outer polysaccharide layer that helps *E. coli* avoid being recognized and killed by phagocytes and protects *E. coli* from lysis by the membrane attack complex of complement. Within these general categories are many specific fine-tuned virulence factors that have evolved through natural selective pressures. Individual distribution of virulence factors in each *E. coli* strain determines which organ system it can target and the type of disease it will cause.

Infections with pathogenic *E. coli* are typically classified as either intestinal or extraintestinal infections. There are five main types of intestinal pathogenic *E. coli*, including: enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and enteroaggregative

E. coli (EAggEC). Of the extraintestinal pathogenic *E. coli* (ExPEC), the most common is uropathogenic *E. coli* (UPEC), but significant disease can be associated with ExPEC infection of the bloodstream, central nervous system, reproductive organs, and other sites within the body.

Intestinal Pathogenic *E. coli*

Enterohemorrhagic *E. coli* (EHEC) include strains of *E. coli*, such as *E. coli* 0157:H7, responsible for the majority of *E. coli* food-borne GI illnesses in human beings. They are also responsible for edema disease in pigs, dysentery in calves and lambs, and are implicated in cutaneous and renal glomerular vasculopathy in greyhounds (CRGV). The main virulence factors present in EHEC are the locus for enterocyte effacement (LEE) carrying the gene for the classic attaching and effacing lesion (eaeA), and shigatoxins. Other suspected virulence factors involved in pathogenicity of EHEC include hemolysin and acid resistance [11]. LEE represents genes mediating attachment of *E. coli* to host cells, architectural and metabolic changes within enterocytes, and effacement of microvilli on the brush border of enterocytes. Shigatoxins have receptors in both the GI tract and kidneys, and shigatoxin 2 (stx2) is the major virulence factor involved in the pathogenesis of hemolytic uremic syndrome (HUS). EHEC initially damages enterocytes, causing dysentery and allowing shigatoxins to be absorbed into the bloodstream.

HUS develops in 2-15% of patients with shigatoxin-producing *E. coli*, and is characterized by dysentery, followed 5-7 days later by microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure (ARF) [12]. The exact pathogenesis of ARF remains unclear but may be directly toxin-mediated or induced by microangiopathic hemolysis and thrombosis [12]. Although most cases of HUS have stx2-positive *E. coli*, some HUS and ARF cases are associated with shigatoxin-negative and non-O157:H7 *E. coli*, suggesting that other virulence factors are involved and need to be elucidated [12, 13]. Genes for shigatoxins are carried on bacteriophages and are often integrated into the bacterium's chromosome. Thus antimicrobial drugs that damage DNA or inhibit DNA synthesis (including fluoroquinolones and sulfonamides) encourage integrated lysogenic bacteriophages to exit the host chromosome, replicate quickly, lyse the host bacterium, and invade new bacteria. This process causes a dramatic increase in toxin production, thus worsening systemic disease and overall prognosis; therefore supportive care is warranted but antimicrobial therapy may be more harmful than beneficial in cases of EHEC [14].

Cattle represent the largest reservoir of EHEC, and management of food contamination from this source is a major public health concern. Adult cattle can carry and intermittently shed *E. coli* O157:H7 as enteric commensal organisms without becoming ill. EHEC may also be found in calves and lambs with diarrhea and dysentery. In pigs, EHEC causes edema disease, which affects the fastest-growing post-weanling piglets, and is characterized by inappetence, severe edema, neurologic disease, and sudden death [11].

Dogs and cats can be healthy carriers or develop diarrhea or HUS from EHEC [15]. A prevalence study documented 3.1% of dogs on feedlots and dairy farms had positive fecal samples for *E. coli* O157:H7 [16]. Another study analyzed canine feces collected from 4 beaches and isolated *E. coli* O157:H7 from 7.8% of samples [17]. A study in urban Argentina isolated stx2-positive EHEC from 3.7% of dogs and 4% of cats but concluded that dogs and cats were not a major reservoir for EHEC in children [18]. Dogs are susceptible to CRGV, a systemic disease similar to HUS involving ARF, vasculitis with pitting edema and ulceration of the distal limbs, microangiopathic anemia, and thrombocytopenia [19]. CRGV has most often been reported in young adult racing greyhounds, and consumption of raw condemned contaminated beef has been implicated as a source. Due to its similarities with HUS, a shigatoxin produced by EHEC is the suspected etiology [11].

Enteropathogenic *E. coli* (EPEC) causes watery secretory diarrhea in infants in developing countries as well as in dogs, rabbits, and young farm animals. Gastroenteritis from EPEC is invasive and triggers a large inflammatory response. Toxins are not a major component of EPEC's virulence, however an enterotoxin, EspC, has been identified in a PAI within EPEC and most likely plays an accessory role in its pathogenesis [20]. Important adhesins include bundle forming pili in dogs and Ral in rabbits. The attaching and effacing lesion has a critical role in EPEC pathogenesis. After loose adhesion, type III secretion system genes are expressed in *E. coli* and inject proteins for tight adhesion and signal transduction into intestinal cells, including a receptor, Tir, for the adhesin intimin. This triggers rearrangement

of underlying actin, villi destruction, and influx of white blood cells. Diarrhea may be caused by destruction of the host cells with subsequent malabsorption and translocation of water and electrolytes or by alteration of the signal transduction pathways causing electrolyte and water loss. EPEC was a common cause of infectious diarrhea in dogs in one study, isolated from 35% of dogs with acute diarrhea, 31% of dogs with chronic diarrhea, and 6% of healthy control dogs [21]. Puppies from 1-3 months of age living in overcrowded pet shops or kennels may be at increased risk for EPEC diarrhea, but dogs of all ages can be affected [11, 21].

Gastroenteritis caused by enterotoxigenic *E. coli* (ETEC) is most often a problem with infants and children in developing countries or "travelers' diarrhea" in adults, but can also affect farm animals and dogs. ETEC are usually acquired from drinking water contaminated with sewage, but the infectious dose is higher than for other strains of *E. coli* [22]. Major virulence factors present in ETEC are adhesins (fimbriae, type 1 pili, adhesin involved in diffuse adherence, and colonization factor antigens) and enterotoxins (various heat-stable and heat-labile toxins). The genes for enterotoxins and adhesin colonization factor antigens are carried on plasmids and can be easily transferred among *E. coli* in the GI tract [23].

Disease caused by ETEC is characterized by a toxin-induced secretory watery diarrhea with mild inflammation and no significant histopathologic changes. Diarrhea is self-limiting in healthy adults; however, in infants or immunosuppressed patients it can cause dehydration, metabolic acidosis, shock, and death. It is the most common cause of diarrheogenic *E. coli* in farm animals, and in pigs it often

progresses to shock and death prior to the development of diarrhea, likely from an acute release of LPS endotoxin [11]. In dogs, studies have shown that 2-31% of diarrhea cases have been associated with ETEC, usually in young animals and most often involving the STa toxin [24]. In contrast, ETEC has low prevalence in healthy dogs and both healthy and diarrheic cats [21, 24].

Enteroinvasive *E. coli* (EIEC) has specific tropism for colonic cells, where it penetrates, invades, and causes destruction. EIEC enters the GI wall via M cells, which are antigen-sampling cells, and it avoids immune destruction by inducing apoptosis of macrophages [25]. Virulence factors include nonfimbrial adhesins that likely involve outer membrane proteins. Transmission is from fecal-oral exposure, either directly or through contaminated food or water, and the infectious dose is as low as 10 organisms [26]. Clinical signs occur within 12-72 hours of ingestion, and include fever, dysentery, and abdominal pain. This presentation is very similar to *Shigella* infection, and the two are often confused clinically. Although EIEC infections are usually self-limiting, complications including HUS can occur, especially in pediatric patients. EIEC has low prevalence in canine GI disease but has been associated with cases of both acute and chronic diarrhea [21].

Enteroaggregative *E. coli* (EAggEC) infection causes persistent diarrhea in children in developing countries, but this virotype has not been identified as causing disease in dogs. Like ETEC, it binds to small intestinal cells, is not invasive, and causes no inflammation or histological changes. Unlike ETEC, it does not bind uniformly, but rather in clumps or aggregates like stacked bricks. Virulence factors

present in EAggEC include very thin fibrillar adhesins, an enterotoxin, and hemolysin. The enterotoxin is similar to the heat-stabile toxin in ETEC. Hemolysin, which is also produced by UPEC, forms pores in host cell membranes. The complete role of both enterotoxin and hemolysin in the virulence of EAggEC has not yet been fully elucidated.

Most pathogenic intestinal *E. coli* causing disease in dogs are EPEC and ETEC, but *E. coli* strains more characteristic of ExPEC have recently been implicated as the cause of histiocytic or granulomatous ulcerative colitis in Boxer dogs [27]. Colonic thickening and ulceration with loss of goblet cells occurs, causing severe chronic large bowel diarrhea with frequent bloody mucoid feces and dramatic weight loss in young Boxer dogs, usually less than 4-years-old. Traditionally, the suspected etiology has been breed-specific immune-mediated inflammatory bowel disease. A recent study using in situ hybridization documented *E. coli* adhering and invading the colonic mucosa and localizing within histiocytes of affected Boxer dogs, while dogs with other types of colitis had no invasive or intracellular *E. coli* [27]. PCR virotyping and phylogenetic analysis found these *E. coli* more characteristic of UPEC than intestinal pathogenic *E. coli* [27]. A second study used immunohistochemistry and found positive immunoreactivity to *E. coli* antibodies, demonstrating presence of *E. coli* antigen, in every section of diseased colon from 10 dogs with ulcerative colitis [28]. Since other enteropathogenic bacteria tested for were not immunoreactive, it was concluded that *E. coli* is the causative agent of this disease in Boxer dogs [28]. Historically, cases of ulcerative colitis in Boxer dogs have been treated with

immunosuppressive therapy with minimal success, and most cases have been euthanized because of this condition; however, recent success with antimicrobial treatment, specifically enrofloxacin and metronidazole, supports the theory of an underlying infectious cause such as *E. coli* [29].

Extraintestinal Pathogenic *E. coli*

Although it traditionally does not cause intestinal disease, extraintestinal pathogenic *E. coli* (ExPEC) can be isolated from the GI tract with commensal *E. coli* strains [30]. ExPEC colonizes the oropharynx, colon, and vagina, and in accordance with the pathogenicity theory, virulence factors help it gain access to normally sterile locations such as the urinary tract [30, 31]. ExPEC can cause a wide variety of disease and be associated with high morbidity, mortality, and healthcare costs [30]. In human medicine, ExPEC infections of major importance include: urogenital tract infections, peritonitis, neonatal meningitis, surgical wound infections, and bacteremia [30]. In veterinary medicine, a prevalence study of nonenteric *E. coli* cultured from dogs found 63% isolates from the urinary tract, 9% from skin, 7.7% from the respiratory tract, 6.7% from the ear, 6.4% from the female reproductive tract, 3.7% from the male reproductive tract, and 3.4% from other organ systems [32]. Other reports describe ExPEC in cats causing UTIs [33, 34], prostatitis [35], endocarditis [36], septic peritonitis [37], and hepatic abscesses [38]. The various combinations of virulence factors present in different ExPEC strains allows them access to and ability

to invade organs throughout the body, making *E. coli* a significant pathogen in both human and veterinary medicine.

E. coli bacteremia can be caused by translocation through an immature or damaged GI tract, secondary to pyelonephritis or pneumonia, or external introduced from intravascular catheters or traumatic wounds. Neonatal puppies and kittens may acquire ExPEC from their mother's vaginal flora, milk, or feces [39]. Parvovirus is a common precursor to septicemia in older puppies and adult dogs, with *E. coli* translocating through the damaged GI tract [24]. ExPEC can persist outside of the GI tract partly because they possess virulence genes for iron acquisition, specifically aerobactin, allowing adaptation to iron-restricted areas of the body [11]. Other virulence factors that contribute to serum resistance and help avoid phagocytosis are the O polysaccharide, capsular antigens, and p fimbriae, while LPS directly triggers septic shock. Isolation of *E. coli* from the bloodstream carries a mortality rate of up to 31% in people, and perhaps higher in animals [30].

Like bacteremia, meningitis caused by *E. coli* is a problem in infants. These ExPEC are acquired from the mother's urogenital tract or environment and are spread from either the nasopharynx or GI tract to the meninges, where they cause inflammation. The major virulence factor involved is the K1 capsular antigen, which is present in 35-38% of GI *E. coli* strains, but is found in 79% of neonatal meningitis strains [7]. The K1 antigen is a poor immunogen, thus bacteria with this capsule avoid phagocytosis and destruction by complement. Protective anti-K1 antibodies are found in adults; however, these antibodies are restricted to an immunoglobulin

subclass that does not readily cross the placenta, leaving neonates unprotected. Antimicrobial resistance has been an increasing problem with neonatal meningitis, with up to 85% of cases being resistant to ampicillin, and case fatality rate ranges between 25-40% [30].

Pneumonia caused by *E. coli* is usually acquired by aspiration of enteric bacteria, however other routes of transmission are also possible, such as inhalation of bacteria or secondary to sepsis from GI translocation. In human beings, *E. coli* is a major cause of nosocomial pneumonia, due to increased oropharyngeal colonization of *E. coli* during times of illness and antimicrobial therapy [30]. In dogs, there are several reports of peracute hemorrhagic pneumonia caused by *E. coli* [40, 41]. Shipment of dogs may increase risk by inducing immunosuppression, stress colitis which can compromise GI integrity and allow translocation, or vomiting from motion sickness followed by aspiration [40]. Cytotoxic necrotizing factor (cnf), hemolysin (hlyD), and pyelonephritis associated pili G allele III (papGIII) have all been identified from ExPEC isolated from these cases and likely contribute to pathogenesis of fulminant fatal pneumonia [40, 41].

E. coli frequently infect the reproductive organs causing mastitis, pyometra, metritis, and prostatitis. Mastitis can be caused by hematogenous spread of *E. coli*, traumatic introduction into the mammary gland, or ascending infection through the teat or nipple. Most women and bitches with mastitis respond well to oral antimicrobial therapy, but some infections progress to necrotic abscesses requiring surgical drainage or removal. Pyometra is accumulation of purulent material within

the uterus of a bitch or queen within 8 weeks of standing heat or induction of ovulation [42]. Estrogen, followed by progesterone production, induces cystic endometrial hyperplasia, which makes the uterus more prone to infection and inflammation. This is followed by bacterial infection, most commonly with *E. coli* ascending from normal vaginal and fecal flora. *E. coli* become trapped in the uterus by functional closure of the cervix and produce endotoxemia leading to septic shock and ARF. Endotoxemia occurs when LPS endotoxins are released from *E. coli* cell walls and stimulate release of local cytokines including tumor necrosis factor, interleukins, and platelet activating factor. These cytokines contribute to hypotension and shock. *E. coli* endotoxins decrease sensitivity of renal tubules to vasopressin, decreasing water reabsorption and causing polyuria and polydipsia and ARF [43]. Glomerulonephritis secondary to antibody-antigen complex deposition in glomeruli may also result from bacterial pyometra [42]. Recommended treatment of pyometra in dogs and cats is ovariohysterectomy and antimicrobial therapy, and prognosis is generally good, although complications including sepsis, shock, and death can occur if not diagnosed and treated promptly. In women, bacterial endometritis is a common complication of cesarean section, with polymicrobial infections including *E. coli* occurring despite prophylactic antimicrobial therapy [44].

In men, prostatitis is most commonly caused by *E. coli*, and hemolysin has been identified as the main virulence factor in its pathogenesis [45]. *E. coli* isolated from prostatitis cases are more likely to produce biofilms than isolates from cystitis or pyelonephritis cases, which likely contributes to the persistent nature of prostatitis

[45]. *E. coli* is the most common cause of prostatitis in dogs and is responsible for the only reported case of bacterial prostatitis in a cat [6, 35].

Uropathogenic *E. coli*

The most common type of ExPEC is uropathogenic *E. coli* (UPEC), which adhere, multiply and persist in the urinary tract causing UTI. *E. coli* is the most prevalent bacterial species isolated from UTIs in both human beings and dogs. UPEC cause up to 95% of uncomplicated cystitis cases and over 90% of uncomplicated pyelonephritis cases in women [30, 46]. In dogs, *E. coli* is isolated from 44% of all UTIs and up to 47% of recurrent and persistent UTIs [47-49]. This high prevalence of *E. coli* UTI corresponds to a significant economic impact, with an estimated 1.6 billion dollars spent annually on community-acquired UTIs in human beings [50]. The tendency of clinicians to treat UTIs empirically with antimicrobial therapy has also contributed to increased antimicrobial-resistant *E. coli* UTIs [30, 51]. For these reasons, there has been a strong research focus on the epidemiology of UPEC, the various manifestations of clinical disease caused by UPEC, and strategies to control emerging antimicrobial resistance within UPEC.

Individual UTIs can be classified as simple or complicated, symptomatic or asymptomatic, community-acquired or nosocomial, and by location within the urinary tract. Simple UTIs occur when there is a temporary breach in the host's local defenses, allowing bacteria to ascend, adhere, multiply, and persist. In contrast,

complicated UTIs occur with an underlying structural, functional, or neurologic defect, involvement of the kidneys or reproductive tract, or a concurrent immunosuppressive disease or medication. Specific factors that predispose development of complicated UTIs in human beings and dogs include: alteration of normal flora with antimicrobial therapy, indwelling urinary catheters, concurrent urologic disease, spinal cord injury, diabetes mellitus, and immunosuppression from either disease (human immunodeficiency virus) or medication (glucocorticoids or chemotherapy) [6, 50]. Although most UTIs are symptomatic, some women and dogs remain asymptomatic, which may occur due to a lack of or decreased expression of adhesin virulence factors within the strain of UPEC [52]. Community-acquired UTIs develop outside of a hospital, and these infections tend to be symptomatic, causing the patient to seek medical attention quickly, and responsive to treatment. Nosocomial infections develop while a patient is hospitalized, may be recognized and treated later in the course of the infection, and tend to be more resistant to antimicrobial therapy. Location within the urinary tract is also important, with bladder infections (cystitis) often being uncomplicated and more responsive to treatment, whereas renal infection (pyelonephritis) or prostatic infections (prostatitis) cause more systemic illness and require longer courses of antimicrobial treatment.

Two etiological theories of bacterial UTI include the prevalence theory and the pathogenicity theory [53, 54]. The prevalence theory suggests that bacteria with high prevalence in the GI tract and perineum are likely to spread horizontally across urogenital mucosa and ascend the distal urethra. Supporting studies have shown that

the strain of UPEC colonizing the bladder of dogs with UTI was also the predominant strain of *E. coli* colonizing the GI tract [55, 56]. Although *E. coli* are highly prevalent in the GI flora, other bacteria with high prevalence, such as *Bacteroides,* are not frequently isolated from UTIs, suggesting other factors are likely involved. The pathogenicity theory suggests that only bacteria possessing virulence factors giving them enhanced ability to colonize and invade the urinary tract will succeed in traveling from the GI tract or urogenital flora to ascend the urinary tract and cause infection [53, 55].

Most strains of UPEC possess multiple virulence factors that contribute to development and severity of UTI. These urovirulence factors are rare in commensal GI *E. coli* strains [30]. Using PCR to test for urovirulence factors in fecal *E. coli* allows differentiation between benign commensal enteric organisms and potential pathogens of the urogenital tract. Urovirulence factors are identified and studied by comparing their presence in infected urine with their presence in the fecal flora of healthy volunteers and patients with UTI [7, 55]. These virulence factors can then be further refined by classifying them by location of UTI and severity of clinical signs with which they are associated. Identifying prevalence of these virulence factors in healthy fecal samples has also been used to search for reservoirs of UPEC, and dogs have been implicated as a potential source [56-58]. Specific virulence factors found in UPEC include: type 1 fimbriae, P fimbriae, S fimbriae, hemolysin, aerobactin, cytotoxic necrotizing factor, capsular polysaccharide antigens, and serum resistance.

The ability to adhere to uroepithelium is a crucial step in colonization and establishing a UTI, because it protects bacteria from mechanical removal during micturition. Fimbriae (from the Latin word for threads) also known as pili (from the Latin word for hairs) are adhesin virulence factors that are long thin extensions from bacteria facilitating attachment to epithelial lining, erythrocytes (hemoagglutination), leukocytes, platelets, spores, sperm, pollen, and latex beads [7]. Adherence is also, in part, determined by host factors [7]. *E. coli* isolates from recurrent UTIs did not adhere more strongly than other *E. coli* strains to control vaginal epithelial cells; however, vaginal cells from patients with recurrent UTIs had greater adherence capacity for standard *E. coli* strains than vaginal cells from control patients, suggesting altered host cells rather than increased bacterial virulence [7].

Type 1 fimbriae are mannose-sensitive adhesins, commonly expressed in both intestinal and extraintestinal *E. coli*. Receptors comprised of mannose residues are widespread throughout the body, and type 1 fimbriae play an important role in *E. coli* adherence within the oral cavity, intestinal cells, vaginal cells, and urinary tract. During UTIs, type 1 fimbriae help trigger host defenses by mediating adherence to phagocytes, stimulating phagocytosis and releasing toxic granules, and ultimately inducing destruction of *E. coli* [59]. *E. coli* with type 1 fimbriae strongly adhere to Tamm-Horsfall proteins within urine, inhibiting their binding to uroepithelial cells and promoting excretion of *E. coli* during micturition [59]. Type 1 fimbriae have also been associated with persistent infections, specifically in patients with long-term indwelling urinary catheters [60].

Expression of type 1 fimbriae is affected by environmental conditions and undergoes phase variation controlled at the transcriptional level [7]. Phase variation is a type of polyphenism, where one genotype can be manifest as several different phenotypes. Phase variation occurs when the promoter for type 1 fimbriae is on an invertible element of DNA. Specifically, two homologous regions of DNA form a loop containing the promoter, allowing two fimbrial genes to come into close contact. Invertase recognizes homologous sites and allows site-specific recombination and inversion of the looped DNA, switching the promoter's direction. When the promoter faces the structural genes, the *E. coli* are phase on and produce type 1 fimbriae, when the promoter is inverted the *E. coli* are phase off and do not produce type 1 fimbriae [61]. Type 1 fimbrial phase variation is regulated by three global regulators: leucine-responsive regulatory protein, integration host-factor, and histone-like proteins [62].

Research aimed at transcriptional manipulation and development of vaccines targeting type 1 fimbriae and their receptors is ongoing to minimize adherence of UPEC and establishment of UTI [62]. D-mannose is used as a nutraceutical to competitively bind FimH on type 1 fimbriae, blocking adherence of UPEC to mannose receptors on the uroepithelium. A benefit of d-mannose is that it can help control UTIs without risk of altering the GI or urogenital flora or inducing selection pressure.

P fimbriae adhere to glycolipids with specific Galactose(α1-4)Galactoseβ (Gal-Gal) moiety receptors on P-blood-group antigens expressed throughout the urinary tract and are considered mannose-resistant adhesins. While type 1 fimbriae

receptors predominate in the urinary bladder, receptors for P fimbriae predominate in the renal uroepithelium, and P fimbriae are thought to facilitate ascending infection to the ureters and kidney [63]. Binding of *E. coli* with P fimbriae to Gal-Gal receptors in the kidneys triggers inflammation, permits renal damage, and can result in pyelonephritis. While type 1 fimbriae are commonly found on commensal *E. coli* in the GI tract of healthy human beings, P fimbriae are more often found on *E. coli* strains in the GI tract of human beings with pyelonephritis than from fecal flora of healthy individuals [7]. For this reason, detection of P fimbriae is a common marker of urovirulence in *E. coli* isolates. The genes for P fimbriae are contained on a chromosomal multicistronic gene cluster named pyelonephritis-associated pili (*pap*) [7]. Various subunits are named Pap and designated with a letter, such as PapA, which is the major structural subunit, and PapG, which is the major adhesin that binds to Gal-Gal. *PapGIII* has been associated with presence of *cnf, hly*, and *sfa* and has been found on *E. coli* isolated from both dogs and human beings [64-66].

S fimbriae adhesins (sfa), also mannose-resistant adhesins, are named for their binding to sialosyl-oligosaccharide residues on the uroepithelium throughout the urinary tract and other extraintestinal sites [67]. S fimbriae mediate binding to human erythrocytes that express blood group antigens other than blood group P, but they also mediate binding to renal epithelium in dogs with pyelonephritis. In human beings S fimbriae are more frequently associated with neonatal meningitis and bacteremia than with UTI [7].

Alpha-hemolysin is a cytolytic toxin secreted by some strains of *E. coli* and is found with a high prevalence in cases of pyelonephritis in dogs and human beings [67-69]. The toxin damages neutrophils, monocytes, mast cells, basophils, lymphocytes, platelets, and renal epithelial cells [67]. Its production is partially regulated by local iron concentrations, with more hemolysin being produced in low iron conditions, thus making iron available for bacterial metabolism. In UTIs, α-hemolysin causes uroepithelial cell lysis, triggers inflammation, and impairs host defenses [7]. Alpha-hemolysin promotes neutrophil degranulation and release of leukotrienes, mast cells to release histamine, and impairs both phagocytosis and chemotaxis [7].

Aerobactin (aer) is part of a bacterial iron sequestration and transport system that makes iron available for bacterial growth in iron-poor environments. This is beneficial during infections when iron supplies are limited, in dilute urine, and in complement-depleted serum [70]. Iron is a critical nutrient utilized for oxygen transport and storage, DNA synthesis, electron transport, and peroxide metabolism [7]. During inflammation and infection, iron is sequestered within macrophages of the liver, spleen, and bone marrow, in an attempt to inhibit bacterial replication [71]. Aerobactin extracts Fe3+ (oxidized iron) from host iron-binding proteins and delivers it directly to bacterial iron centers [72]. The aerobactin system is commonly found in intestinal strains of *E. coli*, but prevalence is greatest in strains isolated from cases of pyelonephritis, cystitis, and bacteremia [7, 73]. One study identified *aer* in 78% of *E. coli* strains from the blood of human patients with urosepsis [70]. *Aer* are carried on

chromosomal DNA or plasmids. Chromosomal location is more commonly associated with noncompromised patients and concurrent uropathogenic virulence factors such as P fimbriae and hemolysin [70]. Plasmid location is associated with compromised patients and multiple antimicrobial resistance genes [70]. This suggests that antimicrobial therapy may select for both virulent and resistant strains leading to increasingly severe UTI and urosepsis in already compromised human beings [7, 70].

Cytotoxic necrotizing factor (cnf) is a bacterial protein toxin that causes direct uroepithelial cell injury, cytoskeletal rearrangement, and inflammation. It may contribute to progression of a UTI to febrile pyelonephritis or sepsis [69]. While *cnf* is commonly expressed in *E. coli* isolates from human beings with pyelonephritis, it has only recently been found in fecal strains from human beings [67, 74]. *Cnf* is found with high frequency in *E. coli* strains isolated from UTIs of both dogs and cats; however, *cnf* is also expressed in *E. coli* strains from dogs with diarrhea and from feces of healthy cats [75-77].

Capsules are polymers, usually composed of repeating carbohydrate subunits that coat the bacterial cell and protect it from host defense mechanisms. More than 80 antigenic types of capsular polysaccharides have been described in *E. coli* [67]. The K1 capsule is the most common capsule overall and in UPEC. The K1 capsule is often found concurrently with P fimbriae, while the K5 capsule is commonly found with hemolysin [67]. Capsules have a negative charge and are hydrophilic, which help prevent phagocytosis [67]. They block opsonization, interfere with activation of the complementation cascade, and contribute to serum resistance [67]. K1 capsules

are expressed more often in *E. coli* isolated from the urinary tract than from the GI tract. Within the urinary tract, K1 capsules are more prevalent in isolates from pyelonephritis than cystitis [7].

Serum Resistance refers to *E. coli's* ability to avoid being killed by serum complement. The property of serum resistance stems from a combination of factors, such as capsular polysaccharides, O-polysaccharide side chains, and various surface proteins that block the membrane attack complex of serum complement from binding to the outer membrane of *E. coli* [7]. *E. coli* isolates from urine of patients with cystitis and pyelonephritis are often serum resistant, while similar isolates from patients with asymptomatic bacteriuria and isolates from feces are most commonly serum sensitive [7].

Studies involving UPEC isolates from human beings and dogs with UTI have identified virulence characteristics that occur in both species, and dogs have been considered a possible reservoir for UPEC in human beings [57, 58, 66, 78]. The most frequently identified urovirulence factor genes in UPEC isolates from dogs with UTI, are *pap, sfa, hly, aer,* and *cnf* [69, 75, 79]. These same virulence factors play an important role in the pathogenesis of human UTI, and the prevalence of these virulence genes in *E. coli* isolates from urine of human beings and dogs with UTI are comparable [74, 80]. Surprisingly, two studies have shown that isolates from feces of healthy cats have these same urovirulence genes in high frequency, suggesting cats may be reservoirs of UPEC for dogs and human beings [74, 77].

Although evidence of similar virulence factor patterns between species is suggestive of transmission of clonal *E. coli* between species, this could also be from concurrent acquisition from a common source in the environment; therefore, multiplex PCR for virulence factor patterns is not useful for determining clonal relatedness between human and canine strains. Such comparisons require DNA analysis (fingerprinting or sequencing) that includes broader and more conserved portions of the bacterial genome. In the future, virotyping of *E. coli* isolates from canine UTI may be of prognostic value to help determine likelihood of ascension to pyelonephritis or persistence of recurrence of infection. Urovirulence factors may also provide new targets for treating or preventing UTI [81].

Antimicrobial Resistance

Prescription of antimicrobial therapy for treatment of pathogenic *E. coli* infections can be either empirical or based on susceptibility testing. Traditionally, physicians have chosen empirical therapy for UTIs because *E. coli* isolates have been predictably susceptible to common antimicrobial drug choices. Empirical therapy is economical, saving the cost of culture and susceptibility testing. The empirical antimicrobial drug of choice for UTIs in women has been trimethoprim-sulfamethoxazole, but antimicrobial resistance has become a problem, with 18% of human UTI isolates resistant to this drug [82, 83]. Resistance is also increasing to other antimicrobial agents commonly used for UTIs, such as ampicillin and

cephalothin, and for this reason, newer antimicrobial agents such as fluoroquinolones are being prescribed with increased frequency [46, 83]. As a consequence of this shift in antimicrobial choice, resistance to fluoroquinolones is now emerging, especially within UPEC [84-86]. Because of this emerging antimicrobial resistance, clinicians should no longer rely on empirical treatment to be effective, but instead should determine the susceptibility pattern for each isolate from patients with UTI and treat accordingly [51].

Antimicrobial resistance can either develop from a mutation within the bacterial genome or be acquired from other bacteria through genetic transfer. Certain mutations enhance survival when exposed to a specific antimicrobial agent. Clonal replication then creates an entire population of resistant bacteria. In the mixed flora of the GI tract, selection pressure occurs as a response to antimicrobial treatment, whereby susceptible bacteria die, while mutated, resistant clones survive and flourish. This increases the proportion of resistant bacteria within the GI tract that are shed into the environment and can potentially spread to the urinary tract.

General categories of mutations that have been identified and linked with resistance to antimicrobial agents include: decreased uptake or increased efflux of the drug, alteration or destruction of the drug, modification of the drug's target, or bypassing the drug's mechanism of action. Decreased uptake via alteration in porin expression and pumping the drug out of the bacterium via efflux mechanisms have a role in development of resistance to fluoroquinolones by *E. coli* and other *Enterobacteriaceae* [87]. Common examples of bacterial products that alter and

destroy antimicrobial drugs are beta-lactamase and extended spectrum beta-lactamases. Many strains of *E. coli* produce beta-lactamases, and *E. coli* strains able to produce extended-spectrum beta-lactamases are relatively common in healthcare settings [87]. Other enzymatic alterations that can render antimicrobials ineffective are acetylation of aminoglycosides and phosphorylation of chloramphenicol [88]. Modifying the drugs' target can occur by mutations in the penicillin-binding protein for beta-lactam drugs, the ribosome binding sites for tetracyclines and macrolides, or the DNA gyrase or topoisomerase IV for fluoroquinolones. By preventing binding of antimicrobial agents to their receptor and site of action, these mutations protect the bacteria from the actions of these drugs. Finally, some bacteria have developed ways to survive even when the antimicrobial agent reaches sufficient concentrations, is not inactivated, and successfully binds to its target. An example of this bypass mechanism is bacteria that have developed an alternate mechanism for folic acid synthesis in the face of trimethoprim-sulfamethoxazole, an antimicrobial drug that interferes with folic acid metabolism [89].

Once genetic mutations occur within bacteria and are selected for, mutated genetic material can be transferred between commensal and pathogenic bacteria within host species and in the environment. There are three main mechanisms of genetic transfer of antimicrobial resistance: transformation, transduction, and conjugation. These mechanisms have already been discussed at length in the section on acquisition of virulence, and mechanisms of horizontal genetic transfer are the same for virulence factors as for antimicrobial resistance genes. Conjugation is

responsible for the majority of genetic transfer of antimicrobial resistance, and it has been estimated that approximately 85% of therapy failure in treating *Enterobacteriaceae* with ampicillin, trimethoprim-sulfamethoxazole, gentamicin, and chloramphenicol is due to conjugation-induced resistance [90].

Concern regarding emerging antimicrobial resistance often centers on antimicrobial use in livestock and transfer of resistance through the food chain. Antimicrobial therapy is used in livestock for both therapeutic purposes and growth promotion. Bacteria within the GI tracts of livestock may develop mutations causing primary resistance, or livestock may acquire resistant bacteria from the environment, other animals on the farm, or human beings with whom they are in contact. Antimicrobial therapy creates selective pressure within the livestock's GI tract, increasing number and proportion of resistant bacteria [91]. This selective pressure disturbs the host's normal flora and colonization resistance, lowering the minimal infectious dose of further resistant or pathogenic bacteria, and increasing shedding dose and duration of resistant bacteria [92]. By horizontal gene transfer, resistant genes from commensal bacteria can be spread to zoonotic pathogenic bacteria within the GI tract. Resistant commensal and pathogenic bacteria can be shed into the environment and act as a fecal reservoir of resistant genes.

Ultimately, shedding resistant bacteria can result in indirect fecal-oral transmission to other animals on the farm, farmers, slaughterhouse workers, and consumers. Accepting sick animals for slaughter and entry into the food chain, and poor hygiene at slaughterhouses allowing GI contents to spill and contaminate meat

may increase the risk of propagating resistant bacteria. The US Department of Agriculture (USDA) regulations and surveillance have minimized contamination of meat, but ground beef contaminated with *E. coli* continues to reach consumers. Irradiation is highly effective at destroying bacteria within contaminated meat prior to commercial sales without altering flavor or appearance; however, until irradiation becomes routinely performed for ground beef, consumers should take precautions by cooking meat thoroughly and avoiding cross-contamination within the kitchen.

Fresh produce can be a source of resistant bacteria spread from animals to human beings. Produce can be contaminated with *E. coli* on the farm by fertilizing with contaminated animal feces, allowing wild pigs to defecate on crops, and by not providing sufficient restrooms, sanitary sewage disposal, or hand-washing facilities for farm workers resulting in crops contaminated with human fecal material. Other potential routes include contaminated irrigation water or aerosolized bacteria spread by wind, insects, or birds. Although recommendations can be made for proper human hygiene, fertilization, water testing, and fences to minimize wild animals, not all potential sources of contamination can be prevented. It is inevitable that contaminated produce will continue to enter the food supply. As with meat, precautions must be taken by consumers to minimize risk of ingesting contaminated produce, including: washing produce with warm water, scrubbing outer surfaces of produce, discarding outer leaves of lettuce and washing inner leaves carefully, good general kitchen hygiene, and caution to avoid cross-contamination of knives, cutting boards, countertops, and other kitchen areas [93].

A method to monitor and survey antimicrobial resistance in healthy populations of livestock, companion animals, and human communities is to routinely collect and test fecal samples from these hosts. Indicator bacteria, such as *E. coli* and *Enterococcus faecalis*, represent commensal GI bacteria within fecal flora. Antimicrobial use within a host results in increased resistance among these indicator bacteria, and susceptibility patterns give an objective prevalence of resistance. This information helps predict the risk of resistance within pathogenic or zoonotic bacteria within the same host, and the degree to which the host may be a reservoir for cross-species transmission [92]. A study of fecal indicator bacteria from healthy dogs found that multiple drug resistant (MDR) *E. coli* strains were rarely isolated from individually owned dogs compared with strains from kennel dogs [94]. In a separate study, each day of hospitalization in the intensive care unit (ICU) increased a dog's odds of being colonized by an ampicillin-resistant *E. coli* by a factor of 2.6, by an amoxicillin-clavulanate-resistant *E. coli* by a factor of 1.6, or by a strain of *E. coli* resistant to both ampicillin and cephalothin by a factor of 1.5 [95]. There was no significant association between concurrent beta-lactam treatment and development of antimicrobial resistant fecal *E. coli* found in this study; however, dogs that were treated with enrofloxacin were 25.6 times more likely to have fluoroquinolone-resistant fecal *E. coli* than dogs that did not receive any antimicrobial treatment [95]. Similarly, comparison of fecal indicator bacteria from different groups of cats has determined that hospitalized and cattery cats have more resistant indicator bacteria than individually owned cats [96]. These studies help to determine potential risk

factors for development of resistance within the GI tract and document that the flora of healthy dogs and cats can act as reservoirs of resistant genes. Surveys of antimicrobial resistance trends in indicator bacteria also provide objective measures to evaluate efforts to control resistance.

Although antimicrobial use in livestock has been implicated in increasing antimicrobial resistance in human beings, strategies to control this potential problem are widely debated. Swedish legislation now prohibits antimicrobial use for growth promotion and strictly regulates sales of antimicrobial drugs, which has led to a decrease in overall antimicrobial resistance in animals [97]. Similar legislation with strict control and limitation of antimicrobial use in animals has been proposed in the United States, and in 1995 the Food and Drug Administration (FDA) banned the use of enrofloxacin in poultry [98]. The American Veterinary Medical Association Legislative Advisory Committee has opposed further legislation in the United States because banning antimicrobial use for therapeutic purposes presents risk to animals' health and welfare without evidence of protecting or improving human health [99].

In addition to concern about misuse of antimicrobial agents in livestock, misuse of antimicrobial therapy in human beings and companion animals is also recognized. Physicians and small animal veterinarians often prescribe antimicrobial therapy indiscriminately and empirically and often select broad-spectrum rather than narrow-spectrum therapy. Incorrect dose, interval, and duration allow selection pressure and promotion of resistant bacteria. In veterinary medicine, off-label use of antimicrobial agents and over-the-counter purchase and use of antimicrobial agents

are additional methods that contribute to emerging antimicrobial resistance. Finally, pharmaceutical companies may contribute to emerging resistance via marketing pressure of new, expensive, broad-spectrum antimicrobial drugs for infections that may respond equally well to older, less-expensive, narrow spectrum drugs [100]. In light of this problem, many human and veterinary hospitals have developed antimicrobial use guidelines, and while some have seen positive effects including decreased overall antimicrobial therapy and decreased antimicrobial resistance, continued monitoring, analysis, and improvement of protocols will be essential in the future to control emerging resistance [101].

Cross-Species Bacterial Transmission

Although the role of livestock as a reservoir of antimicrobial resistant bacteria has been the subject of extensive research, the role of companion animals and human beings as potential reservoirs is less understood and overlooked [100, 102]. In the United States, 58.3% of all households own pets, with 36% owning dogs and 31% owning cats [103]. The human-animal bond is an important phenomenon, with 99% of dog and cat owners considering their pets to be part of the family [104]. With this relationship between pets and human beings comes the possibility and concern of sharing or transmitting resistant bacteria during daily activities including petting, feeding, grooming, sleeping, and disposal of feces.

Numerous studies have implicated dogs as reservoirs for ExPEC [57, 58, 66, 78, 105]. In the late 1980s, *E. coli* causing UTI in dogs were found to carry similar virulence factors such as P fimbriae, hemolysin, and the O4 and O6 somatic antigens, as UPEC in women, suggesting that canine ExPEC strains may pose an infectious threat to human beings [105]. PCR studies have shown that canine urinary and fecal *E. coli* can possess many urovirulence factors similar to UPEC in women, including *papGIII, sfa, cnf*, capsular antigens, and serum resistance [66]. Molecular fingerprinting using PFGE and RAPD has shown indistinguishable commonality between *E. coli* isolates from fecal and urinary specimens from dogs with UTIs and human urosepsis *E. coli* isolates [66]. A study specifically testing the canine reservoir hypothesis collected environmental canine fecal samples from an urban sidewalk and found *papGIII*-positive *E. coli* in 30%, and over half of these samples showed similar phylogenetic characteristics to human ExPEC strains based on RAPD testing [58]. This study concluded that even healthy dogs in urban environments can carry highly virulent strains of *E. coli* that may pose a human public health risk [58].

These collective data show that dogs and human beings have similar *E. coli* strains in the GI tract and causing UTI, and the potential for cross-species transmission does exist. While most studies implicate dogs as a source or reservoir for human beings, others have found that human beings are the more likely reservoir, and that dogs may acquire antimicrobial resistant and virulent bacteria from human beings [106]. One study comparing prevalence and resistance patterns of *E. coli* isolates from urine of women with cystitis and pyelonephritis with isolates from feces

of healthy women and dogs, found that canine isolates had the least amount of antimicrobial resistance, suggesting that dogs were not a likely reservoir for resistant genes [106]. It is possible that both human beings and dogs are potential reservoirs for pathogenic, virulent, and resistant *E. coli.*

Rather than direct transmission, dogs and human beings could acquire the same strain of bacteria from an environmental reservoir. These sources could include contaminated food, water, or exposure to wildlife. From these potential reservoirs, fomites within the kitchen, household, and yard can become contaminated, providing additional opportunity for transmission to dogs and human beings. Furthermore, conjugation can occur within the environment. Some plasmids and other mobile genetic elements are not highly specific to one bacterial genera or species, thus conjugation can cross bacterial species, carrying antimicrobial resistance and virulence factors to otherwise benign bacteria within the environment.

With many potential reservoirs including environmental sources, animals, and human beings, there is ample opportunity for transmission of resistant and virulent bacteria. Improving our understanding of common routes of transmission and risk factors is warranted. The most common route of bacterial transmission of *E. coli* is fecal-oral transmission. Fecal-oral transmission can occur by accidental ingestion of feces during bathing, grooming, sexual intercourse, diaper changing, or disposal of feces from pets or livestock. Human contact with animal feces has been recognized as a risk factor for infection with *E. coli* 0157 and other enteric pathogenic bacteria [107]. Children may be at greater risk, because they have increased direct contact

with pets, they are physically closer to floors and carpets that may be contaminated by pet feces, and they have less stringent hygiene habits than adults [100, 108]. Good personal hygiene minimizes risk of fecal-oral transmission of bacteria and include frequent hand-washing and discouraging hand-to-mouth activities such as smoking, eating, drinking, or using pacifiers in the presence of animals [109].

Other routes of transmission include indirect fecal-oral transmission, sexual activity, aerosolization, and cutaneous transmission. Indirect fecal-oral transmission, as discussed before with regard to ingesting fecal-contaminated meat and produce, is a common cause of outbreaks of gastroenteritis jeopardizing public health. Direct contact through sexual activity has been associated with concurrent fecal colonization of the same strain of *E. coli* implying transmission between sexually active heterosexual and homosexual partners [110-112]. Aerosolization of urine or fecal material followed by inhalation or ingestion is also a potential route of transmission of UPEC. Other routes of bacterial transmission between species are through skin contact, bite wounds, and from bites from ticks or other insects. These routes can be significant for bacteria such as *Staphylococcus aureus (S. aureus), Bartonella*, and rickettsial organisms such as *Rickettsia rickettsii*, respectively, but they are not significant routes of transmission for *E. coli* [113-115].

Although the concept of cross-species transmission is a major public health concern, it is very difficult to prove transmission of bacteria between two individuals and to determine direction of transmission. Comparing genetic makeup of bacterial strains from two individuals and finding a high percentage of clonality (genetic

relatedness) is supportive of transmission, especially if the individuals have opportunity for bacterial sharing. DNA fingerprinting with PFGE is one method to compare clonal relatedness, through % PFGE profile similarity and via dendrograms. DNA sequencing can also be used to compare bacterial strains and to confirm two strains of *E. coli* are related. Because of increased sensitivity, DNA sequencing may replace fingerprinting for comparing relatedness of bacterial strains for transmission studies in the future.

In observational studies, directionality of transmission can only be speculated through understanding the epidemiology of bacteria and behavior of populations being studied. Comparing antimicrobial drug usage and fecal resistance patterns between species is a useful way to hypothesize directionality of cross-species transmission. A study in western Uganda isolated fecal *E. coli* from chimpanzees, from human beings employed in chimpanzee-directed research and tourism, and from local villagers with no chimpanzee contact [116]. Results confirmed that chimpanzees had fecal *E. coli* genetically more similar to those of human beings who worked in chimpanzee-directed research and tourism than local villagers, suggesting sharing of bacteria via either direct contact or indirect contact through ecological overlap and contact with environmental reservoirs [116]. Although chimpanzees in this national park had never been treated with antimicrobial therapy, 4.4% of chimpanzee *E. coli* isolates were resistant to at least one locally-available antimicrobial drug, suggesting that cross-species transmission, directly or indirectly, was occurring from human beings to chimpanzees [116].

Similarly, genetically related *S. aureus* has been identified from domestic animals and human beings within households and veterinary clinics, and transmission was believed to have occurred [113, 117, 118]. Some argue that pets may be the underlying reservoir of methicillin-resistant *S. aureus* (MRSA), infecting their human housemates; however, since cases of MRSA have a higher prevalence in people who are hospitalized or work in healthcare professions than in the general canine population, these bacteria are more likely transmitted from infected human beings to their pets, rather than from pets to owners [100].

There are only sporadic cases that document sharing of *E. coli* clones between companion animals and human beings within a household, supporting potential cross-species transmission [119-121]. A case report documented a single EPEC *E. coli* clone from the feces of a 3-year-old healthy child and a 3-month-old diarrheic dog in her household [108]. The source and direction of transmission of EPEC in this report remained unknown [108].

A separate case report documented extensive sharing of virulent *E. coli* strains within a household between a heterosexual couple and their cat, based on biweekly fecal, urine, and vaginal cultures over a 4-month period and again a year later [119]. PFGE showed that the strain isolated from the woman's UTI was found repeatedly in the man and cat, and PCR showed that presence of the virulence factors *pap, hly,* and *cnf* was associated with persistent, multiple-site, and multiple-host isolation [119]. Based on this finding, it was speculated that these virulence factors may promote colonization and transmission in addition to virulence [119].

A similar longitudinal study followed a woman with a UTI along with 4 household family members and their pet dog, isolating fecal and urine *E. coli* and comparing them with PFGE and RAPD [120]. Three clones were found extensively among multiple human beings and the dog, including the woman's UPEC clone which had the highest overall prevalence and persistence [120]. Both clones isolated from the pet dog were also isolated from multiple human beings in the household [120]. Epidemiologic study of temporal patterns of isolation of these clones and reported physical contact of the family members led to the conclusion that nonsexual host-to-host transmission of *E. coli* was more likely than acquisition of clonal *E. coli* from an environmental common point source [120]. The woman's UPEC clone was not isolated from her again after fluoroquinolone therapy; however it continued to be isolated in fecal samples from her family members and dog, supporting the theory that housemates and pets can be reservoirs for UPEC [120].

A cross-sectional study evaluated fecal samples from human beings and pets from 5 families and used electrophoresis to determine the extent of strain sharing within households. Eleven percent of strains isolated from a household were shared by two or more family members, with 1 strain found in a mother, father, and dog, and a second strain isolated from a son and his cat [121].

Although these case reports and small cross-sectional study have documented sharing of clonal *E. coli* between human beings and companion animals, prevalence of *E. coli* sharing among healthy dogs and human beings within households remains unknown. The objective of the current study was to determine the extent to which

clonally similar *E. coli* isolates are detected in feces from in healthy dogs and human beings living in the same household. Fecal *E. coli* isolates were compared by genetic fingerprints, presence of virulence factors, and antimicrobial susceptibility patterns. An epidemiologic analysis was performed to identify potential risk factors for cross-species sharing of fecal *E. coli.* Results from this study will help veterinarians, medical doctors, and public health authorities make appropriate husbandry and hygiene recommendations to pet owners to minimize cross-species bacterial sharing and transmission.

Materials and Methods

Study Design and Participants

This was a cross-sectional study evaluating fecal *E. coli* isolates collected from participants at one point in time. Study participants included 61 pairs of human beings and their dogs. Participants were recruited from the faculty, staff, and student body of the University of Tennessee, College of Veterinary Medicine (UTCVM) and the Department of Medical Genetics. Recruitment was done by word of mouth, an internet posting, and Email notifications. Inclusion criteria specified that all human participants must be generally healthy and over 18 years of age to participate. Inclusion criteria specified that the dog must be owned by and have lived in the same household as the human participant for at least 6 months, and only one dog and human per household could participate. Exclusion criteria specified that no human or canine participants could have received oral, topical, or parenteral antimicrobial therapy within two weeks of enrollment.

A control population was recruited and included 34 human beings who did not own a dog or cat, nor had more than 1-hour contact per week with a dog. No restrictions were made on contact with other species of non-human animals. Controls were recruited from the faculty, staff, and student body of the UTCVM, the Department of Medical Genetics at the University of Tennessee, and employees from a local business, GI Associates. Recruitment was done by word of mouth, an internet posting, and Email notifications. Inclusion criteria specified that all human

participants must be healthy and at least 18 years of age. Exclusion criteria specified that no controls could have received oral, topical, or parenteral antimicrobial therapy within two weeks of enrollment.

Compliance

This study was granted approval by both the Institutional Review Board (#6910B) and Institutional Animal Care and Use Committee (#1492) of the University of Tennessee to ensure appropriate treatment of all participants. All human participants were informed of potential risks and benefits of participation as well as confidentiality of participation. Each human participant signed a standardized informed consent form. All human participants received a $20 stipend once participation was complete.

Sample Size Calculation

A sample size of 61 pairs of dogs and owners was calculated based on a McNemar's Chi-Square statistical analysis for paired samples with alpha 0.05, effect size 0.30, and 98% power. The test was two-tailed, which means that an effect in either direction was considered important; thus 98% of studies performed would be expected to yield a significant effect, rejecting the null hypothesis that *E. coli* isolates from dogs and their owners would be equally likely to have a particular variable. Sample size for the control population was calculated based on a Chi-Square analysis

for independent samples with alpha 0.05, effect size 0.30, and power 85% and was determined to require 34 control participants. At the time this study was designed, data were not available for % PFGE similarity of *E. coli* between dogs and owners within households from which to extrapolate an appropriate effect size. Previous prevalence studies have shown that between 0.9-31% of fecal *E. coli* from dogs and owners have virulence factors to be tested in this study; however no previous studies compared virulence factors between isolates from dogs and owners in the same households [74, 122]. Similarly, no data was known for susceptibility results between dogs and owners. Therefore, a conservative effect size was chosen at 0.30, which is Cohen's convention for a medium effect size when using Chi-Square analyses [123].

Sample Collection

Participants were given a packet of materials for the study including confidential identification numbers for each participant, a standard questionnaire, and instructions for sample collection, gloves, disposable plastic bag, a bubble envelope, and sterile culture swabs. A key that associated the identification number of each fecal sample with the appropriate dog or human source was kept strictly confidential in a locked cabinet only accessible to the investigators and was destroyed at the end of this study in August 2008. The investigator performing the laboratory work was

blinded from this information, preventing any potential bias of results due to knowledge of which samples were paired.

Samples collected for this study included one fecal BBL CultureSwab Plus Collection and Transport System (Becton Dickinson and Company, Sparks, MD) from each human and dog participant. Paired samples from dogs and owners were collected from the same 12-hour period and submitted together. Human being participants were asked to submit a swab of their own feces. This was collected by defecating in their home toilet and inserting the culture swab deep into the fecal material to generously soil the swab. The soiled swab was then returned to the transport medium tube. Toilets should not have been cleaned or disinfected immediately prior to sample collection, nor should a continuous-release sanitizer have been used within the toilet bowl. Owners were also asked to bring in a swab of their dog's feces. This sample was taken from an outdoor site shortly after the dog defecated, and the swab was to be inserted into the center of the fecal material to allow for generous soiling of the swab. All samples were collected within 24 hours of when they were submitted for the study and refrigerated if collected more than an hour before submission. They were labeled with the confidential identification numbers provided and stored in a disposable plastic bag inside a bubble envelope until submission.

Each human participant answered a questionnaire. The questionnaire was developed in a focus group of dog owners and was piloted prior to use in the study. Questions were designed to identify demographics, medical and medication histories,

and relationship between dog and owner. The questionnaire aimed to determine history of infection or immunosuppression, antimicrobial usage, and extent of dog-owner interaction. The questionnaire is included as Appendix A.

Questionnaire Analysis

Descriptive statistics were used to summarize demographic data, medical history, medication history, and behaviors between dogs and owners. An independent t-test was used to compare age of owners and controls. Chi-Square analysis was used to compare gender distribution between owners and controls. Chi-Square analyses were used to compare history of antimicrobial therapy in participants prior to enrollment in the study. Chi-Square analysis was used to compare history of UTI with gender of participants. Chi-Square analysis was also used to compare owners' reported disposal of dog's fecal material with gender and with hours spent at dog parks per week.

***E. coli* Isolation**

Fecal swabs were processed within 6 hours of submission, and original swabs were discarded immediately. Fecal samples were diluted in 3ml of 0.1% Peptone (Fisher Scientific, Fair Lawn, NJ), vortexed, and 20µl of diluted feces was streaked for isolation onto two separate plates containing: BBL Eosin Methylene Blue Agar Modified (EMB) (Becton Dickinson and Company, Sparks, MD) with added 0.1g/L

MUG fluorescence crystals (HACH Company, Loveland, CO), and Bacto EC Medium with MUG (Becton Dickinson and Company, Sparks, MD). Eosin and methylene blue are toxic to Gram-positive bacteria, but Gram-negative bacteria are protected by their LPS layer and grow well; thus, this medium selects for Gram-negative bacteria. Bacto EC Medium with MUG (Becton Dickinson and Company, Sparks, MD) contains bile salts that are toxic to Gram-positive bacteria, allowing selection for Gram-negative bacteria.

Four-methylumbelliferyl-β-D-glucuronide (MUG) was used as a differential agent to identify *E. coli* colonies. Most strains of *E. coli* possess the enzyme β-glucuronidase, which hydrolyzes MUG to 4-methylumbelliferone, causing colonies to fluoresce under 366nm ultraviolet light. An exception is *E. coli* 0157 which does not possess β-glucuronidase and does not fluoresce; thus florescence was a way to select against *E. coli* 0157 strains. Although rare, other bacteria can produce β-glucuronidase, including strains of *Shigella*, *Salmonella*, and *Yersinia* [124, 125].

Plates were incubated at 44C for 18-24 hours; incubation at this temperature provides additional selectivity for *E. coli*. Plates were examined under 366nm ultraviolet light for blue-fluorescing colonies. Not all green metallic colonies that were later proven to be *E. coli* on EMB plates fluoresced under ultraviolet light; however all colonies that were later proven to be *E. coli* on EC plates fluoresced under ultraviolet light. Presumptive colonies were counted and their appearance

described. Three presumptive *E. coli* colonies were selected from each sample for further testing.

Presumptive *E. coli* colonies were each separately inoculated into 5 ml BBL Brain Heart Infusion Broth, Modified (BHI) (Becton Dickinson and Company, Sparks, MD), vortexed, and incubated at 44C for 18-24 hours. After incubation, samples were vortexed, and 20μl was streaked for isolation onto an EC plate. Plates were incubated at 44C for 18-24 hours and examined under an ultraviolet light for blue-fluorescing colonies that were presumed to be *E. coli*.

API-20E biochemical test kits (bioMerieux, Inc., Durham, NC) were used to confirm *E. coli* colonies. This test utilizes 20 biochemical reactions in parallel. Isolated colonies presumed to be pure *E. coli* were mixed with 5 ml sterile distilled water and tested with a turbidometer (Oxoid, Hampshire, England), which was calibrated daily, until consistent with the 0.5 McFarland turbidity standard. This dilution was used to inoculate API-20E biochemical test kits according to the manufacturer's instructions followed by incubation, addition of reagents, and interpretation of individual test reactions. Results were entered into the bioMerieux API website (http://apiweb.biomerieux.com) to identify the genus and species and confirm each isolate as *E. coli*. Quality control was performed for the API-20E test strips using *E. coli* reference ATCC 25922.

Due to difficulty interpreting citrate results on the API-20E test, slants containing Simmons Citrate Agar (Becton Dickinson and Company, Sparks, MD) were also inoculated and incubated for each isolate. Slants were inoculated by

stabbing the butt of the media and then inoculating the surface with a serpentine pattern. Slants were incubated at 44C for 3 days. Slants that turned blue were considered positive, demonstrating that the bacteria used citrate as the sole carbon source for growth. Slants that remained green were considered negative and consistent with *E. coli*.

All confirmed pure *E. coli* colonies were grown in 5ml BHI broth at 44C overnight and stored long-term in a refrigerator and freezer. Three refrigerator vials, each containing 1ml vortexed BHI mixture and 1ml Difco Stock Culture Agar (Becton Dickinson and Company, Sparks, MD), were stored at 4C. Two freezer vials, each containing 1ml vortexed BHI mixture and 1ml of 20% Glycerol (Fisher Scientific, Fair Lawn, NJ), were stored in two separate freezers at -85C. Glycerol protects *E. coli* from freezer injury and crystallization.

Antimicrobial Susceptibility Testing

E. coli isolates were tested for susceptibility to 17 antimicrobial agents. Sixteen antimicrobial agents were chosen because enteric bacteria of human beings and animals are routinely monitored through passive surveillance by the National Antimicrobial Resistance Monitoring System (NARMS) for susceptibility to these antimicrobial agents. These antimicrobial agents include amikacin, amoxicillin-clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, imipenem, kanamycin, nalidixic acid,

streptomycin, tetracycline, and trimethoprim-sulfamethoxazole. Although an additional two antimicrobial agents, apramycin and sulfamethoxazole, are also considered standard antimicrobial drugs monitored by NARMS, susceptibility test discs were not commercially available, and these antimicrobial agents were excluded from this study. Alternatively, susceptibility was determined for cefpodoxime, a third generation cephalosporin that had recently been introduced to the veterinary market.

Susceptibility testing of *E. coli* isolates was performed with the Kirby-Bauer disk diffusion method, in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI), formerly known as the National Committee for Clinical Laboratory Standards (NCCLS) [126]. Approximately 0.2ml of *E. coli* from a 2-6-hr-old culture in BHI broth was diluted in 5ml sterile distilled water until its turbidity was consistent with the 0.5 McFarland standard, using a turbidometer to ensure accuracy. A cotton swab was used to inoculate two 150mm Mueller Hinton II agar plates per isolate. A BBL Sensi-Disc 12-Place Dispenser (Becton Dickinson and Company, Sparks, MD) was used to apply antimicrobial discs onto inoculated plates. Since 17 antimicrobials were tested, 9 discs were dispensed onto one plate, and 8 discs were dispensed onto the second plate for each isolate. Standard antimicrobial disc potencies were used: amikacin (30μg), amoxicillin-clavulanic acid (30μg), ampicillin (10μg), cefoxitin (30μg), ceftiofur (30μg), cefpodoxime (10μg), ceftriaxone (30μg), cephalothin (30μg), chloramphenicol (30μg), ciprofloxacin (5μg), gentamicin (10μg), imipenem (10μg), kanamycin (30μg), nalidixic acid (30μg),

streptomycin (10μg), tetracycline (30μg), and trimethoprim-sulfamethoxazole (30μg) (BD BBL Sensi-Disc, Becton Dickinson and Company, Sparks, MD). Plates were incubated inverted at 35C for 18-24 hours.

An electronic digital caliper (Control Company *model 14-648-17*, Friendswood, TX) was used to measure zones of inhibition for each antimicrobial agent. Diameter measurements were taken of zones of complete inhibition, with the exception of trimethoprim-sulfamethoxazole. Antagonists to trimethoprim-sulfamethoxazole in the Mueller Hinton medium may allow slight growth of *E. coli*, therefore CLSI recommends measuring trimethoprim-sulfamethoxazole zones at 80% inhibition, or where there is an obvious margin [126]. Zones were measured to the hundredth of a millimeter, but were rounded to the nearest millimeter for interpretation, in accordance with CLSI recommendations. Diameters of zones of inhibition were interpreted in accordance with current guidelines of CLSI and NCCLS for *E. coli* isolated from human beings and animals, allowing classification of each *E. coli* isolate as susceptible, intermediate or resistant to each of the 17 antimicrobial agents, as illustrated in Appendix B [127, 128]. When interpretive recommendations were not available from CLSI for canine isolates, recommendations were followed for *E. coli* isolates from other animal species (ceftiofur) or human beings (cefoxitin, ceftriaxone, ciprofloxacin, nalidixic acid, and streptomycin) [127, 128]. Plates that had one or more intermediate or resistant zones of inhibition were incubated an additional 48 hours and interpreted daily; however, in all of these cases, zones of inhibition did not change enough daily to alter the original determination of

intermediate or resistant. Quality control was performed prior to the study on each package of antimicrobial discs using *E. coli* American Type Culture Collection (ATCC) 25922 and *Staphylococcus aureus* ATCC 25923 to ensure quality of the antimicrobial discs; acceptable quality control ranges and results appear in Appendix C [127, 128].

Susceptibility was reported as a percentage of participants with isolates that were susceptible, intermediate, and resistant to each antimicrobial agent. Susceptibility was also reported as a percentage of total isolates from each participant that was susceptible, intermediate, and resistant to each antimicrobial agent. These results differed because 3 isolates were taken from each participant and frequently had different susceptibility patterns. A participant was considered resistant to an antimicrobial if any of their 3 fecal isolates was resistant to that antimicrobial agent. The Wilcoxon Signed Ranks test was performed to compare susceptibility between paired dogs and owners, using susceptible, intermediate, and resistant as ordinal data. Data were collapsed, so that intermediate results were considered susceptible, and a McNemar Chi-Square test was used to compare paired dog and owner susceptibility results. Similarly, data were collapsed so that intermediate results were considered resistant, and a McNemar Chi-Square test was used to compare paired dog and owner results. Mann-Whitney tests were used to compare susceptibility results, as ordinal data, between independent dogs and controls, and between owners and controls. Mann-Whitney and Chi-Square analyses were used to compare susceptibility results to variables such as history of antimicrobial therapy. A commercial statistical

software program was used to compute all statistics (SPSS 15.0 for Windows, SPSS Inc, Chicago, IL). The original threshold for significance was set at $p \leq 0.05$. The Bonferroni correction for multiple comparisons (17 antimicrobial agents) was applied, lowering the threshold for significance to $p \leq 0.05/17$ or $p \leq 0.003$, and decreasing the power of the study to 84%.

Pulse Field Gel Electrophoresis

Pulse field gel electrophoresis was performed on 2 *E. coli* isolates from each participant. Frozen *E. coli* isolates were grown on Columbia Agar with 5% Sheep Blood (Fisher Scientific, Fair Lawn, NJ) and MacConkey Agar plates (Fisher Scientific, Fair Lawn, NJ) overnight at 35C. Blood agar is a non-selective medium, and *E. coli* colonies appear grey and moist. *E. coli* α-hemolysin produces large, clear zones of hemolysis around colonies on blood agar plates, and phenotypic presence of hemolysis was recorded. MacConkey agar contains bile salts and crystal violet, which are selective against Gram-positive bacteria, as well as a pH indicator that responds to the acidic environment created by lactose fermentation. Thus using MacConkey agar differentiates lactose-fermenting bacteria such as *E. coli*, because the medium turns red, and the colonies are pink. Lactose negative colonies were streaked onto Triple Sugar Iron (TSI) Agar slants, Urea agar slants, and used to inoculate Motility Indole Ornithine medium (Becton Dickinson and Company, Sparks, MD). Isolates biochemically consistent with *E. coli* were acidic throughout

the TSI slant with hydrogen sulfide gas production, were negative for urease production, had positive motility, were positive for indole production, and were positive for ornithine decarboxylase. Any isolate that did not fit these characteristics was confirmed to be *E. coli* with an API-20E biochemical test kit (bioMerieux, Inc., Durham, NC). A Gram stain was performed on a colony from each blood agar plate to confirm the presence of a pure culture of small gram-negative coccobacilli. One colony was then transferred from each blood agar plate to Luria-Bertani broth (LB) (Fisher Scientific, Fair Lawn, NJ) and incubated overnight at 35C. Pure *E. coli* cultures in overnight LB broth were used for PFGE.

Cultures were concentrated and washed in phosphate buffered saline (PBS) (Fisher Scientific, Fair Lawn, NJ), to remove any potential inhibitors of enzymes required for PFGE. From each 10ml LB broth culture, 2ml were dispensed into each of 4 microcentrifuge tubes and centrifuged at 4C for 10 minutes at 8000 x gravity (G). The supernatant was removed from all 4 tubes. The first pellet was suspended in 1ml PBS, and this mixture was used to suspend the second pellet, then third, and fourth pellets, so that all 4 pellets from the same isolate were combined, concentrating the culture. This culture was centrifuged at 4C for 10 minutes at 8000G. The supernatant was removed, and 1ml of PBS was added to suspend and continue washing the pellet, and the sample was again centrifuged at 4C for 10 minutes at 8000G. Finally, the supernatant was removed, and 0.8ml PBS was added to suspend the pellet.

Washed *E. coli* cultures were used to make plugs for PFGE. First 25µl protease K (Qiagen Inc., Valencia, CA) was added to each *E. coli* sample. 2% agarose gel was prepared by combining 1g pulse-field certified agarose (BioRad Laboratories, Richmond, CA) with 50ml 1x Tris-EDTA buffer (Fisher Scientific, Fair Lawn, NJ), and this mixture was tempered to 55C before use. Then 0.5ml of the *E. coli* sample was mixed with 0.5ml of 2% agarose gel in 1x Tris-EDTA buffer (Fisher Scientific, Fair Lawn, NJ). Quickly, 100µl of the *E. coli* agarose mixture was dispensed into each plug mold. Two plugs were made per *E. coli* isolate. Plugs were refrigerated for 10-15 minutes to allow solidification.

Lysis buffer was made fresh before each use and contained 2.25ml 1M Tris HCl (Fisher Scientific, Fair Lawn, NJ), 4.5ml 0.5M EDTA (Fisher Scientific, Fair Lawn, NJ), 4.5ml 10% sarcosine (Teknova, Hollister, CA), 225µl protease K (Qiagen Inc., Valencia, CA), and 18.5ml sterile distilled water. Protease K is responsible for breaking down the bacterial cell wall. The solidified plug and 5ml of lysis buffer were dispensed into a sterile labeled 50ml tube. The plug and lysis solution were incubated in a 54C water bath overnight (IsoTemp 120 by Fisher Scientific, Fair Lawn, NJ). Before leaving for the day, an additional 15µl protease K (Qiagen Inc., Valencia, CA), was added to each tube containing plugs and lysis buffer.

After incubation in lysis buffer, plugs were washed to remove any remaining lysis buffer and the majority of cellular debris so only DNA remained. Lysis buffer was removed from each 50ml tube, 5ml warmed sterile distilled water was added to

the plugs, and the tube was placed in the 54C water bath for 15 minutes. The water was removed, and 5ml warmed 1x Tris-EDTA buffer (Fisher Scientific, Fair Lawn, NJ) was added, and the tube was placed in the 54C water bath for 15 minutes. This 1x Tris-EDTA buffer (Fisher Scientific, Fair Lawn, NJ) washing was repeated an additional two times. Then 1x Tris-EDTA buffer (Fisher Scientific, Fair Lawn, NJ) was removed from each tube and 5ml room-temperature 1x Tris-EDTA buffer (Fisher Scientific, Fair Lawn, NJ) was added, completing the plug washing procedure. The 50ml tubes containing plugs with 1x Tris-EDTA buffer (Fisher Scientific, Fair Lawn, NJ) could be stored at 4C for up to 2 weeks before restriction digestion.

Prior to restriction, plugs were removed from the 1x Tris-EDTA buffer solution (Fisher Scientific, Fair Lawn, NJ), dried, and halved with a straightedge razor blade. Both plugs were transferred into a 1.5ml microcentrifuge tube containing 200µl pre-restriction buffer and incubated at room temperature for 5 minutes. Pre-restriction buffer contained 20µl 10x buffer D (Promega, Madison, WI) and 180µl Nuclease-Free Water (Ambion, Inc., Austin, TX). After 5 minutes of pre-restriction, plugs were removed, dried, placed in a restriction enzyme solution, and stored in a 37C water bath (Isotemp 202S, Fisher Scientific, Fair Lawn, NJ) for at least 8 hours or overnight. Restriction enzyme solution contained 40µl 10x buffer D (Promega, Madison, WI), 5µl BSA (10mg/ml) (Promega, Madison, WI), 5µl restriction endonuclease XbaI 12u/µl (Promega, Madison, WI), and 351µl Nuclease-Free Water (Ambion, Inc., Austin, TX).

Electrophoresis gels (1%) were made with 400ml 0.5x tris-borate-EDTA (Fisher Scientific, Fair Lawn, NJ) and 4g PFGE agarose (BioRad Laboratories, Richmond, CA). Plugs were removed from restriction enzyme buffer and placed in 200µl 0.5x tris-borate-EDTA (Fisher Scientific, Fair Lawn, NJ) for 5 minutes at room temperature before being loaded into the gel wells. Duplicate plugs of each isolate were run on each gel. Plugs from paired dog and human isolates were run on the same gel to minimize effects of gel-to-gel variability on interpretation of similarity between paired isolates. Low range PFG markers (New England BioLabs Inc., Ipswich, MA), comprised of a mixture of lambda DNA-Hind III fragments and lambda concatemers embedded in 1% LMP agarose, were loaded into wells 1, 8, and 15 of each gel. Plugs were sealed into the gel with additional 1% PFGE agarose (BioRad Laboratories, Richmond, CA).

Gels were electrophoresed in 3L of 0.5x tris-borate-EDTA buffer solution (Fisher Scientific, Fair Lawn, NJ). Electrophoresis was performed with the CHEF-Mapper (BioRad Laboratories, Richmond, CA) with initial switch time 6s, final switch time 40s, gradient 6V/cm, included angle 120°, linear ramping, a run time of 24h, and cooling temperature of 14C. Gels were stained with of 400ml sterile distilled water and 1µl ethidium bromide (10mg/ml) for 30 minutes with shaking, and destained with sterile distilled water for 75 minutes with shaking. Gels were visualized by photographing them with a GelDocXR camera using Quantity One

Software 4.6.3 (BioRad Laboratories, Richmond, CA), and saved as tagged image file format (TIFF) images.

FPQuest Software Version 4.5 (BioRad Laboratories, Richmond, CA) was used to analyze bands from electrophoresis gels, as well as to compare, correlate, and generate similarity indices (cluster analysis) among *E. coli* isolates. A matrix of coefficients was generated, and a correlation analysis was conducted by using the unweighted-pair group method with averaging (UPGMA) based on Dice similarity coefficients and the clustering algorithm to create dendrograms. One percent optimization and 2% position tolerance were used. Genomic fingerprints were compared between each dog-owner pair using similarity matrices.

When comparing *E. coli* isolates by PFGE fingerprint similarity, a variety of definitions have been used for clonal sharing, including % PFGE similarity based on similarity matrices and comparing number of bands in common on DNA fingerprint [84, 120, 129]. To remain most consistent with the current literature in this area of research, *E. coli* isolates with >94% PFGE similarly were considered shared strains or clones. In this study, groups were defined as a cluster of 4 or more *E. coli* isolates with >90% PFGE profile similarity.

Chi-Square analysis was used to compare percent of participants sharing clones with other participants, and demographics of those participants sharing clones. Total number of potential clone-sharing pairs was calculated as the overall number of pair-wise combinations of participants, n(n-1)/2, where n is the total number of participants [129]. The number of potential within-household sharing pairs was the

number of dog-owner pairs enrolled in the study. The number of potential across-household sharing pairs was the difference between the two values. Chi-Square analysis was used to compare within-household versus across-household sharing. Chi-Square, Mann-Whitney U, and Kruskal-Wallis analyses were used to compare demographics and dog-owner behaviors to within-household % PFGE similarity, and the threshold for significance was $p \leq 0.05$.

Virulence Factor Multiplex PCR

Multiplex PCR to test for virulence factors was performed on all 3 *E. coli* isolates per participant. Frozen *E. coli* isolates were grown on Columbia Agar with 5% Sheep Blood (Becton Dickinson and Company, Sparks, MD) overnight at 35C. One colony was transferred from each blood agar plate to LB broth (Fisher Scientific, Fair Lawn, NJ) and incubated overnight at 35C. To remove inhibitors, 1ml of each culture was centrifuged for 1 minute at 14,000rpm at 20C, the supernatant was discarded, and the pellet was suspended in 200ul sterile Nuclease-Free Water (Ambion, Austin, TX). A boiled preparation was used for DNA extraction. Suspensions were boiled at 100C for 5 minutes, vortexed to mechanically disrupt the cell membrane, and then boiled for another 5 minutes. After boiling, suspensions were centrifuged for 1 minute at 14,000rpm at 20C. DNA lysate supernatant was transferred to 2ml storage vials and stored at -80C for future use for PCR.

Spectrophotometry was used to determine nucleic acid concentration and quality in DNA lysate from each *E. coli* isolate (NanoDrop ND-1000, NanoDrop Technologies Inc, Wilmington, DE). This ensured enough DNA was included in each sample to provide a positive PCR result if the isolate was truly positive. Two microliters of DNA lysate from each *E. coli* isolate were sampled. An arbitrary cutoff for minimum DNA concentration was set at 400ng/µl. Established guidelines for DNA purity use the ratio of absorption of UV light at 260nm to absorption at 280nm (260:280), based on the knowledge that DNA absorbs light at 260nm and proteins absorbs light at 280nm. Pure DNA samples have a 260:280 ratio of 1.8; a ratio considerably lower than 1.8 suggests presence of proteins, phenols, or other contaminants that absorb light near 280nm [130].

Multiplex PCR was developed to determine presence or absence of 4 urovirulence factors: cytotoxic necrotizing factor (*cnf*), hemolysin (*hlyD*), S fimbriae adhesin (*sfa*), and pilus associated with pyelonephritis G allele III (*papGIII*). Table 1 lists the primer sequences and base pair sizes of each of the four virulence factors tested in this multiplex PCR assay (all Tables are listed in the Appendix). Positive (J96) and negative (JJ055) control strains for these virulence factors were obtained from Dr. James R. Johnson from the University of Minnesota. *E. coli* strain J96 is positive for all 4 virulence factors in this study, as well as numerous other urovirulence factors. *E. coli* strain JJ055 is negative for all 4 virulence factors in this study. Sterile water was also used as a non-nucleic acid negative control.

A master mix was prepared under a biosafety hood to minimize contamination and kept frozen until use. The master mix included (per reaction): 2.5µl 10x buffer (Applied Biosystems, Foster City, CA), 4µl 25mM magnesium chloride (Applied Biosystems, Foster City, CA), 0.25µl Amplitaq Gold polymerase (Applied Biosystems, Foster City, CA), 2µl 2.5mM mixed deoxyribonucleotide triphosphates (dNTPs) (Bioline, Taunton, MA), 0.075µl of each primer (Invitrogen, Carlsbad, CA), and 13.65µl sterile Nuclease-Free Water (Ambion, Austin, TX). Ninety-six-well PCR plates (BioRad, Richmond, CA) were used, and each well was loaded with 23µl of master mix, followed by 2µl of sample DNA lysate. Plates were sealed with microseal film (BioRad Laboratories, Richmond, CA).

Each sample was run in triplicate to ensure accuracy. Each PCR plate contained positive control strain J96 as well as negative control strain JJ055 and sterile nuclease free water (Ambion, Austin, TX). All plates were loaded under a biosafety hood to minimize contamination, in a room separate from any other DNA or RNA research. Study samples were loaded into the plates first, followed by negative controls and water, followed last by the positive control strain.

PCR plates were run on an iCycler (BioRad, Richmond, CA), and conditions were based on a protocol obtained from Dr. James R. Johnson (personal communication 9/18/2007). Activation was performed at 95C for 12 minutes. Twenty-five cycles were performed of denaturation, annealing, extension. Denaturation was set at 94C for 30 seconds, annealing was set at 63C for 30 seconds,

and extension was set at 68C for 3 minutes. A final extension was performed at 72C for 10 minutes.

Twenty-five μl of each PCR sample was analyzed by electrophoresis using a 2% agarose gel (Promega, Madison, WI). Five μl blue/orange loading dye (Promega, Madison, WI) was added to each 25μl sample prior to loading. A 100bp DNA ladder (Promega, Madison, WI) was used as a standard to control the size of the amplified product, and 1.5μl blue/orange dye was added to 7.5μl DNA ladder prior to loading. Positive and negative controls were included on each gel. Gels were run at 150 volts for 3 hours, with 1x tris-acetate EDTA buffer (Promega, Madison, WI). Gels were stained with ethidium bromide (1μl per 500ml water) for 15 minutes, and destained with water for an additional 15 minutes. Images were photographed using a GelDocXR camera (BioRad, Richmond, CA) with Quantity One Software 4.6.3 (BioRad, Richmond, CA), and saved as tagged image file format (TIFF) images.

FPQuest Software Version 4.5 (BioRad, Richmond, CA) was used to analyze band size and to verify presence or absence of virulence factors. McNemar and Chi-Square tests were used to analyze paired dog-owner and independent dichotomous data, respectively. Kappa measure of agreement was used as a comparison of agreement between virulence factors; a Kappa value of 1 implies perfect agreement, and a Kappa value of 0.8-1 implies very good agreement [131]. Chi-Square analysis was also used to measure associations between virulence factors, between virulence factors and susceptibility results, between virulence factors and hand-washing, and

between virulence factors and history of UTI. The original threshold for significance was set at $p \leq 0.05$. A Bonferroni adjustment was made for multiple comparisons. The original p-value (0.05) was divided by the number virulence factors (4), resulting in a new threshold of $p \leq 0.0125$; this decreased the power of these comparisons to 93%.

Results

Questionnaire Results

Participant Demographics

Sixty-one dog and owner pairs and 34 controls that fit the inclusion criteria volunteered to participate from September to December 2006. Forty-three owners were recruited from the UTCVM. Seven dog owners were recruited from the University of Tennessee, Department of Medical Genetics, and 11 owners volunteered from various other departments in the University of Tennessee Department of Agriculture. Twenty controls were recruited from the UTCVM, 4 from Gastrointestinal Associates, 3 from the University of Tennessee, Department of Medical Genetics, and 7 from various other departments within the University of Tennessee. Owner ages ranged from 21 to 76-years-old, with a mean of 34.3 and a median of 28-years-old. Fifty-one owners were women, and 10 were men. Control ages ranged from 21 to 70-years-old, with a mean of 38.5 and a median of 31.5-years-old. Twenty-three controls were women and 11 were men. There was no difference between age ($p=0.168$) or gender ($p=0.133$) of enrolled owners and controls.

Dogs enrolled in this study varied in age from 6-months-old to 18-years-old, with a mean age of 5.5-years-old, and a median age of 5-years-old. Twenty-nine female spayed dogs, 24 male neutered dogs, 4 intact female dogs and 4 intact male dogs were enrolled. Twenty-three purebred dog breeds were represented, but mixed breed dogs (24/61, 39.3%) were more common than any one breed. The most

common pure breed was Labrador retrievers (6/61, 9.8%), followed by boxer dogs (3/61, 4.9%). Twenty-five dogs (41%) were acquired from breeders, 13 dogs (21.3%) were acquired from shelters, 11 dogs (18%) were previously strays, 5 dogs (8.2%) were acquired from family or friends, 2 dogs (3.3%) were acquired from pet stores, and 5 (8.2%) dogs were acquired from other sources.

Canine Medical Histories

One dog in the study had well-controlled diabetes mellitus, and 4/61 dogs had previously been diagnosed with cancer. Three dogs had a history of cutaneous mast cell tumors. The fourth dog was receiving chemotherapy and was in remission from lymphosarcoma. Eight dogs had a history of UTI, including 6 female dogs and 2 male dogs. One dog had received cephalexin 30 days prior to enrollment for a pyoderma and prednisone for atopy. A second dog had received cyclosporine ophthalmic drops in the past month. Eighteen dogs (29.5%) had received antimicrobial therapy within the year prior to enrollment.

Owner Medical Histories

One owner had well-controlled diabetes mellitus, and 27 owners had a history of UTI. All owners with history of UTI were women. Number of UTI per female owner ranged from 0-10, with a mean of 1.55 and a median of 1. Three owners had each taken 1 course of antimicrobial therapy in the 30 days prior to enrollment:

amoxicillin-clavulanic acid for an unreported infection, ciprofloxacin for otitis, and penicillin for a dental procedure. All antimicrobial therapy had been discontinued 2 weeks prior to enrollment. Twenty-two owners (36%) had received antimicrobial therapy within the year prior to enrollment.

Control Medical Histories

One control had an immunosuppressive disease (skin cancer), and one control was receiving a topical immunosuppressive therapy. Ten controls had a history of UTI, all women, with a range of 0-10 lifetime UTIs and a mean of 0.97 lifetime UTIs per individual. Two controls had taken antimicrobial therapy within 30 days prior to enrollment but had finished two weeks prior to enrollment; one received a sulfa antimicrobial for acne, and one received both nitrofurantoin for a UTI and azithromycin for an upper respiratory infection. Nine controls had received antimicrobial therapy within the year prior to enrollment.

History of Antimicrobial Therapy

Dogs (1/61, 1.6%), owners (3/61, 4.9%), and controls (2/30, 6.7%) were equally likely to have received antimicrobial therapy in the 30 days prior to enrollment (p=0.453). Similarly, dogs (18/61, 30%), owners (22/61, 36%), and controls (9/30, 30%) were equally likely to have received antimicrobial therapy in the year prior to enrollment (p=0.711). There was no difference in the number of courses

of antimicrobial therapy taken by participants within each population within the year prior to enrollment (p=0.885).

Of 18 dogs (29.5%) with a history of receiving antimicrobials within a year of enrollment into the study, 13 dogs had received 1 course, and 5 dogs had received 2 courses of antimicrobial therapy. Of the 22 owners (36.1%) with a history of receiving antimicrobial therapy within a year of enrollment into the study, 18 owners had received 1 course, 3 owners had received 2 courses of antimicrobial therapy, and 1 owner had received 1 course of amoxicillin-clavulanic acid and intermittent courses of minocycline throughout the year. Of the 9 controls (30%) with a history of receiving antimicrobial therapy within a year of enrollment into the study, 6 controls had received 1 course, 2 controls had received 2 courses, and 1 control had received intermittent courses of sulfa therapy throughout the year (at least 4 courses).

Grouping all human participants together, 15 courses of antimicrobial therapy had been prescribed over the previous year for respiratory disease (mostly sinus infections), 13 for skin infections, 6 for UTIs, and 5 courses prophylactically. The most common antimicrobial agents prescribed were beta-lactams (penicillin, amoxicillin, amoxicillin-clavulanic acid, and cephalexin) and fluoroquinolones (see Figure 1, all Figures are listed in the Appendix). Of the 6 courses of fluoroquinolones, 5 were ciprofloxacin received by owners, and 1 was levofloxacin received by a control participant. Antimicrobial agents prescribed for human UTIs in the study population included ciprofloxacin, cephalexin, and an unknown agent.

In the canine population, amoxicillin-clavulanic acid was the most commonly prescribed antimicrobial in the year prior to enrollment, and it was prescribed for skin infections, respiratory infections, and a UTI (see Figure 2). Cephalexin and an unknown antimicrobial agent were also reportedly used to treat UTIs in dogs within the year prior to enrollment. Only one owner reported administering a fluoroquinolone, enrofloxacin, to their dog in the previous year, for a UTI.

History of Urinary Tract Infections

Of the 91 human participants, 37 reported having a history of UTI at some point during their lifetime. All 37 were women, making up 52.1% of the total female population in this study, see Figure 3. Women were significantly more likely to have a history of UTI than men ($p<0.001$). Of the dogs in the study, 6/33 (18.2%) female dogs and 2/28 (7.1%) male dogs had a history of UTI, but the difference was not significant ($p=0.203$).

Nine women (25%) had a UTI only once during their lifetime, while 28/37 (75%) had multiple UTIs. Fourteen women (37.8%) reported 2 UTIs, 8/37 (21.6%) women reported 3 UTIs, and 6/37 (16.2%) women reported 4+ UTIs (see Figure 4). Four dogs (50%) had a UTI only once during their lifetime, and 4 dogs (50%) had multiple UTIs, with 3/8 dogs (37.5%) having 3 or more lifetime UTIs (see Figure 5).

Exposure to Other Animals, Children, and Human Hospitals

Of the 43 faculty, staff, and student owners recruited from the UTCVM, 9 had handled canine patients in the 7 days prior to enrollment. Four owners worked at veterinary clinics other than the UTCVM; 3 had 0-10 hours contact with dogs per week, and 1 had 21-30 hours contact with dogs per week. Six controls worked at veterinary clinics, but none had more than 1 hour of direct contact with dogs per week.

Fifty dog-owner households (82%) had pets in addition to the dog enrolled in the study living in their households (see Figure 6). Twenty-seven households (44%) contained only dogs, while 23/61 (38%) households contained other pets including cats, birds, rabbits, and reptiles. The average household contained a mean of 3.23 pets (including the dog enrolled in the study), with a median of 3 pets. One dog, not enrolled, had diarrhea at the time of the study, and 7 unenrolled pets, from 6 different households, had taken antimicrobial therapy in the 30 days prior to their housemates enrolling in this study. Antimicrobial agents recently administered to pets in households of volunteers included cefazolin, cefpodoxime, ciprofloxacin, clindamycin, metronidazole, and trimethoprim-sulfamethoxazole. Nine dog owners (14.7%) reported spending 3 or more hours a week at a dog park. Owners and their dogs may also have had exposure to other dogs, and potentially their fecal material, when visiting dog parks. Three controls had pets in their household; one control had 2 tree frogs, and 2 controls each had 2 cockatiel birds. None of these pets had diarrhea nor had received antimicrobial therapy within the past 30 days.

Ten owners had children living in their house; one child had diarrhea at the time of the parent's enrollment, but no children were receiving antimicrobial therapy. Seven controls had children in their households. None of these children had diarrhea at the time of the study, but 2 children in the same household had received topical ophthalmic ciprofloxacin 5 days prior to their parent's enrollment.

Eleven owners had household members who worked in human hospitals or medical offices. Nine controls worked at human hospitals or medical offices, but none had occupational exposure to human fecal or urine samples.

Relationship with Dog Results

Several questions addressed the amount of direct contact owners had with their dogs. Fifty-four percent of owners spent more than 30 hours per week of awake-time with their dogs, while 9.8% spent 0-10 hours with their dog per week (see Figure 7). Male owners spent more awake-time with their dogs than female owners ($p=0.036$), although there were only 10 male owners and 51 female owners. Fifty-four percent of owners slept in the same bed with their dogs, and seventy percent of owners allowed their dog to kiss or lick them on the face. There was no difference between male or female owners allowing either of these behaviors. Twenty-five percent of owners reported washing their hands after petting their dog (see Figure 8).

Ninety percent of owners were the primary feeder in their household for their dog (see Figure 9). Only 4/61 (6.6%) owners washed their hands before feeding their

dogs, but 47/61 (77%) washed their hands before eating their own meals. The questionnaire inquired about feeding table scraps and raw food, and asked volunteers to list these foods if applicable. There was considerable overlap in the responses. For example, some owners considered carrots to be table scraps, while others considered carrots to be raw foods. For consistency, results were recategorized into feeding of "people food" in general, feeding raw meat, and feeding raw vegetables. Forty-one percent of owners fed their dogs "people food", including table scraps (pizza crusts, cooked meat fat, licking plates and bowls), breads, crackers, pastas, rice, and fresh fruit and vegetables. Eleven percent of owners reported sharing "everything they ate" with their dog. Five owners (8.2%) fed their dogs raw meat, and 10 owners (16.4%) fed their dogs raw fruits and vegetables. Of owners who fed their dog raw meat, 80% also allowed their dogs to kiss or lick them on the face.

Only 29/61 (47.5%) of owners reported disposing of their dog's feces, while 32/61 (52.5%) owners responded that they never disposed of their dog's feces (see Figure 10). A higher percentage of female (49%) than male (40%) owners reported disposing of their dog's feces, but there was no significant association between owner gender and disposing of feces ($p=0.602$). Of owners who disposed of their dog's feces, 27/29 (93.1%) reported washing their hands afterwards.

Of dog-owner pairs who spent 3 or more hours a week at dog parks, only 6/9 (67%) owners reported ever disposing of their dog's feces. Within this group, all three owners who spent 3 or more hours at dog parks and reported never disposing of their dog's feces were veterinary students. Owners who spent 3 or more hours a week

at dog parks were not more likely to dispose of feces than those who visited dog parks 0-2 hours per week (p=0.213).

Thirteen percent of dogs were reported to drink water out of toilets, and therefore, had potential exposure to their owner's urine and fecal material. Within households reporting dogs drinking out of toilets, 75% of owners allowed their dogs to kiss or lick them on the face, and 50% of owners share their "people food" with their dogs.

E. coli Isolation Results

E. coli was isolated from the feces of 91/95 (96%) human participants and 61/61 (100%) dogs. Enumeration of *E. coli* ranged from 3-1500 colonies per plate. If all fecal culturettes had been soiled with a consistent amount of feces, extrapolation of *E. coli* colony enumeration to quantification of *E. coli* per gram feces may have been possible; however, there was a wide range of degree of soiling of fecal swabs by participants, making accurate extrapolation unrealistic. On EC with MUG plates, most *E. coli* colonies were small smooth creamy white colonies, although rough colonies were also seen. On EMB plates, all *E. coli* colonies were smooth, green, and metallic. Three presumptive *E. coli* colonies were isolated from each fecal sample for further testing, and when possible, colonies with different morphologies were selected from the same participant.

Biochemical testing with API-20E kits resulted in 32 different API-20E profiles confirmed as *E. coli*. The most common profile, 5144572, seen in 141/456 isolates, was characterized as β-galactosidase positive, arginine dihydrolase negative, lysine decarboxylase positive, ornithine decarboxylase positive, citrate utilization negative, H_2S production negative, urease production negative, tryptophan deaminase negative, indole production positive, acetoin production negative, gelatinase negative, and had positive fermentation for glucose, mannitol, sorbitol, rhamnose, saccharose, melibiose, and arabinose, and negative fermentation for inositol and amygdalin. An example of an API-20E original worksheet and web printout with this biochemical pattern is shown in Figure 11. The next most common API-20E profile was 5144552, seen in 94/456 (20.6%) isolates, which had negative saccharose fermentation, followed by 5044572, seen in 51/456 (11.2%) isolates, which were ornithine decarboxylase negative. Table 2 summarizes percentages of positive biochemical reactions for all *E. coli* isolates included in this study.

In 111 participants, all 3 *E. coli* isolates had the same API-20E profile. In 31 participants, 2 different API-20E profiles were seen among the 3 *E. coli* isolates, and in 10 participants each of the 3 *E. coli* isolates had a different API-20E biochemical profile. The wide array of API-20E results demonstrates the variety of *E. coli* strains in the fecal flora of human and canine participants in this study.

Four human fecal samples provided by control participants did not yield *E. coli* isolates. Repeat samples were requested from these participants, and instructions for proper collection, storage, and submission were reviewed. Repeat samples were

processed within an hour of submission, but *E. coli* was not isolated from these specimens. No additional enrichment procedures were attempted. Three fecal samples yielded *Klebsiella pneumoniae*, and one yielded *Serratia liquifaciens*, see Figure 12. Since no *E. coli* was isolated from these participants, they were excluded from the remainder of the study. With a sample size of 30 for the control group instead of 34, and a continued alpha set at 0.05, effect size of 0.30, and two-tailed test, the power decreased from 85% to 82%.

Antimicrobial Susceptibility Results

Comparison of Antimicrobial Susceptibility within Groups

For each group, percentage of participants with *E. coli* susceptible to each of the 17 antimicrobial agents is shown in Table 3. Similarly, for each group, percentage of *E. coli* isolates susceptible to each of the 17 antimicrobial agents is shown in Table 4.

Forty-one percent (75/183) of canine isolates were susceptible to all 17 antimicrobial agents. The remaining isolates had either intermediate susceptibility or resistance to at least one antimicrobial agent. All canine isolates were susceptible to amikacin and imipenem, and 182/183 (99.5%) isolates and 180/183 (98.4%) isolates were susceptible to gentamicin and ciprofloxacin, respectively, see Figure 14. Intermediate susceptibility was seen to cephalothin (63/183, 34.4%), streptomycin (53/183, 29%), and ampicillin (24/183, 13.1%). The greatest amount of resistance in

canine isolates was seen to ampicillin (23/183, 12.6%), followed by cephalothin (13/183, 7.1%).

Thirty-nine percent (71/183) owner isolates were susceptible to all 17 antimicrobial agents. All isolates from owners were susceptible to imipenem, while 181/183 (98.9%) isolates were susceptible to amikacin and cefoxitin. Intermediate susceptibility was seen to cephalothin (47/183, 25.7%) and streptomycin (40/183, 21.9%). The greatest amount of resistance was seen to ampicillin (43/183, 23.5%), followed by streptomycin (19/183, 10.4%) trimethoprim-sulfamethoxazole (19/183, 10.4%), and tetracycline (17/183, 9.3%), see Figure 14.

Resistance to two 3rd generation cephalosporins, ceftiofur and cefpodoxime, was seen in dogs and owners but not in controls. Ceftiofur-resistant isolates were seen in 3/61 (5%) of dogs, and 2/61 (3%) of owners. Only Household #58 had ceftiofur-resistant *E. coli* isolated from both dog and owner. There was overlap in resistance for ceftiofur and cefpodoxime. Cefpodoxime-resistant isolates were seen in 4/61 (7%) dogs and 3/61 (5%) of owners. Again, only Household #58 had cefpodoxime-resistant *E. coli* isolated from both dog and owner. In Household #58, owner identification number (ID#)124 had received a course of amoxicillin-clavulanic acid 30 days prior to enrollment, but dog ID#64 had no history of receiving antimicrobial therapy. Of the other participants with isolates resistant to ceftiofur and cefpodoxime, one dog had received amoxicillin in the past year, and one dog had received cephalexin 4 years previously, while the remaining dogs and owners had no history of receiving antimicrobial therapy.

Thirty-eight percent (34/90) control isolates were susceptible to all 17 antimicrobial agents. All control isolates were susceptible to amikacin, cefoxitin, ceftiofur, cefpodoxime, ceftriaxone, gentamicin, and imipenem. Intermediate susceptibility was commonly seen to cephalothin (25/90, 27.8%) and streptomycin (23/90, 25.6%). The greatest amount of resistance was seen to ampicillin (27/90, 30%), followed by trimethoprim-sulfamethoxazole (13/90, 14.4%), streptomycin (9/90, 10%), and tetracycline (9/90, 10%), see Figure 15.

Comparison of Antimicrobial Susceptibility between Groups

No differences were found with the Wilcoxon signed ranks tests for susceptibility comparisons of *E. coli* isolated from dog-owner pairs, to any of the 17 antimicrobial agents, see Table 5. When the data were collapsed so that intermediate results were considered susceptible, and a McNemar Chi-Square test was used, no differences were found between dog-owner pairs for any of the 17 antimicrobial agents. Similarly, if the data were collapsed so that intermediate results were considered resistant, no differences were found between dog-owner pairs for any of the 17 antimicrobial agents. For 6 pairs, both dog and owner had fecal *E. coli* that were susceptible to all 17 antimicrobial agents tested.

There were no differences in susceptibility results from owners and controls, see Table 6. Differences were found when comparing susceptibility results of dogs and controls, see Table 7. Control isolates were more resistant than canine isolates to

ampicillin ($p<0.001$), chloramphenicol ($p=0.029$), ciprofloxacin ($p=0.029$), tetracycline ($p=0.024$), and trimethoprim-sulfamethoxazole ($p<0.001$). With the Bonferroni adjustment, control isolates were more likely resistant to only ampicillin and trimethoprim-sulfamethoxazole than canine isolates.

Analysis of Antibiograms

Construction of antibiograms provides a way to analyze overall antimicrobial resistance patterns. Antibiogram patterns included concurrent resistance to two or more antimicrobial agents, and prevalence for each pattern was calculated for the isolates in each population (see Table 8). In the canine population, the most common antibiogram pattern of resistance was seen to ampicillin and cephalothin (8/183, 4.4%). In owners, the most common antibiogram pattern of resistance was seen to ampicillin and trimethoprim-sulfamethoxazole (13/183, 7.1%), followed by ampicillin and streptomycin (11/183, 6%). Antibiogram results for controls were similar to owners, with highest resistance to ampicillin and trimethoprim-sulfamethoxazole (10/90, 11.1%), followed by ampicillin and streptomycin (9/90, 10%). The combination of resistance to ampicillin, streptomycin, and trimethoprim-sulfamethoxazole was seen in 7/90 (7.8%) of control isolates.

Multiple Drug Resistance

Multiple drug resistance (MDR) was defined as resistance to 3 or more antimicrobial agents, regardless of class. Overall, 19/152 participants (12.5%) had MDR *E. coli* and 46/456 (10.1%) isolates were MDR. Eight isolates (8/183, 4.4%) from 4/61 (6.6%) dogs were classified as having MDR. Two dogs had 1 MDR isolate each, 1 dog had 3 different MDR isolates, and 1 dog had 3 isolates with the same MDR pattern (see Table 9). Isolates from these 4 dogs were resistant to a range of 7-14 antimicrobial agents. One hundred percent were resistant to ampicillin, and 62.5% were resistant to amoxicillin-clavulanic acid, cefoxitin, ceftiofur, cefpodoxime, cephalothin, streptomycin, and trimethoprim-sulfamethoxazole.

Twenty-five isolates (25/183, 13.7%) from 10/61 (16.4%) owners were classified as having MDR. These isolates were resistant to a range of 3-10 antimicrobial agents, with a median of 4.5. In 7/10 of these owners, all 3 fecal isolates were MDR. Five of these owners had the same MDR susceptibility pattern in all 3 isolates, whereas 2 owners had multiple MDR profiles, see Table 10. Ninety-two percent were resistant to ampicillin, 52% were resistant to trimethoprim-sulfamethoxazole, and 48% were resistant to streptomycin. Percentages of resistance to amoxicillin-clavulanic acid and the cephalosporin antimicrobials was lower than in dogs and ranged from 8-36%.

Thirteen isolates (13/90, 14.4%) from 5/30 (16.7%) controls were classified as having MDR. Isolates from these 5 individuals were resistant to a range of 3-9 antimicrobials, with a mean of 4.2 and median of 3. In 4/5 of these controls, all 3

fecal *E. coli* isolates were MDR, and in 3 of these controls all 3 isolates shared the same susceptibility pattern. One control had 3 MDR isolates that each had different susceptibility patterns, see Table 11. One hundred percent were resistant to trimethoprim-sulfamethoxazole, 79.6% were resistant to ampicillin, and 53.8% were resistant to streptomycin. While 23.1% were resistant to cephalothin, all MDR isolates from controls were susceptible to the remaining cephalosporins.

The percentage of MDR *E. coli* varied between groups and was lowest among dogs, see Figure 16. There was no difference between percentage of paired dogs versus their owners (p=0.109) who had MDR *E. coli*. Controls had more MDR isolates (13/90, 14.4%) than dogs (8/183, 4.4%), (p=0.003). There was no statistical difference between the number of owners and controls who had fecal MDR *E. coli* (p=0.974) or between the total number of MDR *E. coli* isolates from owners and controls (p=0.833).

Resistance patterns varied widely between MDR *E. coli* isolated from dogs versus human beings in this study, see Figure 17. Canine MDR isolates were more resistant than owner and control MDR isolates to cefoxitin (p=0.001), and more resistant than control MDR isolates to ceftiofur (p=0.001), cefpodoxime (p=0.001), and ceftriaxone (p=0.002) see Table 12. Control MDR isolates were more resistant than owner MDR isolates to trimethoprim-sulfamethoxazole (p=0.003).

Comparing history of antimicrobial therapy to MDR results, only 2/4 (50%) dogs with MDR isolates had received antimicrobial therapy: dog ID#6 had received a course of amoxicillin within 30 days of enrollment, and dog ID#19 had received a

course of amoxicillin-clavulanic acid within a year of enrollment. Of the owners with MDR isolates, 2/10 (20%) had received antimicrobial therapy: owner ID#109 had received an unknown antimicrobial agent within a year of enrollment, and owner ID#124 had received a course of amoxicillin-clavulanic acid within 30 days of enrollment. Of the controls with MDR isolates, 2/5 (40%) had received antimicrobial therapy: control ID#145 had received azithromycin and nitrofurantoin within 30 days of enrollment, and control ID#156 had received intermittent sulfa therapy throughout the year prior to enrollment.

For two dog-owner pairs, MDR *E. coli* was isolated from dog and owner, see Table 13. One pair, from Household #2, included a dog ID#6 with *E. coli* resistant to 7 antimicrobial agents and an owner ID#66 with *E. coli* resistant to 3 antimicrobial agents. The dog in Household #2 was a 15yr-old female spayed Weimaraner with a history of 3-4 historical UTIs of unknown bacterial etiology, most recently in May 2006 (3 months prior to enrollment). Although the dog had not received antimicrobial therapy within the month prior to enrollment, she had previously received three courses of amoxicillin during 2005-2006. This dog did not have diarrhea or known endocrine disease, and she was not currently receiving immunosuppressive therapy. The owner in Household #2 was an adult female with no history of UTI, immunosuppressive disease or therapy, and no known antimicrobial history. Both dog and owner had *E. coli* resistant to ampicillin. There were 2 other dogs also living in the home at the time, neither of which had any history

of diarrhea or antimicrobial therapy. There were no children or other family members living in this household.

The second pair, from Household #58, included dog ID#64 with *E. coli* resistant to 9 antimicrobial agents and owner ID#124 with *E. coli* resistant to 10 antimicrobial agents. The dog in Household #58 was a 5-year-old female spayed Labrador retriever with no history of UTI, diarrhea, known endocrine disease, or neither antimicrobial nor immunosuppressive therapy. The owner in Household #58 was a 29 year-old female with no history of UTI, diarrhea, or immunosuppressive disease or therapy. This owner had received a 10-day course of amoxicillin-clavulanic acid that was finished 24 days prior to entry into the study. Both dog and owner had *E. coli* resistant to amoxicillin-clavulanic acid. There were no other pets in this household. There was a 13-year-old child in the household, but this child was healthy and had no antimicrobial history.

Comparison of Antimicrobial Susceptibility to Demographics

There was no association between owners' or controls' gender and susceptibility results for any of the 17 antimicrobial agents. An association was seen between dogs' sex and susceptibility to tetracycline (p=0.003), with female dogs more likely to have tetracycline-resistant *E. coli* than male dogs. Living in a multi-pet vs. single pet household was not associated with susceptibility results of any of the 17 antimicrobial agents for dogs or owners. Owner affiliation with the UTCVM

was associated with susceptibility results to kanamycin (p=0.002), streptomycin (p=0.003), tetracycline (p=0.001), and trimethoprim-sulfamethoxazole (p=0.001). Owners not affiliated with the UTCVM were more likely to have *E. coli* resistant to each of these antimicrobial agents than owners affiliated with the UTCVM. Owner affiliation with human hospitals was not associated with susceptibility results to any of the 17 antimicrobial agents or to carriage of MDR *E. coli*.

Comparison of Susceptibility to Antimicrobial Therapy within 30 Days

Participants who received antimicrobial therapy in the month prior to enrollment had more MDR fecal *E. coli* than those who did not (p=0.005). Dog ID#28 had been administered cephalexin within one month of enrollment into this study, but all 3 fecal *E. coli* isolates were susceptible to all 17 antimicrobial agents; isolates from its owner ID#88 had intermediate susceptibility to cephalothin but were susceptible to the remaining antimicrobial agents.

Three owners had each taken 1 course of antimicrobial therapy in the past 30 days. Owner ID#69 had taken penicillin prophylactically for a dental procedure. *E. coli* isolates from the feces of both owner ID#69 and his dog ID#9 had intermediate susceptibility to ampicillin and cephalothin. Dog ID#9 also had *E. coli* that was resistant to nalidixic acid and intermediately susceptible to streptomycin, while the owner's *E. coli* had intermediate susceptibility to nalidixic acid and was susceptible to streptomycin. Owner ID#72 had received a course of topical ciprofloxacin for

otitis in the 30 days prior to enrollment. This owner's fecal *E. coli* had intermediate susceptibility to ampicillin, cephalothin, and streptomycin, and *E. coli* from his dog, ID#12, also had intermediate susceptibility to cephalothin and streptomycin, but *E. coli* from both were fully susceptible to ciprofloxacin. Owner ID#124 (Household #58) had received amoxicillin-clavulanic acid in the 30 days prior to enrollment for an unspecified infection, and both dog and owner had MDR fecal *E. coli* that were resistant to amoxicillin-clavulanic acid, as well as other beta-lactam antimicrobial agents.

Two controls received antimicrobial therapy within the 30 days prior to enrollment. Control ID#145 took nitrofurantoin for a UTI and azithromycin for an upper respiratory infection. *E. coli* isolated from control ID#145 was MDR and resistant to ciprofloxacin, nalidixic acid, and trimethoprim-sulfamethoxazole, and had intermediate susceptibility to cephalothin and streptomycin. Control ID#156 received sulfa drugs frequently for acne, and *E. coli* isolated from this participant was MDR and resistant to ampicillin, cephalothin, chloramphenicol, ciprofloxacin, kanamycin, nalidixic acid, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole, and had intermediate susceptibility to amoxicillin-clavulanic acid.

Five dog-owner households had pets, other than enrolled dogs, who had received antimicrobial therapy within 30 days of their housemate's enrollment. Although the following descriptions suggest recent antimicrobial therapy in unenrolled household pets may be linked to carriage of MDR *E. coli* in enrolled

owners or dogs, no statistical association was found (p=0.137) and (p=0.205), respectively.

A cat in Household #7 received cefazolin 1 week prior to the dog and owner's enrollment; neither the dog nor the owner had received any antimicrobials within the year prior to enrollment. *E. coli* from the dog in Household #7 had intermediate susceptibility to amoxicillin-clavulanic acid, cephalothin, kanamycin, and streptomycin, but was susceptible to the remaining antimicrobials. *E. coli* from the owner in Household #7 were MDR and resistant to amikacin, cefoxitin, ceftiofur, cefpodoxime, ceftriaxone, cephalothin, kanamycin, nalidixic acid, and streptomycin.

Household #28 had a second dog who had received a sulfa antimicrobial within 30 days. While *E. coli* from the enrolled dog were susceptible to trimethoprim-sulfamethoxazole, *E. coli* from the owner were resistant to trimethoprim-sulfamethoxazole although that owner had not taken any antimicrobial agents in the past year.

Household #30 had 2 unenrolled dogs, one who had received cefpodoxime and one who had received ciprofloxacin in the month prior to the study. Fecal *E. coli* from the enrolled dog in Household #30 (and who had not received any antimicrobials in the past 4 years) were MDR and resistant to 14 antimicrobials, had intermediate susceptibility to ceftriaxone, and were susceptible only to amikacin and imipenem. Fecal *E. coli* from the owner in Household #30 were susceptible to all 17 antimicrobial agents.

Household #37 had one unenrolled dog who had diarrhea during the study and had received metronidazole 1 day prior to his housemates' enrollment. Neither the enrolled dog nor the owner had received antimicrobial therapy within the year prior to enrollment. Fecal *E. coli* from the dog were susceptible to all 17 antimicrobials, and from the owner were resistant to ampicillin and had intermediate susceptibility to cephalothin.

Household #44 had two unenrolled dogs who had received clindamycin in the 30 days prior to the study for dental purposes. Fecal *E. coli* from the enrolled dog, who had not received antimicrobials in the past month, but had 1 course each of metronidazole and cefpodoxime over the past year, were resistant to chloramphenicol and had intermediate susceptibility to ampicillin, cephalothin, and streptomycin. Fecal *E. coli* from the owner in Household #44, who had not received antimicrobials in the past 30 days but had received an unknown antimicrobial within the year prior to enrollment for bronchitis, were MDR with resistance to ampicillin, streptomycin, and trimethoprim-sulfamethoxazole.

Although 16.4% of dog-owning households had children in the house, none of these children had received antimicrobial therapy in the 30 days prior to their parent's and dog's enrollment in the study. Control ID#157 had 2 children in the house who received topical ciprofloxacin ophthalmic medication 5 days prior to their parent's enrollment into the study, however *E. coli* from this participant was susceptible to all 17 antimicrobial agents. Unfortunately, it was not asked whether other family

members in the house, besides children, received antimicrobial therapy in the 30 days or year prior to the study; this was an oversight in the questionnaire design.

Comparison of Susceptibility to Antimicrobial Therapy within a Year

Thirty-two percent (6/19) of participants with MDR isolates had a history of receiving antimicrobial therapy within a year prior to enrollment, but this was not associated with isolation of MDR *E. coli* (p=0.948). Dogs, owners, and controls with MDR *E. coli* were equally likely to have taken antimicrobial therapy within the year prior to enrollment (p=0.512). There was no association between taking 2 or more courses of antimicrobial therapy and having MDR *E. coli* (p=0.172). Of participants who received 2 or more courses of antimicrobials within the previous year, 25% had MDR *E. coli* isolates, and 58% of volunteers had isolates that were not susceptible (intermediate or resistant) to at least one of the antimicrobials which they reported receiving. Twenty-two percent of the paired housemates to a participant who received 2 or more courses of antimicrobials in the year prior to enrollment had MDR *E. coli*, and 33% were not susceptible to one of the antimicrobials their pair reported receiving.

Two participants reported receiving antimicrobial therapy, either in frequent courses intermittently or continuously throughout the year. Owner ID#76, who reported taking a course of amoxicillin-clavulanic acid and intermittent minocycline throughout the year, had *E. coli* susceptible to all beta-lactams and tetracycline;

however dog ID#16 in the same household, who had no history of antimicrobial therapy reported in the past year, had *E. coli* resistant to ampicillin and cephalothin, and intermediate susceptibility to amoxicillin-clavulanic acid. Control ID#156 received continuous sulfa antimicrobial therapy from December 2005 through November 2006, prior to enrolling in late December 2006. *E. coli* isolated from this participant was MDR and resistant to trimethoprim-sulfamethoxazole as well as amoxicillin, cephalothin, chloramphenicol, ciprofloxacin, kanamycin, nalidixic acid, streptomycin, and tetracycline.

Comparison of Antimicrobial Susceptibility to History of UTI

Antimicrobial susceptibility results were compared with reported history of a UTI at any time in the participant's lifetime. In women, an association was found between history of UTI and ampicillin resistance in fecal *E. coli* ($p=0.002$); no associations were found when including male isolates. In dogs, there was an association between history of UTI and harboring fecal *E. coli* with resistance to ciprofloxacin ($p<0.001$) and nalidixic acid ($p=0.002$). History of recurrent UTIs was associated with isolates having increased ampicillin resistance in women ($p=0.002$), and increased cefoxitin ($p=0.002$), ceftiofur ($p=0.002$), ceftriaxone ($p=0.002$), ciprofloxacin ($p<0.001$), gentamicin ($p<0.001$), and nalidixic acid ($p<0.001$) resistance in dogs.

History of reported UTI was associated with receiving antimicrobial therapy both 30 days ($p<0.001$) and 1 year ($p<0.001$) prior to enrollment into the study. Nine percent of participants with reported UTI had received antimicrobial therapy in the 30 days prior to enrollment, compared with only 1% of those without a history of UTI. Forty-four percent of participants with reported UTI had received antimicrobial therapy in the year prior to enrollment, compared with 27% of those without a history of UTI.

Comparison of Antimicrobial Susceptibility to Dog-Owner Behaviors

Twenty-five percent of owners reported washing their hands after petting their dogs, and this behavior was associated with antimicrobial susceptibility results in the owners' fecal *E. coli* to ampicillin ($p=0.003$), amoxicillin-clavulanic acid ($p=0.009$), cephalothin ($p=0.033$), and trimethoprim-sulfamethoxazole ($p=0.009$); only ampicillin is significant when using the Bonferroni corrected threshold. Fifty percent of *E. coli* isolates from owners who did not wash their hands after petting their dogs were resistant to ampicillin, compared with only 4% resistance in isolates from owners who did wash their hands.

An association was found between cephalothin resistance and consumption of raw meat by dogs ($p=0.021$); however this result was not significant using the more rigid Bonferroni threshold of $p\leq0.003$. Eighty percent (4/5) of owners who fed raw meat also allowed their dogs to kiss or lick them on the face, but the power was too

low to find statistical differences between susceptibilities of fecal *E. coli* from these two subpopulations. There were no associations between antimicrobial susceptibility results of dog's fecal *E. coli* and consumption of raw fruits and vegetables.

An association was found between ciprofloxacin resistance and feeding "people food" to dogs ($p=0.037$); however this result was not significant using the more stringent Bonferroni corrected threshold of $p\leq0.003$. There was no association between feeding "people food" to dogs and the owners' fecal *E. coli* susceptibility results.

Hand-washing before meals was associated with susceptibility results of fecal *E. coli* isolated from owners to chloramphenicol ($p<0.001$), ciprofloxacin ($p<0.001$), nalidixic acid ($p<0.001$). Isolates from owners who did not wash their hands were more likely resistant than isolates from owners who did wash their hands to chloramphenicol (7.1% vs. 0%), ciprofloxacin (14.3% vs. 0.7%), and nalidixic acid (14.3% vs. 2.8%).

Washing hands before feeding their dogs was associated with susceptibility results to tetracycline in their dog's fecal *E. coli* ($p=0.002$). Tetracycline resistance in canine isolates was more common from dogs whose owners washed their hands before feeding them than from dogs whose owners did not wash their hands (25% vs. 2.3%).

Washing hands after disposing of dog's feces was associated with streptomycin susceptibility results in owner's fecal *E. coli* ($p=0.050$); however this result would not be significant using the more stringent Bonferroni p-value threshold

of $p \leq 0.003$. Streptomycin resistance was more common in isolates from owners who did wash their hands after disposing of their dog's feces than in those who did not wash their hands (16% vs. 0%).

Drinking water out of the toilet was associated with susceptibility results in dogs' fecal *E. coli* for ciprofloxacin ($p<0.001$) and nalidixic acid ($p=0.002$). Dogs that drank water out of the toilet had *E. coli* isolates that were more likely resistant than those who did not to ciprofloxacin (12.5% vs. 0%) and nalidixic acid (12.5% vs. 1.3%).

No associations were identified between susceptibility results and dog-owner pairs sleeping in the same bed, allowing the dog to kiss or lick the owner on the face, or awake time spent together per week.

Pulse Field Gel Electrophoresis Results

Gel Electrophoresis Image Results

An example of a gel's image after running PFGE is shown as Figure 18. Each gel contained 3 pairs of dog and owners, or 6 controls. Lanes 1, 8, and 15 contained Low Range PFG markers with known base pair bands used for standardization of fingerprints within and between gels. In gels representing dogs and owners, lanes 2, 3 contained *E. coli* from the dog, and lanes 4, 5 contained *E. coli* from the owner in the same household. Similarly, lanes 6, 7 contained with *E. coli* from the dog, and lanes 9, 10 contained *E. coli* from the owner in the same household. Finally, lanes

11, 12 contained *E. coli* from the dog, and lanes 13, 14 contained *E. coli* from the owner in the same household. All gel images were analyzed with FPQuest Software Version 4.5 (Bio-Rad, Richmond, CA), and similarity matrices were determined between each dog-owner pair.

Distribution of Clones

Both *E. coli* isolates from 57/152 participants had >94% similarity. Eleven isolates (3.6%) were untypeable due to degradation of the genomic DNA during preparation; of the untypeable isolates, 4 were canine isolates, 1 was an owner isolate, and 6 were control isolates. Thus, 293 isolates with genomic patterns remained for comparison. From these isolates, PFGE resolved 205 discrete genomic patterns or clones. Of these 205 clones, 169 (82.4%) were isolated from only one participant. Thirty-six *E. coli* clones (17.6%) were shared between multiple participants.

Of the 36 shared clones, 3 clones were shared between 3 hosts, and 33 clones were shared between 2 hosts. Six clones were shared between dogs and owners within households, while 30 clones were shared across households. Seven clones were shared between dogs across households, 9 were shared between dogs and owner across households, 2 were shared between dogs and controls across households, 4 were shared between owners across households, 5 were shared between owners and controls across households, and 3 were shared between controls across households

(see Figure 19). A dendrogram showing the similarity between isolates from all dogs, owners, and controls is shown in Figure 20.

Clonal Sharing By Individuals

Of the 152 total participants, 48 (31.6%) shared ≥ 1 clone with another participant (see Figure 21). Twenty dogs (32.7%) shared a fecal *E. coli* clone with another individual in this study, although only 6/61 (9.8%) shared that clone with their owner as a within-household shared clone. Similarly, 20/61 owners (32.7%) shared a fecal *E. coli* clone with another individual in this study, although only 6/61 (9.8%) shared that clone with their dog as a within-household shared clone. Eight control participants (26.7%) shared a fecal *E. coli* clone with another individual in this study, all across-household sharing.

There was no difference in percentage of individuals sharing ≥ 1 clone for each of the three groups (p=0.812). Among human participants, clone sharing was not different between men (26.3%) and women (31.9%) (p=0.636). Similarly, among dogs, clone sharing was not different between male (32.1%) and female (33.3%) dogs (p = 0.921).

Clonal Sharing Within Households

One dog-owner pair had isolates with 100% PFGE profile similarity, see Figure 22. Six dog-owner pairs (9.8%) had isolates with >94% PFGE profile similarity, and 13 dog-owner pairs (21.3%) had isolates with >90% PFGE profile similarity. Results of the remaining dog-owner pair similarity matrices are available in Table 14.

Comparison of Within and Across-Household Sharing

There were 11,476 total potential clone-sharing pairs, 61 potential within-household clone-sharing pairs, and 11,415 potential across-household clone-sharing pairs. Clonal sharing was seen in 36/11,476 (0.3%) total potential clone-sharing pairs, 6/61 (9.8%) potential within-household clone-sharing pairs, and 30/11,415 (0.26%) potential across-household clone-sharing pairs. Within-household sharing was more prevalent than across-household sharing of fecal *E. coli* ($p<0.001$).

Descriptive Analysis of Clone Sharing Across Households

Nine *E. coli* clones were shared between dogs and owners across households, with one clone sharing 100% PFGE profile similarity. Five of these clones (55.6%) were shared by unpaired dogs and owners affiliated with the UTCVM, including the

clone with 100% similarity. Four clones were shared across households with no known direct, occupational, or environmental contact.

Seven clones were each shared between 2 dogs that lived in separate households. All of the dogs' owners either worked or were students at the UTCVM. Twelve clones were shared between human participants living in separate households. Of these, 4 were shared by participants who worked or studied at the UTCVM, while the remaining 8 clones were shared by participants from a mix of UTCVM, GI Associates, and Department of Genetics. Due to confidentiality limitations, further questioning of the owners regarding contact with each other or between their dogs was not permitted after the study was performed.

Analysis of E. coli Groups of Isolates

Based on analysis of the dendrogram in Figure 20, no strains or groups of strains were identified more commonly in dogs, owners, or controls in this study. Instead, the dendrogram displays the genetic uniqueness of the clones isolated from these participants. Of the 205 *E. coli* clones, seventeen groups of isolates were identified that each shared >90% PFGE profile similarity. All groups contained 5 or fewer participants.

Only 1 group included *E. coli* isolates from all 3 populations, dog ID#28, owner ID#112, owner ID#123, and control ID#146, see Figure 23. The owner of dog

ID#28 was affiliated with the UTCVM, but participants ID#112, ID#123, and ID#146 were not and had no known direct or occupational contact.

Six groups included participants from both the dog and owner population (see Figures 24-29). Since the definition of a group required 4 or more isolates to have >90% PFGE similarity, none of the 6 dog-owner pairs that had 2 clones with >94% within-household sharing met this criteria; however, all 4 isolates from one additional dog-owner pair, dog ID#56 and owner ID#116, did have >90% PFGE similarity (Figure 29). One other group included within-household sharing between dog ID#22 and owner ID#82 (Figure 27). All other groups identified were comprised of across-household clonal sharing exclusively. Three groups included hosts from the owner and control populations, 5 groups included just owners (2-3 owners per group), 1 group included 3 controls, and 1 group included 4 isolates from 2 dogs.

Comparison of PFGE Profile Similarity to Demographics

Percent PFGE similarity between isolates from dogs and owners was compared to demographic information, and no significant associations were found with owner gender (p=0.253), dog sex (p=0.282), dog's age category (<2years, 2-5yrs, 6-9yrs, and 10+) (p= 0.563), breed (purebred or mixed breed dog) (p=0.574), or dog source (breeder, shelter/stray, pet store, other) (p=0.123). Living in a multi-pet vs. single pet household was not associated with % PFGE profile similarity, nor was total number of pets in the house (p=0.226), and (p=0.069), respectively. Owner

affiliation with the UTCVM was not associated with increased *E. coli* clonal sharing based on % PFGE profile similarity (p=0.829).

Comparison of PFGE Profile Similarity to Dog-Owner Behaviors

Percent PFGE profile similarity between isolates from dogs and owners were compared to the following dog-owner behaviors: amount of awake-time spent with dog, sleeping in the same bed, kissing on the face, washing hands after petting, being the primary feeder of the dog, feeding dog raw meat, feeding dog raw fruits and vegetables, feeding dog "all people-food," washing hands before feeding dog, washing hands before eating, washing hands after disposing of dog's feces, dog drinking from toilet, having other pets in household, and weekly time spent at dog parks. No behaviors surveyed were found statistically more often in dog-owner pairs exhibiting within-household clonal sharing; however, disposing of dog's feces was associated with lower % PFGE profile similarity (p=0.013). Owners who disposed of their dog's feces had *E. coli* with a mean % PFGE profile similarity with their dog of 81.48%, while owners who did not dispose of their dog's feces had *E. coli* with a mean % PFGE profile similarity with their dog of 85.78%. Owners who disposed of their dog's feces 5 or more times a week had *E. coli* with the lowest % PFGE profile similarity with their dog (mean 77.2%). Times per week an owner disposed of feces was not associated with whether or not they washed their hands after disposing of

feces (p=0.429), but only 2/29 owners who did dispose of feces did not wash their hands.

Virulence Factor Results

Spectrophotometry Results

Nucleic acid concentration and quality results from spectrophotometry testing of DNA lysates to be used for PCR are in listed in Appendix D. All *E. coli* lysates were found to have sufficient DNA (>400ng/µl), with a range of 454-2862ng/µl, mean of 920ng/µl, and a median of 874ng/µl. Overall quality of DNA lysates was pure with a mean 260:280nm absorbance ratio of 1.81.

Gel Electrophoresis Image Results

An example of a gel image from the multiplex PCR assay is shown in Figure 30. Each gel for PCR samples had two rows of 20 lanes, numbered from left to right starting with the top row. Lanes 1, 20, 21, and 40 contained 100bp DNA ladder for standardization. The largest band in this ladder was 1500bp, followed by 1000bp, and then each band decreased in size by 100bp. Lanes 2, 19, 22, and 39 each contained *E. coli* strain J96, a known positive control for all 4 virulence factors. Lane 31 contained sterile water as a negative control. Lane 32 contained *E. coli* strain JJ055, a known negative control for all 4 virulence factors. The remaining lanes

contained PCR-amplified DNA from participant isolates. Base pair sizes for each virulence factor were: *cnf* 974, *hlyD* 904, *sfa* 410, and *papGIII* 258. Bands for *cnf* and *hlyD* were physically close together due to their similar base pair size. All images were analyzed with FPQuest Software Version 4.5 (BioRad, Richmond, CA), to confirm base pair size of each band.

Prevalence of Virulence Factors

Overall, 45/61 (73.7%) dogs, 50/61 (81.9%) owners, and 24/30 (80%) controls were negative for all 4 virulence factors in all 3 fecal *E. coli* isolates, see Tables 15 and 16. Six dogs (9.8%), 4 owners (6.5%), and zero controls had presence of all 4 virulence factors in all 3 fecal *E. coli* isolates. Figure 31 summarizes the percentage of dogs, owners, and controls that had fecal *E. coli* isolates with presence of each of the 4 virulence factors.

Ten dogs (16.4%), 10 owners (16.4%), and 5 controls (16.7%) had *E. coli* isolates positive for *cnf*. Ten dogs (16.4%), 11 owners (18%), and 6 controls (20%) had *E. coli* isolates positive for *hlyD*. Fifteen dogs (24.6%), 10 owners (16.4%), and 3 controls (10%) had *E. coli* isolates positive for *sfa*. Eight dogs (13.1%), 5 owners (8.2%), and 3 controls (10%) had *E. coli* isolates positive for *papGIII*.

Since not all individuals had similar virulence factor profiles among all 3 isolates, prevalence data for total number isolates was different than prevalence per participant. Figure 32 displays the prevalence of each virulence factor when

including all 3 fecal *E. coli* isolates from each participant. Twenty-eight canine isolates (15.3%), 26 owner isolates (14.2%), and 12 control isolates (13.3%) were positive for *cnf*. Twenty-eight canine isolates (15.3%), 29 owner isolates (15.8%), and 15 control isolates (16.7%) were positive for *hlyD*. Forty canine isolates (21.9%), 26 owner isolates (14.2%), and 6 control isolates (6.7%) were positive for *sfa*. Twenty-three canine isolates (12.6%), 14 owner isolates (7.7%), and 7 control isolates (7.8%) were positive for *papGIII*.

Virulence Factor Patterns

In 142/152 (93.4%) of participants, all 3 *E. coli* isolates shared the same virulence factor profile. The most common virulence factor pattern was negative for all 4 virulence factors, occurring in 78% (119/152) of participants. The next most common was positive for all 4 virulence factors, occurring in 7/61 (11.5%) dogs and 5/61 (8.2%) owners, followed by the combination of *cnf, hlyD,* and *sfa*, occurring in 2/61 (3.3%) dogs, 5/61 (8.2%) owners, and 2/30 (6.7%) controls, see Table 17. The only virulence factors that occurred alone were *sfa* in 6/61 (9.8%) dogs and 1/30 (3.3%) controls, and *hlyD* in 1/61 (1.6%) owners.

Comparison of Virulence Factors between Groups

There was no significant difference in presence or absence of each virulence factor between dog and owner pairs (see Table 18). In 35/61 households, all isolates

from both dog and owner were negative for all 4 virulence factors. Only Household #48 had a dog and owner who both had *E. coli* positive for virulence factors. In this household all 3 isolates from both dog and owner were positive for *cnf*, *hlyD*, and *sfa*, but negative for *papGIII*. In the remaining 25/61 households either the dog or the owner had *E. coli* isolates positive for one or more virulence factor.

There was no difference in presence or absence of any of the 4 virulence factors between owners and controls (see Table 19). More canine isolates were positive for *sfa* than control isolates (p=0.002), (see Table 20).

Agreement between Virulence Factors

Presence of phenotypic hemolysis, as determined by visualizing large zones of clearing around colonies on blood agar plates was compared with presence of *hlyD* genotypically in each *E. coli* isolate and found to have a high measure of agreement (Kappa = 0.884, p <0.001). Thirteen isolates positive for *hlyD* on multiplex PCR did not show the hemolytic phenotype (13/72, 18%).

In the canine population, there was perfect agreement (Kappa=1, p<0.001) between presence of *cnf* and *hlyD*, and very good agreement (Kappa=0.886, p<0.001) between presence of *cnf* and *papGIII* and between *hlyD* and *papGIII*, (see Table 21). In the owner population, there was complete agreement between *cnf* and *sfa* (Kappa=1.000, p<0.001), and very good agreement between *cnf* and *hlyD* (K=0.936, p<0.001), and *hlyD* and *sfa* (Kappa=0.936, p<0.001). Within the control population

there was very good agreement between presence of *cnf* and *hlyD* (Kappa=0.870, p<0.001).

Comparison of Virulence Factor Results to Demographics

There were no differences in presence or absence of virulence factors based on dogs' sex, but isolates from male human participants were more likely to have *papGIII* than isolates from female participants (p=0.010). Presence or absence of virulence factors in canine and owner fecal *E. coli* was not associated with living in a single or multi-pet household, nor was it associated with owner's affiliation with the UTCVM or working in a human hospital.

Comparison of Virulence Factors Results to Dog-Owner Behaviors

No associations were found between presence or absence of virulence factors and amount of awake time spent together, sleeping in the same bed, kissing on the face, or dogs drinking from the toilet. Hand-washing after petting their dogs, before eating their own meals, or before feeding their dogs, was not associated with presence or absence of any of the 4 virulence factors in owners' fecal *E. coli***.** Hand-washing after petting their dogs was associated with *papGIII* in the feces of their dogs (p=0.003); in households where owners did not wash their hands after petting their dogs 16.7% of dogs' fecal *E. coli* had *papGIII*, whereas in households where owners did wash their hands, 0% of dogs fecal *E. coli* had *papGIII*. There was no association

between presence of virulence factors in dogs' fecal *E. coli* and whether or not owners washed their hands prior to feeding the dogs. In the subpopulation of owners who disposed of their dogs feces, hand-washing after disposal was not associated with presence or absence of virulence factors in either dog or owner's feces.

Comparison of Virulence Factor Results to UTI History

No association was found between having a history of UTI and presence or absence of any of the virulence factors in fecal *E. coli* for the human population as a whole. For women alone, history of UTI was associated with presence of *hlyD* ($p=0.048$) and *papGIII* ($p=0.026$) in fecal *E. coli*; however these would not be significant using the more stringent Bonferroni corrected threshold of $p \leq 0.0125$. No association was found between history of UTI and presence of any of the 4 virulence factors in fecal *E. coli* for either the entire dog population or the female dog subpopulation. History of recurring UTIs (reporting 2 or more UTIs) was associated with presence of *papGIII* in women's fecal *E. coli* ($p= 0.008$), but not in dogs' or female dogs' fecal *E. coli*. There was no association between *cnf, hlyD*, or *sfa,* and recurrence of UTI in any subpopulation.

An association was found between women's history of UTI and presence of virulence factors in their dogs' feces: *cnf* ($p<0.001$), *hlyD* ($p<0.001$), *sfa* ($p=0.007$), and *papGIII* ($p<0.001$). There was no association between a dog's history of UTI and

presence or absence of *cnf, hlyD, sfa*, or *papGIII* in their owner's fecal *E. coli*, whether including the entire dog population or just female dogs.

Comparison of Susceptibility, Clonality, and Virulence Factors

Comparison of Susceptibility and Virulence Factors

Mann-Whitney analysis was used to compare ordinal susceptibility results with presence or absence of virulence factors for 456 isolates, see Table 22. Resistance to cephalothin, kanamycin, nalidixic acid, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole was associated with decreased presence of virulence factors if a p-value threshold of 0.05 was used; however with the Bonferroni adjustment for multiple comparisons to $p \leq [0.05/(17*4)]$, or $p \leq 0.0007$, the only remaining association was streptomycin resistance with reduction of *sfa*.

Comparison of Virulence Factors to Percent PFGE Similarity

No significant associations were found between presence or absence of any of the 4 virulence factors in *E. coli* isolated from dogs and their owners and percent PFGE profile similarity. Presence of *papGIII* was closest to a significant association with percent PFGE similarity ($p=0.098$).

Comparison of Susceptibility to Percent PFGE Similarity

No associations were found between antimicrobial susceptibility to any of the 17 antimicrobial agents and percent PFGE similarity. Susceptibility data were collapsed from intermediate to resistant to increase likelihood of detecting a significant difference, and both cephalothin ($p=0.068$) and ciprofloxacin ($p=0.074$) approached a p-value of 0.05, but no associations were found with $p \leq 0.05$ or the Bonferroni corrected threshold of $p \leq 0.003$.

Comparison of Susceptibility within Shared E. coli Clones

Susceptibility patterns varied widely within a single clone of *E. coli.* One clone contained isolates with MDR and isolates susceptible to all antimicrobial agents tested. Isolates within 9/36 (25%) of shared *E. coli* clones had identical susceptibility patterns, while isolates within 27/36 (75%) of shared clones had different susceptibility patterns.

In 7/9 of the shared clones with isolates having identical susceptibility patterns, all isolates in the clones were susceptible to all 17 antimicrobial agents. In one clone, both isolates had intermediate susceptibility to cephalothin and were susceptible to all other antimicrobial agents. In the remaining clone, both isolates had identical MDR susceptibility patterns, with resistance to ampicillin, ciprofloxacin, and nalidixic acid. Two of the shared clones with identical susceptibility patterns

were shared between dog-owner pairs within households, while 7 were shared across households (see Figure 33).

Out of the 27 clones whose isolates had different susceptibility patterns, 8 clones contained an MDR isolate. Three of these clones had one MDR isolate and one isolate susceptible to all 17 antimicrobial agents. One across-household clone contained 5 isolates, with 2 canine isolates (ID#28A and ID#28B) susceptible to all 17 antimicrobial agents, 2 owner isolates (ID#112A and ID#112B) that shared an MDR pattern resistant to ampicillin, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole, and 1 owner isolate (ID#123B) that was MDR with resistance to ampicillin, kanamycin, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole (see Figure 34 and Table 23).

Comparison of Virulence Factors within 36 Shared E. coli Clones

Isolates within 28/36 (77.8%) shared *E. coli* clones had identical virulence factor patterns, while 8/36 (22.2%) clones had different virulence factor pattern results. Twenty-six of these clones had all negative virulence factor results, while 2 of these clones contained only *E. coli* isolates positive for all 4 virulence factors. The remaining 8 clones had mixed virulence factor results. Three of these clones had an isolate positive for all 4 virulence factors and an isolate negative for all 4 virulence factors.

Comparing Susceptibility and Virulence Factors within Shared Clones

Six of the 36 shared *E. coli* clones contained isolates that all had identical susceptibility patterns and identical virulence factor patterns within the clone. The remaining 30 clones contained isolates with either different antimicrobial susceptibility patterns or different virulence factor patterns. There was no agreement between having identical susceptibility patterns and having identical virulence factor patterns (Kappa = -0.087, p=0.355). Five of the 36 shared *E. coli* clones contained isolates with neither identical antimicrobial susceptibility patterns nor identical virulence factor patterns.

Further analysis of the 6 clones with all isolates having both identical susceptibility and virulence factor patterns revealed that none of these clones carried both antimicrobial resistant phenotypes and genes for virulence factors (see Table 24). Of these 6 clones, 2 clones had isolates had 4/4 virulence factors present and were susceptible to all 17 antimicrobial agents. Isolates in two clones had 0/4 virulence factors and were susceptible to all 17 antimicrobial agents. Isolates in 1 remaining clone had 0/4 virulence factors and had intermediate susceptibility to cephalothin but were susceptible to the remaining 16 agents, while isolates in the other remaining clone had 0/4 virulence factors and were resistant to ampicillin, ciprofloxacin, and nalidixic acid but susceptible to the remaining 14 agents. Of these 6 clones, 2 clones were shared dog-dog, 2 clones were shared dog-owner (both across-households), 1 clone was shared owner-owner, and 1 clone was shared owner-control. None of the 6 clones were shared within-household.

Discussion

E. coli Isolation

E. coli is one of the most common bacteria in the GI flora of human beings and dogs, and it was expected to be isolated from all fecal samples in this study; however *E. coli* was not isolated from 4/156 (2.6%) of the participants. It has been reported that a small percentage of fecal samples from healthy human beings and dogs do not yield *E. coli* or any coliforms, and that *Klebsiella* may be the predominant coliform in some individuals [121, 132]. Rather than *E. coli*, *Klebsiella pneumoniae* was isolated from 3 participants and *Serratia liquifaciens* was isolated from 1 participant in our study.

Numerous variables affect bacterial composition of the GI tract, including recent exposure to antimicrobial agents or probiotics, altered GI pH, GI pathogens or inflammation, stress, and dietary choices [3]. Two of the 4 participants who did not have *E. coli* isolated from their feces had received beta-lactam antimicrobial therapy within the past year, but none reported routinely consuming yogurt or other probiotics or having a vegetarian lifestyle. Although all reported to be healthy, stress or other physiologic changes may have altered their GI flora and decreased the prevalence of *E. coli*. Other possibilities such as incorrect sample collection, storage, or submission were also considered, although repeat fecal samples also failed to yield *E. coli*. Using microbiology techniques with increased sensitivity for detecting *E. coli* such as pre-enrichment media may have helped isolate *E. coli* from these 4 participants.

Antimicrobial Susceptibility

Susceptibility to Individual Antimicrobial Agents

In canine and human participants, susceptibility of fecal *E. coli* was lowest to ampicillin, cephalothin, and streptomycin. Our results were consistent with a large study by Srinivasan of phenotypic and genotypic antimicrobial resistance patterns in *E. coli* from many species of animals and human beings that found high resistance to ampicillin, cephalothin, and streptomycin, but susceptibility to amikacin, gentamicin, ceftriaxone, ciprofloxacin, and trimethoprim in isolates from both animals and human beings [133].

In the Srinivasan study, all ampicillin resistant strains carried the gene *ampC,* encoding a beta-lactamase [133]. Genotypic resistance testing was not performed in our study, but presence of *ampC* or an *ampC*-like gene encoding for extended spectrum beta-lactamase production, and spread via plasmids, could explain low ampicillin susceptibility. Beta-lactam therapy may have allowed selection pressure and cross-resistance to other beta-lactam antimicrobial agents as well. In total, 10 courses of penicillin, amoxicillin, and amoxicillin-clavulanic acid were received by human participants, and 7 courses of amoxicillin and amoxicillin-clavulanic acid were received by canine participants in the year prior to the study. Resistant *E. coli* from these participants may have contaminated and acted as a reservoir in the

environment and shared plasmids with genes for resistance with other pathogenic bacteria and normal flora, contributing to overall beta-lactam resistance.

As a first generation cephalosporin, cephalothin is susceptible to most beta-lactamases produced by *E. coli* strains and shared via plasmids; therefore, resistance is expected to be higher than for newer cephalosporins. Ceftiofur is the only extended spectrum cephalosporin approved for use in food animals in the US, and is approved for dogs but is not routinely used in this species; however ceftiofur is not approved for use in human beings. Resistance to ceftiofur was seen in 3/61 (5%) canine isolates and 2/61 (3%) owner isolates, but 0% control isolates. This suggests that transmission of ceftiofur-resistant *E. coli* may have occurred directly from dogs to owners, but only Household #58 had ceftiofur-resistant *E. coli* isolated from both dog and owner. Both owners with ceftiofur resistance had MDR *E. coli*, with concurrent resistance to other beta-lactam antimicrobial agents, suggesting that ceftiofur resistance was more likely due to selection pressure from beta-lactam exposure and extended spectrum beta-lactamases.

Cefpodoxime is a third generation cephalosporin that was new on the veterinary market at the time of the current study, and only 1 dog was reported to have taken a course of cefpodoxime prior to enrollment. Cefpodoxime has been available for use in human beings for a longer period, but no human participant reported receiving cefpodoxime therapy. Cefpodoxime-resistant *E. coli* isolates were found in 4/61 (7%) dogs and 3/61 (5%) owners, but in no controls. Although this suggests spread of cefpodoxime resistance between owner and dog, only Household

#58 had cefpodoxime-resistant *E. coli* isolated from both dog and owner. Cefpodoxime resistance is less likely due to selection pressure from specific cefpodoxime therapy or direct transmission, and more likely due to other beta-lactam use and acquired cross-resistance from mobile genetic units such as plasmids and transposons.

Imipenem was the only antimicrobial agent in the present study to which all *E. coli* isolates were susceptible. No canine or human participants were reported to have received imipenem prior to enrollment in this study. As a carbapenem, imipenem is a beta-lactam antimicrobial agent, but it is stable to most beta-lactamases and extended-spectrum beta-lactamases. Resistance to carbapenems occurs by mutations or acquired structural changes in penicillin binding proteins, metallo-beta-lactamases that can degrade carbapenems, and loss of outer membrane porins allowing changes in membrane permeability [134]. Its use is limited by bioavailability (requires parenteral administration), expense, and potential side effect of renal dysfunction and seizures. Although guidelines in both veterinary and human medicine suggest judicial use of carbapenems to minimize selection pressure, the author's clinical impression is that imipenem and meropenem are being prescribed with increasing frequency for resistant infections in dogs and cats. Resistance to imipenem will need to be monitored in the future if therapeutic use increases.

Although streptomycin is no longer widely used in clinical medicine, it is a naturally produced antibiotic, and natural exposure may maintain selection pressure and resistance. In the Srinivasan study, the genes *strA* and *strB*, encoding for

streptomycin phosphotransferase, and *aadA*, encoding for aminoglycoside adenyltransferase, were found in nearly all streptomycin resistant strains of *E. coli* [133]. Another study found a significant proportion of streptomycin resistant isolates carry the integrase gene *intI* for class-1 integrons [135]. Researchers at the University of Tennessee found that 50% of streptomycin resistance is plasmid-borne in *E. coli* (personal communication with Dr. Ann Draughon). Although molecular testing for presence of these genes was not performed in our study, low streptomycin susceptibility suggests presence of class-1 integrons as well as plasmids carrying genes for streptomycin resistance [136, 137].

Resistance to other aminoglycosides, including amikacin, gentamicin, and kanamycin, was less common in this study. No canine or human participant reported having received aminoglycoside therapy, suggesting selection pressure did not contribute to resistance. Lower kanamycin susceptibility (92-96%) may have been due to use of kanamycin resistance genes as selection markers in molecular genetics testing, which may increase the number of kanamycin-resistant *E. coli* in the environment.

Tetracycline resistance is a well-recognized problem in *E. coli* isolated from farm animals, and was seen in 3.8% of canine isolates and 9.3% and 10% of owner and control isolates in this study [138, 139]. Tetracycline resistance may be high in farm animals due to use for disease prevention, and because it has been shown that bacteria in soil can acquire tetracycline resistance from environmental exposure, possibly creating a reservoir [140]. No owners reported administering tetracycline or

doxycycline to their dogs within the year prior to enrollment. One owner received minocycline intermittently throughout the year for acne, and one control received a course of doxycycline 5 months prior to enrollment. Tetracycline resistance occurs by efflux pumps or alteration of the 50S ribosome to prevent binding, and resistance is often plasmid-mediated. Tetracycline resistance may also be carried on class-1 integrons, genetically linked to streptomycin and trimethoprim-sulfamethoxazole resistance genes, with selection pressure from sulfa antimicrobial use causing cross-resistance to tetracyclines. Other possible routes for acquiring tetracycline resistance could be resistant bacterial spreading through the food chain, contact through occupational exposure at the veterinary or human hospitals, or exposure to waste runoff from a food-producing facility.

Susceptibility to trimethoprim-sulfamethoxazole was highest for canine isolates (97.3%) and lowest for control isolates (85.6%). Although sulfa drugs have been available for a long time, are inexpensive, and have often been the first-line empirical choice for treatment of infections, no dog or owner had a history of receiving sulfa therapy, and only 1 control had received intermittent sulfa treatment over the year prior to enrollment. Veterinarians have become cautious with prescribing sulfa antimicrobials due to side effects including immune-mediated diseases. Resistance to sulfa drugs has increased within human UTI isolates, up to 34% of UPEC, making it less desirable as a first-line empirical choice [82, 83, 141]. Resistance to sulfa drugs can be carried on plasmids or class-1 integrons and spread horizontally through populations of *E. coli* and potentially other bacteria. The higher

resistance rate in control participants may be due to cross-resistance from plasmids or class-1 integrons carrying genes for resistance to other antimicrobial agents. Decreased sulfa use over time may result in decreased selection pressure and resistance.

Chloramphenicol is an older but effective antimicrobial agent that is not used frequently in veterinary medicine and was not reportedly received by any participants in this study. Resistance occurs by reduced membrane permeability, inactivation via an acetyltransferase *cat* gene, and less commonly by mutation of the 50S ribosome to decrease or prevent chloramphenicol binding. Chloramphenicol resistance is usually chromosomal but plasmids can concurrently carry resistance genes for chloramphenicol, ampicillin, co-trimoxazole, and tetracycline.

In the present study, susceptibility remained high (>92%) in all 3 populations for both nalidixic acid and ciprofloxacin. Nalidixic acid is the parent quinolone, active mainly against gram-negative bacteria, from which modifications were made to increase the antimicrobial spectrum creating fluoroquinolones such as enrofloxacin and ciprofloxacin. Enrofloxacin was created specifically for veterinary use and is metabolized to ciprofloxacin in dogs. Only one dog had a history of receiving a fluoroquinolone, enrofloxacin, for a UTI; omitting enrofloxacin from the panel of antimicrobial agents tested was a limitation of the study from a veterinary perspective, but it is not routinely monitored by NARMS. Six human participants had received a course of fluoroquinolones, each receiving ciprofloxacin, over the year prior to enrollment. As may also be true in human medicine, a recent epidemiologic

review has shown that increases in fluoroquinolone resistance among canine UTI isolates correlates with the increase in fluoroquinolone usage in veterinary medicine [84].

Fluoroquinolone resistance can occur by efflux pump (*AcrAB*) and chromosomal mutations in gyrase (*gyrA*) and topoisomerase IV (*parC* and *parE*). Plasmid-mediated conjugation plays a small role in spreading fluoroquinolone resistance, although *qnr* genes encoding fluoroquinolone resistance can be transferred on plasmids with extended-spectrum beta-lactamase-encoding genes, allowing for cross-resistance and selection pressure during administration of beta-lactams or fluoroquinolones [142]. Most fluoroquinolone resistance is from chromosomal mutations, and selection pressure plays an important role in maintaining this resistance.

Fluoroquinolones are being increasingly used as resistance has developed to more traditional antimicrobial agents, and ciprofloxacin is now reported to be the most used antibiotic in the world [86, 143]. In both dogs and human beings, treatment with fluoroquinolones has a profound effect of suppressing the normal aerobic flora, specifically eliminating coliforms [144, 145]. Gupta and colleagues showed that treatment with ciprofloxacin decreased prevalence of rectal colonization of *E. coli* in women treated for cystitis from 94% to <10% [146]. One study showed that with selection pressure from fluoroquinolones, but not without, introduced MDR *E. coli* can persist and shed for long periods in the GI tract of treated dogs [144]. Trott and colleagues suggest this may be one explanation for the occurrence of MDR

E. coli nosocomial infections in veterinary hospitals, because hospitalized dogs carrying MDR *E. coli* shed large numbers of drug-resistant fecal *E. coli* following treatment with fluoroquinolones and contaminate the hospital environment [144].

In this study, prior fluoroquinolone use by participants was unlikely to have affected their susceptibility directly. Only 1 participant had received a fluoroquinolone in the month prior to enrollment, an owner received topical ciprofloxacin for otitis, and neither that owner nor his dog had fecal *E. coli* resistant to ciprofloxacin or nalidixic acid. Although other participants had received fluoroquinolones in the past year, the effects of these antimicrobial agents are transient, and participants' normal GI flora would have returned within two weeks or certainly by the time of study enrollment [147]. Rather than direct selection pressure from individuals receiving fluoroquinolone treatment, fluoroquinolone resistance seen in this study may result from increasing trends of fluoroquinolone treatment in veterinary and human medicine causing increased shedding of fluoroquinolone-resistant *E. coli* into the environment allowing for community-acquired sharing.

Mobile Genetic Elements

Multiple antimicrobial resistance genes can be genetically linked on plasmids, integrons, or transposons, which can be spread and integrated between bacteria and various hosts. Although not completely understood, these genetic linkages may help

explain commonalities found in antibiogram patterns, within-household vs. across-household susceptibility comparisons, and multiple drug resistance.

Plasmids carrying genes for resistance to multiple antimicrobial agents have been identified in fecal *E. coli* from human beings, dogs, and food-producing animals, and plasmids can transfer resistance between bacteria either within a host's GI flora or in the environment [136, 137]. Conjugation studies have found that 60-64% of fecal *E. coli* isolates from dogs were able to transfer resistance on plasmids, and that transfer of ampicillin, streptomycin, and tetracycline occur readily, but transfer of chloramphenicol and fluoroquinolones occur less frequently [136, 137].

The most commonly recognized reservoir for plasmids is bacteria within the GI tracts of food-producing animals [137]. *E. coli* and plasmids may spread from this reservoir to other hosts by direct spread to human beings and animals on the farm, environmental contamination and spread through using fecal material as fertilizer, or by contamination of the food supply. Companion animals and human beings may share plasmids due to close association with each other. Within-household sharing of plasmids is a possible explanation for why no differences were found between dog and owner susceptibility results in this study, whereas dog and control participants had significant differences, with control isolates more resistant than canine isolates.

In addition to plasmids, integrons play a major role in the epidemiology of resistance to antimicrobial agents, especially streptomycin and sulfonamides [135]. A study compared antimicrobial resistance patterns and prevalence of integrons in feces of animals with varying degrees of contact with human beings [148]. Isolates from

animals free of human exposure had no antimicrobial resistance, and there was a steady increase in resistance seen as exposure to human beings increased [148]. Class-1 integrons were not seen in isolates from wild animals but were seen in isolates from farm animals (7% prevalence), pet dogs (16% prevalence), and people (16% prevalence) [148]. Lack of a control group of wild dogs without exposure to human beings and not testing for presence of integrons were limitations of the present study. Regardless, this suggests that finding no differences in the susceptibility results from dogs and owners may be due to sharing of integrons or other resistant genetic material in the direction of human beings to dogs, rather than dogs to human beings. This would also be consistent with finding that human beings had more overall MDR isolates than dogs, and that risk of potential transfer would therefore be in the direction of human being to dog more so than dog to human being.

Analysis of Antibiograms

The most common antibiograms for concurrent resistance in human being *E. coli* isolates were to ampicillin and trimethoprim-sulfamethoxazole, followed by ampicillin and streptomycin, ampicillin and tetracycline, and then ampicillin, streptomycin, and trimethoprim-sulfamethoxazole. Ampicillin, tetracycline, and streptomycin are known to be spread via plasmids, while tetracycline, streptomycin, and trimethoprim-sulfamethoxazole may be linked genetically on class-1 integrons. Six control isolates also had concurrent resistance to ciprofloxacin, nalidixic acid, and

trimethoprim-sulfamethoxazole. Genes for fluoroquinolone resistance are usually chromosomal mutations (*gyrA*) rather than acquired by a mobile genetic element, so this finding may be caused by coincidental mutations concurrent with a class-1 integron carrying sulfa resistance.

Antibiogram analysis showed the most common concurrent resistance in dogs was to ampicillin and cephalothin; however this occurred in only 4.4% of all canine isolates. There was a large number of isolates with intermediate susceptibility to both ampicillin and cephalothin, explaining the low susceptibilities yet infrequent resistance patterns for these antimicrobial agents. Many canine isolates were fully susceptible or were only resistant to one antimicrobial agent, which is why antibiogram patterns had low prevalence in canine isolates. A similar study of fecal *E. coli* in dogs found the most common antibiogram for concurrent resistance was to ampicillin, streptomycin, and tetracycline; however no cephalosporins were included in that study [136]. These data are consistent with suspected genetic linkage of ampicillin, streptomycin, and tetracycline on plasmids, providing cross-resistance to all 3 antimicrobial agents [136, 137].

Comparison of Susceptibility between Dogs and Owners

No differences were found in antimicrobial susceptibility results of *E. coli* isolated from dog and their owners to any of the 17 antimicrobial agents, even when data was collapsed to eliminate intermediate results. These results could be from a

small sample size and low power in the study; increasing the number of participants or number of isolates per participant may have identified significant differences if they existed. Another explanation would be that dogs and owners directly share *E. coli* strains or mobile genetic elements with genes for antimicrobial resistance, leading to similar susceptibility profiles within households. Alternatively, conjugation may occur in reservoirs within their households, and both dog and owner may be exposed to similar plasmids carrying genes for resistance.

In contrast, a study comparing fecal *E. coli* from pig farmers and their pigs found the prevalence of antimicrobial resistance to be significantly lower in *E. coli* from farmers than from their pigs [149]. Swine isolates were more resistant to chloramphenicol, nitrofurantoin, oxytetracycline, streptomycin, and sulfamethoxazole than isolates from farmers [149]. Pigs may have *E. coli* with more resistance due to greater selection pressure from prophylactic antimicrobial use and from living in fecal-contaminated barns where opportunity for environmental plasmid-conjugation is high. Pigs and farmers may have fecal *E. coli* with different susceptibility results compared to dogs and owners due to the differences in their human-animal relationship and opportunity for sharing bacteria and mobile genetic elements.

Comparison of Susceptibility between Owners and Controls

No differences were found in the susceptibility results of *E. coli* isolates from owners and controls. This could be a result of a small sample size and power.

Alternatively, it suggests that ownership of a healthy dog does not increase antimicrobial resistance of a human being's fecal *E. coli,* nor does living with a human being increase the antimicrobial resistance of a dog's fecal *E. coli*. This finding would have important public health implications, especially as concerns arise regarding antimicrobial-resistant bacteria being isolated from pets [95, 100, 106, 120, 136]. Dogs provide psychological and physiological benefits to their owners, especially the elderly and immunosuppressed, and these results may help support the claim that the risk of zoonotic transmission of bacterial disease is minimal [150]. Regardless, proper hygiene recommendations such as washing hands should be followed by all pet owners, especially in households with young children, elderly, or immunosuppressed adults.

Comparison of Susceptibility between Dogs and Controls

Although canine and owner isolates had similar susceptibility profiles, control isolates were more resistant than canine isolates to ampicillin and trimethoprim-sulfamethoxazole. One theory to explain this is differences in antimicrobial prescription selection in veterinary medicine versus human medicine. Veterinarians rely on beta-lactam antimicrobials such as ampicillin but are cautious with sulfa drugs due to side effects of immune-mediated disease. There was no difference in ampicillin prescribed to dogs versus controls in this study, but one control had received numerous courses of sulfa therapy throughout the year (including during the

month prior to enrollment) whereas no dog had received sulfa therapy. Although selection pressure was likely a factor in this control who did have MDR *E. coli* resistant to trimethoprim-sulfamethoxazole, there were also 4 other controls (total 5/30, 17%) with *E. coli* isolates resistant to trimethoprim-sulfamethoxazole, whereas only 3/61 (5%) of dogs had *E. coli* isolates resistant to this agent.

A second theory to explain more resistance in control isolates compared with canine isolates is that enrolled dogs are younger than the control population and likely had been exposed to fewer antimicrobial agents over their lifespan. While this may be true, review of antimicrobial history within a month or year of enrollment could not link antimicrobial exposure and selection pressure to differences in resistance between species. This theory does not explain why dogs and their owners had no differences in susceptibility results, since owners were also older than their dogs and had opportunity for more lifetime exposure to antimicrobial agents and environmental reservoirs. A third theory is that there was sampling bias from the small number of controls enrolled in the study (N=30). It remains unclear why control isolates were more resistant than canine isolates.

Multiple Drug Resistance

Both owners and controls had significantly more MDR *E. coli* isolates than dogs in this study. One potential bias is that 27.8% (17/61) of owners and 20% (6/30) of controls worked in veterinary clinics, and 18% (11/61) of owners and 30% (9/30)

of controls worked in human hospitals, allowing for possible occupational exposure to MDR strains. It is unknown whether sampling the general public would have yielded the same results. Risk factors for carrying MDR isolates include immunosuppression and treatment with broad-spectrum antimicrobials, but there were no differences between the 3 groups for history of immunosuppressive disease or antimicrobial treatment history during the past year [151]. Human beings in this study may have carried more MDR *E. coli* because of differences in selection pressure from antimicrobial therapy or environmental exposure.

Among MDR isolates, resistance patterns varied between species, with canine MDR isolates showing significantly more resistance than human being MDR isolates to beta-lactam antimicrobial agents. This may reflect veterinarians' tendencies to choose beta-lactams as first-line treatments for antimicrobial therapy [100]. Twenty-five percent of dogs with MDR *E. coli* received a beta-lactam, amoxicillin-clavulanic acid, in the month prior to enrollment, compared with 6.7% of human beings with MDR *E. coli* that received a beta-lactam, also amoxicillin-clavulanic acid, in the month prior to enrollment. Canine MDR *E. coli* isolates were more resistant to amoxicillin-clavulanic acid than human being MDR isolates. These data support previous studies suggesting use of beta-lactam antimicrobial agents is one contributing factor in the emergence of antimicrobial-resistant *E. coli* [138, 139].

MDR isolates from human beings had highest resistance to ampicillin and trimethoprim-sulfamethoxazole, with 100% of control MDR isolates showing trimethoprim-sulfamethoxazole resistance. This may have been selection error due to

a relatively small sample size, but only 20% of controls with MDR *E. coli* received sulfa therapy in the month or year prior to enrollment, compared with none of the dogs or owners.

Prevalence of MDR *E. coli* in fecal flora of dogs varies with sample population and stress level of dogs. A study sampling healthy dogs from a humane society found 79% of fecal *E. coli* had MDR; dogs were not receiving antimicrobial therapy, and MDR was defined as resistance to 3 or more antimicrobial agents [136]. In another study, healthy dogs living in breeding kennels had 13% MDR fecal *E. coli* isolates, whereas healthy dogs living with individual owners had only 2% MDR fecal *E. coli* isolates [94]. These data are consistent with our finding of 4% of canine fecal *E. coli* isolates having MDR. Dense living conditions in shelters and breeding facilities may increase fecal sharing of bacteria and genes for resistance, via exposure to feces and grooming. Stress also plays a role in increasing shedding of resistant fecal *E. coli* [89]. This may explain the higher prevalence of MDR in these populations compared to individually owned dogs. Good hygiene, separation of animals, and stress reduction in kennels and shelters may help minimize further spread of antimicrobial resistance in these locations.

MDR *E. coli* was isolated from both dog and owner from two separate households. Household #2 had a dog ID#6 with a history of UTI and frequent amoxicillin use; selection pressure and extended-spectrum beta-lactamases may explain cross-resistance to all beta-lactams tested except imipenem in this dog. The dog shedding resistant *E. coli* into the household environment could explain MDR

isolates found in its owner ID#66, however the MDR isolate from the owner was clonally different (77% PFGE similarity) than the dog's and had a different susceptibility pattern, with resistance to ampicillin, ciprofloxacin, and nalidixic acid but susceptibility or intermediate susceptibility to all other beta-lactams. There was no reported fluoroquinolone use by the owner or any household dogs, making direct selection pressure less likely to explain fluoroquinolone resistance. Alternatively, cross-resistance would be possible through transmission of a plasmid carrying both the *qnr* genes encoding fluoroquinolone resistance and extended-spectrum beta-lactamase-encoding genes, allowing selection pressure during administration of beta-lactams [142].

Household #58 involved a dog ID#64 and owner ID#124 with MDR *E. coli* isolates resistant to 9 and 10 antimicrobial agents. The owner had finished an empirical course of amoxicillin-clavulanic acid 24 days prior to enrollment, but neither dog nor owner had any further antimicrobial treatment history. Both the dog and owner MDR isolates in this household had cross-resistance to beta-lactams (except imipenem). The canine *E. coli* was also resistant to streptomycin and trimethoprim-sulfamethoxazole, while the owner's *E. coli* had additional resistance to aminoglycosides (gentamicin and kanamycin), and both ciprofloxacin and nalidixic acid. Increased fecal shedding of resistant strains of *E. coli* during treatment with beta-lactams, followed by contaminating the household and fecal-oral exposure is one mechanism to explain the dog's acquisition of MDR *E. coli*. This should result in identical clones of *E. coli* isolated from both owner and dog, as measured by PFGE

and susceptibility testing. Since these isolates shared only 80% PFGE profile similarity, direct transmission of *E. coli* was less likely. Alternatively, plasmids containing genes for beta-lactamases and extended-spectrum beta-lactamases may have been transmitted between dog and owner explaining the common cross-resistance to beta-lactam antimicrobial agents.

Selection Pressure and MDR Isolation

The association found between a participant's history of receiving antimicrobial therapy within 30 days of enrollment and isolation of fecal MDR *E. coli* supports the theory of selection pressure leading to resistance. Antimicrobial use is considered by some to be the most important factor in the emergence, selection, and dissemination of antibiotic-resistant bacteria [152]. This includes antimicrobial use for treatment of infections in human beings, companion animals, or livestock, or for growth promotion or disease prevention in livestock. A study in Mexico found 20% of fecal *E. coli* isolates from children were resistant to 5 or more antimicrobial agents, and resistance rate was higher in children who had received antimicrobial therapy within 60 days of enrollment [153]. In healthy swine, herds that receive the most antimicrobial therapy also had the highest number of resistant fecal nonpathogenic *E. coli* [154]. Schroeder suggests using tetracycline derivatives, sulfa drugs, cephalosporins, and penicillins, either therapeutically or preventatively, may be a key driving force in the selection of antimicrobial resistance in *E. coli* [138, 139].

Without antimicrobial selection pressure, MDR *E. coli* do not compete well with resident coliforms; MDR isolates are less prevalent and are shed for briefer periods from dogs without antimicrobial treatment than from treated dogs [144].

In this study, no significant associations were found between history of recent antimicrobial therapy in other pets or children and isolation of MDR *E. coli* from participants. Isolates that have undergone selection pressure due to antimicrobial therapy in these housemates' GI tracts may become resistant, shed, and pose a public health risk to others. Further investigation in this area is needed.

Summary of Antimicrobial Susceptibility of Dogs, Owners, and Controls

No differences were found between the susceptibility results of dogs and their owners, suggesting they may share genes for antimicrobial resistance. An obvious manifestation of this occurred in the two households with MDR *E. coli* isolated from both dog and owner. In these households, PFGE profiling proved the isolates were not clonal, suggesting that plasmids or other mobile genetic elements were responsible for spreading resistant genes between the two hosts.

Overall, dogs had fewer MDR isolates than human beings. Not all households with MDR isolates from owners had MDR isolates from dogs, so horizontal gene transfer within the household environment is not 100% efficient. Owners had a similar number of MDR isolates to controls, suggesting that dog ownership did not increase risk of harboring resistant *E. coli*. Alternatively, as has been suggested by

Sannes, human beings may be more likely to spread MDR *E. coli* to dogs than dogs are to spread MDR *E. coli* to human beings [106].

Comparison of Antimicrobial Susceptibility to History of UTI

Fifty-two percent of women participants reported being treated for at least one UTI in their past, and 75% reported multiple episodes of UTI. Thirteen percent of dogs had a history of UTI with a 50% recurrence rate. History of UTI was positively associated with antimicrobial therapy within 30 days and 1 year prior to enrollment into the study. UTI is a common indication for prescribing antimicrobial therapy, which may cause selection pressure allowing survival of resistant *E. coli* in the GI tract. Women with a history of UTI had fecal *E. coli* with increased resistance to ampicillin, but dogs with UTI history had fecal *E. coli* with increased resistance to ciprofloxacin and nalidixic acid. This may reflect antimicrobial therapy choices by physicians and veterinarians, however both beta-lactams and fluoroquinolones are prescribed commonly by both types of clinicians [84, 143, 146]. Cooke's study of enrofloxacin resistance in canine UPEC found the increase in resistance paralleled increase in fluoroquinolone prescriptions from their pharmacy during the same time period [84]. A study of UPEC from dogs with indwelling catheters by Ogeer-Gyles found resistance to ampicillin and cephalothin was most common and may be related to antimicrobial use both within the ICU and prior to admission [95]. Although beta-lactams and fluoroquinolones continue to be used for treating UTIs and other

infections, clinicians should recognize the selection pressure these antimicrobial agents may induce and prescribe them judiciously [144].

Comparison of Antimicrobial Susceptibility to Dog-Owner Behaviors

An association was found between feeding dogs raw meat and isolation of cephalothin-resistant *E. coli* from dogs. Commercial raw diets designed for dogs and cats can contain zoonotic pathogens including *E. coli*, *Salmonella,* and *Clostridium*, but feeding bones and raw meat can also contain *E. coli* 0157:H7 and *Campylobacter* [155]. These bacteria may be resistant to various antimicrobial agents or contain plasmids with genes for resistance. Raw diets not intended for human consumption may also contain antimicrobial residues that could induce selection pressures within the dogs' GI tracts. Food-producing animals most often carry *E. coli* resistant to sulfamethoxazole, tetracycline, and streptomycin, but resistance to beta-lactam antimicrobials is less consistent [133, 138, 139]. One study found 54% of *E. coli* isolates from swine were resistant to cephalothin; therefore, if contamination of pork with GI contents occurred during processing, feeding a dog raw bacon or other pork products may increase risk of cephalothin-resistant *E. coli* [138].

In this study, several dogs reportedly ate raw deer meat. One study found 11.8% of fecal *E. coli* isolated from farmed deer were resistant to cephalothin [152]. It is unclear how deer have acquired cephalothin-resistant *E. coli*. Explanations include contact with natural *Cephalosporium* molds in the wild, or contact with feces

of livestock containing resistant *E. coli*. Dogs who eat raw deer meat and their GI contents may consume cephalothin-resistant *E. coli*, thus influencing their own fecal flora.

Feeding "people food" to dogs may involve hand-feeding dogs, sharing plates or utensils, or placing food normally intended for human consumption into dog bowls. Hand-feeding or sharing plates or utensils may increase risk of sharing bacteria or mobile gene elements between species. Paired dogs and owners had similar susceptibility results to ciprofloxacin (overall susceptibility was 98.4% in dogs vs. 96.2% in owners), but dogs who were fed "people food" had fecal *E. coli* with more resistance to ciprofloxacin than those not fed "people food" (4% vs. 0%). Susceptibility results of owners' fecal *E. coli* were not associated with feeding or sharing "people food" with their dogs. The association between ciprofloxacin-resistant *E. coli* in dog's fecal samples and consuming people food may be due to sharing of ciprofloxacin-resistant *E. coli* from owner to dog during these behaviors.

Hand-washing is an important way to minimize potential spread of bacteria. An association was found between owners who did not wash their hands after petting their dogs and increased owners' fecal *E. coli* resistance to ampicillin. Petting dogs provides direct contact between two individuals and provides a mechanism for transfer of bacteria or mobile genetic elements from one host to the other, in either direction. During grooming, dogs may spread fecal *E. coli* throughout their hair, exposing owners to fecal *E. coli* while petting them. In this study, canine fecal *E. coli* had lowest susceptibility to cephalothin, streptomycin, ampicillin, and amoxicillin-

clavulanic acid; sharing genes for resistance for these antimicrobials through petting is a plausible explanation for this finding.

Hand-washing before meals may reduce transmission of resistant bacteria or mobile gene elements acquired from contact with dogs or other environmental sources and spread via hands to the owner's oral cavity and GI tract. Owners who did not wash before meals had *E. coli* isolates with increased resistance to chloramphenicol, ciprofloxacin, and nalidixic acid. Although these resistant-*E. coli* could have been acquired from multiple environmental sources, it is possible this increased resistance was acquired through contact with their dogs and may have been avoided by washing their hands after contact with their dogs and before meals. Similarly, owners washing their hands prior to feeding their dogs may decrease transmission of resistant bacteria or resistance genes from owners to dogs.

Toilets may be a reservoir for resistant bacteria and genes for antimicrobial resistance originating from the feces of human beings. Dogs that drank out of the toilet had *E. coli* with increased resistance to ciprofloxacin and nalidixic acid. This suggests fecal-oral transmission of ciprofloxacin-resistant *E. coli* from owners' feces to their dogs, via toilet water. Fluoroquinolone resistance is usually chromosomal, suggesting that dogs may be exposed to resistant bacteria, rather than plasmids or transposons, while drinking toilet water. In this study, owners were asked not to clean toilets prior to sample collection, so that a sample representative of a typical day could be acquired. It is unknown if the cleanliness of household toilets of participants in this study are representative of the general public. Regardless, these

results suggest that dogs may be at risk of acquiring fluoroquinolone-resistant *E. coli* from drinking toilet water, and we recommend owners discourage their dogs from drinking from toilets.

Environmental Reservoirs for Resistant E. coli and Resistance Genes

Outside of the household, resistant *E. coli* have been isolated from many environmental sources such as human septage, rivers, wildlife, and farm environments [152, 156-158]. Spread of resistance genes via plasmids in a fecal-infested environment and fecal-oral ingestion may be as important as conjugation occurring within the GI tract [90]. Similar to our findings, highest resistance in these environmental *E. coli* was to cephalothin, streptomycin, tetracycline, and sulfisoxazole [152]. In one study, cephalothin resistance was higher in isolates from farm environments (manure storage facilities and animal housing areas) than from livestock fecal isolates, and water samples were only resistant to cephalothin [152]. Exposure to rivers, wildlife, and farm animals was not asked on our questionnaire, but it is known that some participants and their dogs spent time in the Smoky Mountains near streams and wildlife, and some participants affiliated with the UTCVM may have had exposure to livestock or live on farms. These environmental areas may have been a common source of exposure for shared *E. coli* strains or mobile genetic elements between participants in this study.

Contaminated water supplies may be an important reservoir and source of common exposure to resistant *E. coli*. Specifically, 18% of *E. coli* isolates from irrigation water and sediments were resistant to cephalothin, and 16-96% of gram-negative bacteria from water samples across 22 US rivers were resistant to cefotaxime [156, 157]. Integrons have been identified in *E. coli* isolated from irrigation water and sediments and likely play an important role in maintaining and spreading resistance in the environment [157]. In our study, potential sites of contaminated water supplies could have included the Tennessee River or the Veterinary Teaching Hospital bathroom sinks. One caveat is that if a contaminated water supply was a major reservoir for our participants, we would expect to see one or few common clones shared by many participants rather than a wide array of clones in the population.

The human food chain could be a source of common exposure for resistant *E. coli* strains or plasmids. Antimicrobial agents are used in food-producing animals for growth promotion, prophylaxis, and treatment of infections. Strict regulations are enforced by the USDA requiring antimicrobial withdrawal prior to slaughter to prevent antimicrobial residues from entering the human food chain. Contamination has been minimized by improving hygiene at slaughterhouses and ensuring that GI contents do not mix with meat. This study did not inquire about human being dietary habits but also did not find a single clone consistent with a typical food-borne outbreak.

Future Strategies for Minimizing Resistance

Our results show that antimicrobial resistance occurred even in fecal *E. coli* from healthy human beings and dogs. Supporting existing programs and implementing new strategies may help slow the progression of antimicrobial resistance. On the national level, in 2001 the Center for Disease Control (CDC) organized the Public Health Action Plan as a task force of 11 governmental agencies, with a goal of decreasing unnecessary antimicrobial prescriptions, to decrease selection pressure and development of resistance in both the medical and veterinary professions (www.cdc.gov/drugresistance). On the local level, many human hospitals and some veterinary hospitals have developed antimicrobial use guidelines, both to help clinicians select appropriate antimicrobials and to restrict use of certain antimicrobials such as vancomycin [101]. These guidelines are only as effective as they are appropriately developed and followed. Each hospital should create a committee to keep up-to-date with the Public Health Action Plan recommendations and current literature, to amend the hospital's antimicrobial use guidelines as needed, and to be available to clinicians for consultation on antimicrobial selection.

Clonal Analysis of *E. coli* Isolates

Distribution of Clones

A wide array of 205 *E. coli* strains was isolated from participants enrolled in this study. Eighty-two percent of these clones were identified from only 1 host.

These findings agree with results from a similar study performed concurrently by Johnson et al. which enrolled 63 households with a total of 152 human beings and 76 pets (mainly dogs and cats) [159]. In their study, 335 unique fecal *E. coli* clones were identified, 73% were identified from 1 host only [159]. These data suggest there is a great variety of *E. coli* strains shed in the feces of healthy human beings and dogs, as well as other pets, into the environment. This diversity of clones may be beneficial allowing immunity to form to *E. coli* strains in both animals and human beings.

Thirty-six clones (17.6%) were shared between hosts either within or across-households, but prevalence data showed within-household sharing occurred more often (9.8%) than across-household sharing (0.26%). In Johnson's study, 27% of clones were identified from 2-11 (median 2) hosts, and they too found significantly more within-household sharing (27%) than across-household sharing (0.8%) [159]. Johnson's study included more isolates (up to 20) from each participant, as well as including multiple human beings and pets from one household, allowing higher sensitivity for finding bacterial sharing; this may explain the higher within-household prevalence found in their study [159]. Despite this difference, both studies provide evidence that bacterial sharing was more a household-specific occurrence than an across-household phenomenon.

In our study, 31.6% of participants shared an *E. coli* clone with another participant, but human beings were as likely as dogs to share an *E. coli* clone with another participant. This occurred within households between dogs and their owners, and across households between participants with no known contact. Of across-

household clonal sharing pairs in our study, sharing occurred most often between unpaired dogs and owners (30%) followed by sharing between 2 dogs in separate households (23%). Johnson's study found 52% of participants shared a clone with another participant, and his data corroborated that strain sharing was statistically no more frequent for pets (52%) than for human beings (53%) [159].

Despite nearly 1/3 of participants sharing a clone with another participant, no clone was shared by more than 3 participants. Rather than one or several clones of *E. coli* being shared between the majority of participants, there were many unique clones being shared by 2-3 participants each. Many of the participants sharing *E. coli* clones came from different households, and the route of bacterial sharing for these participants remains unknown. Although participants' occupations may provide exposure to common environmental reservoirs of *E. coli*, no one clone was seen in a large proportion of participants as would be expected from a point source, such as a specific location from within the UTCVM.

Analysis of dendrograms did not identify any large clusters of related *E. coli* strains that could be traced to one species (dogs vs. human beings) or occupational exposure (UTCVM vs. Department of Genetics). When the dendrogram was analyzed for groups with 4 or more isolates having >90% PFGE similarity, 17 groups of *E. coli* were identified with 2-5 hosts each (median 3 hosts). These groups included mixed participants from all 3 populations of dogs, owners, and controls, with mostly across-household sharing of similar *E. coli* strains. The dendrogram and group analysis suggest that while there is a very large variety of *E. coli* found in the

feces of human beings and dogs, some genetically similar strains of *E. coli* are shared by individuals with no known contact. Further investigation into how this sharing occurs and how to minimize its occurrence is warranted.

Across-Household Sharing

Thirty clones were identified that represented across-household sharing, occurring between all combinations of unpaired participants. Occupational contact may be a contributing factor, but only 1/3 of the human-human sharing occurred between people working in the same location. The majority (5/9) of unpaired dog and owner sharing occurred between participants associated with the UTCVM, including the unpaired dog-owner clone with 100% PFGE profile similarity. Many staff and students bring their dogs to the UTCVM for medical care, dog baths, and boarding. It is possible that either direct contact may have occurred between participants during these visits, or indirect contact through exposure to common reservoirs within the college such as cages or bathtubs. Common exposure may also occur between unpaired dogs and human being participants outside of the UTCVM if their owners socialize at dog parks or other community locations. Other environment sources such as water supply, the food chain, or spreading of *E. coli* strains throughout the community by birds or insects may also play a role in the epidemiology of across-household sharing, but further investigation is required in this area.

Within-Household Sharing

Only one dog-owner pair in this study shared *E. coli* with 100% PFGE profile similarity, but within-household clonal sharing was found with 9.8% prevalence when defined as two strains sharing >94% PFGE profile similarity. Clones are defined in the literature in various ways, including % PFGE profile similarity calculated by similarity matrices as was performed here, or by number of bands in common between strains [84, 159, 160]. For this study, visually comparing differences in number and location of bands between each profile would have been very subjective and time-consuming with the number of PFGE samples included; this technique is more practical for studies with few samples. Comparing samples by similarity matrices was more objective for this study, and the 94% cutoff was chosen to be conservative and consistent with current literature in this field [159]. Interestingly, when the more liberal definition of >90% PFGE profile similarity was used to identify similar strains, 21.3% of dog-owner pairs had similar *E. coli* strains, suggesting that definition of sharing is an important factor, and that by increasing the number of participants and power in the study a higher prevalence of sharing may be found.

Although our study only evaluated within-household fecal *E. coli* sharing between dog and owner, Johnson's study included all household family members and pets and found that within-household sharing was highest between pet-pet (22/38, 58%), followed by human-human (47/154, 31%), and pet-human (31/179, 17%)

(Johnson). The highest sharing prevalence was between cat-cat (82%), followed by dog-dog (79%), adult-child (34%), child-child (33%), and adult sexual partners (31%) (Johnson). These results support within-household bacterial transmission more so than point source acquisition from an environmental reservoir in the household. Sharing of strains between similar species of pets most likely occurs during fecal-oral spread of bacteria during grooming. Sharing of bacteria between adults and children may occur during diaper changes, assisting with bathroom visits, or laundry, and sharing between children most likely occurs due to poor hygiene habits. Sexual activity between heterosexual or homosexual adults has been recognized as a potential route of transmission or sharing of *E. coli* clones [110-112]. Further investigation of bacterial sharing including all household members, human beings and pets, will be important to develop recommendations for minimizing bacterial transmission.

In Johnson's study, pet-human sharing was more common with cats (12/47, 26%) than with dogs (19/115, 17%) [159]. Indoor cats can spend many contact hours with owners each week, including sleeping in the same bed. Cat hair could be contaminated with fecal bacteria after grooming, and shedding could spread it throughout the household. Petting could directly transfer contaminated hair to human beings. Cleaning of the litter box would also be a potential source of bacterial sharing. A limitation of our study was not including cats; however, a similar study investigating cross-species sharing of bacteria between cats and owners is proposed. A detailed questionnaire would help to determine the differences in the relationships

and human-animal bond between cat and owner versus dog and owner and how this may relate to cross-species bacterial sharing.

Age of owner also was a factor in cross-species sharing in the Johnson study, with a higher prevalence of sharing between cats and adult human beings (12/36, 33%) than cats sharing with children (0/11, 0%) [159]. Children are closer to the ground and tend to have less stringent hygiene habits, and so it is expected that they would have increased bacterial sharing with pets. However, while children may have increased direct contact with dogs, they may have less direct contact with cats that can be more aloof.

Human-Animal Bond and Within-Household E. coli Sharing

Dog ownership continues to increase nationally, with 37.2% of US-households owning at least one dog in 2006, compared with 31.6% in 1996 [161, 162]. With this increase in ownership is a concurrent increase in the perceived strength of the human-animal bond, with 99% of owners considering the pets to be part of the family [104]. Increased dog ownership and closeness of relationship between dogs and owners creates concern of risk of bacterial sharing.

Dog owners in this study were more likely to purchase their dogs from a breeder (41%) than the average US dog owner (27%) [163]. One study showed that owners who purchased their dogs had stronger bonds with their dogs than owners who acquired their dogs at no cost from family or friends [163]. This increase in

bond may translate to increased direct contact and potential for bacterial sharing; however dog owners in this study who purchased their dogs from breeders did not have significantly more sharing of fecal *E. coli* based on % PFGE similarity than owners who acquired their dogs from other sources.

Based on a national survey, dog owners spend an average of 45.3 hours per week with their dog, and 40% of dog owners spent >30 hours per week with their dogs [163]. Results of this study were slightly higher, with 54% of owners spending >30 hours of awake-time with their dog per week and 9.8% of owners spending ≤10 hours of awake-time per week with their dog; however, awake-time spent with dog was not associated with sharing of fecal *E. coli* based on % PFGE similarity ($p=0.779$).

That same study showed that female owners were more bonded with their dogs than male owners [163]. Our study had an uneven distribution of female (51) and male (10) owners, which reflects the general population of veterinary students, who made up 60% of the human participants in this study. Although many indicators of strength of the human-animal bond used in their study were different than the behaviors asked in our relationship, time spent awake with dog was assessed in both studies. In the general population survey, women were found to spend more time with their dogs, while in our study, male owners spent significantly more awake-time with their dogs than female owners ($p=0.036$). Other behaviors suggesting a strong human-animal bond, such as sleeping in the same bed and kissing on the face were not different between male and female owners in our study.

Fifty-nine percent of US pet-owning households own >1 pet, and households with dog, cats, or both, own on average 2.48 pets [163], while in this study 82% of dog-owning households had >1 pet, with an average of 3.23 pets per household. This high percentage of multi-pet households would be expected for the population of veterinary students, staff, and faculty that were recruited from for this study, and our study did find more multi-pet households from participants working or studying at the UTCVM than those participants recruited outside of the college ($p=0.044$). However, neither number of pets in household nor affiliation with the UTCVM was associated with within-household sharing of fecal *E. coli* between enrolled dogs and owners based on % PFGE profile similarity.

Johnson's study enrolled all members from 63 households, isolating fecal *E. coli* from 152 human beings and 76 pets, and found that within-household sharing of *E. coli* increased linearly with household size [159]. Households with 6 or more individuals (including human beings and pets) had 100% probability of clonal sharing of fecal *E. coli* [159]. In that study, pet-pet sharing (58%) of fecal *E. coli* occurred more commonly than human-human (31%) sharing or human-pet (17%) sharing, and 72% of households with ≥1 pet had evidence of pet-pet sharing of clonal *E. coli* [159].

Increased household size may result in increased direct contact between individuals, as well as common exposure to potential environmental reservoirs of *E. coli* such as bathrooms. High prevalence of pet-pet sharing of *E. coli* clones is likely from fecal oral ingestion after sniffing rectums, grooming, and coprophagy.

Alternatively, human-human sharing is most associated with sexual active adults, but may also occur as fecal-oral spread during diaper changes, and between kids or adults who do not wash their hands after using the restroom and proceed to contaminate their environment [110, 111].

Sharing of bacteria between dogs and owners within households may occur by direct contact or contaminated environment reservoirs, and certain behaviors hypothesized to pose increase risk included: sleeping in the same bed, kissing on the face, sharing of food, handling dog's feces, and dogs drinking from toilets. Although sharing food, handling feces, and drinking from the toilet were associated with susceptibility results, none of these behaviors was associated with increased within-household sharing of fecal *E. coli* based on PFGE profiling. There may be no true association present or these results may be from low sample size and low number of isolates sampled per participant.

The risk of cross-species bacterial transmission during sleeping in the same bed may be low under most circumstances, but risk may increase if the owner or dog has diarrhea, if bedding is not routinely cleaned, or if the owner maintains poor hygiene. Allowing a dog to kiss or lick an owner on the face also has potential for cross-species bacterial sharing and zoonotic spread, especially if the dog grooms other dogs, drinks from toilets, and eats raw foods. The risk of disease transmission from dogs drinking from toilets would depend on the cleanliness of the toilet and frequency of drinking from it. Most water dogs drink from flushed toilets where water is only very dilutely contaminated with fecal material or urine, but risk of fecal-

oral transmission is possible. A dog kissing or licking an owner right after drinking from the toilet could also cause fecal-oral transmission to the owner, with bacteria either from the owner's own feces or from another human being in the household. Likewise, sharing table scraps and eating together after a dog drinks from the toilet could contaminate the owner's mouth with dog's saliva and cause fecal-oral transmission by that mechanism as well.

Forty-one percent of dog owners reported sharing "people food" with their dogs, which is consistent with a recent survey of dogs in the US and Australia that found 45.4% of dog and cat owner feed their pets table foods at least 1 or more times a week [164]. Although no association was found in this study, behaviors such as hand-feeding dogs and allowing dogs to lick plates or utensils intended for human use could in theory pose a higher risk for zoonotic or anthropozoonotic transmission of bacteria than placing "people food" into a dog bowl. Further investigation into the risk of these specific behaviors may be warranted.

Raw meat was fed by 8.2% of dog owners in this study, which was consistent with the recent survey finding in the US and Australia that 7.4% of dogs received raw meat or bones as a treat or snack at least weekly [164]. The motivation behind feeding raw meat was not asked in our questionnaire, but pet owners may feed bones and raw meat for various reasons including perceived health benefits, because they are a more natural and less processed food, or as a special treat for their pets. Several owners provided information that they fed their dogs raw deer meat scraps while

hunting and this may have been a bonding or rewarding behavior for these participants.

Although feeding raw meat was associated with cephalothin resistance, it was not associated with increased clonal sharing by PFGE profiling in this study. Eighty percent of owners who fed their dogs raw meat allowed their dogs to kiss or lick them on the face, but this behavior too was not associated with increased clonal sharing. Despite these findings, raw meat can be contaminated with bacteria and parasites that can make both dog and owner sick, and so proper hygiene measures should be followed when handling raw meat, and behaviors such as eating or feeding raw meat to dogs is not advised.

Hand-washing is often overlooked by owners of companion animals, perhaps because they are considered members of the family [104]. In this study, only 25% of owners reported washing their hands after petting their dogs, but this behavior was not associated with sharing of *E. coli* clones. Washing their hands before meals has become routine for many human beings, with 77% of owners washed their hands before eating their own meals, but only 6.6% of owners reported washing their hands before feeding their dogs. Hand-washing by owners prior to their own meals may minimize spread of bacteria from dogs to human beings but not washing prior to feeding the dogs leaves opportunity for spread of bacteria from human beings to dogs through contact with their food. It is also recommended that owners wash their hands after feeding their dogs, because contaminated dry dog food has now been identified as a source of human *Salmonella* infections [165]. According to the CDC, dog

owners should wash their hands for 20 seconds in warm water with soap after handling dry pet food, treats, and supplements, before eating their own food, and they should keep children under the age of 5 away from all pet food and treats [165].

Forty-eight percent of dog owners reported disposing of their dog's feces, while 52% of owners never disposed of their dogs feces. One possibility for the low number of dog owners who disposed of feces may be due to assigned chores in households, with another household member carrying primary responsibility for this chore. Interestingly, an association was found between disposing of dog's feces and % PFGE similarity, with those owners who disposed of their dog's feces 5+ times a week having the lowest % PFGE similarity between dog and owner fecal *E. coli* compared with owners who disposed of their dog's feces less frequently or never. One explanation for this finding may be that owners who handle their dog's feces more frequently also are more careful about personal hygiene and wash their hands more consistently than other owners, but this association was not proven statistically. Despite these results, it would be irresponsible to suggest that handling canine feces is protective against cross-species bacterial sharing. Good hygiene with hand-washing after disposing of feces is recommended.

Virulence Factor Results Discussion

Prevalence of Virulence Factors

Twenty-two percent of participants harbored *E. coli* with one or more virulence factors, while the majority of participants (118/152, 78%) carried *E. coli* negative for all four virulence factors. Low prevalence of virulence factors in fecal *E. col*i was not unexpected, because these virulence factors were chosen for their urovirulence but were tested in isolates from feces of healthy participants. A study by Usein et al. found carriage of urovirulence factors, including *cnf, hly, sfa*, and *pap*, was only 35% in *E. coli* from fecal flora of healthy adults compared with 70% in UPEC strains [166]. Finding urovirulence factors in 22-35% of fecal strains from healthy adults supports the hypothesis that intestinal *E. coli* serve as reservoirs for UTIs.

Cytotoxic necrotizing factor (*cnf*) was present in 15.3% of canine isolates, 14.2% of owner isolates, and 13.3% of control isolates. Other studies have found a prevalence of 12-30% in healthy canine fecal *E. coli* isolates and 0.9-10% in healthy human being fecal isolates [55, 74, 122, 167]. In uropathogenic *E. coli* strains, prevalence of *cnf* is higher, ranging from 41-45% in canine isolates, and between 39% (cystitis), 47% (pyelonephritis), and 78% (prostatitis) in human being isolates [55, 75, 168].

Hemolysin (*hlyD*) was present in 15.3% of canine isolates, 15.8% of owner isolates, and 16.7% of control isolates. *HlyD* is one gene in the operon, *hlyCABD*,

encoding alpha-hemolysin. Other studies have found *hly* prevalence of 6-31% in healthy canine fecal *E. coli* isolates and 2.7-20% in healthy human being fecal isolates [55, 74, 122, 169]. In uropathogenic *E. coli* strains, prevalence of *hly* ranges from 44-50% in canine isolates [55, 75], and from 43% (cystitis), 47% (pyelonephritis), and 78% (prostatitis) in human being isolates [168]. Genes for hemolysin are included in most PAIs identified thus far in *E. coli* [10].

S fimbriae adhesin (*sfa*) was present in 21.9% of canine isolates, 14.2% of owner isolates, and 6.7% of control isolates. Other studies have found *sfa* prevalence of 6-27% in healthy canine fecal *E. coli* isolates and 14% in healthy human being fecal *E. coli* isolates [55, 74]. In uropathogenic *E. coli* strains, prevalence of *sfa* ranges from 57-60% in canine cases [55, 75], and from 61% (cystitis), 53% (pyelonephritis), and 83% (prostatitis) in human being isolates [168].

Pilus-associated with pyelonephritis G allele III (*papGIII*) was present in 12.6% of canine isolates, 7.7% of owner isolates, and 7.8% of control isolates. Other studies have found *papGIII* prevalence of 6-30% in healthy canine fecal *E. coli* isolates and 4-8.2% in healthy human being fecal *E. coli* isolates [55, 58, 160]. In uropathogenic *E. coli* strains, prevalence of *papGIII* ranges from 22% (cystitis), 35% (pyelonephritis), and 34% (prostatitis) in human being isolates [168].

Virulence Factor Patterns

The most common virulence factor pattern was the presence of all 4 virulence factors (*cnf, hlyD, sfa,* and *papGIII*) in isolates from 7/61 (11.5%) dogs and 5/61 (8.2%) owners. This virulence factor pattern has also been noted by Feria et al. from 60% of canine UPEC isolates and 68% of feline UPEC isolates [75]. In Drazenovich's study, 70% of UPEC clones isolated from dogs with persistent UTIs were positive for one or more virulence factors, with *cnf, hly,* and *sfa* occurring most often [79]. The *pap* gene was present less commonly but was always present in combination with *cnf, hly,* and *sfa* [79]. Usein et al. compared healthy human adults' fecal *E. coli* isolates with clinical UPEC strains and found the combination of *cnf, hly, sfa,* and *pap* in 5% fecal strains but 55% UPEC strains [166]. *E. coli* with concurrent presence of all 4 virulence factors in ours and other studies suggests genetic linkage on a PAI, but this has not been conclusively proven at this time; PAIs have been identified with *cnf* and *hlyD,* and *cnf, hlyD,* and *papGIII*, but not all 4 [65, 170]. Higher prevalence of these virulence factors from UPEC than from fecal samples supports association of these particular genes with urovirulence.

Comparison of Virulence Factors between Groups

There was no difference in presence or absence of any of the four virulence factors between paired dogs and owners. The low overall prevalence of virulence factors in this population influenced this comparison. Lack of significant difference

cannot be explained by cross-species sharing of bacteria or mobile genetic elements, since only 1 household with positive results had a similar virulence factor pattern between dog and owner. Although not significant, there may be clinical importance to the 25/61 households containing either a dog or owner (but not both) with *E. coli* positive for at least one virulence factor. This suggests that even if *E. coli* are carrying PAIs with virulence factors, these PAIs are not being shared routinely between dog and owner.

There was also no significant difference in virulence factor results between owners and controls, suggesting that dog ownership did not affect presence or absence of virulence factors in a human being's fecal *E. coli*. On the contrary, more canine isolates (21.9%) were positive for *sfa* than control isolates (6.7%). Yuri et al. study's included healthy dogs and human beings in separate households, and also found a higher prevalence of *sfa* in fecal isolates from dogs (27%) than human beings (14%), but no p-value was provided [74]. It is possible *sfa* is transferred on plasmids, transposons, or phages more frequently among the canine population than human population, although within-household sharing would be expected if this were the case. Many plasmids and PAIs also contain other virulence factors, such as *cnf, hlyD,* and *papGIII*, and so transfer of *sfa* alone would be unexpected with plasmid or PAI acquisition.

Agreement between Virulence Factors

The hemolytic phenotype of *E. coli* isolates on blood agar plates agreed significantly with the hemolytic genotype, or presence of *hlyD*. Eighteen percent of isolates had negative phenotypes but positive genotypes. The absence of a hemolytic phenotype in the presence of *hlyD* can be due to defects in the hemolysin operon including the *hlyBCD* genes or to defects in the transcriptional activator *rfaH* [171].

Results of this study were consistent with previous findings of a close association between the two toxins encoded by *hlyD* and *cnf* [122]. Cnf was found in approximately 92% of hemolytic isolates in our study, but never in non-hemolytic isolates; only 2 participants had isolates that were *cnf*-negative but *hlyD*-positive. *HlyD* can be located either on the chromosome or on a transmissible plasmid, whereas, *cnf* is only a chromosomally-inherited gene [122]. Therefore, perfect agreement between these two virulence factors is best explained by linkage in a chromosomal gene cluster, such as a PAI, that concurrently governs the synthesis of both toxins [122]. Blum et al. have documented the presence of both *cnf* and *hly* on chromosomal PAI II on UPEC strain J96 [170]. Bingen-Bidois et al. have also shown genetic linkage between *papGIII*, *hly*, and *cnf* [65].

The strong agreement between *cnf* and *sfa* and other combinations of virulence factors in this study provides further support for genetic linkage of these virulence factors, whether on a chromosome and controlled by the same promoter or on mobile genetic elements such as PAIs or plasmids. No isolated association between *cnf* and *sfa* was found in the literature, although they are recognized to occur

in combination with other virulence factors with high frequency and are suspected to be included in PAIs [75, 166]. Johnson et al. identified an association between *papGIII, hly,* and *sfa* but did not assess *cnf* prevalence in their study [64]. Alternatively, Hilali et al. found associations between *papC, hlyC,* and *cnf* and believed them to be associated on a PAI, perhaps PAI V [167]. In theory, housing virulence genes together in the same chromosomal cluster or mobile genetic elements may allow coordinated expression of different toxins and adhesins and more efficient establishment of extraintestinal infection.

Virulence Factor Association with Hand-Washing

One would expect the effect of owners not washing their hands to be on their own fecal flora, so the association between their hand-washing and virulence factors in their dog's fecal *E. coli* is difficult to explain. Since prevalence of virulence factors was low overall in the population studied here, these associations may have been found by chance alone. All dogs with *papGIII* in fecal *E. coli* lived with owners who did not wash their hands after petting their dogs. This may suggest that these owners have poor hygiene, and perhaps the owners are transmitting the *papGIII* gene to the dogs.

Urinary Tract Infection History and Urovirulence Factors

Although self-reporting of UTI has inherent potential for error, the high prevalence of women participants in this study with a history of UTI (52.1%) is consistent with national data that approximately 50% of all women will have a UTI during their lifetimes [50]. Only14% of all dogs develop a UTI at some point during their lifetime, and our canine population had a similar overall prevalence (13%) [172]. Aerobic urine culture is the gold standard for diagnosing a UTI, but physicians and veterinarians may treat empirically based on clinical signs [6, 46, 173]. Although self-reporting was a limitation of the study, without reviewing medical records, the authors had no way to verify whether reported UTIs were confirmed by culture.

The sex predilection for UTIs seen in our participants (100% women vs. 0% men, and 18.2% female dogs vs. 7.1% male dogs) was consistent with females being more susceptible than males to UTIs [50]. This may in part be due to anatomical differences; compared with females, the male urethral opening is located anatomically distant from the rectum and is longer, forcing bacteria to travel farther to reach the bladder and before establishing an infection. Specific risk factors for women to develop UTIs include sexual intercourse, spermicidal use, urinary incontinence, and diabetes mellitus, which are similar to risk factors in dogs including underlying urinary anatomical defects, uroliths, incontinence, indwelling urinary catheters, and endocrine disorders [6, 174]. Men are at increased risk of UTI if homosexually active, sexually active with an infected woman, or if not circumcised, whereas male dogs are at increased risk if they are sexually intact [6, 46].

In women, there is a high recurrence rate of UTIs, with >25% recurring within the first 6 months [52]. In our subpopulation of women with history of UTI, 75% reported more than 1 UTI in their lifetimes, while 50% of our dogs with a UTI history had more than 1 UTI. It is unclear how many of these were relapses of the same strain of *E. coli* vs. reinfections with a new strain or new bacterium. Up to 68% of UTI recurrences in women are caused by *E. coli* identical to the original strain [52]. Results of this study confirmed previous studies' results that antimicrobial patterns can differ extensively within a single PFGE clone; therefore, determination of relapse vs. reinfection should be based on PFGE profile similarity instead of antimicrobial susceptibility patterns [79, 84, 175]. This information may prove useful in determining the underlying cause of the UTI and preventing future recurrence.

Numerous virulence factors have been associated with UTI in women and dogs in previous studies [53, 56, 66]. Without enrolling participants with active UTIs, our questionnaire enabled us to compare history of UTI in our participants with presence or absence of virulence factors in fecal *E. coli*. We recognize the potential for error that exists because *E. coli* in the fecal flora at the time of sampling for the study may not represent what was present in the flora at the time of the UTI.

For women, having a history of UTI was associated with presence of *hlyD* and *papGIII* in fecal *E. coli*, and having a history of recurrent UTIs was associated with presence of *papGIII* in fecal *E. coli*. Of the 4 virulence factors analyzed in this study, *hlyD* and *papGIII* were the most specific to the urogenital tract [7]. It is possible that significant associations were not found in the canine population due to small number

of enrolled dogs with a history of UTI; a future study could be designed to further investigate the role of these virulence factors in dogs.

The association found between presence of all 4 virulence factors in a dog's fecal *E. coli* and an owner's history of UTI has not been reported in the literature. One interpretation of these results is that dogs may harbor *E. coli* with urovirulence factors that may be shared with their human housemates, thus increasing the owner's risk of developing UPEC UTIs. Two longitudinal studies found extensive within-household sharing of UPEC among family members and pets [119, 120]. Presence of virulence factors *pap*, *hly*, and *cnf*, were associated with persistence of these strains [119]. In both studies the women's UPEC isolates persisted in the feces of the family members and pets once the woman's UTI was treated and cleared, indicating that both other housemates and pets could be reservoirs for reinfecting the women in the future [119, 120]. We recognize associations found between historical UTIs and dog's fecal virulence factors identified in our study, but we caution against over interpreting these data since owners did not have active UTIs during fecal sample collection. Further investigation is needed enrolling owners with current UTIs and testing fecal samples from both human beings and dogs in the households to determine true association between owners UTIs and virulence factors in canine fecal *E. coli*.

Comparison of Susceptibility, Clonality, and Virulence Factors

Comparison of Susceptibility and Virulence Factors

Our study found that streptomycin resistance was associated with presence of fewer virulence factors in fecal *E. coli*. These data correspond with other studies that have shown that resistance to quinolones, fluoroquinolones, and trimethoprim-sulfamethoxazole was associated with reduced virulence traits in UPEC [176, 177]. It has also been shown that quinolone-resistance can induce loss of PAIs containing virulence factors from *E. coli* strains [176]. Without the Bonferroni adjustment, both the quinolone in our study, nalidixic acid, and trimethoprim-sulfamethoxazole were associated with decreased virulence factors but the fluoroquinolone, ciprofloxacin, was not associated with any virulence factor.

One potential explanation for our finding is therapeutic use of antimicrobial agents causing selection pressure and allowing for survival of resistant commensal strains within the GI tract which are then isolated from fecal samples of healthy individuals or are able to migrate to the urinary tract and cause infection due to decreased host defenses rather than increased bacterial virulence. Soto et al demonstrated one mechanism for quinolone-induced PAI loss is by induction of the error-prone SOS pathway of DNA repair and suggested that this deletion may be an adaptation of UPEC strains to gain selective advantages over less flexible organisms [176].

The potential association between quinolones and fluoroquinolones with urovirulence factors is important as guidelines now recommend fluoroquinolones as first-line empirical treatment for UTIs in women in regions where trimethoprim-sulfamethoxazole resistance is >10-20% [178]. Enrofloxacin usage has increased in veterinary medicine as well, and enrofloxacin one of the most commonly used antimicrobial agents for UTIs in dogs [47, 84]. Although >80% of UPEC in dogs still show susceptibility to enrofloxacin, there is evidence that fluoroquinolone resistance is emerging that will affect UTIs of both women and dogs [84-86]. Increased use of fluoroquinolones in human and veterinary medicine may lead to a continued rise in resistance but loss of PAIs and virulence factors within UPEC. This is an area of research that will need to be followed closely in both fields of medicine.

Comparison of Susceptibility within Shared E. coli Clones

This study corroborated others' results that susceptibility patterns can vary widely within a single clone of *E. coli* [79, 84, 175]. In our study, isolates in 25% of shared *E. coli* clones had identical susceptibility patterns, while isolates in 75% of shared clones had different susceptibility patterns. Similarly, the Freitag study found that antimicrobial isolates in 33.3% of shared *E. coli* clones had identical susceptibility patterns, while isolates in 66.7% of shared *E. coli* clones had different susceptibility patterns [175]. This finding suggests that horizontal gene transfer is occurring between *E. coli* strains spreading genes for antimicrobial resistance on

plasmids or integrons. This knowledge may be useful to clinicians who treat recurring UTIs. These data have shown that basing classification of relapse versus reinfection on antimicrobial susceptibility pattern is not accurate. Although technically difficult and expensive, PFGE can accurately differentiate between relapse and reinfection and can be a beneficial tool for diagnosing clinical cases as well as continued research studies.

Comparison of Virulence Factors within Shared E. coli Clones

In contrast to the variety of susceptibility patterns seen within a single clone, virulence factors were more consistent within a clone. Isolates within 78% of shared clones had identical virulence factor patterns, while isolates within 22% of clones had different virulence factor patterns. This was also seen in Drazenovich's study, where PCR assays for urovirulence factors almost always found the same results each time the same PFGE clone was observed; the same virulence factors were found each time the same clone was found in the same dog, except for in two clones [79]. In contrast, in the Usein study, 14 human UPEC clones were identified and isolates within 9 (64%) clones had different virulence profiles [166]. Different virulence factor patterns may occur within a single clone due to horizontal gene transfer of PAIs containing various virulence factors.

Comparing Susceptibility and Virulence Factors within Shared Clones

Only 6/36 shared clones had isolates with identical susceptibility patterns and identical virulence factors, but no clones carried both an antimicrobial resistant phenotype and genes for virulence factors. In one clone with MDR *E. coli*, ciprofloxacin and nalidixic acid resistance may have contributed to decreased presence of virulence factors, as reported elsewhere [176, 177]. These results suggest genes for resistance and these 4 virulence factors may not be linked together on plasmids or integrons, but studies including antimicrobial genetic testing would be required to further investigate this issue. None of these shared clones were shared within households; all were shared between various combinations of participants across-households, suggesting these *E. coli* strains may found in environmental reservoirs rather than being transmitted by direct contact between hosts.

Limitations

A longitudinal study design may have increased the sensitivity for detecting within-household sharing over a cross-sectional design, since *E. coli* clones can be detected inconsistently in a particular individual [120]. Limitations of the study population were including only one dog and owner from each household rather than including all family members and pets, and including only adults rather than children who may have closer contact with pets and less stringent hygiene habits [100, 108]. Another limitation was the possibility of Type II error in stating that dogs and owners did not have different susceptibility or virulence factor results; increasing the sample

size and number of isolates per participant would have increased the overall power of the study and may have allowed identification of differences if they existed. It is also acknowledged that the population sampled in this study did not represent the general population because most human participants had occupational exposure to either human or veterinary hospitals.

The questionnaire was piloted prior to use, but in retrospect several questions needed more clarification and other questions could have been added. As discussed in the Results section, under Relationship with Dog Results, the question regarding feeding of raw foods was too vague and should have specified raw meat vs. vegetables or fruits. In light of linking cases of human *Salmonella* to handling dry dog food, the questionnaire should have asked about washing hands after feeding dogs as well as before feeding dogs [165]. Participants should have been asked if they routinely wash their hands after using the restroom. Participants who work at veterinary clinics should have been asked if they routinely clean up feces of dogs other than their own.

Disc diffusion susceptibility testing could have been complimented by molecular testing for presence of resistance genes. In the Srinivasan study not all *E. coli* displaying phenotypic resistance to an antimicrobial carried a gene conferring resistance to that particular antimicrobial, and many susceptible strains did carry genes for resistance [179]. This suggests that mechanisms of resistance exist beyond recognized resistance genes, and that not all genes for resistance are expressed [179]. In our study, knowing which isolates carried resistance genes would have allowed

further comparison between dog and owner isolates and provided more information regarding linkage of antimicrobial agents on mobile genetic elements.

Antimicrobial susceptibility testing included those agents routinely monitored by the NARMS, but for veterinary application adding enrofloxacin would have provided useful information. Specific benefits would have included knowing the level of enrofloxacin-resistant *E. coli* in the normal flora of dogs and their owners, knowing the association of resistance with historical use of enrofloxacin, and knowing the association of resistance with presence of urovirulence factors. One limitation of the questionnaire was participant recall with history of antimicrobial therapy; participants reported 13 courses of therapy with unknown antimicrobial agents which they or their dogs had received, which may have altered statistical analyses.

A limitation of PFGE analysis was that it was only performed on 2 isolates per participant due to financial and time limitations. Other researchers have increased the power of detecting within-household sharing by comparing up to 20 isolates from each household member [159]. While XbaI is a commonly used restriction enzyme for PFGE of *E. coli*, adding a second restriction enzyme such as BlnI may help improve discriminating ability between fingerprints from study participants. Directly comparing each isolate between owners and controls and between dogs and controls using similarity matrices rather than analysis of dendrograms may have found additional across-household sharing clones.

The 4 virulence factors tested were chosen because they have been identified previously from both human being and canine UPEC isolates; however testing for a larger panel of virulence factors would have provided additional useful information [57, 58, 66, 78, 120]. Direct testing for presence of PAIs using PCR would have supplemented the study as well [10]. Multiplex PCR is one way to test for multiple virulence factors, but microarray may be the best technology for this objective because it can test for significantly more virulence factors at one time [9].

Conclusion

There was a wide diversity of *E. coli* strains identified in fecal samples from both human beings and dogs. Sharing of fecal *E. coli* between dog and owner pairs occurred within households with 9.8% prevalence, but no difference was found in susceptibility patterns or virulence factor prevalence between paired dogs and their owners. No specific risk factors were identified that were associated with increased sharing of fecal *E. coli* clones between dogs and their owners. Direct contact between dogs and owners may be responsible for sharing of bacteria and mobile genetic units within households, and good hygiene, including frequent hand-washing, is recommended. Sharing of fecal *E. coli* also occurred across-households with 0.26% prevalence, suggesting environmental reservoirs of *E. coli* may exist to which participants may have common exposure. Both direct contact and environmental reservoirs may be important routes allowing sharing of bacteria or horizontal transfer

of genes for antimicrobial resistance and virulence factors between species. Public health concerns regarding cross-species sharing of *E. coli* are warranted, and efforts such as proper hygiene measures to minimize transmission should be taken.

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Appendices

Appendix A. Questionnaire for study on *E. coli* organisms in dogs and their owners

Dog Owner Participants

Date_____

Dog Information: (this should only reflect the dog participating in the study)

Dog's Study ID# _____ Breed _____ Age _____ years

Is your dog: Male Intact □ Female Intact □
Male Neutered □ Female Spayed □

How old was your dog when you adopted him/her?
Puppy (<1 year old) □
1-4 years old □
4-7 years old □
7-10 years old □
>10 years old □
unknown □

From where did you get your dog?
Animal Shelter □
Breeder □
Pet Store □
Other _____

Dog's Health

Does your dog currently have loose stool or diarrhea? Yes □ No □

Has your dog ever been diagnosed with any of the following diseases?

Diabetes mellitus Yes □ No □

Hyperadrenocorticism (Cushing's Disease) Yes □ No □

Cancer Yes □ No □
If yes, what type (if known) _____
If yes, when was the diagnosis made? _____month/year

Urinary Tract infection Yes □ No □
If yes, on how many different occasions? _____
When was the most recent urinary tract infection? ____month/year

Which bacteria was it caused by: *Enterococcus*□ *Streptococcus* □
E. coli □ Other □
Proteus □ Unknown □
Staphylococcus □

Medications:

Has your dog received antibiotics in the past month? Yes ☐ No ☐

If yes, reason for administration:

Dental disease	☐	Skin/trauma/wound	☐
Gastrointestinal infection	☐	Urinary tract infection	☐
Respiratory infection	☐	Other/Unknown	☐

If yes, type of antibiotic:

Amikacin	☐	Doxycycline	☐
Amoxicillin	☐	Enrofloxacin (Baytril®)	☐
Azithromycin	☐	Erythromycin	☐
Cefpodoxime (Simplicef®)	☐	Gentamicin	☐
Cephalexin	☐	Marbofloxacin	☐
Chloramphenicol	☐	Metronidazole	☐
Clavamox®	☐	Sulfa antibiotic	☐
Clindamycin	☐	Other/unknown	☐

How were these antibiotics administered?

Oral	☐	Into eyes	☐
Injectable	☐	Onto skin	☐
Into ears	☐	Other	☐

When was the last date of administration? _____mm/dd/2006

To the best of your memory: please summarize your dog's antibiotic history over the past year by providing the month/year, type of antibiotic, route of administration, number of days the antibiotic was given, and reason for antibiotic usage.

Month/Year	Type of Antibiotic	Route	#Days	Reason
Example: Feb 2004	*Clavamox*	*Orally*	*14 days*	*abscess*

Has your dog received chemotherapy in the past month? Yes ☐ No ☐

Has your dog received prednisone in the past month? Yes ☐ No ☐

If yes, please list reason if known_____

Has your dog received any other known immunosuppressive medication in the past month? Yes ☐ No ☐

If yes, please list medications _____

Owner Information:

Study ID # _____ Age _____years Male □ Female □

Faculty _____ Student_____ or Staff _____
Note: "staff" includes house officers, technicians and veterinary assistants.

Has your role at the University of Tennessee Veterinary Teaching Hospital included routine handling of canine patients in the previous 7 days?

Yes ☐ No ☐

If you are a veterinary student, in which year of study are you?

First □ Second □ Third □ Fourth □

Do you work or volunteer at another veterinary clinic other than the University of Tennessee Veterinary Teaching Hospital? Yes ☐ No ☐

If so, how many hours per week do you work at this other clinic?

0-10 hours □
11-20 hours □
21-30 hours □
30+ hours □

Is there anyone in your household that works or volunteers regularly in a human hospital? Yes ☐ No ☐

Has any human from your household been hospitalized in the past month?

Yes ☐ No ☐

Are there any children in your household? Yes ☐ No ☐

If so, please list their ages, if diarrhea is currently present, and any antibiotics they have received in the past 30 days, including the last date this medication was given.

	Age	Diarrhea?	Antibiotic	Last Date Given
Example	*3 years old*	*no*	*amoxicillin*	*7 days ago*

Owner's Medical History

Have you been diagnosed with an immunosuppressive disease (such as HIV, diabetes mellitus, or cancer)? Yes □ No □

Are you currently taking any immunosuppressive therapy (such as prednisone or chemotherapy)? Yes □ No □

Have you ever been diagnosed with a urinary tract infection? Yes □ No □

If yes, on how many different occasions? _____

When was the most recent urinary tract infection?_____month/year

Do you currently have loose stool or diarrhea? Yes □ No □

Does any other human in your household have loose stool or diarrhea? Yes □ No □

Owner's Medications:

Have you received antibiotics in the past month? Yes □ No □

If yes, reason for administration:

Dental disease	□	Skin/trauma/wound	□
Gastrointestinal infection	□	Urinary tract infection	□
Respiratory infection	□	Other	□

If yes, type of antibiotic:

Amikacin	□	Doxycycline	□
Amoxicillin	□	Erythromycin	□
Augmentin®	□	Gentamicin	□
Azithromycin	□	Metronidazole	□
Cephalexin	□	Sulfa antibiotic	□
Chloramphenicol	□	Other	□
Ciprofloxacin	□	Unknown	□

How were these antibiotics administered?

Oral	□	Onto skin	□
Injectable	□	Into eyes	□
Into ears	□	Other	□

When was the last date of administration? _____mm/dd/2006

To the best of your memory: please summarize your antibiotic history for the past year by providing the month/year, type of antibiotic, route of administration, number of days the antibiotic was given, and reason for antibiotic usage.

Month/Year	Type of Antibiotic	Route	#Days	Reason
Example: Feb 2004	*Augmentin*	*Orally*	*14 days*	*abscess*

Relationship with Dog

How much awake time per week do you spend with your dog?

0-10 hours	□	21-30 hours	□
11-20 hours	□	30+ hours	□

Does your dog sleep in your bed at night? Yes □ No □

Does your dog lick/kiss you on the face? Yes □ No □

Do you wash your hands after petting your dog? Yes □ No □

Are you the primary person who feeds your dog? Yes □ No □

Does your dog eat table scraps? Yes □ No □
If yes, please list:_____

Does your dog eat raw foods? Yes □ No □
If yes, please list:_____

Do you wash your hands before feeding your dog? Yes □ No □

Do you wash your hands before eating? Yes □ No □

How many times per week do you personally dispose of your dog's feces?

Never	□	3-4 times	□
1-2 times	□	5+ times	□

Do you wash your hands after disposing of your dog's feces? Yes □ No □

Does your dog drink out of the toilet? Yes □ No □

How much time do you and your dog spend at dog parks per week?

0-2 hours	□	7-10 hours	□
3-6 hours	□	11+ hours	□

Do you have other pets in the household? Yes □ No □

If so, please list the species and age of these pets, if diarrhea is currently present, and any antibiotics they have received in the past 30 days, including the last date this medication was given.

	Species	Age	Diarrhea?	Antibiotic	Last Date Given
Example	*cat*	*2 yrs*	*no*	*none*	*not applicable*

Questionnaire for study on *E. coli* organisms in dogs and their owners
Control Participants

Date _____

Study ID # _____ Age _____years Male □ Female □

Do you work at a human hospital or medical office? Yes □ No □
If yes, do you have routine exposure to human fecal material at work?
Yes □ No □
If yes, do you have routine exposure to human urine samples at work?
Yes □ No □

Do you work or volunteer at a veterinary clinic? Yes □ No □
Do you have regular contact (>1 hour/week) with any dogs? Yes □ No □

Do any animals live in your household? Yes □ No □
If so, please list the species and age of these pets, if diarrhea is currently present, and any antibiotics they have received in the past 30 days, including the last date this medication was given.

	Species	Age	Diarrhea?	Antibiotic	Last Date Given
Example	*cat*	*2 yrs*	*no*	*none*	*not applicable*

Are there any children in your household? Yes □ No □
If so, please list their ages, if diarrhea is currently present, and any antibiotics they have received in the past 30 days, including the last date this medication was given.

	Age	Diarrhea?	Antibiotic	Last Date Given
Example	*3 years old*	*no*	*amoxicillin*	*7 days ago*

Have you been diagnosed with an immunosuppressive disease (such as HIV, diabetes mellitus, or cancer)? Yes □ No □

Are you currently taking any immunosuppressive therapy (such as prednisone or chemotherapy)? Yes □ No □

Have you ever been diagnosed with a urinary tract infection? Yes □ No □

If yes, on how many different occasions? _____

When was the most recent urinary tract infection?_____month/year

Do you currently have loose stool or diarrhea? Yes □ No □

Does any other person in your household have loose stool or diarrhea? Yes □ No □

Have you taken antibiotics in the past month? Yes □ No □

If yes, reason for administration:

Dental disease	□	Skin/trauma/wound	□
Gastrointestinal infection	□	Urinary tract infection	□
Respiratory infection	□	Other	□

If yes, type of antibiotic:

Amikacin	□	Doxycycline	□
Amoxicillin	□	Erythromycin	□
Augmentin®	□	Gentamicin	□
Azithromycin	□	Metronidazole	□
Cephalexin	□	Sulfa antibiotic	□
Chloramphenicol	□	Other	□
Ciprofloxacin	□	Unknown	□

How were these antibiotics administered?

Oral	□	Onto skin	□
Injectable	□	Into eyes	□
Into ears	□	Other	□

When was the last date of administration? _____mm/dd/2006

To the best of your memory: please summarize your antibiotic history during the past year by providing the month/year, type of antibiotic, route of administration, number of days the antibiotic was given, and reason for antibiotic usage.

Month/Year	Type of Antibiotic	Route	#Days	Reason
Example: Feb 2004	*Augmentin*	*Orally*	*14 days*	*abscess*

Appendix B. Antimicrobial disc diffusion zone diameter interpretive standards [127, 128].

Antimicrobial Agent		Isolate A	Isolate B	Isolate C	Resistant	Intermediate	Susceptible
amikacin	AN				<14mm	15-16	>17
ampicillin	AM				<13	14-16	>17
amoxicillin-clavulanic acid	AmC				<13	14-17	>18
cefoxitin	FOX				<14	15-17	>18
ceftiofur	XNL				<17	18-20	>21
cefpodoxime	CPD				<17	18-20	>21
ceftriaxone	CRO				<13	14-20	>21
cephalothin	CF				<14	15-17	>18
chloramphenicol	C				<12	13-17	>18
ciprofloxacin	CIP				<15	16-20	>21
gentamicin	GM				<12	13-15 dogs	>16 dogs
					<12	13-14 people	>15 people
imipenem	IPM				<13	14-15	>16
kanamycin	K				<13	14-17	>18
nalidixic acid	NA				<13	14-18	>19
streptomycin	S				<11	12-14	>15
tetracycline	Te				<14	15-18	>19
trimethoprim-sulfamethoxazole	TMS				<10	11-15	>16

Appendix C. Quality control testing results of antimicrobial susceptibility test discs using ATCC *E. coli* 25922 and *S. aureus* 25923, performed on 8/30/06 [127, 128].

Antimicrobial Agent		***E. coli* 25922**	**Range for *E. coli* 25922**	***S. aureus* 25923**	**Range for *S. aureus* 25923**
amikacin	AN	26mm	19-26	21	20-26
ampicillin	AM	21	16-22	34	27-35
amoxicillin-clavulanic acid	AmC	23	18-24	29	28-36
cefoxitin	FOX	29	23-29	28	23-29
ceftiofur	NXL	30	26-31	30	27-31
cefpodoxime	CPD	28	23-28	24	19-25
ceftriaxone	CRO	35	29-35	27	22-28
cephalothin	CF	20	15-21	33	29-37
chloramphenicol	C	27	21-27	26	19-26
ciprofloxacin	CIP	40	30-40	24	22-30
gentamicin	GM	26	19-26	22	19-27
imipenem	IPM	32	26-32	34	-
kanamycin	K	25	17-25	24	19-26
nalidixic acid	NA	28	22-28	10	-
streptomycin	S	20	12-20	14	14-22
tetracycline	Te	25	18-25	30	24-30
trimethoprim-sulfamethoxazole	TMS	28	23-29	32	24-32

Table 1. Primers for multiplex virulence factor polymerase chain reaction assay.

Gene	Virulence Factor	Primer sequence (5'-3')	Size of product (bp)
cnf	cytotoxic necrotizing factor	F atcttatactggatgggatcatcttgg	974
		R gcagaacgacgttcttcataagtatc	
hlyD	hemolysin	F ctccggtacgtgaaaaggac	904
		R gccctgattactgaagcctg	
sfa	S fimbriae adhesin	F ctccggagaactgggtgcatcttac	410
		R cggaggagtaattacaaacctggca	
papGIII	pilus associated with pyelonephritis; GIII allele	F ggcctgcaatggatttacctgg	258
		R ccaccaaatgaccatgccagac	

F=forward, R=reverse

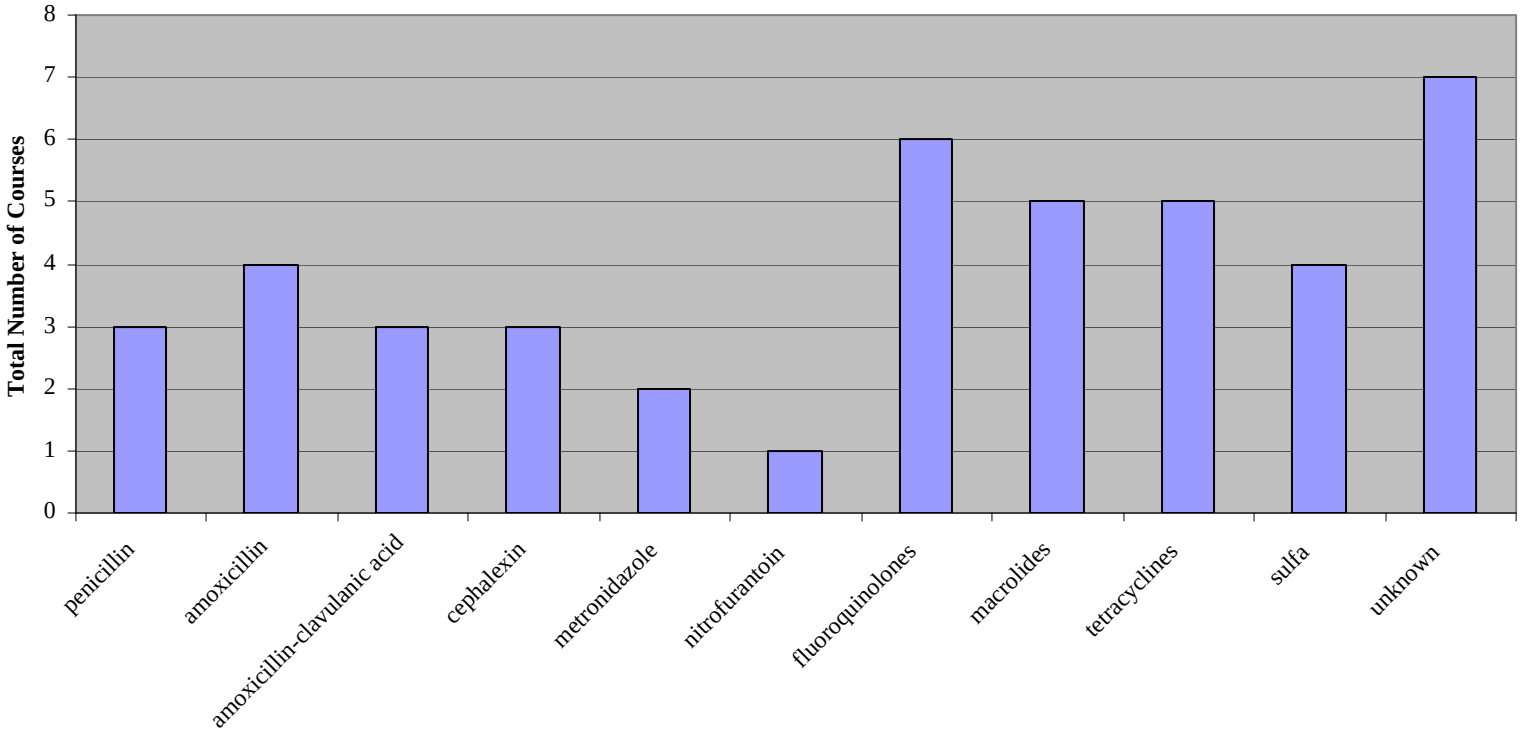


Figure 1. Type and total number of antimicrobial courses taken by all human participants in the year prior to enrollment.

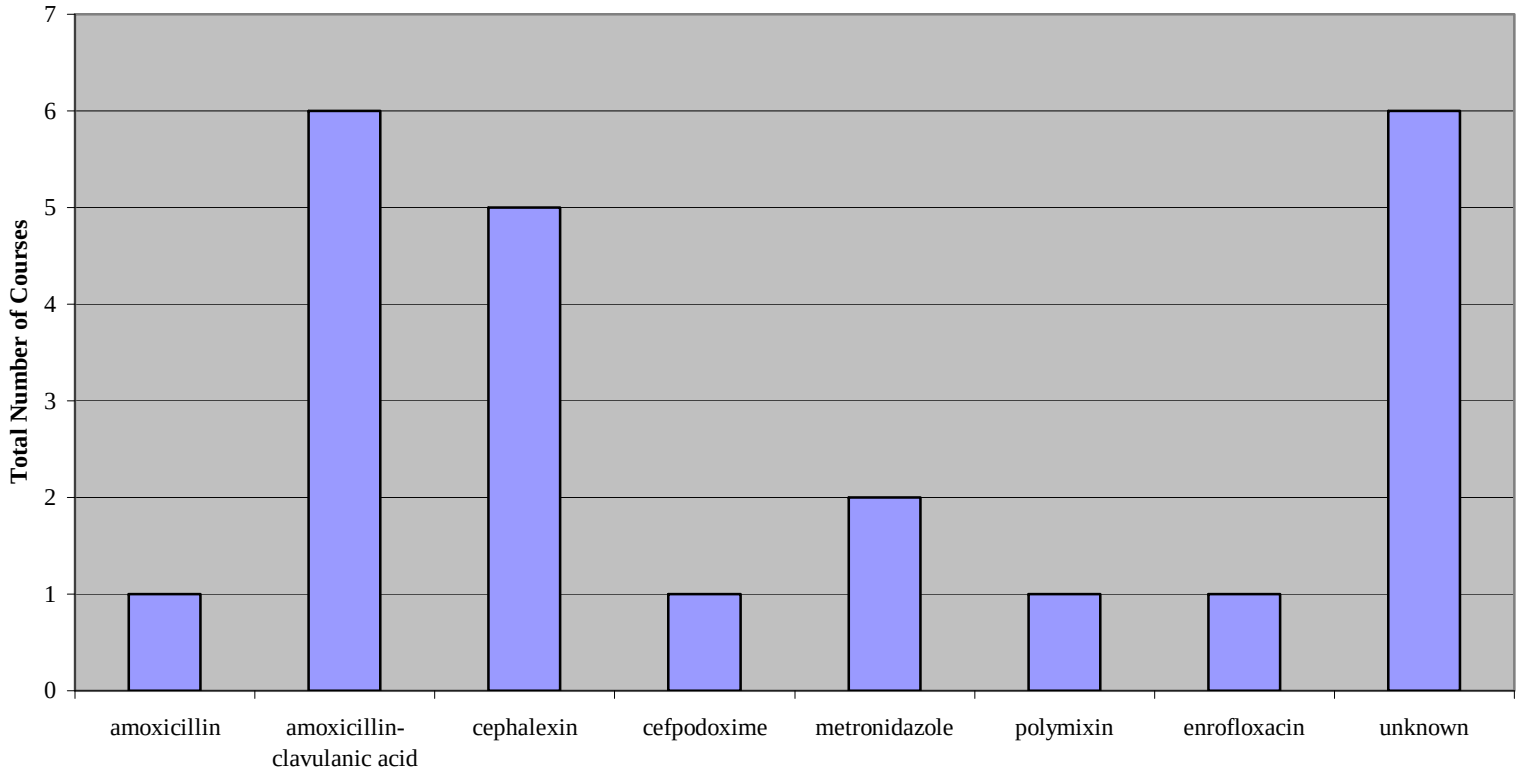


Figure 2. Type and total number of antimicrobial courses taken by all canine participants in the year prior to enrollment.

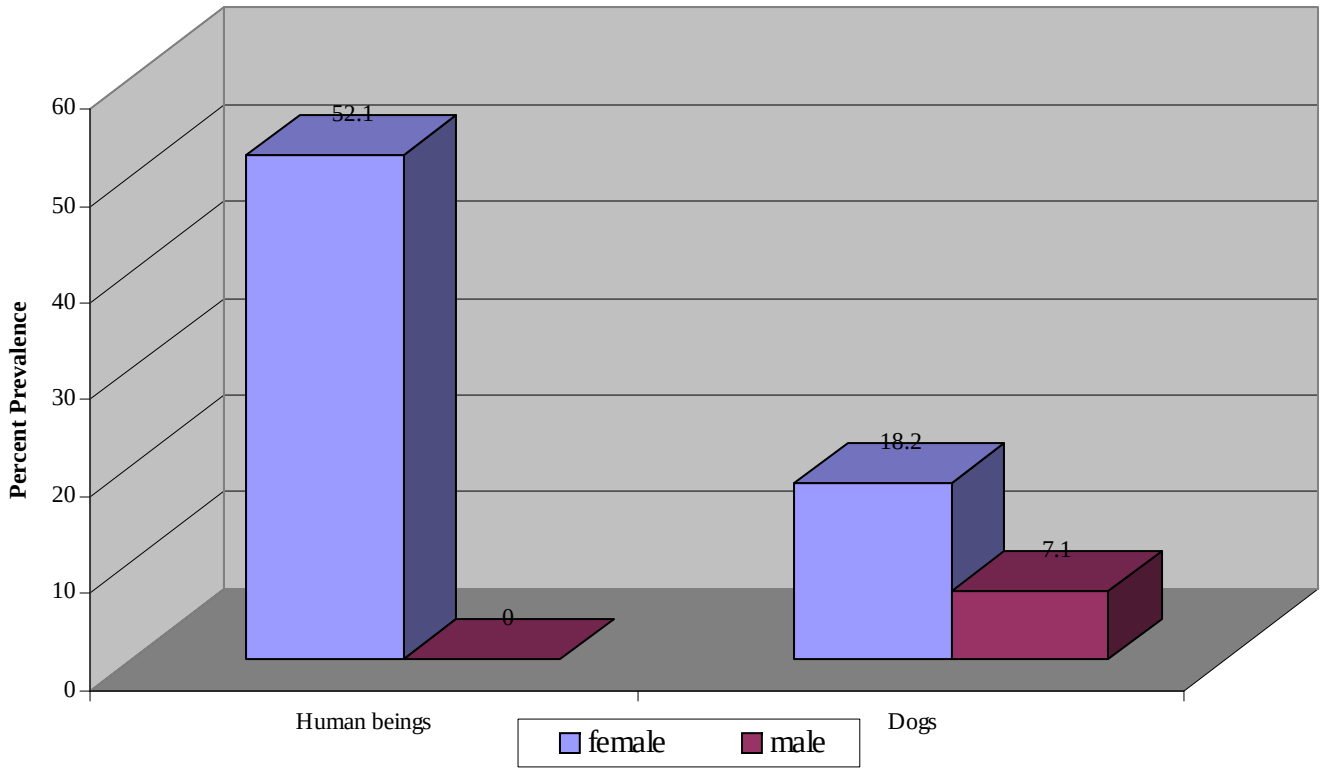


Figure 3. Prevalence of human and canine participants with history of urinary tract infections. More women had a history of UTI than men (p<0.001). There was no difference in number of female dogs vs. male dogs with a history of UTI (p=0.203).

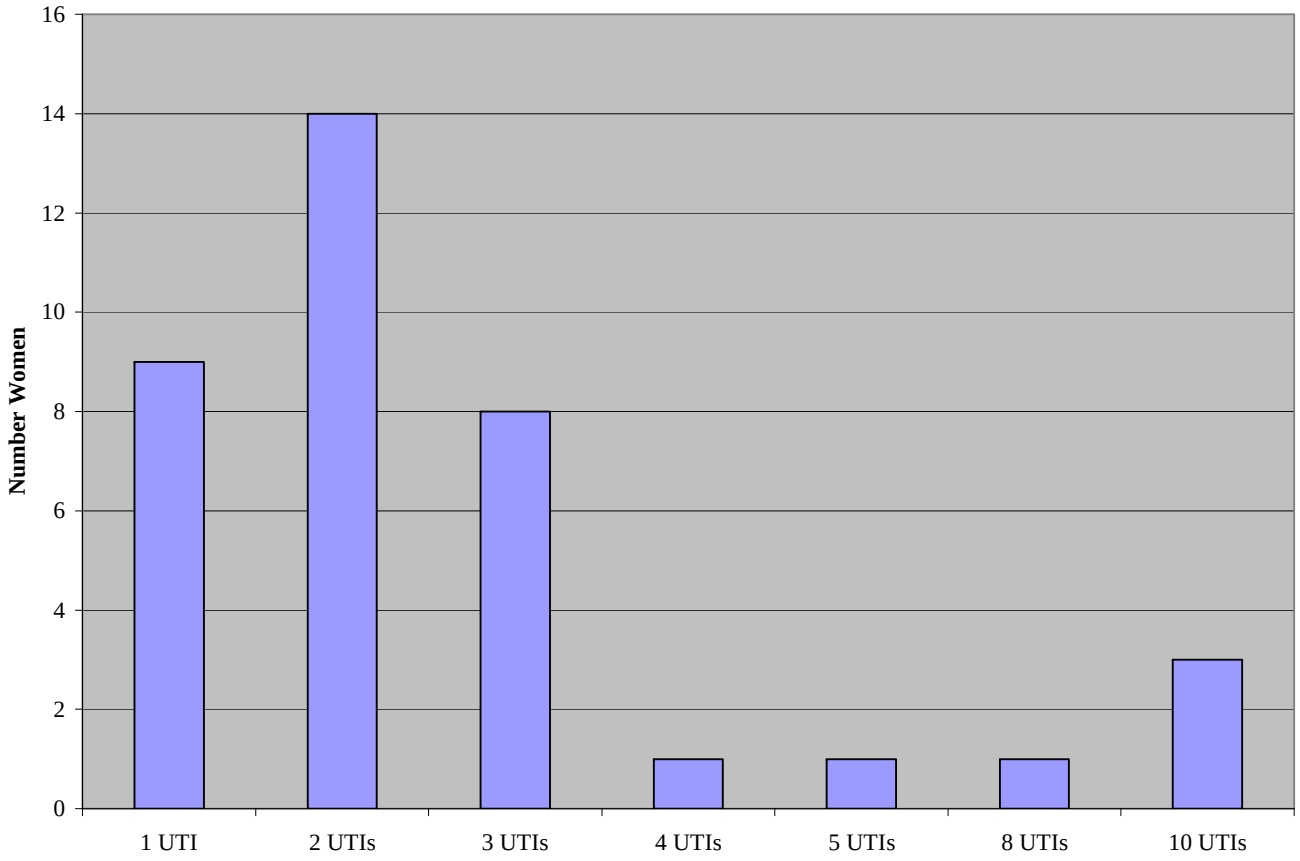


Figure 4. Number of lifetime episodes of UTI in women with reported UTI history. Seventy-five percent of women with a history of UTI had recurrent UTIs.

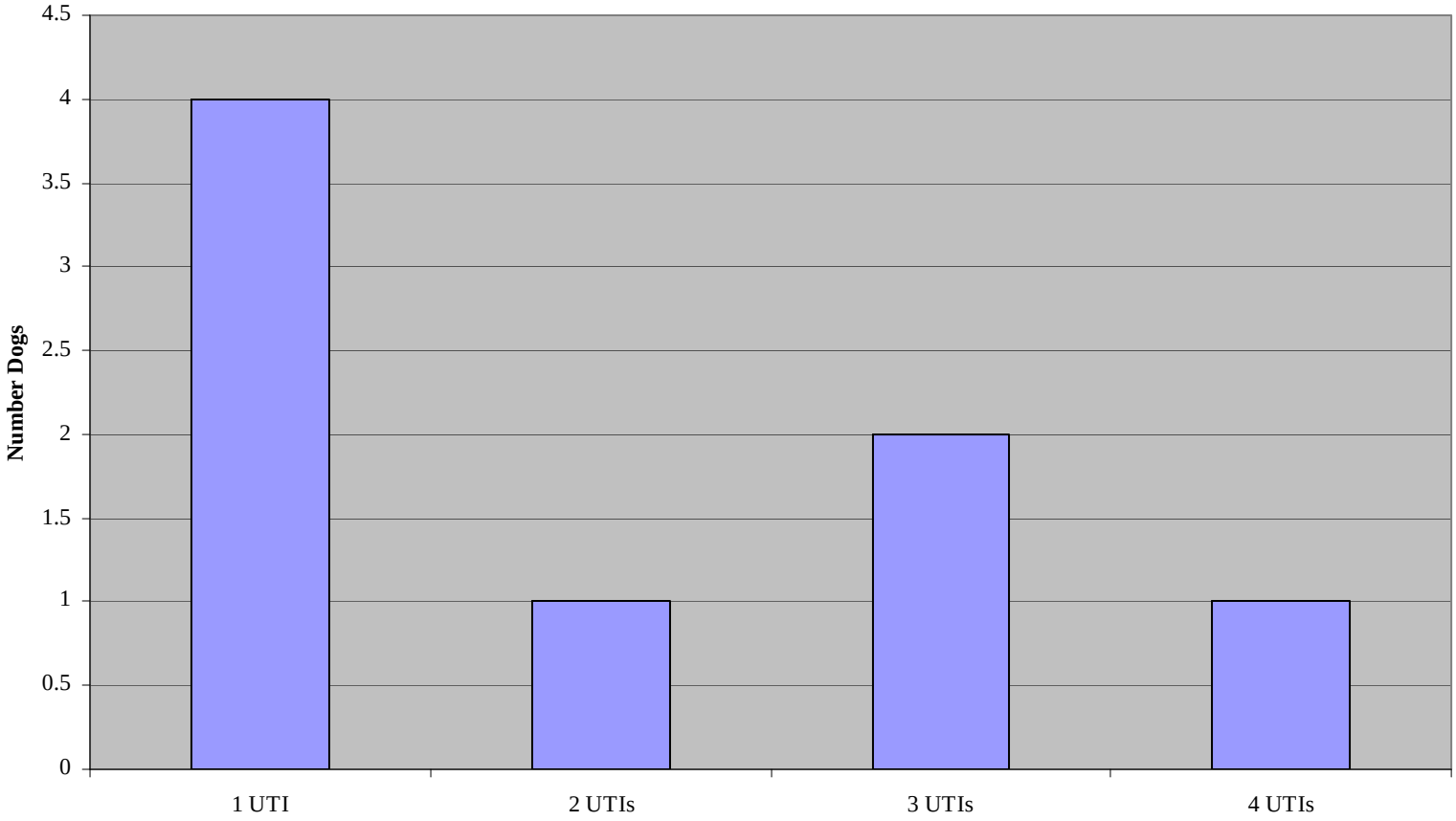


Figure 5. Number of lifetime episodes of UTI in dogs with reported UTI history. Fifty percent of dogs with history of UTI had recurrent UTIs.

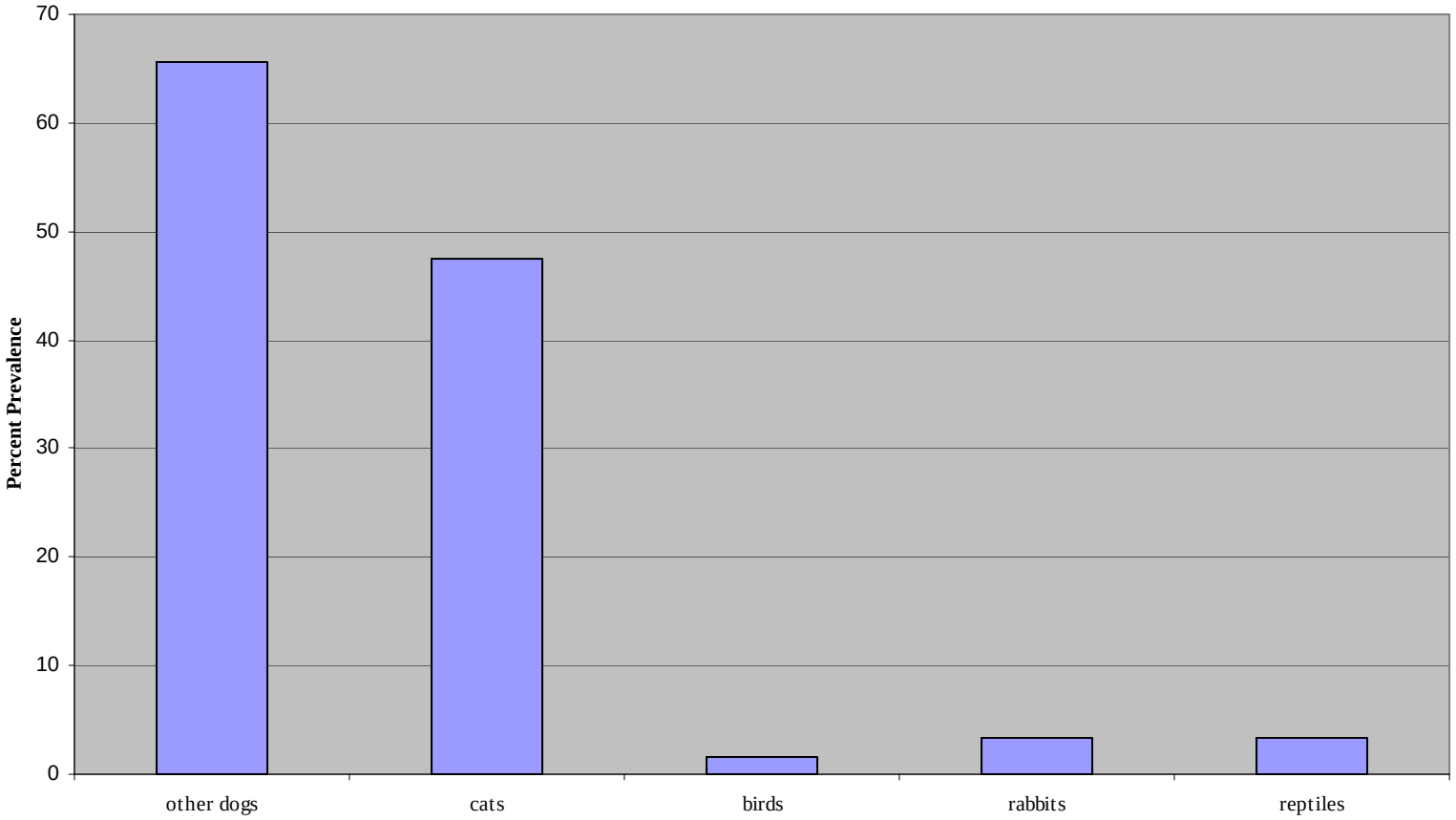


Figure 6. Prevalence of dog-owner households owning other pets. Eighty-two percent of households owned pets in addition to the dog enrolled in the study. Owning a second dog was most common.

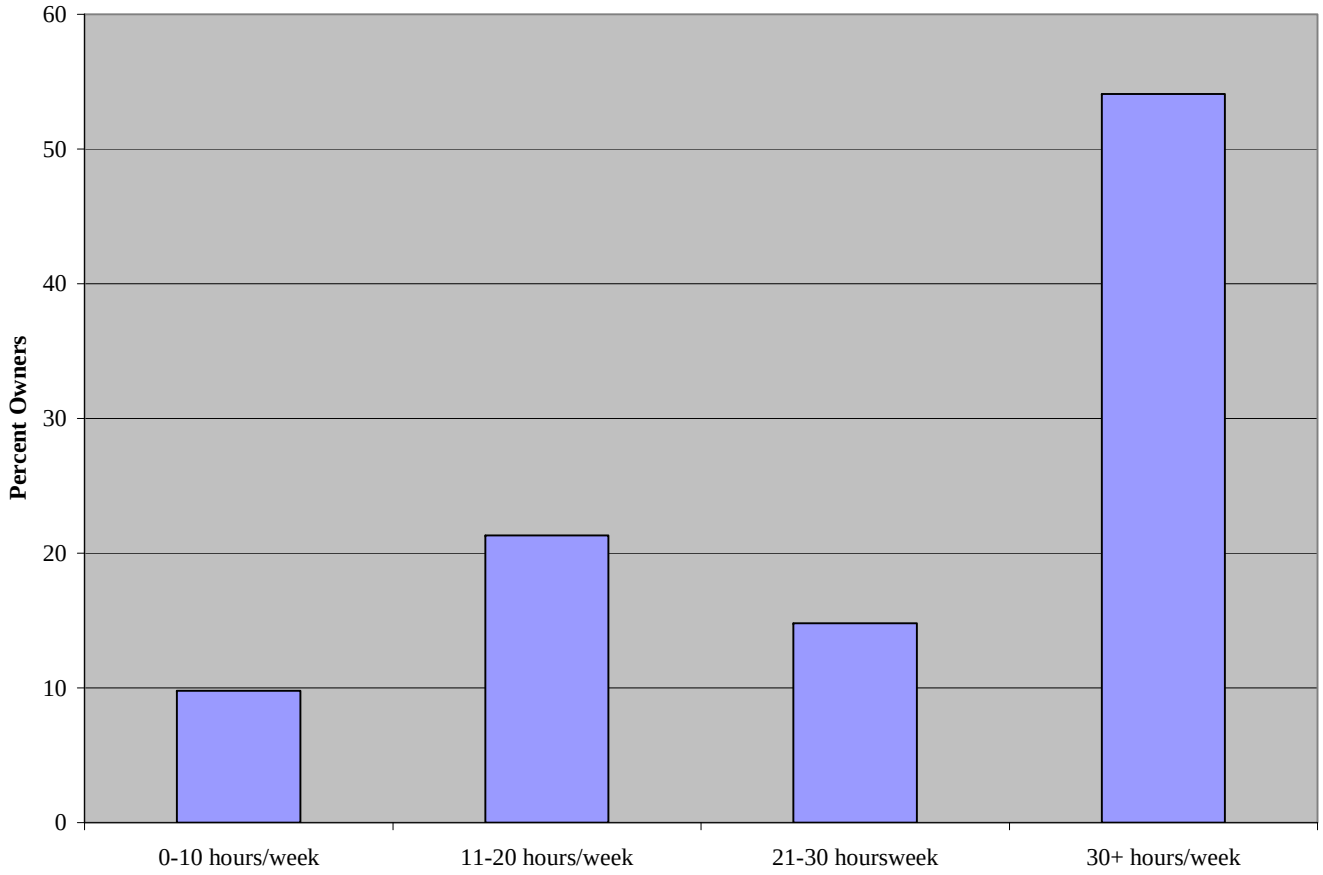


Figure 7. Average awake-time owner spends with dog per week. Fifty-four percent of owners spent 30 or more hours per week of awake-time with their dog.

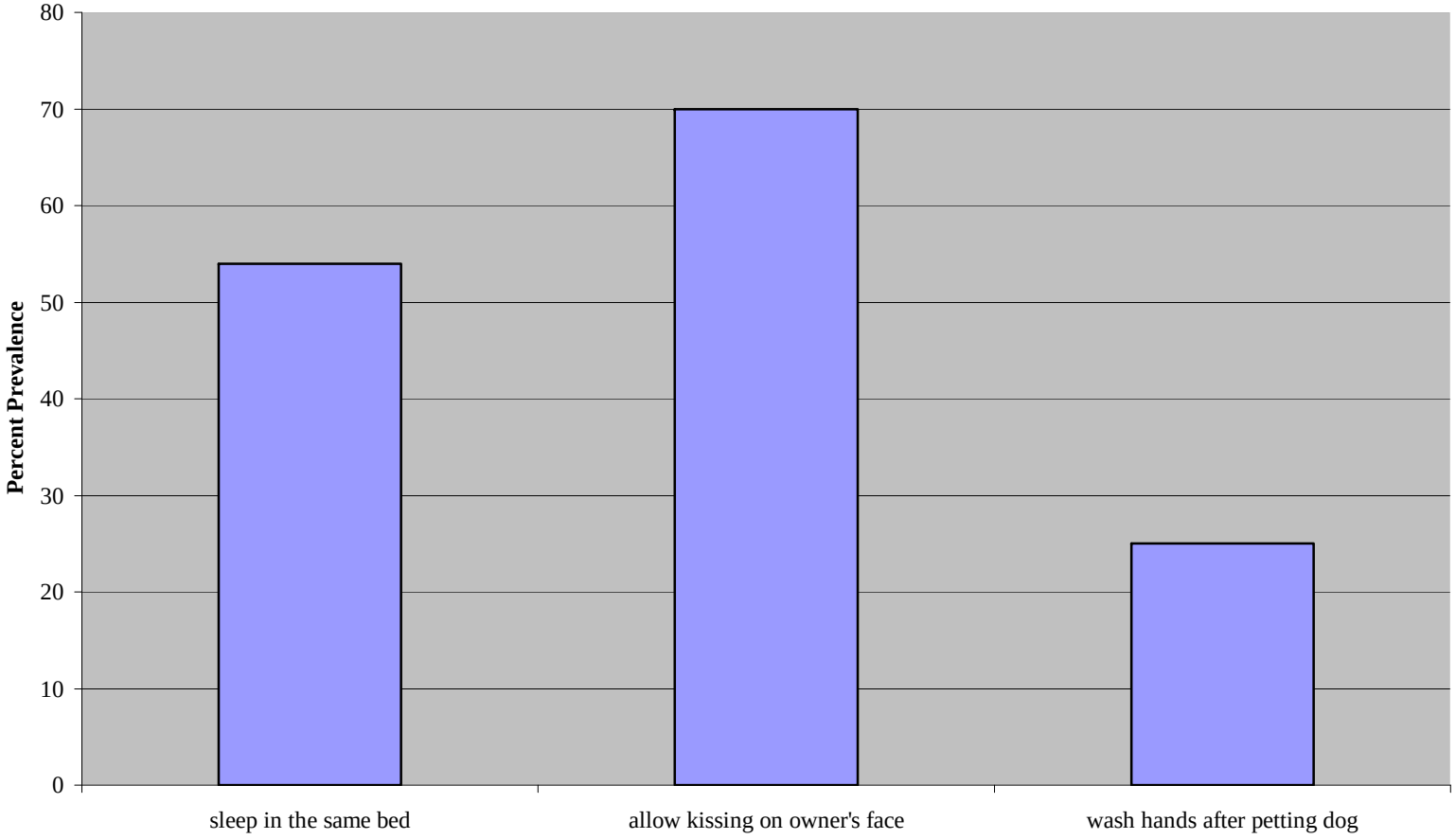


Figure 8. Prevalence of contact behaviors between dogs and owners.

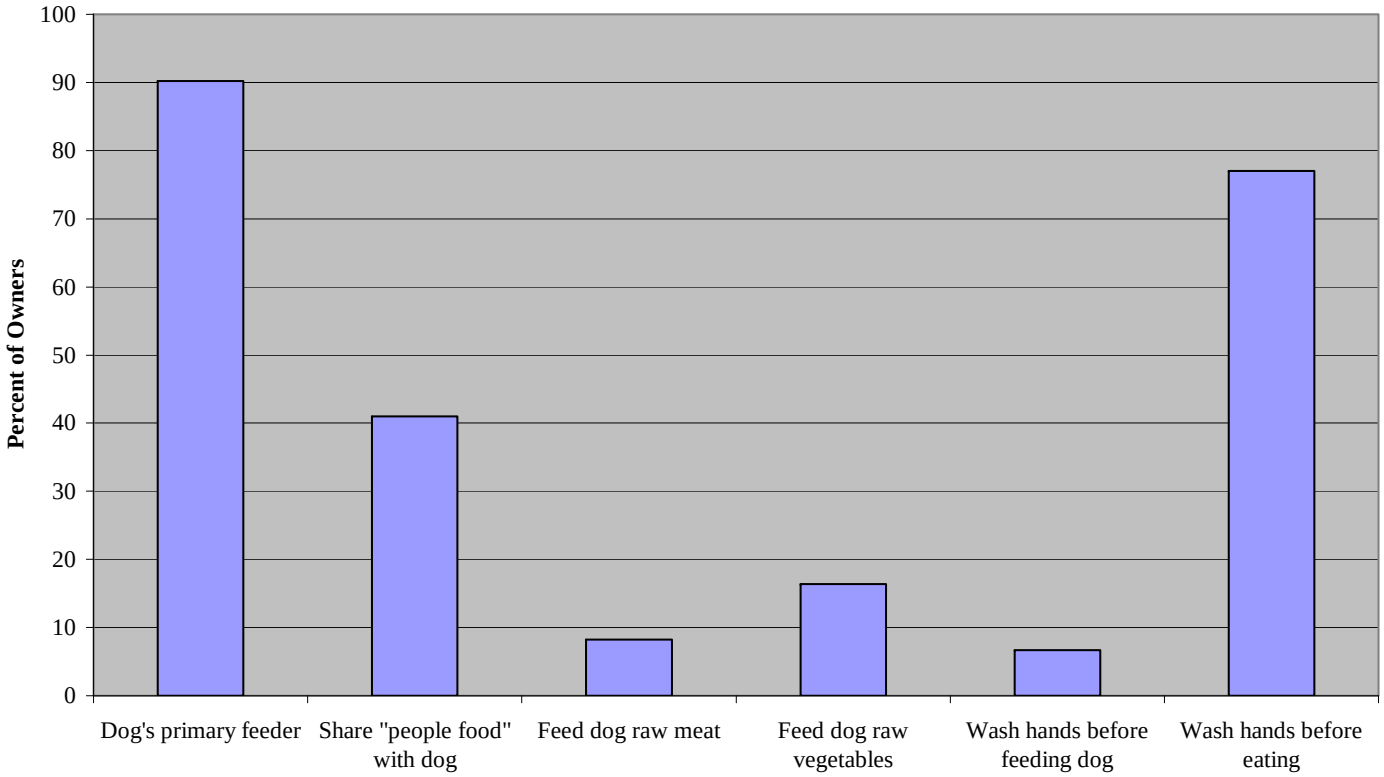


Figure 9. Dog-owner feeding behaviors.

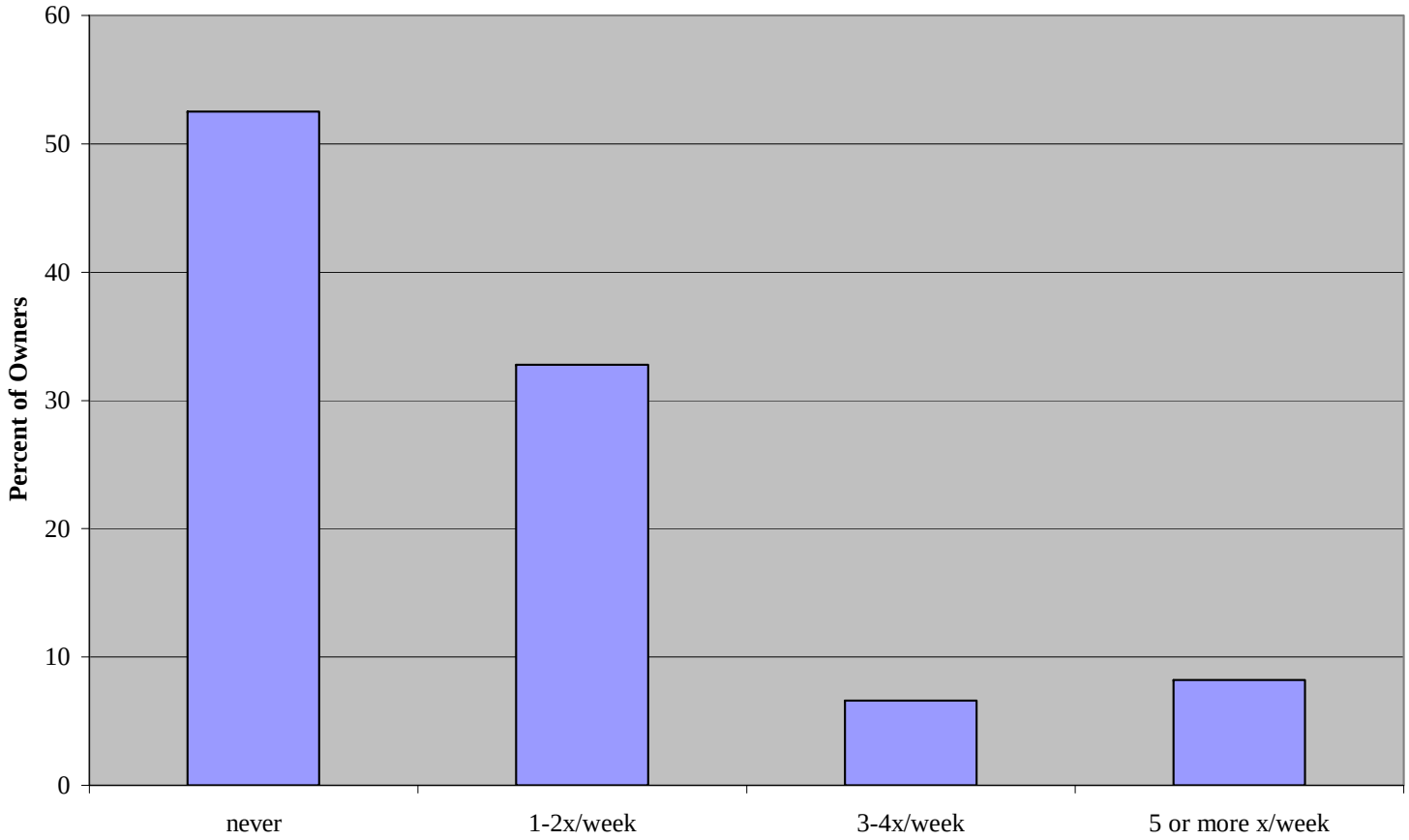
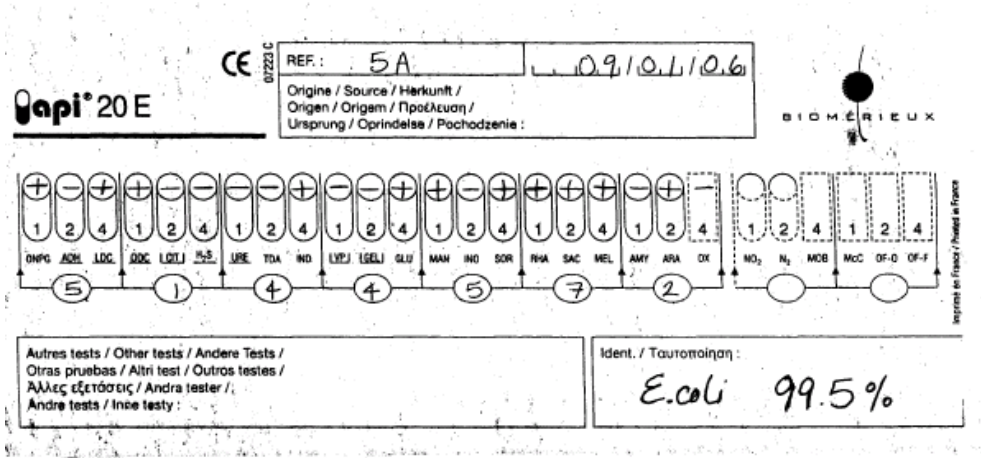


Figure 10. Times per week owner disposed of dog's feces. Forty-seven percent of owners reported disposing of their dogs' feces at least once during an average week.



apiweb™ - Identification result Page 1 of 1

U of Tennessee College of Veterinary Medicine - Knoxville

apiweb

API 20 E V4.1

ONPG ADH LDC ODC CIT H_2S URE TDA IND VP GEL GLU MAN INO SOR RHA SAC MEL AMY ARA OX

5 1 4 4 5 7 2

NO_2 N_2 MOB McC OF-O OF-F

REFERENCE **DATE**
5A 9/1/06
COMMENT

VERY GOOD IDENTIFICATION

Strip	API 20 E V4.1
Profile	5 1 4 4 5 7 2
Note	

Significant taxa	**% ID**	**T**	**Tests against**
Escherichia coli 1	**99.5**	**0.96**	

Next taxon	**% ID**	**T**	**Tests against**				
Kluyvera spp	**0.4**	**0.53**	**LDC**	**25%**	**SOR**	**25%**	**AMY**	**99%**

Close Print

Figure 11. Original and website printout results of API-20E for an *E. coli* isolate. (bioMerieux, Durham, NC).

Table 2. Number (%) of biochemical reactions results found in *E. coli* isolates. N=456.

β-galactosidase	456 (100%)
Arginine Dihydrolase	87 (19.1%)
Lysine Decarboxylase	434 (95.2%)
Ornithine Decarboxylase	355 (77.9%)
Citrate Utilization	0 (0%)
H_2S Production	0 (0%)
Urease Production	3 (0.6%)
Tryptophane Deaminase	0 (0%)
Indole Production	455 (99.8%)
Acetoin Production	0 (0%)
Gelatinase	0 (0%)
Glucose Fermentation	456 (100%)
Mannitol Fermentation	456 (100%)
Inositol Fermentation	1 (0.2%)
Sorbitol Fermentation	453 (99.3%)
Rhamnose Fermentation	437 (95.8%)
Saccharose Fermentation	270 (59.2%)
Melibiose Fermentation	434 (95.2%)
Amygdalin Fermentation	8 (1.8%)
Arabinose Fermentation	456 (100%)
Cytochrome Oxidase	0 (0%)
Lactose Fermentation	288 (94.7%)

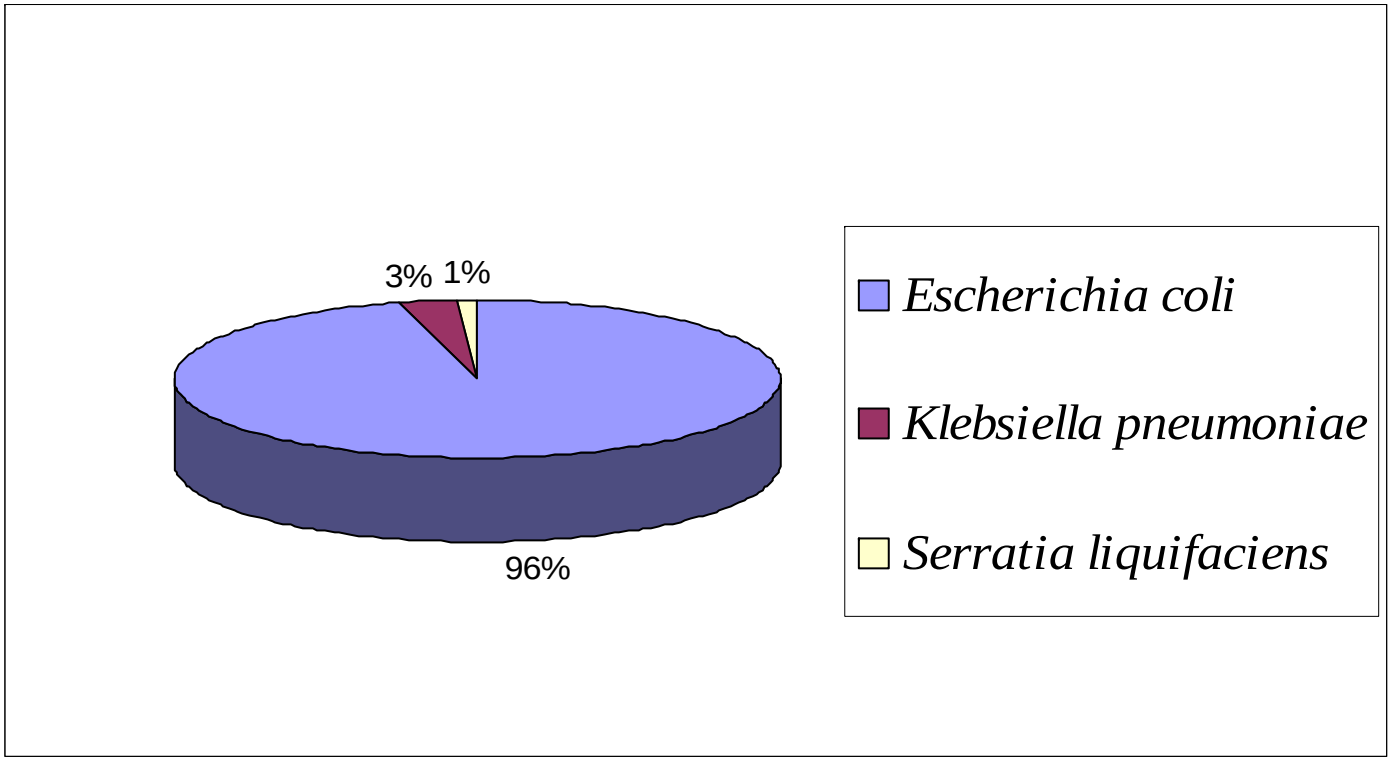


Figure 12. Predominant fecal coliform isolated from human participants. *E. coli* was isolated from 96% of human participants and from all canine participants.

Table 3. Number (%) of participants with *E. coli* susceptible, intermediate, or resistant to each antimicrobial agent.

		Canine (N=61)			Owner (N=61)				Control (N=30)	
	Susceptible	**Intermediate**	**Resistant**		**Susceptible**	**Intermediate**	**Resistant**		**Susceptible**	**Intermediate**	**Resistant**
AN	61 (100%)	0	0		60 (98%)	0	1 (2%)		30 (100%)	0	0
AM	41 (67%)	10 (16%)	10 (16%)		37 (61%)	9 (15%)	15 (25%)		15 (50%)	6 (20%)	9 (30%)
AmC	49 (80%)	9 (15%)	3 (5%)		53 (87%)	6 (10%)	2 (3%)		24 (80%)	6 (20%)	0
FOX	58 (95%)	0	3 (5%)		60 (98%)	0	1 (2%)		30 (100%)	0	0
NXL	58 (95%)	0	3 (5%)		59 (97%)	0	2 (3%)		30 (100%)	0	0
CPD	57 (93%)	0	4 (7%)		58 (95%)	0	3 (5%)		30 (100%)	0	0
CRO	58 (95%)	1 (2%)	2 (3%)		59 (97%)	0	2 (3%)		30 (100%)	0	0
CF	29 (48%)	25 (41%)	7 (11%)		36 (59%)	18 (30%)	7 (11%)		18 (60%)	11 (37%)	1 (3%)
C	58 (95%)	0	3 (5%)		59 (97%)	1 (2%)	1 (2%)		28 (93%)	0	2 (7%)
CIP	60 (98%)	0	1 (2%)		58 (95%)	0	3 (5%)		28 (93%)	0	2 (7%)
GM	60 (98%)	0	1 (2%)		58 (95%)	1 (2%)	2 (3%)		30 (100%)	0	0
IPM	61 (100%)	0	0		61 (100%)	0	0		30 (100%)	0	0
K	56 (92%)	3 (5%)	2 (3%)		54 (89%)	4 (7%)	3 (5%)		28 (93%)	0	2 (7%)
NA	58 (95%)	0	3 (5%)		55 (90%)	1 (2%)	5 (8%)		28 (93%)	0	2 (7%)
S	34 (56%)	23 (38%)	4 (7%)		39 (64%)	15 (25%)	7 (11%)		18 (60%)	9 (30%)	3 (10%)
Te	56 (92%)	1 (2%)	4 (7%)		54 (89%)	1 (2%)	6 (10%)		26 (87%)	1 (3%)	3 (10%)
TMS	58 (95%)	0	3 (5%)		54 (89%)	0	7 (11%)		25 (83%)	0	5 (17%)

Table 4. Number (%) of *E. coli* isolates susceptible, intermediate, or resistant to each antimicrobial agent.

		Canine (N=183)			Dog Owner (N=183)				Control (N=90)	
	Susceptible	**Intermediate**	**Resistant**	**Susceptible**	**Intermediate**	**Resistant**	**Susceptible**	**Intermediate**	**Resistant**
AN	183 (100%)	0	0	181 (98.9%)	0	2 (1.1%)	90 (100%)	0	0
AM	136 (74.3%)	24 (13.1%)	23 (12.6%)	117 (63.9%)	23 (12.6%)	43 (23.5%)	49 (54.4%)	14 (15.6%)	27 (30%)
AmC	159 (86.9%)	19 (10.4%)	5 (2.7%)	164 (89.6%)	15 (8.2%)	4 (2.2%)	76 (84.4%)	14 (15.6%)	0
FOX	178 (97.3%)	0	5 (2.7%)	181 (98.9%)	0	2 (1.1%)	90 (100%)	0	0
NXL	178 (97.3%)	0	5 (2.7%)	180 (98.4%)	0	3 (1.6%)	90 (100%)	0	0
CPD	177 (96.7%)	0	6 (3.3%)	177 (96.7%)	0	6 (3.3%)	90 (100%)	0	0
CRO	178 (97.3%)	1 (0.5%)	4 (2.2%)	180 (98.4%)	0	3 (1.6%)	90 (100%)	0	0
CF	107 (58.5%)	63 (34.4%)	13 (7.1%)	120 (65.6%)	47 (25.7%)	16 (8.7%)	62 (68.9%)	25 (27.8%)	3 (3.3%)
C	180 (98.4%)	0	3 (1.6%)	177 (96.7%)	3 (1.6%)	3 (1.6%)	84 (93.3%)	0	6 (6.7%)
CIP	180 (98.4%)	0	3 (1.6%)	176 (96.2%)	0	7 (3.8%)	84 (93.3%)	0	6 (6.7%)
GM	182 (99.5%)	0	1 (0.5%)	177 (96.7%)	2 (1.1%)	4 (2.2%)	90 (100%)	0	0
IPM	183 (100%)	0	0	183 (100%)	0	0	90 (100%)	0	0
K	177 (96.7%)	4 (2.2%)	2 (1.1%)	169 (92.3%)	8 (4.4%)	6 (3.3%)	84 (93.3%)	0	6 (6.7%)
NA	178 (97.3%)	0	5 (2.7%)	170 (92.9%)	3 (1.6%)	10 (5.5%)	84 (93.3%)	0	6 (6.7%)
S	123 (67.2%)	53 (29%)	7 (3.8%)	124 (67.8%)	40 (21.9%)	19 (10.4%)	58 (64.4%)	23 (25.6%)	9 (10%)
Te	173 (94.5%)	3 (1.6%)	7 (3.8%)	163 (89.1%)	3 (1.6%)	17 (9.3%)	78 (86.7%)	3 (3.3%)	9 (10%)
TMS	178 (97.3%)	0	5 (2.7%)	164 (89.6%)	0	19 (10.4%)	77 (85.6%)	0	13 (14.4%)

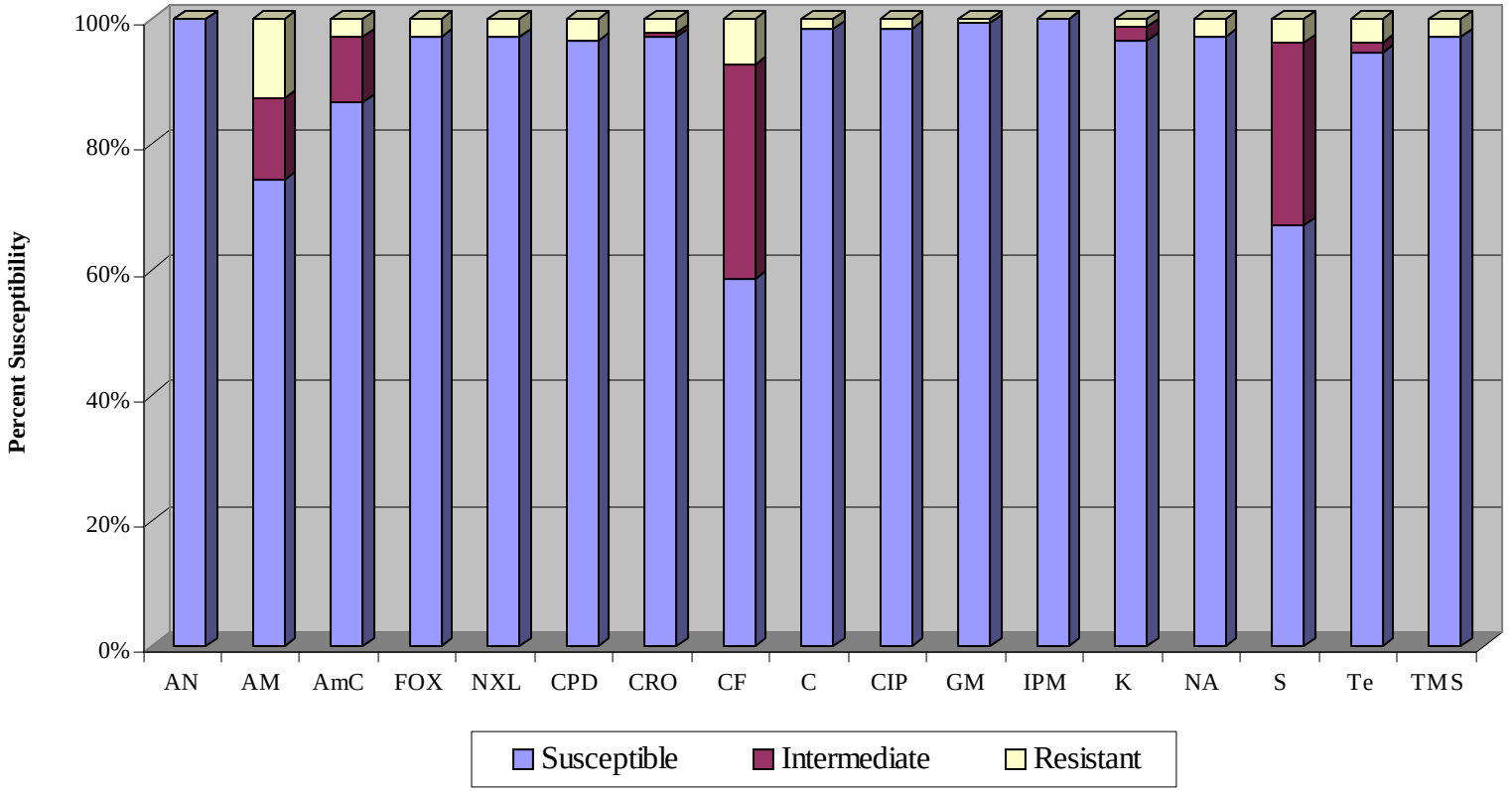


Figure 13. Susceptibility results of canine *E. coli* isolates.

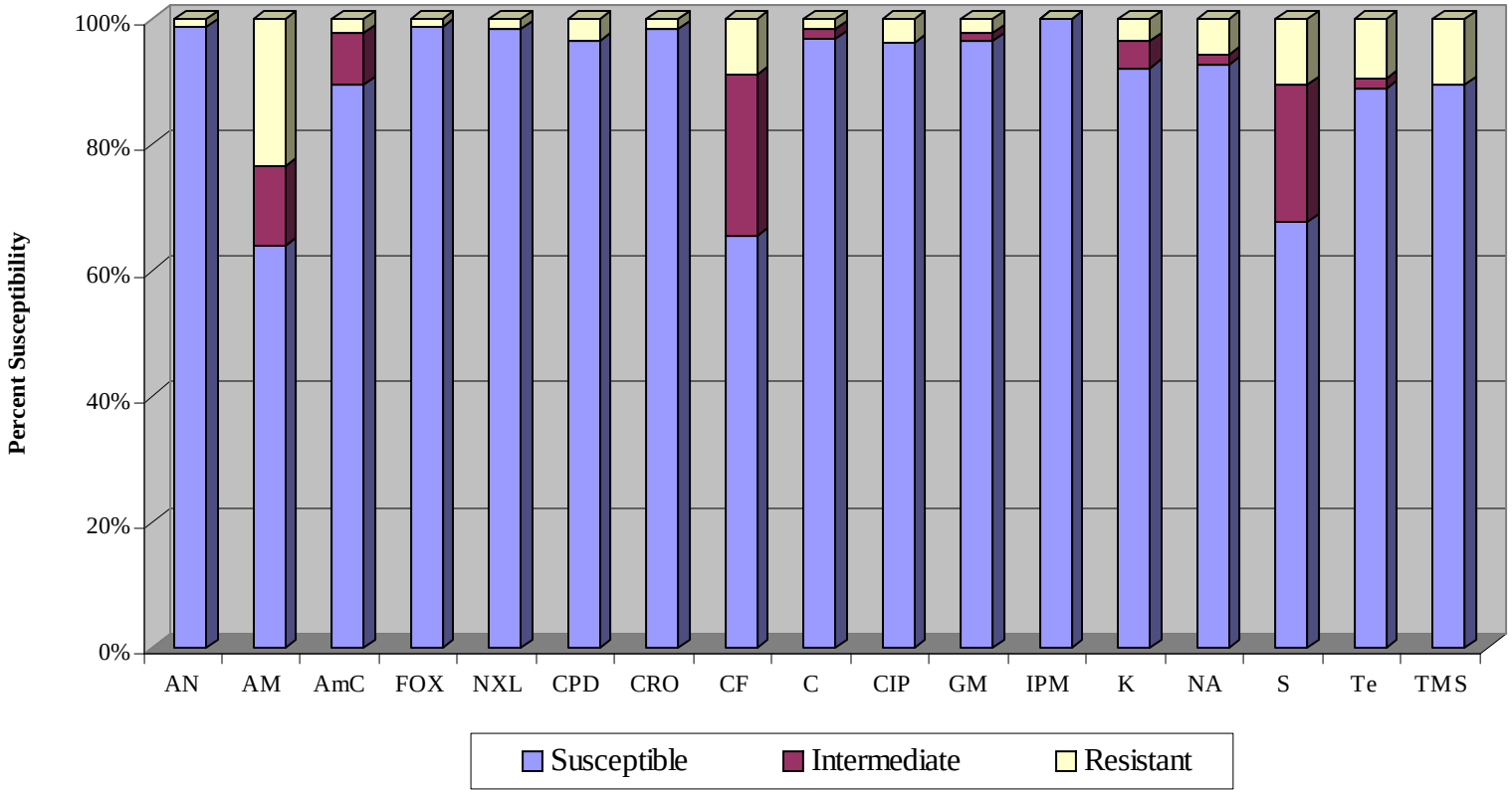


Figure 14. Susceptibility results of owner *E. coli* isolates.

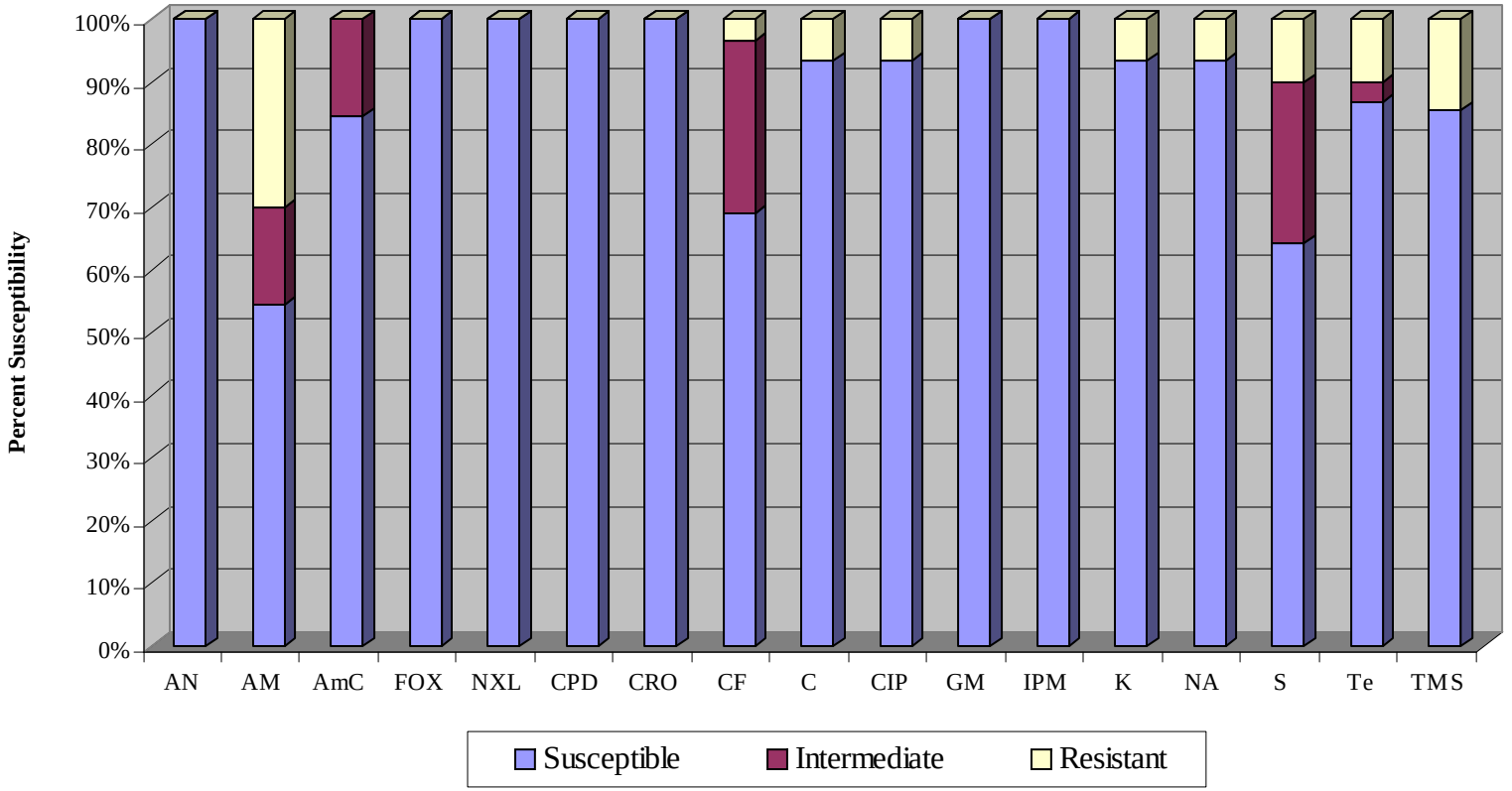


Figure 15. Susceptibility results of control *E. coli* isolates.

Table 5. Wilcoxon signed ranks test results comparing antimicrobial susceptibility between dog-owner pairs. There were no differences in susceptibility results between dog owner pairs for any of the 17 antimicrobial agents.

Antimicrobial Agent	Z-score	p value
amikacin	-1.000	0.317
ampicillin	-1.038	0.299
ampicillin-clavulanic acid	-1.031	0.302
cefoxitin	-1.000	0.317
ceftiofur	-0.577	0.564
cefpodoxime	-0.447	0.655
ceftriaxone	-0.272	0.785
cephalothin	-0.895	0.371
chloramphenicol	-0.849	0.396
ciprofloxacin	-1.000	0.317
gentamicin	-0.756	0.450
imipenem	0.000	1.000
kanamycin	-0.548	0.584
nalidixic acid	-1.055	0.291
streptomycin	-0.253	0.800
tetracycline	-0.807	0.420
trimethoprim-sulfamethoxazole	-1.265	0.206

Table 6. Mann-Whitney test results comparing antimicrobial susceptibility between owners and controls. There were no differences in susceptibility results between owners and controls for any of the 17 antimicrobial agents.

	% owners and controls			% isolates from owners and controls	
Antimicrobial Agent	**Z-Score**	**p value**		**Z-Score**	**p value**
amikacin	-0.701	0.483		-0.994	0.320
ampicillin	-0.883	0.377		-1.484	0.138
amoxicillin-clavulanic acid	-0.769	0.442		-1.148	0.251
cefoxitin	-0.701	0.483		-0.994	0.320
ceftiofur	-0.997	0.319		-1.219	0.223
cefpodoxime	-1.228	0.219		-1.734	0.083
ceftriaxone	-0.997	0.319		-1.219	0.223
cephalothin	-0.373	0.709		-0.793	0.428
chloramphenicol	-0.761	0.447		-1.322	0.186
ciprofloxacin	-0.342	0.732		-1.034	0.301
gentamicin	-1.228	0.219		-1.734	0.083
imipenem	0.000	1.000		0.000	1.000
kanamycin	-0.652	0.514		-0.206	0.837
nalidixic acid	-0.482	0.630		-0.100	0.920
streptomycin	-0.266	0.790		-0.460	0.645
tetracycline	-0.231	0.817		-0.544	0.587
trimethoprim-sulfamethoxazole	-0.684	0.494		-0.979	0.328

Table 7. Mann-Whitney test results comparing antimicrobial susceptibility between dogs and controls. Control isolates were more resistant than canine isolates.

	% dogs and controls			% isolates from dogs and controls	
Antimicrobial agent	**Z-Score**	**p value**		**Z-Score**	**P value**
amikacin	0	1.000		0	1.000
ampicillin	-1.679	0.093		-3.559	<0.001**
amoxicillin-clavulanic acid	-0.073	0.942		-0.451	0.652
cefoxitin	-1.228	0.219		-1.580	0.114
ceftiofur	-1.228	0.219		-1.580	0.114
cefpodoxime	-1.427	0.154		-1.734	0.083
ceftriaxone	-1.228	0.219		-1.580	0.114
cephalothin	-1.322	0.186		-1.768	0.077
chloramphenicol	-0.342	0.732		-2.183	0.029*
ciprofloxacin	-1.256	0.209		-2.183	0.029*
gentamicin	-0.701	0.483		-0.701	0.483
imipenem	0.000	1.000		0.000	1.000
kanamycin	-0.201	0.841		-1.336	0.182
nalidixic acid	-0.342	0.732		-1.551	0.121
streptomycin	-0.217	0.829		-0.760	0.448
tetracycline	-0.758	0.448		-2.250	0.024*
trimethoprim-sulfamethoxazole	-1.850	0.064		-3.659	<0.001**

*** p<0.05, **p<0.003**

Table 8. Antibiogram patterns for *E. coli* isolates from dogs, owners, and controls.

	Number of isolates resistant to antimicrobial agents (%)			
Antibiogram Pattern	**Total (n=456)**	**Dog (n=183)**	**Owner (n=183)**	**Control (n=90)**
ampicillin and amoxicillin-clavulanic acid	9(2.0%)	5(2.7%)	4(2.2%)	0
ampicillin and cephalothin	21(4.6%)	8(4.4%)	10(5.5%)	3(3.3%)
ampicillin and chloramphenicol	8(1.8%)	2(1%)	0	6(6.7%)
ampicillin and kanamycin	12(2.6%)	2(1%)	4(2.2%)	6(6.7%)
ampicillin and streptomycin	27(5.9%)	7(3.8%)	11(6%)	9(10%)
ampicillin and tetracycline	20(4.4%)	5(2.7%)	9(4.9%)	6(6.7%)
ampicillin and trimethoprim-sulfamethoxazole	28(6.1%)	5(2.7%)	13(7.1%)	10(11.1%)
cephalothin and streptomycin	12(2.6%)	4(2.2%)	5(2.7%)	3(3.3%)
ciprofloxacin and nalidixic acid	16(3.5%)	3(1.6%)	7(3.8%)	6(6.7%)
ampicillin, cephalothin, and streptomycin	10(2.2%)	4(2.2%)	3(1.6%)	3(3.3%)
ampicillin, streptomycin, and trimethoprim-sulfamethoxazole	22(4.8%)	5(2.7%)	10(5.5%)	7(7.8%)
ampicillin, tetracycline, and trimethoprim-sulfamethoxazole	14(3.1%)	2(1%)	9(4.9%)	3(3.3%)
ciprofloxacin, nalidixic acid, and trimethoprim-sulfamethoxazole	7(1.5%)	1(0.5%)	0	6(6.7%)
ampicillin, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole	11(2.4%)	2(1%)	6(3.3%)	3(3.3%)

Table 9. Susceptibility results of canine MDR fecal *E. coli* isolates.

Antimicrobial Agent	**Dog #6B**	**Dog #19A**	**Dog #34A**	**Dog #34B**	**Dog #34C**	**Dog #64A,B,C**
amikacin	S	S	S	S	S	S
ampicillin	R	R	R	R	R	R
amoxicillin-clavulanic acid	R	S	R	I	S	R
cefoxitin	R	S	R	S	S	R
ceftiofur	R	S	R	S	S	R
cefpodoxime	R	S	R	S	S	R
ceftriaxone	R	S	I	S	S	R
cephalothin	R	I	R	I	I	R
chloramphenicol	S	R	R	S	S	S
ciprofloxacin	S	S	R	R	R	S
gentamicin	S	S	R	S	S	S
imipenem	S	S	S	S	S	S
kanamycin	S	R	R	S	S	S
nalidixic acid	S	R	R	R	R	S
streptomycin	I	R	R	S	S	R
tetracycline	S	R	R	S	S	S
trimethoprim-sulfamethoxazole	S	R	R	S	S	R
Number Resistant	**7**	**7**	**14**	**3**	**3**	**9**

S= Susceptible, I= Intermediate, R= Resistant MDR=resistant to 3 or more agents.

Table 10. Susceptibility results of owner MDR *E. coli* isolates.

Antimicrobial	Owner #66A/B	Owner #66C	Owner #71A	Owner #71B	Owner #85A	Owner #85B/C	Owner #89A,B,C
amikacin	S	S	R	R	S	S	S
ampicillin	R	R	S	I	R	R	R
amoxicillin-clavulanic acid	S	I	S	S	S	S	R
cefoxitin	S	S	R	R	S	S	S
ceftiofur	S	S	R	R	S	S	S
cefpodoxime	S	S	R	R	S	S	R
ceftriaxone	S	S	R	R	S	S	S
cephalothin	I	I	R	R	I	I	R
chloramphenicol	S	S	S	S	S	S	S
ciprofloxacin	R	R	S	S	R	R	S
gentamicin	S	S	I	I	R	R	S
imipenem	S	S	S	S	S	S	S
kanamycin	S	S	R	R	I	I	S
nalidixic acid	R	R	R	R	R	R	S
streptomycin	S	S	R	R	S	I	S
tetracycline	S	S	S	S	S	S	S
trimethoprim-sulfamethoxazole	S	S	S	S	S	S	S
Number Resistant	**3**	**3**	**9**	**9**	**4**	**4**	**4**

S= Susceptible, I= Intermediate, R= Resistant

Table 10 (continued). Susceptibility results of owner MDR *E. coli* isolates.

Antimicrobial	Owner # 91C	Owner # 109 A,B,C	Owner # 111 A,B,C	Owner # 112A,B,C	Owner # 123 A,B,C	Owner # 124A
amikacin	S	S	S	S	S	S
ampicillin	R	R	R	R	R	R
amoxicillin-clavulanic acid	I	S	I	I	S	R
cefoxitin	S	S	S	S	S	S
ceftiofur	S	S	S	S	S	R
cefpodoxime	S	S	S	S	S	R
ceftriaxone	S	S	S	S	S	R
cephalothin	I	S	R	S	I	R
chloramphenicol	S	S	S	S	S	S
ciprofloxacin	S	S	S	S	S	R
gentamicin	S	S	S	S	S	R
Imipenem	S	S	S	S	S	S
kanamycin	S	S	S	S	R	R
nalidixic Acid	S	S	S	S	S	R
streptomycin	R	R	R	R	I	S
tetracycline	S	S	R	R	R	S
trimethoprim-sulfamethoxazole	R	R	R	R	R	S
Number Resistant	**3**	**3**	**5**	**4**	**4**	**10**

S= Susceptible, I= Intermediate, R= Resistant

Table 11. Susceptibility results of control MDR *E. coli* isolates.

Antimicrobial	Control #145A	Control #145B	Control #145C	Control #151C	Control # 156A,B,C	Control # 158A,B,C	Control # 166A,B,C
amikacin	S	S	S	S	S	S	S
ampicillin	S	S	S	R	R	R	R
amoxicillin-clavulanic acid	S	S	S	I	I	S	S
cefoxitin	S	S	S	S	S	S	S
ceftiofur	S	S	S	S	S	S	S
cefpodoxime	S	S	S	S	S	S	S
ceftriaxone	S	S	S	S	S	S	S
cephalothin	S	I	I	I	R	S	S
chloramphenicol	S	S	S	S	R	S	S
ciprofloxacin	R	R	R	S	R	S	S
gentamicin	S	S	S	S	S	S	S
imipenem	S	S	S	S	S	S	S
kanamycin	S	S	S	S	R	S	R
nalidixic acid	R	R	R	S	R	S	S
streptomycin	S	I	S	R	R	R	I
tetracycline	S	S	S	S	R	I	S
trimethoprim-sulfamethoxazole	R	R	R	R	R	R	R
Number Resistant	**3**	**3**	**3**	**3**	**9**	**3**	**3**

S= Susceptible, I= Intermediate, R= Resistant

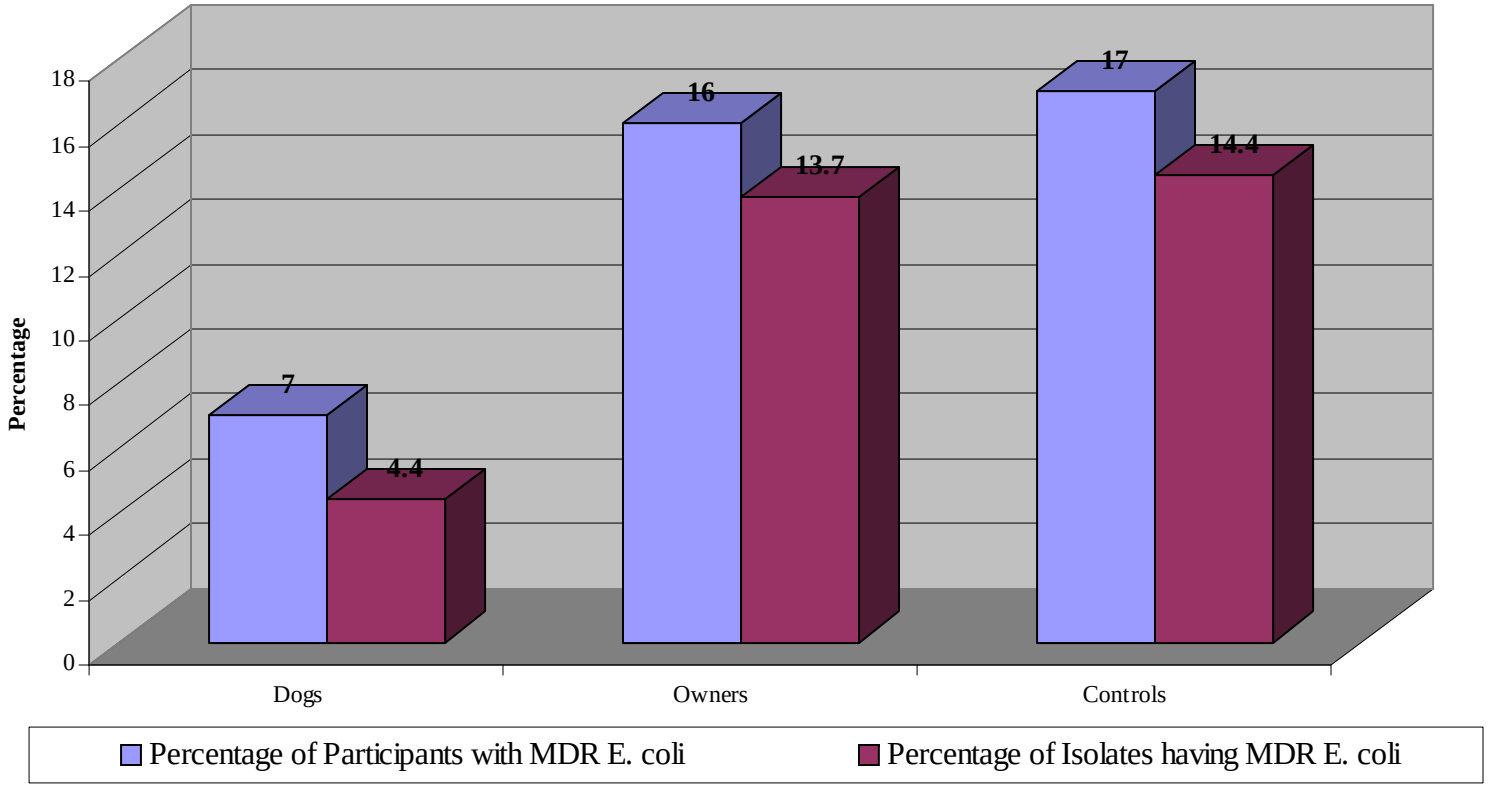


Figure 16. Comparison of multiple drug resistant *E. coli* between groups. There was no difference in number of MDR isolates between dogs and owners (p=0.109), but controls had more MDR *E. coli* isolates than dogs (p=0.003). There was no difference in number of MDR isolates between owners and controls (p=0.833).

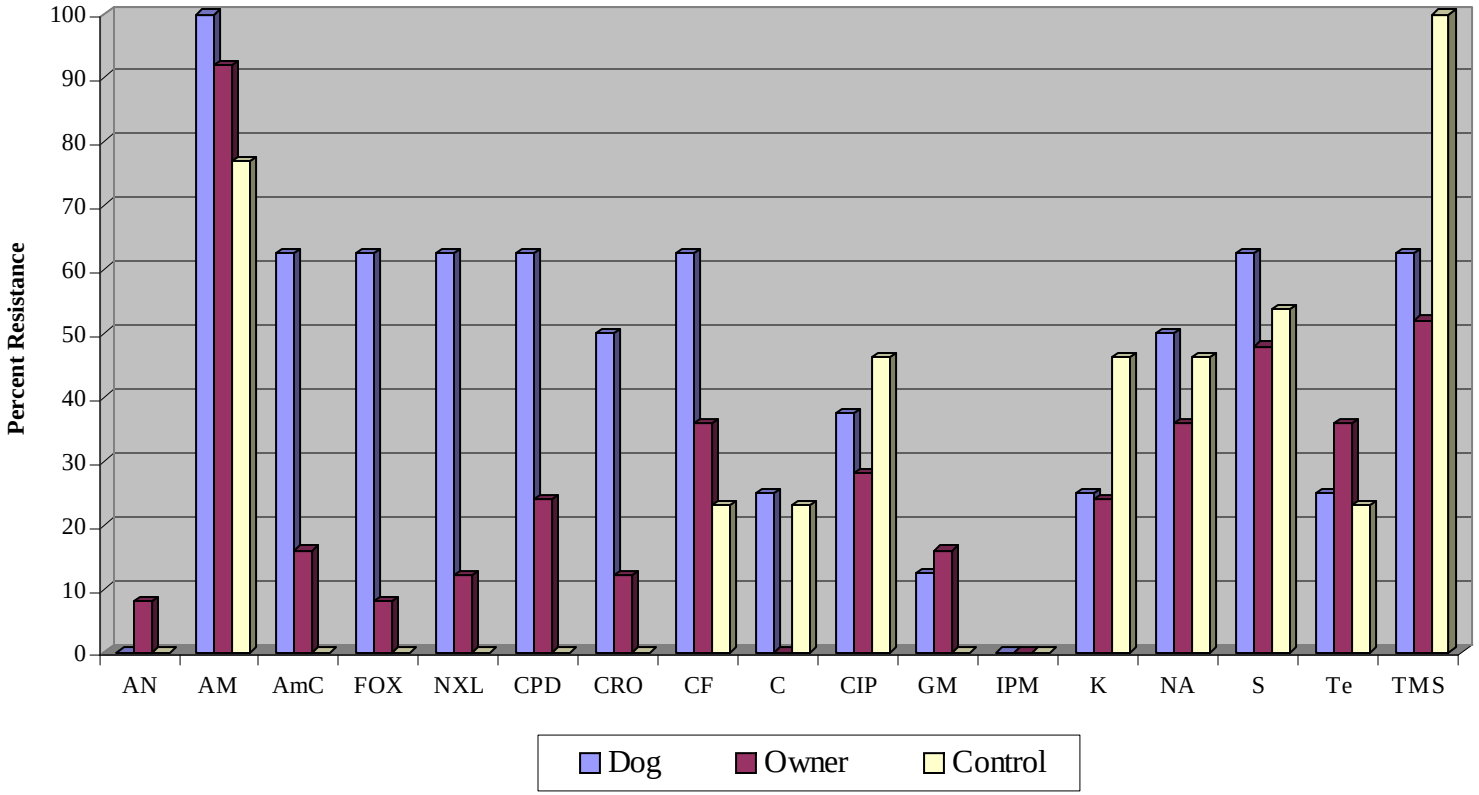


Figure 17. Percent resistance among MDR *E. coli* isolates. Canine MDR isolates were more resistant to beta-lactam antimicrobial agents, especially cephalosporins than human MDR isolates. Control MDR isolates were 100% resistant to trimethoprim-sulfamethoxazole.

Table 12. Mann-Whitney comparisons of antimicrobial susceptibility of multiple drug resistant *E. coli* isolates. Canine MDR isolates were more resistant than owner and control MDR isolates to cefoxitin, and more resistant than control isolates to ceftiofur, cefpodoxime, and ceftriaxone. Control MDR isolates were more resistant than owner MDR isolates to trimethoprim-sulfamethoxazole.

	Dogs and Owners	Dogs and Controls	Owners and Controls
amikacin	p=0.416	p=1.000	p=0.301
ampicillin	p=0.417	p=0.152	p=0.173
amoxicillin-clavulanic acid	p=0.042	p=0.009	p=0.208
cefoxitin	*p=0.001	*p=0.001	p=0.301
ceftiofur	p=0.004	*p=0.001	p=0.199
cefpodoxime	p=0.048	*p=0.001	p=0.058
ceftriaxone	p=0.006	*p=0.002	p=0.199
cephalothin	p=0.107	p=0.017	p=0.125
chloramphenicol	p=0.011	p=0.922	p=0.014
ciprofloxacin	p=0.616	p=0.704	p=0.269
gentamicin	p=0.536	p=0.202	p=0.058
imipenem	p=1.000	p=1.000	p=1.000
kanamycin	p=0.686	p=0.344	p=0.364
nalidixic acid	p=0.487	p=0.867	p=0.549
streptomycin	p=0.534	p=0.903	p=0.492
tetracycline	p=0.572	p=0.503	p=0.915
trimethoprim-sulfamethoxazole	p=0.609	p=0.020	*p=0.003

*p≤0.003

Table 13. Susceptibility and virulence factor results of MDR *E. coli* isolates from 2 households.

	Household #2			Household #58	
	Dog ID#6	**Owner ID#66**		**Dog ID#64**	**Owner ID#124**
amikacin	S	S		S	S
ampicillin	**R**	**R**		**R**	**R**
amoxicillin-clavulanic acid	**R**	I		**R**	**R**
cefoxitin	**R**	S		**R**	S
ceftiofur	**R**	S		**R**	**R**
cefpodoxime	**R**	S		**R**	**R**
ceftriaxone	**R**	S		**R**	**R**
cephalothin	**R**	I		**R**	**R**
chloramphenicol	S	S		S	S
ciprofloxacin	S	**R**		S	**R**
gentamicin	S	S		S	**R**
imipenem	S	S		S	S
kanamycin	S	S		S	**R**
nalidixic acid	S	**R**		S	**R**
streptomycin	I	S		**R**	S
tetracycline	S	S		S	S
trimethoprim-sulfamethoxazole	S	S		**R**	S
cnf	-	-		-	-
hlyD	-	-		-	-
sfa	+	-		-	-
papGIII	-	-		-	-

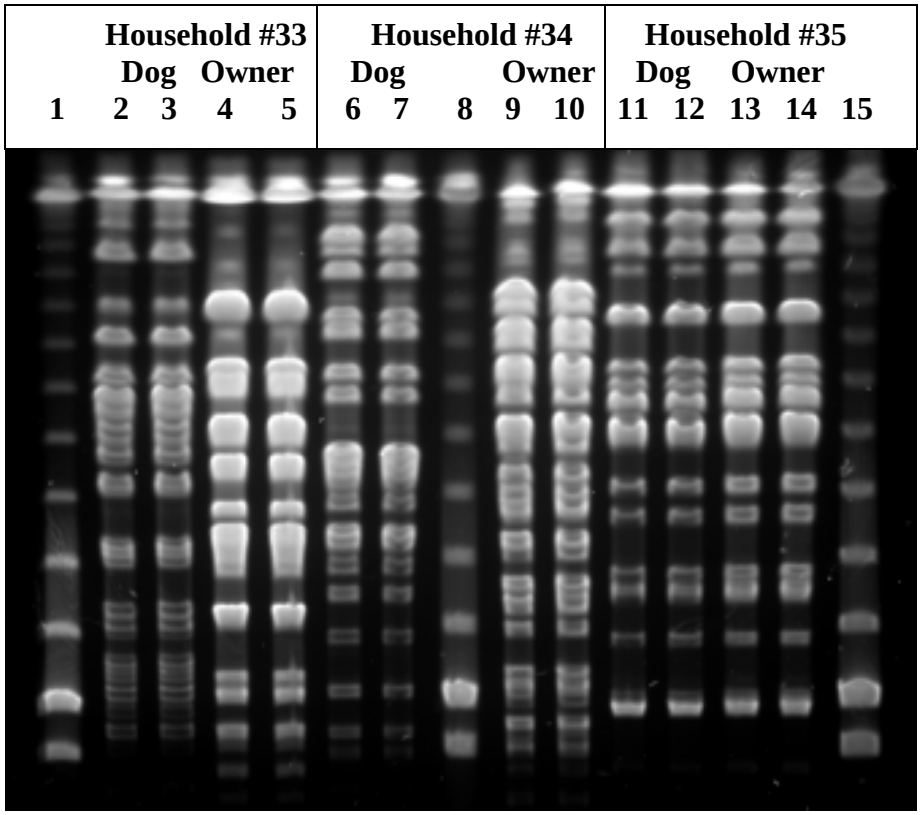


Figure 18. Representative agarose gel showing PFGE patterns of XbaI-digested genomic DNA from *E. coli* isolates from dog-owner pairs in 3 households. Lanes 1, 8, and 15 contain Low Range PFG markers. Household #35 had dog and owner isolates with 100% PFGE similarity.

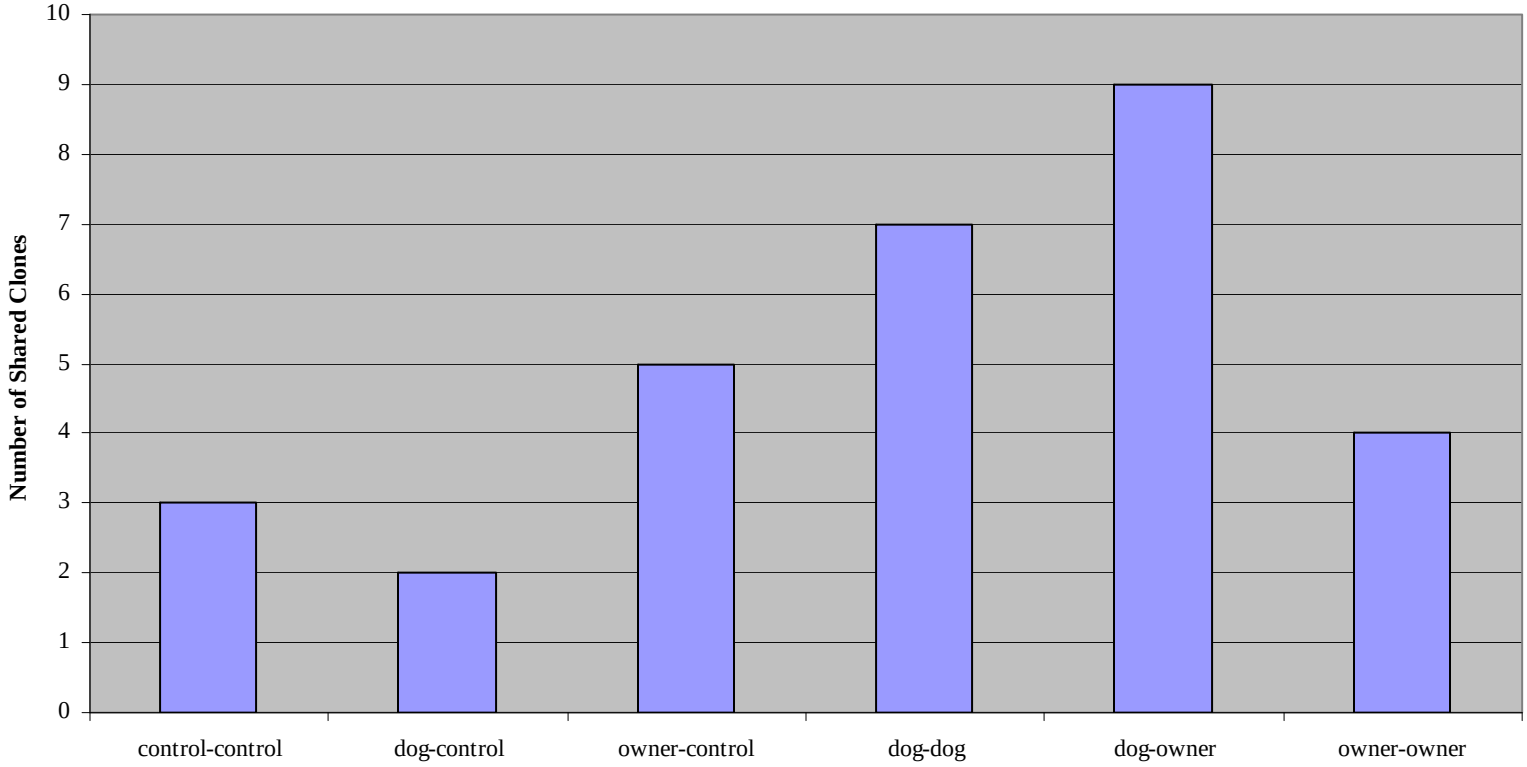


Figure 19. Distribution of across-household sharing of *E. coli* clones. Cross-species sharing of *E. coli* clones between unpaired dogs and owners occurred most commonly, followed by sharing between two dogs with no known contact.

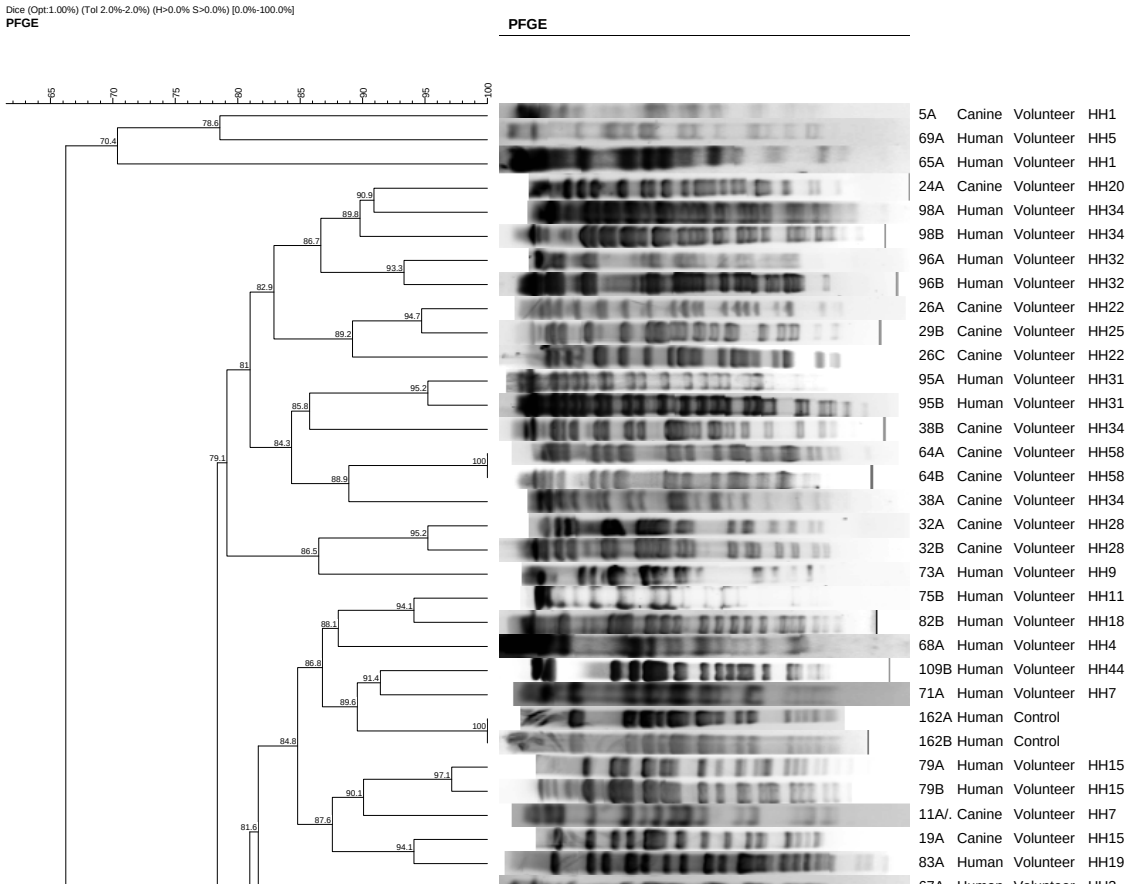


Figure 20. Dendrogram of PFGE profiles from *E. coli* isolated from dogs, owners, and controls.

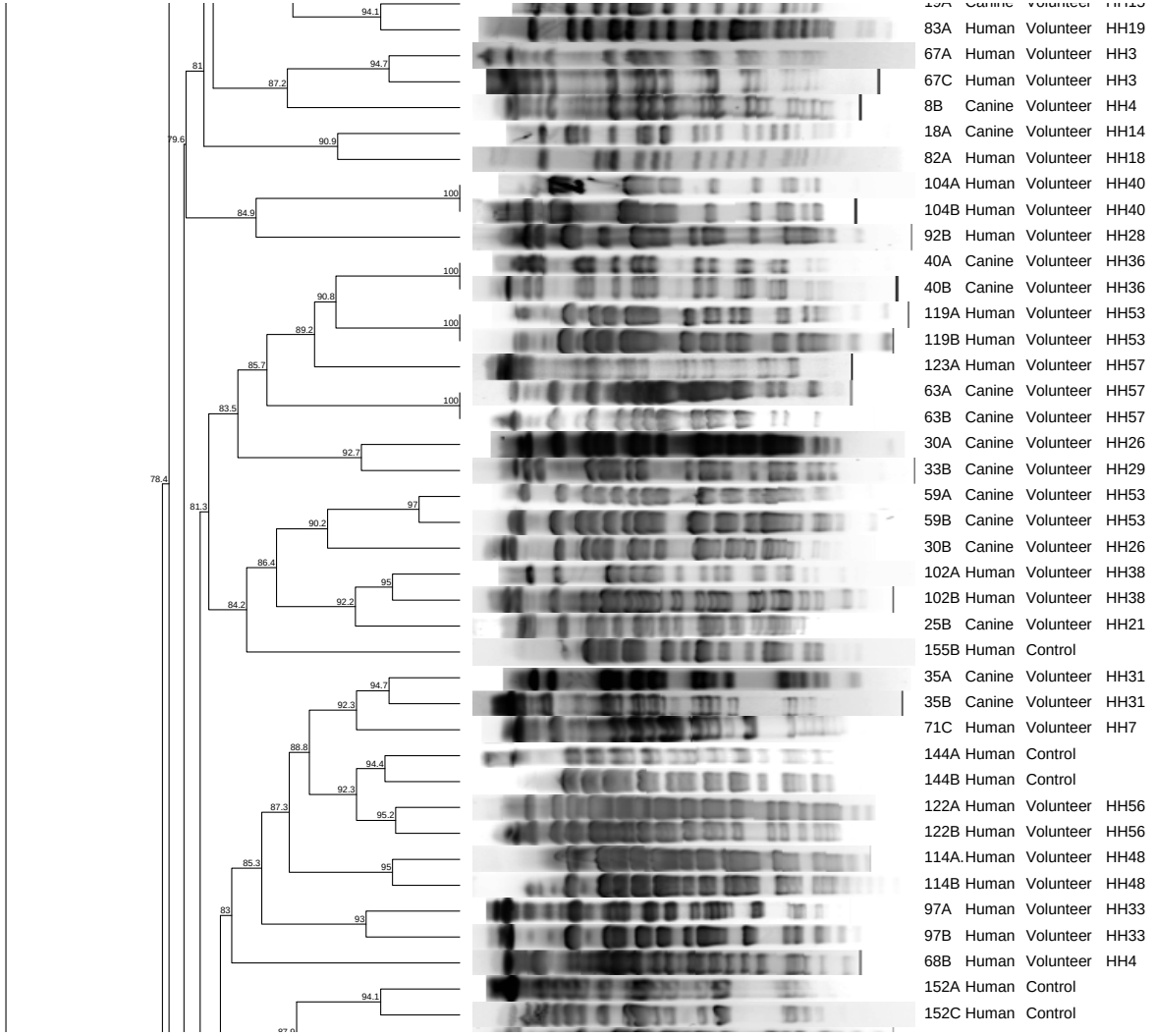


Figure 20. Continued. Dendrogram of PFGE profiles from *E. coli* isolated from dogs, owners, and controls.

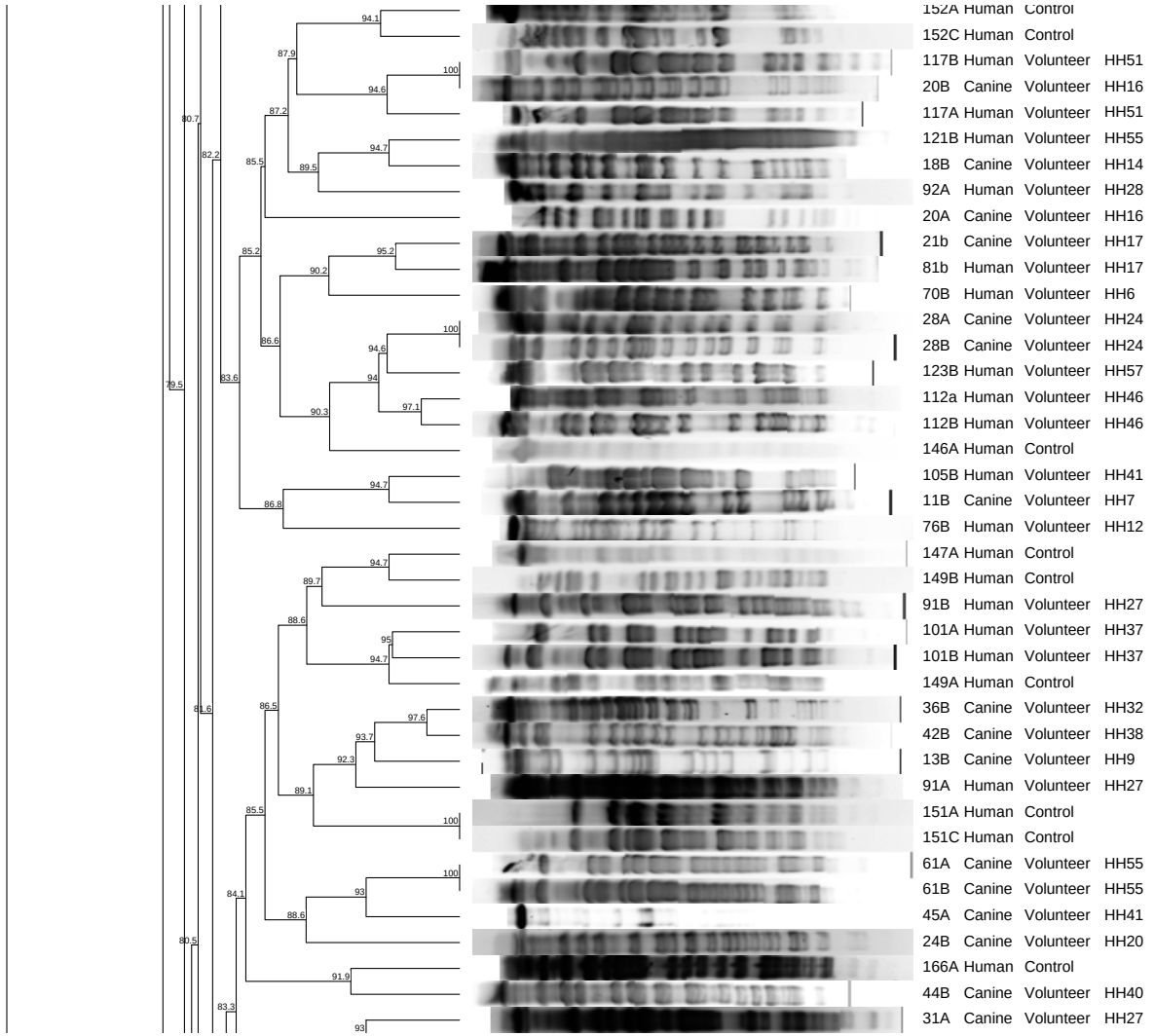


Figure 20. Continued. Dendrogram of PFGE profiles from *E. coli* isolated from dogs, owners, and controls.

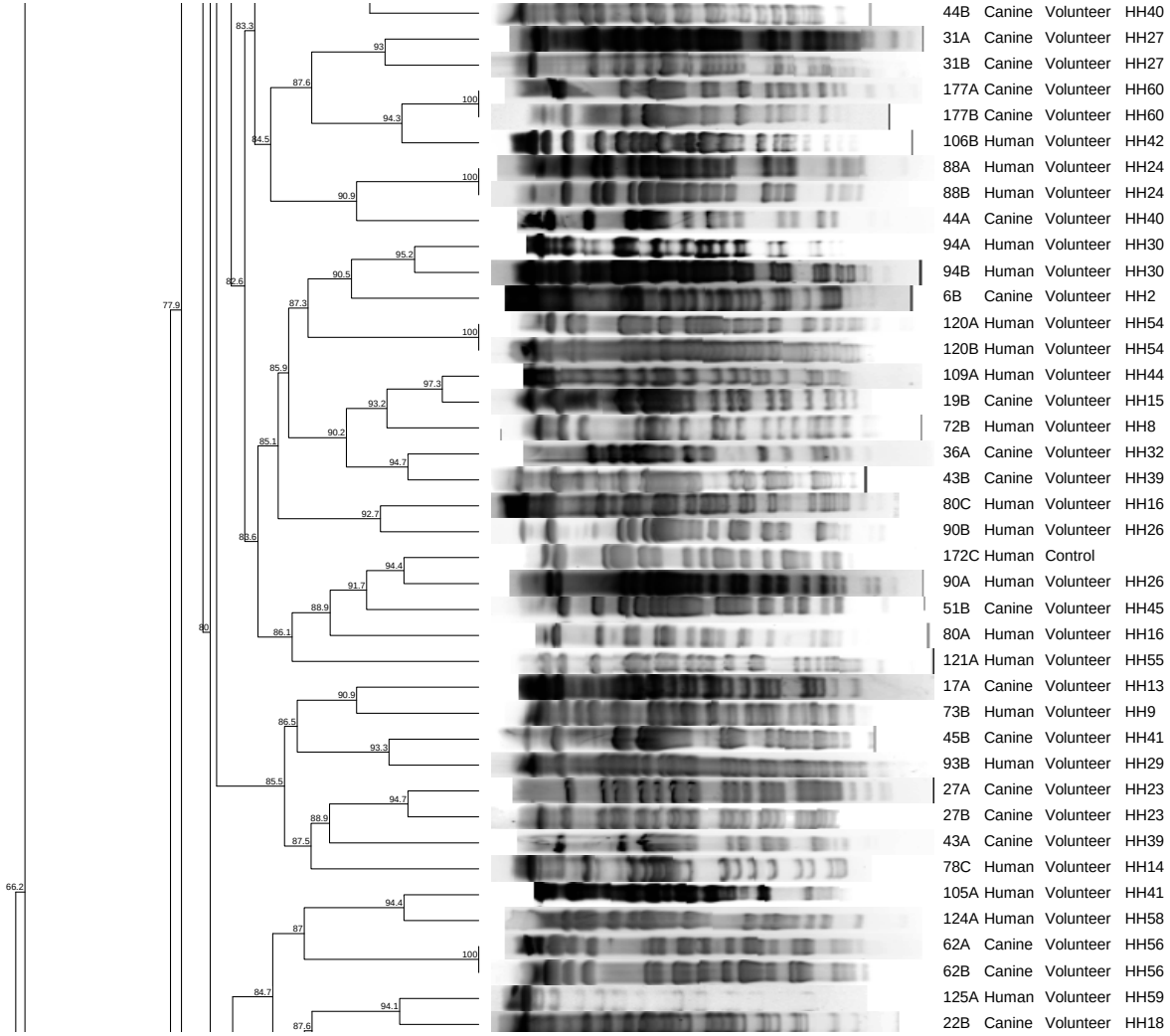


Figure 20. Continued. Dendrogram of PFGE profiles from *E. coli* isolated from dogs, owners, and controls.

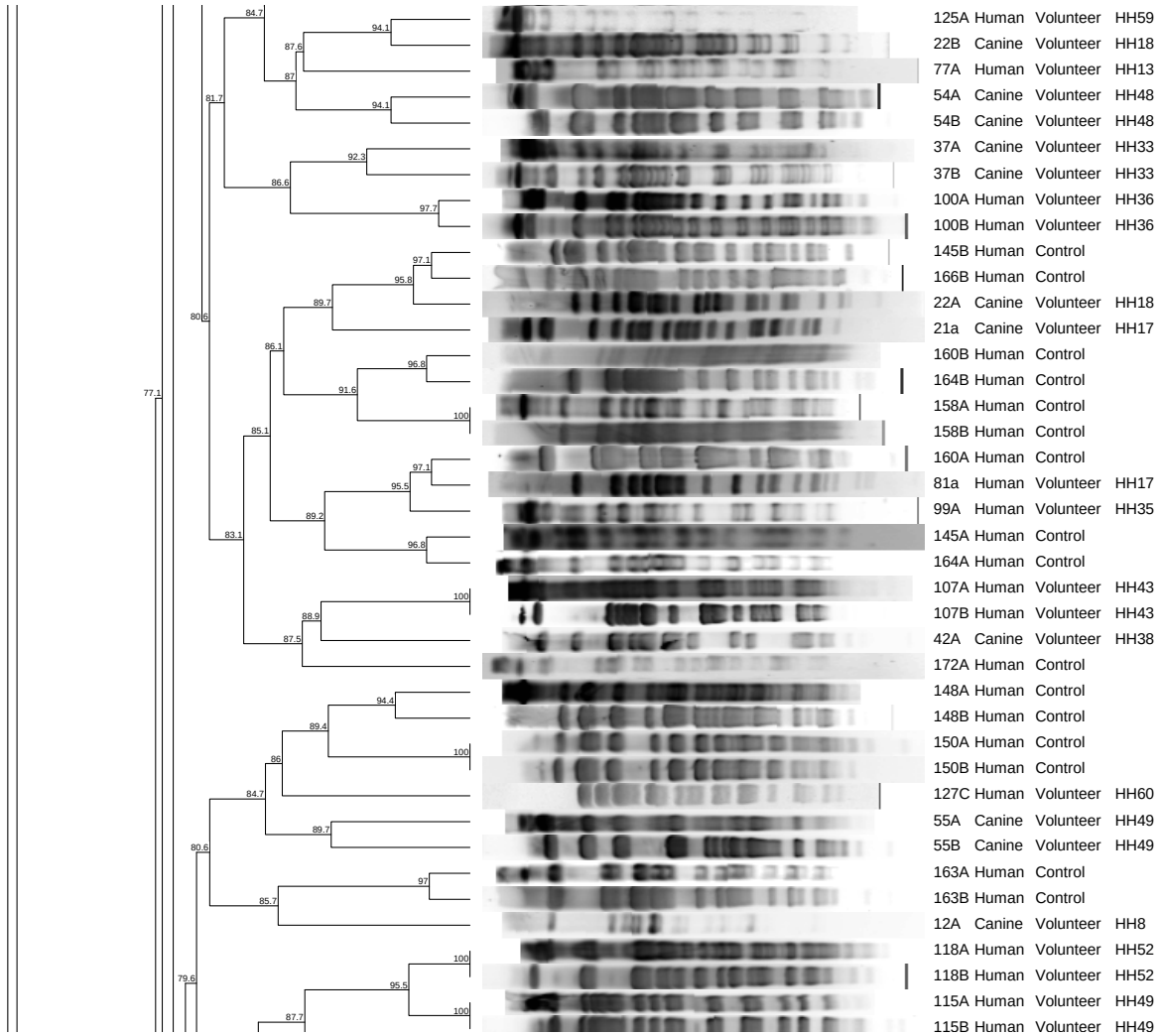


Figure 20. Continued. Dendrogram of PFGE profiles from *E. coli* isolated from dogs, owners, and controls.

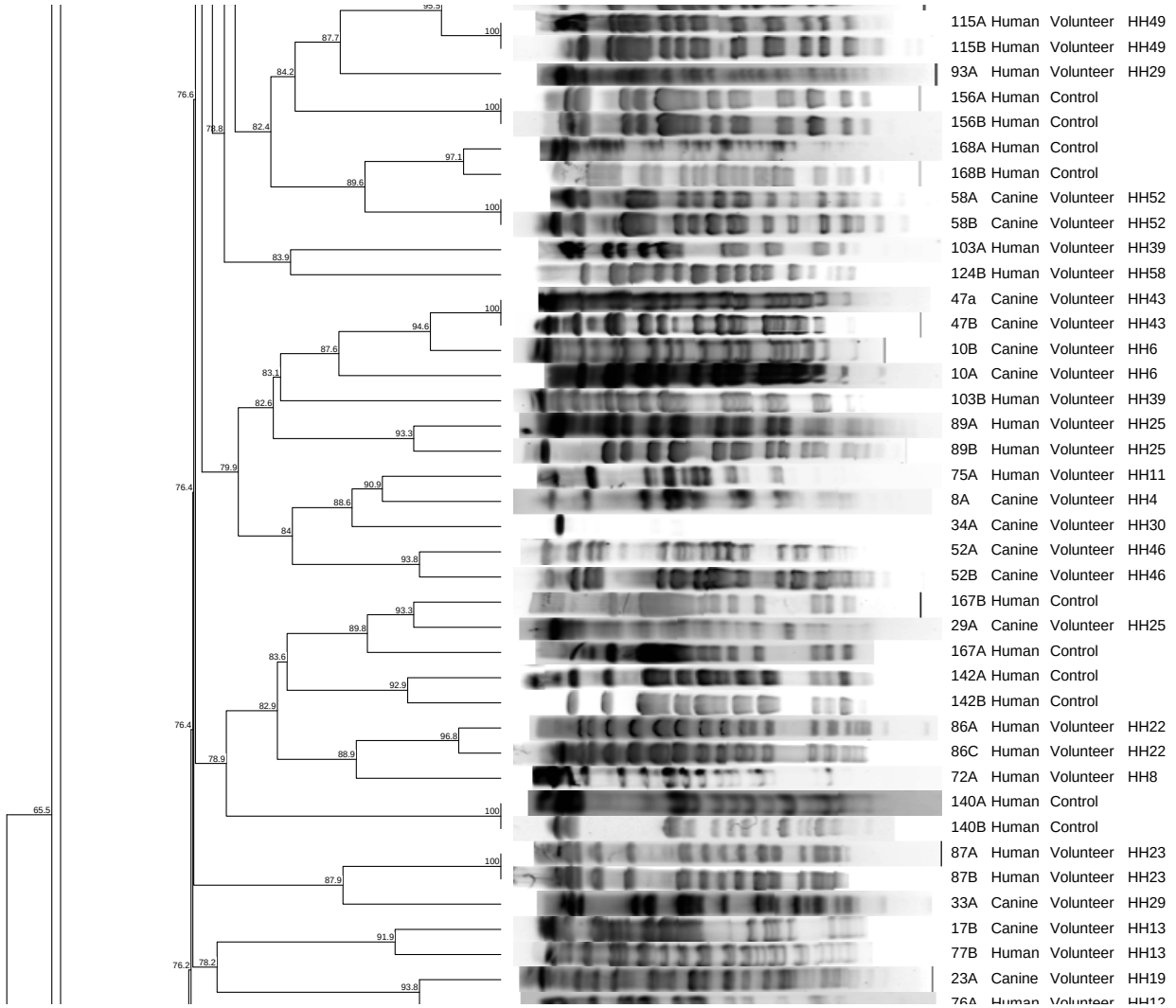


Figure 20. Continued. Dendrogram of PFGE profiles from *E. coli* isolated from dogs, owners, and controls.

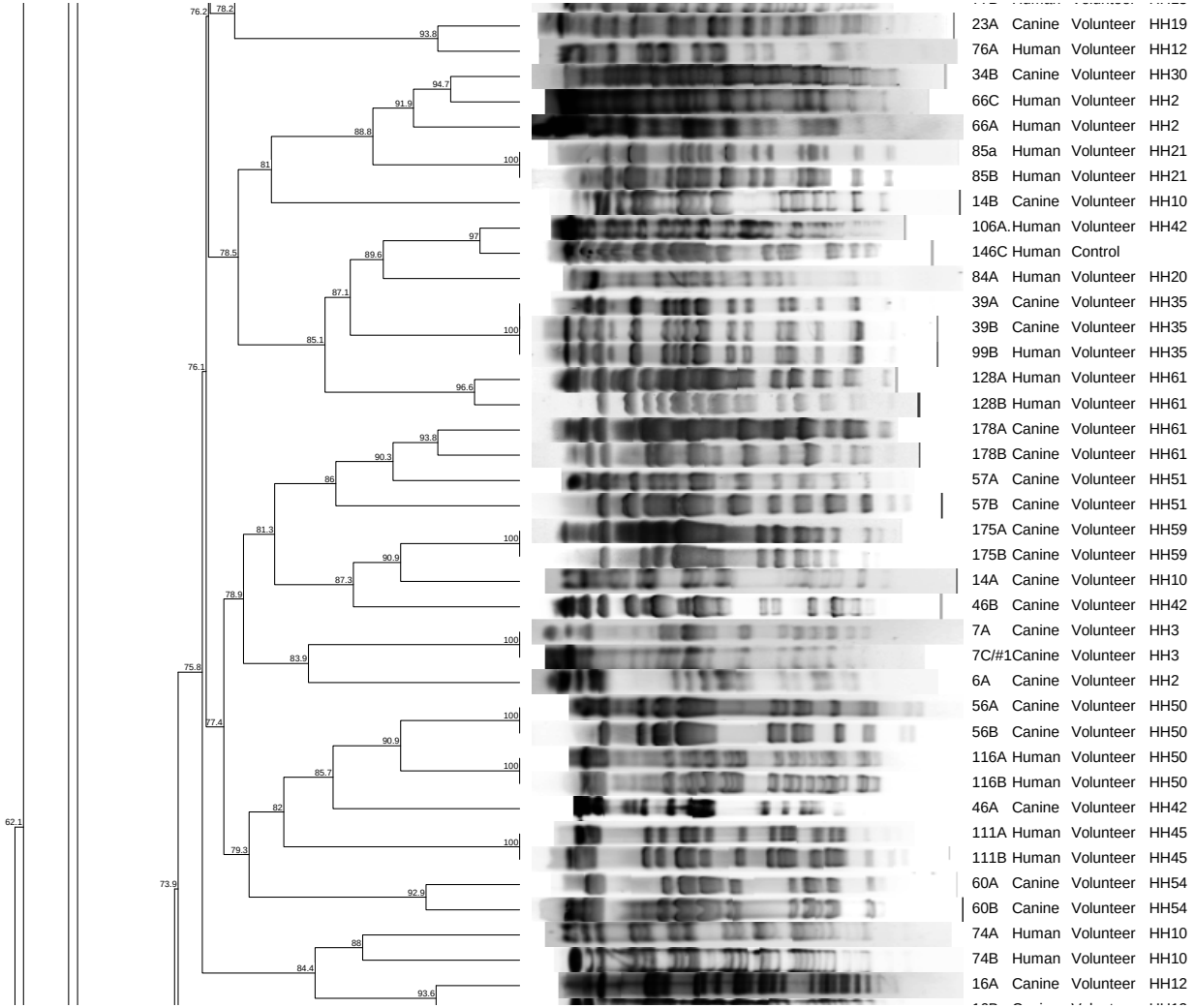


Figure 20. Continued. Dendrogram of PFGE profiles from *E. coli* isolated from dogs, owners, and controls.

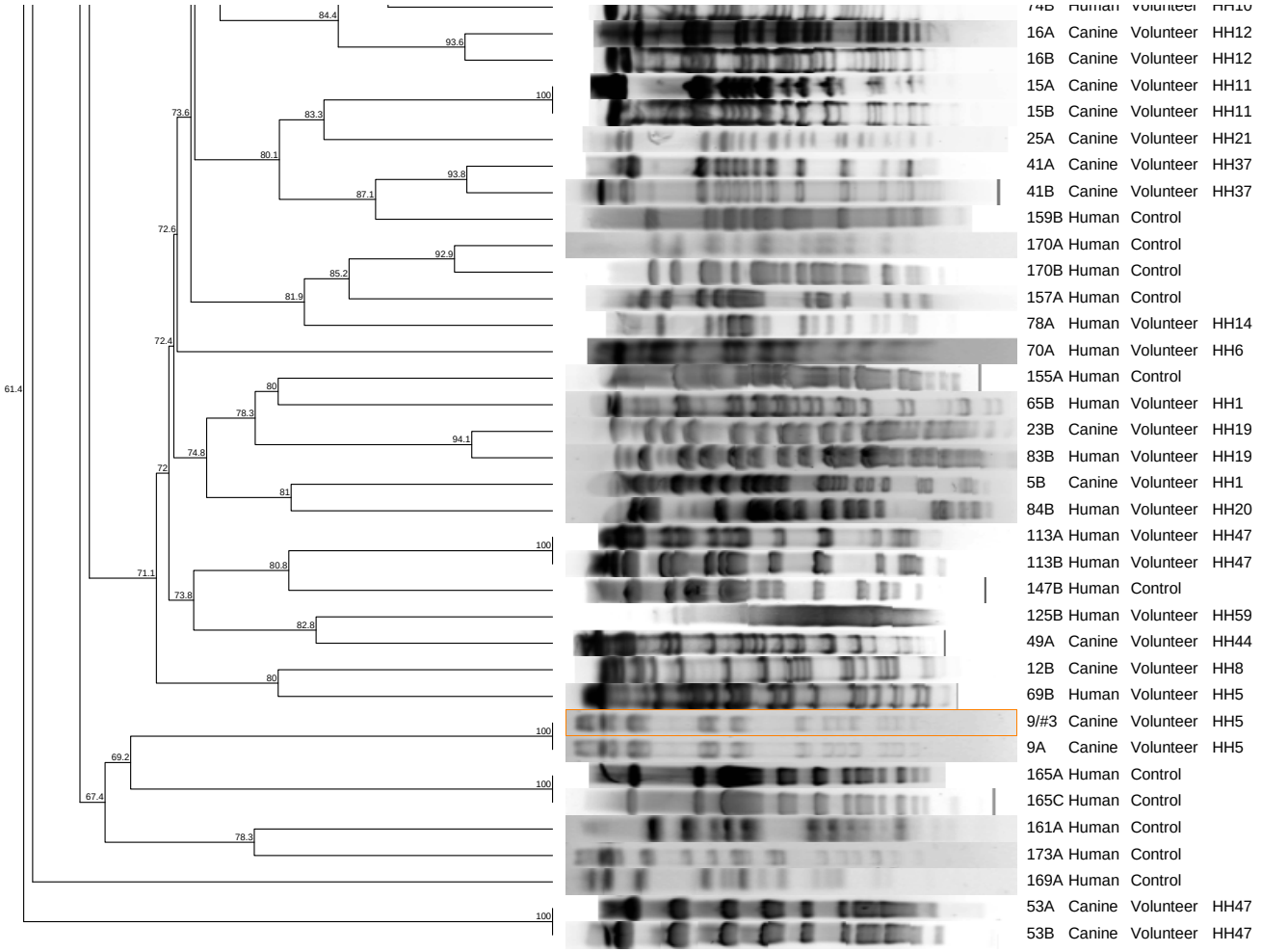


Figure 20. Continued. Dendrogram of PFGE profiles from *E. coli* isolated from dogs, owners, and controls.

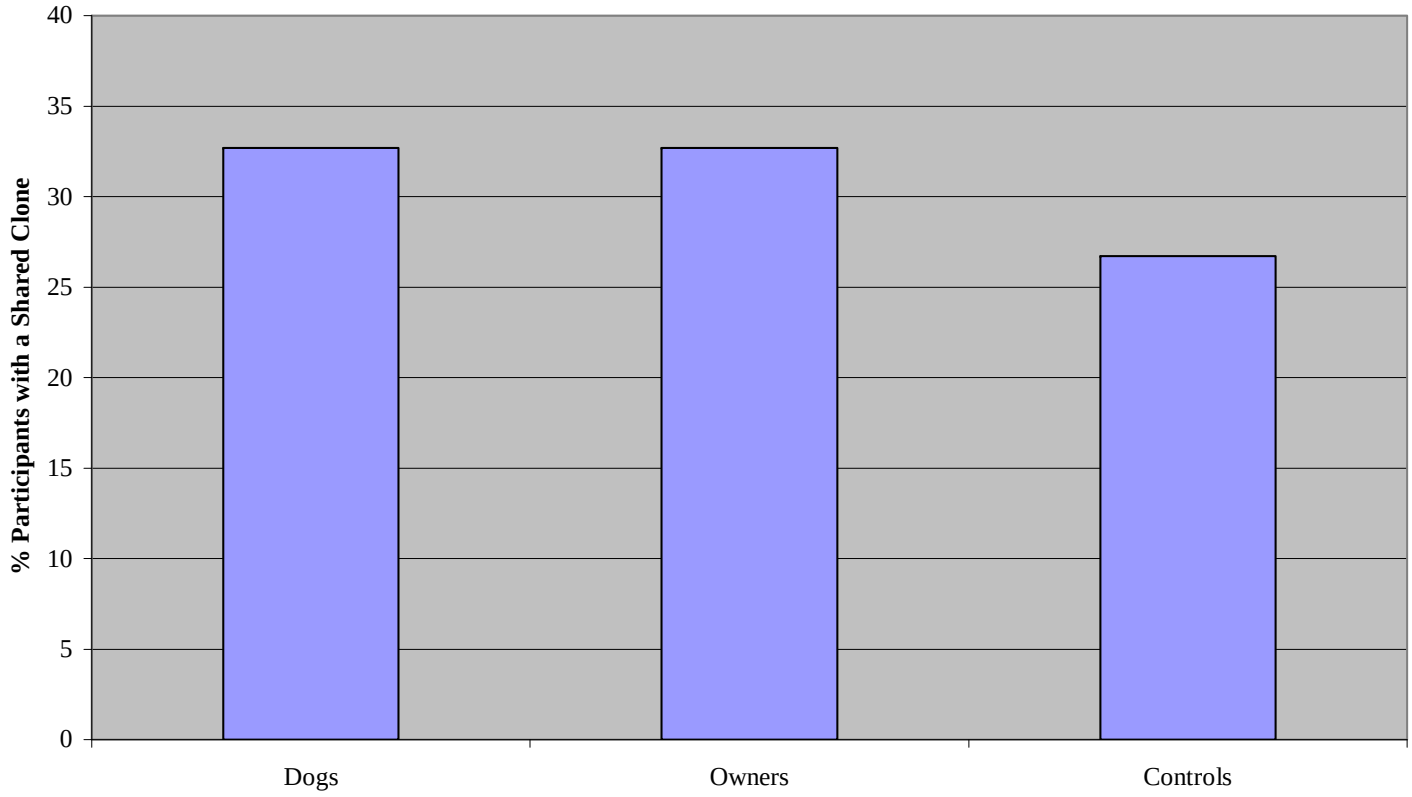


Figure 21. Percent of participants that shared an *E. coli* clone with another participant.

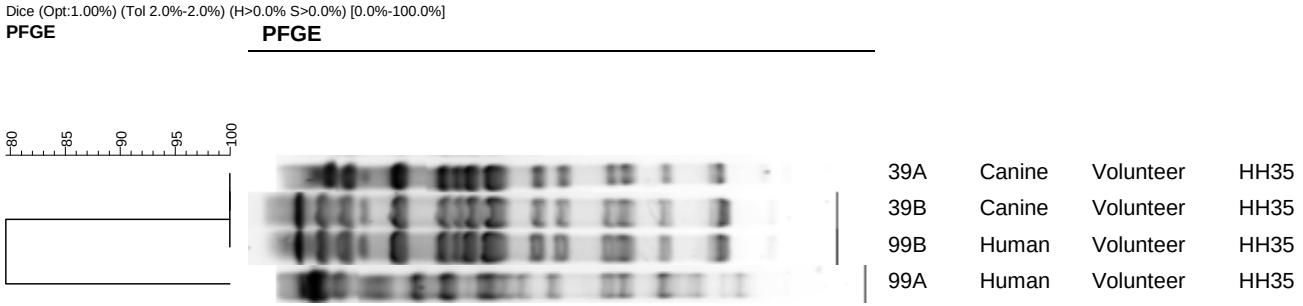


Figure 22. Dendrogram of *E. coli* isolated from Household #35 with 100% PFGE profile similarity between dog and owner isolates.

Table 14. Percent PFGE profile similarity between dog and owner fecal *E. coli.*

Household #	Dog ID#	Owner ID#	Dog-owner % PFGE similarity
1	5	65	74
2	6	66	77
3	7	67	78
4	8	68	77
5	9	69	71
6	10	70	92
7	11	71	92
8	12	72	84
9	13	73	88
10	14	74	82
11	15	75	74
12	16	76	78
13	17	77	92
14	18	78	84
15	19	79	97
16	20	80	87
17	21	81	95
18	22	82	94
19	23	83	94
20	24	84	82
21	25	85	78
22	26	86	79
23	27	87	74
24	28	88	78
25	29	89	84
26	30	90	84
27	31	91	88
28	32	92	80
29	33	93	89
30	34	94	78
31	35	95	78
32	36	96	83

Table 14, continued.

Household #	Dog ID#	Owner ID#	Dog-owner % PFGE similarity
33	37	97	85
34	38	98	88
35	39	99	100
36	40	100	83
37	41	101	78
38	42	102	92
39	43	103	80
40	44	104	84
41	45	105	85
42	46	106	85
43	47	107	83
44	49	109	77
45	51	111	79
46	52	112	85
47	53	113	70
48	54	114	84
49	55	115	88
50	56	116	91
51	57	117	75
52	58	118	94
53	59	119	91
54	60	120	75
55	61	121	88
56	62	122	84
57	63	123	91
58	64	124	80
59	175	125	88
60	177	127	73
61	178	128	87

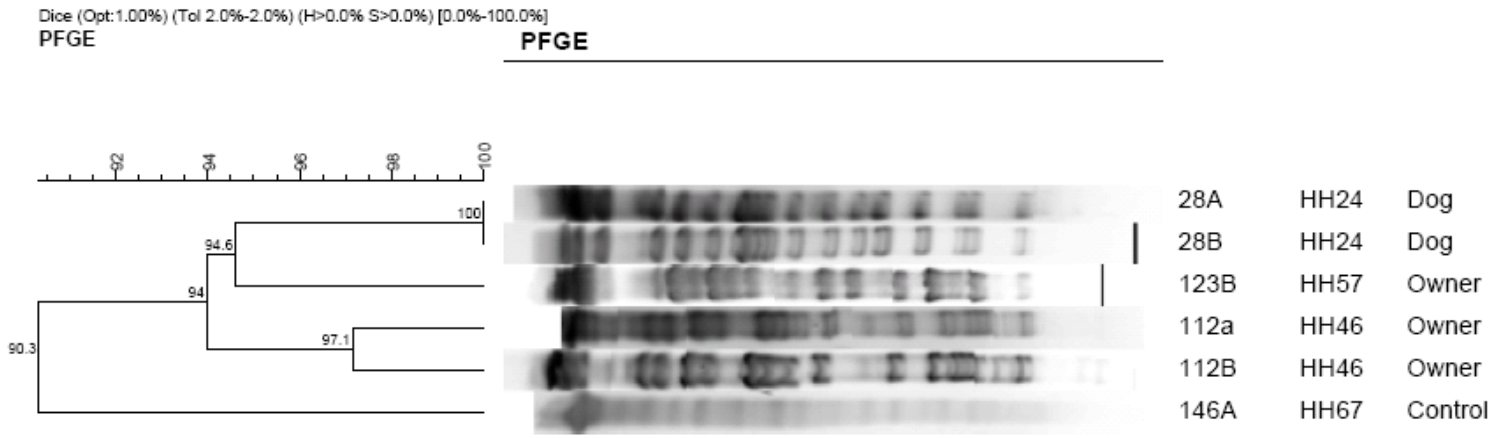


Figure 23. Dendrogram of PFGE profiles from an *E. coli* group comprised of a dog, 2 owners, and a control participant.

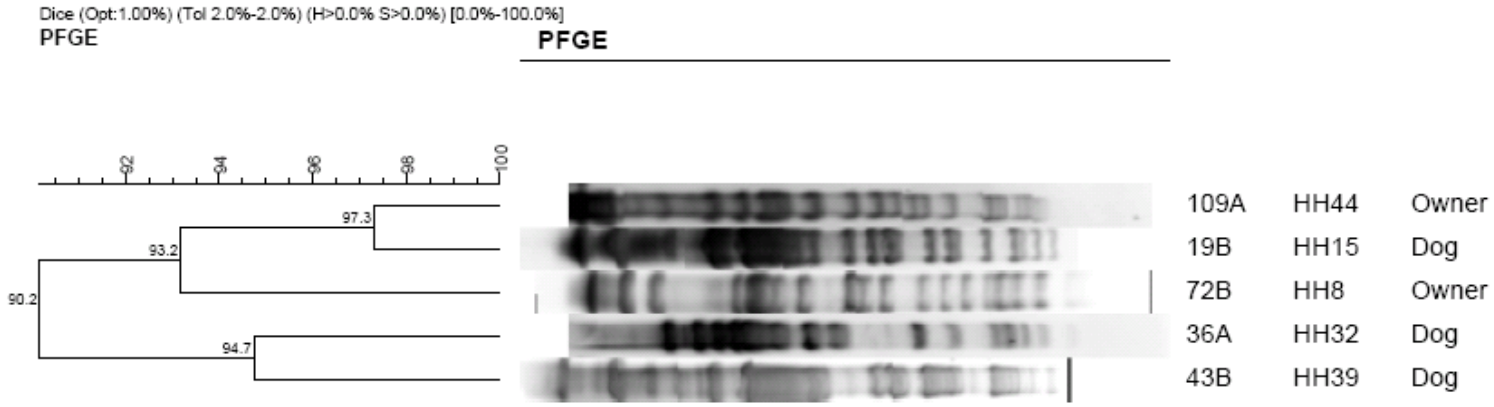


Figure 24. Dendrogram of PFGE profiles from an *E. coli* group comprised of 5 across-household participants.

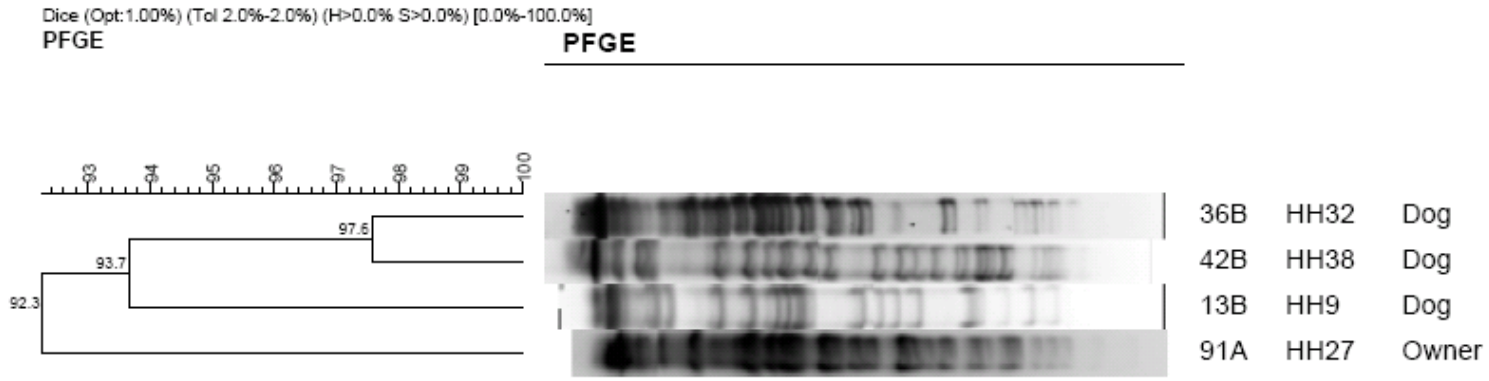


Figure 25. Dendrogram of PFGE profiles from an *E. coli* group comprised of 4 across-household participants.

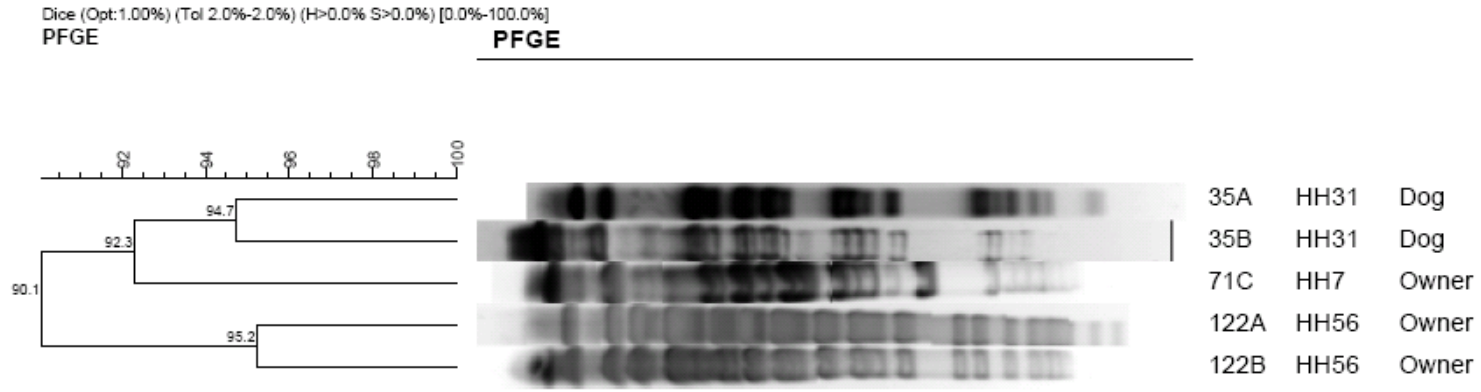


Figure 26. Dendrogram of PFGE profiles from an *E. coli* group comprised of 3 across-household participants.

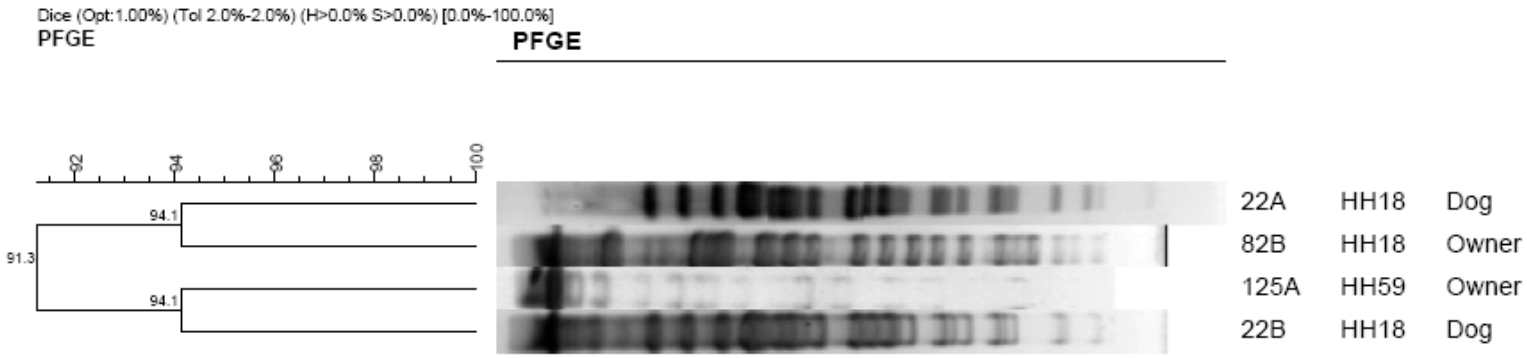


Figure 27. Dendrogram of PFGE profiles from an *E. coli* group with 3 participants, including a within-household pair.

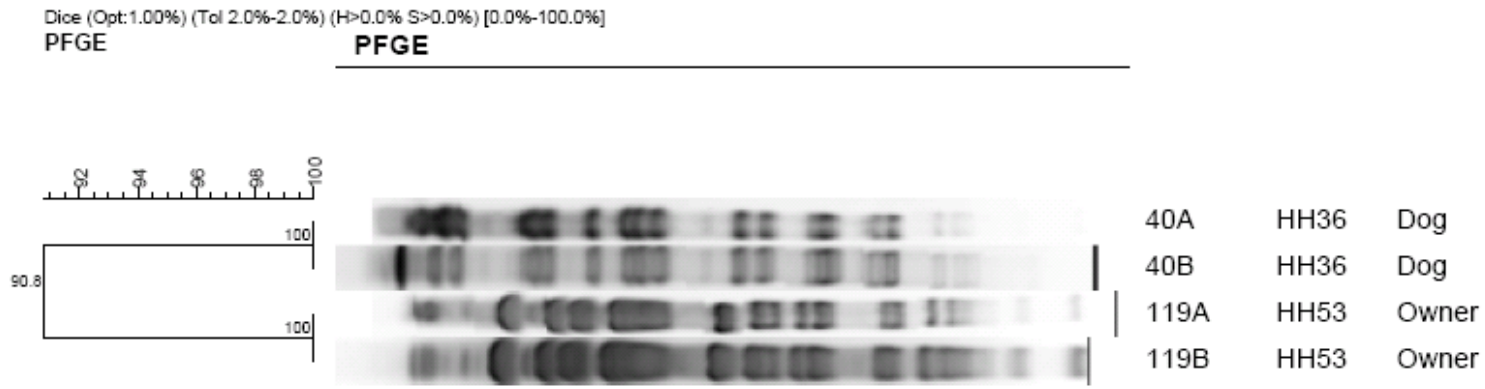


Figure 28. Dendrogram of PFGE profiles from an *E. coli* group comprised of 2 across-household participants.

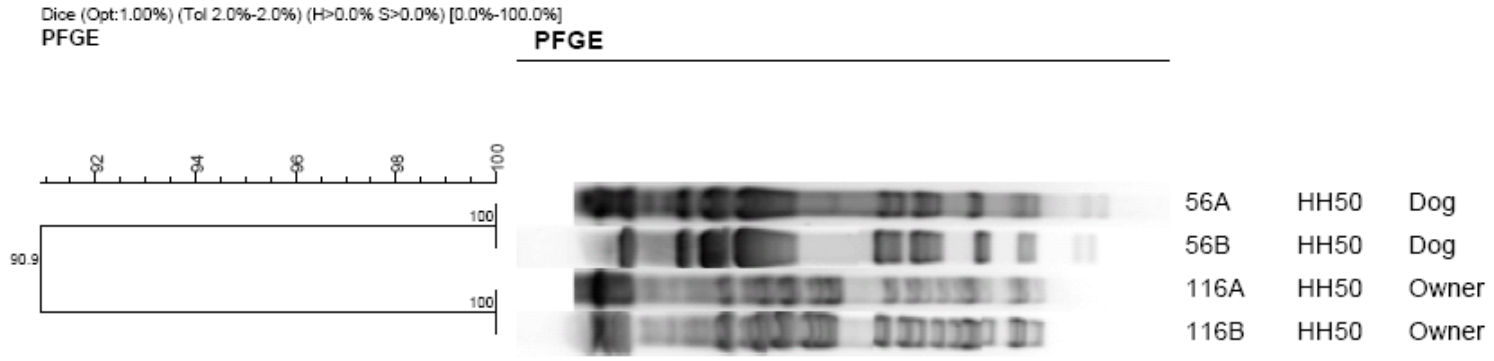


Figure 29. Dendrogram of PFGE profiles from an *E. coli* group comprised of a within-household dog-owner pair.

Appendix D. Spectrophotometry results for nucleic acid concentration and quality of DNA lysates

Isolate	Nucleic Acid Concentration (ng/µl)	Ratio of Sample Absorbance (260/280)
5A	684	1.90
5B	651	1.83
5C	1150	1.81
6A	635	1.94
6B	940	1.68
6C	1521	1.82
7A	870	1.90
7B	669	1.86
7C	939	1.68
8A	592	1.74
8B	734	1.69
8C	1108	1.81
9A	544	1.87
9B	848	1.81
9C	1076	1.87
10A	1131	1.95
10B	1089	1.67
10C	1803	1.82
11A	1972	1.96
11B	1053	1.73
11C	671	1.84
12A	852	1.95
12B	1417	1.74
12C	584	1.78
13A	995	1.79
13B	1039	1.75
13C	2003	1.83
14A	991	1.87

Isolate	Nucleic Acid Concentration (ng/μl)	Ratio of Sample Absorbance (260/280)
14B	830	1.76
14C	2158	1.73
15A	1158	1.84
15B	1468	1.81
15C	2863	1.82
16A	805	1.82
16B	986	1.81
16C	1010	1.85
17A	1109	1.88
17B	1053	1.77
17C	1500	1.74
18A	1011	1.87
18B	732	1.75
18C	1880	1.81
19A	760	1.82
19B	738	1.80
19C	1245	1.82
20A	1543	1.98
20B	1044	1.89
20C	970	1.77
21A	1176	1.90
21B	938	1.85
21C	624	1.82
22A	1046	1.95
22B	724	1.90
22C	1297	1.81
23A	1075	1.87
23B	647	1.87
23C	1156	1.78
24A	1352	1.88
24B	714	1.92
24C	830	1.80
25A	1461	1.90
25B	745	1.91

Isolate	Nucleic Acid Concentration (ng/μl)	Ratio of Sample Absorbance (260/280)
25C	732	1.78
26A	867	1.78
26B	565	1.77
26C	767	1.95
27A	1008	1.82
27B	1115	1.79
27C	805	1.84
28A	1202	1.84
28B	889	1.89
28C	678	1.77
29A	1037	1.83
29B	735	1.81
29C	686	1.72
30A	1368	1.88
30B	946	1.78
30C	638	1.79
31A	1151	1.82
31B	919	1.77
31C	558	1.71
32A	857	1.82
32B	1030	1.77
32C	746	1.74
33A	1338	1.77
33B	528	1.78
33C	790	1.85
34A	974	1.85
34B	797	1.96
34C	788	1.78
35A	1329	1.88
35B	938	1.81
35C	765	1.79
36A	1552	1.82
36B	659	1.86
36C	672	1.78

Isolate	Nucleic Acid Concentration (ng/μl)	Ratio of Sample Absorbance (260/280)
37A	929	1.81
37B	1112	1.77
37C	870	1.86
38A	872	1.84
38B	956	1.70
38C	769	1.81
39A	826	1.87
39B	790	1.84
39C	669	1.86
40A	988	1.82
40B	1050	1.77
40C	809	1.86
41A	811	1.80
41B	900	1.84
41C	884	1.82
42A	983	1.83
42B	1138	1.77
42C	743	1.77
43A	929	1.88
43B	1182	1.78
43C	820	1.81
44A	1031	1.87
44B	1055	1.76
44C	962	1.92
45A	1093	1.87
45B	1224	1.73
45C	721	1.91
46A	954	1.78
46B	840	1.86
46C	750	1.88
47A	1311	1.93
47B	1569	1.74
47C	657	1.82
49A	1290	1.82

Isolate	Nucleic Acid Concentration (ng/μl)	Ratio of Sample Absorbance (260/280)
49B	513	1.72
49C	1053	1.72
51A	1114	1.79
51B	1323	1.72
51C	645	1.81
52A	1514	1.79
52B	1188	1.69
52C	744	1.85
53A	1404	1.75
53B	1095	1.84
53C	874	1.80
54A	2063	1.92
54B	749	1.90
54C	865	1.81
55A	926	1.88
55B	984	1.68
55C	592	1.67
56A	1331	1.86
56B	1129	1.75
56C	888	1.82
57A	1046	1.82
57B	1162	1.76
57C	861	1.88
58A	1346	1.83
58B	981	1.85
58C	742	1.77
59A	1017	1.82
59B	1271	1.75
59C	776	1.80
60A	1304	1.92
60B	1041	1.75
60C	933	1.89
61A	1183	1.83
61B	1095	1.75

Isolate	Nucleic Acid Concentration (ng/μl)	Ratio of Sample Absorbance (260/280)
61C	942	1.87
62A	1208	1.90
62B	1004	1.74
62C	792	1.80
63A	1433	1.77
63B	997	1.68
63C	698	1.75
64A	1246	1.84
64B	837	1.85
64C	651	1.75
175A	884	1.86
175B	960	1.88
175C	748	1.78
177A	901	1.83
177B	848	1.89
177C	736	1.78
178A	925	1.82
178B	1072	1.90
178C	568	1.79
65A	937	1.80
65B	929	1.70
65C	660	1.82
66A	645	1.73
66B	557	1.68
66C	509	1.56
67A	898	1.75
67B	513	1.69
67C	1041	1.68
68A	1027	1.81
68B	1087	1.72
68C	651	1.69
69A	926	1.88
69B	1001	1.91
69C	681	1.78

Isolate	Nucleic Acid Concentration (ng/μl)	Ratio of Sample Absorbance (260/280)
70A	799	1.89
70B	805	1.86
70C	632	1.80
71A	792	1.88
71B	496	1.78
71C	584	1.83
72A	1095	1.86
72B	554	1.80
72C	686	1.85
73A	900	1.88
73B	865	1.82
73C	872	1.89
74A	796	1.82
74B	642	1.80
74C	786	1.69
75A	742	1.77
75B	465	1.71
75C	733	1.77
76A	571	1.69
76B	676	1.82
76C	630	1.72
77A	1092	1.85
77B	1034	1.71
77C	648	1.74
78A	979	1.88
78B	552	1.74
78C	751	1.81
79A	920	1.83
79B	700	1.78
79C	783	1.79
80A	1156	1.80
80B	539	1.77
80C	1055	1.80
81A	1229	1.88

Isolate	Nucleic Acid Concentration (ng/μl)	Ratio of Sample Absorbance (260/280)
81B	869	1.88
81C	703	1.77
82A	1031	1.97
82B	912	1.84
82C	894	1.81
83A	1140	1.83
83B	1142	1.72
83C	928	1.87
84A	1060	1.89
84B	1468	1.68
84C	836	1.84
85A	1156	1.90
85B	1047	1.80
85C	734	1.79
86A	1064	1.91
86B	1237	1.90
86C	753	1.81
87A	1193	1.91
87B	1194	1.86
87C	877	1.76
88A	699	1.92
88B	903	1.90
88C	722	1.80
89A	925	1.88
89B	1000	1.78
89C	635	1.84
90A	891	1.91
90B	974	1.90
90C	724	1.83
91A	805	1.82
91B	1383	1.80
91C	684	1.89
92A	1053	1.88
92B	1191	1.79

Isolate	Nucleic Acid Concentration (ng/μl)	Ratio of Sample Absorbance (260/280)
92C	917	1.85
93A	960	1.86
93B	1221	1.80
93C	760	1.85
94A	465	1.81
94B	733	1.81
94C	690	1.77
95A	796	1.79
95B	740	1.71
95C	590	1.72
96A	936	1.91
96B	1154	1.69
96C	695	1.85
97A	849	1.89
97B	1492	1.65
97C	690	1.74
98A	837	1.85
98B	1140	1.72
98C	674	1.78
99A	929	1.80
99B	902	1.76
99C	682	1.80
100A	947	1.91
100B	1295	1.82
100C	710	1.83
101A	745	1.94
101B	938	1.84
101C	765	1.80
102A	1036	1.94
102B	1507	1.69
102C	808	1.94
103A	993	1.86
103B	1551	1.72
103C	771	1.77

Isolate	Nucleic Acid Concentration (ng/μl)	Ratio of Sample Absorbance (260/280)
104A	920	1.82
104B	1376	1.77
104C	913	1.75
105A	788	1.86
105B	1129	1.64
105C	714	1.79
106A	738	1.82
106B	852	1.71
106C	495	1.75
107A	787	1.80
107B	995	1.69
107C	565	1.72
109A	1111	1.88
109B	1532	1.73
109C	806	1.85
111A	758	1.74
111B	789	1.70
111C	663	1.75
112A	842	1.89
112B	1707	1.73
112C	843	1.80
113A	825	1.92
113B	1577	1.71
113C	515	1.77
114A	948	1.81
114B	1174	1.75
114C	815	1.83
115A	800	1.88
115B	712	1.85
115C	578	1.68
116A	1176	1.90
116B	1090	1.90
116C	703	1.78
117A	841	1.85

Isolate	Nucleic Acid Concentration (ng/μl)	Ratio of Sample Absorbance (260/280)
117B	786	1.89
117C	546	1.72
118A	1261	1.92
118B	651	1.83
118C	649	1.66
119A	902	1.81
119B	1027	1.93
119C	714	1.80
120A	996	1.89
120B	887	1.94
120C	700	1.78
121A	1251	1.98
121B	1055	1.97
121C	572	1.75
122A	675	1.79
122B	1185	1.75
122C	663	1.85
123A	930	1.76
123B	1230	1.72
123C	749	1.86
124A	784	1.86
124B	578	1.80
124C	717	1.79
125A	958	1.86
125B	1145	1.87
125C	740	1.84
127A	928	1.78
127B	518	1.73
127C	992	1.80
128A	1130	1.86
128B	1128	1.88
128C	583	1.78
140A	780	1.83
140B	709	1.76

Isolate	Nucleic Acid Concentration (ng/μl)	Ratio of Sample Absorbance (260/280)
140C	1067	1.87
141A	937	1.86
141B	834	1.89
141C	951	1.81
142A	765	1.84
142B	1146	1.95
142C	769	1.80
144A	884	1.75
144B	940	1.87
144C	574	1.70
145A	614	1.80
145B	1598	1.87
145C	693	1.80
146A	758	1.85
146B	454	1.66
146C	1430	1.74
147A	943	1.88
147B	1076	1.89
147C	505	1.74
148A	662	1.74
148B	740	1.64
148C	676	1.77
149A	759	1.87
149B	1348	1.90
149C	680	1.88
150A	711	1.83
150B	523	1.80
150C	505	1.77
151A	894	1.78
151B	538	1.75
151C	735	1.75
152A	776	1.84
152B	575	1.68
152C	1108	1.78

Isolate	Nucleic Acid Concentration (ng/μl)	Ratio of Sample Absorbance (260/280)
155A	845	1.81
155B	941	1.81
155C	715	1.74
156A	787	1.80
156B	1091	1.75
156C	778	1.79
157A	1008	1.80
157B	824	1.63
157C	801	1.77
158A	710	1.83
158B	1466	1.71
158C	568	1.69
159A	919	1.83
159B	1110	1.84
159C	775	1.75
160A	1016	1.86
160B	1144	1.85
160C	706	1.76
161A	780	1.79
161B	736	1.80
161C	793	1.84
162A	897	1.84
162B	717	1.82
162C	640	1.83
163A	560	1.73
163B	770	1.72
163C	541	1.69
164A	848	1.91
164B	1198	1.79
164C	670	1.79
165A	988	1.81
165B	713	1.80
165C	948	1.73
166A	891	1.71

Isolate	Nucleic Acid Concentration (ng/μl)	Ratio of Sample Absorbance (260/280)
166B	787	1.67
166C	510	1.72
167A	887	1.84
167B	1178	1.75
167C	894	1.85
168A	874	1.85
168B	588	1.75
168C	558	1.72
169A	972	1.86
169B	976	1.68
169C	818	1.79
170A	1472	1.82
170B	706	1.71
170C	578	1.74
172A	698	1.89
172B	671	1.81
172C	1046	1.85
173A	734	1.80
173B	803	1.86
173C	591	1.69
J96	940	1.87
J55	1075	1.94

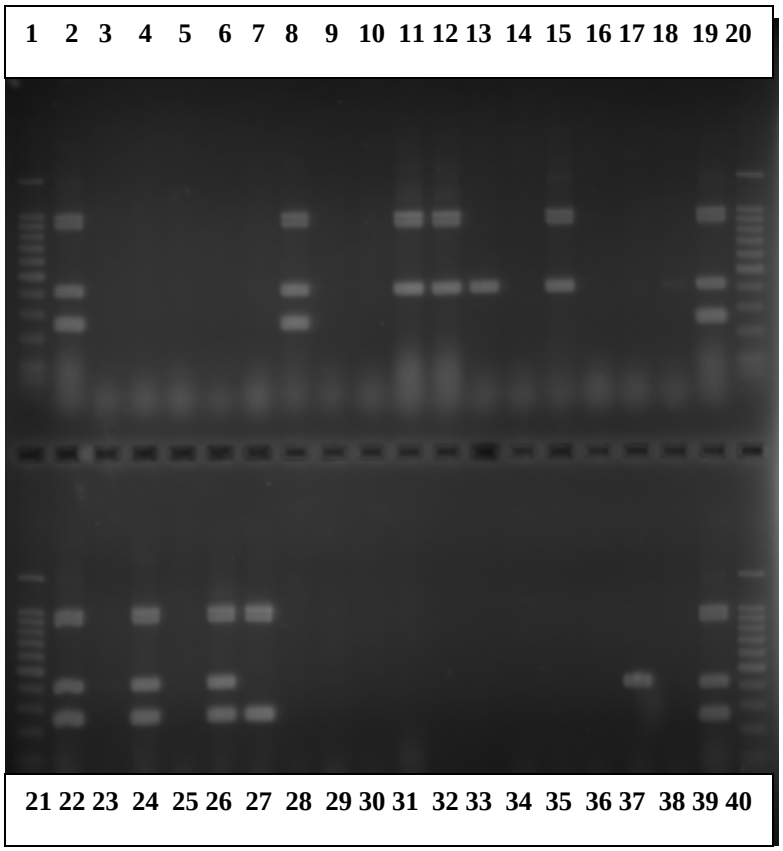


Figure 30. Example of a gel electrophoresis image from multiplex PCR. Lanes 1, 20, 21, and 40 contain 100bp DNA ladder. Lanes 2, 19, 22, and 39 contain a positive control for all 4 virulence factors. Lanes 31 and 32 contain negative controls. The remaining lanes contain participant samples. Band sizes for each virulence factor are: *cnf* 974bp, *hlyD* 904bp, *sfa* 410bp, and *papGIII* 258bp.

Table 15. PCR results for presence or absence of virulence factors in *E. coli* isolates, with dog-owner pairs in the same rows.

Dog ID#	*cnf*	*hlyD*	*sfa*	*papGIII*		Owner ID#	*cnf*	*hlyD*	*sfa*	*papGIII*
5A	neg	neg	neg	neg		65A	pos	pos	pos	neg
5B	neg	neg	neg	neg		65B	pos	pos	pos	neg
5C	neg	neg	neg	neg		65C	pos	pos	pos	neg
6A	neg	neg	neg	neg		66A	neg	neg	neg	neg
6B	neg	neg	pos	neg		66B	neg	neg	neg	neg
6C	neg	neg	neg	neg		66C	neg	neg	neg	neg
7A	neg	neg	neg	neg		67A	neg	neg	neg	neg
7B	neg	neg	neg	neg		67B	neg	neg	neg	neg
7C	neg	neg	neg	neg		67C	neg	neg	neg	neg
8A	neg	neg	neg	neg		68A	neg	neg	neg	neg
8B	neg	neg	neg	neg		68B	neg	neg	neg	neg
8C	neg	neg	neg	neg		68C	neg	neg	neg	neg
9A	neg	neg	neg	neg		69A	neg	neg	neg	neg
9B	neg	neg	neg	neg		69B	neg	neg	neg	neg
9C	neg	neg	neg	neg		69C	neg	neg	neg	neg
10A	pos	pos	pos	pos		70A	neg	neg	neg	neg
10B	pos	pos	pos	pos		70B	neg	neg	neg	neg
10C	pos	pos	pos	pos		70C	neg	neg	neg	neg
11A	neg	neg	neg	neg		71A	neg	neg	neg	neg
11B	neg	neg	neg	neg		71B	neg	neg	neg	neg
11C	neg	neg	neg	neg		71C	neg	neg	neg	neg
12A	neg	neg	neg	neg		72A	neg	neg	neg	neg
12B	neg	neg	neg	neg		72B	neg	neg	neg	neg
12C	neg	neg	neg	neg		72C	neg	neg	neg	neg
13A	neg	neg	pos	neg		73A	neg	neg	neg	neg

Table 15, continued.

Dog ID#	*cnf*	*hlyD*	*sfa*	*papGIII*		Owner ID#	*cnf*	*hlyD*	*sfa*	*papGIII*
13B	neg	neg	neg	neg		73B	neg	neg	neg	neg
13C	neg	neg	neg	neg		73C	neg	neg	neg	neg
14A	neg	neg	neg	neg		74A	neg	neg	neg	neg
14B	neg	neg	neg	neg		74B	neg	neg	neg	neg
14C	neg	neg	neg	neg		74C	neg	neg	neg	neg
15A	pos	pos	pos	neg		75A	neg	neg	neg	neg
15B	pos	pos	pos	neg		75B	neg	neg	neg	neg
15C	neg	neg	pos	neg		75C	neg	neg	neg	neg
16A	neg	neg	neg	neg		76A	neg	neg	neg	neg
16B	neg	neg	neg	neg		76B	neg	neg	neg	neg
16C	neg	neg	neg	neg		76C	neg	neg	neg	neg
17A	neg	neg	neg	neg		77A	neg	neg	neg	neg
17B	neg	neg	neg	neg		77B	neg	neg	neg	neg
17C	neg	neg	neg	neg		77C	neg	neg	neg	neg
18A	neg	neg	neg	neg		78A	neg	neg	neg	neg
18B	neg	neg	neg	neg		78B	neg	neg	neg	neg
18C	neg	neg	neg	neg		78C	neg	neg	neg	neg
19A	neg	neg	neg	neg		79A	neg	neg	neg	neg
19B	neg	neg	neg	neg		79B	neg	neg	neg	neg
19C	neg	neg	neg	neg		79C	neg	neg	neg	neg
20A	pos	pos	pos	pos		80A	neg	neg	neg	neg
20B	pos	pos	pos	pos		80B	neg	neg	neg	neg
20C	pos	pos	pos	pos		80C	neg	neg	neg	neg
21A	neg	neg	neg	neg		81A	neg	neg	neg	neg
21B	neg	neg	neg	neg		81B	neg	neg	neg	neg
21C	neg	neg	neg	neg		81C	neg	neg	neg	neg

Table 15, continued.

Dog ID#	*cnf*	*hlyD*	*sfa*	*papGIII*		Owner ID#	*cnf*	*hlyD*	*sfa*	*papGIII*
22A	pos	pos	pos	pos		82A	neg	neg	neg	neg
22B	pos	pos	pos	pos		82B	neg	neg	neg	neg
22C	pos	pos	pos	pos		82C	neg	neg	neg	neg
23A	pos	pos	neg	pos		83A	neg	neg	neg	neg
23B	pos	pos	neg	pos		83B	neg	neg	neg	neg
23C	pos	pos	neg	pos		83C	neg	neg	neg	neg
24A	neg	neg	neg	neg		84A	neg	neg	neg	neg
24B	neg	neg	neg	neg		84B	neg	neg	neg	neg
24C	neg	neg	neg	neg		84C	neg	neg	neg	neg
25A	neg	neg	neg	neg		85A	neg	neg	neg	neg
25B	neg	neg	neg	neg		85B	neg	neg	neg	neg
25C	neg	neg	neg	neg		85C	neg	neg	neg	neg
26A	neg	neg	neg	neg		86A	pos	pos	pos	pos
26B	neg	neg	neg	neg		86B	pos	pos	pos	pos
26C	neg	neg	neg	neg		86C	pos	pos	pos	pos
27A	neg	neg	neg	neg		87A	neg	neg	neg	neg
27B	neg	neg	neg	neg		87B	neg	neg	neg	neg
27C	neg	neg	neg	neg		87C	neg	neg	neg	neg
28A	neg	neg	neg	neg		88A	neg	neg	neg	neg
28B	neg	neg	neg	neg		88B	neg	neg	neg	neg
28C	neg	neg	neg	neg		88C	neg	neg	neg	neg
29A	neg	neg	neg	neg		89A	pos	pos	pos	pos
29B	neg	neg	neg	neg		89B	pos	pos	pos	pos
29C	neg	neg	neg	neg		89C	pos	pos	pos	pos
30A	neg	neg	neg	neg		90A	neg	neg	neg	neg
30B	neg	neg	neg	neg		90B	neg	neg	neg	neg

Table 15, continued.

Dog ID#	*cnf*	*hlyD*	*sfa*	*papGIII*		Owner ID#	*cnf*	*hlyD*	*sfa*	*papGIII*
30C	neg	neg	neg	neg		90C	neg	neg	neg	neg
31A	neg	neg	pos	neg		91A	neg	neg	neg	neg
31B	neg	neg	pos	neg		91B	neg	neg	neg	neg
31C	neg	neg	pos	neg		91C	neg	neg	neg	neg
32A	neg	neg	neg	neg		92A	neg	neg	neg	neg
32B	neg	neg	neg	neg		92B	neg	neg	neg	neg
32C	neg	neg	neg	neg		92C	neg	neg	neg	neg
33A	neg	neg	neg	neg		93A	neg	neg	neg	neg
33B	neg	neg	neg	neg		93B	neg	neg	neg	neg
33C	neg	neg	neg	neg		93C	neg	neg	neg	neg
34A	neg	neg	neg	neg		94A	neg	neg	neg	neg
34B	neg	neg	neg	neg		94B	neg	neg	neg	neg
34C	neg	neg	neg	neg		94C	neg	neg	neg	neg
35A	neg	neg	neg	neg		95A	neg	neg	neg	neg
35B	neg	neg	neg	neg		95B	neg	neg	neg	neg
35C	neg	neg	neg	neg		95C	neg	neg	neg	neg
36A	neg	neg	neg	neg		96A	neg	neg	neg	neg
36B	neg	neg	neg	neg		96B	neg	neg	neg	neg
36C	neg	neg	neg	neg		96C	neg	neg	neg	neg
37A	neg	neg	neg	neg		97A	pos	pos	pos	pos
37B	neg	neg	neg	neg		97B	pos	pos	pos	pos
37C	neg	neg	neg	neg		97C	pos	pos	pos	pos
38A	pos	pos	pos	pos		98A	neg	neg	neg	neg
38B	pos	pos	pos	pos		98B	neg	neg	neg	neg
38C	pos	pos	pos	pos		98C	neg	neg	neg	neg

Table 15, continued.

Dog ID#	*cnf*	*hlyD*	*sfa*	*papGIII*		Owner ID#	*cnf*	*hlyD*	*sfa*	*papGIII*
39A	neg	neg	neg	neg		99A	pos	pos	pos	neg
39B	neg	neg	neg	neg		99B	neg	neg	neg	neg
39C	neg	neg	neg	neg		99C	neg	neg	neg	neg
40A	neg	neg	neg	neg		100A	pos	pos	pos	neg
40B	neg	neg	neg	neg		100B	pos	pos	pos	neg
40C	neg	neg	neg	neg		100C	pos	pos	pos	neg
41A	neg	neg	neg	neg		101A	neg	neg	neg	neg
41B	neg	neg	neg	neg		101B	neg	neg	neg	neg
41C	neg	neg	neg	neg		101C	neg	neg	neg	neg
42A	neg	neg	neg	neg		102A	pos	pos	pos	neg
42B	neg	neg	neg	neg		102B	neg	neg	neg	neg
42C	neg	neg	neg	neg		102C	neg	neg	neg	neg
43A	neg	neg	neg	neg		103A	neg	neg	neg	neg
43B	neg	neg	neg	neg		103B	neg	neg	neg	neg
43C	neg	neg	neg	neg		103C	neg	neg	neg	neg
44A	neg	neg	neg	neg		104A	neg	neg	neg	neg
44B	neg	neg	neg	neg		104B	neg	neg	neg	neg
44C	neg	neg	neg	neg		104C	neg	neg	neg	neg
45A	neg	neg	neg	neg		105A	neg	neg	neg	neg
45B	neg	neg	neg	neg		105B	neg	neg	neg	neg
45C	neg	neg	neg	neg		105C	neg	neg	neg	neg
46A	neg	neg	neg	neg		106A	neg	neg	neg	neg
46B	neg	neg	neg	neg		106B	neg	neg	neg	neg
46C	neg	neg	neg	neg		106C	neg	neg	neg	neg
47A	pos	pos	pos	pos		107A	neg	neg	neg	neg
47B	pos	pos	pos	pos		107B	neg	neg	neg	neg

Table 15, continued.

Dog ID#	***cnf***	***hlyD***	***sfa***	***papGIII***		**Owner ID#**	***cnf***	***hlyD***	***sfa***	***papGIII***
47C	pos	pos	pos	pos		107C	neg	neg	neg	neg
49A	neg	neg	neg	neg		109A	neg	neg	neg	neg
49B	neg	neg	pos	neg		109B	neg	neg	neg	neg
49C	neg	neg	pos	neg		109C	neg	neg	neg	neg
51A	neg	neg	neg	neg		111A	neg	neg	neg	neg
51B	neg	neg	neg	neg		111B	neg	neg	neg	neg
51C	neg	neg	neg	neg		111C	neg	neg	neg	neg
52A	neg	neg	pos	neg		112A	neg	neg	neg	neg
52B	neg	neg	pos	neg		112B	neg	neg	neg	neg
52C	neg	neg	pos	neg		112C	neg	neg	neg	neg
53A	neg	neg	neg	neg		113A	neg	neg	neg	neg
53B	neg	neg	neg	neg		113B	neg	neg	neg	neg
53C	neg	neg	neg	neg		113C	neg	neg	neg	neg
54A	pos	pos	pos	neg		114A	pos	pos	pos	neg
54B	pos	pos	pos	neg		114B	pos	pos	pos	neg
54C	pos	pos	pos	neg		114C	pos	pos	pos	neg
55A	pos	pos	pos	pos		115A	neg	neg	neg	neg
55B	pos	pos	pos	pos		115B	neg	neg	neg	neg
55C	neg	neg	pos	neg		115C	neg	neg	neg	neg
56A	neg	neg	neg	neg		116A	neg	neg	neg	neg
56B	neg	neg	neg	neg		116B	neg	neg	neg	neg
56C	neg	neg	neg	neg		116C	neg	neg	neg	neg
57A	neg	neg	neg	neg		117A	pos	pos	pos	pos
57B	neg	neg	neg	neg		117B	pos	pos	pos	pos
57C	neg	neg	neg	neg		117C	pos	pos	pos	neg

Table 15, continued.

Dog ID#	*cnf*	*hlyD*	*sfa*	*papGIII*		Owner ID#	*cnf*	*hlyD*	*sfa*	*papGIII*
58A	neg	neg	pos	neg		118A	neg	neg	neg	neg
58B	neg	neg	pos	neg		118B	neg	neg	neg	neg
58C	neg	neg	pos	neg		118C	neg	neg	neg	neg
59A	neg	neg	neg	neg		119A	neg	neg	neg	neg
59B	neg	neg	neg	neg		119B	neg	neg	neg	neg
59C	neg	neg	neg	neg		119C	neg	neg	neg	neg
60A	neg	neg	neg	neg		120A	neg	pos	neg	neg
60B	neg	neg	neg	neg		120B	neg	pos	neg	neg
60C	neg	neg	neg	neg		120C	neg	pos	neg	neg
61A	neg	neg	neg	neg		121A	neg	neg	neg	neg
61B	neg	neg	neg	neg		121B	neg	neg	neg	neg
61C	neg	neg	neg	neg		121C	neg	neg	neg	neg
62A	neg	neg	neg	neg		122A	pos	pos	pos	pos
62B	neg	neg	neg	neg		122B	pos	pos	pos	pos
62C	neg	neg	neg	neg		122C	pos	pos	pos	pos
63A	pos	pos	pos	pos		123A	neg	neg	neg	neg
63B	pos	pos	pos	pos		123B	neg	neg	neg	neg
63C	pos	pos	pos	pos		123C	neg	neg	neg	neg
64A	neg	neg	neg	neg		124A	neg	neg	neg	neg
64B	neg	neg	neg	neg		124B	neg	neg	neg	neg
64C	neg	neg	neg	neg		124C	neg	neg	neg	neg
175A	neg	neg	neg	neg		125A	neg	neg	neg	neg
175B	neg	neg	neg	neg		125B	neg	neg	neg	neg
175C	neg	neg	neg	neg		125C	neg	neg	neg	neg
177A	neg	neg	neg	neg		127A	neg	neg	neg	neg
177B	neg	neg	neg	neg		127B	neg	neg	neg	neg

Table 15, continued.

Dog ID#	***cnf***	***hlyD***	***sfa***	***papGIII***		**Owner ID#**	***cnf***	***hlyD***	***sfa***	***papGIII***
177C	neg	neg	neg	neg		127C	neg	neg	neg	neg
178A	neg	neg	neg	neg		128A	neg	neg	neg	Neg
178B	neg	neg	neg	neg		128B	neg	neg	neg	Neg
178C	neg	neg	neg	neg		128C	neg	neg	neg	neg

Table 16. PCR results for presence or absence of virulence factors in *E. coli* isolates from controls

Control ID#	*cnf*	*hlyD*	*sfa*	*papGIII*
140A	neg	neg	neg	neg
140B	neg	neg	neg	neg
140C	neg	neg	neg	neg
141A	neg	neg	neg	neg
141B	neg	neg	neg	neg
141C	neg	neg	neg	neg
142A	pos	pos	pos	neg
142B	pos	pos	pos	neg
142C	pos	pos	pos	neg
144A	neg	neg	neg	neg
144B	neg	neg	neg	neg
144C	neg	neg	neg	neg
145A	neg	neg	neg	neg
145B	neg	neg	neg	neg
145C	neg	neg	neg	neg
146A	pos	pos	pos	neg
146B	pos	pos	pos	neg
146C	neg	neg	neg	neg
147A	neg	neg	neg	neg
147B	neg	neg	neg	neg
147C	neg	neg	neg	neg
148A	pos	pos	neg	pos
148B	pos	pos	neg	pos
148C	pos	pos	neg	pos
149A	neg	neg	neg	neg
149B	neg	neg	neg	neg

Table 16, continued.

Control ID#	*cnf*	*hlyD*	*sfa*	*papGIII*
149C	neg	neg	neg	neg
150A	neg	neg	neg	neg
150B	neg	neg	neg	neg
150C	neg	neg	neg	neg
151A	neg	neg	neg	neg
151B	neg	neg	neg	neg
151C	neg	neg	neg	neg
152A	neg	neg	neg	neg
152B	neg	neg	neg	neg
152C	neg	neg	neg	neg
155A	neg	neg	neg	neg
155B	neg	neg	neg	neg
155C	neg	neg	neg	neg
156A	neg	neg	neg	neg
156B	neg	neg	neg	neg
156C	neg	neg	neg	neg
157A	neg	neg	neg	neg
157B	neg	neg	neg	neg
157C	neg	neg	neg	neg
158A	neg	pos	neg	neg
158B	neg	pos	neg	neg
158C	neg	pos	neg	neg
159A	neg	neg	neg	neg
159B	neg	neg	neg	neg
159C	neg	neg	neg	neg

Table 16, continued.

Control ID#	*cnf*	*hlyD*	*sfa*	*papGIII*
160A	neg	neg	neg	neg
160B	neg	neg	neg	neg
160C	neg	neg	neg	neg
161A	neg	neg	neg	neg
161B	neg	neg	neg	neg
161C	neg	neg	neg	neg
162A	neg	neg	neg	neg
162B	neg	neg	neg	neg
162C	neg	neg	neg	neg
163A	neg	neg	neg	neg
163B	neg	neg	neg	neg
163C	neg	neg	neg	neg
164A	neg	neg	neg	neg
164B	neg	neg	neg	neg
164C	neg	neg	neg	neg
165A	neg	neg	neg	neg
165B	pos	pos	pos	pos
165C	neg	neg	neg	neg
166A	neg	neg	neg	neg
166B	neg	neg	neg	neg
166C	neg	neg	neg	neg
167A	neg	neg	neg	neg
167B	neg	neg	neg	neg
167C	neg	neg	neg	neg
168A	neg	neg	neg	neg
168B	neg	neg	neg	neg

Table 16, continued.

Control ID#	***cnf***	***hlyD***	***sfa***	***papGIII***
168C	neg	neg	neg	neg
169A	neg	neg	neg	neg
169B	neg	neg	neg	neg
169C	neg	neg	neg	neg
170A	pos	pos	neg	pos
170B	pos	pos	neg	pos
170C	pos	pos	neg	pos
172A	neg	neg	neg	neg
172B	neg	neg	neg	neg
172C	neg	neg	neg	neg
173A	neg	neg	neg	neg
173B	neg	neg	neg	neg
173C	neg	neg	neg	neg

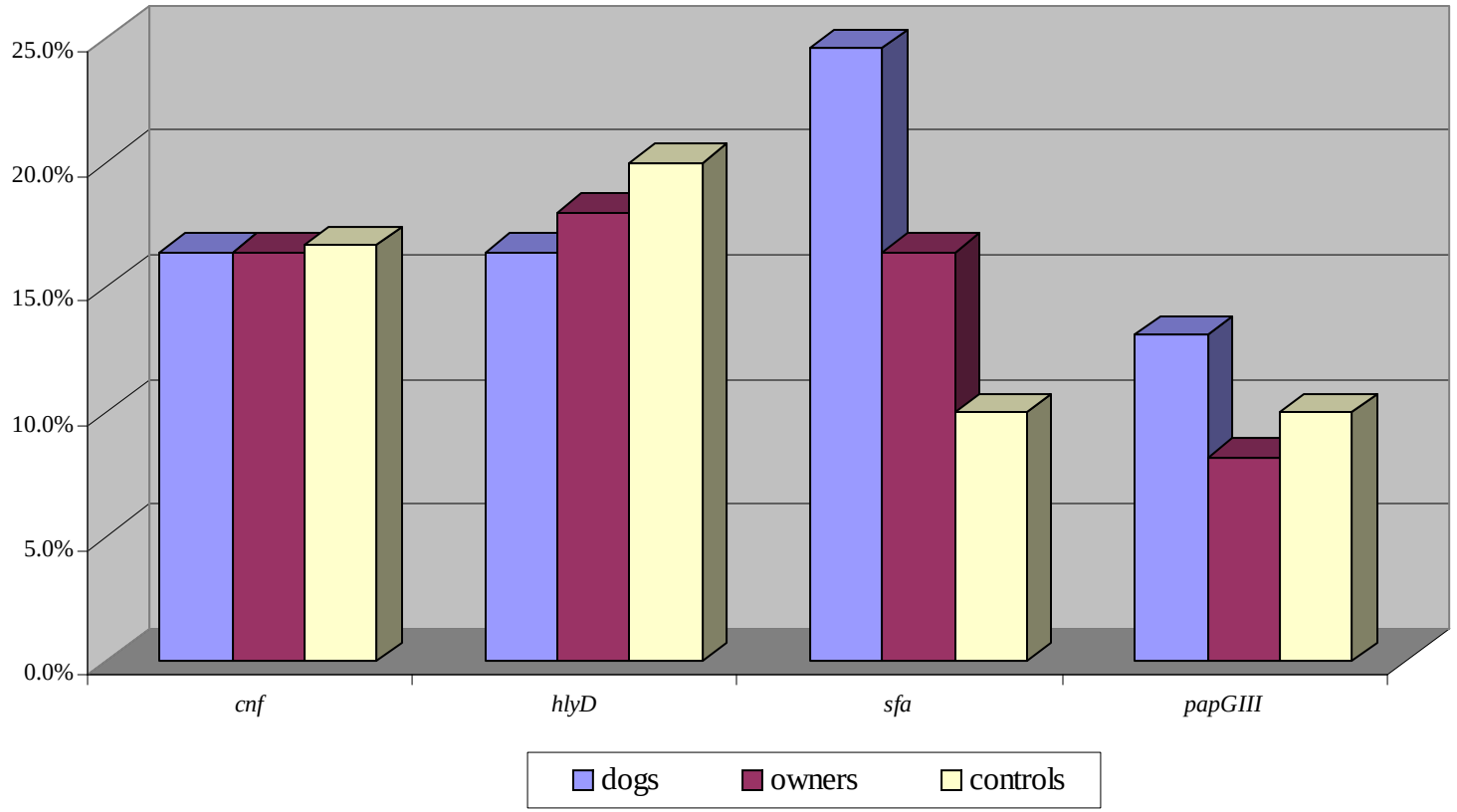


Figure 31. Percent participants with fecal *E. coli* positive for each virulence factor.

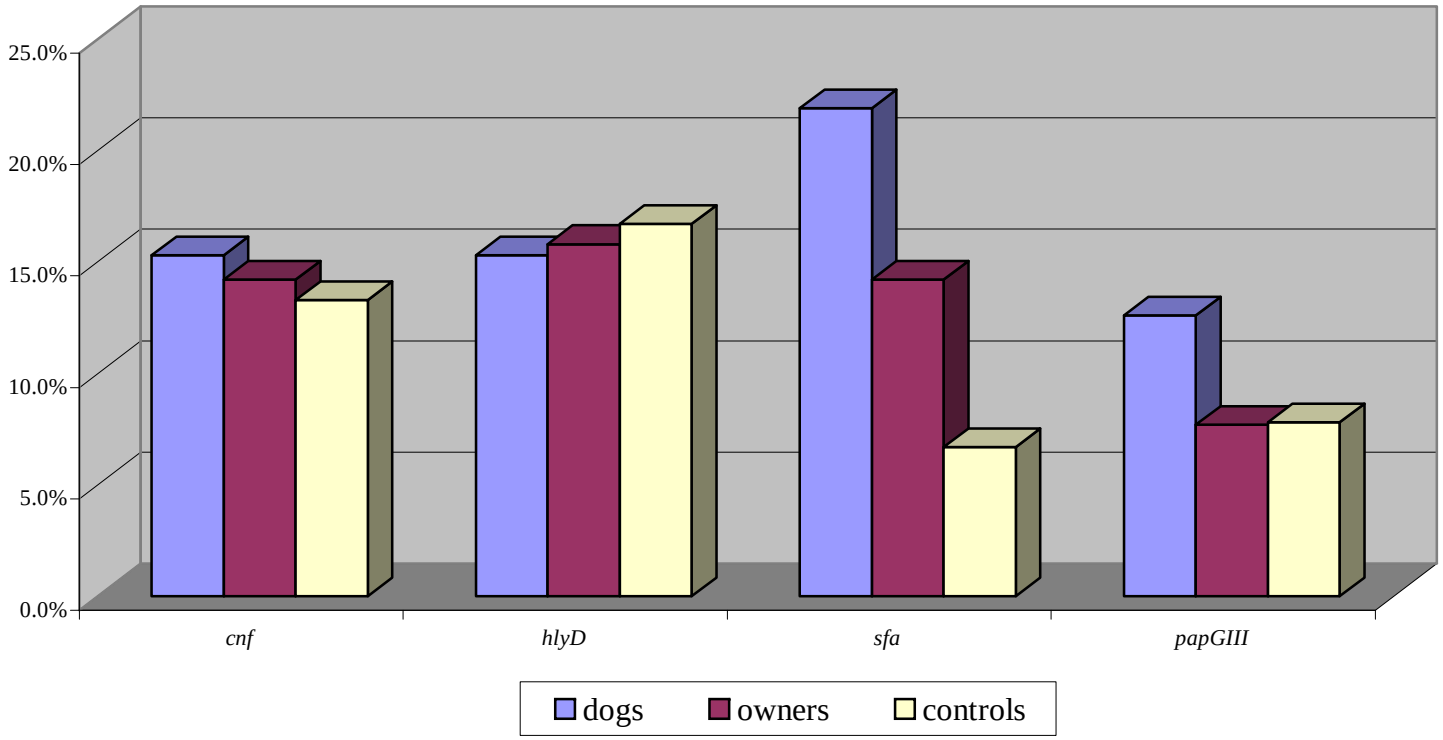


Figure 32. Percent of total *E. coli* isolates positive for each virulence factor.

Table 17. Number (%) dogs, owners, and controls with *E. coli* displaying each virulence factor pattern.

Virulence Factor Pattern	dogs	owners	controls
	N=61	N=61	N=30
hlyD	0	1(1.6%)	0
sfa	6(9.8%)	0	1(3.3%)
cnf+hlyD+sfa	2(3.3%)	5(8.2%)	2(6.7%)
cnf+hlyD+papGIII	1(1.6%)	0	2(6.7%)
cnf+hlyD+sfa+papGIII	7(11.5%)	5(8.2%)	1(3.3%)

Table 18. McNemar Chi-Square results comparing virulence factor prevalence within dog-owner pairs. There was no difference in presence or absence of any of the 4 virulence factors in dogs and owners in the same households.

Virulence Factor	McNemar
cnf	p=1.000
hlyD	p=1.000
sfa	p=0.405
papGIII	p=0.581

Table 19. Chi-Square results comparing virulence factor prevalence between owners and controls. There was no difference in presence or absence of virulence factors between owners and controls.

	Prevalence in owners vs. controls			Prevalence in owner isolates vs. control isolates	
Virulence Factor	**Chi-Square**	**p-value**		**Chi-Square**	**p-value**
cnf	0.001	0.974		0.038	0.844
hlyD	0.026	0.872		0.030	0.863
sfa	0.671	0.413		3.315	0.069
papGIII	0.082	0.775		0.001	0.970

Table 20. Chi-Square results comparing virulence factor prevalence between dogs and controls. Canine isolates were *sfa* positive more often than control isolates.

	Prevalence in dogs vs. controls			Prevalence in canine isolates vs. control isolates	
Virulence Factor	**Chi-Square**	**p-value**		**Chi-Square**	**p-value**
cnf	0.001	0.974		0.187	0.666
hlyD	0.001	0.974		0.085	0.771
sfa	2.698	0.100		9.937	*0.002
papGIII	0.184	0.668		1.415	0.234

*$p \leq 0.0125$

Table 21. Comparisons of virulence factors within groups using the Kappa measure of agreement. There was perfect agreement between *cnf-hlyD* in canine isolates, and between *cnf-sfa* in owner isolates.

	Dogs	**Owners**	**Controls**	**All Participants**
cnf-hlyD	K=1.000, p<0.001	K=0.936, p<0.001	K=0.870, p<0.001	K=0.949, p<0.001
cnf-sfa	K=0.667, p<0.001	K=1.000, p<0.001	K=0.634, p<0.001	K=0.795, p<0.001
cnf-papGIII	K=0.886, p<0.001	K=0.667, p<0.001	K=0.708, p<0.001	K=0.774, p<0.001
hlyD-sfa	K=0.667, p<0.001	K=0.936, p<0.001	K=0.526, p<0.001	K=0.753, p<0.001
hlyD-papGIII	K=0.886, p<0.001	K=0.611, p<0.001	K=0.593, p<0.001	K=0.726, p<0.001
sfa-papGIII	K=0.566, p<0.001	K=0.667, p<0.001	K=0.088, p=0.400	K=0.549, p<0.001

K=Kappa; Poor agreement: K<0.2; Fair agreement: K=0.2-0.4; Moderate agreement: K=0.4-0.6; Good agreement: K=0.6-0.8; Very good agreement: K=0.8-1.0; Perfect agreement: K=1.0 [131]

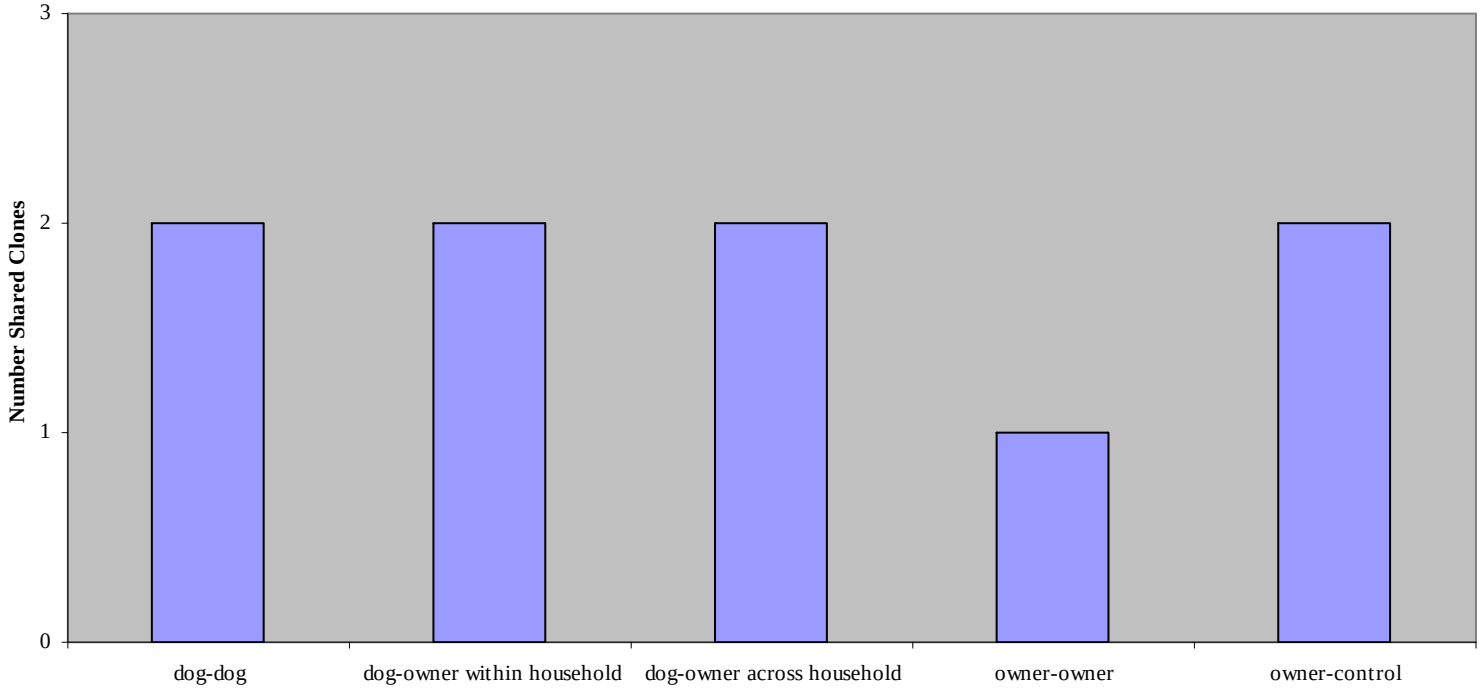


Figure 33. Participants who shared *E. coli* clones with isolates having identical susceptibility patterns.

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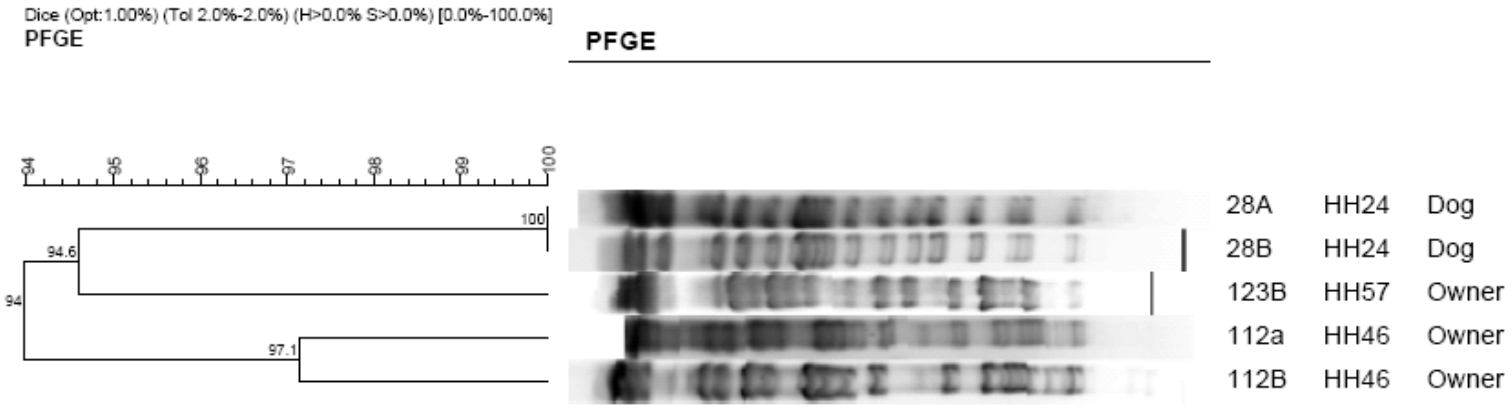

Figure 34. Dendrogram of an *E. coli* clone containing 5 isolates with differing susceptibility patterns.

Table 22. Mann-Whitney comparison (p-values given) between virulence factors and antimicrobial susceptibility of 456 *E. coli* isolates. Associations were found between antimicrobial resistance and decreased virulence.

		Virulence Factors		
Antimicrobial agents	***cnf***	***hlyD***	***sfa***	***papGIII***
AN	0.560	0.540	0.540	0.644
AM	0.765	0.939	0.314	0.205
AmC	0.684	0.495	0.799	0.565
FOX	0.273	0.249	0.913	0.384
NXL	0.241	0.217	0.797	0.352
CPD	0.294	0.376	0.092	0.068
CRO	0.241	0.217	0.799	0.352
CF	0.224	0.080	0.058	*0.006
C	0.527	0.639	0.089	0.163
CIP	0.094	0.078	0.078	0.184
GM	0.273	0.249	0.249	0.384
IPM	1.000	1.000	1.000	1.000
K	*0.031	*0.023	*0.023	0.087
NA	*0.039	*0.030	*0.030	0.100
S	*0.001	*0.008	**<0.001	*0.003
Te	0.358	0.919	0.258	*0.027
TMS	*0.009	0.182	*0.006	*0.038

*p≤0.05, **p≤0.0007

Table 23. Susceptibility patterns of an *E. coli* clone containing susceptible isolates and MDR isolates.

	ID#28A	ID#28B	ID#112A	ID#112B	ID#123B
amikacin (AN)	S	S	S	S	S
ampicillin (AM)	S	S	**R**	**R**	**R**
amoxicillin-clavulanic acid (AmC)	S	S	I	I	S
cefoxitin (FOX)	S	S	S	S	S
ceftiofur (NXL)	S	S	S	S	S
cefpodoxime (CPD)	S	S	S	S	S
ceftriaxone (CRO)	S	S	S	S	S
cephalothin (CF)	S	S	S	S	I
chloramphenicol (C)	S	S	S	S	S
ciprofloxacin (CIP)	S	S	S	S	S
gentamicin (GM)	S	S	S	S	S
imipenem (IPM)	S	S	S	S	S
kanamycin (K)	S	S	S	S	**R**
nalidixic acid (NA)	S	S	S	S	S
streptomycin (S)	S	S	**R**	**R**	I
tetracycline (Te)	S	S	**R**	**R**	**R**
trimethoprim-sulfamethoxazole (TMS)	S	S	**R**	**R**	**R**

Table 24. Clones with isolates sharing identical virulence factors and susceptibility patterns.

Isolate ID#s	Type of Participants	Within or Across-Household	Percent PFGE Similarity	Virulence Factors present	Antimicrobial Susceptibility
10B, 47A, 47B	Dog-Dog	Across	94.6%	*cnf, hlyD, sfa, papGIII*	Susc. to all agents
20B, 117A, 117B	Dog-Owner	Across	94.6%	*cnf, hlyD, sfa, papGIII*	Susc. to all agents
26A, 29B	Dog-Dog	Across	94.7%	none	Int. to CF; Susc. to remaining agents
34B, 66C	Dog-Owner	Across	94.7%	none	Res. to AM, CIP, NA; Int. to AmC, CF; Susc. to remaining agents
75B, 82B	Owner, Owner	Across	94.1%	none	Susc. to all agents
90A, 172C	Owner, Control	Across	94.4%	none	Susc. to all agents

Susc.=Susceptible, Int.=Intermediate Susceptibility, Res.=Resistant

Vita

Dr. Kate Stenske is originally from the Chicago area and earned a BS degree in biology from the University of Notre Dame in 1998 and a DVM degree from the University of Minnesota in 2002. Her veterinary training included an internship at Tufts University and a residency in small animal internal medicine and a PhD in bacteriology and public health at the University of Tennessee. She is a Diplomate in the American College of Veterinary Internal Medicine. She will continue her veterinary career in academia as a clinician, teacher, and researcher on faculty at Kansas State University in Manhattan, Kansas.