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Prevalence of Potential Zoonotic Enteric Bacterial Pathogens in Dogs and Cats and Factors Associated with Potential Transmission Between Animals and Humans

Omaima Maamoun Ahmed
University of Tennessee, Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Omaima Maamoun Ahmed entitled "Prevalence of Potential Zoonotic Enteric Bacterial Pathogens in Dogs and Cats and Factors Associated with Potential Transmission Between Animals and Humans." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

F. Ann Draughon, Major Professor

We have read this thesis and recommend its acceptance:

Dr. Joseph W. Bartges, Dr. John C. New

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Prevalence of Potential Zoonotic Enteric Bacterial Pathogens in Dogs and Cats and Factors Associated with Potential Transmission Between Animals and Humans

A Thesis Presented for the Master of Science Degree

The University of Tennessee, Knoxville

Omaima Maamoun Ahmed

August 2004

DEDICATION

This dissertation is dedicated to my husband

Mohamed Moustafa Abd -Eldaim

Whose hard work, patience, sacrifices, and love has made

my accomplishments possible.

To my Father Maamoun Ahmed Atta,

Whose encouragement was the spiritual fuel to start and finish this degree and to

my mom Ekram Azab, I am still going in this life only by

her huge love and prayers.

To my wonderful daughters: Nada, Safa, and Hana. They are my pretty lovely

flowers that make me enjoy my life.

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ABSTRACT

With the discovery of the human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS), concerns about dangers of pet ownership have increased. Zoonotic organisms associated with cats and dogs, may cause life-threatening infections in immuosuppressed human beings. The objectives of this project were to determine the prevalence of potential zoonotic enteric pathogens (*Salmonella*, *Listeria*, and *Campylobacter*) in feces of dogs and cats (diarrheic, healthy, and hospitalized), to evaluate the association of diarrhea in dogs and cats with diarrhea in human beings sharing the same household, and to evaluate the antimicrobial susceptibility of *Salmonella*, *Listeria*, and *E.coli* to 18 antimicrobials of human and veterinary importance. Methods of bacterial isolation and identification followed conventional FDA BAM protocols (Bacteriological Analytical Manual). Bacterial isolates were tested for their susceptibility using the disk diffusion assay in accordance with NCCLS guidelines**.** Owners of pets with diarrhea participating in the study were interviewed using a phone questionnaire that focused on identifying association of diarrhea in human beings living in the same household with affected pets. *Salmonella* and *Campylobacter* spp. were isolated from 1 each of 95 dogs having acute or chronic diarrhea (1.1%). *Listeria* species was isolated from 12 of 353 (3.4%) total dogs and cats. Generic *E.coli* was isolated from feces in 70.8% of all dogs and cats sampled (250 of 353). E.coli isolated from healthy dogs and cats showed the highest resistance rate to the antibiotics followed by diarrheic dogs and cats. Most *E.coli* isolates (79.7%) were multidrug resistant (MDR). Imipenem was the only antibiotic which none of

the *E.coli* isolates were resistant to. *Listeria* spp. isolated from dogs were most resistant to nalidixic acid (88.9%) followed by cefoxitin (77.8%). The low incidence of enteric pathogens in dogs and cats having acute or chronic diarrhea shows that the risk is low for transmission to human beings. However, individuals who are immunocompromised should have animals with acute or chronic diarrhea checked by a veterinarian. High prevalence of MDR bacteria is a serious problem and the search for alternative therapeutic compounds is needed especially for the immunocompromised, infants and elderly people.

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INTRODUCTION

With the discovery of numerous factors affecting immune response including the human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS), concern about dangers of pet ownership has increased considerably. Zoonotic pathogens (i.e. infectious pathogens shared by people and animals) are associated with cats and dogs, many of which can cause potentially life-threatening infections in immunosuppressed human beings (Greene 1998) (Cone et al., 2003) (Nair et al., 1985). There are reports of transmission of zoonotic enteric bacteria from dogs and cats to immunosuppressed human beings including those with HIV- infection, young children, elderly, and cancer patients undergoing chemotherapy and/or radiation therapy (Glaser et al., 1994) (Sato et al., 2000). There are no epidemiological surveys on prevalence of zoonotic enteric bacteria in healthy dogs and cats compared to those with diarrhea. *Salmonella*, *Campylobacter*, *Listeria*, and *Escherichia coli* can cause gastroenteritis in dogs and cats mostly accompanied with diarrhea. The cases of gastroenteritis in dogs and cats are mostly self-limiting and administration of antibiotics is usually unnecessary; however, for immunocompromised, infants, and elderly people, antibiotic therapy may be needed. In these situations, the prevalence of multidrug resistant bacteria is critical and the search for alternative therapeutic compounds is needed (Szych et al., 2001). A recent study showed that the prevalence of antimicrobial resistance to first-line therapy in human beings exceeded 20% in bacteria in many North

American regions. This is a serious problem since it may result in clinical failure in humans when these antibiotics are used (Talan et al., 2004).

The purpose of this study is to determine the prevalence and antibiotic susceptibility of potential zoonotic enteric bacteria isolated from a convenience sample of healthy dogs and cats, hospitalized animals*, and animals with acute and chronic diarrhea. We hypothesize that there will be a relationship between diarrhea and occurrence of zoonotic enteric bacterial pathogens in dogs and cats with diarrhea.

The objectives of this project:

1. To determine the prevalence of *Salmonella*, *Campylobacter*, *Listeria*, and generic *E.coli* pathogens in feces from healthy, hospitalized (non-diarrheic, but unhealthy), and animals with diarrhea.
2. To evaluate the association of diarrhea and enteric pathogens in dogs and cats with pet handling practices by human beings sharing the same household.
3. To determine antimicrobial susceptibility of enteric isolates to 18 antimicrobials of human and veterinary importance (using NARMS).

* Animals refers to dogs and cats only

Part I. LITERATURE REVIEW

PREVALENCE OF ZOONOTIC BACTERIAL PATHOGENS

Campylobacter

Campylobacters are curved unicellular microorganism, spiral rods 1.5-3.5 *μ*m long by 0.2-0.4 *μ*m wide. They are gram-negative, non-spore forming microaerophilic rods, and motile with a polar flagellum at one or both ends of the cell. Cells move quickly across a microscopic field twirling or rotating in a spiral-like motion. (Smibert 1978)

There are many species of Campylobacters but the most common zoonotic pathogens (i.e. infectious diseases shared by people and animals) are: *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter upsaliensis*.

C.jejuni has been recognized as an important zoonotic enteric pathogen of human beings causing acute and subacute gastrointestinal illness (Blaser and Reller 1981). *C. coli* is also associated with human illness, but is less common (Torre and Tello 1993) (Altekruse et al., 1994). *C.upsaliensis* transmission from pets to humans could be very important, as the disease associated with this species of bacteria may be severe (Burnens and Nicolet 1992). Most *Campylobacter* infections have a zoonotic cause that is associated with: consumption of contaminated food and water (indirect contact) or by infected animals (direct contact) (Altekruse et al., 1994). Therefore, both direct and indirect contacts are possible modes of transmission of the pathogen from animals to human. Since small numbers (500 cells) of *C.jejuni* can cause infection in human beings, the possibility of acquiring an infection from contact with feces from animals is a valid concern (Dillon and Wilt 1983).

Campylobacter-associated diarrhea has a wide clinical spectrum in dogs as well as human beings, ranging from mild, loose feces to watery diarrhea to bloody mucoid diarrhea. Anorexia, vomiting, and elevated body temperature may also be present. Erythromycin is the drug of choice for Campylobacteriosis in human beings, and it may be effective in animals (Greene 1998).

Consumption of contaminated food and water (indirect contact):

Poultry are considered to be an important source for transmission of Campylobacters. *C. jejuni* was isolated from chicken flocks at slaughter in 1983, in the United States (Prescott and Gellner 1984). In 1987, a strong association was shown between sporadic Campylobacter infections and handling and processing of raw chicken. The juices, which drain from frozen chickens on thawing are frequently contaminated with Campylobacters, and is an ideal medium for transferring Campylobacters from chicken to the hands of operatives (Coates et al., 1987)

In 100 slaughtered beef cattle, 50 animals were positive for *C.jejuni*; only one was positive for *C.coli* (Garcia et al., 1985). The distribution pattern of *C.jejuni*-positive animals was steers (55%), bulls (40%), and cows (22%). *C.jejuni* serogroups encountered in slaughter cattle were similar to those commonly isolated from human sources (Garcia et al., 1985).

In Canada, *C. jejuni* has an ecologic cycle involving water, animals, and foods. During the years 1983-1986, samples were collected from federally inspected abattoirs across Canada and tested for Campylobacters. Thermophilic Campylobacters were isolated from 16.9% of pork, 22.6% of beef, 43.1% of veal,

73.7% of turkey, and 38.2% of chicken carcasses. *C. jejuni* was the most frequent isolate from beef, veal, poultry, and pork. *C. coli* was also frequently isolated from pork (Lammerding et al., 1988)

C.jejuni was isolated from three (1.5%) of 200 retail mushroom samples, which suggests an increased relative risk of developing Campylobacteriosis in individuals who consume fresh uncooked mushrooms (Doyle and Schoeni 1986). This occurrence is probably due to the substrate (animals feces) used for mushroom cultivation.

In a Tennessee study (Rohrbach et al., 1992), milk samples from dairy farm bulk tanks were analyzed for *C.jejuni*. Frequency of *Campylobacter* isolation was 12.3%. Consumption of raw milk was reported by 34.9% of dairy producers and their household (Rohrbach et al., 1992).

C.jejuni has been isolated from fresh and salt water and has been shown to survive in fresh water at a range of temperatures (5-37°C) (Chen et al., 1995). As feces from infected animals or people may contain viable organisms, it could be a source for contamination of the environment. Campylobacters were recovered from small rodents inhabiting alpine meadows and *C.jejuni* was also isolated from bear fecal samples collected from a watershed. Because these animals may carry human pathogens; they should be included as a health risk associated with mountain watersheds (Pacha et al., 1987).

One study found that campylobacters suspended in 0.1 % peptone water and dried on fingertips survived for different periods of time ranging from <1 to >4 min. However, campylobacters suspended in chicken liquor or blood survived for

more than 4 minutes. Great attention to thoroughly washing and completely drying the hands after washing may help reduce the incidence of sporadic *Campylobacter* infection in human beings (Coates et al., 1987).

Infected animals contact (direct contact):

Zoonoses are most common in human beings because of poor hygienic practice especially hand-to-mouth behavioral patterns and close contact between human beings and animals or their excretions (Kahrs et al., 1978).

There are a number of public health concerns associated with feeding raw meat diets to dogs. Dogs are susceptible to a variety of foodborne infections. The risk of foodborne diseases in pet dogs is a major concern, but of more importance is the public health risk of zoonotic infections. To improve the health of pets and their owners: owners should never permit animals to be fed raw meat, fish, or eggs, and limit access to carrion or hunting. Pet food should be stored and served in a clean container and uneaten food should be discarded. Owners and families should practice personal hygiene when feeding and interacting with pets (LeJeune and Hancock 2001). However, the behavior of owners is not always consistent with public health recommendations.

C. jejuni and *C.coli* have been isolated from both clinically normal and diarrheic dogs and cats with a higher incidence in dogs with diarrhea than normal healthy ones (Bruce et al., 1980) (Nair et al., 1985). Campylobacter were isolated from 49% of feces from 144 dogs examined from premises handling stray animals. Only two of the dogs had diarrhea at the time of sampling and *C. jejuni/coli* were isolated from one of the animals. In a total of 80 puppies

examined from a veterinary practice, 38 clinically normal animals yielded 15 (39.5%) positives for Campylobacters and 16 of 42 (38.1%) diarrheic puppies were positive for Campylobacter. A family of two adults and two children (a boy aged six and a girl aged seven) acquired a seven-week-old puppy with diarrhea from premises handling stray animals. Six days after taking the animals home, the boy developed abdominal pain and acute enteritis. The symptoms lasted for four days during which time his sister complained of the same symptoms. *C jejuni/coli* were recovered from the feces of both children and the puppy (Bruce et al., 1980).

Campylobacter was also isolated from dogs with chronic diarrhea. A 12-year-old spayed female dog was referred to the University of Minnesota, Veterinary Teaching Hospital with a 2-month history of diarrhea. The dog had access to lakes and wooded areas where it was known to eat dead fish and ducks. *Campylobacter spp* was isolated from the small intestine. In domestic dogs *C. jejuni* was recovered from 21.7% of dogs with diarrhea as compared with 3.1% of normal healthy dogs (Davies et al., 1984).

C.jejuni was frequently isolated from puppies between birth and six months of age (Torre and Tello 1993; Nair et al., 1985) *Campylobacter* spp. were detected in the majority of puppies in a closed breeding colony by eight weeks of age with a corresponding rise in Campylobacter specific serum antibody (Newton et al., 1988). Also isolation of *Campylobacter* SPP. was greater in dogs aged six to twelve months (19.7%) compared with adult dogs (8.83%) in another setting (Torre and Tello 1993).

Several stress factors have been reported to initiate the onset of Campylobacteriosis in a wolf (Harwell et al., 1985). Stress can increase the excretion of Campylobacters (Newton et al., 1988). In 1999, in a study of the temporal distribution of environmental isolates of *C.jejuni* infection in the United States, isolation was most frequently accomplished in warmer months (Thomas et al., 1999). In another study which compared isolation rate during different seasons, *C.jejuni* was isolated more frequently in autumn and summer (Kahrs et al., 1978), than in winter (Doyle and Schoeni 1986) (Chen et al.,1995). Two pregnant adult Beagles (about two weeks from whelping) were vaccinated and were treated with an intestinal anthelmintic and shipped to The Ohio State University. Two days after the bitches arrived, both developed a sudden onset of diarrhea that was diagnosed as Campylobacteriosis (Harwell et al., 1985). In another study, rectal swabs were collected from apparently healthy dogs of different origin. The largest number of *C.jejuni* was recovered from dogs in breeding kennels (20.8%), compared with those in small animals clinics (13.7%) and veterinary practices (0%) (Torre and Tello 1993). The prevalence of *C.jejuni* was significantly greater in apparently healthy dogs living in high density and cohabitation housing for long periods (Torre and Tello 1993). A study in South Australia found that intensive housing and open drains increased the carriage rate of *Campylobacter* spp. by 2 and 2.6 times, respectively (Baker et al., 1999).

C.upsaliensis transmission from pets to human beings would be very important, as the disease associated with these bacteria may be severe (Burnens and Nicolet 1992). In a clinical and experimental study, fecal samples

were collected from 54 dogs with diarrhea and 54 healthy control dogs. In diarrheic dogs 16 were positive (29.6%) for Campylobacters. Most of the isolates were *C.upsaliensis* (18.5%) and *C.jejuni/coli* (11.1%). In healthy control dogs the prevalence of *Campylobacter* spp. was 24.1%, composed of *C.upsaliensis* (5.6%) and *C.jejuni/coli* (11.1%). Another group of dogs were infected experimentally with both *C.jejuni* (three dogs) and *C.upsaliensis* (three dogs). Clinical signs of diarrhea were seen only in one dog infected with *C.jejuni* (Olson and Sandstedt 1987). *C. upsaliensis* has been reported as frequently as *C.jejuni* in dogs and cats (Burnens and Nicolet 1992). In the same study from 397 diarrheic dogs and cats, 72 Campylobacter isolates were recovered. Approximately half were *C.upsaliensis* and half were *C.jejuni*. *C.upsaliensis* was found in 11% of cats. *C.coli* was not isolated from cats and only 4% were positive for *C.jejuni* (Burnens and Nicolet 1992). A study in South Australia reported *C. upsaliensis* present in 34% of dogs. (Baker et al., 1999).

Approximately half of 56 clinically normal cats examined yielded Campylobacter (Bruce et al., 1980). *C. jejuni* has also been associated with chronic diarrhea in cats. *C.jejuni* was isolated from the feces of a 10-month-old domestic, sexually intact female cat with a 5-week history of diarrhea (Fox et al., 1986). *Campylobacter jejuni/coli/upsaliensis* were isolated more frequently from dogs compared with cats (Baker et al., 1999).

Salmonella

A variety of *Salmonella* serotypes have been isolated from dogs. In a 1975 study, the most commonly isolated *Salmonella* were *S.* Typhimurum and *S.*

Anatum. Dogs are considered as a source of salmonellosis for human beings and other animals, since dogs may remain carriers. The sources of canine diseases can include consumption of infected rodents, rabbits, and small game or their feces (Morse and Duncan 1975).

Salmonella were found in apparently healthy dogs in Tehran, Iran. Household dogs (472 dogs) yielded *Salmonella* (4.4% positives) of 13 different serotypes, most commonly *S.* Entertidis and *S.* Typhimurium (Shimi et al., 1976).

Salmonella is considered a common problem in sled dogs and was isolated from 26 normal asymptomatic dogs. Most of the isolates were *S.* Typhimurium (69%). During the race, the dogs developed diarrhea and most of the isolates were *S.* Typhimurium (63%) (Cantor et al., 1997). In a separate study, *Salmonella* species were found in 3 out of 26 healthy dogs (Dahlinger et al., 1997).

A study of the occurance of *Salmonella* in commercial raw meat used in diets of racing greyhounds, found that 45% of the 112 commercial raw meat samples were positive for *Salmonella*. *S.* Typhimuruim was the most frequently isolated serotype (48%) followed by *S.* Nepwort (12.76%) (Chengappa et al., 1993). In dairy products, *Salmonella* was isolated from 8.9% of bulk tank milk samples (Rohrbach et al., 1992). Thirteen of 110 birds (11.8%) yielded Salmonella Enteritidis from chicken flocks at slaughter (Prescott and Gellner 1984).

Human beings may get salmonellosis from contaminated water of terrapins and the fecal pellets of tortoises. Most cases of reptile associated salmonellosis have been reported in young children (Borland 1975).

Research was done on fecal shedding of *Salmonella* in the Knoxville, Tennessee Zoo in wild cats. Two separate groups were both fed a raw feed diet. The first group was fed a commercial horsemeat- based diet and the other group was fed raw chicken. *Salmonella* was cultured from animals fed the raw chicken and the raw horsemeat diet. *Salmonella* was isolated from more than 90% of the animals fecal samples and *S.* Typhimurium was the most frequently isolated serotype (Clyde et al., 1997).

In a small animals hospital, *S.* Travis was isolated from all clinical cases of dogs. *S.* Travis infection occurred in 2% of hospitalized dogs during a 5-month period (Ketaren et al., 1981). *S.* Typhimurium was isolated from 12 week-old puppies and resulted in 32 cases of salmonellosis and 8 deaths. *S.* Virchow infection has been associated with zoonotic transmission from a household dog to a 4-month-old male infant. The infant manifested diarrhea and *S*. Virchow was isolated from his stool (Sato et al., 2000).

S. Arizonae gastroenteritis has been reported in a kitten (Krum et al., 1977). Cats may be exposed to Salmonellae infection because they catch mice and birds. In Tehran, Iran, cats play a very important role in the epidemiology of salmonellosis in human beings and animals. *Salmonella* was isolated from 18.4% of 141 pet cat fecal samples, and eight of the serotypes isolated were most frequently reported in cases of salmonellosis in human infants (Shimi and Barin

1977). Salmonellosis in cats is mostly manifested by gastroenteritis (Dow et al., 1989).

Clinical signs associated with *Salmonella* gastroenteritis in dogs and cats are fever, malaise, anorexia followed by vomiting, abdominal pain, and diarrhea. Cats often hypersalivate. The diarrhea in dogs and cats may vary in consistency from watery to mucoid, and fresh blood is present in severe cases. Antibiotics reported to be effective against *Salmonella* include chloramphenicol, trimethoprim-sulfonamide, amoxicillin, aminoglycosides, quinolones, and imipenem (Greene 1998).

Listeria

In 1924, E.G.D. Murray first isolated *Listeria* from the blood of laboratory rabbits under the name *Bacterium monocytogenes*. He could not assign these pathogenic microorganisms to any known bacterial genus at this time (Hof 2003). *Listeria monocytogenes* is a pathogenic, beta-hemolytic, facultative anaerobic, gram-positive rod (Greene 1998). It is a non-spore forming rod that can occur singly or in short chains. The organism grows between one and 45ºC and grows well at refrigerator temperatures (5 to10˚C) (DiMaio 2000). Cold storage to prolong food product shelf life has opened an ecological window for the growth of *L*. *monocytogenes* in fresh and processed foods and feeds (Notermans and Hoornstra 2000). *L. monocytogenes* is also considered an environmental contaminant and has been isolated from human beings, animals, and foods (Iida et al., 1998).

L. monocytogenes differs from non-pathogenic *Listeria* species in that it possesses hemolytic toxins. *Listeria* in dogs and cats is uncommon. When it occurs it is usually associated with ingestion of contaminated meat or meat-by-products. Clinical signs are due to the degree of intestinal inflammation. Fever, diarrhea, vomiting, neurologic signs, and abortion occur most frequently reported. Antimicrobial agents effective against *Listeria* are trimethoprim-sulfonamide, aminoglycosides, penicillin, ampicillin, erythromycin, and chloramphenicol (Greene 1998).

L. monocytogenes is considered a serious foodborne pathogen that has been isolated from many food products. Contaminated food products are responsible for approximately 2000 cases of listeriosis in the U.S. each year, which account for approximately 425 deaths each year (DiMaio 2000).

L. moncytogenes is recognized as a significant pathogen, that could be fatal especially in immunosuppressed patients (Abram et al., 2003), (Iida et al. 1998). It could be associated with gastroenteritis (Hof 2001). Central nervous system involvement can follow bacteremia because of invasion by *L. moncytogenes.* Meningitis is the most common manifestation followed by cerebral abscess in about 1% of immunosuppressed patients (Cone et al., 2003). Pregnant women also represent a high-risk group for listeriosis. Abortion, stillbirth, and severe neonatal infection can be a serious outcome of *L. monocytogenes* infection (Abram et al., 2003).

L. moncytogenes has been isolated from dairy products including 3 to 4% of raw milk. Low numbers of the organisms (less than 15 cfu/g) have been

isolated from frozen dairy products *(i.e., ice cream*) due to contamination of the finished product through post-pasteurization contamination (Kozak et al., 1996). Soft cheese made from raw milk is considered to be a high-risk product for causing human listeriosis (Bemrah et al., 1998). *Listeria* was isolated from approximately 4% of the raw bulk tank milk samples in one study (Rohrbach et al., 1992).

Listeria has been isolated in as high as 34.2% of retail sliced beef, 36.4% of pork, and 90% to 100% of fresh and processed fish and shellfish samples (Iida et al., 1998). Generally 1 to 7% of deli meats and salads have been reported to contain *Listeria* (Gombas et al., 2003).

In Portugal, *L. monocytogenes* was isolated from various commercial food products. In vegetables, it was found in 17% of frozen sliced courgette, 16.2% of frozen broccoli, 22.6% of frozen sliced green pepper, and 14.8% of frozen peas. *L. monocytogenes* was isolated from 60% of raw chicken, 17.7% of raw meat, 25% of ham, and 12% of raw fish. Also it was found in 18.5% of flour and 4.1% of pastry samples (Kozak et al., 1996).

L. monocytogenes has been reported to be responsible for many serious complications in women such as endocarditis and neonatal infections (DiMaio 2000). In Japan, *L*. *moncytogenes* has been isolated from fecal specimens of healthy animals. The prevalence was found to be 1.9% in cattle, 0.6% in pigs, and 6.5% in rats (Iida et al., 1991). In Germany, *L. monocytogenes* was isolated from 33.3% of fecal samples of healthy cattle, 8% from hens, 8% from sheep, 5.9% of pigs, and 4.8% from horses (Weber et al., 1995)

The prevalence of *L. moncytogenes* in Japan was found to be 0.9% in fecal samples of healthy dogs and zero from cats. Approximately 50% of the isolates were associated with human listeriosis (Iida et al., 1991). In Germany, *L. moncytogenes* was isolated from 1.3% of fecal samples from 300 healthy dogs and from 0.4% of fecal samples of 275 healthy cats (Weber et al., 1995).

L. monocytogenes has been reported as a cause of abortion in a bitch and caused general neurological disorders in dogs that consumed raw meat products contaminated with the organism. Foodborne listeriosis is considered a direct risk to pets with little risk of secondary transmission to human beings from pets (LeJeune and Hancock 2001).

The sources of *L. monocytogenes* infections in pet dogs and cats could be from the transmission of the infectious agent via feed by feeding raw meat, offal, unsterilized milk products and contaminated feed products. Listeriosis can be transmitted from human infections to dogs and cats through contact (Mayr 1989).

Escherichia coli

The natural habitat of *E. coli* is the enteric tract of human beings and warm-blooded animals (Staats et al., 2003). The presence of *E. coli* in food is an indicator of direct and indirect fecal contamination; therefore, *E. coli* has been used as an indicator of food sanitation and cleanliness. Pathogenic *E.coli* are one of the major types of enteric pathogens causing diarhhea through contaminated food and environmental vectors. They can result in serious environmental and foodborne disease outbreaks (Strachan et al., 2001). Water contaminated with pathogenic *E.coli* is responsible for approximately 1.7 million deaths a year

worldwide, mainly through infectious diarrhea (Ashbolt 2003). Foodborne *E.coli* O157 was also responsible for Britain's worst outbreak of hemorrhagic colitis, which affected nearly 500 people, and killed 21 (Rubery 2003).

E.coli has been isolated from many different types of food. In Argentina, *E.coli* was isolated from 93.3% of a total of 94 different ready-to-eat food samples (Gonzalez et al., 2003). *E.coli* O157 has been also isolated from fresh sausage (Normanno et al., 2004) and contaminated beef products (Li and Mustapha 2004). Pathogenic *E.coli* were found in fresh seafood, beef, lamb, pork, and poultry collected from grocery stores in Seattle, Washington (Samadpour et al., 1994).

Pathogenic *E.coli* was isolated from raw milk, cultured pasteurized milk, and naturally soured raw milk at levels of 4.5, 7.1, and 7.8 $\log_{10}$ CFU ml^{-1}, respectively, in Zimbabwe (Gran et al., 2003). *E.coli* is often detected in vegetable foods (Ercole et al., 2003). In Greece, pathogenic *E.coli was* isolated from 1% of ewes' milk samples, and 1.3% of fresh sausages (Dontorou et al., 2003).

Healthy domestic animals may serve as a reservoir of *E.coli*. General *E.coli* was found in fecal samples from healthy cattle (21.1%), sheep (66.6%), goats (56.1%), pigs (7.5%), cats 13.8% and dogs 4.8% (Beutin et al., 1993). Haemolytic *E.coli* was also isolated from feces of healthy cats (Blanco et al., 1993) and 21% of feces of healthy bitches (Chen et al., 2003). *E.coli* shiga toxins (*stx1,*and *stx2*) were present in 3% and 36%, respectively, of non-diarrheic greyhounds (Staats et al., 2003). Fecal swabs from 52 healthy dogs in a

midwestern research kennel were examined for *E.coli*. Enterotoxin-producing *E.coli* were isolated and belonged to serogroups O42, O170, and O-negative (Holland et al., 1999).

Diarrhea is one of the main causes of morbidity and mortality and a large proportion is caused by diarrheagenic *E. coli* (Clarke 2001). Until the late 1950s *E.coli* was considered a non-pathogenic normal cohabitant of all warm-blooded animals. However, certain strains are now known to cause different diarrheal diseases such as enterotoxigenic *E.coli*, enteropathogenic *E.coli,* enteroinvasive *E.coli, and* enterohemorrhagic *E.coli* (Greene 1998). Food originating from warm-blooded animals may contain *E.coli.* Several outbreaks have been associated with consumption of meat and meat products. Food could be a route for spreading pathogenic organisms to human beings. Human beings differ in their risk of *E.coli* infection. Important factors that affect risk are the immunological and nutritional status of the host. *E.coli* strains have been implicated in disease in persons with AIDS (Olsvik et al., 1991).

In dogs, *E.coli* was isolated from the feces of a six-year-old dog with a chronic diarrhea associated with intestinal anomalies (Zenger et al., 1992). *E.coli* shiga toxins (*stx1,* and *stx2*) were present in 15% and 23%, respectively, of diarrheic fecal samples of greyhound dogs (Staats et al., 2003). Haemolytic *E.coli* has also been isolated from other dogs with diarrhea (Starcic et al., 2002).

Histopathologic and electron microscopic examination of intestines of two cats revealed enteropathogenic *E.coli* in ileum, cecum, and colon (Pospischil et

al., 1987). Among *E.coli* strains isolated from diarrheic cats, most strains were hemolytic with cytotoxin activity (Abaas et al., 1989).

Flies play an important role in transmission of enteric bacteria. In one study flies were trapped from 10 dog breeding kennels in the region around Abilene, KS. Flies were examined for the prevalence of Gram (-) enteric bacteria. Blowflies were twice as likely to be contaminated with enteric bacteria as other flies. The apparent high incidence of enteric contamination of flies clearly implicates them as a vector of enteric diseases in kennels (Urban and Broce 1998).

ANTIMICROBIAL SUSCEPTIBILITY OF ENTERIC BACTERIA

Human sources

Numerous studies have been conducted on antimicrobial susceptibility of *Salmonella*. Antimicrobial sensitivity test results for *S.*Typhi and *S*.Paratyphi serotypes isolated from human patients in ten European countries showed multi-drug-resistance (MDR) to 4 antimicrobial drugs or more. For *S.*Typhi, 22 to 29% of the isolates were MDR, 11% of strains were sensitive to nalidixic acid. For *S.* Paratyphi, MDR increased from 1999 to 2001 from 9 to 25% and ciprofloxacin susceptibility decreased (Threlfall et al., 2003).

S.enterica strains were isolated from stool cultures of Italian children hospitalized for acute diarrhea and tested for susceptibility to seven antimicrobial agents. A total of 67.9% were resistant to one antibiotic and 26.1% were MDR. The rates of resistance were 60.6% for tetracycline, 46.8% for ampicillin, 21.6% for chloramphenicol, 1.8% for ceftriaxone, 8.7% for ciprofloxacin and ceftriaxone (Chiappini et al., 2002).

In Poland, during 1998-1999, *Salmonella* was isolated from human stool samples. The highest prevalence of MDR strains to two or more antibiotics was among serotypes *S*.Typhimurium. Approximately 93% of *S*. Virchow serotypes were resistant to furazolidone, none were resistant to ciprofloxacin, and only one strain (*S*. Mbandaka) was resistant to cefotaxime (Szych et al., 2001).

From hospitalized adults and children in Riyad, Saudi Arabia, 153 *Salmonella* strains were isolated and tested for antimicrobial susceptibility. The overall resistance was 16% for ampicillin, 13% for nalidixic acid, and 11% for chloramphenicol and trimethoprim/sulphamethoxazole. All isolates were susceptible to the second and third generations of cephalosporins, fluoroquinolones, and gentamicins although 16% of the isolates were MDR (Kambal 1996).

In Pennsylvania, *S.enterica* subsp. *enterica* Serovar Newport strains, which were isolated from animals, showed resistance to cephalosporins antibiotics and MDR (Rankin et al., 2002).

E.coli is often associated with symptomatic urinary tract infections (UTIs), which constitute a major health problem throughout the western world. The resistance of *E.coli* to trimetoprime/sulphamethoxazole, the first-line therapy for UTIs, exceeds 20% resulting in clinical failure associated with the resistance (Talan et al., 2004).

Concern has arisen over the increasing resistance of Campylobacters to new-generation antibiotics. Campylobacter isolated from foodborne disease patients were tested for antimicrobial susceptibility to the fluoroquinolones,

ciprofloxacin. Approximately 11% of the isolates were resistant and 42% of isolates from patients were fluoroquinolones resistant (Kassenborg et al., 2004).

The susceptibility of *C. jejuni* isolated from stool samples of patients with diarrhea against 9 antibiotics was determined. High susceptibility (>84%) was found to ampicillin, tetracycline, gentamycin, chloramphenicol, ciprofloxacin, and erythromycin (Oncul et al., 2003).

In Egypt, from a rural pediatric population with diarrhea, antimicrobial susceptibility was conducted on *C. jejuni* and *C. coli* isolates from 1995 through 2000. *C. jejuni* and *C. coli* showed decreasing ciprofloxacin susceptibility with a higher degree of susceptibility of *C. coli* compared to *C. jejuni* (Putnam et al., 2003).

In Jakarta, Indonesia, bacterial pathogens were isolated from 14% of hospitalized patients diagnosed with diarrhea. Bacterial isolates were susceptible to quinolones with the exception of *C. jejuni,* which was resistant to ciprofloxacin, nalidixic acid, and norfloxacin (Oyofo et al., 2002).

Listeria are considered a high-risk pathogen for cancer patients. From 736 clinical isolates from cancer patients, garenoxacin was compared with ciprofloxacin for activity against *Listeria*. Garenoxacin was the most active agent over all against Gram-positive organisms with potent activity against *L. monocytogenes* (Rolston et al., 2002).

L. monocytogenes was isolated from patients (n=84) with systemic listeriosis as a complication in undergoing treatment for cancer. The results showed susceptibility to penicillin (97.6%), ampicillin (90.7%), erythromycin

(98.8%), tetracycline (96.9%), and gentamicin (98.0%) and high resistance (96.2%) to clindamycin (Safdar and Armstrong 2003).

Food sources

Among the *Salmonella* isolated from imported foods and tested for their susceptibility for 17 antimicrobial agents, 8% were resistant to one antimicrobic, and 2.7% were MDR to three or more. Out of 187 total isolates, nine were resistant to tetracycline, four from seafood were resistant to nalidixic acid, seven were resistant to sulfonamides, and one (*S.* Derby) isolated from frozen anchovies was resistant to six antibiotics (Zhao et al., 2003).

E.coli isolated from retail meat and poultry was found to be resistant to at least one antimicrobial. Resistance was frequently associated with trimethoprim-sulphamethoxazole, fluoroquinolones, and cephalosporins (Schroeder et al., 2004).

Strains of *Campylobacter spp* isolated from poultry carcasses in a Swiss poultry slaughterhouse were tested for their antimicrobic susceptibility by the disk diffusion method. Resistance was found in 31.3%; 41 strains exhibited single resistance to streptomycin, ampicillin, or ciprofloxacin and18 strains revealed MDR to erythromycin and streptomycin. None of the isolates were resistant to tetracycline (Frediani-Wolf and Stephan 2003).

In French slaughterhouses, antimicrobial susceptibility testing was carried out for six different antimicrobial agents for *Campylobacter* isolates isolated from broilers. For *C. jejuni* the results showed 23% resistance to ampicillin, 25% to nalidixic acid, 17% to enrofloxacin, 57% to tetracycline, 0.3% to erythromycin,

and 0% to gentamycin. For *C. coli* the resistance percentage was 29% for ampicillin, 43% for nalidixic acid, 40% for enrofloxacin, 70% for tetracycline, 31% for erythromycin, and 0% for gentamycin (Avrain et al., 2003).

In Asturias, Spain, 38 isolates of *L. monocytogenes* and 18 of *Listeria* spp obtained from ripened chesses were tested for antimicrobial susceptibility. Low-level resistance to streptomycin, kanamycin, and gentamycin was found and a high percentage resistance was found for fosfomycin (Margolles et al., 2001).

In Porto, Portugal, 63 *Listeria spp* and *Listeria monocytogenes* isolated from poultry carcasses were tested for their antimicrobial susceptibility. For clindamycin, enrofloxacin, tetracycline, streptomycin, and erythromycin, *Listeria spp* showed resistance percentages of 54, 43, 15, 7, and 2% and *Listeria monocytogenes* showed resistance percentage of 35, 58, 0, 4, and 0% respectively (Antunes et al., 2002).

Animal sources

Antimicrobial susceptibility testing for 16 antimicrobial agents was performed on 581 clinical *E.coli* isolated from pigs, dairy cattle, and from urinary tract infections in dogs and cats in Switzerland. Resistance was most frequently found for sulfonamides, tetracycline, and streptomycin (Lanz et al., 2003).

Drug resistance was examined using isolates of *E.coli* obtained from dogs and cats in community practice in the UK. MDR was identified to clavulanate-amoxycillin and streptomycin (Normand et al., 2000).

The antimicrobial susceptibility of *L.monocytogenes* strains isolated from sheep and tested by the disk diffusion method showed resistance to tetracycline (7.3%) and doxycycline (4.9%) in *L.monocytogenes* strains of animals origins.

Multiple sources

From Ireland and Northern Ireland, a collection of 112 isolates of S. Enterica serotypes was collected from animals, food, and human sources. Approximately 74% of isolates were susceptible to all antimicrobial agents, 21% were resistant to sulfonamide and trimethoprim, and only one isolate (.9%) was MDR to five antimicrobial agents (Cormican et al., 2002).

Recent studies showed MDR *S.* Enterica Serotype Newport was resistant to expanded-spectrum cephalosporins in the United States (Gupta et al., 2003).

Salmonella isolates (178) were collected from California and came from dairy cattle, human clinical samples, bulk tank milk, fecal samples from preweaned calves, and waterways. The isolates were resistant to cephalosporins and florfenicol and were general sensitive to kanamycin and neomycin (Berge et al., 2004). In *S. enterica* Serotype Newport isolates obtained from human beings and dairy cattle, the prevalence of ceftrioaxone resistance increased from 0.05% in 1998 to 2.4% in 2001(Gupta et al., 2003).

In Japan, a total of 221 isolates of *Salmonella enterica* serovar Typhimurium from human and nonhuman sources including cattle, poultry, pigs, and environmental were characterized as MDR (Izumiya et al., 2001).

In France, from 1995 to 1996, 309 isolates of *Salmonella enterica* subsp. Enterica serotype Typhimurium strains were isolated from human beings, cattle,

pig, and poultry. Nalidixic acid resistance increased from 8.5% in 1995 to 18.6% in 1996 (Heurtin-Le Corre et al., 1999).

Antibiotic activity of 13 antibiotic substances against 60 *E.coli* with verocytotoxin-producing *E.coli* (VTEC) associated virulence factor were isolated from food, animals, and human fecal samples. All strains were susceptible to quinolones, gentamycin, trimethoprim/sulfamethoxazole and nitrofurantoin. Resistance was observed in *E.coli* isolates to cephalothin, tetracycline, and cefazolin. No MDR was observed (Klein and Bulte 2003).

Antibiotic susceptibility was investigated in 474 *E.coli* isolates isolated from animals feces, human feces, and food products of animals origin. A high frequency of resistance to ciprofloxacin, naldixic acid, and gentamycin was observed in broilers (38, 88, and 40%, respectively) and from food (13, 53, and 17%, respectively). High levels of resistance to trimethoprim-sulfamethoxazole and tetracycline were found in *E.coli* isolated from broilers, pigs, and foods (Saenz et al., 2001).

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PART II. PREVALENCE OF POTENTIAL ZOONOTIC ENTERIC BACTERIAL PATHOGENS IN DOGS AND CATS

ABSTRACT

With the discovery of the human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS), concerns about dangers of pet ownership have increased. Zoonotic organisms associated with cats and dogs, may cause life-threatening infections in immunosuppressed human beings. The objectives of this project were to determine the prevalence of potential zoonotic enteric pathogens (*Salmonella*, *Listeria*, and *Campylobacter*) in feces of dogs and cats with diarrhea and feces of healthy dogs and cats, and to evaluate the association of diarrhea in dogs and cats with diarrhea in human beings sharing the same household. Feces and fecal swabs were collected from dogs and cats during a chronic or acute episode of diarrhea by their veterinarian using conventional office practices and placed into transport tubes. Methods of bacterial isolation and identification followed conventional FDA BAM protocols (Bacteriologica Analytical Manual). Owners of pets with diarrhea participating in the study were interviewed using a phone questionnaire that focused on identifying association of diarrhea in human beings living in the same household with affected pets. *Salmonella* and *Campylobacter* spp. were isolated from 1 each of 95 dogs having acute or chronic diarrhea (1.1%). *Listeria* species was isolated from 12 of 353 (3.4%) total dogs and cats. Generic *E.coli* was isolated from feces in 70.8% of all dogs and cats sampled (250 of 353). The low incidence of enteric pathogens in dogs and cats having acute or chronic diarrhea indicates that the risk is low for transmission to human beings. However, individuals who are immunocompromised should have animals with acute or chronic diarrhea

checked by a veterinarian and should follow sound sanitary practices with companion animals.

INTRODUCTION

With the discovery of numerous factors affecting immune response including the human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS), concern about dangers of pet ownership has increased considerably. Zoonotic pathogens are associated with cats and dogs, many of which can cause potentially life-threatening infections in immunosuppressed human beings (Greene 1998) (Cone et al., 2003) (Nair et al., 1985). There are reports of transmission of zoonotic enteric bacteria from dogs and cats to immunosuppressed human beings including those with HIV- infection (Glaser et al., 1994), young children, elderly, and cancer patients undergoing chemotherapy and/or radiation therapy (Sato et al., 2000). There are no epidemiological surveys on prevalence of zoonotic enteric bacteria in healthy dogs and cats compared to those with diarrhea.

The purpose of this study was to determine the prevalence of zoonotic enteric bacteria isolated from healthy dogs and cats, hospitalized dogs and cats and animals with acute and chronic diarrhea. We hypothesized that there would be relationship between diarrhea and occurrence of zoonotic enteric bacterial pathogens in dogs and cats with diarrhea.

The objectives of this project were (1)To determine the prevalence of *Salmonella*, *Campylobacter*, *Listeria*, and generic *E.coli* pathogens in feces from healthy, hospitalized (non-diarrheic, but unhealthy), and animals with diarrhea

and (2)To evaluate the association of diarrhea and enteric pathogens in dogs and cats with pet handling practices by humans sharing the same household.

MATERIALS AND METHODS

Experimental design

Client-owned dogs and cats, presenting to the Small Animals Veterinary Teaching Hospital, The University of Tennessee (SAVTHUT) or to a variety of private veterinary clinics in Tennessee, were evaluated. A total of 353 fecal swabs were collected from dogs and cats for bacterial isolation and identification. Fecal swabs (n= 95) were collected from animals with acute (one episode of less than 7 days' duration) and chronic diarrhea (multiple episodes or a single episode lasting longer than 7 days). Fecal swabs were collected from healthy dogs and cats (n=188) owned by faculty, staff, and students of SAVTHUT. Fecal swabs (n=70) of hospitalized (non-diarrheic, but unhealthy) dogs and cats were also collected from patients of the SAVTHUT. All samples were analyzed in the Food Safety and Processing Building laboratories at the Food Safety Center of Excellence (FSCOE) at the University of Tennessee.

Sample collection

Fecal swabs were collected by a veterinarian or an assistant from dogs and cats. Samples were refrigerated and transferred to the laboratory within 24h. Any recent history (within the previous 5 days) of antibiotic therapy resulted in the exclusion of animals from the study. Permission was obtained from the University of Tennessee IACUC (Institutional Animals Care and Use Committee) to conduct this work with animals.

Questionnaire

The owner questionnaire and interview process was approved by the Department of Comparative Medicine Institutional Review Board and filed with the UT Office of Research. Owners of pets with diarrhea participating in the study were interviewed by phone using a questionnaire that focused on identifying association of diarrhea in human beings living in the same household with affected pets. Examples of the dog and cat owner questionnaires are presented in Appendix.

Microbial analysis

Isolation and identification of Salmonella

Culture media, reagents, equipment and materials used for isolation and confirmation of *Salmonella* are described in the *Food and Drug Administration* (FDA) *Bacteriological Analytical Manual* (BAM) *Protocols* (Pangloli et al., 2003).

Samples (fecal swabs) in sterile test tubes were mixed with 10 ml of tetrathionate selective enrichment broth and incubated at 42° C for 24 h (Becton, Dickinson and Company, Sparks, MD). After incubation, a loopful of sample was streaked onto xylose-lysine-tergitol 4 (XLT4) selective plates (Becton, Dickinson and Company, Sparks, MD). The plates were incubated at 35° C for 24 h (Table1). Single non-lactose fermenting black or yellow colonies were selected and streaked onto triple sugar iron agar (TSI) slants (Becton, Dickinson and Company, Cockeysville, MD) (Pangloli et al., 2003). *Salmonella* isolates were confirmed by biochemical tests: indole, and urea (Becton, Dickinson and

Company, Sparks, MD) then confirmed by API. E (Analytical Profile Index for Enterobacteriaceae family) (Becton, Dickinson and Company, Sparks, MD).

Isolation and identification of E.coli

Samples for *E.coli* isolation were enriched in10 ml of trypticase soy broth (mTSB; TSB plus 20 mg of novobiocin per liter) enrichment medium (Becton, Dickinson and Company, Franklin Lakes, NJ) and incubated at 37ºC for 24 h (Murinda et al., 2002). A loopful of the enriched samples was streaked onto eosin methylene blue (EMB) plates (Becton, Dickinson and Company, Sparks, MD) for isolation. Plates were incubated at 35ºC for 24 h (Table1). Typical; sorbitol fermenting *E. coli* colonies were picked from each plate and inoculated on trypticase soy agar (TSA) slants (Becton, Dickinson and Company, Sparks, MD). Cultures were incubated at 37ºC for 24 h. Isolates on TSA were used for biochemical testing. Suspected colonies were typed biochemically with indole_methyl red, Voges-Proskauer, citrate tests (Becton, Dickinson, France) (Murinda et al., 2002).

Isolation and identification of Listeria

Samples (fecal swabs) were added and mixed with 10 ml of buffered *Listeria* enrichment broth (LEB; plus 0.5% acriflavin, 0.5% naladixic acid, 10% pyruvic acid, and 2.5% cyclohexamide) (Oxoid LTD., Hampshire, England) and incubated at 30°C for 48 h. After incubation, a loopful of sample was streaked onto PALCAM. *Listeria* selective agar base (Becton, Dickinson and Company, Sparks, MD). The PALCAM plates were incubated at 35° C for 48 h (Table1). Single gray-green colonies with a black halo or black background were streaked

onto triptocase soy agar with yeast extract (TSA-YE) plates (Becton, Dickinson and Company, Sparks, MD). Identification of the species was made by the observation of the sugar fermentation of 1% mannitol, rhamnose (Becton, Dickinson, le Pont de Claix, France), and xylose (Becton, Dickinson and Company, Sparks, MD) incubated at 35°C for 48 h (Iida et al., 1991) and catalase test. Isolates were confirmed by motility test examined by wet mount, using 0.85% saline for suspending medium and oil immersion objective of phase-contrast microscope (BAM, FDA).

Isolation and identification of Campylobacter

Samples were enriched in *Campylobacter* enrichment Bolton Broth (BB with lysed horse blood) (Oxoid LTD., Hampshire, England) and incubated at 42° C for 48 h under *Campylobacter* environment (BAM, FDA). After enrichment, samples were streaked onto *Campylobacter* blood-free selective agar plates, with supplements (Oxoid LTD., Hampshire, England). The plates were incubated under microaerophilic conditions with a clinical N_2-CO_2 mixture (balanced-10) to give a final O_2 concentration of 5% at 42°C for 48 h (Table1). Suspect colonies were individually subcultured on blood agar plates for further testing. Negative glucose fermentation, positive-catalase, positive-oxidase reactions, and inability to tolerate oxygen were tentatively considered to be *Campylobacter* species.

Data analysis

Prevalence of *Salmonella, Campylobacter, E. coli,* and *Listeria* in dogs and cats was calculated as the number of positive fecal samples divided by the total

Table1. Enrichment and plating media used to isolate *Salmonella, Escherichia coli, Campylobacter, and Listeria*

Organism	Enrichment	Incubation time and temperature	Plating media	Incubation time and temperature
Salmonella	TT42[c]	24 h, 42° C	XLT4[d]	24 h, 35° C
E.coli	mTSB[a]	24 h, 37° C	EMB[b]	24 h, 37° C
Campylobacter	BB[e] with blood	48 h, 42° C	CCDA[f]	48 h, 42° C
Listeria	LEB[g]	48 h, 30° C	PALCAM[h]	48 h, 35° C

[a] Modified trypticase soy broth
[b] Eosin methylene blue
[c] Tetrathionate
[d] Xylose-lysine-tergitol 4
[e] Bolton broth
[f] *Campylobacter* blood-free selective agar
[g] Buffered *Listeria* enrichment broth
[h] PALCAM selective agar media

number of animals tested. Only one fecal sample was collected from each animal. Data from questionnaires were organized by demographic variables and analyzed for potential exposure factors, which may be associated with diarrhea in dogs and cats with diarrhea this was performed using Microsoft ® Excel 2000[1].

RESULTS

Fecal samples were collected from dogs and cats with diarrhea, starting in February 6, 2003, and ending April 16, 2004. Of 95 animals fecal samples from dogs and cats with diarrhea, 1 dog (1.1%) was cultured positive for *Campylobacter* and 1 different dog tested positive for *Salmonella*. *Campylobacter* and *Salmonella* were not isolated from healthy and hospitalized animals (Table 2). Generic *E.coli* was found in 63.2% of dogs and cats with diarrhea. *E.coli* was recovered from 75.5% of healthy animals (n=188) and in 67% of hospitalized animals (n=70) (Table 2).

L. moncytogenes was isolated from 1 dog with diarrhea (1.1%). In healthy animals (n=188), 9 dogs (4.8%) were cultured positive for *Listeria* spp. Of 70 hospitalized animals, 2 dogs (2.9%) were positive.

Questionnaires

A total of 95 owners of dogs or cats with diarrhea participated in the study by providing a fecal swab from their dog or cat (fecal swabs were collected by owners or animal's veterinarian). Of these owners, 77 completed questionnaires (70.5%) administrated by phone and 18 did not.

[1] Microsoft Corporation Redmond, WA, USA

Table 2. Frequency of *Salmonell*a, *Escherichia coli, Campylobacter*, and *Listeria* recovered from dogs with diarrhea, healthy and hospitalized dogs [a]

Sample category [b]	Number of dogs sampled	Number (%) of positive pathogens by microbial type			
		Salmonella [c]	*Escherichia coli* [c]	*Campylobacter* [c]	*Listeria*
Diarrhea	61	1 (1.6)	23 (37.7)	1 (1.6)	1 (1.6)
Healthy	109	0 (0.0)	85 (78.0)	0 (0.0)	9 (8.3)
Hospitalized	63	0 (0.0)	43 (68.3)	0 (0.0)	2 (3.2)
Total	233	1 (0.4)	151 (64.8)	1 (0.4)	12 (5.2)

[a] None of the enteric pathogens of interest except *E.coli* were isolated from cats.
[b] The number of cats in the study was: 34 diarrhea, 79 healthy, and 4 hospitalized.
[c] All species/serovars of *Salmonella*, *Campylobacter*, and *Listeria*

Questionnaires were not completed for various reasons: lack of information (samples were submitted from the clinics without adequate information for owner contact), and owner-related reasons (unable to contact after multiple attempts).

Data from questionnaires were organized by demographic variables (Table 3) and analyzed for potential exposure factors, which may be associated with risk of exposure to agents of diarrhea in human beings, dogs and cats with diarrhea. These data are presented in Table 4 (dogs), Table 5 (cats), and Table 6. Data were analyzed also for behaviors that could increase exposure of children (> 3 years of age) in households with dogs and cats with diarrhea (Table 7).

Case descriptions

Listeria

Listeria moncytogenes was isolated from an intact 6-week-old female dog with acute diarrhea. The dog lived with another companion dog in the same household. Both dogs were fed a commercial diet and neither commercial nor table treats were given. The owner reported feeding raw meat to the dog but type of meat was not provided. Also the dog was not exposed to raw eggs or raw milk. When the dog developed diarrhea, fenbendazole[2] and pyrantel pamoate[3] were dispensed. The owners reported that the dogs were kept indoors. The dog (testing positive fro *Listeria*) slept in the bedroom of the owners who were an adult male and female (50-60 years of age), but not on the bed.

[2] Panacur®, DPT Laboratories, San Antonio, TX.

[3] Strongid®, Antihelminthic, Pfizer INC, NY, NY

Table 3: Demographics of dogs and cats by acute or chronic diarrhea

Animal	**Type of Diarrhea**	**N[a]**	**Age in years (SD[b])**	**Sex by neuter status No. (%)**			
				Male		Female	
				Neutered	Intact	Spayed	Intact
Dogs	Acute	29	4.8 (± 3.7)	13 (44.8)	4 (13.8)	10 (34.5)	2 (6.9)
	Chronic	23	5.8 (± 4.3)	4 (17.4)	5 (21.7)	12 (52.2)	2 (8.7)
	Total	52	N/A	17 (32.7)	9 (17.3)	22 (42.3)	4 (7.7)
Cats	Acute	4	2.7 (± 2.5)	2 (50.0)	0 (0.0)	0 (0.0)	2 (50.0)
	Chronic	21	3.4 (± 4.2)	10 (47.6)	3 (14.3)	7 (33.3)	1 (4.8)
	Total	25	N/A	12 (48.0)	3 (12.0)	7 (28.0)	3 (12.0)
Total mean		77	4.6 (± 4.0)	29 (37.7)	12 (15.6)	29 (37.7)	7 (9.0)

[a] Number of samples
[b] Standard deviation

Table 4: Frequency of potential exposure factors to enteric pathogens: Dogs with diarrhea

Factor	Yes #(%)	No # (%)	Total # (%)
Exposure to raw meat	2 (3.8)	50 (96.2)	52 (100)
Allowed to catch prey	20 (38.5)	32 (61.5)	52 (100)
Other animals in the household	33 (63.5)	19(36.5)	52 (100)
Provided treats	37 (71.2)	15 (28.8)	52 (100)
Medications for diarrhea	34 (65.4)	17 (32.7)	51 (100)
Medications for pre-existing conditions (i.e., prior to start of diarrhea)	24 (46.2)	28 (53.8)	52 (100)
Known to get in the litter box	5 (9.8)	46 (90.2)	51 (100)
Drinking from toilets	7 (13.5)	45 (86.5)	52 (100)
Access to outside water sources	30 (57.7)	22 (42.3)	52 (100)
Drinking from rain water	24 (46.2)	28 (53.8)	52 (100)
Drinking from lake or pond	11 (21.2)	41 (78.8)	52 (100)
Drinking from river or stream	4 (7.7)	48 (92.3)	52 (100)

Table 5: Frequency of potential exposure factors to enteric pathogens: Cats with diarrhea

Factor	Yes # (%)	No # (%)	Total # (%)
Exposure to raw meat	1 (4)	24 (96)	25 (100)
Allowed to catch prey	10 (40)	15 (60)	25 (100)
Having litter box	23 (92)	2 (8)	25 (100)
Other animals in the household	21 (84)	4 (16)	25 (100)
Provided treats	9 (36)	16 (64)	25 (100)
Cat refuses to use the litter box	5 (20)	20 (80)	25 (100)
Medications for diarrhea	21 (84)	4 (16)	25 (100)
Medications for pre-existing conditions (i.e., prior to start of diarrhea)	11 (44)	14 (56)	25 (100)
Drinking from toilets	7 (28)	18 (72)	25 (100)
Access to outside water sources	9 (36)	16 (64)	25 (100)
Drinking from rain water	4 (16)	21 (84)	25 (100)
Drinking from lake or pond	0 (0)	25 (100)	25 (100)
Drinking from river or stream	0 (0)	25 (100)	25 (100)

Table 6: Proximity measures of potential exposure of humans to dogs and cats with diarrhea

Animals	Factor	Yes # (%)	No # (%)	Total # (%)
	Inside all of the time	19 (36.5)	33 (63.5)	52 (100)
	Inside most of the time	24 (46.2)	28 (53.8)	52 (100)
	Occasionally inside the house	6 (11.5)	46 (88.5)	52 (100)
Dogs	Sleeping outside	6 (11.5)	46 (88.5)	52 (100)
	Sleeping inside the house	46 (88.5)	6 (11.5)	52 (100)
	Sleeping in bedroom	27 (51.9)	25 (48.1)	52 (100)
	Sleeping on bed	21 (40.4)	31 (59.6)	52 (100)
	Inside all of the time	21 (84)	4 (16)	25 (100)
	Inside most of the time	3 (12)	22 (88)	25 (100)
	Occasionally inside the house	0 (0)	25 (100)	25 (100)
Cats	Sleeping outside	1 (4)	24 (96)	25 (100)
	Sleeping inside the house	24 (96)	1 (4)	25 (100)
	Sleeping in bedroom	16 (64)	9 (36)	25 (100)
	Sleeping on bed	18 (72)	7 (28)	25 (100)

Table 7: Parent-reported behaviors that could increase exposure of children (> 3 years of age) in households with dogs and cats with diarrhea

Factor	**Yes** **# (%)**	**No** **# (%)**	**Total** **# (%)**
Kissing the dog or cat	7 (26.9)	19 (73.1)	26 (100%)
Touching the mouth of the dog or cat.	11 (42.3)	15 (57.7)	26 (100%)
Touching the bottom (i.e. rectal area) of the dog or cat	2 (7.7)	24 (92.3)	26 (100%)

Both of the owners developed diarrhea around the same time the dog developed it but they did not seek medical attention. Consequently, the cause of their diarrhea was not determined. The water source for the household was a municipal water supply. Dogs were not allowed to catch prey nor drink from any outside water sources such as a river, stream, or puddles of rainwater. No *Listeria* was isolated from cats in the household and no other animals including the other dogs were reported to have diarrhea around the same time.

Listeria moncytogenes was also isolated from a neutered 6-year-old female healthy dog. The dog lived with another companion dog and cat in the same household. Dogs were fed a commercial diet and commercial treats that included raw meat. Animals were not exposed to raw eggs or milk. The owner reported that the dog was only occasionally indoors (i.e., less than 10 hours a day). The dog was reported to have been in the cat litter box and did not receive any medications prior to diarrhea. The infected dog slept in a dog bed in the bedroom of the owners who were an adult male and female (40-50 years of age), but not on the bed. The water source for the household was a municipal water supply. The dog was not allowed to catch prey but she was allowed to drink from outside water sources such as lake, pond, puddles of rainwater, and also was known to drink from toilets. Besides the one dog described, the other animals and humans did not develop diarrhea. No *Listeria* was isolated from cats

Salmonellae

Salmonellae Arizona was isolated from a 2-year-old neutered male dog with acute diarrhea. There were no other animals in the household and the

affected dog was fed a commercial diet and given treats, but was reportedly never exposed to raw meat, eggs or milk prior to developing diarrhea.

The dog was treated with Cleocin[4], an antibiotic, at the time he developed diarrhea and received flea medication (name not provided) just before the diarrhea started. The owner reported that the dog was only occasionally indoors (i.e., less than 10 hours a day). However, they let the dog sleep inside on his own pillow in the bedroom of a 20-30 year old female but not on bed. The owner of the dog did not develop diarrhea at the time of the dog's illness. The water source was municipal city water. Dogs were allowed to catch prey such as frogs and insects and drink from outside water sources such as a lake, pond, or puddles of rainwater. No *Salmonella* was isolated from the cats.

Campylobacter

Campylobacter was isolated from an intact 10.5-year-old male dog with chronic diarrhea. The dog lived with no other animals in the house. He was fed commercial diet, treats, and had never been allowed to eat raw meat, milk, or raw eggs. The dog was treated when he developed the diarrhea with metronidazole[5] for Giardia and given bismuth-subsalicylate[6] for upset stomach and diarrhea. The owner reported that the dog was mostly indoors, and let the dog sleep in the basement. None of the human beings developed diarrhea around the time the dog developed it. The water source was municipal city water.

[4] Clindamycin®, American Society of Health-System Pharmacists, Inc., Bethesda, Maryland

[5] Falgyl ®, Aventis, Bridgewater,NJ

[6] Pepto-Bismol ®, Procter&Gamble, Cincennati, Ohio

The dog was not allowed to catch prey but was allowed to drink from outside water sources such as puddles of rainwater. No *Campylobacter* was isolated from cats.

All four of the dogs from which pathogens were isolated were fed a commercial diet and they received medications because of diarrhea at the time they developed the diarrhea. Their owners let the dogs sleep inside. The main water source was municipal city water. *L. monocytogenes* were isolated from healthy and diarrheic dogs and both were exposed to raw meat and none of them were exposed to raw eggs or milk.

DISCUSSION

The data collected in this study represent a convenience sample of dogs and cats whose owners consented to be in the study. Consequently, general inference to other populations cannot be made. The animals in this survey included healthy, diarrheic, and hospitalized dogs and cats at SAVTHUT and private veterinary clinics. The selection of this study group was biased because random sampling of dogs and cats was not feasible. One of the difficulties with attempting a survey of domestic pets such as dogs and cats is the difficulty in finding enough owners to cooperate in sampling their animals. Added to this are the logistical problems in collecting and processing samples within an acceptable time period.

A sample size of 52 for dogs with diarrhea (if they have been randomly selected) would have the power to detect at least one positive animal if pathogens were present at a level of 6% prevalence or greater with 95%

confidence interval (Cannon and Roe, 1982). For *Salmonella*, *Campylobacter*, and *Listeria* the sample size was powerful enough to detect one positive pathogen. However, since the samples were not randomly selected, we cannot assume this is a reliable measure of prevalence.

An infection rate 1.1% for *Salmonella* and *Campylobacter* in dogs with diarrhea was found, which supported the view that privately owned adult dogs and cats generally have a lower isolation rate for *Campylobacter* or *Salmonella* than dogs housed in high density (animals shelters) or stray animals (Fox 1982). These results were lower than the prevalence rates of 21.7% of *Campylobacter* and 4.6% of *Salmonella* reported by others (Davies et al., 1984) (Adesiyun et al., 1997).

Salmonella was not isolated in our study from healthy and hospitalized dogs and cats, although other studies isolated *Salmonella* (4.4%) from healthy dogs (Shimi et al., 1977).

We also did not isolate *Campylobacter* from healthy and hospitalized dogs and cats. Previous studies reported *Campylobacter* isolation in 3.1% of normal healthy dogs and approximately 50% of clinically normal cats examined yielded *Campylobacter* (Bruce et al., 1980), (Davies et al., 1984).

Foodborne listeriosis (originating from raw meat) is considered a direct risk to pets with a potential risk for secondary transmission to humans (LeJeune and Hancock 2001) (Mayr 1989). Our results suggest this as well since *L*. monocytogenes was recovered from a female dog with acute diarrhea (1.1%) that consumed raw meat, and her owners also developed diarrhea concurrently.

However, we have no data on the causes of the human cases. We also recovered *L. monocytogenes* from a healthy female dog that consumed commercial treats containing raw meat which agrees with previous studies that found 0.9% in fecal samples of healthy dogs (Iida et al., 1991).

A sample size of 25 for cats with diarrhea (if they have been randomly selected) could have had the power to detect at least 1 infected cat at a prevalence of 11% or greater with 95% confidence interval (Cannon and Roe, 1982). Our results showed no positives in cats, but we cannot assume that cats are not infected, as the sample size was not large enough nor selected in a way to support such a conclusion.

Listeria, *Salmonella*, or *Campylobacter* isolated from the dogs with diarrhea may not be the cause of the diarrhea. Dogs may have developed the diarrhea due to non-microbial causes. Exposure factors (showed in table 4 and 5) could have exposed animals to agents other than bacterial. Some behaviors such as catching prey, receiving treats, receiving medications for preexisting conditions, and drinking from potentially contaminated water sources were associated with the isolation of *Salmonella* from dogs. The *Salmonella* positive dog was allowed to catch prey such as birds and mice, which has been reported as sources of *Salmonella* (Shimi and Barin 1977). The dog was provided treats, which could have been stored or served in unclean containers, and this could be a mode of transmission (LeJenue and Hancock, 2001). The dog received medications for pre-existing conditions, which could have changed the microenvironment of the gastrointestinal tract to favor a pathogen (Harwell et al.,

1985). The dog drank from potentially contaminated water sources. Contaminated water of terrapins and fecal pellets of tortoises can be a source of salmonellosis (Borland 1975). However, the dog in our study that was positive for *Salmonella* infection was not exposed to raw meat. In previous reports raw meat ingestion was associated with *Salmonella* infection in dogs (Chengappa et al., 1993).

The *Campylobacter* positive dog with chronic diarrhea was provided treats, which could have been stored or served in unclean containers, and this could be a mode of transmission (LeJenue and Hancock, 2001) or it could be contaminated by flies which have been reported as carriers for *Campylobacters* (Urban et al., 1998). The *Campylobacter* positive dog was also allowed to drink from rainwater which potentially could be contaminated by wild animals, reported as carriers of *Campylobacter* (Dillon and Wilt 1983). *Campylobacter* can survive in fresh and salt water (Chen et al., 1995). This finding of *Campylobacter* is similar to previous work (Davies et al., 1984). However, our results differ in that raw meat consumption was not a characteristic of recovery of *Campylobacter* (Garcia et al., 1985).

Both dogs with *L*. *moncytogenes* were exposed to raw meat, which agrees with previous study (Iida et al., 1998). Pets sleeping on beds are a common practice that could be a way for transmission of zoonotic bacteria between pets and humans (Bruce et al., 1980).

Drinking from toilets can be a significant risk in transmitting microbial pathogens (Briggs and Carling 2004). This behavior was reported from a

household where *L*. *monocytogenes* was isolated from a clinically healthy dog. However, a high percentage of dogs and cats included in our study (13.5% from dogs and 28% of cats) drank from toilets but were culture negative for *Campylobacter* and *Salmonella*.

The question is, are dogs and cats the source of infection or can they acquire the enteric pathogens from the same sources as people or from infected people. Regardless of the means by which dogs and cats become carriers, animals carrying *Listeria* are certainly a potential source of infection for people in contact with them.

The low prevalence of enteric pathogens in dogs and cats having acute or chronic diarrhea suggested that the risk may be low for transmission to humans. However, it may possible to minimize the risk of zoonotic spread from companion animals to humans by encouraging pet owners to follow good hygienic practices particularly when young children are handling pets. Also immunocompromised individuals should have their animals with acute or chronic diarrhea checked by a veterinarian and consistently follow sound sanitary practices with companion animals.

The risk of foodborne diseases in pet dogs is a major concern, but of more importance is the public health risk of zoonotic infections. To improve the health of pets and their owners: owners should never permit animals to be fed raw meat, fish, eggs, and limit access to carrion or hunting. Pet food should be stored and served in a clean container and uneaten food should be discarded properly.

Owners and families should practice personal hygiene when feeding and interacting with pets.

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Part III. ANTIMICROBIAL SUSCEPTIBILITY BY A STANDARD DISK DIFFUSION METHOD

ABSTRACT

The prevalence of bacterial resistance to first-line antibiotic therapy exceeds 20% in many American regions. Resistance may lead to clinical failure and increased morbidity and mortality. The aim of the study was to evaluate the antimicrobial susceptibility of *Salmonella*, *Listeria*, and *E.coli* bacterial isolates recovered from the feces of healthy, diarrheic, and hospitalized dogs and cats. Bacterial isolates were tested for their susceptibility to 18 antimicrobials of human and veterinary importance using the disk diffusion assay in accordance with NCCLS guidelines. Most *E.coli* isolates (79.7%) were multidrug resistant (MDR) (i.e. resistant to two or more antimicrobials) including *E.coli* isolated from dogs and cats with diarrhea (53.3% MDR), and from hospitalized dogs and cats (89.4% MDR). Prevalence of MDR was unexpectedly high for *E.coli* isolated from healthy animals (68.6%). Salmonella Arizona exhibited no resistance to any of the 18 antibiotics, but over 88% of the *Listeria* isolates showed MDR. The only antimicrobial that none of the *E. coli* isolates was resistant to was imipenem. The overall highest resistance for *E.coli* isolates were associated with dogs 89.4% to cephalothin, 58.9% to ampicillin, 51.9% to streptomycin, 41.1% to nalidixic acid, 41.7% to enrofloxacin, 40.4% to ciprofloxacin, 41.1% to tetracycline and 39.7% to cefoxitin. The overall highest resistance for *Listeria* isolates was 88.9% to nalidixic acid, 77.8% to cefoxitin, and 66.7% to cephalothin, ceftiofur, ampicillin, gentamycin, and tetracycline. High prevalence of MDR bacteria is a serious problem and the search for alternative therapeutic compounds is needed especially for the immunocompromised, infants and elderly people.

INTRODUCTION

Salmonella, *Campylobacter*, *Listeria*, and *E.coli* are among the bacteria most frequently isolated from healthy and sick dogs and cats. *Salmonella* and *Campylobacter* are the two most common causes of human foodborne infections. Foodborne gastroenteritis is mostly self-limiting and administration of antibiotics is usually unnecessary. Listeriosis has a much lower incidence but a much higher fatality (Rolston et al., 2002). For immunocompromised people, infants, and elderly people, antibiotic therapy may be needed. In these situations, the prevalence of multidrug resistant (MDR) bacteria may be a critical problem (Szych et al., 2001). Recent studies show that the prevalence of bacterial resistance to first-line antibiotic therapy exceeds 20% in many North American regions and there is concern that this may lead to clinical failure associated with MDR (Talan et al., 2004).

The aim of the study was to evaluate the antibiotic susceptibility of *Salmonella*, *Listeria*, and *E.coli* isolated from the feces of healthy, diarrheic, and hospitalized dogs and cats.

MATERIAL AND METHODS

Bacterial isolates

The study was carried out using 232 *E.coli*, 9 *Listeria*, and 1 *Salmonella* isolates. Isolates were recovered from a total of 353 fecal swabs obtained from the Small Animals Veterinary Teaching Hospital, The University of Tennessee and analyzed in the Food Safety and Processing Building laboratories. Of the

232 *E.coli* isolates, 45 were isolated from dogs and cats with diarrhea, 140 isolated from healthy dogs and cats, and 47 were isolated from hospitalized dogs and cats.

Antimicrobial susceptibility testing

The susceptibility of the 242 bacterial isolates (232 *E.coli*, nine *Listeria*, and one *Salmonella*) was determined by the disk diffusion method in accordance with the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (Lorian 2004). The bacterial isolates were tested for their susceptibility to 18 antibiotics of human and veterinary importance (Becton, Dickinson and Company, Sparks, MD) including: amikacin, ampicillin, amoxicillin with clavulanic acid, cefoxitin, ceftiofur, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, imipenem, kanamycin, nalidixic acid, streptomycin, sulfamethoxazone with trimethoprim, trimethoprim, and enrofloxacin (Table 8).

The inhibitory zones were measured and scored as sensitive, intermediate, and resistant according to the NCCLS guidelines (Saenz et al., 2001).

Statistical analysis

A chi-square analysis was used to find differences in resistance to different antimicrobial agent. The three responses (resistant, intermediate, and susceptibile) to different antimicrobial were calculated as categorical data. SAS proc FREQ (SAS 9.0 version) was used for data analysis (Kambal, 1996). The chi-square analysis used is shown below:

$$X^2 \text{ statistic} = \Sigma (f - F)^2 / F, \text{ taken over all cells}$$

Table 8. Antibiotics, codes, and concentrations used in the disk diffusion assay

Antimicrobial	Code	Concentration (µg)
Amikacin	AN	30 µg
Ampicillin	AM	10 µg
Amoxicillin with clavulanic acid	AMC	30 µg
Cefoxitin	FOX	30 µg
Ceftiofur	XNL	30 µg
Ceftriaxone	CRO	30 µg
Cephalothin	CF	30 µg
Chloramphenicol	C	30 µg
Ciprofloxacin	CIP	5 µg
Gentamicin	GM	10 µg
Imipenem	IPM	10 µg
Kanamycin	K	30 µg
Nalidixic acid	NA	30 µg
Streptomycin	S	10 µg
Sulfamethoxazone with Trimethoprim	SXT	23.75 µg 1.25 µg
Tetracycline	TE	30 µg
Trimethoprim	TMP	5 µg
Enrofloxacin	EN	5 µg

f is the observed frequency in the cell, and F is the expected frequency in the cell.

For the *E. coli* samples isolated from dogs and cats with diarrhea, the value of X^2 = 558.16 for degree of freedom (DF=34) at a significance level (P <. 0001) was used to reject the null hypothesis. For the *E. coli* samples isolated from healthy dogs and cats, the value of X^2 = 1235.9 for degree of freedom (DF=51) at a significance level (P < .0001) was used to reject the null hypothesis. For the *E. coli* samples isolated from hospitalized dogs and cats, the value of X^2 = 299.6 for degree of freedom (DF=34) at a significance level (P < .0001) was used to reject the null hypothesis.

For the *E. coli* samples isolated from dogs with diarrhea, the value of X^2 = 214.42 for degree of freedom (DF=34) at a significance level (P <. 0001) was used to reject the null hypothesis. For the *E. coli* samples isolated from healthy dogs, the value of X^2 = 735.6 for degree of freedom (DF=34) at a significance level (P < .0001) was used to reject the null hypothesis. For the *E. coli* samples isolated from hospitalized dogs, the value of X^2 = 281.5 for degree of freedom (DF=34) at a significance level (P < .0001) was used to reject the null hypothesis. For the overall *E. coli* samples isolated from dogs, the value of X^2 = 1069.2 for degree of freedom (DF=34) at a significance level (P < .0001) was used to reject the null hypothesis.

For the *E. coli* samples isolated from cats with diarrhea, the value of X^2 = 395.6 for degree of freedom (DF=34) at a significance level (P <. 0001) was used to reject the null hypothesis. For the *E. coli* samples isolated from healthy cats,

the value of X^2 = 508.7 for degree of freedom (DF=34) at a significance level (P < .0001) was used to reject the null hypothesis. For the *E. coli* samples isolated from hospitalized cats, the value of X^2 = 37.0 for degree of freedom (DF=34) at a significance level (P =0.33) was used which did not reject the null hypothesis of no differences. For the overall *E. coli* samples isolated from cats, the value of X^2 = 798.3 for degree of freedom (DF=34) at a significance level (P < .0001) was used to reject the null hypothesis.

For the *Listeria* samples isolated from dogs and cats, the value of X^2 = 48.776 for degree of freedom (DF=34) at a significance level (P <. 05) was used to reject the null hypothesis.

RESULTS

E.coli from dogs

Bacterial isolates of *E.coli* (n= 151) from fecal samples of dogs were evaluated during 2003-2004. The isolate sources included 23 from dogs with diarrhea, 85 from healthy dogs, and 43 from hospitalized dogs (non-diarrheic, but unhealthy). Table 9 summarizes the resistance of all *E.coli* isolates to 18 antimicrobial agents.

The only antimicrobial which none of the *E.coli* isolates were resistant to was imipenem. High levels of resistance were found (in declining order) for cephalothin 89.4%, ampicillin 58.9%, streptomycin 51.9%, enrofloxacin 41.7%, tetracycline 41.1%, nalidixic acid 41.1%, and ciprofloxacin 40.4%.

Table 9. Antibiotic resistance of *E.coli* spp. isolated from dogs during the period 2003-2004

Dogs with:	Number of isolates	Resistance (%) among different types of antimicrobial agents [a]																
		AN	AM	AmC	FOX	XNL	CRO	CF	C	CIP	GM	IPM	K	NA	S	SXT	TE	TMP	ENO
Diarrhea	23	0.0	43.5	17.4	8.7	8.7	8.7	78.3	4.4	8.7	17.4	0.0	13.0	13.0	30.4	17.4	26.1	13.0	8.7
Healthy	85	5.9	49.4	23.5	32.9	12.9	20.0	89.4	5.9	35.3	20.0	0.0	23.5	31.8	48.2	29.4	29.4	31.8	37.7
Hospitalized	43	18.6	86.1	65.1	69.8	60.5	58.1	95.4	27.9	67.4	58.1	0.0	69.8	74.4	67.4	69.8	72.1	65.1	67.4
Over all average	151	8.6	58.9	34.4	39.7	25.8	29.1	89.4	11.9	40.4	30.5	0.0	35.1	41.1	51.9	39.1	41.1	38.4	41.7

The resistance, intermediate, and susceptibility responses to different antimicrobial were calculated as categorical data. A chi-square analysis ($p<0.0001$) was used to find differences in resistance to different antimicrobial agent.

[a] abbreviations:
AN: Amikacin
AM: Ampicillin
AmC: Amoxicillin with clavulanic acid
FOX: Cefoxitin
XNL: Ceftiofur
CRO: Ceftriaxone
CF: Cephalothin
C: Chloramphenicol
CIP: Ciprofloxacin
GM: Gentamicin
IPM: Imipenem
K: Kanamycin
NA: Nalidixic acid
S: Streptomycin
SXT: Sulfamethoxazone with Trimethoprime
TE: Tetracycline
TMP: Trimethoprim
ENO: Enrofloxacin

Percent resistance of *E.coli* to other antibiotics included: cefoxitin (39.7%), sulfamethoxazone with trimethoprim (39.1%), trimethoprim (38.4%), kanamycin (35.1%), Amoxicillin with clavulanic acid (34.4%), and gentamycin (30.5%). Lower resistance to the following antibiotics was found: ceftriaxone (29.1%), ceftiofur (25.8%), chloramphenicol (11.9%), and amikacin (8.6%).

A total of 23 *E.coli* were isolated from dogs with diarrhea. None of the *E. coli* isolates were resistant to imipenem and amikacin. High levels of resistance were found (in declining order) for cephalothin 78.3%, ampicillin 43.5%, streptomycin 30.4%, and tetracycline 26.1%. Resistance of *E.coli* to amoxicillin with clavulanic acid, gentamycin, and sulfamethoxazone with trimethoprime was 17.4% each. Resistance to kanamycin, nalidixic acid, and trimethoprime was found in 13% of *E.coli* isolates from dogs with diarrhea. Resistance to cefoxitin, ceftiofur, ceftriaxone, ciprofloxacin, and enrofloxacin was found in 8.7% of *E.coli* isolates. *E.coli* was found resistant in 4.4% for chloramphenicol.

A total of 85 *E.coli* were isolated from healthy dogs. No isolates were resistant to imipenem. Higher levels of resistance were found (in declining order) for cephalothin (89.4%), ampicillin (49.4%), streptomycin (48.2%), enrofloxacin (37.7%), ciprofloxacin (35.3%), and cefoxitin (32.9%). Resistance of *E.coli* to nalidixic acid and trimethoprim was 31.8%. Fewer *E.coli* were resistant to tetracycline and sulfamethoxazone with trimethoprim (29.4%), kanamycin and amoxicillin with clavulanic acid (23.5%). Lower resistance to the following antibiotics was found: ceftriaxone and gentamycin (20%), ceftiofur (12.9%), chloramphenicol and amikacin (5.9%). (Table 9)

A total of 43 *E.coli* were isolated from hospitalized dogs. No isolates were resistant to imipenem. Antibiotic resistance of *E.coli* was found (in declining order) for cephalothin (95.4%), ampicillin (86.1%), nalidixic acid (74.4%), tetracycline (72.1%), cefoxitin, sulfamethoxazone with trimethoprim, and kanamycin (69.8%), ciprofloxacin and enrofloxacin (67.4%), streptomycin (67.4), amoxicillin with clavulanic acid, and trimethoprim (65.1%), and ceftiofur (60.5%). Fewer *E.coli* isolates were resistant to the following antibiotics: ceftriaxone and gentamycin (58.1%), chloramphenicol (27.9%), and amikacin (18.6%). (Table 9)

E.coli from cats

Bacterial isolates of *E.coli* (n=81) from fecal samples of cats were evaluated during 2003-2004. The sample types included 22 from cats with diarrhea, 55 from healthy cats, and 4 from hospitalized catss (non-diarrheic, but unhealthy). Table 10 summarizes the resistance of all *E.coli* isolates to 18 antimicrobial agents.

The only antimicrobial to which none of the *E.coli* isolates were resistant was imipenem. High levels of resistance were found (in declining order) for cephalothin 87.7%, ampicillin 40.7%, streptomycin 34.6%, nalidixic acid 29.6%, ciprofloxacin 25.9%, enrofloxacin 24.7%, tetracycline and cefoxitin 23.5%. Percent resistance of *E.coli* to other antibiotics included: trimethoprim (21%), Sulfamethoxazone with trimethoprim (19.8%), amoxicillin with clavulanic acid (17.3%). Lower resistance to the following antibiotics was found: ceftiofur, ceftriaxone and kanamycin (16.1%), gentamycin (13.6%), chloramphenicol (7.4%), and amikacin (1.2%).

Table 10. Antibiotic resistance in *E.coli* spp. isolated from cats during the period 2003-2004

Cats with:	Number of isolates	% Resistance within different type to antimicrobial agents [a]																
		AN	Am	AmC	FOX	XNL	CRO	CF	C	CIP	GM	IPM	K	NA	S	SXT	TE	TMP	ENO
Diarrhea	22	0.0	27.3	0.0	0.0	0.0	0.0	86.4	0.0	0.0	0.0	0.0	0.0	9.1	22.7	4.6	9.1	4.6	0.0
Healthy	55	1.8	41.8	21.8	30.9	18.2	20.0	89.1	7.3	34.6	18.2	0.0	20.0	36.4	40.0	23.6	27.3	25.5	32.7
Hospitalized	4	0.0	100.0	50.0	50.0	75.0	50.0	75.0	50.0	50.0	25.0	0.0	50.0	50.0	25.0	50.0	50.0	50.0	50.0
Overall average	81	1.2	40.7	17.3	23.5	16.1	16.1	87.7	7.4	25.9	13.6	0.0	16.1	29.6	34.6	19.8	23.5	21.0	24.7

The resistance, intermediate, and susceptibility responses to different antimicrobial were calculated as categorical data. A chi-square analysis ($p<0.0001$) was used to find differences in resistance to different antimicrobial agent.

[a] abbreviations:
AN: Amikacin
AM: Ampicillin
AmC: Amoxicillin with clavulanic acid
FOX: Cefoxitin
XNL: Ceftiofur
CRO: Ceftriaxone
CF: Cephalothin
C: Chloramphenicol
CIP: Ciprofloxacin
GM: Gentamicin
IPM: Imipenem
K: Kanamycin
NA: Nalidixic acid
S: Streptomycin
SXT: Sulfamethoxazone with Trimethoprime
TE: Tetracycline
TMP: Trimethoprim
ENO: Enrofloxacin

A total of 22 *E.coli* were isolated from cats with diarrhea. High levels of susceptibility were found. *E.coli* isolates were susceptible to imipenem, amikacin, Amoxicillin with clavulanic acid, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamycin, kanamycin, and enrofloxacin. High levels of resistance were found (in declining order) for cephalothin 86.4%, ampicillin 27.3%, and streptomycin 22.7%. Resistance of *E.coli* to tetracycline and nalidixic acid was 9.1%, trimethoprim and Sulfamethoxazone with trimethoprim was 4.6%.

A total of 55 *E.coli* were isolated from healthy cats. No isolates were resistant to imipenem. Higher levels of resistance were found (in declining order) for cephalothin (89.1%), ampicillin (41.8%), streptomycin (40%), nalidixic acid (36.4%), ciprofloxacin (34.6%), enrofloxacin (32.7%), and cefoxitin (30.9%). Fewer *E.coli* were resistant to tetracycline (27.3%), trimethoprim (25.5%), sulfamethoxazone with trimethoprime (23.6%), and amoxicillin with clavulanic acid (21.8%). Lower resistance to the following antibiotics was found: ceftriaxone and kanamycin (20%), gentamycin and ceftiofur (18.2%), chloramphenicol (7.3%), and amikacin (1.8%). (Table 10)

Only 4 *E.coli* were isolated from hospitalized cats. No isolates were resistant to imipenem and amikacin. Antibiotic resistance of *E.coli* was found (in declining order) for ampicillin (100%), cephalothin and ceftiofur (75%). Resistance of *E.coli* to amoxicillin with clavulanic acid, cefoxitin, ceftriaxone, chloramphenicol, ciprofloxacin, kanamycin, nalidixic acid, sulfamethoxazone with

trimethoprim, tetracycline, trimethoprim, and enrofloxacin was 50%. Resistance to gentamycin and streptomycin was 25%. (Table 10).

Approximately 79% of overall *E.coli* isolated from dogs and cats exhibited MDR (defined as resistance to two or more antibiotics) for two or more antimicrobials. High prevalence (over 53%) of MDR *E.coli* was observed among *E.coli* isolated from dogs and cats with diarrhea. Two isolates had no resistance to any of the antibiotics tested. *E.coli* isolated from healthy dogs and cats unexpectedly showed higher levels of resistance compared to the ones isolated from dogs and cats with diarrhea. Over 68.5% of these *E.coli* isolates were MDR. The highest prevalence of multiple drug resistance (89.4%) was found in *E.coli* isolated from hospitalized dogs and cats, with only one isolate that was susceptible to all antibiotics tested. (Table 11)

Salmonella

In our study, only one S*almonella* was isolated from a dog with diarrhea. The isolate was susceptible to amikacin, ampicillin, amoxicillin with clavulanic acid, cefoxitin, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, imipenem, kanamycin, nalidixic acid, sulfamethoxazone with trimethoprime, tetracycline, trimethoprim, and enrofloxacin. *Salmonella* showed intermediate resistance to ceftiofur, and streptomycin.

Listeria

A total of 9 *Listeria* were isolated from dogs. Higher levels of resistance were found (in declining order) for nalidixic acid (88.9%), cefoxitin (77.8%), cephalothin, ceftiofur, ampicillin, gentamycin, and tetracycline (66.7%).

Table 11. Number (%) of multidrug resistance (MDR)[a] *E.coli* isolates from dogs and cats fecal samples

Isolates origin	Numbers	No. (%) of susceptible isolates	No. (%) resistant to one antibiotic	No. (%) of MDR[a]
Diarrhea	45	2 (4.5)	19 (42.2)	24 (53.3)
Healthy	140	9 (6.4)	35 (25.0)	96 (68.6)
Hospitalized	47	1 (2.1)	4 (8.5)	42 (89.4)
Total	232	12 (5.2)	38 (15.1)	185 (79.7)

[a]MDR, defined as resistance to two or more antibiotics

Listeria isolates (55.6%) were resistant to amoxicillin with clavulanic, sulfamethoxazone with trimethoprim, chloramphenicol, kanamycin, and streptomycin. *Listeria* (44.4%) were resistant to amikacin, ceftriaxone, and trimethoprim and 33.3% were resistant to ciprofloxacin. Only 22.2 % of *Listeria* were resistant to enrofloxacin, and imipenem (Table 12). Eight of the *Listeria* isolates showed MDR of over 88%.

DISCUSSION

Cephalosporins are an important class of antimicrobial agents in use today for both humans and animals. Four generations of cephalosporins have evolved, all of which contain the beta-lactam sub-structure first found in penicillin (Lorian 2004). Third-generation cephalosporins (ceftiofur, and ceftriaxone), second generation (cefoxitin), and first generation cephalosporins (cephalothin) have been developed strictly for veterinary use and were evaluated in our study (Hornish and Kotarski 2002). Different use patterns of antimicrobial agents are expected to have some impact on the distribution of antimicrobial resistance (Lanz et al., 2003). Our data on the distribution of resistance phenotypes in the dogs and cats support this hypothesis. For instance, cephalosporins, especially first generation cephalosporins (cephalothin), were heavily used for the treatment of *E.coli* bacterial infections, particularly urinary tract infections in dogs and cats (Rogers et al., 1988; Thoresen et al., 2002) .

Table 12. Antibiotic resistance (%) in *Listeria* isolated from fecal samples from dogs

Antimicrobial agents	**% Resistant [a] (N=9)**
NA	88.9
FOX	77.8
XNL	66.7
CF	66.7
GM	66.7
AM	66.7
TE	66.7
AmC	55.6
C	55.6
K	55.6
S	55.6
SXT	55.6
TMP	44.4
AN	44.4
CRO	44.4
CIP	33.3
IPM	22.2
ENO	22.2

[a] abbreviations;
AN: Amikacin
AM: Ampicillin
AmC: Amoxicillin with clavulanic acid
FOX: Cefoxitin
XNL: Ceftiofur
CRO: Ceftriaxone
CF: Cephalothin
C: Chloramphenicol
SXT:Sulfamethoxazone with Trimethoprime
CIP: Ciprofloxacin
GM: Gentamicin
IPM: Imipenem
K: Kanamycin
NA: Nalidixic acid
S: Streptomycin
TE: Tetracycline
TMP: Trimethoprim
ENO:Enrofloxacin

This use is clearly reflected in the higher resistance rate for dogs (89.4%) and cats (87.7%) for cephalothin observed in total *E.coli* recovered from these animals, including high resistance level for *E.coli* isolated from hospitalized dogs (95.4%), healthy dogs (89.4%), healthy cats (89.1%), and cats with diarrhea (86.4%).

In vitro, third-generation cephalosporins (ceftiofur) showed potent activity and wide spectra against veterinary clinical isolates of *E. coli* resistant to ampicillin aminoglycosides (Deshpande et al., 2000). Resistance to ceftiofur observed in *E.coli* that were recovered from hospitalized cats (75%) and hospitalized dogs (60.5%) and lower resistance (8.7%) in *E.coli* recovered from dogs with diarrhea and high sensitivity (0% resistance) of isolates from cats with diarrhea that not hospitalized reflects the widespread use of ceftiofur in the clinical environment.

Quinolones, which include nalidixic acid, ciprofloxacin, and enrofloxacin are well-established broad–spectrum antibiotics with potent bactericidal activity against clinically important pathogens responsible for a variety of infections including urinary tract infections (UTIs), gastrointestinal infections, and respiratory tract infection (Appelbaum and Hunter 2000).

Our results revealed that the overall *E.coli* isolated from dogs (40.4%) and cats (25.9%) had a high resistant rate to ciprofloxacin, especially those *E.coli* isolated from hospitalized dogs (67.4%) and hospitalized cats (50%). Resistance of *E.coli* to ciprofloxacin was also high in healthy dogs (35.3%) and healthy cats (34.6%). These results do not support the recommendation of a recent study,

which recommends ciprofloxacin as a first choice for treatment of uncomplicated UTIs due to *E.coli* (Talan et al., 2004), since, the resistance to trimethoprime/sulphamethoxazole (the first line therapy for UTIs) was lower than ciprofloxacin. The percent resistance for trimethoprim/sulphamethoxazole was 39% for the overall *E.coli* isolates from dogs and only 19.8% for cats (69.8% for *E.coli* isolated from hospitalized dogs and 50% from hospitalized cats, 29.4% isolated from healthy dogs and 23.6% from healthy cats, and 17.4% isolated from dogs with diarrhea and 4.6% from cats with diarrhea).

High levels of resistance to enrofloxacin were found in overall *E.coli* isolated from fecal samples of dogs (41.7%). Our results support data of a related study on several strains of enrofloxacin-resistant *E.coli* isolated from urine from dogs with UTIs (Cooke et al., 2002). High levels of resistance to enrofloxacin were found in overall *E.coli* isolated from fecal samples of cats (24.7%). On the other hand, cats with diarrhea showed high sensitivity to enrofloxacin, which agrees with the previous studies (Spreng et al., 1995).

The similarities in resistance patterns to enrofloxacin and ciprofloxacin and the multiple antibiotic resistance patterns accompanying ciprofloxacin and enrofloxacin resistance of *E.coli* is of great concern, since these are two of the most powerful antibiotics currently available for treatment of *E.coli* infections.

Nalidixic acid was the first quinolone heavily used for many infections especially UTIs in human beings (Appelbaum and Hunter 2000). However, it is not commonely used in veterinary medicine. This may explain the higher resistance of *E.coli* to this antibiotic in comparison to the other fluoroquinolones

(ciprofloxacin and enrofloxacin). Resistance to nalidixic acid by overall *E.coli* isolated from fecal samples of dogs and cats was 41.1%, 29.6%, respectively, with the highest percentage by *E.coli* isolated from hospitalized dogs (74.4%) and hospitalized cats (50%).

Fluoroquinolones have the potential for providing the small animal veterinary practitioner a potent antibacterial tool. However, without thoughtful use, selection of resistant organisms dramatically reduces the clinical effectiveness of this class of antimicrobial agents with a concern for the future use.

Beta-lactam antibiotics include penicillin derivative and ampicillin-like drugs including: ampicillin, amoxicillin with calvulanic acid (AMC). Clavulanic acid is an inhibitor of beta-lactamase (penicillinase) and when used with amoxicillin the resulting combination becomes active against most bacteria resistant to amoxicillin through production of beta-lactamase. In our study, resistance to amoxicillin with clavulanic acid was 34.4% overall for *E.coli* isolated from dogs which agrees with a study by Bywater et al., (1985). Amoxicillin with clavulanic acid was used in the treatment of bacterial cystitis in cats; this may explain the high resistance rate of overall *E.coli* isolated from cats (17.3%) and hospitalized cats (50%) (Senior et al., 1985).

The resistance level for ampicillin (58.9% overall for dogs and 40.7% for cats) was higher than amoxicillin with clavulanic acid, in *E.coli*, with a very high resistance in *E.coli* isolated from hospitalized dogs (86.1%) and hospitalized cats

(100%, 4 of 4). Ampicillin is a well-known, heavily used antibiotic, which may reflect the high level of resistance by *E.coli*.

Aminoglycoside (amikacin, gentamycin, kanamycin, and streptomycin) are bactericidal agents and exhibit a rapid lethal effect on susceptible aerobic Gram-negative bacilli (Lorian 2004). Amikacin exhibited high sensitivity with no resistance for *E.coli* isolated from dogs and cats with diarrhea, and relatively lower resistance for other *E.coli* isolated from the healthy (5.9% for dogs, 1.8% for cats) and the hospitalized animals (18.6% for dogs, 0% for cats). Resistance to aminoglycosides has increased in dogs compared to a study in 1988, which demonstrate high efficacy in *E.coli*–related complications (Rogers et al., 1988).

The resistance of *E.coli* to streptomycin was 51.9%, 30.4%, 48.2%, and 67.4% respectively for overall *E.coli*, diarrheic, healthy, and hospitalized dogs. In cats the resistance rate was 34.6%, 22.7%, 40%, and 25% respectively for overall *E.coli*, diarrheic, healthy, and hospitalized cats. Streptomycin is considered among the least effective antibiotics in the small animals clinic (Ndung'u and Buoro 1994).

A high level of resistance to gentamycin (30.5%) and kanamycin (35.1%) was observed for overall *E.coli* isolated from dogs, with high resistance in the *E.coli* isolated from hospitalized dogs (gentamycin 58.1%, and kanamycin 69.8%). Lower level of resistance was observed for overall *E.coli* isolated from cats (gentamycin 13.6%, and kanamycin 16.1%) with high sensitivity (0% resistance) to gentamycin and kanamycin in *E.coli* isolated from cats with diarrhea.

It had been reported that in severe infections; the use of aminoglycosides is commonly administered in the small animal clinics (Ndung'u and Buoro 1994). This widespread use of this drug, may have led to development of resistance among the bacteria.

Imipenem is an extremely active antibiotic with a broad-spectrum of activity against almost all gram-positive and gram-negative organisms, both aerobic and anaerobic. Our results emphasize the importance of the drug, as it was the only antimicrobial to which none of the *E. coli* isolates were resistant.

Based on these results imipenem may be the most effective drug, for use against *E.coli*-related complications in dogs and cats. However, the drug should not be widely used, as most strains of methicillin-resistant staphylococci are resistant to imipenem. Loss of sensitivity to imipenem due to heavy use would be extremely unfortunate.

Salmonellae Arizona resistance data were not particularly informative, since there was only one isolate of salmonellae. This particular isolate was sensitive to the majority of antibiotics evaluated.

Listeria spp, including *L.monocytogenes* were resistant at some level to all of the antibiotics used. The isolates showed higher levels of resistance to cephalosporins and quinolones especially nalidixic acid (88.9%), which agrees with recent studies (Poros-Gluchowska and Markiewicz Z. 2003). The lowest resistance recorded was to imipenem and enrofloxacin (22.2%). The combination of ampicillin and gentamycin has been used as a therapy of choice for the treatment of human listeriosis (Lorber 1997). This use may be reflected in the

higher resistance rate (66.7%) for both of them. Second-choice therapy involves the combination of trimethoprim with a sulfonamide, which cannot be recommended based on our results.

In general, the highest resistance of *E.coli* isolated from dogs and cats was to cephalothin (first generation cephalosporins) followed by ampicillin. Such high resistance could be explained by the heavy use of these antibiotics in small animals clinics. Imipenem was the only antimicrobial to which none of the *E. coli* isolates were resistant.

Cats were less resistant to antibiotics because of cats may be exposed less to potentially pathogenic bacteria in comparison with dogs. Also it has been reported that there are a lower bacterial counts in felines compared to canines (Wooley and Blue, 1976). Lower bacterial counts mean fewer disease frequencies and less antibiotics uses.

The highest resistance rate was associated with *E.coli* isolated from hospitalized animals followed by healthy animals, then dogs and cats with diarrhea. The resistance pattern of *E.coli* isolated from healthy dogs and cats (except imipenem) tested in this study were a major concern. How do these healthy animals develop enteric microflora with such high antibiotic resistance? The use of antibacterial drugs in feeds may be one source of drug-resistant *E.coli* in healthy animals (Nazer 1978). However, based on our data showing that hospitalized animals had higher overall resistance to antibiotics, clinical use of antibiotics also likely contributes to resistance.

The heavy uses of antibiotics is expected to increase resistance rate to the antibiotics, so it would be better for clinical uses to switch between the potent antibiotics and not focus on one. This could extend the potency of antibiotics and reduce pressure on bacterial populations to develop resistance over time.

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APPENDIX

DOG OWNER QUESTIONNAIRE

Good (morning), my name is ------- and I am a graduate student at the university of TN.

I understand you have a dogs/cats with diarrhea and have agreed to participate in our study. We are trying to determine some of the causes of diarrhea in dogs/cats and we need your help with some information for our study.

Is this a convenient time for me to ask you some questions, it will take about 10 min.

1. I know you have a dog; do you have any other animals in and around the home, including other dogs?

Yes___ No____

_____

2. Now, I would like to ask you more information about the dogs and cats you own?

Name	Type		Age	Sex		Neutered Spay		Diarrhea In the last 60 d	Raw meat		Raw eggs		Raw milk	
	D	C		M	F	Y	N		Y	N	Y	N	Y	N
Case:								Yes						

3. What brand of food do you feed *dog name*?

_____

Any other brands? _____

4. Have you ever give *name* commercial pet treats in the last 60 d period (such as milk bone, etc.)?

Yes_____ No_____

If yes, what is the name?_____

Now, I would like to ask more about *dog name*?

5. During the average 24 h period, how many hours is *name* in the house?

_____

6. Does *name* sleep inside at night?

□ □

Yes No (outside)

□

What room does *name* sleep in?

□ Kid room

□ Bedroom

□ other

Where does *name* sleep?

□ Dog's house

□ Garage

□ under the house

Bed room Yes

□

Does he \ she sleep on bed at night?

□ □

Yes No

□ Stop

Whose bed?

□ Children □ Parents

7. Does *name* ever catch

Birds		Rodent Mice-rat		Reptiles Lizard-snakes		Amphibians Frogs		Insects		Rabbits	
Y	N	Y	N	Y	N	Y	N	Y	N	Y	N

8. *In case of presence of cat,* About the cat, do you have litter box?

□

Yes No

□ Stop

A) Who takes care of the litter box?

_____

B) Does *name* ever refuse to use the litter box?

□ □

Yes No

□ Stop

C) Does the cat have an accident (soil) in the house?

□ □

Yes No

□

D) Where does she have it?

_____

Who cleans it up?

_____

*Does your dog ever play in the litter box?

_____Yes _____No

9. Did you give ***name*** any medicine for diarrhea?

□ □

Yes No

□

Non-Prescription_____

Prescription (name)_____

Other _____

10. Was any medication given for other conditions before ***name*** developed diarrhea, such as antibiotics, etc.?

□ □

Yes No

□

What? Give a specific name or class of drug

_____

_____

Now, I would like to ask more about the people in your household.

11. How many people in your household? _____

First name	Age*	Sex	Did anyone in your household have a diarrhea in the last 60 ?		
			No	Yes, did he/she see the physician?	Cause

12. What is your main source of drinking water?

□ Municipal city water

□ Well

□ Bottled water

□ other

13. In addition to the main drinking water sources, does your animals ever drink from these sources of water?

	Yes	No	Don't know
Lake/Pond	_____	_____	_____

River/ stream _____ _____ _____

Rain water _____ _____ _____

Toilet _____ _____ _____

*<u>*Only asked if the children 3 years or less:*</u>

14.I would like to ask you a few more questions about your children;

Are any children in diaper at least part of the time?

□ □

Yes No

□ (Stop)

What is the type of diaper?

□ Cloth

□ Disposable

15. Have you ever found your child within the last 6-month doing any of the following?

	Yes	No
Playing in a litter box	_____	_____
In the garbage	_____	_____
In the diaper pail	_____	_____
Playing in the toilet	_____	_____

<u>For all children</u>:

	Dog		Cat	
	Y	N	Y	N
Kissing the dog/cat				
Touching the dog/cat mouth				
Touching the animals's bottom				

-This completes the study, do you have any questions?

-Thank you, I really appreciate your participation in this important study. We hope this study will help assure that you and your pets stay healthy.

-Do you have any questions about the study or the results?

CAT OWNER QUESTIONNAIRE

Good (morning), my name is -------- and I am a graduate student at the university of TN.

I understand you have a dog/cat with diarrhea and have agreed to participate in our study. We are trying to determine some of the causes of diarrhea in dogs/cats and we need your help with some information for our study.

Is this the convenient time for me to ask you some questions, it will take about 10 minutes.

1. I know you have a cat; do you have any other animals in and around the home, including other cats?

Yes_____ No_____

_____

Now, I would like to ask you more information about the dog and cats you own?

Name	Type		Age	Sex		Neutered Spay		Diarrhea In the last 60 d	Raw meat		Raw eggs		Raw milk	
	D	C		M	F	Y	N		Y	N	Y	N	Y	N
Case:								Yes						

3. What brand of food do you feed *name*?

_____

Any other brands? _____

4. Have you ever give *name* commercial pet treats in the last 60 d period (such as Pounce, etc.)?

Yes_____ No_____

If yes, what is the name?_____

Now, I would like to ask more about *cat name*:

5. During the average 24 h period, how many hours is *name* in the house?

_____

6. Does *name* sleep inside at night?

□ ↓

Yes No

□

Where does *name* sleep?

□ Kid room

□ Bedroom

□ other

Bed room Yes

□

Does he \ she sleep on bed at night?

□

Yes No

□ Stop

Whose bed?

□ Children □ Parents

7. Does ***name*** ever catch:

Birds		Rodent Mice-rat		Reptile Lizard-snakes		Amphibians Frog		Insects		Rabbits	
Y	N	Y	N	Y	N	Y	N	Y	N	Y	N

8. Do you have litter box?

□ □

Yes No

□ Stop

A) Who takes care of the litter box?

_____

_____

B) Does ***name*** ever refuse to use the litter box?

□ □

Yes No

□ Stop

C) Does the cat have an accident (soil) in the house?

□ □

Yes No

□ Stop

D) Where does she have it?

_____

E) Who cleans it up?

_____

_____

F) *Incase of presence of dog,* Does your dog ever play in the litter box?

_____Yes _____No

9. Did you give ***name*** any medicine for diarrhea?

□ □

Yes No

□

Non-Prescription_____

Prescription (name)_____

Other _____

10. Was any medication given for other conditions before ***name*** developed diarrhea, such as antibiotics, etc.?

□ □

Yes No

□

What? Give a specific name or class of drug

_____

_____

11.Now, I would like to ask more about the people in your household.

How many people in your household? _____

First name	Age*	Sex	Did anyone in your household have a diarrhea in the last 60 d?		Cause
			No	Yes, did he/she see the physician?	

12. What is your main source of drinking water?

□ Municipal city water

□ Well

□ Bottled water

□ other

13. In addition to the main drinking water sources, does your animals ever drink from these sources of water?

	Yes	No	Don't know
Lake/Pond	_____	_____	_____

River/ stream _____ _____ _____

Rain water _____ _____ _____

Toilet _____ _____ _____

Asked only if the children 3 years or less:

14. I would like to ask you a few more questions about your children;

Are any children in diaper at least part of the time?

□ □

Yes No

□ (Stop)

What is the type of diaper?

□ Cloth

□ Disposable

Have you ever found your child within the last 6-month doing any of the following?

	Yes	No
Playing in a litter box	_____	_____
In the garbage	_____	_____
In the diaper pail	_____	_____
Playing in the toilet	_____	_____

For all children:

	Cat		Dog	
	Y	N	N	Y
Kissing the dog/cat				
Touching the dog/cat mouth				
Touching the animals's bottom				

-This completes the questionnaire. Do you have any question?

- Thank you, I really appreciate your participation in this important study.

We hope this study will help assure that you and your pets stay healthy.

Do you have any questions about the study or the results?

VITA

Omaima Maamoun Ahmed was born in Ismailia, Egypt on September 3rd, 1975. She started and finished her alimentary, meddle, and high school educations at Ismailia. She then enrolled at Suez Canal University, Ismailia, Egypt. She received a Bachelor of Science degree in Veterinary Medicine 1997. She worked for about 2 years as a veterinarian in poultry field as she was responsible for birds' vaccinations. She was accepted into the Food Science and Technology program under the direction of Dr. F. Ann Draughon at the University of Tennessee, USA and began working toward the Master Degree in 2002. Her education during this period was focused on food microbiology, epidemiology, and antimicrobial susceptibility. Upon completion of her graduate research in August 2004, she was awarded her Master of Science Degree.