Antimicrobial Resistance and Enteric Pathogens in Companion Animals

Thesis submitted in accordance with requirements of the University of Liverpool for the degree of Doctor of Philosophy

By

Jennifer Siân Wilson

This thesis is based on research carried out in the department of Medical Microbiology and Department of Veterinary Pathology at the University of Liverpool. Except for the help and assistance acknowledged, this thesis is my unaided work.

Jennifer Sian Wilson

Abstract

The aims of this study were to investigate the prevalence of antibiotic resistant and potentially enteropathogenic bacteria in dog faecal samples and the potential for transmission of these bacteria to humans. The general prevalence of *E. coli*, *Salmonella* spp., *Campylobacter* spp., VRE, MRSA, and their antimicrobial susceptibilities were primarily investigated by conducting a cross-sectional survey, obtaining dog faecal samples from parks, boarding and rescue kennels and households. This revealed a high prevalence of healthy dogs excreting antibiotic resistant *E. coli* and *E. coli* carrying virulence determinants. There was generally a higher prevalence of antibiotic resistant *E. coli* isolated from boarding kennels and rescue home dogs. A significantly higher prevalence of antibiotic resistant *E. coli* were isolated from dogs on antibiotic therapy for kennel cough, suggesting antibiotic therapy select for antibiotic resistant *E. coli* in the gut flora. The highest prevalence of *E. coli* carrying virulence determinants were isolated from parks, although a high prevalence were also isolated from rescue and boarding kennels. *Salmonella* Typhimurium were isolated only from an outbreak in a dog rescue home. A low prevalence of VRE and *C. perfringens* were isolated from dogs and MRSA was not isolated from any faecal samples.

Overall *Campylobacter* spp. were isolated from 9% dogs in the cross sectional study. There was no significant association between *Campylobacter* spp. isolation and the presence of diarrhoea in dogs referred to the Small Animal Hospital for GI disease, suggesting that this bacterium is not a cause of diarrhoea in dogs. The most frequently isolated species of *Campylobacter* from dog faecal samples was *C. upsaliensis*. Although it is not isolated very frequently from human infection, dogs may be a source for a significant number of human cases. The prevalence of *C. upsaliensis* may be under estimated in both human and dog infections due to the nature of media used which is inhibitory to this species of *Campylobacter*.

A longitudinal study was carried out to investigate the transmission of *Campylobacter* spp., commensal *E. coli* and *E. coli* carrying virulence determinants between dogs and their owners. Primarily a questionnaire was designed and distributed to obtain information on how healthy people would prefer to collect faecal samples if given a choice. The preferred method was putting used toilet paper into a sterile diluent and a preliminary trial showed that this method was viable. Volunteers were recruited to participate in a six-month study collecting feacal samples from both dogs and owners. This study revealed that dogs within the same household are able to carry the same strain of commensal *E. coli* as their human owners, suggesting transmission between dogs and owners or vice versa. The results from this study were very interesting and further work should be carried out to assess fully the transmission of pathogenic bacteria to humans from dogs.

There have been few previous studies investigating *E. coli* carrying known virulence determinants, and also antibiotic resistant *E. coli* and the resistance genes responsible from healthy dogs and to our knowledge this is the first study in the UK. The results from the longitudinal study were extremely interesting and suggest that dogs may pose as a zoonotic risk for humans.

Acknowledgements

Firstly, I would like to acknowledge and thank my supervisors, Prof Tony Hart and Prof Malcolm Bennett for giving me the opportunity and encouragement to work towards my Ph.D. and whose help and wisdom has been valued over the last 4 years.

Secondly I would like to thank Nicola Williams and Sarah Hazel for introducing me into the fascinating world of research. Nicola has been fantastic, both as a friend and as a work colleague. I can only say I hope to be like her some day as she knows everything about everything, although how she remembers I will never know! I would also like to thank Thelma Roscoe without whom, making media would have taken twice as long and also Chris McCracken who was always there to sort out my problems.

I would like to acknowledge the great friends that I have met at Leahurst over the last few years and the great times we have had and I will never forget. In particular, Helen, for making me go to the gym when I really didn't want to. Trev, for providing me with hours of endless amusement (and yes it was me who kept putting the windscreen wipers up on your car). Jo and Richy for being great friends and allowing me to stay with them, for which I will be eternally grateful! Jez, for sorting out my endless computer problems and scanning numerous pictures. I would also like to thank Simon and Helen for their help with my endless questions about statistics before it all actually sunk in!

Finally, I would like to acknowledge my parents, without whom I would not have had the help or (financial!?) support to get through the last 3 years.

This PhD is dedicated in loving memory of my Grandad.

Contents

List of Tables

List of figures

Chapter 1

1.1 Introduction

Gastroenteritis in humans can be caused by a variety of bacteria including *Campylobacter* spp., *Salmonella* spp., pathogenic strains of *Escherichia coli, Clostridium perfringens* and *Staphylococcus aureus.* In developed countries, *Campylobacter* spp. infection is the most common cause of gastroenteritis in humans, followed by *Salmonella* spp. (www.hpa.org.uk). Infection with these pathogens is typically regarded as food-borne, although other risk factors have also been identified such as contaminated drinking water, foreign travel and transmission from pets (Mermin *et al.,* 1997), especially puppies (Williams *et al,* 1987). Companion animals as a source of human infection are becoming an important issue as humans, especially children, have frequent close contact with their pets. Infection with these pathogens is often self-limiting and does not usually warrant antimicrobial therapy. However, when more serious infections do occur and antimicrobial therapy is required, treatment options are becoming limited owing to the development of resistance to antibiotics. The number of pathogens becoming resistant to antimicrobials has increased over the last few decades and is a serious concern for public health.

The aim of the work in this thesis was to investigate the prevalence of potentially zoonotic enteric bacteria in dog faeces to determine whether or not dogs pose a significant risk for humans. There have been many studies investigating the prevalence and epidemiology of antimicrobial resistance genes present in bacteria isolated from humans, but very few studies have been carried out investigating resistance genes from veterinary bacterial isolates (Normand *et al.,* 2002). The distribution of antibiotic resistant bacteria is not well documented from veterinary isolates in the UK (Normand *et al.,* 2002, Lanz *et al.,* 2003). Therefore, in this study, a cross sectional study was undertaken to investigate the prevalence and dissemination of pathogenic and antibiotic resistant bacteria from faecal samples obtained from household dogs, parks, dogs resident in rescue and boarding kennels, farm dogs and cats. Genetic determinants of pathogenicity and antibiotic resistance were determined using the polymerase chain reaction (PCR). A longitudinal study

was also undertaken, investigating potential transmission of pathogenic bacteria from dogs to owners.

1.2 Zoonotic enteric pathogens

The zoonotic potential of pathogenic enteric organisms is a serious concern for Public Health, especially when bacteria are resistant to antibiotics. However, this is not always the case, for example human infection with *E. coli* O157 is not treated with antibiotics, as this would make symptoms worse. Most surveillance and research effort has focused on food animal products as a source of infections for humans. However, companion animals may also be important as they are present in the home and most humans have much closer contact with them than with other animals. Companion animals such as cats and dogs have been implicated in the direct or indirect transmission of at least 30 infectious agents to humans, including *Salmonella* spp. and *Campylobacter* spp. (Tan, 1997, Kozak *et al.*, 2003, Damborg *et al.*, 2004), and many of these pathogenic organisms are carried asymptomatically by the dog (Skirrow, 1977, Blaser *et al.*, 1979).

1.3 Antibiotics

The earliest known chemotherapeutic agents were of plant origin. The Ancient Greeks used the extract of male fern to treat worm infections and the South American Indians used extracts of cinchona bark to treat malaria. Mercury was also used for the treatment of syphilis until the beginning of the twentieth century, (Williams *et al.*, 1996). The discovery of antibiotics, in particular penicillin in 1928 by Alexander Fleming, was a huge advance in the treatment of bacterial infections. Antibiotics are secondary metabolites, naturally produced by bacteria and fungi as a mechanism of killing off competition for food. Antibiotics may also be produced by bacteria when subjected to stressful conditions (Brock *et al.*, 1997). Since the discovery of penicillin many more naturally occurring and synthetic antimicrobials have become available.

Resistance can emerge in bacteria by gene mutation or by the acquisition of resistance genes from other bacteria. The presence of antimicrobial resistance genes on mobile genetic elements such as conjugative plasmids and transposons facilitates the spread of resistance genes between many different bacteria. These genetic elements may also confer resistance to several antibiotics. Thus selection for resistance against one antibiotic can lead to the rapid development of multi-resistance through horizontal transfer within and between populations of bacteria. It is generally acknowledged that overuse of antimicrobial agents is a major factor contributing to the emergence of resistant bacterial strains (Sanders and Sanders, 1992), although resistance has been found in coliforms isolated from glacial ice, estimated at 2000 years old (Dancer *et al.*, 1997). Disinfectants, heavy metals and other non-antimicrobial substances such as products in soap and toothpastes are also thought to have a small part to play in the development of antimicrobial resistance (Aarestrup and Hasman, 2004).

Antimicrobials are widely used in both human and veterinary medicine and approximately 50% produced are used for veterinary and agricultural use (Teuber, 2001). However, the contribution of veterinary medicine and agriculture to antimicrobial resistance is still debated (Teuber, 2001). Antimicrobials have been widely used in food animals to treat or prevent disease (prophylactic use) and also for growth promotion. In the early 1970's Britain banned the use of many growth promoters over concerns of increasing antibiotic resistance in bacteria. The EU then followed suit and, in 1997, banned the use of growth promoter avoparcin. In 1999 they also banned the use of four others (tylosin, spiramycin, bacitracin and virginiamycin). Imported food animals or products, contaminated with antibiotic resistant bacteria may be a source or the overuse of antibiotics in human medicine could be selecting for resistant bacteria (Phillips *et al.*, 2004).

The correlation between consumption of antimicrobials and the emergence of resistance in bacteria is complex and it has proved difficult to establish with absolute certainty. Besides antimicrobial use, there are other contributory factors involved in selecting antibiotic resistance in bacteria, such as cross-species transfer of antimicrobial resistance genes, both in hospitals and in the community (Cristino, 1999). In human medicine, the extent of antimicrobial resistance varies between the hospital and community environment, the greatest proportion of antimicrobial use

being in community practice where approximately 95% of antimicrobials are prescribed. This compares with 5% prescribed in hospitals (BSAC, 2002) and it is estimated that of the antimicrobials prescribed in the community, 60-70% are prescribed unnecessarily (BSAC, 2002). Infections with multi-drug resistant bacteria such as methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant enterococci (VRE) are predominantly associated with hospital-acquired infections but these, and other resistant organisms are now appearing in the community (Fey *et al.*, 2003). A study in the USA revealed striking differences in the geographical patterns of antimicrobial resistance that did not necessarily correlate with heavy use of antimicrobials in particular areas suggesting that there are other important factors for human acquisition of antibiotic resistant bacteria (Gaynes, 1997).

There are many potential sources for humans to be infected with antibiotic resistant bacteria. As discussed above, current attention is on food producing animals as a major source and studies from many different countries have reported a high prevalence of antibiotic resistant bacteria on raw meat products from supermarkets (Del Grosse *et al.*, 2000). Although there is no direct evidence for the transmission of antimicrobial resistant bacteria in this way, there are strong epidemiological links and other additional circumstantial evidence that suggest this (van den Bogaard and Stobberingh, 2000). Antibiotic resistant bacteria have also been found in wildlife (Gilliver *et al.*, 1999) and environmental samples, for example soil (N. Williams pers comms), and these are also potential sources of human infection.

Companion animals have also been implicated in the transmission of antibiotic resistant bacteria to humans (Guardabassi *et al.*, 2004). Nearly 50% of the 24.5 million households in Britain own a pet, the most popular being cats and dogs. Figures from the Pet Food Manufacters Association (2001) show that an estimated 4.8 million households own a dog and 21.5% of these have more than one dog. Dogs and cats have been also been suggested to be potential reservoirs of highly resistant organisms such as MRSA and VRE (Manian, 2003, Guardabassi *et al.*, 2004). Horses also carry antibiotic resistant bacteria and may be a potential source for humans (Seguin *et al.*, 1999, M. Omar pers comms).

1.4 Antibiotic groups

There are many different classes of antibiotics that can be either bactericidal or bacteriostatic. The β-lactam antibiotics are the most common group of antibiotics used in both human and veterinary medicine. This family of antibiotics includes the penicillins, methicillin, cephalosporins, monobactams, cephamycins and carbapenems. They disrupt cell wall synthesis by acting as substrate analogues for the penicillin binding proteins (PBP). PBP's are the extra-cellular or periplasmic enzymes found in bacteria that are responsible for the final stages of peptidoglycan synthesis. The β-lactam ring of the antibiotic binds strongly to the transpeptidases (PBP) so they can no longer catalyze the final cross-linking reactions of peptidoglycan synthesis.

Quinolones are synthetic, broad-spectrum antibiotics and were first described as a new class of drug in 1962. The targets of quinolone activity are the bacterial DNA gyrase and topoisomerase IV, enzymes essential for DNA replication and transcription. They bind to DNA gyrase via the carboxy group at C3 in the 4-quinolone ring. The first generation of quinolones include nalidixic acid, oxolinic acid and cinoxacin. Second generation quinolones contain fluorine atoms to create the fluoroquinolones (ciprofloxacin, lomefloxacin, norfloxacin), which have increased activity against gram-negative bacteria. Third generation quinolones such as moxifloxacin are currently becoming available for human use.

Tetracycline is a broad spectrum, inexpensive, low toxicity antibiotic that has been widely used in both human and veterinary medicine. The tetracyclines were first discovered in the 1940's and only a few analogues are used. First generation drugs include chlortetracycline. Second-generation drugs such as deoxytetracyline and third-generation drugs (gylcylcyclines) such as minocycline are both semi-synthetic compounds (Schnappinger and Hillen, 1996). Tetracyclines are bacteriostatic and penetrate susceptible organisms by active transport through the cell wall, inhibiting protein synthesis at the ribosome. *In vitro*, tetracycline inhibits both bacterial and eukaryotic ribosomes but *in vivo*, the active uptake mechanisms present in bacteria make them much more susceptible to the antibiotic. Studies have shown that there are low and high affinity binding sites on the ribosome to which tetracycline binds. The

high affinity site is located on the 30S subunit while low affinity sites are located on both subunits (Tritton, 1977). Tetracycline competes with tRNA for the A site, binding to the ribosomes and in doing so impairing protein synthesis.

Trimethoprim is a synthetic, broad-spectrum antibiotic, first used in the UK in 1962 (Huovinnen, 1987). Trimethoprim is largely excreted unmetabolised in the urine and is useful for treating urinary tract infections. Trimethoprim can be regarded as an antifolate, a structural analogue of folic acid competitively inhibiting the reduction of dihydrofolate to tetrahyrofolate by dihyrofolate reductase (DHFR) in all living cells (Burchall and Hitchings, 1965). Cells, including mammalian cells, depend on this enzymic reaction for the synthesis of DNA thymine. Trimethoprim selectively acts on prokaryotic cells, because the affinity of mammalian DHFR for trimethoprim is so low, that the concentrations of drug needed to inhibit micro-organisms have little effect on the eukaryote host. X-ray crystallographic studies have shown that this difference in affinity is due to trimethoprim not fitting into the nucleotide binding site of mammalian dihydrofolate reductase, but doing so easily with *E. coli* dihyrofolate reductase (Matthews *et al.*, 1987).

Chloramphenicol is a broad-spectrum, bacteriostatic antibiotic, first discovered in 1947 following the screening of *Streptomyces venezuelae* (in soil and compost) for antimicrobial activity. This drug is active against both Gram-negative and Gram-positive bacteria, both aerobic and anaerobic. Despite this, the use of chloramphenicol has declined due to adverse reactions in humans (Wareham and Wilson, 2002), although claims have been made that adverse affects have been exaggerated (Wareham and Wilson, 2001). Its usage in veterinary medicine has also declined due to concerns over toxicity (Bischoff *et al.*, 2002). Chloramphenicol is still useful for human patients who are allergic to β-lactam antibiotics and for eye infections. Chloramphenicol binds to the 50S subunit of the prokaryotic ribosome, preventing the normal binding of tRNA complexes, and thus, inhibiting protein synthesis.

Marolides include the antibiotics erythromycin, azithromycin, roxithromycin and clarithyromycin. Tylosin is available for veterinary use. They are bacteriostatic and inhibit bacterial synthesis by binding to the 23S rRNA of the 50S ribosome, preventing protein synthesis. They have fairly narrow spectrum of activity and gram-

negative bacilli are often resistant. There are a few exceptions, examples being *Campylobacter* spp. and *Helicobacter* spp. (Williams *et al.*, 1996).

Aminoglycosides and aminocyclitols are broad spectrum, highly potent antibiotics produced by actinomycetes. The first aminoglycoside to be produced was streptomycin in 1944, others include kanamycin and gentamicin. Examples of aminocyclitols are spectinomycin and apramycin, the latter being licensed for veterinary use only. Aminocyclitols are closely related to aminoglycosides and have a similar mode of action. Structurally, streptomycin is not strictly an aminoglycoside, but it is often included in this group because of the drug's activity. In the 1970's the semi-synthetic aminogylcosides were produced, these included dibekacin, amikacin and netilmicin, and these have activity against organisms that had acquired resistance to earlier aminoglycosides. This group of antibiotics impair protein synthesis by binding to the 30S subunit of the prokaryotic ribosome, and freezing the initiation complex on the mRNA strand. Low levels of aminoglycosides slow protein synthesis (due to the prevention of the ribosome transversing the mRNA strand) and mismatch codons at the A site, due to the distortion of the ribosome. High levels of aminoglycoside bind very strongly to the ribosome and prevent the initiation complex transversing the mRNA (Williams *et al.*, 1996).

Glycopeptide antibiotics include vancomycin, ristocetin and teicoplanin. Gram-negative bacteria, with the exception of some isolates of *N. gonorrhoeae,* are not susceptible to this group of antibiotics as the drugs' molecules are large and unable to penetrate the Gram-negative outer membrane. Glycopeptides are active against *Staphylococci, Streptococci* and other Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA). They act by blocking peptidoglycan synthesis. The toxicity of vancomycin and ristocetin precludes their usefulness and they are not used except in severe infections that fail to respond to other antibiotics. Teicoplannin is a newer drug and supposedly has lower toxicity (Williams *et al.*, 1996).

1.5 Specific enteric pathogens and antibiotic resistance mechanisms

Escherichia coli

Escherichia coli is the most intensively studied and best understood of all bacteria. It was first described in 1885 by Theodor Escherich who noted its high prevalence in the intestinal flora, and its ability to cause disease when inoculated into extra-intestinal sites. *E .coli* is the predominant facultative anaerobe of the commensal bacteria present in the intestinal tract of most warm blooded animals. After birth, the bacterium usually colonises an infant's intestinal tract in a matter of hours. *E. coli* normally remains harmlessly in the intestinal tract. However, in immuno-suppressed or debilitated patients, even non-pathogenic strains can cause disease, namely sepsis/meningitis or urinary tract infections. *E. coli* is also present on most uncooked foods and is widely spread throughout the environment. Pathogenic strains are differentiated from non-pathogenic strains by the acquisition of virulence determinants. The virulence determinants of each *E. coli* strain are distinct between different strains, although they can all generally be characterised as colonisation factors, secreted toxins and type III secretion systems (Nataro and Kaper, 1998).

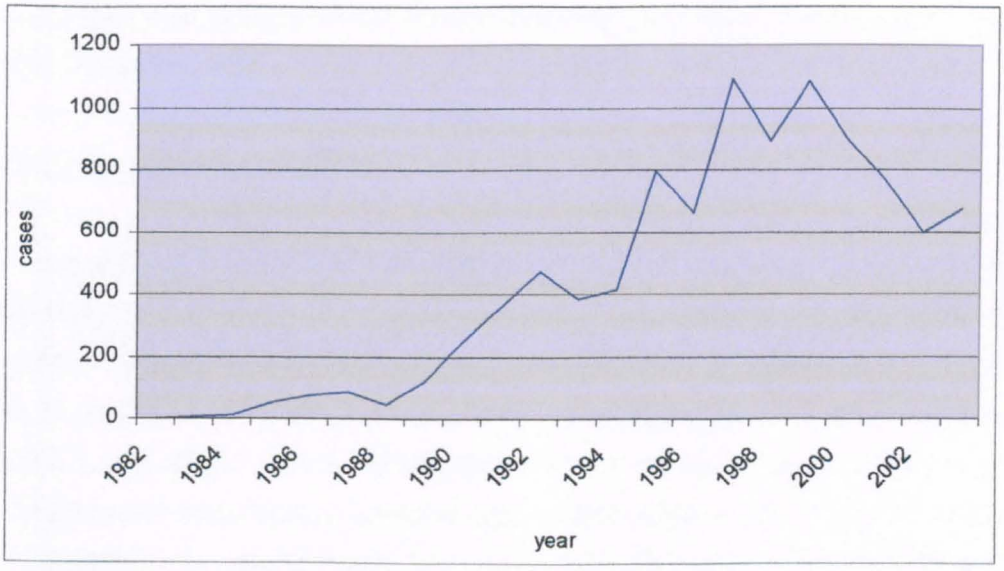
Verocytotoxic E. coli (VTEC)

Verocytotoxic *E. coli* were first described in 1977 and named according to their ability to cause damage to cultured Vero cells (Konowalchuk *et al.*, 1977), this being the virulence determinant that identifies this strain of *E. coli* as being pathogenic to humans. There are two main types of verocytotoxins, VT1 and VT2, and these are encoded in and expressed by temperate bacteriophages (Scotland *et al.*, 1983). VTEC serotypes commonly isolated from human infection include 026, 0145 and O157. *E. coli* O157 is the most commonly isolated strain of VTEC from human infections, and is one of the serotypes referred to as enterohaemorrhagic *E. coli* (EHEC).

VTEC can cause haemorrhagic colitis (HC), haemolytic uraemic syndrome (HUS) and thrombocytic thrombocytopenic purpura (TTP) in humans, which have high

mortality rates, especially in children and the elderly. Approximately 2-7% of patients affected with VTEC develop systemic complications, mostly HUS. Necrosis of the large intestine can also occur in severe cases. Only a small number of bacteria are needed to cause disease and outbreaks are largely confined to industrialised countries. The associations between *E. coli* O157 and HUS were established in the early 1980's (Karmali *et al.*, 1983). Since then it has been the cause of both outbreaks and sporadic cases of diarrhoea and HUS involving thousands of cases and numerous deaths (Mead *et al.*, 1999) and the number of cases has been rising (see figure 1).

Figure 1.1 Verocytotoxin-producing *E. coli* O157 isolated from humans in England and Wales 1982-2003 (reproduced from www.hpa.org.uk)



VTEC can carry a number of virulence factors including two genetically unrelated haemolysins, on a 60 Mda plasmid, and intimin (Beutin *et al.*, 1993, Schmidt *et al.*, 1995). Intimin is a 94kDa outer membrane protein that mediates the attachment of the bacterium to epithelial cells and causes the characteristic attaching and effacing lesions. Intimin is the product of the *eaeA* gene (Jerse *et al.*, 1990), and lesions associated with the presence of *eaeA* have been reported in humans, rabbits, calves, horses, lambs, cats and dogs (Janke *et al.*, 1989, Moxley *et al.*, 1986, Broes *et al.*, 1988). The genetic determinants for the production of attaching and effacing lesions

are located in the locus of enterocyte effacement (LEE), a pathogenicity island that contains genes, not only encoding for intimin (eaeA), but also a type III secretion system and a number of secreted transduction (Esp) proteins. The cluster of genes present on this locus are needed to cause lesions on human and animal epithelium (Jores *et al.*, 2004).

VTEC carry one or both of the *vt* genes that are important in human medicine. The difference in virulence potential of strains carrying one or both genes, plus the *eaeA* gene is unclear. Strains of *E. coli* O157 that carry *vt2* and *eaeA* are most commonly isolated from human patients who have developed haemorrhagic colitis (Beautin *et al.*, 1994, Werber *et al.*, 2003), and this is also the strain most frequently isolated from cattle. Cattle are regarded as the major human source of infection for VTEC and over 100 different serotypes have been isolated, including *E. coli* O157 (Montenegro *et al.*, 1990, Beautin *et al.*, 1997).

Enteropathogenic E. coli (EPEC)

Enteropathogenic *E. coli* (EPEC) mostly effect children, in whom it causes infantile diarrhoea (Hart *et al.*, 1993). They are particularly common in developing countries, while in industrialised countries the frequency of these organisms has decreased. However, they do still continue to be an important cause of gastrointestinal infection (Nataro and Kaper, 1998). Adult infections are usually associated with other conditions and the increased resistance seen in older children and adults may be due to immunity or the loss of receptors for specific adhesion factors (Nataro and Kaper, 1998).

EPEC adhere to the intestinal mucous membrane producing characteristic 'attaching and effacing' lesions of the microvilli or brush border, also seen in VTEC infection. Studies have shown that the *eaeA* genes of EPEC and VTEC are functionally the same (Donnenberg *et al.*, 1992), although the gene can differ between the two and they can be classified into distinct types or subtypes (Adu-Bobie *et al.*, 1998).

EPEC also carries the *bfpA* gene. This is responsible for the formation of the bundle forming pili, which are member of the type IV pilin family. The pili interconnect

bacteria within the micro-colonies of bacteria forming a pattern called localised adherence (LA), and thus, promoting their stabilisation. The *bfpA* gene is located on a high molecular plasmid, termed the EPEC adherence factor (EAF; Nataro *et al.*, 1985), which may also be present in certain serotypes of VTEC. This plasmid is not essential for the formation of A/E lesions, although its presence may enhance their efficiency of production (Knutton *et al.*, 1987).

Enterotoxigenic E. coli (ETEC)

ETEC are a major cause of children's and traveller's gastroenteritis, causing a watery, cholera-like diarrhoea (Nataro and Kaper, 1998). This is again largely confined to developing countries and people who visit them. ETEC can also cause similar symptoms in young farm animals (Nagy and Fekete, 1999). ETEC can produce one or two enterotoxins, heat stable enterotoxin (ST) and heat labile enterotoxin (LT). Both of the enterotoxins are plasmid encoded and stimulate intestinal fluid secretion by a cascade of complex mechanisms. There are two sub-types of each toxin, namely, STa and STb, and LT-I and LT-II respectively. ETEC strains are very diverse and express different colonising fimbriae that determine their host specificity. The plasmids that carry one or more ETEC enterotoxins are also able to carry colonisation factor antigens (CFAs). These are able to be subdivided based on morphological characteristics. CFA/I is a rigid rod-shaped fimbria composed of a single protein (Jann and Hoschutsky, 1991), CFA/III is a bundle-forming pilus and CFA/II and CFA/IV are composed of multiple fimbrial structures (Nataro and Kaper, 1998). CFA/I, CFA/II or CFA/IV are believed to be expressed in approximately 75% of human ETEC isolates (Wolf, 1997).

Enteroinvasive E. coli (EIEC)

EIEC are able to invade intestinal cells and can cause dysentery in a similar manner to that produced by *Shigella* spp. Infection occurs in humans of all ages and is more common in less developed countries, however, it is still a minor cause of gastrointestinal disease. EIEC carry genes for invasion of the epithelial cells in the colon on plasmids. After invasion, EIEC multiply and eventually cause cell death, the bacterium then being released to invade other cells. This process causes inflammation

and ulceration of mucosa. EIEC infection is usually milder than dysentery caused by *Shigella* spp. (Hart *et al.*, 1993).

Enteroaggregative E. coli (EAEC)

These strains produce persistent diarrhoea due to inflammation of the intestine and cytotoxic changes in enterocytes. Initially, EAEC were thought to be largely confined to children in less developed countries, and travellers to those countries. However, many studies are now suggesting that it is an important cause of diarrhoea in all ages, both in developing and industrialised countries (Okeke and Nataro, 2001). EAEC show a distinctive aggregative pattern of adherence to epithelial cells and early studies often referred to them as EPEC due to lack of toxins or other factors being identified, relating to their pathogenicity (Levine *et al.*, 1984). Genotypic definition is difficult for EAEC as adhesins, toxins and other factors that contribute to its pathogenicity are not unique to the EAEC category (Okeke and Nataro, 2001). EAEC can also be isolated from humans that do not have diarrhoea (Echeverria *et al.*, 1992), but evidence from human volunteer studies (Mathewson *et al.*, 1986) and a number of outbreaks (Smith *et al.*, 1997) has shown that some EAEC strains are a cause of human disease. EAEC are also pathogenic for rabbits (Vial *et al.*, 1999).

Pathogenic E. coli in dogs

Enteropathogenic *E. coli* are a well known cause of gastroenteritis in humans, but their role as a cause of gastrointestinal disease in dogs is unclear. There are a number of pathogenic *E. coli* serotypes that have been associated with diarrhoeal illness in dogs (Beutin, 1999). These include enterotoxigenic *E. coli* (ETEC), verotoxigenic *E. coli* (VTEC;), including enterohemorrhagic *E. coli* (EHEC) and enteropathogenic *E. coli* (EPEC; Beutin *et al.*, 1993, Beaudry *et al.*, 1996, Beautin, 1999, Goffaux *et al.*, 2000, Neiger *et al.*, 2002). However, virulence determinants attributed to pathogenic strains of *E. coli,* usually associated with diarrhoea in dogs have also been found in healthy dogs (Holland *et al.*, 1999).

All reports regarding heat stable toxin carrying ETEC isolates concern diarrhoeic dogs (Wasteson *et al.*, 1988, Hammermueller *et al.*, 1995) and there are no reports of

heat labile toxin ever being present (Hammermueller *et al.*, 1995). Canine serotypes of ETEC are not usually the same as those found in humans and one study has shown that ETEC from human and dog sources carry their enterotoxin genes on plasmids of different sizes (Wolf, 1997). Colonisation factors have been found in ETEC isolates from dogs (Droplet *et al.*, 1994), but again not with types commonly found in human isolates. It has also been observed that haemolysin is rarely detected in human strains of ETEC and EPEC, but frequently among ETEC strains from animal origin (Parada *et al.*, 1991, Starčič *et al.*, 2002).

From published data on the prevalence of VTEC genes isolated from dogs, there seems to be no significant difference between diarrhoeic and asymptomatic dogs (Beutin *et al.*, 1995, Tuck *et al.*, 1998, Holland *et al.*, 1999). However, a study by Hammerueller *et al.* (1995), did find an association between diarrhoea in dogs and *vt2* producing VTEC (Hammermueller *et al.*, 1995). VTEC has also been isolated from cats, both healthy and diarrhoeic (Beautin *et al.*, 1993, Smith *et al.*, 1998). VTEC O157:H7 phage type 4 has also been isolated from dog faeces (Trevena *et al.*, 1996).

EPEC are considered an important cause of diarrhoea in dogs, particularly in puppies (Droplet *et al.*, 1994, Beaudry *et al.*, 1996, Sancak *et al.*, 1997, Goffaux *et al.*, 2000). Attaching and effacing lesions caused by the *eaeA* gene and PCR detection of the *eaeA* gene is fairly well documented in dogs (Broes *et al.*, 1988, Janke *et al.*, 1989, Drolet *et al.*, 1994, Beaudry *et al.*, 1995, Turk *et al.*, 1998, Goffaux *et al.*, 2000, Neiger *et al.*, 2002), although canine strains of EPEC have been found to produce a different intimin (*eaeA*) from that isolated from human and other animal EPEC strains (An *et al.*, 1997). In dogs, the LEE locus has been observed in EPEC isolates (Nakazato *et al.*, 2004), and strains closely related to those found in human cases have been isolated (Goffaux *et al.*, 2000). The EAF plasmid has also been found in dog EPEC isolates (Droplet *et al.*, 1994). A study by Sancak *et al.*, (2004) found a higher prevalence of EPEC and VTEC in kennelled dogs than dogs in private households.

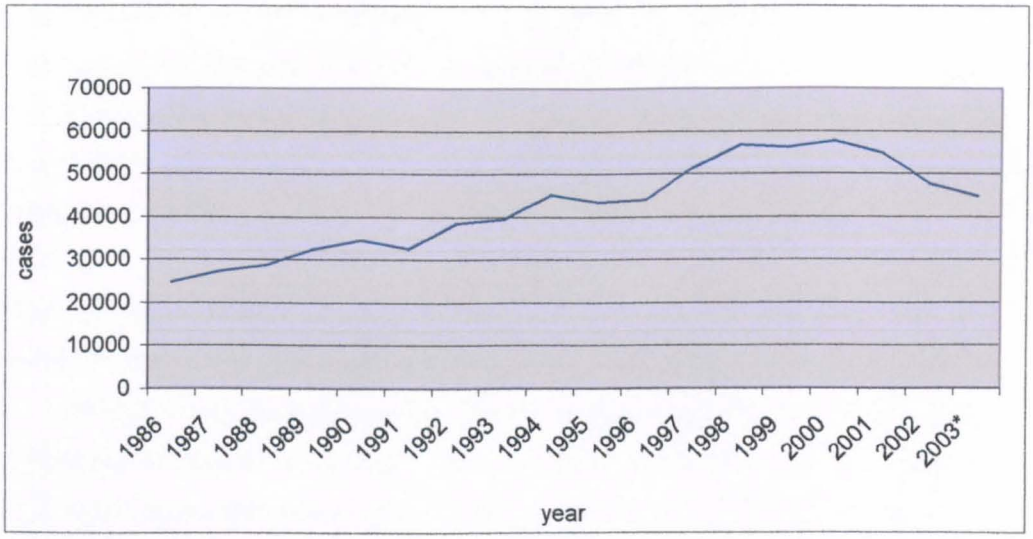
Dogs have been proposed as a possible reservoir of virulent *E. coli* strains that cause both intestinal and extraintestinal disease in humans including diarrhoea, cystitis, pyelonephritis, bacteraemia or meningitis (Johnson *et al.*, 2001). Much effort has been put into the characterisation of pathogenic strains of *E. coli* involved in intestinal

infections in poultry, sheep, pigs and cattle, but the role of virulence factors in gastrointestinal disease in dogs is less well defined. Further investigation of the role of pathogenic strains of *E. coli* is required to learn more about the role of their virulence determinants in dogs.

Campylobacter spp.

Campylobacter spp. are the most common cause of bacterial gastroenteritis in Britain, being responsible for over 50 000 cases of human gastroenteritis every year (FSA 2001), and it is thought that many more cases go unreported (Sethi *et al.*, 1999). Over 90% of infections are believed to be caused by *C. jejuni* and the majority of the remainder by *C. coli* (Skirrow, 1994). It is also the most common cause of traveller's diarrhoea. Symptoms can include abdominal cramps, fever and frequent, often bloody diarrhoea. These symptoms are usually self-limiting with a duration of 2-5 days but can persist for 2 weeks or longer. Recent studies have indicted that 10% of cases of campylobacteriosis in the UK each year are hospitalised (Frost, unpublished data). In developing countries where high levels of malnutrition are experienced, infection with *Campylobacter* spp. has a high rate of mortality, especially in children. Extraintestinal manifestations occur primarily in the young, elderly and immuno-copromised. These include bacteraemia, pancreatitis and reactive arthritis. *Campylobacter* spp. infection is now recognised as the most identifiable infection preceding Guillain-Barrè syndrome (GBS, Nachamkin, 2002). GBS is an immune mediated disorder that affects the peripheral nervous system causing flaccid paralysis and sensory loss.

Figure 1.2. Laboratory reports of *Campylobacter* spp. isolation from human faeces in England and Wales (reproduced from www.hpa.org.uk)



Campylobacter spp. are considered predominantly, a food borne pathogen and sources that are most frequently associated with both epidemics and sporadic cases are un-pasteurised milk, contaminated drinking water and inadequately cooked meat, especially poultry and poultry products (Allos, 2001, Gillespie *et al.*, 2003).

Warm-blooded animals such as cattle, sheep, rodents, poultry and wild birds are suggested to be the natural reservoir of *Campylobacter* spp. (Frost, 2001). *Campylobacter* spp. do not necessarily cause disease in these animals. Domestic animals and pets such as dogs can also carry the bacterium asymptomatically (Skirrow, 1977, Blaser *et al.*, 1979).

Campylobacter spp. in dogs

Campylobacter spp. are not considered a primary pathogen in dogs, which can carry the organism in their intestinal tracts without any ill effect (Baker *et al.*, 1999). A number of studies have found higher excretion rates of *Campylobacter* spp. in diarrhoeic dogs (Nair *et al.*, 1985, Balucinska *et al.*, 1997) but other studies have found no significance between diseased and healthy dogs (Olsen and Sandstedt, 1987, Figura 1991, Burnens *et al*, 1992, Adesiyun *et* al, 1997, Baker *et* al, 1999). The

prevalence of excretion of *Campylobacter* spp. among healthy dogs ranges between 5%-48% (Sandberg *et al.*, 2002). Studies on the prevalence of *Campylobacter* spp. in dogs with diarrhoea have found rates between 5%-66% (Chattopadhyay *et al.*, 2001, Hald *et al.*, 1997, Burnens *et al.*, 1992). It is believed that *Campylobacter* spp. may be a secondary pathogen and may only cause disease when another pathogen has already disrupted the gastrointestinal tract such as viruses and parasites (Fox, *et al*, 1983 Brown *et al*, 1999).

Cases of dog-associated human enteritis from contact with diarrhoeic and non-diarrhoeic dogs have been reported (Blaser *et al.*, 1979, Hoise *et al.*, 1979, Bruce *et al.*, 1980) and various risk analysis studies have shown that pet ownership is a significant risk factor for *Campylobacter* spp. infection (Adak *et al.*, 1995, Neimann *et al.*, 2003), especially puppies (Neal *et al.*, 1997). A study in Calcutta suggested that domesticated animals such as dogs and goats served as the source of *Campylobacter* spp. infection for humans as the animals shared the same bathing water and slept under the same roof (Chattopadhyay *et al.*, 2001). A study in Los Angeles, USA found that *C. upsaliensis* was the second most frequently isolated species from patients with Campylobacteriosis. Eighty-three percent of the patients had pets at home and 33% had dogs from which *C. upsaliensis* was isolated. Molecular typing by pulse field gel electrophoresis (PFGE) did not show clonality, although the pets' faeces were not cultured until 3-6 months after isolates had been recovered from the pet owners (Labarca *et al.*, 2002). Another study from Denmark reported that the same quinolone-resistant *C. jejuni* strain, was isolated from both a dog and a 2 year old child in the same household (Damborg *et al.*, 2004)

Studies have shown that dogs kept in communal situations and dogs that are below one year of age have a higher rate of carriage of *Campylobacter* spp. (Fleming *et* al., 1980, Blaser *et al.*, 1980, Nair *et al.*, 1985, Mailk and Love, 1989, Adesiyun *et al.*, 1996, Bruce *et al.*, 1983, López *et al.*, 2002). A study by Lopez *et al.*, (2002) not only found that dogs below one year of age had a higher prevalence of *Campylobacter* spp., but that the prevalence was higher in the summer.

Household dogs are found to have a much lower incidence of *Campylobacter* spp. carriage than those in dog rescue homes and kennels (Malik and Love, 1989, Bruce *et*

al., 1993, Balucinska *et al.*, 1997, Cantor *et al.*, 1997). This is presumably due to splashing of faecal material contaminating food or drinking bowls or dogs drinking from drains. Dogs in a closed breeding colony were found to excrete *Campylobacter* spp., asymptomatically by eight weeks old (Newton *et al.*, 1988). A recent study by Hald *et al.*, (2004) observed *Campylobacter* spp. excretion over a 2 year period.

The most frequently reported species of *Campylobacter* isolated from dogs is *C. jejuni* (Moreno *et al*, 1993, Hald and Madsen, 1997, López *et al.*, 2002). However, this may be due to under reporting of *C. upsaliensis*, this species being sensitive to the antibiotics incorporated into most Campylobacter selective agar (Steinhauserova *et al.*, 2000, Byrne *et al.*, 2000, Labarca *et al.*, 2002, Modolo and Giuffrida, 2004). *C. upsailensis* was first isolated from dogs in Sweden in 1983. It is different to common serotypes isolated clinically from humans, namely *C. jejuni* and *C. coli*, although *C. upsaliensis* is occasionally found in human disease (Frost *et al.*, 1998). *C. upsaliensis* is recognised as causing mild enteritis particularly in children and travellers (Goossens *et al.*, 1990, Lindblom *et al.*, 1995) and also in HIV infected patients (Jenkin and Tee, 1998).

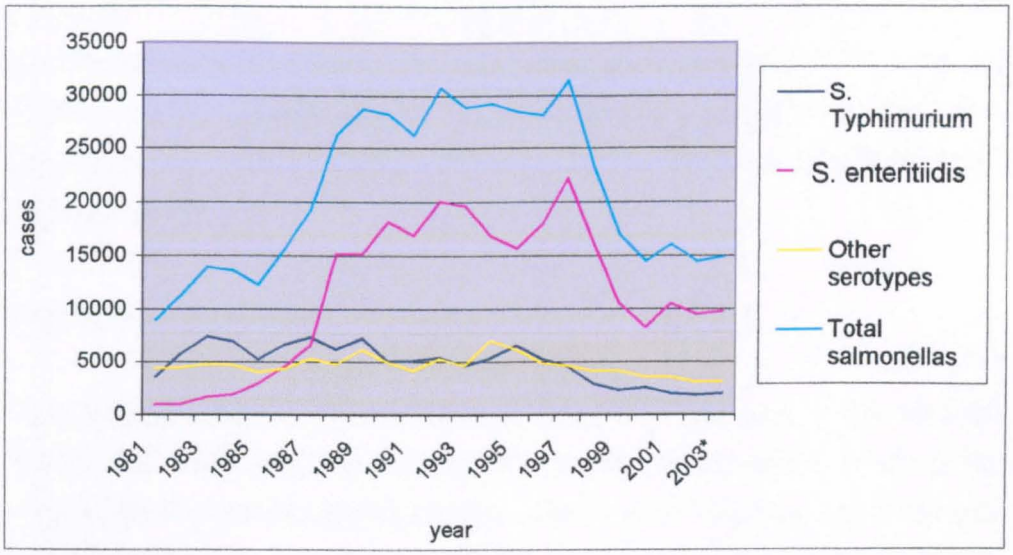
Studies have found carriage rates of *C. upsaliensis* to be as high as 75% in dogs (Hald *et al.*, 2004). *C. upsaliensis* is rarely found in animals other than cats and dogs (Hald and Madsen, 1997) suggesting they are the reservoir for this species of *Campylobacter*. Simultaneous infection with multiple *Campylobacter* species in dogs has also been reported (Koene *et al.*, 2004), which may also lead to underreporting of *C. upsaliensis*.

Salmonella spp.

Non-typhoidal *Salmonella* spp. (NTS) have been well documented as important pathogens in humans (Lance *et al.*, 1992). NTS were, for many years the most common cause of gastroenteritis in humans, in particular *S. enterica* ser Typhimurium and *S.* enteriditis but in recent years *Campylobacter* spp. have superseded them. Clinical features of infection include sudden headache, chills, vomiting and diarrhoea, followed by a fever. Gastrointestinal infection does not usually warrant antimicrobial

therapy but persistent symptoms can be treated with antibiotics. In developing countries NTS are becoming increasingly important as a cause of bacteraemia and other invasive disease that require antimicrobial treatment (Graham *et al.*, 2000).

Figure 1.3. *Salmonella* isolated from humans in England and Wales – faecal and unknown reports excluding *S.* Typhi and *S.* Paratyphi (reproduced from www.hpa.org)



(* - provisional data)

Reservoirs of NTS are thought to be poultry and birds, and human infection is most commonly associated with consumption of contaminated food of animal origin such as infected meat, poultry, eggs and milk. Direct contact with livestock has also been documented as a source of infection (Wall *et al.*, 1995). Outbreaks of human Salmonellosis directly traceable to contact with farm animals have been reported from as far back as the 1960's (Fish *et al.*, 1967, Fey *et al.*, 2000). Reports of NTS on chicken pieces from supermarkets and on packaging have been made (Sackey *et al.*, 2001) and in the USA it is estimated that 95% of NTS infections are related to food-borne transmission (Mead *et al.*, 1999).

The PHLS has been monitoring the current antimicrobial resistance trends seen in NTS infections from humans. The most common serotypes of NTS isolated from human clinical cases in 2000 were, in descending order, *S.* enteritidis, *S.* typhimurium,

S. hadar and *S.* virchow. There were a further 248 different serotypes isolated, but these were represented in very small numbers. 35% of all isolates were drug resistant and resistance was seen most commonly in *S.* typhimurium with 67% of isolates being resistant to 4 drugs or more. The numbers of *S.* typhimurium phage type DT 104 isolated also increased from 1999 – 2000, mainly due to an outbreak in the West Midlands with over 300 cases being reported. This was thought to be due to imported lettuce (Threlfall, 2002).

All of the known 2460 serotypes of *Salmonella* are infectious to humans (McClelland and Pinder, 1994), although only 2000 are associated with gastroenteritis. Each is also infectious to susceptible animals, including companion animals such as dogs (Urban and Broce, 1998).

Salmonella spp. from dogs

Dogs are rarely a source of human infection with NTS, although it is estimated that between 10% and 27% of dogs are infected with NTS, usually with serotypes similar to those affecting humans (James and Tan, 1997). NTS have been implicated as a cause of diarrhoea in dogs, but can also be isolated from the faeces of clinically healthy dogs (Kwaga, 1989, Hackett, 2003, Kozak *et al.*, 2003). NTS have also been isolated from cats (Ball, 1951, Shimi and Barin, 1977), but is not thought to be associated with diarrhoea (Dow *et al.*, 1989). Nosocomial infection has also been documented as a risk factor for dogs (Uhaa *et al.*, 1988). It is believed that the majority of dogs infected with NTS get infected from the environment, although it is also possible for humans to pass NTS to companion animals via contaminated human food (Joffe and Schlesinger, 2002). Dogs are not usually treated for NTS infection with antibiotics as this can cause persistent shedding of the bacterium that can last for several months. Carrier states can be induced fairly easily as shown by experiments where dogs were given *S.* typhimurium orally and were shedding still over 5 days later indicating colonisation of the intestinal tract by the bacteria. (Tanka *et al.*, 1976). NTS isolates from dogs faeces include *S.* typhimurium (Tanka et al., 1979), *S.* infantis (Sato and Kuwamoto, 1992) and *S.* krefeld (Uhaa et al., 1988). More recently in Trinidad (Seepersadsingh et al., 2004) *S.* javiana, *S.* newport, *S.* arechavaleta and *S.* heidelberg have been isolated from dogs.

Outbreaks of NTS infection are often associated with communal kennels and breeding colonies. This is maybe due to cross-contamination between dogs as they are living so closely together. Poor hygiene may also be partly responsible for this by animal attendants spreading infection via boots, clothing and feed bowls (BSAVA, 1997).

The published prevalence of NTS infection in faecal samples from non-diarrhoeic dogs ranges from 0.5% to 30%. Low prevalences of NTS have been reported from Slovakia (1.2%, Kozak *et al.*, 2003), Nigeria (1%, Kwaga *et al.*, 1989), Italy (2.4%, Nastasi *et al.*, 1986) and Germany (3.45%, Webber *et al.*, 1995). In the USA a low prevalence of 1.2% has been reported in Washington but higher prevalences of up to 15% in Florida (Gorham *et al.*, 1951, Galton *et al.*, 1952, Mackel *et al.*, 1952). An even higher prevalence of NTS in asymptomatic dogs has been reported in racing sled dogs in Alaska (57%, Cantor *et al.*, 1997). This is much higher than in other studies involving sled dogs that have found the asymptomatic prevalences of NTS excretion to be 0%-7% (Butler *et al.*, 1965, Grumbles *et al.*, 1955). Sanitation is of a low standard in rural Alaska where such races take place especially where running water is concerned. There have been cases of salmonellosis occasionally seen in mushers and villagers along the sled trail (Cantor *et al.*, 1997).

Salmonellosis is known to be common in greyhounds. NTS prevalence rates as high as 36.5 % have been reported from dogs with diarrhoea (Galton *et al.*, 1952). A perennial problem suffered by breeding kennels is a high incidence of morbidity and mortality among greyhound puppies from intestinal infections. Breeders traditionally feed dogs raw meat and meat obtained frozen from commercial renderers, and is frequently contaminated with enteric organisms including various serotypes of NTS. During thawing, rendered meat may also be exposed to numerous filth flies that have been documented as vectors of enteric and other pathogenic bacteria (Urban and Broce, 1998). Batches of dog chews and snacks have also been reported to be contaminated with NTS (Christensen *et al.*, 1999, Badger, 2000, Willis, 2001, Pitout *et al.*, 2003) making pet foods an important route of NTS infection for dogs.

An outbreak of human *S.* typhimurium infection from an animal shelter in Minnesota, USA, implicated cats in the transmission of this bacterium to humans. All isolates were indistinguishable by pulsed-field gel electrophoresis (PFGE) of macrorestricted chromosomal DNA (MMWR, 2001). There have been sporadic reports of dogs

transmitting *Salmonella* spp. to humans. A report in Japan implicated a dog in transmitting *S.* virchow to a 4 month old infant, the PFGE patterns of the two isolates were identical (Sato *et al.*, 2000).

The prevalence of NTS in dogs from the UK is not well documented and detail on clinical cases from veterinary hospitals in the UK is not widely available (BSAVA news, 1997).

Vancomycin-Resistant Enterococci (VRE).

Enterococcus spp. are a gram positive, aerobic bacteria, considered to be part of the normal gastrointestinal flora of humans and a variety of other animals. They are ubiquitous and can be present in soil, surface water and on plants and vegetables. They can also be part of the flora on various foods (Franz *et al.*, 1999). Since the isolation of multi-resistant strains in the late 1970's, they have emerged as an important cause of nosocomial infections and, more recently, community-acquired infections. Enterococci have been known for over a century to cause urinary tract infections, endocarditis, septicaemia and intra-abdominal infections (Cetinkaya *et al.*, 2000).

The ability of enterococci to acquire antibiotic resistance genes has made them a therapeutic challenge in human medicine and the emergence of vancomycin resistance is causing particular concern as this is the drug of last resort for the treatment of multiple resistant, gram-positive infections such as methicillin resistant *S. aureus* (MRSA). The first report of vancomycin resistant enterococci was in 1986 in Europe and 1987 from the United States (Uttley *et al.*, 1988). Since then VRE have spread rapidly and are now encountered in most hospitals both in the UK and the United States (Cetinkaya *et al.*, 2000). VRE are considered of low virulence in the healthy human population and only tend to cause problems in transplant, intensive care and dialysis patients. VRE were first isolated outside the health care setting in 1993 when vancomycin resistant *Enterococcus faecium* was isolated from sewage treatment plants in urban areas of England, thus suggesting a community reservoir (Bates *et al.*, 1993). In most human clinical cases *E. faecalis* or *E. faecium* are frequently isolated

species. Less frequently isolated species include *E. gallinarum, E. casseliflaus, E. durans* and *E. avium* which together account for approximately 5% of clinical isolates (Cetinkaya *et al.*, 2000).

Animals have been implicated in the transmission of VRE to humans via the food chain, and there are strong epidemiological links to support this claim (van den Bogaard and Stobberingh, 2000). Vancomycin resistance in *Enterococcus* spp. from animals is thought to have arisen from the use of avoparcin, a growth promoter that was used in animal feed from the 1970's but was banned from use in 1999. Avoparcin is a glycopeptide antibiotic structurally similar to vancomycin and which induces cross-resistance. Association with the use of avoparcin in animal husbandry and the occurrence of VRE was established in 1995 (Aarestrup, 1995, Kruse *et al.*, 1999, van den Bogaard and Stobberingh, 1999), however, alternative sources of infection have also been recognised such as pets, wild rodents (van Belkum *et al.*, 1996, Mallon *et al.*, 2002) and contaminated vegetables (Bager, 1997).

Much higher isolation rates of VRE are seen on farms that have used avoparcin in comparison to farms where the antibiotic has not been used. High levels of VRE have also been found in the faeces of workers on farms where avoparcin was used. Similar resistance patterns and genes have been detected among human, broiler and pig isolates, suggesting that there is a potential for transmission of VRE between them (Aarestrup *et al.*, 2000). Since avoparcin was banned the levels of VRE isolated from slaughterhouses and food products have declined, but several studies have shown that VRE are still prevalent (Borgen *et al.*, 2001, Aarestrup *et al.*, 2001).

In the USA avoparcin was never used in animals and it is believed that high levels of resistance have emerged from over use of vancomycin in human medicine. In the late 1980's, the prevalence of MRSA was increasing in US hospitals (Schaberg *et al.*, 1991), this resulted in increased use of vancomycin. In Europe, glycopeptide use in humans is generally much lower than in the USA (Wittle, 1999) and there have been fewer human clinical cases of VRE infection (Schouten *et al.*, 1999, Wittle, 1999). In contrast, there have been no reports of VRE from food producing animals in the US (Coque *et al.*, 1996).

VRE can be found in the faeces of both healthy individuals and hospital patients. Faecal colonisation rates of 2% - 28% have been reported in communities of healthy people (Endtz *et al.*, 1997, Van der Auwera *et al.*, 1996), although faecal carriage rates can be as high as 86% in some occupations such as hospital staff (Linden *et al.*, 1994). In the United States much less is known about the presence of VRE in the community. Limited data available in the USA show that in contrast to European data, VRE are absent or very rare in healthy people outside of the hospital environment (Coque *et al.*, 1996, McDonald *et al.*, 1997, Silverman *et al.*, 1998).

Enterococci can be readily isolated from foods such as raw meat products and ready to eat foods. Enterococci seem to play an important role in the flavour and quality of cheeses. High isolation rates of VRE have been reported from slaughterhouses for pigs and chickens (Wegener *et al.*, 1997). A study in Spain showed that VRE were present in over 27% of raw chicken products purchased from a supermarket (Robredo *et al.*, 2000). A similar study found VRE in pork (Lemcke and Bulte, 2000). VRE has also been isolated from Italian cheeses (Giraffa *et al.*, 2000). Similar PFGE patterns have been found in multiple resistant enterococci isolated from French cheeses and clinical cases in a hospital suggesting that animal products may serve as a reservoir and vehicle for these antibiotic resistant bacteria (Bertrand *et al.*, 2000).

Treatment for an enterococcal infection usually combines a β-lactam antibiotic such as ampicillin or a glycopeptide in combination with an aminoglycoside. The appearance of aminoglycoside and glycopeptide resistance has presented a major therapeutic problem for enterococcal infection that was increased when vancomycin resistance appeared. Enterococci can show a broad innate resistance towards many antibiotics including cephalosporins, semi-synthetic penicillins, macrolides and low concentrations of aminoglycosides (Endtz *et al.*, 1999, Facklam *et al.*, 1999). Vancomycin is one of the few drugs that can treat infections with *Enterococcus* spp.

Methicillin-resistant Staphylococcus aureus (MRSA).

Staphylococcus aureus is a cause of gastroenteritis and nosocomial infections in humans. It is a gram positive, pus-producing bacterium that can infect wounds and

cause blood poisoning, pneumonia and toxic shock syndrome. In England and Wales *S. aureus* it is the second most common cause of bacteraemia after *E. coli*, accounting for 20% of human cases (Reacher *et al.*, 2000). The prevalence of MRSA in the clinical setting in Europe ranges between 5-20% (Tiemersma *et al.*, 2004).

S. aureus can be carried on moist skin in the nose, axillae and perineum and approximately one third of the healthy human population are carriers. Higher rates of carriage are observed in injecting drug users, people with insulin-independent diabetes, patients with dermatological conditions and healthcare workers. It also survives well on skin facilitating cross contamination and infection between people.

S. aureus can also be a cause of gastroenteritis in humans and is often reported in Japan and the United States. Reports from Japan concern mostly processed foods such as rice, pork, sushi and eggs (Shimizu *et al.*, 2000). In Taiwan, *S. aureus* has contributed to 30% of the food-borne outbreaks between 1986 and 1995 (Pan *et al.*, 1997). Symptoms can include fever, nausea, vomiting, diarrhoea and abdominal pain. It has a rapid onset of 30 minutes to 8 hours after ingesting contaminated food (Holmberg and Blake, 1984). Causes of infection can also be due to contaminated meat, poultry, puddings and cakes and creamy salad dressings (Jones *et al.*, 2002). Enterotoxins produced by this bacterium are responsible for the food poisoning symptoms, and are also involved in wound infections, septicemia and toxic shock syndrome, although additional virulence determinants may be involved in the latter diseases.

S. aureus produces several enterotoxins that are released onto food products causing nausea, vomiting and diarrhoea. Six types of enterotoxin have been well characterised, these being named A, B, C_1, C_2, D and E. In recent years many other enterotoxin genes have also been identified, G, H, I, J, K, L, N, O, P, G, R and U (Lovseth *et al.*, 2004). All are genetically related. Enterotoxin A is a small single peptide that has a molecular weight of 30,000 and is most frequently associated with outbreaks of *S. aureus* food poisoning Enterotoxin A is chromosomally encoded by the *entA* gene. The B and C type enterotoxins may be plasmid or transposon encoded.

Methicillin resistant *S. aureus* (MRSA) first emerged as nosocomial pathogens in the early 1960's (Jorgensen, 1986) just one year after the launch of methicillin. Methicillin, which is a penicillinase-stable β-lactam, was the drug of choice for *S. aureus* infection but was replaced by cloxacillin and flucloxacillin because of its toxicity, however, resistance quickly arose to all three of these antibiotics and all other β-lactams. Until recently most infections of MRSA were acquired primarily in hospital settings, but now infection with MRSA can occur in the community, in both rural and urban settings. The first reports emerged in the early 1980's, primarily in people who had a history of drug abuse (Saravolatz *et al.*, 1993). Since then reports of community infections with MRSA have been increasing and studies show that it is circulating beyond hospital settings (Groom *et al.*, 2001, Fey *et al.*, 2003). Studies have shown that many hospital infections with MRSA were actually acquired outside the hospital setting. One hospital-based study found that up to 40% of MRSA infections in adults were acquired before admission (Layton *et al.*, 1995).

A recent outbreak of gastroenteritis in the USA was found to be related to coleslaw from a supermarket being contaminated with MRSA (Jones *et al.*, 2002). It is thought that this is the first report of community-acquired gastroenteritis caused by this bacterium.

MRSA diarrhoea is rarely reported although it is becoming increasingly more common (Pasha *et al.*, 2001). In recent years the incidence level of Staphylococcal food poisoning has decreased although it is still a major cause of outbreaks (Chiou *et al.*, 2000).

The origins of community-acquired MRSA are subject to debate. It would be expected that they might have evolved from the hospital environment, however, studies have shown that if this is indeed the case then they have undergone considerable change due to distinct differences in PFGE patterns in hospital isolates compared with those found in the community (Chambers, 2001, Fey *et al.*, 2003). Another possibility is that *S. aureus* could have acquired resistance genes by horizontal transfer of methicillin resistance genes. This method could also account for the different PFGE patterns and the lack of resistance to multiple antibiotics. Horizontal transfer of *mecA*, the gene responsible for methicillin resistance, is thought

to be relatively rare, accounting for only a handful of all clinical isolates worldwide (Kreiswirth *et al.*, 1993).

S. aureus is not just a pathogen for humans but for many other animal species. There have been reports of MRSA infection in animals although not to the levels of MRSA being reported from humans. MRSA has been found in dairy cows and chickens (Lee, 2003), companion animals (Pak *et al.*, 1999) and horses (Seguin *et al.*, 1999). Humans have been implicated in the passage of MRSA to horses in an equine hospital due to the fact the MRSA is fairly common in humans and much less so in animals (Seguin *et al.*, 1999), however, there are very few studies carried out in this area so there is little evidence to suggest transmission either way.

MRSA infection in dogs

There have been few reports of MRSA from dogs. Published reports are usually from skin and wound infections (Gortel *et al.*, 1999, van Duijkeren *et al.*, 2003, Rich and Roberts, 2004). Dogs have also been implicated in the transmission of MRSA to humans (Manian 2003) and humans have been implicated in transmission to dogs (O'Rourke 2002). There have been no reports of MRSA isolation from dog faecal samples and *S. aureus* is not known to cause diarrhoea in dogs.

Clostridium perfringens

Clostridium perfringens is an anaerobic, gram positive, spore-forming bacterium, although some strains are aerotolerant (Sainsbury, 2000). It is a natural inhabitant of the soil and intestinal tract of many warm-blooded animals and humans, however, it is also associated with disease in certain circumstances. *C. pefringens* has been associated with diarrhoea in humans, livestock, dogs and horses (Griffiths, 1996, Marks *et al.*, 2002). It has also been implicated in gas gangrene in humans and in birds it has been linked with enterotoxaemia and necrotic enteritis. Enterotoxaemia is due to the systemic effects of several *C. perfringens* toxins, being released into the small intestine. It can cause sudden death in birds, cattle, sheep, goat, pig and foals (Pritchett, 1991).

It humans *C. perfringens* can cause two different types of diarrheal disease, Type A which is relatively mild and the most common cause of outbreaks in industrialised countries, and Type C, a rare necrotic enteritis. Type C has not been reported in Europe for over a decade (Brynestad and Granum, 2002). Most human outbreaks occur in restaurants, hospitals and homes for the elderly and fatalities are frequently reported (Labbé, 2000). There are no data on the number of reported cases in England and Wales, data from Northern Ireland show that reports of gastrointestinal infections caused by *C. perfringens* have doubled in the last two years (see table 1) and the majority of cases are in elderly people (www.cnscni.org.uk).

Table 1.1. Number of laboratory reports of gastrointestinal infections due to *C. perfringens* in Northern Ireland

Year	Number of *C. perfringens* cases
1992	8
1993	10
1994	7
1995	2
1996	11
1997	5
1998	12
1999	6
2000	10
2001	12
2002	20
2003	20

C. perfringens isolates are classified as 1 of 5 toxigenic types (A-E) based on the production of 1 or more of 4 major toxins namely, alpha, beta, epsilon and iota. Each bacterium may also express a variety of other toxin types and a *Clostridium perfringens* enterotoxin (CPE).

Table 1.2.Major lethal toxins of *C. perfringens* for type determination (Nilo, 1980)

Type	Toxins			
	Alpha	Beta	Epsilon	Iota
A	++	-	_	-
B	+	++	+	-
C	+	++	-	-
D	+	-	++	-
E	+	-	-	+

++ = Produced as a predominant toxic fraction.

\+ = Produced in smaller quantities

\- = Not produced

Type A is the most common of all *C. perfringens* types and is the most variable in toxigenic properties. Type A, possessing the *cpe* gene is associated with outbreaks of gastroenteritis in humans and livestock (Petit *et al.*, 1999). All 5 toxigenic types of *C. perfringens* are capable of producing CPE but it is mostly associated with Type A strains. CPE is a single polypeptide with a molecular weight of 3.5kDa and has the greatest effect in the small intestine. It has been implicated as one of the major virulence factors in food-borne disease and was first isolated in the 1970's (Stark and Duncan, 1971). CPE has been found to be highly conserved in Type A strains (Billington *et al.*, 1998). The production of CPE is regulated by sporulation (McClane 2001) and it has been recently shown that cells with a copy of CPE are also more heat tolerant (Sarker *et al.*, 2000).

C. perfringens has been isolated from antibiotic-associated diarrhoea in humans. The first report appeared in the mid 1980's, mainly from immuno-compromised patients. Antibiotics are assumed to remove the factors that usually prevent *C. perfringens* from colonising and a similar incidence of *C. perfringens* producing CPE and *C. difficile* has been reported (Hancock, 1997). The majority of studies concerning antimicrobial resistance in *C. perfringens* are from poultry.

C. perfringens in dogs

C. perfringens has been associated with diarrhoeal disease in dogs, ranging from mild, self-limiting illness to a potentially fatal acute haemorrhagic syndrome (Cassutto and Cook, 2002, Cave *et al.*, 2002, Marks *et al.*, 2002). However, the clinical significance of the presence of *C. pefringens* in dogs is distorted by the presence of these organisms as normal inhabitants of the intestinal flora. Enterotoxigenic *C. perfringens* has been associated with canine nosocomial diarrhoea (Kruth *et al.*, 1989), haemorrhagic enteritis (Rood *et al.*, 1991, Sasaki *et al.*, 1999) and acute and chronic large bowel diarrhoea (Carman and Lewis, 1983, Twedt, 1993). There is also a report of *C. perfringens* isolated from a UTI infection in dogs, although it is rarely a cause of UTI infection and there may have been an underlying cause (Gilardoni *et al.*, 1999).

A clear association between CPE and diarrhoea in dogs has been reported from various studies. A study by Marks *et al.* (2002) found that diarrhoea could result from a change in the intestinal environment, inducing sporulation of commensal *C. perfringens*, thus causing the production of CPE. The diet of the dog has been found to affect the prevalence of *C. perfringens* in the intestinal tract (Zentek *et al.*, 2003). These findings agree with those of Weese *et al.* (2001b), who detected CPE in 28% dogs with diarrhoea and 5% of dogs without diarrhoea. These studies suggest that *C. perfringens* is an opportunistic pathogen, only causing disease secondary to intestinal disruption by another pathogen. Enteric *C. perfringens* frequently proliferates in dogs with parvoviral enteritis, and studies have found *C. perfingens* to be present in 69% of dogs with parvovirus (Turk *et al.* 1992).

1.1.5 Antibiotic resistance mechanisms in specific pathogens

β-lactams

There are many different mechanisms by which bacteria are resistant to antibiotics. The predominant mechanism for resistance to β-lactam antibiotics is the production of β-lactamase enzymes, which can be produced by both gram-negative and gram-positive bacteria (Livermore, 1995). These enzymes are able to hydrolyse β-lactam

antibiotics, rendering them inactive. Two different schemes have been used to classify β-lactamases. Amblers's scheme (1980) involves four classes of β-lactamase based on amino acid sequence; classes A, B, C and D. Classes A, C and D have a serine residue in the active site whereas class B enzymes are less abundant and require a catalytic zinc for activity (Petrosino and Palzkill, 1996) The scheme developed by Bush, Jacoby and Medeiros (Bush[a] *et al.*, 1995) is based on four classes of β-lactamase, labelled 1 – 4. These are determined according to their substrate and inhibitor profiles. Class 1 are cephalosporinases that are not generally inhibited by clavulanic acid or related inhibitors, group 2, broad-spectrum β-lactamases that can be inhibited by clavulanic acid, group 3 metallo-β –lactamases and group 4 penicillinases that are not inhibited by clavulanic acid. Over 225 unique β-lactamases have now been identified (Bush and Miller, 1995), although only a few occur commonly. These are traditionally encoded by the *tem* and *shv* genes. TEM-type β-lactamases are the main mechanism of β-lactam resistance in enteric gram-negative bacteria (Blazquez *et al.*, 2000).

Extended spectrum cephalosporins and monobactams have traditionally been used in cases where β-lactamases are a problem but now extended spectrum β-lactamases have emerged (ESBLs). Of the 225 β-lactamases identified, 114 of these are ESBLs. ESBLs differ from the classical TEM-1, -2 and SHV-1 β-lactamases by their ability to hydrolyse third generation cephalosporins e.g. ceftazidime, cefotaxime and monobactams e.g. aztreonam, but do not affect cephamycins e.g., cefoxitin and cefotetan or carbapenems e.g., meropenem or imipenem (CDR Weekly, 2002). They typically have between 1-7 amino acid changes from TEM-1 and SHV-1 (Essack, 2000). The first report of a cephalosporin-hydrolysing β-lactamase was from Klebsiellae in 1982 (Hart and Percival, 1982). In 1983, a cephalosporin-hydrolysing β-lactamase, found to be mutant of SHV-1 β-lactamase, was reported in Germany. Four years later the first mutant of TEM β-lactamase was reported in France (Payne *et al.*, 1992).

ESBLs emerged gradually after the introduction of new β-lactamases but the number and variety has increased and disseminated at an unpredictable rate. To complicate matters ESBL's are usually encoded on plasmids that also carry genes conferring

resistant to aminoglycosides, chloramphenicol, sulphonamides, trimethoprim and other antimicrobials (Livermore and Williams, 1995).

Other mechanisms of resistance to β-lactam antibiotics include the production of AmpC β-lactamase. This is an inducible system that is usually responsible for low-level β-lactam resistance. Most members of the *Enterobacteriaceace* family contain chromosomally mediated AmpC-type β-lactamases. Occasionally, *E .coli* will hyper-produce AmpC β-lactamase which results in high level resistance to β-lactam antibiotics and combinations of β-lactams that have β-lactamase inhibitors (Normark *et al.*, 1980). Gene amplification or mutations at either the promoter and/or the attenuator of the structural β -lactamase gene result in AmpC hyperproduction (Caroff *et al.*, 1999). Nevertheless, AmpC is also being increasingly encoded on plasmids that may facilitate the spread of AmpC mediated resistance to other pathogenic bacteria (Philippon *et al.*, 2002).

The most common mechanism of β-lactam resistance in *E. coli* is the production of β-lactamase, TEM-1 being the most commonly encountered in *E. coli*, responsible for ampicillin resistance in over 90% of isolates (Baker, 1999). A study following resistance mechanisms in *E. coli* isolated from human clinical isolates over a three year period in France found that the two most frequent resistance mechanisms were hyper-production of chromosomal class C β-lactamases and the production of inhibitor resistant TEM (IRT) enzymes (Leflon-Guibout *et al.*, 2000). ESBL types can vary considerably between different countries. TEM-type ESBLs are relatively common in France and the US but SHV-type ESBLs seen to be more common in UK surveys (Bush and Miller, 1995).

E. coli (and other bacteria) can have the ability to produce broad-spectrum efflux pumps, these are generally involved in low-level resistance to multiple antibiotics including to some β-lactams, quinolones, chloramphenicol, rifampicin and tetracyclines. This results in a MAR (multiple antibiotic resistant) phenotype. Efflux pumps can also be responsible for resistance to some disinfectants such as pine oil and triclosan, and organic solvents such as cyclohexane (Alekshun and Levy, 1997, Moken *et al.*, 1997). Expression of *marA* and *soxS* is also inducible by a number of structurally unrelated substances including the antibiotics tetracycline and

chloramphenicol and both organic and inorganic substances such as dinitrophenol, paraquat and sodium salicylate (Seone *et al.*, 1995). Tolerance to cyclohexane found in *E. coli* indicates broad-spectrum efflux activity (Mazzariol *et al.*, 2000). This system may act as a 'stepping stone' to higher levels of resistance (Randall and Woodword, 2002).

NTS can posses a multiple antibiotic resistance (*mar)* locus that is structurally and functionally similar to that seen in *E. coli.* The *mar* locus is again, implicated in low-level resistance to β-lactams, cholamphenicol, quinolones and tetracycline (Randall and Woodward, 2001).

Multidrug-resistance phenotypes of NTS have been increasingly described worldwide. A recent 7 year study on NTS in Spain revealed that over the 7 year time period, ampicillin resistance increased from 8 – 44%, tetracycline resistance from 1 – 42%, chloramphenicol resistance from 1.7 – 26% and nalidixic acid from 0.1 – 11% (White *et al.*, 2002). Similar observations have been made in the UK (Threlfall *et al.*, 1993).

The most predominant strain isolated from humans in the UK until 1998 was *S.* Typhimurium DT 104 (Threlfall, 2000). This strain was first identified in the UK in 1984 (Threlfall *et al.*, 2000). This strain is multi-drug resistant and is typically associated with resistance to five antibiotics, ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline, although not all DT 104 isolates demonstrate this penta-resistant phenotype. It first emerged among cattle in England and Wales (Akkina *et al.*, 1999) and was subsequently isolated from poultry, pigs, sheep and then humans. It is largely responsible for epidemics throughout Europe, the USA and Canada. A recent outbreak in Singapore was due to contaminated imported dried anchovy. The outbreak lasted 3 months (Ling *et al.*, 2002), and involved 33 patients. Two people died in Denmark from *S.* Typhimurium DT 104 acquired from pork (Mølbak *et al.*, 1999). Cattle are recognised as a major reservoir for *S.* Typhimurium DT 104, although increasing numbers are being reported from other sources such as porcine and avian populations, especially poultry (Ridley and Threlfall, 1998). The observed high prevalence of this serotype is attributed to chromosomal integration of resistance genes (Threlfall *et al.*, 1994).

TEM appears to be the most commonly reported enzyme mediating β-lactam resistance in NTS although OXA-type and CARB-type β-lactamases have also been reported (Gallardo *et al.*, 1999). The appearance of resistance in NTS has been mainly attributed to TEM-type β-lactamases encoded on plasmids (Ling *et al.*, 1998). ESBLs are rarely found in NTS, although reports are increasing. Most ESBLs in NTS are attributed to mutations in common TEM-1, -2 and SHV-1 β-lactamases (Weill *et al.*, 2004). Until recently, NTS did not possess the *ampC* gene encoding AmpC β-lactamase. It was believed that the *ampC* gene may have been be too big a burden for NTS, having to sacrifice other attributes such as growth rate and invasiveness (Morosini *et al.*, 2000). There has been evidence to suggest that NTS gained a plasmid-mediated *ampC* β-lactamase from *E. coli* (Winokur *et al.*, 2001).

Methicillin resistance in *S. aureus* is a generally recognised as a marker for complete β-lactam antibiotic resistance. In many cases strains are also multi-resistant to many commonly used antibiotics such as macrolides, clindamycin and tetracycline. Figures from the PHLS show that clinical isolates of *S. aureus* from blood and cerebral fluid that are resistant to methicillin rose from 1.5% in 1989 – 1991 to 25% in 1998. In 2000, 42% of bacteraemic infections caused by *S. aureus* were MRSA. Simultaneous rises in resistance to aminoglycosides, macrolides and quinolones have also occurred.

High-level resistance to methicillin is a result of expression of the *mecA* gene. This encodes for a 78-kDa protein; penicillin binding protein 2A (PBP-2a) that has a much lower affinity for β-lactam antibiotics. *mecA* has been identified on a 40 kb mobile genetic element which is encoded by an extra gene rather than a mosaic gene. The gene shows high levels of homology between MRSA strains but is absent from methicillin susceptible strains. The *mecA* gene is often integrated on the chromosome by the genetic element encoding recombinases that can catalyse its excision from, and integration into, the chromosome (Wielders *et al.*, 2001). This gene can be spread horizontally, which is how *S. aureus* is thought to have first acquired the gene. The gene can be easily transferred to methicillin susceptible strains, facilitating the worldwide spread of MRSA (Moore and Lindsay, 2001, Wielders *et al.*, 2001).

There are three other mechanisms that have been reported to produce low-level resistance (MIC 4-8 $mg\text{-}l^{-1}$) occurring in the absence of *mecA*. These include over production of PBP-4 with lower affinity for β-lactams than PBP-1-3; modification of PBP–2 to a lower affinity molecule and over expression of β-lactamase.

Quinolone resistance

The most common mechanisms of quinolone resistance are the alteration of the drug targets i.e., DNA gyrase or topoisomerase IV (encoded by *gyrA, gyrB,* and *parC, parE* respectively). Hotspots called 'quinolone resistance determining regions' or QRDR, are where mutations in *gyrA* are most frequently located, even in very different bacteria such as *E. coli, Mycobacterium tuberculosis* and *E. faecalis* (Cabral *et al.*, 1997). Resistance to nalidixic acid can develop readily, and results from a point mutation in the QRDR and is usually non-transferable. Recently, plasmids (*qnr*) mediating low level resistance to the quinolones have been reported (Martinez-Martinez *et al., 1998*, Jonas *et al.*, 2005). Plasmids encode for a protein that directly protects gyrase from quinolone inhibition (Tran and Jacoby, 2002). The presence of *qnr* contributes to high level resistance through facilitating the selection of chromosomal mutations in QRDR (Martinez-Martinez *et al.*, 1998).

The number of reports of quinolone and fluoroquinolone resistant *E. coli* in humans and animals is increasing (Orden *et al.*, 2000). In Beijing from 1997-1999, 60% of infections *E. coli* strains isolated from human hospital acquired-infections were resistant to ciprofloxacin (Zhang *et al.*, 1997). Quinolone resistance in *E. coli* has been associated mostly with genomic mutations in regions of the *gyrA* and the *parC* genes (Vila *et al.*, 1996) with mutations involving *gyrB* and *parE* being less common (Nakamura *et al.*, 1989, Quabdesselam *et al.*, 1995).

Fluoroquinolones and erythromycin are the drugs of choice for *Campylobacter* spp. infections (Saenz *et al.*, 2000) but there are now increasing reports of resistance among *Campylobacter* spp. (Thwaites and Frost, 2000). Foreign travel, prior treatment and consumption of imported poultry are risk factors for acquisition or development of fluoroquinolone resistant strains in man (Hooper, 1999). High rates of

ciprofloxacin resistance of up to 62%, have been found in broilers (Van Looveren *et al.*, 2001), although recent studies have found much higher levels of fluoroquinolone resistance in human *Campylobacter* spp. isolates than in chicken isolates, thus suggesting acquisition of resistant *Campylobacter* spp. from another source (Cox, 2001, Moore *et al.*, 2002). Fluoroquinolone resistance has also been encountered in human isolates of *Campylobacter* spp. from countries where the antimicrobials have not been approved for use in food animals (Sjögren *et al.*, 1993, Gaudreau *et al.*, 1997).

Resistance among human clinical isolates of *Campylobacter* spp. has been followed by the PHLS between 1993 and 2001. In *C. coli*, ciprofloxacin resistance has remained steady though out the years with 22%-27% of the isolates showing resistance, whereas in *C. jejuni* ciprofloxacin resistance has increased steadily from 10% before 1997 to 14.8% in 1999/2000. The incidence of erythromycin resistance was consistently higher in *C. coli* than *C. jejuni*. In *C. jejuni* resistance remained constant between 1% and 3%, whereas in *C. coli* resistance increased during the late 1990's to 19.2% but decreased to 17.8% in 1999/2000 (Frost, 2001). *Campylobacter* spp. resistance to the fluoroquinolones also involves mutations in *gyrA* (Thwaites and Frost, 2000). Mutations in *gyrB* are rare and mutations have not been reported in *parC* or *parE* (Piddock, 2002).

Flouroquinolones are often the recommended drug of first choice for treating NTS infections and are useful for the treatment of multiple-resistant strains. The first report of the failure of ciprofloxacin therapy in clinical isolates of *Salmonella* spp. was in 1990 and since then reports have been rising (Piddock *et al.*, 1990, Frost et al., 1996). The high number of fluoroquinolone resistant NTS observed is thought to be due to overuse in animal husbandry, as with the case of *Campylobacter* spp. Quinolone resistance seen in NTS is again, usually due to mutations in *gyrA*, analogous to those seen in *E. coli*. All of the mechanisms of quinolone resistance so far described for NTS are chromosomally encoded and thus, the number of quinolone resistant bacteria can only increase in two ways, either the selection of quinolone resistance after exposure to quinolones or the spread of quinolone resistant bacteria. There is some evidence to suggest that quinolone resistant NTS arise in animals after fluoroquinolone exposure, following studies where resistance has arisen on farms

after the antibiotic was used (Fluit *et al.*, 2001, Walker *et al.*, 2000). An increase in veterinary NTS isolates that were resistant to nalidixic acid increased up to the year 1998 and a similar increase was also seen in human isolates (Threlfall *et al.*, 2000).

Flouroquinlone resistance in *S. aureus* involves modification of target enzymes (Nakamura, 1997) being similar to that seen in *E. coli*, involving the *gyrA* enzymes.

Tetracycline resistance

When resistance arises to tetracycline it is usually to all analogues of the antibiotic. Tetracycline resistance determinants are widespread among many bacterial species and three specific resistance mechanisms have so far been identified, tetracycline efflux, ribosome protection and enzymatic tetracycline modification. The genes responsible are often acquired and are associated with conjugative plasmids or transposons (Roberts, 1996).

Resistance genes associated with tetracycline efflux are, most commonly, *tetA, B, C, D, E, G, I, M. K.* Resistance genes associated with a ribosomal protection mechanism and/or efflux mechanism are mainly *tetK, L, M, O, S, P, Q, B, D, H* and *C*. The only gene associated with causing the enzymatic alteration of tetracycline is *tetX* (Roberts, 1996). These genes have been found in both gram-negative and gram-positive bacteria (Levy *et al.*, 1999), although *tetK, L* and *P* are mostly associated with gram-positive bacteria (Schnappinger and Hillen, 1996). There have now been over 29 different *tet* resistance genes identified (Chopra and Roberts, 2001), the most common being those mentioned above.

There are 2 types of efflux pumps involved in tetracycline resistance, multi-drug resistance pumps and tetracycline specific transporters. The majority of efflux pumps identified in both gram negative and gram-positive bacteria specifically transport tetracycline (Ma *et al.*, 1994). Most of the work on ribosomal protection has been carried out on *tetM*. The ribosome is able to continue with protein synthesis in the presence of tetracycline and the drug is still able to bind to the resistant ribosomes, but to no effect (Burdett, 1993).

High levels of tetracycline resistance in *E. coli* are seen in pigs and cattle. Sub-therapeutic levels of oxytetracycline had often been used as food additives for growth promotion (Ley *et al.*, 1994, DANMAP, 2002). Efflux mechanisms appear to be the major cause of resistance among gram-negative bacteria (Chopra and Roberts, 2001) and there have been many *tet* genes described in *E. coli* including *tetA, B, C, D, E, I* and *Y*.

Several tetracycline resistance genes have been reported in NTS including *tetA, B, C, D* and *G* (Frech and Schwarz, 1998, Chopra and Roberts 2001). *TetG* has been identified as being responsible for tetracycline resistance in *S.* Typhimurium DT104, being present on *Salmonella* genomic island 1 (Cloeckaert and Schwarz, 2001).

Tetracycline resistance encoded by *tetO* (ribosomal protection) has been described on plasmids in *C. jejuni* and *C. coli* (Taylor *et al.*, 1987). Plasmids encoding tetracycline resistance in *Campylobacter* spp. have also been shown to transfer horizontally (Avrain *et al.*, 2004). Tetracycline would not normally be used for the treatment of campylobacteriosis, however, because resistance is plasmid mediated (Taylor *et al.*, 1987) it is useful to monitor it.

Two main mechanisms of tetracycline resistance have been described in *S. aureus*. Active efflux, resulting from the acquisition of plasmid encoded *tetK* and *tetL* genes, and ribosomal protection, mediated by chromosomally or transposon encoded *tetM* and *tetO* (Trzcinski *et al.*, 2000).

There have been many reports of tetracycline resistance in *C. perfringens* isolates from veterinary sources (Rood *et al.*, 1991, Alexander *et al.*, 1995, Lyras *et al*, 1996). Published reports on tetracycline resistance genes include *tetA, tetB, tetP* and *tetM* (Sloan *et al.*, 1994, Lyras and Rood, 1996). *Tet* genes have been found in *C. perfringens* both alone (Sasakiet *et al.*, 2001, Lyras *et al.*, 1996) and with a second different resistance gene (Lyras *et al.*, 1996).

Trimethoprim resistance

Bacterial resistance to trimethoprim is due to a variety of mechanisms and can be chromosomally or plasmid encoded. The main mechanism of resistance is due to reduction in the sensitivity of dihydrofolate reductase (DHFR) to trimethoprim. This is encoded by over 20 different genes, which are divided into class A and class B. Class A are termed *dfrA1* onwards. The exact origin of these enzymes is not known but they are believed to have spread horizontally. The majority of *dfr* genes occur within gene cassettes, these being inserted into class 1 and class 2 integrons which can also harbour either sulphonamide (*Sul1*) or aminoglycoside resistance, the most frequently encountered is *aadA1* (Shaw *et al.*, 1993). They are extremely diverse through a wide variety of organisms (Sundström *et al.*, 1995), although class 1 integrons are most commonly located in clinical isolates of gram-negative bacteria (Yu *et al.*, 2003). The Class B family includes IIa, IIb, IIc encoding for variants of DHFR and being completely unrelated to other DHFR.

The most prevalent resistance gene seen in gram-negative bacteria appears to be *dfrA1* (Sköld, 2002). Chromosomally encoded resistance to trimethoprim involves increased production of chromosomal DHFR and similar mechanisms have been in seen in a variety of organisms (Flensberg and Sköld, 1987, de Groot *et al.*, 1996). Low level resistance to trimethoprim has also been reported through the ability of the bacteria to loose their ability to methylate deoxyuridylic acid to thymidylic acid, making them dependant on an external supply of thymine (King *et al.*, 1983, Hamilton-Miller, 1984).

Trimethoprim resistance in human isolates of *E. coli* has been increasing (Livermore *et al.*, 2000). The most common mechanism of resistance to trimethoprim in *E. coli* is acquisition of the *dfr* genes, and there have been many *dfr* genes described in *E. coli* isolates from both hospitalised and healthy humans, including *dfrA1, dfrA5, dfrA7, dfrA9, dfrA12, dfrA17* (Adrian *et al.*, 1995, Lee *et al.*, 2001). d*frA9* was originally found in *E. coli* isolates from swine, being located on transferable plasmids (Jansson and Sköld, 1991). This gene has been found in *E. coli* isolates from veterinary sources but rarely from human sources (Jansson *et al.*, 1992). *Dfr* genes in *E. coli* have often been described on integrons and cassettes, often conferring resistance to streptomycin

and spectinomycin (*aadA* genes, Chang *et al.*, 2000) and the combination of trimethoprim-sulfamethoxazole (Paulsen *et al.*, 1993). The most frequent gene cassettes located in *E. coli* are *dfrA17-aadA5* and *dfrA12-aadA2* (Yu *et al.*, 2003).

Trimethoprim was the primary antibiotic of choice but resistance has arisen to it, especially in *C. jejuni*, and now *Campylobacter* spp. are considered intrinsically resistant to this antibiotic. Trimethoprim resistance in *C. jejuni* has been shown to be linked to the acquisition of foreign genes into the chromosome. The most commonly occurring genes in human strains of *Campylobacter* spp. are *dfrA*1 and *dfrA*9 encoding resistance variants of the dihydrofolate reductase enzyme (Sköld, 2002).

Chloramphenicol resistance

Resistance to chloramphenicol occurs either enzymatically, encoded by the *cat* genes (I-III) or non-enzymically through drug efflux pumps, thought to be encoded by the *cmlA* gene. The most common cause of resistance to chloramphenicol is enzymatically through acetyltransferases. Chloramphenicol acetyltransferase (CAT) catalyses the transfer of an acetyl group from acetyl-CoA to both hydroxyl groups on the drug, preventing its binding to the ribosome and rendering the drug inactive. *catI* was the first gene to be described (Alton and Vapnek, 1979) and is the most widespread in gram-negative bacterium such as *E. coli* and NTS *cat* genes can be chromosomally or plasmid encoded.

Non-enzymatic resistance to chloramphenicol was first noted when bacterial cells were exposed to sub-inhibitory levels of chloramphenicol (Naghi and Mitsuhashi, 1972, Gaffney *et al.*, 1981) and the *cml* gene was first isolated from plasmids from *Pseudomonas aeruginosa* (Rubens *et al.*, 1979). Different variants of the *cml* gene have now been identified in gram-negative bacteria, examples being, *cmlA2* (Ploy *et al.*, 1998) and *cmlA4* (Poirel *et al.*, 2000). The *flo* gene also encodes an efflux pump that confers resistant to both chloramphenicol and florfenicol and shares 57% amino acid sequence identity to *cmlA*.

Chloramphenicol resistance in *C. perfringens* has been reported to be due to acetylases, *catP* and *catQ*,

Macrolide resistance

Resistance to macrolides emerged soon after their launch in 1956, first being seen in *Staphylococcus* spp. (Weisblum, 1995). Three different mechanisms have been identified; these include target modification, efflux of the antibiotic and drug inactivation.

The most common cause of resistance to macrolides is by modification of its target on the ribosome by methylation. This leads to cross-resistance to macrolides, lincosamides and streptogramins B, which has been given the name of the MLS_B phenotype (Weisblum, 1995). Expression of the MLS_B phenotype can be inducible or constitutive (Leclercq, 2002). When resistance is inducible, mRNA unable to encode methylase is produced which becomes active in the presence of erythromycin. Resistance seen in this phenotype is encoded by a number of *erm* (erythromycin ribosome methylase). Erm proteins methylate a single adenine residue in 23S rRNA, so binding of erythromycin, and other antibiotics is impaired.

Nearly 40 *erm* genes have so far been identified (Roberts *et al.*, 1999), the majority being encoded on plasmids and self-transmissible. There are four major groups in pathogenic bacteria, these being *ermA, ermB, ermC* and *ermF* (Weisblum, 1995, Roberts *et al.*, 1999).

In *Campylobacter* spp. few studies have focused on mechanisms of resistance to the macrolides. Mutations have been identified in the 23S rRNA genes, in erythromycin resistant *C. jejuni* and *C. coli* (Trieber and Taylor, 2001, Vacher *et al.*, 2003). A recent study by the VLA on *C. coli* isolates from slaughterhouses showed that 80% of *C. coli* were resistant to erythromycin. A sharp decline in erythromycin resistance was seen in pigs in Denmark between 1998 and 1999 following the decline in use of the macrolide tylosin as a growth promoter (DANMAP 2000).

Erythromycin resistance in *C. perfringens* is most commonly due to *ermP* gene (Farrow *et al.*, 2002).

Resistance seen in *S. aureus* is encoded by *ermA* in MRSA strains. This gene is present on transposons. *ermC* in methicillin strains is often present on plasmids (Leclercq, 2002).

Aminoglycoside resistance

The main mechanisms of resistance to aminoglycosides include decreased uptake and /or accumulation of drug, or expression of enzymes that that modify the antibiotic and render it inactive. Decreased uptake mostly seen in gram-negative bacteria is due to membrane impermeabilisation. Underlying mechanisms are still largely unknown (Mingeot-Leclercq *et al.*, 1999).

Aminoglycoside modifying enzymes catalyze the covalent modification of amino or hydroxyl groups, leaving the drug unable to bind properly to the ribosome. There are three main enzymes, acetyltransferases (AAC) that affect amino groups, and nucleotidyltransferases (ANT) and phosphotransferases (APH) that affect hydroxyl groups. The enzymes act on different groups at different positions on the structure of the drug. The groups affected in typical aminogylcosides are positions 3, 2', and 6' for ACC, 4' and 2" for ANT and 3' and 2" for APH (Mingeot-Leclercq *et al.*, 1999). Enzymes are usually plasmid encoded but are also associated with transposable elements. Enzymatic modification is the most common mechanism of resistance in both gram negative and gram-positive bacteria.

In *E. coli*, many variants of the enzyme modifying enzymes described above have been identified and aminoglycoside resistant *E. coli* may also carry genes for trimethoprim resistance (being inserted on gene cassettes) as also mentioned above. Combinations of common gentamicin modifying enzymes with ACC(6')-I, an enzyme that acetylates tobramycin, netilmicin and amikacin are frequently found in *E. coli* and the frequency of combinations is often found to vary within geographical regions (Miller *et al.*, 1997).

Genes encoding enzyme modifying enzymes have also been reported in NTS including *aadA1*, *aadA2*, *aacC2*, *Kn*, *aph(3)-IIa*, and *aac(3)-Iva*. *aadA2* has been found to be responsible for resistance seen in *S.* Typhimurium DT104 (Briggs and Fratamico, 1999).

The main mechanism of resistance to aminoglycosides in *S. aureus* is again, drug inactivation by cellular aminoglycoside-modifying enzymes, acetyltransferase (AAC), although adenylyltransferase (ANT) and phosphotransferase (APH) have been reported from clinical isolates of *S. aureus*. Resistance to tobramycin and kanamycin are mediated by AAC(6') and APH(2") (Ubukata *et al.*, 1984) whereas resistance to neomycin, kanamycin, tobramycin and amikacin is mediated by an ANT(4')-I enzyme (Bryne *et al.*, 1991).

Anaerobic bacteria such as *C. perfringens* are intrinsically resistant to aminoglycosides due to low membrane potential (Bryan *et al.*, 1979).

Glycopeptide resistance

Resistance to glycopeptide antibiotics is mediated by the *van* genes. This group of genes encode for ligases, enzymes that catalyse the peptidoglycan precursors. Resistance to antibiotics usually arises fairly quickly after introduction into clinical use, whereas for vancomycin very little resistance was seen for 30 years, first reports emerging in 1988 (Leclercq *et al.*, 1988). Since then glycopeptide resistance has become widespread and is worrying, as these antibiotics are drugs of last resort for MRSA infection.

There have now been reports of *S. aureus* becoming resistant to vancomycin. In 1996 the first report of *S. aureus* with reduced susceptibility to vancomycin was documented from a patient who had a surgical wound infected (Hiramatsu *et al.*, 1996). These isolates are named VISA, vancomycin intermediate *S. aureus*, or now GISA, glycopeptide intermediate *S. aurues*. Isolates soon appeared in the USA, France (Barker, 1999), Korea (Kim *et al.*, 2000) and Thailand (Trakulsomboon *et al.*,

2001). The first report of vancomycin-resistant *S. aureus* (vancomycin MIC>128mg/L) was from Michigan, USA being isolated from the tip of a dialysis catheter (MMWR, 2002). This was due to the acquistion of high-level resistance, *vanA* genes mostly associated with vancomycin resistance in enterococci and conjugative transfer of *vanA* from enterococci has been demonstrated in vitro (Noble *et al.*, 1992).

Vancomycin resistance in enterococci is encoded by several genes – *van*A, *van*B, *van*C1, *van*C2, *van*C3, *van*D and *van*E. In several cases they have been reported to be located on conjugative plasmids or transposons (Aarestrup *et al.*, 2000, Rice *et al.*, 1998).

The *van*A associated phenotype consists of high level resistance to vancomycin (MICs 64->1000μg/ml) and teicoplanin (MICs 16-512μg/ml) and is often found to be located on transposons, the most commonly described being Tn*1546*. *van*A is the most commonly found gene among farmyard animals and is the predominant type of resistance reported in clinical isolates throughout Europe (Cetinkaya *et al.*, 2000). The 38 kDa *vanA* resistance protein of *E. faecium* is homologous with gram negative D-ala-D-ala ligases. Ligases are the enzymes that catalyse the synthesis of the terminus of peptidoglycan precursors. Expression of the *vanA* gene and other genes involved in the expression of vancomycin results in abnormal synthesis of peptidoglycan precursors terminating in D-ala-D-lactate, rather than D-ala-D-alanine for which vancomycin has a much lower affinity. The vanA protein cannot confer resistance alone and enterococci must acquire all the genes within the *vanA* operon for the system to operate properly.

The *vanB* associated resistance phenotype consists of variable levels of vancomycin resistance (MICs 4-1024μg/ml) and no teicoplanin resistance (MICs ≤0.5μg/ml) and is more commonly located on the host chromosome. Resistance is also mediated by an abnormal ligase that is structurally related to the *vanA* encoded ligase also producing D-ala-D-lactate rather than D-ala-D-ala, hence lowering the ability of vancomycin to bind. The *vanB* phenotype is still susceptible to teicoplanin as this antibiotic induces the synthesis of *vanA* related proteins but not those of *vanB*. *vanB* strains are fairly common in the USA but *vanA* still predominates. This is possibly due to the *vanA*

gene cluster often being located on a transposon that can be part of transferable plasmids, whereas the *vanB* cluster is located on the host chromosome, however, it can still be transferred on plasmids as part of large mobile elements.

The *vanA* and *vanB* resistance phenotypes are both primarily described in *E. faecalis* and *E. faecium*, although the *vanA* ligase has also been found in other enterococcal species as well as other bacteria such as *Lactococcus* spp. and *Corynebacterium* spp. (Teuber *et al.*, 1999, Power *et al.*, 1995).

The *vanC* genes are usually intrinsic to species of enterococci that are not as commonly isolated such as *E. gallinarum* and *E. casseliflavus*. This encodes for low-level resistance to vancomycin with MICs of around 2-32μg/ml. The nucleotide sequences of the *vanC-1* gene in *E. gallinarum*, the *vanC-2* gene in *E. casseliflavus* and the *vanC-3* gene in *E. flavescens* have been published but there is still some disagreement over whether *E. flavescens* is a legitimate enterococci species (Clark *et al.*, 1998). The *vanC* ligase of *E. gallinarum* produces an additional ligase, producing D-ala-D-ser to which vancomycin binds weakly. It is thought that the presence of variable amounts of both D-ala-D-ala and D-ala-D-ser produce the different levels of vancomycin resistance. Resistance can be inducible or constitutive (Sahm *et al.*, 1995). The *vanC-2* gene of *E. casseliflavus* also produces an additional ligase that shows 66% homology to *vanC-1*. There is extensive homology of 98% between the gene sequences of *vanC-2* and the *vanC-3* of disputed *E. flavescens* (Clark *et al.*, 1998).

The *vanD* resistance phenotype was first described from a patient in a New York hospital in 1991 (Perichon *et al.*, 1997) appearing in *E. faecium*. It appears located on the chromosome and partial sequencing shows that it is similar to the *vanA* and *vanB* ligase enzymes. It is not transferable to other enterococci species.

The *vanE* resistance phenotype has been described in *E. faecalis* conferring low levels of resistance to vancomycin (MICs 16μg/ml) but susceptibility to teicoplanin. It shows similarities to the intrinsic *vanC* type of resistance found in less commonly isolated species of enterococci.

1.1.5 Antibiotic resistant bacteria among companion animals

Antibiotic resistant E. coli from dogs

A high prevalence of antimicrobial resistance in *E. coli* has been found in companion animals such as dogs and cats (Monaghan *et al.*, 1981, Moss and Frost, 1984, Normand et al., 2000), and multi-drug resistant *E. coli* have been reported from nosocomial infections (Sanchez *et al.*, 2002). There have been many studies investigating antibiotic resistant *E. coli* from UTI infections in the dog (Teshager *et al.*, 2000, Cooke *et al.*, 2002, Férina *et al.*, 2002, Sanchez *et al.*, 2002, Lanz *et al.*, 2003, Drazenovich *et al.*, 2004) but fewer studies have investigated the faeces of healthy dogs (Moss and Frost, 1984). A recent study by De Graef *et al.* (2004) observed much lower levels of multi-drug resistant *E. coli* in faeces from dogs in kennels compared to privately owned dogs.

Resistance to enrofloxacin has been observed in *E. coli* isolates from UTI infections in dogs (Cooke *et al.*, 2002) and *E. coli* possessing *tem*-1, *shv*, *oxa*-1 and *AmpC* genes encoding β-lactamases have been isolated (Teshager *et al.*, 2000, Féria *et al.*, 2002). Ampicillin resistant *E. coli* have also been isolated from cats (Moss and Frost, 1984).

Studies investigating tetracycline resistance in *E. coli* have observed *tetA* and *tetB* genes from urinary tract infections in both dogs and cats (Lanz *et al.*, 2003). A study by Bryan *et al.* (2004) detected the presence of the *tetA*, *tetB* and *tetC* gene in *E. coli* isolates from healthy dog faecal samples and a small percentage of *E. coli* isolates contained two tetracycline resistance genes. This study also found *tetA* and *tetB* in *E. coli* isolated from cat faecal samples. Chloramphenicol resistant *E. coli* has also been isolated from wound and UTI infections (Sanchez *et al.*, 2002). Trimethoprim-sulfamethoxazole resistant *E. coli* have also been observed in UTI infections in dogs and the same study found significantly different PFGE patterns in isolates, indicating horizontal acquisition of resistance plasmids or integrons (Drazenovich *et al.*, 2004). Studies have found *dfrA17* to be present on a gene cassette in *E. coli* from UTI and wound infections in dogs (Sanchez et al., 2002).

Antibiotic resistance in Campylobacter spp from dogs

Higher rates of antibiotic resistant *Campylobacter* spp. have also been found in stray dogs when compared to pet dogs. A study from Trinidad reported that 26.2% of stray dogs, compared to 5.4% of pet dogs had *Campylobacter* spp. isolates resistant to 1 or more of the antimicrobials they were tested against (Adeynsin *et al.*, 1999). A study by Preston *et al.* (1990) has reported isolates of *C. upsaleinsis* from dogs that were resistant to trimethoprim and teicoplanin.

Antibiotic resistant NTS from dogs

S. Typhimurium DT 104 represents roughly one third of NTS isolates from cats and dogs (BSAVA News, 1997, Van Immerseel *et al.*, 2004). There are very limited reports on antibiotic resistant NTS from dogs. Outbreaks of multi-drug resistant *S.* Typhimurium have been reported from Veterinary centres in Idaho, Minnesota and Washington (MMWR, 2001). A recent study in Trinidad (Seepersadsingh et al., 2004) found 85.1% NTS isolates from non-diarrhoeic dogs were resistant to one or more antimicrobial agents tested against.

VRE in dogs

In the USA, VRE has never been isolated from pets (Coque *et al.*, 1996), although isolates have been found in companion animals from countries outside of the USA. Studies have found carriage rates as high as 48% (Deveriese *et al.*, 1996, van Belkum *et al.*, 1996) from dogs on farms and hospitalised dogs in Europe. VRE have also been isolated from dry dog food sold in the USA (Dunne *et al.*, 1996). There have been many reports stressing the absence of VRE from companion animals (Harward *et al.*, 2001, Wagenvoort *et al.*, 2003). Published data from a study by Willems *et al.*, (2000) found similarities between VRE isolates from human patients and cats and dogs, using amplified length polymorphism analysis. VanA is the most common *E. faecium* phenotype isolated dogs from dogs (van Belkum *et al.*, 1996).

Antibiotic resistant C. perfringens in dogs

There have been few reports of antibiotic resistant *C. perfringens* from dogs. Resistance to erythromycin, tylosin and metronidazole, has been documented but the prevalence of resistant *C. perfringens* isolates was very low (Marks et al., 2003).

In conclusion, the presence of pathogenic and antibiotic resistant bacteria from dog faecal samples is not well documented in the UK. Therefore, the aims of this Ph.D. study were:

- To conduct a cross sectional study to investigate the prevalence and antimicrobial susceptibility of *E. coli, Campylobacter* spp., *Salmonella* spp., *C. perfringens*, VRE and MRSA in dog faecal samples. Faecal samples will be collected from parks and dogs resident in boarding kennels, rescue kennels and households to assess communal situations as a risk factor for carriage of the above bacteria. Faecal samples will also be from both healthy dogs and dogs with diarrhoea to assess the above bacteria as a cause of diarrhoea in dogs.
- To carry out a longitudinal study to assess dogs as a potential zoonotic risk for humans, by investigating transmission of pathogenic and commensal bacteria between them.

Chapter 2

Materials and Methods

2.1 Collection of dog faecal samples

Dog faecal samples were collected in a sterile universal container using a scoop (Greiner Bio-one, Stonehouse, UK). All samples were graded for consistency using a faecal consistency chart, grade 1 being solid faeces and grade 5 being diarrhoea/ liquid faeces. The chart used was published in the Waltham magazine (appendix, A1.5, permission kindly given by Glyn Moxham)

2.2 Processing of faecal samples

Approximately 2 grams of faeces was placed into a sterile bijou bottle and an equivalent volume of brain heart infusion broth (LABM, LAB51) containing 5% glycerol. Samples were mixed to create a faecal emulsion the remainder of which was stored at –80°C for future use.

2.2.1 Isolation of *E. coli* from faecal samples

Two drops of faecal emulsion were added to 3 ml of Brilliant Green Bile broth (LABM LAB51, Bury, UK). Broths were incubated at 37°C for 24 hours under aerobic conditions, then streaked onto Eosin Methylene Blue agar (EMBA), (LabM, LAB61) and incubated under the same conditions. *E. coli* ATCC 10536 was used as a positive control.

Plates were examined for the presence of typical *E. coli* colonies, 1-3 mm round colonies with a metallic sheen. Three suspect colonies were then selected and subcultured onto nutrient agar (LABM, LAB8) and incubated as above for 24 hours.

Biochemical identification

Each isolate was subjected to the following tests for identification of *E. coli.*

Gram stain

A smear of the isolate was prepared in sterile water, air dried and heat fixed. The smear was stained by flooding the slide with crystal violet for 1 minute, Lugol's iodine (Pro-lab Diagnostics, Neston, UK) for 1 minute, then destained with acetone and counter stained with safranin for 2 minutes. The slide was examined under oil immersion using x100 magnification.

E. coli cells are small, Gram negative rods.

Catalase and Oxidase test

Catalase- A drop of 3% hydrogen peroxide (Sigma, Basingstoke, UK) was placed a clean glass slide and a colony was added and emulsified. The production of bubbles was indicative of catalase production.

Oxidase- A colony was smeared onto an oxidase strip (Mast, Bootle, UK, ET04). A colour change to purple within 30 seconds was indicative of oxidase activity.

E. coli should be catalase positive and oxidase negative.

Suspect *E. coli* isolates were subcultured onto Tryptone Soy agar (LabM, LAB11) and MacConkey agar (LabM, LAB30), and incubated at 37°C for 24 hours. Isolates were also subcultured onto Simmon's Citrate agar (LabM, LAB69) and incubated at 37°C for 48±4 hours.

Indole test

A small strip of filter paper was flooded with Kovac's reagent (bioMerieux, Basingstoke, UK, 55631) and placed onto colonies of the suspect isolate which had been grown on Tryptone soy agar. *E. coli* isolates turns the filter paper pink due to indole production.

Lactose fermentation

E. coli ferments lactose which is indicated by the colonies being pink on MacConkey agar.

Citrate test

E. coli cannot utilise citrate, therefore *E. coli* isolates will grow poorly on Simmon's Citrate agar.

Due to time limitations, from January 2002 biochemical testing was discontinued and PCR for the β-glucouronidase gene, present in all *E. coli* strains was used as a replacement (see 2.6.4).

2.2.2 Isolation of *Campylobacter* spp. from faecal samples

Two drops of faecal emulsion were added to 3 ml of Campylobacter Enrichment Broth (LABM, LAB135) containing 5% lysed horse blood (Southern Laboratories Group, Corby, UK). No antibiotic supplement was added as this can inhibit the growth of some species of *Campylobacter* such as *C. upsaliensis* (Koene *et al.*, 2004). Laboratory strain LS002 (*C. coli*) and LS 017 (*C. upsaliensis*) were used as positive controls. Broths were incubated at 37°C for 24 hours under microaerophilic conditions (BOC, Guildford, Surrey). Broths were then used to inoculate Campylobacter Selective agar (LABM, LAB21) containing Cefoperazone, Amphotericin (CA) antibiotic supplement (LabM, X112), and incubated as above for 3-5 days. Three suspect colonies were taken from each plate and subcultered onto Columbia agar (LABM, LAB1) containing 5% defribinated horse blood (Southern Laboratories Group).

Biochemical identification

Each isolate was subjected to the following biochemical tests for presumptive *Campylobacter* spp. identification.

Gram stain

This was carried out as previously described.

Campylobacter spp. are small, gram negative curved rods

Catalase and oxidase tests

These were carried out as previously described.

Campylobacter spp. should be positive for both catalase and oxidase activity, although *C. upsaliensis* can produce weak or negative catalase reactions.

Growth in O_2

Isolates were subcultured onto Columbia agar supplemented with blood and incubated aerobically at 37°C for 48 hours. *Campylobacter* spp. should not grow aerobically.

Identification of Campylobacter genus and species

This was carried out using PCR, as described later in the chapter (2.6.3.).

2.2.3 Isolation of NTS from faecal samples

One ml of faecal emulsion was added to 10ml of buffered peptone water (LABM LAB46) and incubated at 37C for 24 hours under aerobic conditions. *S.* Typhimurium DWC 2578 was used as a positive control.

Initially, 1ml of this solution was added to 10ml of Rappaport-Vassiliadis broth (LABM, LAB86) and incubated at 42°C for 48 hours. Broth was streaked out onto Xylose Lysine Decarboxylase (XLD) agar (labM, LAB032-A) and incubated as above for 24 hours. Plates were then examined for suspect colonies: i.e. round and glossy with a pink halo. Later, due to time limitations, 0.1ml of buffered peptone water was added to the centre of semi-solid Rapapor-Vasilius semi-solid medium (LABM, LAB150) and incubated as above for 24 hours. A spreading growth over plates indicates the presence of *Salmonella* spp. Suspect isolates from both methods were subcultured onto nutrient agar and incubated for 24 hours as above.

Biochemical identification

Each isolate was subjected to the following biochemical tests for presumptive NTS identification.

Lactose fermentation

This was carried out as above. NTS do not ferment lactose and therefore yellow growth will indicate a positive result.

Agglutination tests

Three drops of normal saline (0.8%) were placed on a clean glass slide and an emulsion of the suspect isolate was prepared in each drop. A drop of polyvalent O (Pro-lab Diagnostics) antisera was added to the first bacterial emulsion and a drop of polyvalent H antisera (Pro-Lab diagnostics) was added to the second drop. The slide was then gently rocked from side to side and each drop observed for the presence of agglutination.

NTS should be positive for both O and H antigens tests, although sometimes NTS may only be positive for O antigen only. The drop without any antisera should not agglutinate.

Urea utilisation

Suspected isolates were subcultured onto Christensen's urea agar slopes (LABM, LAB130), containing 40% urea, turning red/pink. NTS should ferment urea and be positive for this test.

API

All suspected NTS were further confirmed to be *Salmonella* spp. using the Api 20E test strips (bioMerieux).

Serotyping

All suspected isolates were sent to the Department of Medical Microbiology (University of Liverpool) for specific anti-O and anti-H antisera agglutination tests for serotyping (Kaufmann and White typing scheme, Murex).

2.2.4 Isolation of vancomycin-resistant *Enterococci* (VRE) from faecal samples

Two drops of faecal emulsion were added to 3ml of Kanamycin Aesculin Azide broth (LABM LAB107) supplemented with 0.5mg/l of bile salts (LABM). Broths were incubated at 37°C for 24 hours in aerobic conditions. Broths were then used to inoculate Kanamycin Aesculin Azide agar (LABM, LAB106) with a 5μg vancomycin disc (MAST) placed on the middle using a sterile needle. Plates were incubated as above for 48 hours. VRE 149 *E. faecalis* (kindly provided by Dept Medical Microbiology) was used as positive control.

VRE colonies are small, black and round with a halo, exhibiting growth to the vancomycin disc. Three suspect isolates were taken and subcultured onto Columbia agar containing 5% horse blood and incubated as above for 24 hours.

Biochemical identification
Each isolate was subjected to the following biochemical tests for presumptive VRE identification.

Gram stain
This was carried out as previously described.
Enterococci should appear as Gram positive cocci.

Species identification
This was carried out in a multiplex PCR reaction together with the van genes responsible for vancomycin resistance. This is described later in the chapter (see 2.6.7).

2.2.5 Isolation of methicillin-resistant *Staphylococcus aureus* (MRSA) from faecal samples

Faecal emulsion was used to inoculate Mannitol Salt agar (LABM LAB007) containing 2mg/l oxacillin (Mast Diagnostics MS29). EMRSA 15 and 16 were used

as positive controls. Plates were incubated at 37°C for 48 hours under aerobic conditions. Suspect colonies appear as yellow, circular and shiny. Three colonies were subcultured onto Columbia agar supplemented with 5% horse blood and incubated as above for 24 hours.

Biochemical identification

Each isolate was subjected to the following biochemical tests for presumptive MRSA identification.

Catalase and oxidase tests

These were carried out as previously described.

S. aureus should be positive for both of the tests.

Methicillin resistance

Suspect colonies were subcultured onto Columbia agar suplemented with 5% defribinated blood and a methicillin strip placed across the culture using a sterile needle. Plates were incubated as above for 24 hours. Methicillin resistance was indicative of growth to the methicillin strip.

API

All suspect MRSA isolates were further confirmed using the API Staph test strips (bioMerieux).

2.2.6 Isolation of *Clostridium perfringens* from dog faecal samples

Half ml of faecal emulsion was added to pre-reduced cooked meat granules (Lab M, LAB71) which was prepared with fastidious anaerobe broth (Lab M, LAB09) and incubated for 24-48 hours at 37°C under anaerobic conditions (MACS VA anaerobic workstation, N_2: 80%, CO_2: 10%, H_2: 10%, BOC gas). Strain DWC 2485 (kindly provided by Don Whitley Scientific, Shipley, UK) was used as a positive control. Broths were then plated out onto Shahidi-Ferguson Perfringens agar (Oxoid) containing 10% egg yolk emulsion and incubated as above. Typical colonies of *C. perfringens* have black centres and opalescent surrounding zones.

Plates which were covered with swarming organisms were discarded and the sample's original broth containing the sample was heat shocked by heating in a water bath at 70°C for 10 minutes, and then plated onto Perfringens agar and incubated as above. Typical colonies of *C. perfringens* were then plated onto Columbia agar supplemented with 5% horse blood.

Biochemical identification

Each isolate was subjected to the following biochemical tests for presumptive *C. perfringens* identification.

Gram staining

This was performed as previously described. *C perfringens* appear as large gram positive rods (brick shaped), although cultures can appear gram negative or with granules if not fresh.

Urease tests

These were performed using Christensen's urea agar slopes containing 40% urea. Slopes were inoculated with the suspect isolate and incubated under anaerobic conditions as above for 48 hours. *C. perfringens* should be negative for this test.

Indole test

This was performed using anaerobic identification media (AIMS, appendix A2.1) with the addition of 4g tryptone (Lab M, MC5). Suspect isolates were inoculated onto plates and incubated under anaerobic conditions for 24 hours at 37°C. Five microlitres of Kovac's reagent was dropped onto each grown colony. *C. perfringens* should be negative for this test.

Lactose fermentation test

This was performed using AIMS agar supplemented with 10% lactose. Agar in petri dishes was cut into 6 sections and each section inoculated with a suspect isolate. One section on each plate was left as a negative control. Plates were incubated as above. Plugs of agar were then removed and placed into the wells of a microtitre plate. Three drops of 0.004% aqueous Bromophenol Blue was added to each well. If fermentation

had occurred the solution turned yellow, pale green for weak fermentation and dark green for no fermentation. *C. perfringens* should ferment lactose.

2.3 Antibiotic sensitivity testing

2.3.1 Antibiotic sensitivity testing of *E. coli* and NTS isolates

All *E. coli* isolates were tested for antibiotic susceptibility using the disc diffusion method according to the British Standards of Antimicrobial Chemotherapy (BSAC) guidelines (2002).

An overnight growth of each *E. coli* isolate on nutrient agar was suspended in of 3ml sterile distilled water consistent with a 0.5 McFarlands standard. A sterile swab was dipped into the suspension and used to inoculate Isosensitest agar plates (Lab M) evenly using a spiral plater. Six different antibiotics (Mast Diagnostics) were placed on the inoculated agar using a sterile needle, ampicillin (10μg), apramycin (30μg), chloramphenicol (30μg), nalidixic acid (30μg), ciprofloxacin (1μg), tetracycline (30μg) and trimethoprim (2.5μg). Plates were incubated at 37°C in aerobic conditions for 24 hours. Resistance was indicted by growth reaching the antibiotic disc within the acceptable range as according to BSAC guidelines. Zone sizes were read with a ruler.

2.3.2 β-lactamase production

Ampicillin resistant *E. coli* isolates were tested for β-lactamase production using nitrocefin (LAB M). A positive result is indicated by a colour change from yellow to pink within 10 minutes.

2.3.3 Extended spectrum activity

Ampicillin resistant *E. coli* isolates were tested against a range of β-lactams and cepholosporins to test for extended spectrum activity.
This was carried out as above but using the antibiotic discs (Mast diagnostics) aztreonam (30μg), co-amoxyclav (30μg), ceftazidime (30μg), cefoxitin (30μg), cefuroxime (30μg), piperacillin and tazobactaum (75μg and 10μg).

2.3.4 Spectinomycin, streptomycin and sulphamethoxazole resistance

Trimethoprim resistant *E. coli* isolates were tested for susceptibility to spectinomycin, streptomycin, and sulphamethoxazole. This was carried out as previously described (section 2.3.1) using the above antimicrobial discs.

2.3.5 *Campylobacter* spp. antibiotic susceptibility testing

This was carried out as advised by Leatherbouger (pers comms.). Muller-Hinton agar supplemented with 5% lysed horse blood was used in place of iso-sensitest agar, and the following antibiotic discs used; co-amoxyclav (30μg), ampicillin (10μg), erythromycin (5μg), nalidixic acid (30μg) and trimethoprim (2.5μg). If isolates were resistant to nalidixic acid they were also tested against ciprofloxacin (1μg).

2.3.6 Vancomycin resistant enterococci antibiotic sensitivity testing

This was carried out in accordance to BSAC (2002) as previously described (section 2.1) with the following exceptions. Iso-sensistest agar was used, supplemented with 5% lysed horse blood. The following antibiotic discs were used; ampicillin (10μg), azithromycin (15μg), gentamicin (200μg), imipenem (10μg), linezolid (10μg), meropenem, (10μg) synercid (15μg) and teicoplanin (30μg).

2.3.7 *Clostridium perfringens* susceptibility testing

This was carried out in accordance to BSAC (2002) as previously described (section 2.1.). Wilkins Chalgren agar (Mast, DM235D) was used in place of iso-sensitest agar, and the following antibiotic disc concentrations were used ampicillin (10μg), chloramphenicol (30μg), clindamycin (2μg), erythromycin (5μg), metronidazole (50μg), tetracycline (30μg) and tylosin (30μg).

2.4 Method for the determination of the minimum inhibitory concentration (MIC) of antimicrobials using agar incorporation tests for *E. coli.*

Overnight cultures of *E. coli* isolates were grown on nutrient agar and were used to make a suspension equivalent to that of 0.5 McFarlands standard. Half a millilitre of this suspension was then added to 4.5ml sterile water to make a 1 in 10 dilution. A multi-point inoculator was then used to transfer bacteria onto the iso-sensitest agar containing different concentrations of antibiotics (See Appendix A1.3).

2.5 *E. coli* bacterial conjugation

Conjugation experiments were carried out using a nalidixic acid resistant K12 strain or rifampicin resistant strain (developed using *E. coli* NCTC 10536). *E. coli* K12 was a recipient strain.

E. coli K12 recipient strain was inoculated into 20ml nutrient broth (LabM) and incubated overnight aerobically at 37°C. Resistant *E. coli* isolates (donor strains) were used to inoculate separate 3ml nutrient broths and also incubated overnight. Four ml of recipient strain was then added to 3ml of the donor strain and incubated as above for one hour. Broths were then streaked onto agar either containing nalidixic acid (30μg/ml) or rifampicin (16μg/ml), plus ampicillin (8μg/ml), (See appendix A1.3). Plates were incubated as above for twenty four hours. Successful transconjugants were subcultured onto nutrient agar for antibiotic sensitivity testing as previously described (see 2.3.1).

2.6 Molecular methods

2.6.1 DNA Extraction

E. coli, Campylobacter spp.

Boil preparations - A 5µl loop of each isolate was suspended in 0.5 ml sterile water in an eppendorf tube, and heated for 20 minutes at 100°C. DNA templates were stored at 4°C for up to 2 weeks.

C. perfringens, VRE and MRSA (method adapted from Holmes and Quigley, 1981)

1. Overnight growth from blood agar plates (16-18 hours) was harvested into 1ml sterile water and centrifuged at 12000g for 4 minutes and the supernatant discarded.
2. The pellet was washed twice in 1 ml sterile water, then resuspended in 100µl of STET buffer (8% sucrose, 5% triton X-100, 50 mM EDTA, 50 mM TRIS-HCl) and 10 µl lysozyme (20mg/ml) added. The tube was incubated at 70°C for 5 minutes.
3. The tube was centrifuged at 12000g to pellet cell debris. The supernatant was removed and added to 250µl 0f 100% ethanol and 30µl of 3M sodium acetate.
4. The DNA was precipitated at either -20°C for 30 minutes or by snap-freezing in liquid nitrogen and then centrifuged at 12000g for 10 minutes.
5. The supernatant was removed and the pellet washed with 500µl of 70% ethanol then placed in an incubator to dry, before being resuspended in 200µl of sterile water.
6. DNA templates were then frozen at –20 °C until used for PCR.

2.6.2 PCR

PCR Primers and reagents

All primers were purchased from MWG (Ebersberg, Germany) and are listed, alongside amplicon sizes in appendix A1.4. All PCR reagents were purchased from Abgene (Epsom, UK).

2.6.3 Identification of the genus *Campylobacter* and *Campylobacter* species

The PCR protocol used to identify the genus *Campylobacter* and assigning *Campylobacter* to species was based on that published by Linton *et al.*, (1996). All assays are specific for regions in the 16S rRNA that differ from other species of *Campylobacter*. PCR reactions were used for *C. upsaliensis, C. hyointestinalis, C. lari, C. fetus and C. helveticus*. Reactions were carried out in 25µl volumes containing 20mM Tris HCl (pH 8.3), 50mM KCl, 2.5mM $MgCl_2$, 0.2mM DNTP's, 0.4µM each primer, 0.625 U *taq* DNA polymerase and 1µl DNA template. PCR conditions for assay 1 were as follows: 30 cycles of 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute. PCR conditions for assay's 2-4 were the same but with different annealing temperatures. Assay 2 had an annealing temperature of 60°C, assay 3, 65°C and assay 4, 64°C. The positive control for *C. lari* was strain NCTC 11352. Positive controls for other strains were lab strains *C. upsaliensis* LS0017, *C. hyointestinalis* LS0026, *C. fetus* LS0032 and *C. helveticus* LS0042.

The PCR protocol used to detect *C. jejuni* and *C. coli* was based on that published by Houng *et al* (2001) and is based on distinctive *ceu*E (iron-chelating protein) genes between the two species. Reactions were carried out as single reactions in 25µl volumes containing 25 nM each primer, 0.1 mM of each DNTP, 1.5mM $MgCl_2$, 1x PCR buffer, 1.25U *taq* DNA polymerase and 1µl DNA templates. PCR conditions were as follows; 35 cycles of 95°C for 15 seconds, 55°C for 15 seconds and 72°C for 1 minute. Positive controls used were *C. coli* DWC 2304 and *C. jejuni* NCTC 11351.

2.6.4 Detection of *E coli* virulence genes

The PCR protocol used to detect virulence genes in *E. coli* was based on a paper published by Rappelli *et al.* (2001). It is based on 3 separate multiplex assays, assay 1 contains primers for the *elt, sta* and *uidA* genes; assay 2 contained primers for the *eae* and *bfpA* genes; assay 3 contained primers for the *stx1, stx2* (*vt1, vt2*) genes. PCR was performed in a 25µl reaction containing 50pM of each primer and 23µl 1.1xReddyMix PCR MasterMix (AB-0575/LD/A), and 1µl DNA template. PCR conditions were as follows, 94°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute for 30 cycles, followed by 72°C for 1 minute for 1 cycle. Positive controls used were Enterohaemorhagic *E. coli*, Enteropathogenic *E. coli* and Enteropathogenic *E. coli* supplied by Department of Medical Microbiology. All strains were confirmed as toxigenic by PCR and sequencing.

2.6.5 Detection of *E. coli* antimicrobial resistance genes

Ampicillin resistance

Ampicillin resistant *E. coli* isolates were subjected to PCR to investigate the presence of the *tem* and *shv* genes. PCR was carried out accordance to the protocol published by Pitout *et al.* (2001). Each 25µl reaction consisted of 0.2mM of each primer, 23µl 1.1xReddyMix PCR MasterMix and 1µl DNA template. The PCR program consisted of an initial denaturation at 96°C for 15 seconds followed by 24 cycles of 96°C for 15 seconds, 50°C for 15 seconds and 72°C for 2 minutes. Positive control for the *tem* gene was from a Laboratory strain 6167 that had been sequenced. The positive control for the *shv* gene was an isolate that was also consistently positive.

Tetracycline resistance

Tetracycline resistant *E. coli* isolates were subjected to PCR to investigate the presence of the *tet* genes. Two multiplex PCR protocols were followed in accordance to that published by Ng *et al* (2001), for 6 different tetracycline resistance genes, *tetA*,

tetB, tetC, tetD, tet E and *tetG*. Each 25µl PCR reaction mix for Assay 1 consisted of 1µl DNA template, 2.5 U *taq* polymerase, 0.3mM DNTP's, 4mM $MgCl_2$, and 0.25µM each primer (*tetA, tetG* and *tetD*). Each 25µl assay 2 reaction was as above but with 3mM $MgCl_2$ and 1µM each primer. Later, because of *tetB* and *tetD* have a similar amplicon size and therefore difficult to differentiate during electrophoresis, *tetD* was excluded and run in a separate PCR reaction. This was also done with *tetE* with assay 2. PCR conditions were as follows, 1 cycle of 94°C for 5 minutes followed by 35 cycles of 94°C for 1 minute, 55°C for 1minute, 72°C for 1 minute and 30 seconds. Positive controls were Laboratory strains 5035 (*tetA*), 7062 (*tetB*), 7336 (*tetC*), and 5820 (*tetD*) that had been sequenced previously. Positive controls for *tetE* and *tetG* gene were isolates that were consistently positive.

Trimethoprim resistance

dfr1 and *dfr9*

PCR was carried out as previously described by Gibreel and Sköld, (1998) in separate assays. PCR's were performed in 25µl volumes containing 50pM each primer, 23µl 1.1xReddyMix, PCR MasterMix and 1µl DNA template. PCR conditions were 30 cycles of 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes. Positive controls were isolates that were consistently positive.

dfrA5, dfrA7, dfrA8, dfr12, dfr13, dfrA14 and *dfr17*

This PCR protocol was carried out as described by Lee *et al.*, 2001. PCR reactions were carried out in 25µl volumes containing 1µl of each DNA template, 50pM of each primer, 1.1xReddyMix, PCR MasterMix and 1µl DNA template. PCR conditions for assay 1 consisted of 30 cycles of 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 1 minute and a single cycle of 72°C for 1 minute. PCR cycle for assay 2 was as above but with an annealing temperature of 51°C. Positive controls were Laboratory strains 6137 (*dfr14*), 7071 (*dfr12*), 7082a (*dfr17*) and 366 (*dfr8*). Other controls were isolates that were consistently positive.

Enzyme digests

Products for the *dfrA17, dfrA12, dfrA13* and *dfrA5* and *dfrA14* genes were co-amplified and therefore needed to be cleaved. PCR products *dfrA7*and *dfrA17, dfrA12* and *dfrA13,* and *dfrA5* and *dfrA14* were cleaved using 20 U *pst1, EcoRV* and *EcoR1* (Sigma) respectively, following the manufacturers instructions. Products were separated by electrophoresis (see below).

Chloramphenicol resistance

PCR for *catI, catII* and *catIII* was carried out in accordance to that published by Vassort-Bruneau *et al,* (1996). Reactions were carried out in 25 µl volumes containing 25pM of each primer, 23µl 1.1xReddyMix PCR MasterMix and 1µl DNA template. PCR conditions were as follows: 5 mins at 30°C followed by 30 cycles of 94°C for 30 seconds, 50°C for 30 seconds and 72°C for 1 minute and 30 seconds. Positive controls were isolates that were consistently positive.

PCR for the *cmlA* gene was carried out as described by Keyes *et al.* (2000). Reactions were carried out in 25µl volumes containing 50 pmol of each primer, 23µl 1.1xReddyMix PCR MasterMix and 1µl DNA template. PCR conditions were as follows; 30 cycles of 94°C for 1 minute, 40°C for 1minute and 72°C for 15 seconds.

Nalidixic acid resistance

PCR amplification for the *gyrA* gene was carried out in accordance with the method of Oram and Fisher (1991). Reactions were carried out in 100µl volumes containing 4µl of each DNA template, 50pM of each primer, 50µl 2x 1.5 $MgCl_2$ PCR MasterMix (AB-0575/DC/A), made up to 100µl volume with sterile water. PCR conditions were as follows, 30 cycles of 92°C for 25 seconds, 64°C for 1 minute and 74°C for 2 minutes 30 seconds.

PCR amplification for the *parC* gene was carried out in accordance with Vila *et al.* (1996). Reactions were carried out in 100µl volumes containing 4µl DNA template, 2.5 U *taq* polymerase, 0.3mM DNTP's, 3mM $MgCl_2$, and 50pM of each primer. PCR conditions were as follows, 30 cycles of 94°C for 1 minutes, 55°C for 1 minute and 72°C for 1 minute.

PCR products were purified using an Amplipure kit (BG-AP/50, Biogene) and sent for sequencing at the Advanced Biotechnology Centre, Imperial College Medical School London, UK.

2.6.6 Detection of *C. perfringens* toxin genes

PCR was used in accordance to that used by N Griffiths (Ph.D. thesis, 1996).

PCR reactions were carried out in 25µl volumes containing 50pM of each primer 75mM, Tris-HCl, 20mM $(NH_4)_2SO_4$, 1.5mM $MgCl_2$; 0.2mM of each DNTP, 2.5U of *taq* DNA polymerase and 1µl DNA template. PCR conditions were as follows: 94°C for 1 minute, 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds, with a final elongation of 72°C for 7 minutes. Strains DWC 2587, 2487, 2585 and 2794 were used as positive controls.

2.6.7 Vancomyin resistant enterococci species and resistance genes

PCR was carried out in accordance to that published by Dutka-Malen *et al.*, (1995). Each 25µl reaction contained 10 pM of each primer, 0.75U *taq* polymerase, 1.25mM DNTP's, 3.5mM $MgCl_2$ and 1µl DNA template. PCR conditions were as follows, 94°C for 2 minutes then 30 cycles of 94°C for 1 minute, 54°C for 1 minute and 72°C for 1 minute. This was finished by a final elongation stage of 72°C for 10 minutes.

2.6.8 Electrophoresis of PCR products

To visualise PCR products, 20 µl of each product was mixed with 2 µl of gel loading buffer (Sigma, unless ReddyMix was used then no gel loading buffer was needed) and loaded into a well of 1.5% agarose gel containing 1 x Tris Acetate EDTA (TAE) buffer and 6.2µl ethidium bromide (10mg/ml). Gels were subjected to electrophoresis at 120V for 90 mins. A 100bp molecular weight marker (Abgene) was used to estimate the molecular weight of the PCR products. PCR products were visualised with UV light.

2.7 Restriction Fragment Length Polymorphism (RFLP), Pulsed Field Gel Electrophoresis (PFGE)

2.7.1 Rapid *E. coli* and *Salmonella* spp. PFGE Method

This was carried out in accordance to the PulsedNet Standard Protocol (www.cdc.gov/pulsenet).

Preparation of agarose plugs

Overnight growth of the various isolates on nutrient agar, was harvested into 1ml 1 x TE buffer in a sterile eppendorf tube. Suspensions were diluted 1:10 (2.7ml 1xTE buffer + 300µl original suspension) and fresh 200µl suspensions made, adjusted to OD_{610}=1.35 using a spectrophotometer, in eppendorf tubes. Ten microlitres of proteinase K (20mg/ml) was then added to each suspension and mixed gently. 200µl of agarose mixture (see appendix A1.1) was then added, mixed by pipetting and immediately transferred into duplicate plug molds. These were left to set at 4°C. Duplicate blocks were made in case the digestion enzyme did not cut the first set of blocks, or a comparison was needed with a different restriction enzyme.

Three ml of cell lysis buffer (CLB, see appendix A1.2) was deposited into bijoux bottles and 15µl proteinase K added. The set blocks were immersed into the buffer

and incubated with shaking (175-200 rpm) at 54°C for 2 hours. The buffer was then removed and 3ml pre-heated sterile water added. Blocks were incubated at 54°C for 15 minutes with shaking as above. This was carried out twice. After the second wash, 3ml pre-heated 1xTE buffer was added and this was incubated as above. This was repeated 4 times. After this stage blocks can be stored in fresh buffer at 4°C until ready for enzymic digestion.

Restriction enzyme digests

TE buffer was removed and one agarose block transferred to a sterile eppendorf tube containing 200µl, 1x restriction buffer (supplied with enzyme). Incubation was at 37°C for 15 minutes with shaking as above to equilibrate blocks. The remaining block was covered in 1ml 1xTE buffer and stored at 4°C. After incubation, restriction buffer was removed and 200µl fresh 1x restriction buffer containing 50U *Xba*1 or *Spe*I added. This was incubated at 37°C for a minimum of 2 hours but could be left overnight. The reaction was stopped by putting blocks in the fridge at 4°C.

Gel electrophoesis

Digested blocks were run on a 1% agarose 27 well gel made with 0.5 x TBE buffer. Blocks were cut in half and half inserted into the gel, the other half being kept in 1ml of 1xTBE buffer and returned to the fridge. A (size!!) Lamda genomic molecular weight marker (New England Biolabs) was used for band size. Gels were run on a Bio-Rad CHEF DRIII system with 0.5x TBE buffer at 14°C for 20 hours. Intial switch time was 2.2s and final switch time was 54.2s (gradient of 6 V/cm and angle of 120).

2.7.2 Rapid *Campylobacter* PFGE Method

This was carried out in accordance with the method of Ribot *et al.*, (2001).

Preparation of agarose plugs

Forty-eight hour growths of *Campylobacter* spp. on columbia agar supplemented with 5% horse blood, were harvested into 1ml sterile PBS in a sterile eppendorf tube. A 1:10 dilution was made as described above and a fresh 400µl suspension made to OD_{610}=0.4. 25µl. Proteinase K (20mg/ml) was then added and mixed gently. 400µl of 1% agarose in 1xTE buffer was mixed by pipetting into each eppendorf tube and transferred into duplicate plug molds and allowed to set as above. Blocks were transferred to 3ml CLB as above with 25µl (20mg/ml) proteinase K and incubated with shaking at 54°C for 15 minutes. Blocks were washed 4 times, once with 3ml pre-heated sterile water and 3 times with 3ml pre-heated 1xTE buffer. Blocks can then be stored in fresh buffer at 4C until ready for restriction enzyme digest.

Restriction enzyme digest

One x TE buffer was removed and one block transferred to an eppendorf tube containing 0.5ml 0.1xTE buffer. This was incubated at 25C for 20 minutes. The remaining block was covered with fresh 1xTE buffer and stored at 4°C. Blocks were then equilibrated in 200µl 1x restriction buffer (supplied with enzyme) and incubated as above. Restiction buffer was then removed and fresh 1x restriction buffer containing 40U *Sma*I and incubated at 25°C for 2 hours with agitation, or 4 hours without. Digests were stopped again by putting blocks into the fridge at 4°C.

Gel electrophoresis

Electrophoresis was carried out as above at 14°C but with an initial switch time of 6.75 s and a final switch time of 38.35 s (gradient of 6 V/cm and angle of 120), for 16 hours.

Analysis of banding patterns

Bands were analysed using Biorad Molecular analyst software. Genetic relatedness was assessed following criteria published by Tenover *et al.* (1995). This involves isolates considered indistinguishable if there is no difference in banding pattern, closely related if there is 2-3 bands difference in banding pattern, and possibly related if there is 4-6 difference in banding pattern.

Chapter 3

The prevalence of potentially zoonotic bacteria in dogs; a cross-sectional study

3.1 Introduction

The aim of this study was to investigate the prevalence of *Escherichia coli*, *Campylobacter* spp., *Salmonella* spp., *Clostridium perfringens*, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) in dog faeces from boarding kennels, rescue homes, farm environments and parks in the UK. A small number of cat faecal samples were also collected. These bacteria, as well as potentially causing disease in dogs, can also infect human beings (James and Tan, 1997, Kozak *et al.*, 2003, Damborg *et al.*, 2004). This study was carried out as part of a larger investigation into the role of companion animals as a potential source of antibiotic resistant and pathogenic bacteria for humans.

Although there have been a number of studies on the prevalence of zoonotic organisms in dog and cat faeces, very few have been conducted in the UK (Normand *et al.*, 1999). The prevalence of *Campylobacter* spp. in dogs has been reported to be between 5%-77% (Gondrosen *et al.*, 1985, Burnens *et al.*, 1992, Sandberg *et al.*, 2002, Engvall *et al.*, 2003). However, there are many factors which can affect the incidence of *Campylobacter* in dogs. Risk factors associated with a higher prevalence of *Campylobacter* spp. in dogs include communal housing (Malik and Love, 1989, Bruce *et al.*, 1993, Cantor *et al.*, 1997), and the presence of diarrhoea (McOrist and Browing, 1982, Chattopadhyay *et al.*, 2001). However, the latter is still a subject of debate as many studies have not found a significant difference in the presence of *Campylobacter* spp. from healthy and diarrhoeic dogs (Olsen and Sandstedt, 1987, Figura 1991, Burnens *et al*, 1992, Adesiyun *et al*, 1997, Baker *et al*, 1999). Studies have also found a higher prevalence of *Campylobacter* spp. in dogs below one year of age (Fleming *et* al., 1980, Blaser *et al.*, 1980, Bruce and Flemming 1983, López *et al.*, 2002, Engvall *et al.*, 2003, Hald *et al.*, 2004) and a higher prevalence has also been observed in cats below one year of age (Spain *et al.*, 2001). The majority of studies of excretion of *Campylobacter* spp. in dogs have been cross sectional. Longitudinal

investigations into the carriage of *Campylobacter* spp. in dogs have rarely been done (Hald *et al.*, 2004).

C. jejuni is the most frequently isolated species of *Campylobacter* in dogs (Woldehiwet *et al.*, 1990, Moreno *et al*, 1993, Hald and Madsen, 1997, López *et al.*, 2002). However, this may have been due to under reporting of *C. upsaliensis*, which is sensitive to the antibiotics used in most Campylobacter selective media (Steinhauserova *et al.*, 2000, Modolo and Giuffrida, 2004). A recent study by Hald *et al.*, (2004) found that 75% of *Campylobacter* isolates from dogs were *C. upsaliensis*. *C. upsaliensis* has also been reported in cats (Hald and Madsen, 1997, Moser *et al.*, 2001) and at present cats and dogs are the only known carriers of *C. upsaliensis*. The prevalence of *Campylobacter* spp. in cats has been reported to be similar, or slightly lower than that found in dogs (Baker *et al.*, 1999, Hill *et al.*, 2000, Moser *et al.*, 2001). The prevalence of *Campylobacter* spp. from dogs in the UK is not widely known (Hoise 1979, Flemming 1980, Holt 1980, Bruce *et al.*, 1980, Bruce and Flemming, 1983, Moreno *et al.* 1993).

The prevalence of *Salmonella* spp. in dogs has been reported to be up to 15% in the USA (Gorham *et al.*, 1951, Galton *et al.*, 1952, Mackel *et al.*, 1952), but a lower prevalence has been found in studies in Europe (Weber *et al.*, 1995, Nastasi *et al.*, 1986, Kozak *et al.*, 2003) and Japan (Fukata *et al.*, 2002). *Salmonella* spp. infection in dogs is often reported in outbreaks (Tillotson *et al.*, 1997, Uhaa *et al.*, 1998), and the main risk factor associated with *Salmonella* spp. isolation is communal housing (BSAVA, 1997). *Salmonella* spp. have also been reported in cats (Hill *et al.*, 2000).

Very few studies have investigated vancomycin resistant enterococci (VRE) or methicillin resistant *Staphylococcus aureus* (MRSA) in dogs. Carriage rates for VRE are reported to be as high as 48% from healthy dogs in Europe (Devriese *et al.*, 1996, Belkum *et al.*, 1996), although other studies have reported the absence of VRE from dog faecal samples (Harward *et al.*, 2001, Wagenvoort *et al.*, 2003). MRSA has been isolated mainly from wound and skin infections in dogs (Gortel *et al.*, 1999, van Duijkeren *et al.*, 2003, Rich and Roberts, 2004), and dogs have been implicated in the transmission of this organism to humans (Manian, 2003). There has been little investigation of the faecal carriage of MRSA in dogs.

The prevalence of *C. perfringens* in dogs been has reported to be between 5-28% (Weese *at al.*, 2002b). There has been a clear association between diarrhoea in dogs and *C. perfringens* enterotoxin (CPE) produced by certain strains of *C. perfringens* (Marks *et al.*, 2002, Weese *et al.*, 2002a, Weese *et al.*, 2002b), however, the role of this organism as a causative agent of diarrhoea is still unclear as *C. perfringens* is present in the normal gut flora.

This chapter, therefore, describes cross-sectional studies of British dogs from a variety of backgrounds to determine the prevalence of a range of potentially zoonotic, enteric pathogens in dog faeces, as the first step to determining whether or not dogs pose a significant zoonotic risk.

3.2 Materials and methods

3.2.1 Cross sectional study of *E. coli*, *Campylobacter* spp., *Salmonella* spp., MRSA and VRE in dog faeces.

Collected faecal samples are listed in appendix A2.

Dog faecal samples were collected from parks, dog rescue homes, boarding kennels and households in Cheshire (NW England) to determine the prevalence of *E. coli*, *Campylobacter* spp., *Salmonella* spp., MRSA and VRE. All dog faecal samples were collected in sterile universal containers and processed in the laboratory on the same day as collection using the methods described in chapter 2. Samples were graded for faecal consistency according to the Waltham Chart (Vol. 11, 2001, see appendix A1).

Eighty dog faecal samples were collected, from five different boarding kennels. Samples were collected early in the morning before the kennels were cleaned out. A sample of the freshest looking faeces was collected and samples were not taken if the sample was disturbed (e.g. trodden around the kennel), or appeared contaminated with urine. Samples were collected between October 2001-July 2003.

Seventy-eight dog faecal samples were collected from four different dog rescue homes (3 RSPCA, 1 privately owned) using the same criteria as above.

Dog faecal samples were obtained from eighty-four dogs resident in private households. Samples were collected by their owners, who were also asked to complete a short questionnaire (see appendix A2) regarding the dog's age, gender, diet, previous antibiotic therapy and where the dog was exercised.

Sixty-seven dog faecal samples from public parks and pathways were collected. Only fresh-looking samples that had not been disturbed were collected.

Twenty-one faecal samples were collected from dogs resident on farms. Samples were again, collected by the dogs' owners.

Twenty-nine dog faecal samples were collected from dogs being treated for canine upper respiratory tract disease ('kennel cough'). These were collected on two separate occasions, fourteen samples in October 2001 (group 1) and fifteen samples in November 2002 (group 2). All the dogs were housed in the same block in an RSPCA rescue home. Group 1 were treated with Synulox (co-amoxyclav) and deoxycycline, and group 2 were treated with Ronaxan (deoxycycline).

Faecal samples from 24 cats were also collected. Eight samples were from boarding kennels, three samples were from household cats, nine samples were from cats in rescue homes and five samples were from cats referred to the small animal hospital, University of Liverpool with signs of gastrointestinal disease.

Statistical analysis

Data were examined using the Chi-squared and Fishers' exact test. The Cochrane-Armitage test was used to test for trends.

3.2.2 Investigation of *Clostridium perfringens* in dog faeces.

A random sample of twenty-five dog faecal samples from each of the four groups sampled (boarding kennels, rescue homes, households and parks) were investigated for the presence of *Clostridium perfringens*. Samples were thawed at room temperature from -80°C and a single loop-full of sample put into an enrichment broth as described in chapter 2.

3.2.3 Longitudinal investigation of pathogenic bacteria in dog faeces.

Faecal samples from dogs' resident at a dog re-homing centre were collected weekly, fortnightly or monthly from the same dogs to investigate the prevalence of pathogenic bacteria in faeces over a period of time. The prevalence of *E. coli*, *Campylobacter* spp. and *Salmonella* spp. were investigated. Samples were collected by the Centre's staff and information on the breed, approximate age and treatment (if any) was

obtained for each dog. The genetic relatedness between strains was examined using pulsed field gel electrophoresis (PFGE) where appropriate as described in chapter 2 (2.7).

Table 3.1.Isolation of *E. coli, Campylobacter* spp., *Salmonella* spp., MRSA and VRE from faecal samples, obtained from dogs in boarding kennels, rescue homes, households, public parks, farm environments and cats from households and a catery

Origin of samples	No. samples Collected	No. samples with *E. coli*	No. samples with *Campylobacter* spp.	No. samples with VRE	No. samples with MRSA	No. samples with *Salmonella* spp.
Boarding kennels	80	76 (95%)	8 (10%)	0	0	0
Rescue homes	79	74 (95%)	2 (3%)	2 (3%)	0	0
Households	84	74 (88%)	13 (15%)	1 (2%)	0	0
Parks	67	63 (94%)	4 (6%)	0	0	0
Farm dogs	21	20 (95%)	0 (0%)	0	0	0
Cats	24	22 (92%)	1 (4%)	0	0	0
Dogs on antibiotics	29	28 (97%)	3 (10%)	0	0	0

E. coli

E. coli was isolated from the majority of samples collected from each group (see table 3.1). The lowest prevalence was from faecal samples collected from dogs in households (88%). The prevalences of *E. coli* isolation from boarding kennels, rescue homes, parks, farms, cats and dogs on antibiotic treatment were virtually the same (95%, 95%, 94%, 94%, 92%, 97%, respectively). There were no significant differences in prevalence of *E. coli* isolated between the different groups (Fisher's exact test, P= 0.697).

Campylobacter spp.

The prevalence of *Campylobacter* spp. was highest in faecal samples from household dogs (15%), the second highest prevalence being from faecal samples from dogs on antibiotic treatment (10%, table 3.1). The lowest prevalence of *Campylobacter* spp. was in faecal samples from dogs in rescue homes (3%) and farm dogs (0%). Overall, *Campylobacter* spp. were isolated from 9% of faecal samples. There was a significant difference between the prevalence of *Campylobacter* spp. isolated from the different groups (Fisher's Exact test, P=0.05).

The age of the dog was known for 88% (N=74) of dogs in the household group (returned questionnaires, see appendix A3). *Campylobacter* spp. were isolated from four samples from dogs under the age of one year (40%) and from nine dogs (14%) above the age of one year, which was found to be marginally significant (P= 0.067). The age of the dog (or cat) was not known in any of the other groups.

Using the chi-squared test, there was no significant difference in the isolation of *Campylobacter* spp. between dogs been given kitchen scraps and those which had not received kitchen scraps (P=0.13). The significance of and differences between the locations where dogs were walked and contact with other dogs was not tested due to small numbers, and no trends were evident.

The majority of *Campylobacter* isolates were *C. upsaliensis*. *C. jeuni* was isolated from single faecal samples obtained from dogs in boarding kennels and cats and dogs

on antibiotic treatment. The species of six *Campylobacter* isolates were not able to be determined.

Table 3.2.Isolation of *Campylobacter* spp.

Origin of samples	*C. upsaliensis*	*C. jejuni*	Not determined	Total no *Campylobacter* Isolates
Households (84)	10	0	3	13
Boarding kennels (80)	7	0	1	8
Dogs on antibiotic treatment (29)	2	1	0	3
Parks (67)	3	0	1	4
Rescue home (79)	1	0	1	2
Cats (24)	0	1	0	1

VRE

VRE were isolated from two faecal samples from dogs in a rescue home (samples 116 and 118) and 1 faecal sample from a household dog (sample 32). All isolates were *E. faecium*. This represented an overall prevalence of 3% from rescue home dogs and 2% from household dogs.

Neither NTS nor MRSA were isolated from any of the dog or cat faecal samples in this cross sectional study.

3.3.2 Prevalence of *Clostridium perfringens* in dog faecal samples

The prevalence of *C. perfringens* in dog faecal samples was very low. It was isolated from two samples from household dogs (samples 34 and 468). Sample 34 had a faecal consistency of 2 and sample 468 had a faecal consistency of 3, indicating diarrhoea was not present. This represented an overall prevalence of 2%.

3.3.3 Faecal consistency

There was little correlation between faecal consistency and the isolation of *Campylobacter* spp. The number of *Campylobacter* spp. isolates from rescue homes, and cats and dogs on antibiotic treatment group was not large enough to carry out any significance testing. There was no evidence of trends within the groups, although the test for trend in the household group was mildly significant.

Where there were few or no samples of a particular faecal consistency, they were not included in significance testing.

Table 3.3.Faecal consistency and *Campylobacter* spp. isolation

Origin of sample	Faecal consistency	No samples	No samples *Campylobacter* spp.	Fisher's exact test	Cochran-Amitage test for trend	
Parks	1	0	0	P=0.845	X_1^2 =0.004	P =0.950
	1.5	1	0			
	2	10	1			
	2.5	23	1			
	3	17	1			
	3.5	7	0			
	4	8	1			
	4.5	2	0			
	5	0	0			
Household dogs	1	0	0	P=0.013	X_1^2 =2.927	**P =0.087**
	1.5	0	0			
	2	13	6			
	2.5	23	2			
	3	21	1			
	3.5	11	3			
	4	14	1			
	4.5	0	0			
	5	0	0			

3.3.4 Longitudinal investigation of pathogenic bacteria in dog faeces

Overall, 184 samples from 107 dogs were collected over 8 months from dogs resident in a re-homing centre.

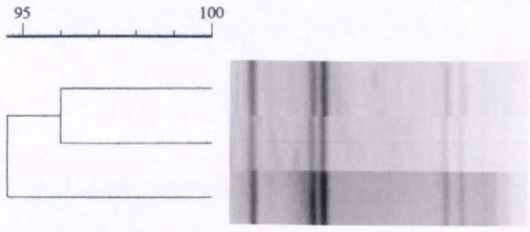
Campylobacter spp.

Campylobacter spp. were only isolated from 3 faecal samples (240, 242, 248) in this study. *Campylobacter* spp. were isolated from two samples from dogs under 10 months of age, and all three samples were collected within the same two-week period (13/1/02-20/1/02). Six isolates from the 3 faecal samples were all *C. upsaliensis*.

Pulsed field gel electrophoresis (PFGE) of Campylobacter upsaliensis isolates

The genetic relatedness of *C. upsaliensis* isolates was determined using pulsed field gel electrophoresis following digestion with *sma*I. Isolates from all 3 samples collected were identical.

Figure 3.1.PFGE of *C. upsaliensis* digestion with *Sma*I

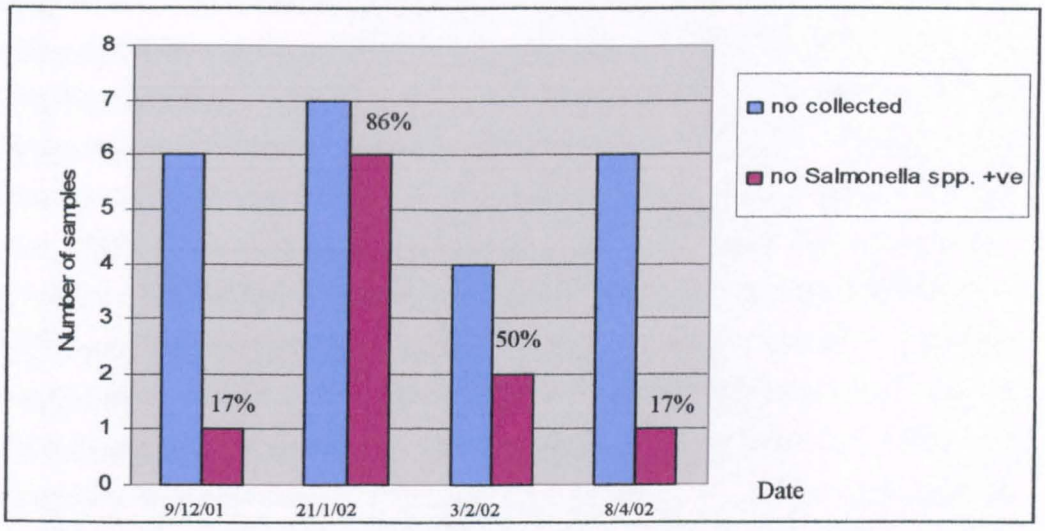


Salmonella spp.

All NTS were isolated from the faecal samples of 10 different dogs over a period of five months (see figure 3.2). NTS were isolated from one faecal sample (17% of samples collected) on 9/12/01, six samples (86%) on the 21/1/01, two samples (50%) on 3/2/02, and from one final sample (17%) on 8/4/02. None of the dogs were

diarrhoeic or showing any signs of salmonellosis. All samples were from dogs that had recently joined the re-homing centre and were housed in the admissions block.

Figure 3.2.Number of samples from which *Salmonella* spp. isolated on each occasion



Pulsed-field gel electrophoresis (PFGE) of S. Typhimurium

The genetic relatedness of each of the *S.* Typhimurium isolates was determined by pulsed-field gel electrophoresis analysis following digestion with two restriction enzymes, *Xba*I and *Spe*I. All isolates obtained over the five-month period showed identical profiles with both enzymes, suggesting they were all the same strain. An example of banding patterns seen with both enzymes is shown below (figures 3.3 and 3.4).

Figure 3.3.*Xba*I profile of *S.* Typhimurium isolates

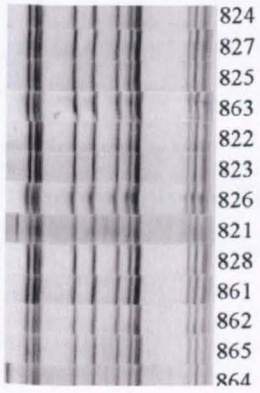
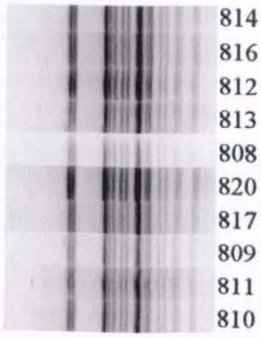


Figure 3.4.*Spe*I profile of *S.* Typhimurium isolates



3.4. Discussion

There was a significant difference in the prevalence of *Campylobacter* spp. between the groups of dogs from which samples were collected (Fishers exact test, P=0.05). A higher prevalence of *Campylobacter* spp. was observed from household dogs (13%) than from boarding kennel dogs (10%) and rescue homes (3%), in contrast to other studies that have identified communal settings as a risk for factor for *Campylobacter* isolation in dogs (Malik and Love, 1989, Bruce *et al.*, 1993, Cantor *et al.*, 1997, Baker *et al.*, 1999). The most common species of *Campylobacter* isolated in this study was *C. upsaliensis*. Studies from Switzerland (Burnens *et al.*, 1992), Sweden (Engvall *et al.*, 2003), and Denmark (Hald *et al.*, 2004) have observed similar findings. *C. jejuni* has been the most common species reported overall in other studies but this may be due to under-reporting of *C. upsaliensis:* studies that have not used inhibitory media have observed a higher prevalence of *C. upsalinesis* in dogs as mentioned above (Hald *et al.*, 2004). The results of this study suggest that dogs are not likely to be a significant source of *C. jejuni* infection for humans.

The reasons for the higher prevalence of *Campylobacter* spp. from household dogs are not known. Many of the dogs that were sampled for the household group were local to the area, having access to farmland, but this is an unlikely source of *C. upsaliensis* and there were no *Campylobacter* spp. isolated from farm dogs. Kitchen scraps given to dogs may also be a source of infection with *Campylobacter* spp. but again, this would be an unlikely source of *C. upsaliensis*. Using the questionnaires returned with samples from household dogs there was no correlation between dogs being given kitchen scraps and isolation of *Campylobacter* spp. The faecal consistency and the prevalence of *Campylobacter* spp. were compared using the chi-squared test. We incorporated a test for trend to ensure any differences were not due to a linear trend in the faecal consistency from the samples. The test for trend in the household group was just significant but as the chi-squared test was not significant, this is unlikely to be meaningful.

The low prevalence of *Campylobacter* spp. from rescue homes, boarding kennels and the dog re-homing centre may be due to the heavy use of disinfectants and cleaning

agents in such establishments. Samples from rescue homes were collected at different periods over the whole year, suggesting there was no evidence for the seasonal variation in prevalence of *Campylobacter* spp. in dogs that has been observed in other studies (Lopez *et al.*, 2002). *C. upsalinesis* was isolated from 3 samples from the re-homing centre, all of which had identical PFGE patterns. Samples were collected on consecutive weeks suggesting either transmission of this strain between dogs or infection from the same source. It is unknown why there was such a low prevalence from the dog re-homing centre over the five-month sampling period.

Half of the *Campylobacter* spp. isolates from household dogs were obtained from dogs below one year of age, a result which was marginally significant ($p=0.067$). This is consistent with other studies that have also found that dogs below one year of age have a higher rate of *Campylobacter* spp. excretion (Fleming *et* al., 1980, Blaser *et al.*, 1980, Bruce *et al.*, 1983, López *et al.*, 2002, Engvall *et al.*, 2003, Hald *et al.*, 2004).

Only low prevalences of VRE and *C. perfringens* were found, and MRSA was not isolated from any of the samples. VRE and MRSA are typically regarded as nosocomally acquired in humans, although a community presence is now being recognized in both humans and companion animals (O'Rourke, 2002, Groom *et al.*, 2001, Fey *et al.*, 2003). VRE have also been isolated from wildlife (Mallon *et al.*, 2002) and more recently from horses (Mohammed Omar, pers comms). There have been limited reports of VRE isolation from dogs and many studies have not isolated this organism (Harwood *et al.*, 2001, Wagenvoort *et al.*, 2003). However, a study by Willems *et al.*, (2000) found similarities between isolates from human patients, cats and dogs, using amplified length polymorphism analysis which suggests that cats and dogs may be a reservoir of VRE for humans, or vice versa. Nevertheless, the low prevalence of VRE isolated in this and other studies suggest that dogs should not pose as a significant risk for VRE infection in humans.

NTS were isolated from faecal samples collected in the longitudinal study, but not from the cross sectional study. This suggests that NTS are only isolated in epidemics in dogs as has been suggested by other studies (Tillotson *et al.*, 1997, Uhaa *et al.*, 1998). When NTS were first isolated and the re-homing centre informed, further samples were not given from any of the dogs from which the bacterium was first

isolated, nor from dogs being housed in the block where the outbreak appeared centered. This may have had a significant effect on the results. If NTS had been present in all of the dogs housed in the admissions block, this would suggest transmission of NTS between the dogs, or an environmental source. If NTS were not isolated from other dogs, it would suggest that they were infected before they entered the home. These results would have been very interesting. None of the dogs showed signs of diarrhoea, but dogs can carry this organism asymptomatically. NTS were isolated from dogs of all ages being housed in the admissions block, indicating that length of stay in the centre and age of the dog were not risk factors for NTS infection. PFGE banding patterns of each of the *S.* Typhimurium isolates obtained over the five-month period were identical, as demonstrated by digestion with 2 different enzymes. This strain therefore has persisted over this period of time through either transmission between the dogs, or in the environment. It was not known if staff were affected by the NTS.

In conclusion, the results from this study demonstrate that *C. upsaliensis* is very common in dogs and at present, no other significant reservoir of this species is known, other than cats. *C. upsaliensis* is known to cause clinical disease in humans (Jimenez *et al.*,. 1995, Lindblom *et al.*, 1995), and although it is not isolated very frequently from human infection, dogs may be the source of a significant number of human cases. Furthermore, the prevalence of *C. upsaliensis* may be under-estimated in human infections due to due the nature of media used which is inhibitory to this species of *Campylobacter,* a factor that probably also explains the low prevalence of *C. upsaliensis* in some previous canine studies. The prevalence of *Salmonella* spp., *C. perfringens* and VRE was low, but its presence in dogs may be a source of zoonotic infection for humans. Alternatively, humans may be the source of canine infections, or both have common other sources of infection. The transmission of the agents between host clearly needs to be investigated further.

Chapter 4

Molecular characterisation of antibiotic resistance and pathogenic virulence determinants in *E. coli, Campylobacter* spp., *Salmonella* spp., VRE and *C. perfringens* from dogs

4.1 Introduction

The aim of the work described in this chapter is to examine and characterise antibiotic resistance genes and virulence determinants present in bacteria isolated from dog faeces. The presence and mechanisms of antibiotic resistance are well documented in human bacterial isolates but much less is known about mechanisms of resistance in veterinary isolates, and very few studies have been carried out in the UK (Normand *et al.*, 2000a). *E. coli, Campylobacter* spp., NTS, *C. perfringens* and VRE isolated from dog and cat faecal samples collected from boarding kennels, rescue homes, parks, households, farms and dogs on antibiotics as previously described in chapter 3, were subjected to antimicrobial resistance testing. Antibiotic resistance and virulence genes were also determined for the bacteria isolated.

A high prevalence of antimicrobial resistance in *E. coli* has been found in companion animals such as dogs and cats, and particularly in *E. coli* from urinary tract infections in dogs (Teshager *et al.*, 2000, Cooke *et al.*, 2002, Féria *et al.*, 2002, Briņas *et al.*, 2002, Sanchez *et al.*, 2002, Lanz *et al.*, 2003, Drazenovich *et al.*, 2004). Multi-drug resistant *E. coli* have also been reported from nosocomial (wound) infections in the dog (Sanchez *et al.*, 2002). The majority of reports concerning antibiotic resistant *E. coli* in the dog are from clinical cases and do not give a wider picture of the dissemination of antibiotic resistance genes within the healthy canine population (Monaghan *et al.*, 1981, Moss and Frost, 1984, Normand *et al.*, 2000).

There have been numerous reports of antibiotic resistance in human and cattle isolates of *Salmonella* spp. and *Campylobacter* spp. isolates (Busani *et al.*, 2004), but few reports of antibiotic resistant isolates from dogs. Multi-drug resistant *Salmonella* spp. have been isolated from cats (Van Immerseel *et al.*, 2004) and Preston *et al.* (1990) found *C. upsaliensis* isolates from dogs (various geographical locations) were

resistant to trimethoprim. Reports suggest that *Salmonella* Typhimurium DT 104 may represent roughly a third of *Salmonella* isolates from cats and dogs (BSAVA News, 1997). VRE have been widely isolated from farm animals, humans, the environment and foodstuffs (Aarestrup *et al.*, 2000, Devriese *et al.*, 1996, Robredo *et al.*, 2000), however, there are very limited data on the presence of VRE and the genes responsible for resistance from dogs. There are also very limited data on *C. perfringens* in dogs and studies concerning *C. perfringens* toxin genes and antimicrobial susceptibility concentrate on human food-borne outbreaks (Adak *et al.*, 2002) and farm animals (Tschirdewhen *et al.*, 1991, Sipos*et al.*, 2003), including poultry (Engstrom *et al.*, 2003).

A number of enteropathic *E. coli* types have been isolated from dogs with GI disease. These include enterotoxigenic *E. coli* (ETEC), verotoxigenic *E. coli* (VTEC), including enterohemorrhagic *E. coli* (EHEC) and enteropathogenic *E. coli* (EPEC); (Beutin *et al.*, 1993, Beaudry *et al.*, 1996, Beautin, 1999, Goffaux *et al.*, 2000, Neiger *et al.*, 2002). These types are known to cause disease in humans but it is unclear to what extent they cause disease in dogs as virulence determinants attributed to human enteropathic strains of *E. coli*, can also be found in healthy dogs (Holland *et al.*, 1999).

Although much is known about the mechanisms and epidemiology of antibiotic resistant bacteria in human medicine, little is known about isolates from companion animals (Normand *et al.*, 2000a), especially in the UK (Lanz *et al.*, 2003). This chapter describes antibiotic resistance and virulence genes in bacterial isolates from dogs, continuing the investigation into whether dogs pose a significant zoonotic risk for humans.

4.2 Methods

4.2.1 Cross-sectional survey

Faecal samples were collected from dogs resident in boarding kennels, rescue homes, households, parks, farm dogs, dogs on antibiotic treatment and from cats, as described in the chapter 3. Questionnaires (see appendix A2) were distributed to owners of dogs in households.

Antibiotic susceptibility testing

All bacterial isolates were investigated for antimicrobial susceptibly as described in chapter 2 (2.3). *E. coli* and VRE isolates were investigated for the presence of antibiotic resistance genes responsible using PCR (2.6).

The Minimum Inhibitory Concentration (MIC) of each antibiotic was determined for resistant *E. coli* isolates (2.4). Ampicillin resistant isolates were investigated for extended spectrum activity (2.3.2.). Trimethoprim resistant isolates were investigated for resistance to aminoglycosides and sulphamethoxazole (2.3.4).

The transferability of ampicillin resistance determinants in *E. coli* from dogs being treated with antibiotics was investigated using conjugation (2.5.) The genetic relatedness of isolates was also assessed using pulsed field gel electrophoresis (2.7).

E. coli virulence determinants

All *E. coli* isolates were investigated for the presence of enteropathic virulence determinants using PCR (2.6.4)

C. perfringens toxin genes

C. perfringens isolates were investigated for the presence of *C. perfringens* toxin genes (2.6.6).

Statistical analysis

Data were analysed using the Chi-squared and Fishers' exact test. The Cochrane-Armitage test was used to test for trends.

4.2.2 Investigation of antibiotic resistant and pathogenic bacteria in dog faecal samples obtained from dogs in a re-homing centre, over a period of 8 months.

Faecal samples from dogs resident at a re-homing centre were collected weekly or fortnightly from the same dogs to investigate the prevalence of antibiotic resistance and potentially pathogenic bacteria over a period of time. The prevalences of enteropathic and antibiotic resistant *E. coli* were investigated.

4.3 Results

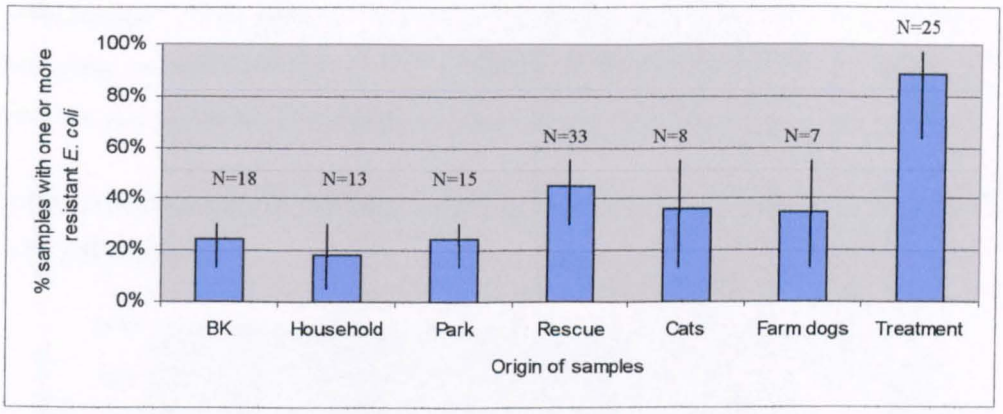
4.3.1 Antibiotic resistance in *E. coli*

All *E. coli* isolates were classified as sensitive or resistant using the disc diffusion assay (BSAC, 2002). Percentages used in this chapter are expressed from the proportion of samples from which *E. coli* was isolated (see table 3.1), not the number of samples that were collected.

Prevalence of antibiotic resistant E. coli

Overall there was a high prevalence of *E. coli* resistant to one or more of the antibiotics tested. Resistance was seen mostly to ampicillin, tetracycline and trimethoprim, with the prevalence of *E. coli* resistant to chloramphenicol and nalidixic acid being the lowest. The highest prevalence of ampicillin resistant *E. coli* was found in faecal samples from dogs on antibiotic treatment for kennel cough (89%, figure 4.1). There was a significant difference, using the chi-squared test, between antibiotic resistant *E. coli* isolated from dogs samples collected from different environments. The 95% binomial confidence levels overlap for all groups, the exception being the antibiotic treatment group. This is suggestive of a higher proportion of ampicillin resistant *E. coli* in the antibiotic treatment group, but no difference in the proportions of ampicillin resistant *E. coli* in any of the other groups. This trend was also seen in *E. coli* isolated from dogs on antibiotic treatment that were resistant to other antibiotics (table 4.1 below). A trend was seen of decreasing prevalence of ampicillin resistance in *E. coli* from rescue homes, cats and farm dogs (45%, 36%, and 35% respectively) and then boarding kennels and parks (24%) and household dogs (18%).

Figure 4.1.Percentage of dog faecal samples from which one or more ampicillin resistant *E. coli* were isolated

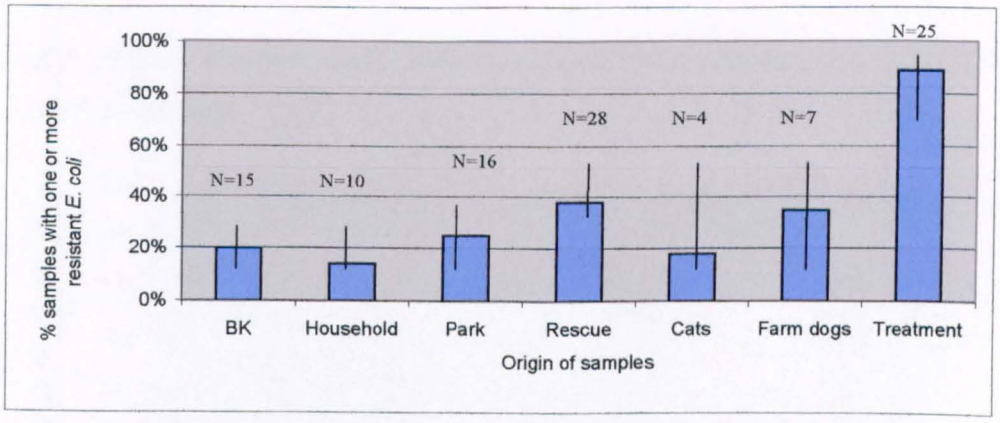


(BK – boarding kennels)

(Bars plot 95% confidence interval levels, N = number of samples containing *E. coli*)

The highest prevalence of tetracycline resistant *E. coli* was in faecal samples obtained from dogs receiving antibiotic treatment (89%, figure 4.2). The next highest prevalences were from samples from dogs in rescue homes (38%) and samples from farm dogs (35%). The remaining groups had lower prevalences of tetracycline resistant *E. coli* (Parks – 25%, BK – 20%, Cats – 18%, Households – 14%).

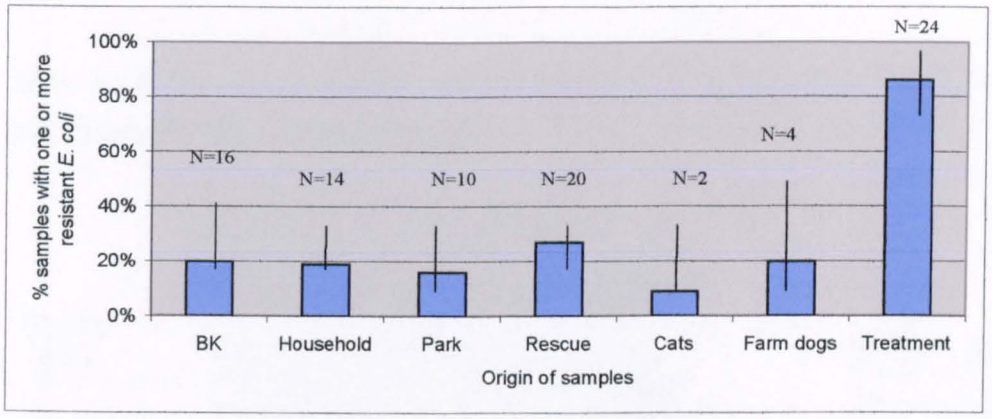
Figure 4.2.Percentage of faecal samples from which one or more tetracycline resistant *E. coli* were isolated



(Bars plot 95% confidence interval levels, N = number of samples containing *E. coli*)

The prevalences of trimethoprim resistant *E. coli* were fairly similar between all faecal samples obtained from all of the groups (figure 4.3; BK – 20%, Household – 19%, Rescue – 27%, parks – 16%, farm dogs – 20%), with the exception of samples from dogs on antibiotic treatment that had a much higher prevalence of trimethoprim resistant *E. coli* (86%). The lowest prevalence was from cats (9%).

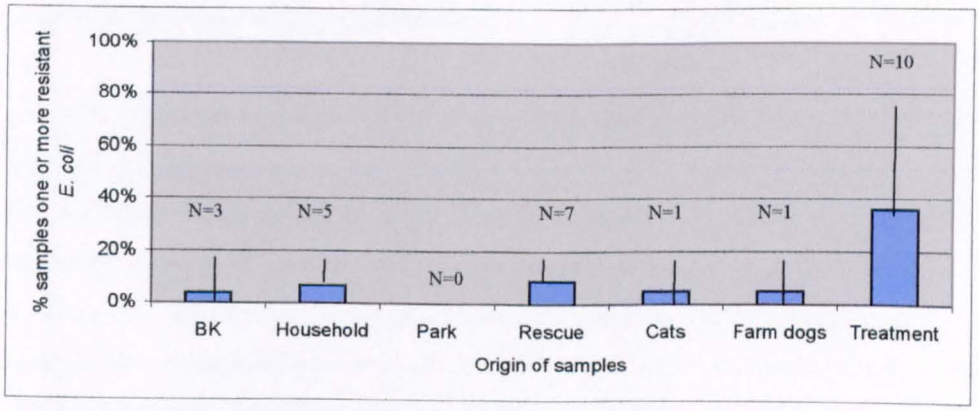
Figure 4.3.Percentage of samples from which one or more trimethoprim resistant *E. coli* were isolated



(Bars plot 95% confidence interval levels, N = number of samples containing *E. coli*)

The highest prevalence of chloramphenicol resistant *E. coli* was in samples from dogs on antibiotic treatment (36%, figure 4.4,). All other groups had a low prevalence of chloramphenicol resistant *E. coli*.

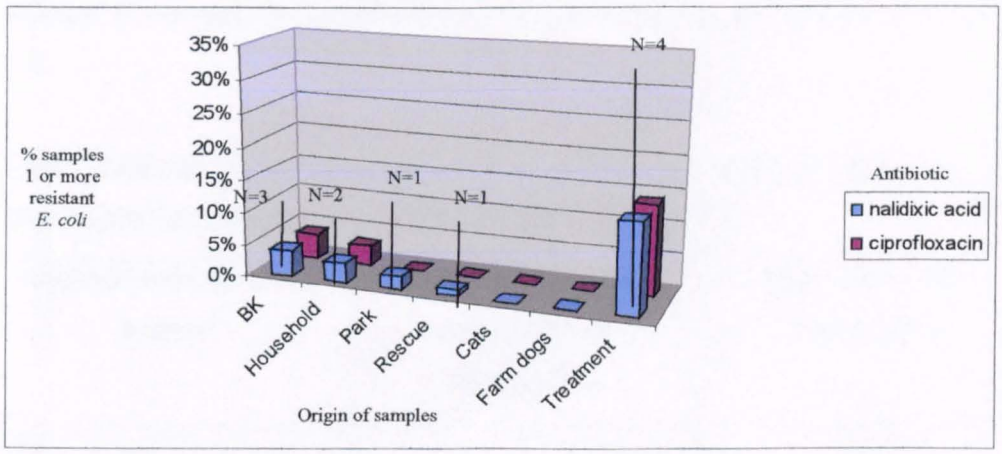
Figure 4.4.Percentage of samples from which one or more chloramphenicol resistant *E. coli* were isolated



(Bars plot 95% confidence interval levels, N = number of samples containing *E. coli*)

There was very little resistance to nalidixic acid and ciprofloxacin in *E. coli* isolates obtained in this study (figure 4.5). The highest prevalence of nalidixic acid resistant isolates was from dogs being treated with antibiotics (14%), and each of these isolates was also resistant to ciprofloxacin. There was a similar prevalence of nalidixic acid resistant *E. coli* from samples from the other groups (exceptions being from cats and farm dogs), although isolates from parks and rescue homes were not resistant to ciprofloxacin.

Figure 4.5.Percentage of samples from which one or more nalidixic acid and ciprofloxacin resistant *E. coli* were isolated



(Bars plot 95% confidence interval levels, N = number of samples containing *E. coli*)

4.3.2 Prevalence of antibiotic resistant *E. coli* from dogs that had received current or previous antibiotic treatment

The 95% binomial confidence level seen in the dogs on antibiotic treatment group does not overlap with any of the others groups for the majority of antibiotics tested (the exception being nalidixic acid). This is suggestive of a higher proportion of antibiotic resistant *E. coli* in this group. Using the chi-squared test there is also evidence of a statistically strong association between the use of antibiotic therapy in dogs and the isolation of one or more resistant *E. coli* from dog faecal samples, when compared between the different groups of dogs ($P= 6.627 \times 10^{-8}$, table 4.1).

Table 4.1.Number of dog faecal samples from which one or more resistant *E. coli* were isolated from dogs currently receiving and not receiving antibiotic treatment

Antibiotic resistant *E. coli* isolated?	Dogs on antibiotic treatment	Dogs not on antibiotic treatment
Yes	25	113
No	3	194

There was also evidence of a significant association between the isolation of antibiotic resistant *E. coli* and the use of different antibiotic therapy in dogs (P=0.004, table 4.2).

Table 4.2.Number of dog faecal samples from which one or more resistant *E. coli* were isolated from dogs receiving different antibiotic treatment

Antibiotic resistant *E. coli* isolated?	Dogs treated with co-amyoxyclav and deoxycycline	Dogs treated with deoxycycline
Yes	41	37
No	0	8

Antibiotic resistant E. coli from household dogs with previous antibiotic therapy

The chi-squared test was used to test if there was any significant difference between previous but not current antibiotic treatment and the isolation of antibiotic resistant *E. coli* from faecal samples from dogs in the household group. From the 84 dog faecal samples collected from household dogs, 61 were returned with a completed questionnaire. The analysis was only applied if antibiotics had been taken in the previous year as the numbers were too small to test significance between antibiotic treatment in the last month and isolation of antibiotic resistant *E. coli*. There was no

evidence of an association between antibiotic resistant *E. coli* being present and antibiotic usage in the last year (P=0.459, table 4.3).

Table 4.3.Number of faecal samples obtained from dogs in the household group from which one or more resistant *E. coli* was isolated, and had or had not received antibiotic treatment in the last year

Antibiotic resistant *E. coli* isolated?	Antibiotic treatment in the last year	No antibiotic treatment in the last year
Yes	5	11
No	26	31

4.3.3 Molecular characterisation of *E. coli* antibiotic resistance

Dogs being treated with antibiotics

Further characterisation was carried out on isolates from dogs being treated with antibiotics to try and determine if similar strains of ampicillin resistant *E. coli* appeared to be prevalent and if resistance determinants in such isolates were transferable.

A total of 71 ampicillin resistant isolates were tested and five transconjugants expressed resistance to ampicillin when subjected to antibiotic sensitivity testing. One of the ampicillin resistant transconjugants (no 349) was also resistant to tetracycline, chloramphenicol and trimethoprim.

The genetic relatedness of ampicillin resistant *E. coli* isolates was also determined using pulsed-field gel electrophoresis following digestion with *Xba*I. Genetic relatedness was assessed following criteria published by Tenover *et al.* (1995) and many of the isolates did appear to be related (2-3 banding pattern difference). Samples were obtained from dogs housed in the same block on both sampling occasions, samples from group 1 being obtained in October 2001, and isolates from group 2 being obtained in November 2002. Isolates exhibited similar antibiotic resistant

profiles, 49% (n=20/41) of resistant isolates from group 1 and 70% (n=24/34) isolates from group 2 being resistant to ampicillin, tetracycline and trimethoprim (see appendix A2). Isolates marked out in red also appear to have similar banding patterns between the 2 groups.

Figure 4.6.Group 1 *E. coli* isolates following digestion with *Xba*I

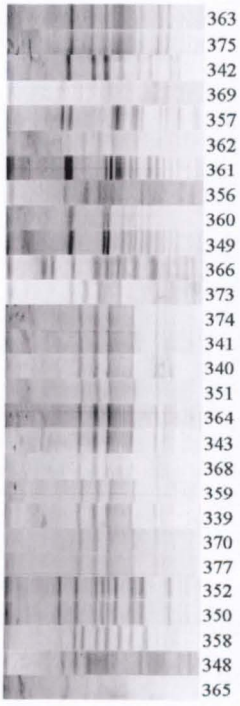
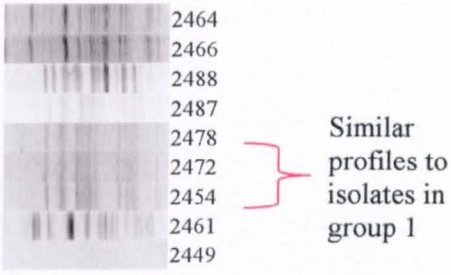
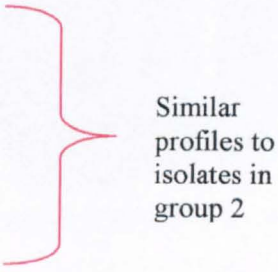


Figure 4.7.Group 2 *E. coli* isolates following digestion with *Xba*I



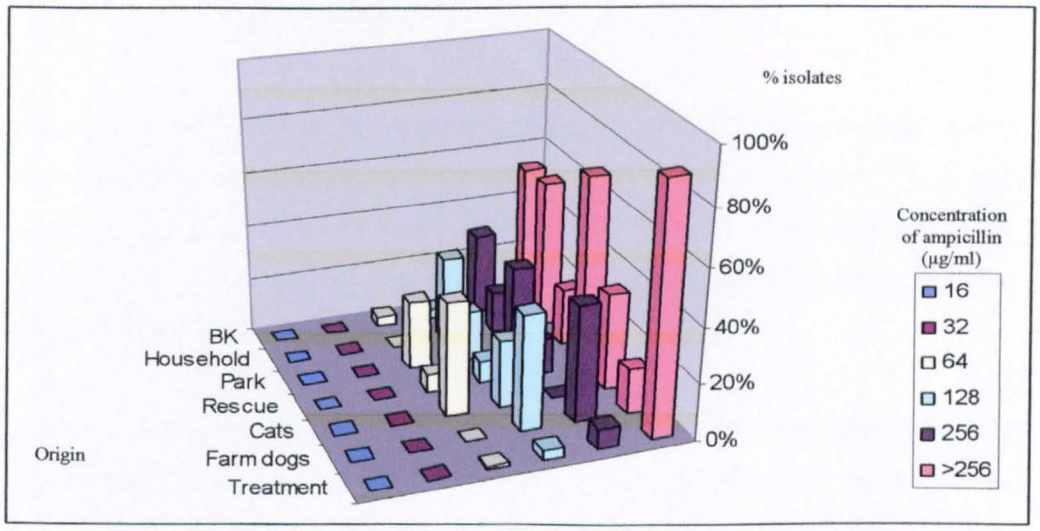


Ampicillin resistance

The MICs of ampicillin were determined for all *E. coli* isolates found to be resistant by disc diffusion (BSAC 2002). BSAC guidelines place the breakpoint concentration of ampicillin indicative of resistance in *E. coli* at 16µg/ml, and NCCLS guidelines at 32µg/ml. The MIC of ampicillin for the majority of dog isolates was 64µg/ml or

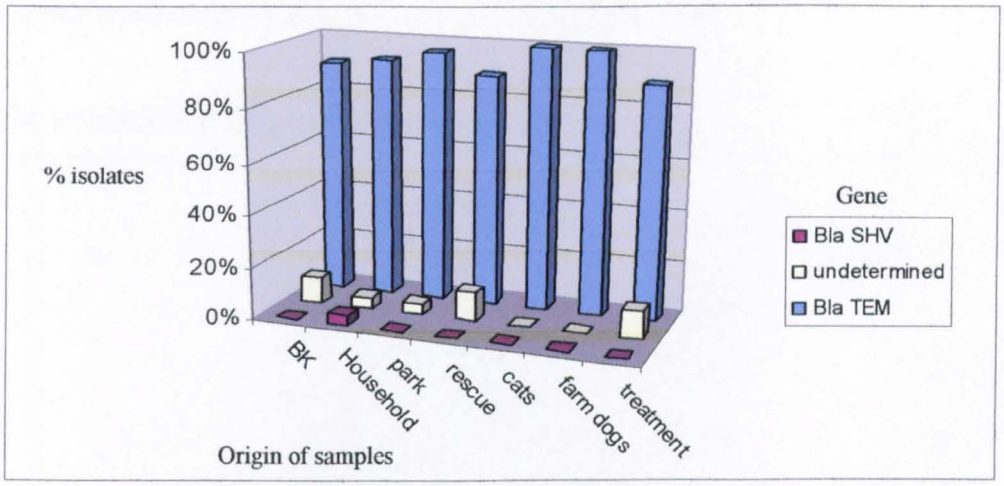
above (figure 4.8). One isolate from boarding kennels had a MIC of ampicillin of 8μg/ml, and 2 isolates from rescue homes had MICs of ampicillin of 1- and 2μg/ml (not shown on figure 4.8) and thus would be classified as sensitive. Isolates from dogs on antibiotic treatment had the highest proportion with an MIC of ampicillin of greater than 56μg/ml (n=64/72), the next highest group being isolates from dogs in rescue homes (n=45/64). MICs of ampicillin in isolates from household dogs, cats and samples from parks were more evenly distributed between higher and lower concentrations of ampicillin.

Figure 4.8.MICs of ampicillin for *E. coli* isolates



The majority of resistant *E. coli* isolates from all groups carried the *tem* gene (figure 4.9; BK – n=27/30, household – n=24/26, park – n=27/28, rescue – n=57/64, cats – n=17/17, Farm dogs – n=12/12, dogs on treatment – n=64/72). The isolate from boarding kennels with a MIC of ampicillin of 8μg/ml did not carry either the *tem* or *shv* genes, whereas two isolates from rescue homes with ampicillin MICs of 1- and 2μg/ml both carried *tem*. The *shv* gene was only found in one isolate from a household dog sample.

Figure 4.9.Ampicillin resistance genes present in *E. coli* isolates



The majority of isolates were sensitive to all additional β-lactam antibiotics tested. One isolate from a household dog faecal sample and one isolate from a farm dog faecal sample expressed resistance to cefoxitin. Both isolates possessed the *tem* gene. The farm dog isolate had an MIC of ampicillin of 256μg/ml and the household isolate had an MIC of ampicillin of >256μg/ml.

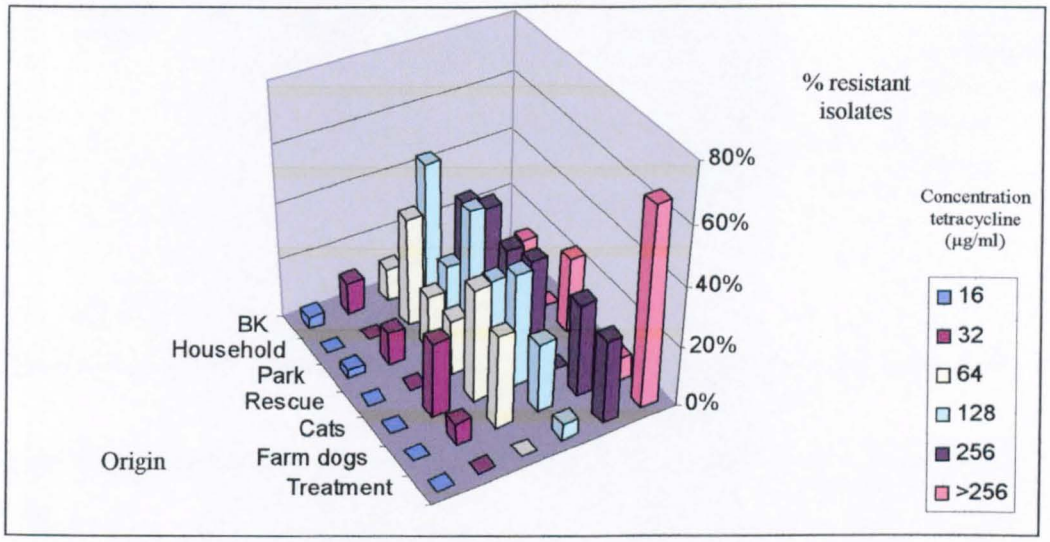
The 8% (n=19) of isolates that carried neither the *tem* or *shv* gene were not characterised further. The MIC of ampicillin for the majority of these isolates (n=18/19) was 128μg/ml or above, and they were all sensitive to the additional β-lactam antibiotics tested.

Tetracycline resistance

The breakpoint concentration of tetracycline indicative of resistance is 2μg/ml (BSAC) or 16μg/ml (NCCLS). MICs of tetracycline were highest for *E. coli* isolates from dogs on antibiotic treatment (n=46/69 isolates MICs >256μg/ml, figure 4.10). Isolates from boarding kennels, households and parks demonstrated the highest proportion of MICs of tetracycline at 128μg/ml (BK – n=12/27, park – n=12/29), whereas isolates from household dogs, farm dogs, rescue dogs and cats, MICs of tetracycline were spread relatively evenly over 256μg/ml, 128g/μml and 64μg/ml. One isolate from a sample obtained from boarding kennels had a MIC of tetracycline

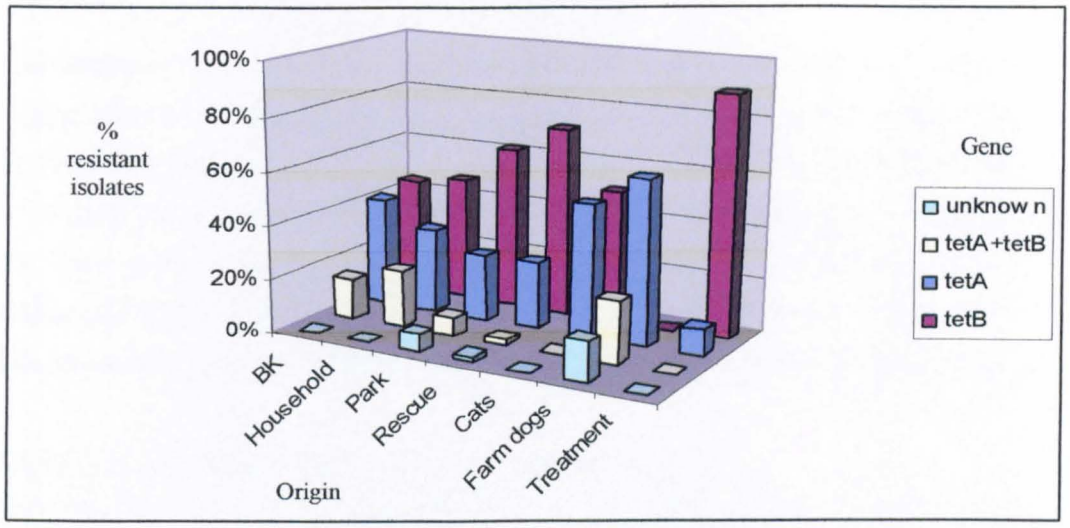
of 1µg/ml, classing it as sensitive according to both BSAC and NCCLS guidelines, but still it possessed the *tetB* gene (not shown on figure 4.10).

Figure 4.10.MICs of tetracycline for *E. coli* isolates



The most prevalent tetracycline resistance gene was *tetB* in the majority of groups (figure 4.11, Treatment – n=62/72, rescue homes – n=36/51). An equal number of *E. coli* isolates from cat faecal samples were positive for either the *tetA* or *tetB* gene by PCR (n=4/8). *E. coli* isolated from farm dogs demonstrated the opposite trend with *tetA* accounting for 62% (n=8/15) of tetracycline resistant strains. A small number of isolates which carried both the *tetA* and *tetB* genes were present in dog samples from households (n=4/19), boarding kennels (n=4/27), parks (n=2/29) and rescue homes (n=1/51). A small proportion of isolates did not carry any of the *tet* genes investigated by PCR.

Figure 4.11.Tetracycline resistance genes present in *E. coli*



Does the presence of tetA or tetB make a difference to the MIC of tetracycline in E. coli?

There was a significant association between isolates with an MIC of tetracycline of >256µg/ml and the expression of the *tetB* gene, in comparison to expression of the *tetA* gene (P=<0.001) using the chi-squared test (table 4.4); isolates that expressed the *tetB* gene had higher MIC's of tetracycline than isolates that expressed the *tetA* gene. Isolates that expressed both the *tetA* and *tetB* genes were excluded because of the low numbers making the chi-squared approximations inaccurate. Isolates from dogs on antibiotic treatment were also excluded. Isolates that expressed MICs of tetracycline of 64µg/ml and below were included in one category, again because of low numbers.

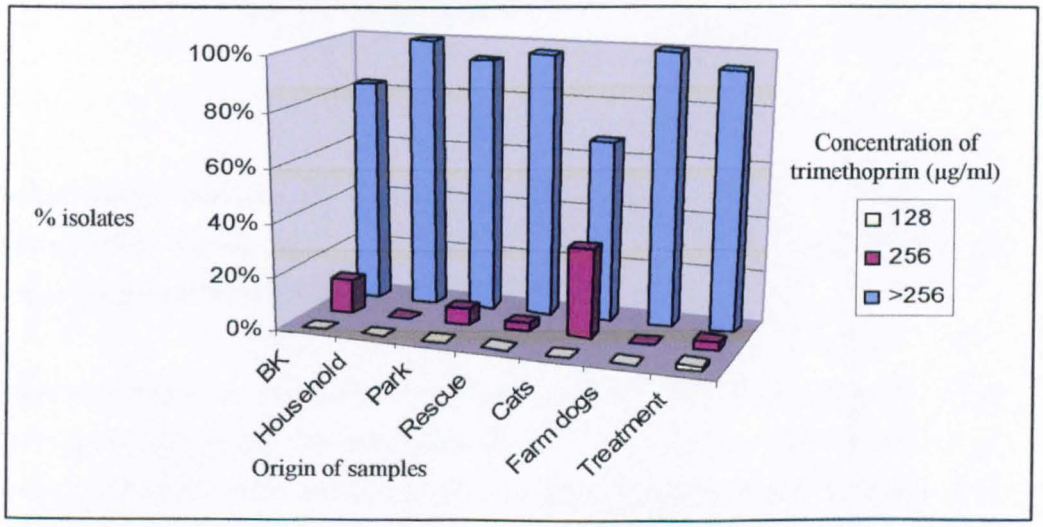
Table 4.4.Proportion of *tetA* and *tetB* isolates for MICs of tetracycline

Tetracycline MIC	*TetA*	*TetB*
>256µg/ml	4	16
256µg/ml	45	63

Trimethoprim resistance

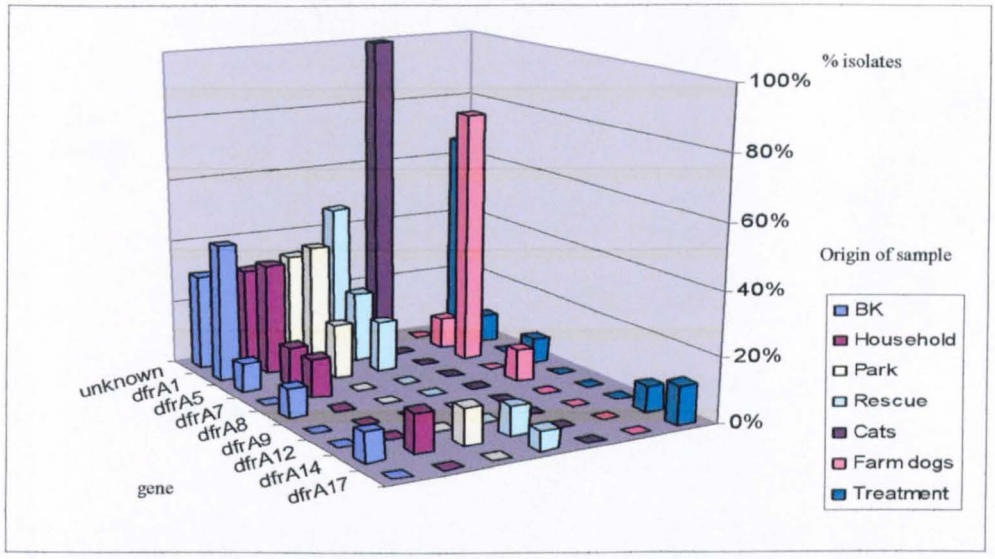
The breakpoint concentration of trimethoprim indicative of resistance in *E. coli* is 4μg/ml (BSAC) or 16μg/ml (NCCLS). The majority of isolates from all groups had an MIC of trimethoprim of >256μg/ml (BK – n=20/25, Household – n=28/28, Rescue – n=32/33, Parks – n=17/18, Cats, n=2/2, Farm dogs, n=10/10, treatment – n=60/63, see figure 4.12). One isolate from boarding kennels and one isolate from rescue homes had MICs of trimethoprim of 4μg/ml and 8μg/ml respectively, making them resistant according to BSAC guidelines but sensitive according to NCCLS guidelines.

Figure 4.12.Trimethoprim MICs for *E. coli* isolates



There were many different trimethoprim resistance genes present in *E. coli* isolates from all of the different groups and resistance genes were unable to be determined using PCR for many isolates as seen in figure 4.13. The most prevalent gene was *dfrA1* (households 32%, n=9/28, BK 40%, n=10/25, rescue 21%, n=7/33, parks 39%, n=7/18), with *dfrA5* being the second most prevalent gene (farm dogs 80%, n=8/10, parks 17% n=3/18, rescue 12%, n=4/33, dogs on antibiotics 8%, n=5/63, BK 8%, n=2/25, households 10%, n=3/29).

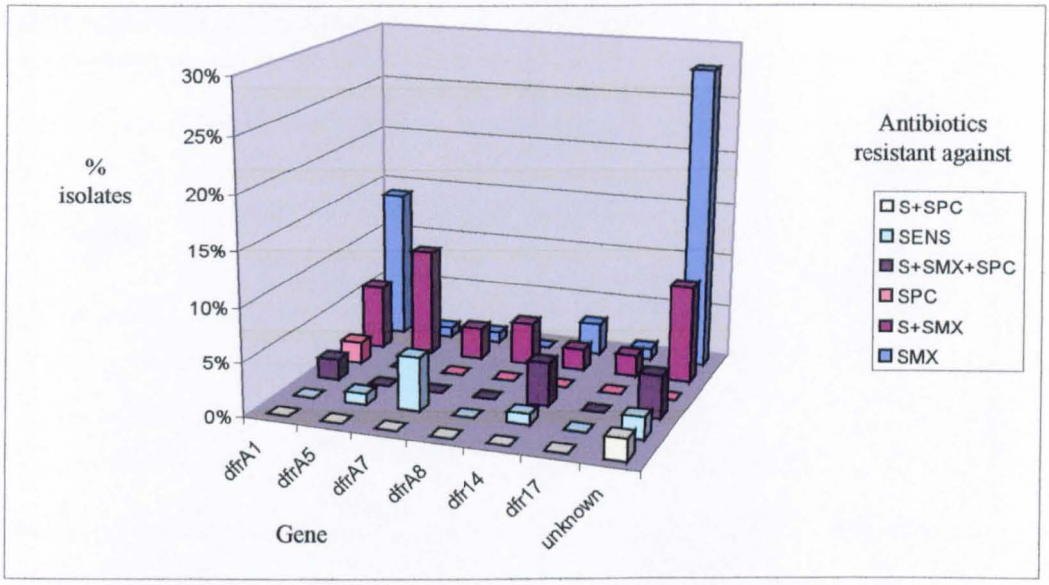
Figure 4.13. Trimethoprim resistance genes in *E. coli* isolates



Trimethoprim resistant *E. coli* isolates were also subjected to antimicrobial susceptibility testing with streptomycin, spectinomycin (aminoglycosides) and sulphamethoxazole (sulphonamide).

Resistance was most common to sulphamethoxazole and streptomycin in *E. coli* carrying all *dfr* genes, the exceptions being *E. coli* isolates carrying *dfr14*, the majority of which were resistant to all 3 antibiotics and *dfrA7* when isolates were mostly sensitive to all antibiotics (figure 4.14). Resistance to sulphamethoxazole was most common in isolates in which the mechanism of trimethoprim resistance could not be determined. Resistance to this antibiotic was also most common in isolates possessing the *dfr1* gene, with resistance to streptomycin and sulphamethoxazole being the second most common resistance pattern. All isolates that were sensitive to all three antibiotics had MICs of tetracycline of 128μg/ml and above.

Figure 4.14.Resistance to aminogylcosides and sulphonamides

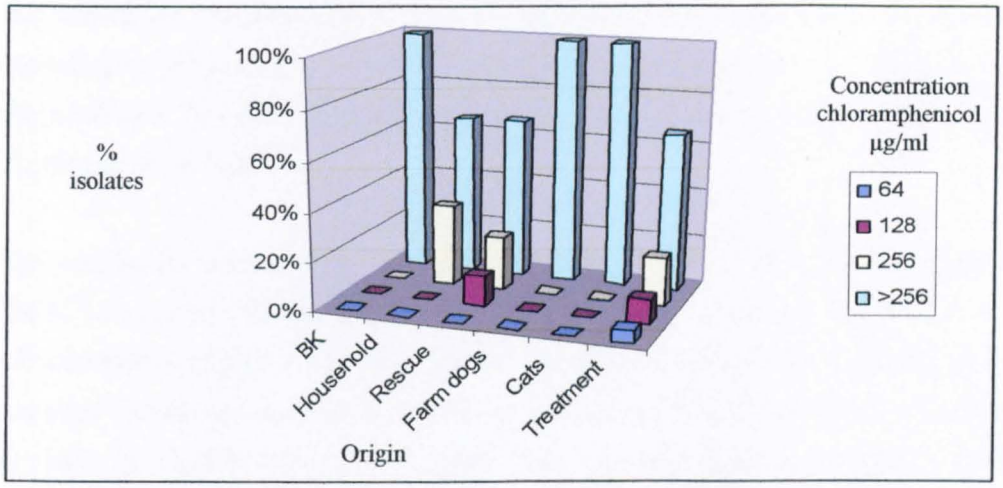


(S – streptomycin, SMX – sulphamethoxazole, SPC – spectinomycin, SENS – sensitive to all 3 antibiotics)

Chloramphenicol resistance

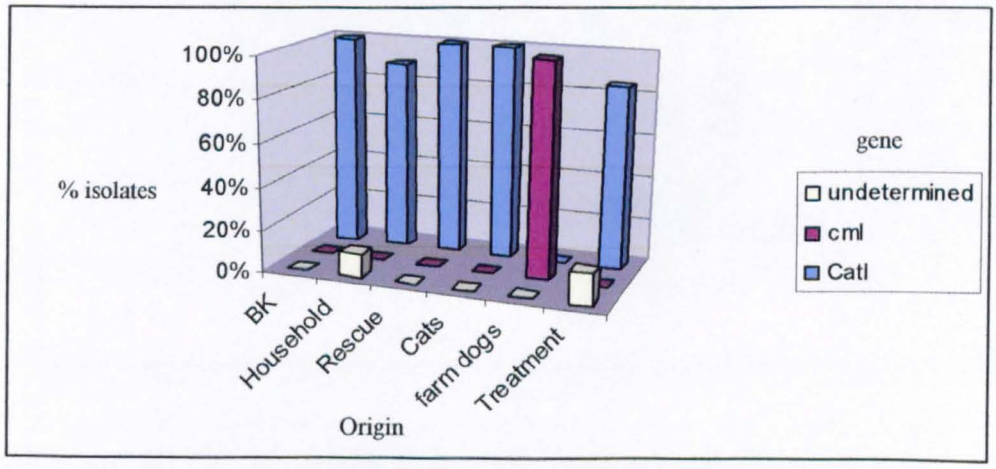
The breakpoint concentration of chloramphenicol indicative of resistance in *E. coli* is 16µg/ml (BSAC) or 32µg/ml (NCCLS). The majority of chloramphenicol resistant *E. coli* had MIC's of chloramphenicol of 256µg/ml or above (figure 4.15). All chloramphenicol resistant isolates from dog faecal samples from boarding kennels (n=4) and cat faecal samples (n=2) had MICs of chloramphenicol of >256µg/ml, whereas *E. coli* isolates obtained from dog faecal samples from households, rescue homes and dogs on treatment were slightly more varied and a small number of isolates exhibited MICs of chloramphenicol of 256µg/ml (Households n=3/9, rescue n=2/9, treatment n=3/18) and 128µg/ml (rescue n=1/9, treatment n=2/18). The single isolate from the farm dog sample had an MIC of chloramphenicol of 128µg/ml. The number of chloramphenicol resistant isolates was too small for any correlations between MIC and genotype to be detected.

Figure 4.15.MICs of chloramphenicol in *E. coli* isolates



The majority of chloramphenicol resistant *E. coli* contained the *catI* gene (figure 4.16; BK – n=4/4, Household n=8/9, Rescue – n=9/9, cats – n=2/2, dogs on treatment – n=17/18). The *cmlA* gene was present in one isolate (n=1/1) from a farm dog. Resistance determinants were undetermined from four isolates, one of nine from a household dog and three from eighteen dogs on treatment.

Figure 4.16.*E. coli* chloramphenicol resistance genes

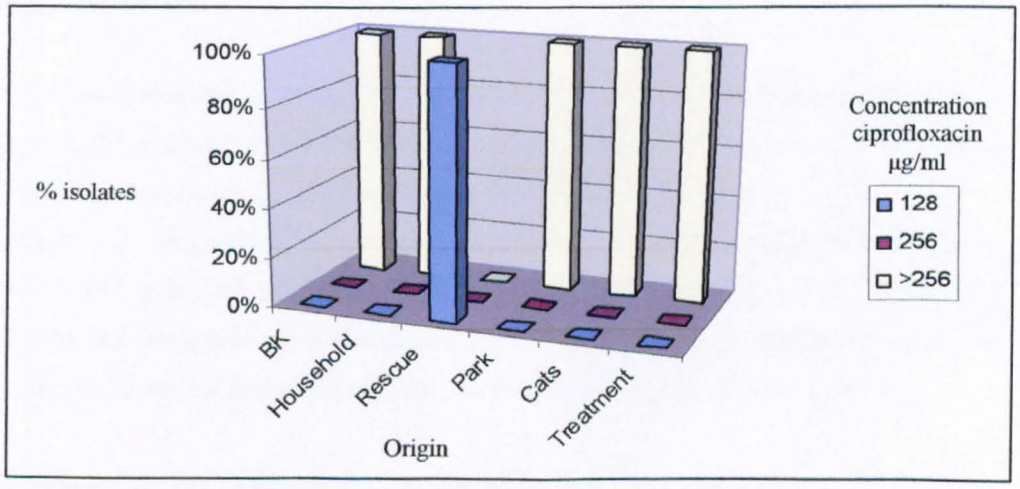


Nalidixic acid and ciprofloxacin resistance

The breakpoint concentration of nalidixic acid indicative of resistance is 16μg/ml according to NCCLS guidelines. BSAC does not have a breakpoint concentration for this antibiotic. However, the MICs of nalidixic acid were greater than 256μg/ml for all resistant *E. coli* isolates.

The breakpoint concentration of ciprofloxacin indicative of resistance is 1μg/ml (BSAC) or 4μg/ml (NCCLS). High MICs of ciprofloxacin were also observed in the *E. coli* isolates (figure 4.17). Sixty percent (n=3/5) of isolates from samples from boarding kennels had an MIC of ciprofloxacin of 64μg/ml and the others (n=2/5) had an MIC of ciprofloxacin of 128μg/ml. Fifty percent (n=2/4) of isolates from household dogs had MICs of ciprofloxacin of 64μg/ml whereas the others (n=2/4) had lower MICs of 4μg/ml. Isolates from dogs on antibiotic treatment had ciprofloxacin MICs that were evenly spread between 64μg/ml, 32μg/ml and 4μg/ml (n=3/9 for each).

Figure 4.17.MICs of ciprofloxacin in resistant isolates



For one or two *E. coli* isolates from each group, genomic DNA sequencing was performed to investigate mutations in the quinolone resistance determining region (QRDR) of *gyrA* and the analogous region of the *parC* gene. Sequences were

compared to quinolone sensitive strains, *E. coli* K12 (accession number U0096) and *E. coli* 0157 (accession number AP002560). Aligned sequences are in appendix A3.

Table 4.5.Mutations in *gyrA* and *parC* of quinolone resistant *E. coli* isolates

Isolate and Origin	Nal MIC	Cipro MIC	Amino acid change			
			GyrA		ParC	
			Ser-83	Asp-87	Ser-80	Glu-84
BK – 213	>256μg/ml	2μg/ml	Leu	Asp/Asn	Ile	Glu/Gly
BK – 243	>256μg/ml	64μg/ml	Leu	Asn	Ile	Gly
T – 371	>256μg/ml	4μg/ml	Leu	Asn	Ile	Glu
R – 2532	128μg/ml	2μg/ml	Ser	Asp	Ser	Glu
P – 2597	>256μg/ml	4μg/ml	Leu	Asn	F	F
H – 94	>256μg/ml	4μg/ml	F	F	Ser/Ile	Glu
H – 284	4μg/ml	2μg/ml	F	F	Ser	Glu

(BK – boarding kennels, T – dogs on antibiotics, R – rescue homes, P – parks, H – household, F – PCR product failed to be sequenced)

(Leu – Leucine, Asp – Aspartic acid, Asn – Asparagine, Ile – Isoleucine, Glu – Glutamic acid, Gly – glycine, ser - serine)

All isolates that had mutations in *gyrA* had mutations at both amino acid codon ser 83 and asp 87 and all isolates had MIC of nalidixic acid of >256μg/ml. All mutations at ser 83 were to leucine and exhibited a C→T conversion (table 4.5). Mutations at asp 84 had a G→A substitution leading to a substitution of aspartic acid for asparagine. Isolate 213 exhibited an indistinguishable amino acid change of an A or G at asp 87, due to the sequencing of the forward and reverse strands not matching at this particular base and therefore the amino acid is either aspartic acid or asparagine.

Mutations in *parC* were seen in serine at codon 80. In isolates 213, 243 and 371, isoleucine was substituted for serine, resulting from a nucleotide change of C→T. Isolate 94 exhibited an indistinguishable nucleotide change of G or T, again due to the forward and reverse sequences not matching, therefore the amino acid could either be serine or isoleucine. At Glu 84 in isolate 94, there was a nucleotide conversion of GAA→GAG not resulting in an amino acid change. Only isolate 243 exhibited a

codon change with a nucleotide substitution of GAA for GGA this resulted in the substitution of glutamic acid for glycine. None of the other isolates exhibited a nucleotide change at this site.

All isolates that exhibited mutations in *gyrA* also exhibited mutations in *parC* at ser 80. Only one isolate had a complete double mutation in *parC*, this resulted in a high MIC (64μg/ml) of ciprofloxacin.

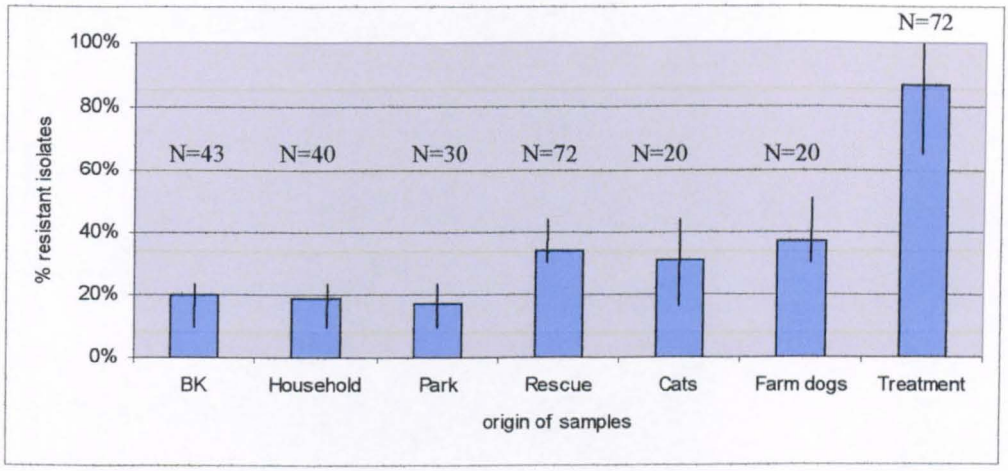
Apramycin resistance

No apramycin resistant *E. coli* were isolated from any samples in this cross sectional study.

4.3.4 Multi-drug resistant *E. coli*

E. coli were classed as multi-drug resistant (MDR) if isolates were resistant to 2 or more different antibiotics. The prevalence of MDR *E. coli* (figure 4.18) was significantly higher in dogs receiving antibiotic treatment than in the other groups (n=72/84), in which the prevalences were fairly similar; boarding kennels (n=43/217), parks (n=30/176) and household (n=40/206) samples. The prevalence was higher for farm dogs (n=20/54), cats (n=20/66) and rescue home samples (n=72/213). The binomial confidence level from the dogs on antibiotic treatment, again, does not overlap with any of the others groups suggesting a higher proportion of MDR *E. coli* from this group. There was a significant association between dogs on current antibiotic treatment and the isolation of MDR *E. coli* using the chi-squared test ($p=<0.0001$, table 4.6)

Figure 4.18.Percentage of *E. coli* isolates resistant to 2 or more antibiotics



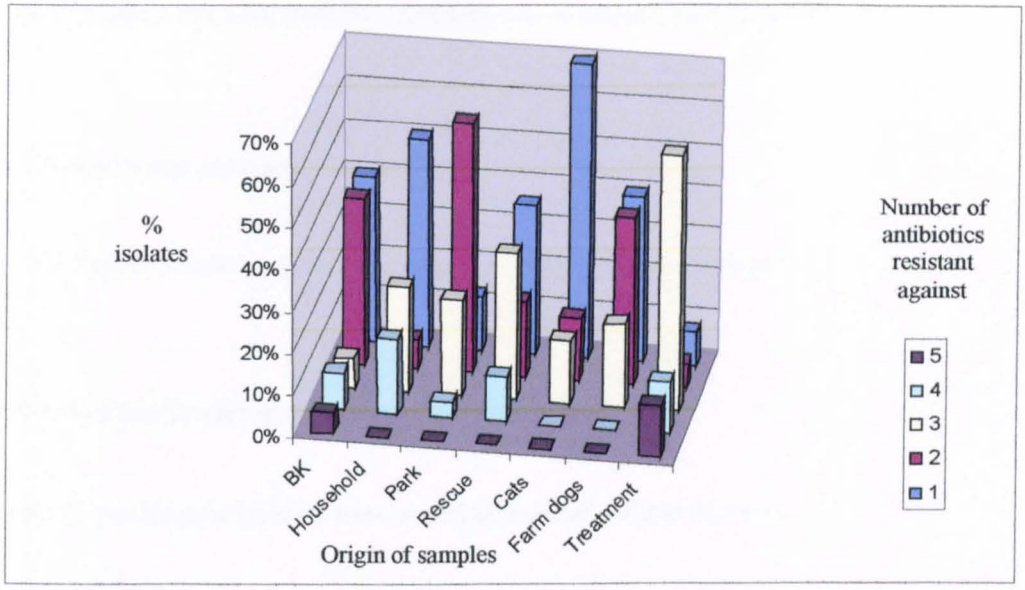
(Bars plot 95% confidence interval levels, N = number of samples containing *E. coli*)

Table 4.6.Number of MDR *E. coli* isolates obtained from dogs currently on antibiotic treatment and from dogs not on treatment

Currently on antibiotic treatment?	MDR *E. coli* isolated	Non MDR *E. coli* isolated
Yes	72	14
No	225	707

As can be seen from figure 4.19, the majority of MDR isolates from all groups were resistant against 1-3 different antibiotics and there was a low prevalence of isolates resistant to 4 or 5 different antibiotics. Almost the same number of isolates from boarding kennels and farm dogs were resistant to 1 or 2 antibiotics (n=17/43, n=8/20 respectively), whereas the majority of isolates from households (n=20/20), and cats (n=14/20) were resistant to just one antibiotic. The majority of isolates from dogs on antibiotics were resistant to 3 different antibiotics (n=44/72).

Figure 4.19.Percentage of *E. coli* isolates resistant against 1-5 different antibiotics



4.3.5 Antibiotic susceptibility in vancomycin resistant Enterococci

Isolates (n=2/74) obtained from household dogs both exhibited resistance to teicoplanin and azithromycin. The isolates obtained from the rescue (n=2/74) home samples exhibited resistance to teicoplanin and synercid.

4.3.6 Vancomycin resistance genes in enterococci

The *vanA* gene was found to be present in all 4 *E. faecium* isolates from rescue homes and household dogs.

4.3.7 Antibiotic susceptibility in *Campylobacter* spp.

Thirty isolates of *Campylobacter* spp. were tested against a panel of antibiotics and very little resistance was observed (although all were resistant to trimethoprim). Two *Campylobacter* spp. isolates (n=2/74 samples from household dogs) that could not be determined to species level were resistant to ampicillin. Two *C. upsaliensis* isolates

(n=2/76 samples, from boarding kennels) were also resistant to ampicillin. One *C. jejuni* isolate from a cat (n=1/22 samples) was resistant to tetracycline.

4.3.8 Antibiotic susceptibility in NTS.

All *S.* Typhimurium isolates were sensitive to all antibiotics tested.

4.3.9 Antibiotic susceptibility in *C. perfringens*

Both *C. perfringens* isolates were susceptible to all antibiotics tested.

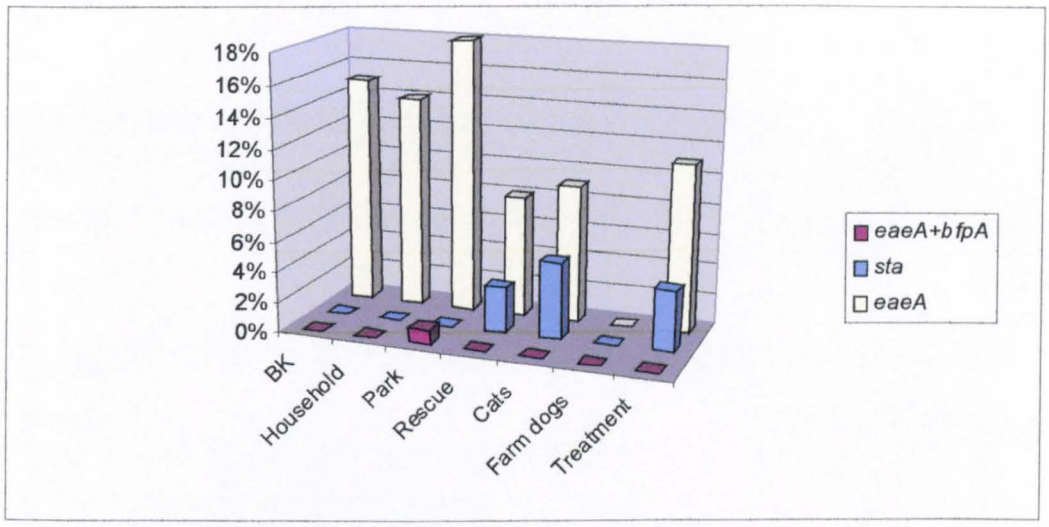
4.3.10 *C. perfringens* toxin genes

From the two *C. perfringens* isolates from household dogs, alpha toxin was shown to be present in one isolate from one sample (34) and beta-toxin in the other isolate from the other sample (468).

4.3.11 *E. coli* virulence genes

The most common virulence gene found in all groups (8% - 18%) using PCR was the *eaeA* gene (figure 4.20). One percent (n=1/76) of samples from parks also carried the *bfpA* gene, indicative of EPEC. Four percent (n=1/28) of samples from dogs on treatment, 3% (n=2/28) of dog samples from rescue homes and 5% (n=1/22) of faecal samples from cats possessed *E. coli* that was positive for the *sta* gene by PCR.

Figure 4.20.Percentage of faecal samples from which one or more *E. coli* possessing one or more virulence gene were isolated



Antibiotic resistant E. coli also carrying virulence determinants

E. coli resistant to one or more antibiotics, and also positive for the *eaeA*, *sta* or *eaeA* and *bfpA* gene together, were isolated from 25% (n=12/48) samples overall. Three *E. coli* isolates (4% of samples) positive for the *eaeA* or *sta* gene from parks and from dogs on antibiotic treatment were resistant to one or more antibiotics, whereas one isolate (2% samples) from a dog in boarding kennels, positive for the *eaeA* gene was resistant to a single antibiotic. Six isolates from rescue homes (8% samples) positive for the *eaeA* or *sta* gene were resistant to one or more antibiotics. No *E. coli* isolates positive for the *eaeA* or *sta* gene from cats or household dogs were resistant to antibiotics.

4.3.12 Faecal consistency, virulence factors and antibiotic resistance

There was no evidence of an association between the faecal consistency and the isolation of *E. coli* possessing virulence determinants. The Cochran-Amitage test for trend was carried to out to test whether any significant associations found were due to

a larger number (larger proportion) of samples at a particular faecal consistency. There was no evidence of linear trends in any of the groups.

Table 4.7.Number of *E. coli* isolated at different faecal consistencies

Origin of samples	Faecal consistency	No samples *E. coli*	No antibiotic resistant *E. coli*	Fisher's Exact test	Cochran-Amitage test for trend	
Boarding kennels	2	17	9	P=0.073	X^2_1= 1.597	P= 0.206
	2.5	21	3			
	3	23	6			
	3.5	6	3			
	4	5	0			
	4.5	1	0			
Household	2	12	4	P= 0.302	X^2_1= 0.458	P= 0.499
	2.5	21	2			
	3	19	5			
	3.5	10	4			
	4	11	3			
Rescue homes	2	14	8	P= 0.384	X^2_1= 0.011	P= 0.916
	2.5	15	7			
	3	17	5			
	3.5	16	10			
	4	11	5			
Parks	2	9	1	P= 0.100	X^2_1= 1.943	P= 0.163
	2.5	23	11			
	3	16	3			
	3.5	7	3			
	4	8	4			
	4.5	2	1			

Table 4.7.Cont.Number of *E. coli* isolated at different faecal consistencies

Cats	1.5	2	1	P=	X^2_1=	P=
	2	5	1	0.785	2.193	0.139
	2.5	4	1			
	3	5	3			
	3.5	0	0			
	4	4	2			
	4.5	1	1			
Farm dogs	2	5	3	P=	X^2_1=	P=
	2.5	5	3	0.805	0.115	0.73
	3	6	2			
	3.5	1	0			
	4	3	2			
Dogs on antibiotics	2	7	6	P=	X^2_1=	P=
	2.5	5	5	>0.999	1.619	0.203
	3	6	6			
	3.5	3	3			
	4	6	6			

4.3.13 Investigation of virulence determinants and antibiotic susceptibility in *E. coli* from dogs in a re-homing center over a period of 8 months.

There appeared to be no trend in the prevalence of *E. coli* carrying virulence genes or antibiotic resistant *E. coli* being isolated from repeated samples from the same dogs. One hundred and eighty four faecal samples were collected from 107 different dogs over a period of eight months. Only one sample was obtained from the majority of dogs (64%, n=68) as shown in table 4.8. Overall, *E. coli* was isolated from 91% of samples (n=167).

Table 4.8.Number of samples collected from dogs resident in the re-homing center

	No. of samples from each individual dog					
	1	2	3	4	5	6
No. of dogs	64% (n=68)	18% (n=20)	8% (n=9)	4% (n=4)	2% (n=2)	4% (n=4)

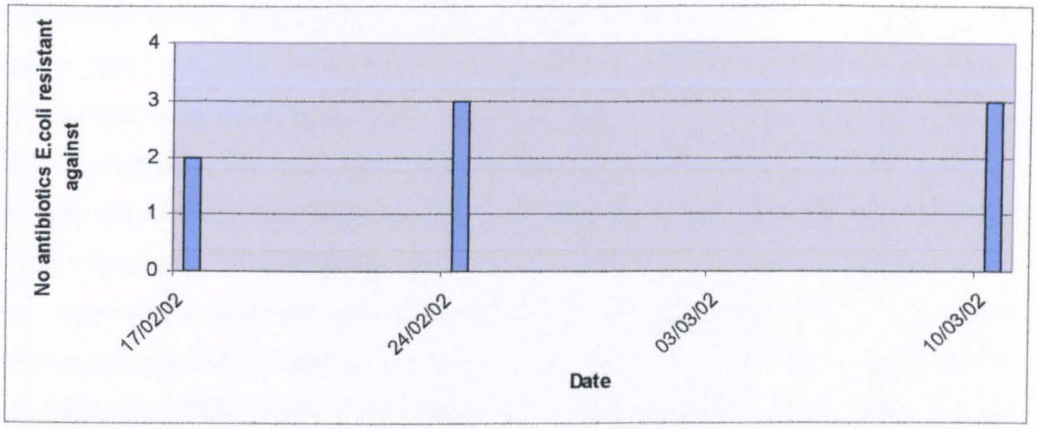
Prevalence of E. coli carrying virulence determinants

Overall, the *eaeA* gene was detected in 14% (n=23) dog faecal samples from which *E. coli* were isolated. Thirty nine percent (n=9) of these samples were from dogs aged 1 year or less. *E. coli* possessing the *eaeA* gene were only isolated on more than one sampling occasion from two dogs. Samples, from which *E. coli* were isolated, were collected from 'Speedy' on 1/1/02 and 10 days later on 11/2/02, and from 'Preston' on 13/3/02 and nearly 3 months later on 10/6/02 (appendix A2). The *sta* gene was isolated from one dog faecal sample (219) taken on 9/12/01.

Prevalence of antibiotic resistant E. coli

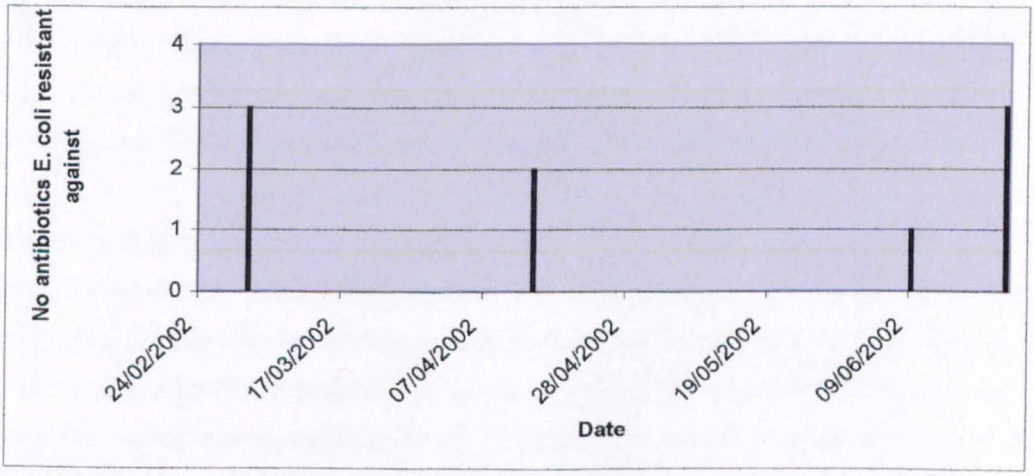
Overall, *E. coli* resistant to one or more antibiotics was isolated from 41% samples (n=68). There appeared to be no trend in the prevalence of antibiotic resistant *E. coli* isolated from faecal samples obtained from the same dogs. The graphs below (figures 4.21 and 4.22) show examples of repeated samples collected from the same dog over different periods of time. Graphs show *E. coli* isolates that were resistant to the highest number of antibiotics from each individual sample. Unfortunately PFGE was not carried out to determine relatedness of isolates.

Figure 4.21.Graph to show antibiotic resistant *E. coli* isolated from faecal samples obtained from Holly (lab-cross) over 3 weeks



(17/2/02 – *E. coli* resistant to ampicillin and tetracycline, 24/2/02 and 10/3/02 – *E. coli* resistant to ampicillin, tetracycline and trimethoprim)

Figure 4.22.Graph to show antibiotic resistant *E. coli* isolated from faecal samples collected from Preston (Neopolitan) over a period of 4 months



(4/3/02 and 13/3/02 – *E. coli* resistant to ampicillin and trimethoprim, 14/4/02 – *E. coli* resistant to ampicillin, tetracycline and trimethoprim, 10/6/02 and 24/6/02 – *E. coli* resistant to ampicillin and trimethoprim)

4.4 Discussion

The prevalence of dogs carrying antibiotic resistant *E. coli* in this study was much higher than expected. Interestingly, this study observed a significant association between the isolation of antibiotic resistant *E. coli* from dog faecal samples and the use of antibiotics for the treatment of kennel cough. There was also a significant association between dogs treated with one or two antibiotics and the prevalence of antibiotic resistant *E. coli* isolated from faecal samples from within this group. This may suggest that treatment with an increased number of antibiotics may increase the number of antibiotic resistant *E. coli* in the dog gut flora. The 95% binomial confidence level from dogs in this group did not overlap with the other groups for the majority of antibiotics (exception being the quinolones), suggesting that there is a higher proportion of antibiotic resistant *E. coli* in this group. PFGE analysis of several ampicillin resistant *E. coli* isolates obtained from different dogs that were being treated with antibiotics had very similar banding patterns suggesting possible transmission of the same *E. coli* strain between dogs, or the presence and spread of the same *E. coli* strain through an environmental source, for example, drains. However, only a small number of isolates were subjected to PFGE, and analysis was carried out following digestion with just one enzyme. Had more isolates been subjected to PFGE and also digested with another enzyme, isolates may have demonstrated different banding patterns, suggesting an uncommon source.

PFGE banding patterns from a number of ampicillin *E. coli* isolates obtained from dogs being treated with antibiotics were also very different. Several studies have suggested that the consumption of oral antibiotics may select for antibiotic resistant bacteria in the gut flora (Levy, 2000) and a recent study by Trott *et al.* (2004), found dogs that were given an oral dose (5mg) of enrofloxacin for 21 days had higher levels of multi-drug resistant *E. coli* in their faeces compared with dogs that were not treated. In this study however, in contrast to dogs currently being treated with antibiotics, there was no significant association between treatment with antibiotics over the proceeding year and the isolation of antibiotic resistant *E. coli* from dogs in the household group. This may suggest that the consumption of antibiotics does not increase the prevalence of antibiotic resistant *E. coli* in the gut flora long-term, although the number of samples able to be subjected to this test from the household

group, were small and owners who have had their dogs for a number of years, may have forgotten about prior antibiotic treatment. The prevalence of antibiotic resistant *E. coli* from dog faecal samples collected from the same dog over various time periods demonstrates that antibiotic resistant *E. coli* is isolated sporadically from faecal samples. Dogs are most likely to be colonised with and shed many different strains of *E. coli*, some being resistant and some not. In this study, only 3 colonies of *E. coli* were selected from each dog and therefore resistant *E. coli* may have been present in faecal samples but not selected.

There was also little evidence of mobile genetic elements being present in ampicillin resistant *E. coli* isolates from dogs being treated with antibiotics as demonstrated by conjugation studies. Due to time constraints the development of *E. coli* NCC 10536 as a rifampicin resistant strain, was not completed and therefore, the concentration of rifampicin that *E. coli* was resistant against may not have been high enough to enable selection of resistant transconjugants. A rifampicin resistant strain was necessary for selection of nalidixic resistant strains that would not be selected for using nalidixic acid resistant K12 *E. coli* strain. Further work would need to be undertaken to examine the relatedness of isolates, and the presence of mobile genetic elements further.

The findings from this study appear to show the prevalence of antibiotic resistant *E. coli* differing between dogs housed in different situations. A recent study by De Graef *et al.* (2004) observed a higher prevalence of multi-drug resistant (MDR) *E. coli* from dogs in kennels than from privately owned dogs. In my study there was generally a higher prevalence of *E. coli* isolates resistant to both a single antibiotic, and 2 or more antibiotics (MDR) from samples obtained from kennels (both boarding and rescue) than household dogs and other groups (exception being chloramphenicol resistance from *E. coli* isolates from boarding kennels). Dogs in kennels have contact with a greater number of dogs than they would normally, the dogs being from varied backgrounds and thus, increasing the potential reservoir of different antibiotic resistant *E. coli*. Splashing of faecal material when kennels are cleaned out, and dogs being stressed and therefore having diarrhoea, may also increase the possibility of transmission of antibiotic resistant *E. coli*, as well as other bacteria between dogs. There also appeared to be a higher prevalence of resistant *E. coli* being isolated from

rescue homes than boarding kennels. Dogs resident in rescue kennels were mostly stray dogs and the behavior of stray dogs, e.g. scavenging in bins, may increase their potential to acquire antibiotic resistant *E. coli* when compared to boarding kennel dogs that are usually resident in households, and have contact with a relatively small number of dogs outside of this household. Antibiotic resistant *E. coli* can be isolated from soil (Dr N. Williams, pers comms), and although great care was taken when samples were collected, it is not possible to completely exclude the possibility that dog faecal samples had been contaminated by soil.

There have been few studies on the genes responsible for antibiotic resistance in bacterium from faecal samples from the healthy canine population (Sáenz *et al.*, 2003, Bryan *et al.*, 2004), especially in the UK (Normand *et al.*, 2000b). Many of the genes responsible for antibiotic resistance in *E. coli* were, unfortunately, not determined in this study. PCR is used to investigate the presence of genes that are carried most frequently in resistant *E. coli* or gram-negative bacterium, however, genes accountable for resistance that are observed less frequently may have been responsible but due to time constraints, their presence could not be investigated. This is still interesting, as you would expect the most common genes that we found in human *E. coli* isolates are also carried by dog isolates if there was transmission between the two. This may suggest that there is limited transmission of *E. coli* between humans and dogs. When resistance genes could not be determined and a low antibiotic MIC is observed, resistance may be due to a multi-drug resistance (MDR) phenotype, for example expression of *marA* causing increased efflux of unrelated antibiotics.

A number of genes that were carried by *E. coli* in this study have been observed in dog *E. coli* isolates in previous studies. This includes *tem* (Teshager *et al.*, 2000), *tetA*, *tetB* and *tetC* (Bryan *et al.*, 2004), *dfr17* and mutations in the QRDR of *gyrA* and *parC* (Sàenz *et al.*, 2003). These genes are widespread among *E. coli* and/or other gram-negative bacteria from human and veterinary sources (Baker *et al.*, 1999, Chopra and Roberts, 2001, Lee *et al.*, 2001, Everett *et al.*, 1996, Vila *et al.*, 1996, Sáenz *et al.*, 2003, Chaniotaki *et al.*, 2004). The majority of *E. coli* isolates carrying antibiotic resistant genes from previous studies in dogs were isolated from wound or urinary tract infections, and to our knowledge, this is the first time *E. coli* carrying

many of the antibiotic resistance genes observed in this study, have been reported in faecal samples from the healthy canine population in the UK. This may suggest that wound and urinary tract infections in dogs are caused by the dogs own faecal contamination although further work would be needed to support this theory.

The majority of ampicillin resistant *E. coli* isolates in this study were sensitive to additional β-lactam antibiotics. This suggests that the *tem* and *shv* genes possessed by *E. coli* isolates in this study have acquired few mutations and isolates do not express extended spectrum β-lactamases. The *tem* gene may have acquired one or more mutations (one or more amino acid substitutions) from the classic *tem*-1 in isolates that were also resistant to cefoxitin, however, these isolates were not investigated further.

Interestingly, there was a significant association between isolates that expressed *tetB* and an had an MIC of tetracycline of >256μg/ml. High MICs of tetracycline associated with the presence of *tetB* have been seen before in *E. coli* isolates from swine and cattle (Lee *et al.*, 1993, Blake *et al.*, 2003). This observation has been suggested as an effect of previous antibiotic usage and the transmission and persistence in the farm environment. Why this effect is seen in *E. coli* isolates from healthy dogs in this study is unknown, however, the number of isolates subjected to this test was fairly small.

The most common *dfr* gene responsible for trimethoprim resistance in *E. coli* isolates found in this study was *dfrA1*, and this also appears to be the most prevalent gene found in Gram-negative bacteria (Sköld, 2002). This has been attributed to the success of its carrier transposon Tn7 that can readily insert itself into the *E. coli* chromosome (Craig, 1991). In this study, resistance was most frequently seen to either sulphamethoxazole (sulphonamide) alone or to both sulphamethoxazole and streptomycin in trimethoprim resistant isolates. Although no further investigation into the presence of integrons or gene cassettes was carried out on these isolates, these findings suggest that the *sul1* or *sul2* gene encoding for sulphonamide resistance may be fairly widespread within trimethoprim resistant *E. coli* isolates from healthy dogs. A recent study in the UK found that the *sul2* gene was more commonly found in human clinical isolates (Enne *et al.*, 2001).

The prevalence of chloramphenicol, nalidixic acid and ciprofloxacin resistant *E. coli* was lower than that for ampicillin, tetracycline and trimethoprim. Chloramphenicol resistant *E. coli* have been isolated from wound and UTI infections in veterinary isolates from dogs and the *cmlA* homologue *flo* was found to be responsible (Sanchez *et al.*, 2002). The *flo* gene shares 57% amino acid sequence identity to that of *cmlA* (Cannon *et al.*, 1991).

In this study, only one of the ciprofloxacin isolates sequenced had an MIC to ciprofloxacin of 64μg/ml and also possessed double mutations in both *gyrA* and *parC*. All other isolates exhibited single, if any mutations in *parC*, resulting in ciprofloxacin MIC's of 2-4μg/ml. The additional amino acid change in *parC* may account for the higher MIC's seen to ciprofloxacin in this isolate. Previous studies have associated double mutations in both *gyrA* and *parC* with an increased ciprofloxacin MIC (Vila *et al.*, 1994, Vila *et al.*, 1996). Substitution with leucine at ser-83 (*gyrA*) and isoleucine for ser-80 (*parC*) as seen in this study has been reported in both human and veterinary isolates, including dogs (Everett *et al.*, 1996, Vila *et al.*, 1996, Sáenz *et al.*, 2003, Chaniotaki *et al.*, 2004). One isolate had an MIC of nalidixic acid of 128μg/ml and an MIC of ciprofloxacin of 4μg/ml but did not show any QRDR mutations in *gyrA* or *parC*. Mechanisms involving drug permeation or drug efflux (e.g. *marA*) may account for resistance in this case. *gyrB* and *parE* were not investigated in this study as mutation in these genes are considered of minor importance in resistance to the quinolones.

E. coli carrying virulence determinants

The high prevalence of the *eaeA* gene from healthy cats and dogs suggests that the presence of this gene is not necessarily associated with diarrhoea. Surprisingly, the *eaeA* gene was not present in *E. coli* from samples obtained from farm dogs. Cattle are known to be major reservoirs of VTEC (Montenegro *et al.*, 1990, Beautin *et al.*, 1997) and it would be probable that dogs on farms would have frequent contact with cattle and therefore have increased risk of infection. Studies in cattle have suggested that the *eaeA* gene may be involved as a colonisation factor in *E. coli*. Mutants of *E. coli* 0157:H7 that were *eaeA* negative were not shed in such great numbers or for as a

long a duration as wild-type *E. coli* O157:H7 that were *eaeA* positive (Cornick *et al.*, 2002, Cookson and Woodward, 2003). Therefore the role of the *eaeA* gene in *E. coli* from dogs may be more involved in bacterial colonisation. A high prevalence of *E. coli* carrying the *eaeA* gene was seen in samples obtained from households and parks. *E. coli* possessing the *eaeA* gene have been isolated from soil (H. Leatherbarrow, pers comms) and gardening or garden play have been implicated as risk factors for infection with *E. coli* O157 (Coia *et al.*, 1998). Although care was taken over the collection of samples from parks, this may represent possible environmental contamination with soil, although this is highly unlikely. Samples from household dogs were usually collected by the owner and therefore care may not have taken when obtaining samples. *E. coli* possessing the *eaeA* gene were also isolated from cats. This gene has been isolated from healthy cats in a previous study and has not been associated with diarrhoea (Scaletsky *et al.*, 1984).

The *eaeA* gene is situated on the locus of enterocyte effacement (LEE), a pathogenicity island that also contains genes needed to cause lesions on human and animal epithelium (Jores *et al.*, 2004). The LEE locus has been found in EPEC isolates from dogs, although reports are limited (Goffaux *et al.*, 2000, Nakazato *et al.*, 2004). The occurrence of the *eaeA* gene in *E. coli* isolates is usually indicative of LEE being present (Jorres *et al.*, 2004), although in this study the presence of LEE or colonisation factors were not investigated. EPEC strains closely related to those found in human cases have been isolated from dogs (Goffaux *et al.*, 2000, Nakazato *et al.*, 2004).

There was a low prevalence of *eaeA* gene together with the *bfpA* gene (indicative of EPEC), and the *sta* gene. The low prevalence of these genes may suggest that they are involved in causing disease in dogs and previous studies have associated EPEC (Drolet *et al.*, 1994, Beaudry *et al.*, 1996, Sancak *et al.*, 1997, Goffaux *et al.*, 2000) and ETEC (Richer *et al.*, 1987, Hammermueller *et al.*, 1995) with diarrhoea in dogs. However, in this study all isolates carrying such virulence determinants were isolated from apparently healthy dogs. Interestingly, all isolates carrying the *sta* gene were from samples collected from dog rescue homes. *E. coli* possessing this gene were also isolated from the dog re-homing centre (longitudinal study). This suggests that communal conditions may be a risk factor for ETEC infection in dogs. *E. coli*

carrying this gene was also isolated from a sample from a household cat, also not having diarrhoea. There is very little known about ETEC infection in cats (Beautin, 1999) and the *sta* gene has not been reported in previous studies (Abaas *et al.*, 1989). However, due to a small sample size there are few conclusions that can be drawn from this information.

Other antibiotic resistance

All *S.* Typhimurim isolates in this study were sensitive to all the antibiotics tested and there have been few previous reports of antibiotic resistant *Salmonella* spp. from dogs (Gray *et al.*, 2004, Seepersadsingh *et al.*, 2004). *S.* Typhimurium isolated from clinical cases in humans are usually resistant to one or more antibiotics (Guardabassi *et al.*, 2004) which suggests that dogs are not a major reservoir for *Salmonella* spp. infection for humans.

All *Campylobacter* spp. isolates were resistant to trimethoprim, against which, *Campylobacter* spp. is considered intrinsically resistant. Two *C. upsaliensis* isolates, 2 isolates of an undetermined *Campylobacter* species and one *C. jejuni* isolate were also resistant to ampicillin, all isolates being from samples collected in boarding kennels, although on separate occasions. A study by Adeynsin *et al.*, (1999) observed a higher prevalence of antibiotic resistance in *Campylobacter* spp. isolates from boarding kennels than household dogs which agrees with the findings of our study. There have been few other reports on antibiotic resistance on *Campylobacter* spp. from dogs (Preston *et al.*, 1990).

C. perfringens was only isolated from 2 dogs, both showing no signs of diarrhoea. CPE was not found in either isolates and these findings agree with previous studies that have found *C. pefringens* as a causative agent of diarrhoea in dogs, but only when CPE is produced (Weese *et al.*, 2001, Marks *et al.*, 2002).

VRE was only isolated from 4 isolates, all being resistant to multiple antibiotics. All isolates exhibited the *vanA* genotype, this being the most common genotype isolated from dogs (van Belkum *et al.*, 1996). The prevalence of VRE in this study is much

lower than that reported in other studies (Deveriese *et al.*, 1996, van Belkum *et al.*, 1996), although there been many reports of the absence of VRE from dog faecal samples (Harward *et al.*, 2001, Wagenvoort *et al.*, 2003).

The results of this study suggest that dogs are not major carriers of either *C. pefringens* or VRE.

The results of this study demonstrate that a high prevalence of dogs excrete antibiotic resistant *E. coli*, and *E. coli* carrying virulence determinants. The limited number of resistance genes determined in this study are similar to those found in human isolates (Guardabassi *et al.*, 2004), however, since the majority of genes carried were not determined, this may suggests that there is limited transmission of *E. coli* between dogs and humans. To our knowledge this is the first time that many of these genes have been reported from a healthy canine population. The prevalence of *Salmonella* spp., *C. perfringens* and VRE were low suggesting that dogs are not a major reservoir of these bacteria. However, dogs do carry bacteria that could be potentially harmful for humans and further investigation is needed to determine precisely how great a zoonotic risk for humans dogs do pose.

Chapter 5

The prevalence of *Campylobacter* spp., enteropathogenic *E. coli* and antimicrobial resistance in *E. coli* in dogs with and without diarrhoea.

5.1 Introduction

The aim of this study was to determine the prevalence of *Campylobacter* spp. and pathogenic *E. coli* in dogs with and without diarrhoea. The antimicrobial susceptibility of these pathogens, and also the presence of commensal *E. coli* were also investigated. In humans, *Campylobacter* spp. and pathogenic *E. coli* are well documented as causes of diarrhoea (www.hpa.org.uk, Swartz, 2002); however, their role as gastrointestinal pathogens in dogs is unclear.

Campylobacter spp. have been isolated from dogs with and without diarrhoea with prevalences being reported between 5% and 66% (Chattopadhyay *et al.*, 2001, Hald *et al.*, 1997, Burnens *et al.*, 1992) and 5% - 48% (Sandberg *et al.*, 2002) respectively. While some studies have reported a higher prevalence of *Campylobacter* spp. from dogs with diarrhoea (McOrist and Browning, 1982, Chattopadhyay *et al.*, 2001), many studies have not (Olsen and Sandstedt, 1987, Figura 1991, Burnens *et al*, 1992, Adesiyun *et al*, 1997, Baker *et al*, 1999, Sanberg *et al.*, 2002). Experimental infections have produced similarly mixed results (Prescott and Barker, 1978, Prescott et al., 1981). It has been suggested that *Campylobacter* spp. may act as secondary pathogens, following infection with, for example, parvovirus, rotavirus, hookworms, whipworms or coccidia (Fox, *et al*, 1983, Sandstedt *et al*, 1983, Olsen and Sandstedt, 1987, Brown *et al*, 1999).

A higher prevalence of *Campylobacter* spp. has been reported in dogs below one year of age although it is not associated with diarrhoea in this age group (Fleming *et* al., 1980, Blaser *et al.*, 1980, Bruce *et al.*, 1983, López *et al.*, 2002, Engvall *et al.*, 2003, Hald *et al.*, 2004).

The predominant *Campylobacter* species reported from both healthy and diarrhoeic dogs is *C. jejuni*. This, however, may be due to the under reporting of other species such as *C. upsaliensis* (see chapter 3). The majority of *Campylobacter* isolation media are optimised for the isolation of better known species of *Campylobacter*, such as *C. jejuni, C. coli* and *C. lari*, and contains antibiotic supplements which may inhibit the growth of other species (Byrne *et al.*, 2001). Studies that have not used selective supplements in media have found *C. upsaliensis* to be the most common *Campylobacter* species isolated from dog faeces (Hald *et al.*, 2004).

There are a number of pathogenic *E. coli* serotypes that have been associated with diarrhoeal illness in dogs (Beutin, 1999). These include enterotoxigenic *E. coli* (ETEC), verotoxigenic *E. coli* (VTEC), including enterohemorrhagic *E. coli* (EHEC) and enteropathogenic *E. coli* (EPEC); (Beutin *et al.*, 1993, Beaudry *et al.*, 1996, Beautin, 1999, Goffaux *et al.*, 2000, Neiger *et al.*, 2002). However, virulence determinants attributed to pathogenic strains of *E. coli*, have also been found in healthy dogs (Holland *et al.*, 1999), and of course these virulence factors have been defined on the basis of the properties in humans, not dogs. Pathogenic strains of *E. coli* involved in intestinal infections in sheep, pigs and cattle have been well characterised and studied (Beutin *et al.*, 1997, Leung *et al.*, 2001), but the roles of virulence factors involved in gastrointestinal disease in dogs is less well defined.

ETEC with heat stable toxin have been reported only in dogs with diarrhoea (Hammermueller *et al.*, 1995, Richer *et al.*, 1987), and there are no reports of heat labile toxin present in canine *E. coli* (Hammermueller *et al.*, 1995). VTEC, however, have been reported in both diarrhoeic, and healthy cats and dogs (Beutin *et al.*, 1995, Tuck *et al.*, 1998, Holland *et al.*, 1999, Smith *et al.*, 1998), although Hammerueller *et al.* (1995) reported an association between diarrhoea in dogs and VT2-producing VTEC. EHEC 0157:H7 phage type 4 has also been isolated from dog faeces, although it was not reported whether diarrhoea was present (Trevena *et al.*, 1990). Sancak *et al.*, (2004) reported a higher prevalence of VTEC in healthy kenneled dogs than healthy dogs in

private households. EPEC are considered an important cause of diarrhoea in puppies (Drolet *et al.*, 1994, Beaudry *et al.*, 1996, Sancak *et al.*, 1997, Goffaux *et al.*, 2000). There have been reports of attaching and effacing lesions present in intestinal epithelium in dogs, and these lesions were usually associated with the presence of *E. coli* carrying the *eaeA* gene, which encodes intimin (Broes *et al.*, 1988, Janke *et al.*, 1989, Droplet *et al.*, 1994, Beaudry *et al.*, 1995, Goffaux *et al.*, 2000, Neiger *et al.*, 2002). The *eaeA* gene has been found to be expressed in *E. coli* isolates from dogs with and without diarrhoea. Canine strains of EPEC have been found to produce a different intimin from that detected in human and other animal EPEC strains (An *et al.*, 1997). The *eaeA* gene is located in the locus of enterocyte effacement (LEE) and in dogs, the LEE locus has been found in all EPEC isolates (Nakazato *et al.*, 2004). EPEC strains closely related to those found in human cases have been isolated from dogs (Goffaux *et al.*, 2000). The EPEC adherence factor (EAF) plasmid has also been found in dog EPEC isolates (Droplet *et al.*, 1994). The *bfpA* gene is located on the EAF plasmid, this gene encodes the bundle forming pili which are involved in the organisation of EPEC into micro-colonies and promoting their stabilisation.

The prevalence and clinical significance of antibiotic resistance genes is well known in bacterial isolates from human clinical cases, but the dissemination of antibiotic resistance genes and organisms within the vet visiting canine veterinary population is not widely known (Normand *et al.*, 2000b). The consequences of antibiotic therapy in small animal practice does not appear to differ from that observed in human medicine (Guardabassi *et al.*, 2004). Rising trends in antibiotic resistance have been observed in dog clinical isolates of *E. coli* and Staphylococci from dogs in the UK (Lloyd *et al.*, 1996, Normand *et al.*, 2000a) and other European countries (Pellerin *et al.*, Wissing *et al.*, 2001).

Due to the close contact that humans have with dogs, it has been suggested that dogs may act as a reservoir of infection for antibiotic resistant and pathogenic bacteria for humans (Blaser *et al.*, 1978, Hoise *et al.*, 1979, Bruce *et al.*, 1980, Adak *et al.*, 1995, Beautin 1999, Johnson *et al.*, 2001 Neimann *et al.*, 2003, Hald *et al.*, 2004).

The present study has investigated *Campylobacter* spp., and pathogenic *E. coli* as a cause of diarrohea in dogs, and also the prevalence of antibiotic resistance genes within commensal flora of dogs. This was done as part of wider study investigating dogs as a possible zoonotic risk through the transmission of pathogenic and antibiotic resistant bacteria to humans.

5.2 Methods

5.2.1 Sample collection

Faecal samples were collected from dogs with and without diarrhoea, on admission to the Small Animal Hospital referral clinic, University of Liverpool, between November 2002 and October 2003 (listed in appendix A3). Thirty-one samples were collected from 28 dogs with chronic diarrhoea and 31 samples were collected from 22 dogs with acute diarrhoea. Diarrhoea was diagnosed as chronic if it lasted for longer than 5 days. A further 45 samples were collected from dogs without signs of gastro-intestinal (GI) disease including 9 healthy staff dogs and 34 non-GI cases (4 oncology, 4 orthopaedic, 2 cardiovascular, 2 endocrine, 2 urogenital, 3 respiratory, 1 neurology, 1 oesophagial, and 16 not stated).

Three dogs in the control group and three dogs with chronic diarrhoea were being treated with antibiotics at the time of sampling. All dogs were visiting with the exception of 2 dogs with chronic diarrhoea that stayed in the hospital overnight.

5.2.2 Bacterial isolation

All faecal samples were collected in sterile universal containers and processed in the laboratory for isolation of *E. coli* and *Campylobacter* spp. within 48 hours of collection, using the methods described in chapter 2 (2.2.1 and 2.2.2).

5.2.3 Molecular characterisation

E. coli virulence gene PCR

All *E. coli* isolates were investigated for the presence of pathogenic virulence determinants using PCR, as described in chapter 2 (2.6.4)

Antibiotic susceptibility

All *Campylobacter* spp. isolates were subjected to antimicrobial susceptibility testing using disc diffusion according to BSAC guidelines (section 2.3.4).

All *E. coli* isolates were subjected to antimicrobial susceptibility testing using disc diffusion according to BSAC guidelines (section 2.3.1)

The Minimum Inhibitory Concentration (MIC) of each antibiotic was determined for resistant *E. coli* isolates as described in chapter 2 (section 2.4). Isolates were also investigated for the presence of antibiotic resistance genes responsible using PCR (section 2.6.5).

Ampicillin resistant *E. coli* were investigated for extended spectrum activity as described in chapter 2 (section 2.3.3). Trimethoprim resistant isolates were also investigated for resistance to specific aminoglycosides, indicating the possibility of resistance genes being present on integrons (section 2.3.4).

Pulsed field gel electrophoresis

Campylobacter spp. isolates were compared by pulsed field gel electrophoresis as previously described in chapter 2 (2.7.2).

Statistical analysis

Analysis was carried out using the chi squared and Fisher's Exact tests.

5.3 Results

5.3.1 Prevalence of *Campylobacter* spp.

Campylobacter spp. were isolated from seven samples, five from dogs with diarrhoea and two from dogs without diarrhoea (table 5.1). There was no significant association between the isolation of *Campylobacter* spp. and the presence of diarrhoea in dogs using Fishers exact test (P=0.44).

Table 5.1 *Campylobacter* spp. isolated from dog faecal samples obtained from dogs with and without GI signs

Campylobacter spp. isolated?	Control group	GI groups
Yes	2	5
No	43	48

Both isolates from dogs without diarrhoea were *C. upsaliensis*. Two isolates from dogs with diarrhoea were *C. upsaliensis* and two *C. jejuni*. The seventh isolate could not be identified to the species level by PCR with the primers used. All *Campylobacter* spp. isolates were sensitive to all the antibiotics against which they were tested.

5.3.2 Pulsed-field gel electrophoresis of *Campylobacter* spp.

Pulsed field gel electrophoresis of macroresticted DNA from all seven *Campylobacter* isolates, following digestion with *Sma*I and *Xho*I demonstrate that they were not the same strain type. Figures 5.1 and 5.2 are examples of four patterns of *C. upsaliensis* isolates from the GI and non-GI groups following digestion with *Sma*I and *Xho*I.

C. upsaliensis

Figure 5.1.Digestion with *SmaI*

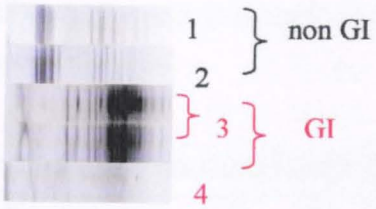
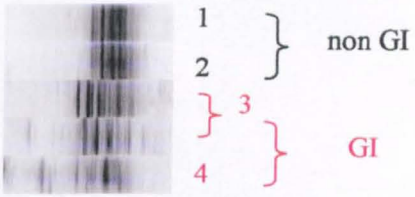


Figure 5.2.Digestion with *XhoI*

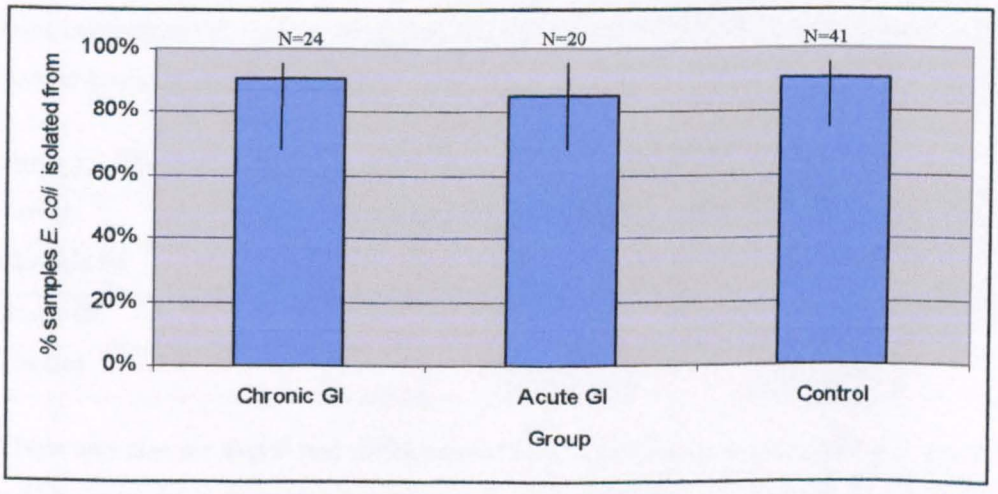


(Numbers 1-4 refer to each sample, 2 isolates were obtained from sample 3)

5.3.3 Prevalence of *E. coli*

Whether or not dogs had diarrhoea did not make any difference to the prevalence of *E. coli* isolated as shown in figure 5.3 (Chronic GI - %, acute GI - %, control - %).

Figure 5.3.Proportion of samples positive for *E. coli* from dogs with acute and chronic diarrhoea, or without GI disease



(Bars plot 95% confidence interval levels, N = number of samples)

5.3.4. Proportion of *E. coli* carrying virulence determinants

E. coli from four control dogs and 5 dogs with GI disease were positive by PCR for the *eaeA* gene (table 5.2). This gave an overall prevalence of 9%. *E. coli* positive for both the *eaeA* and *bfpA* gene (indicative of EPEC), these were isolated from 2 control dogs and 1 dog with GI signs.

Table 5.2.Virulence genes present in *E. coli* isolates from dog faecal samples

Virulence genes	Control dogs	Dogs with GI disease
bfpA and *eaeA*	2	1 chronic GI
eaeA	4	3 chronic GI 2 acute GI

5.3.5 Antimicrobial susceptibility of *E. coli* isolates

All *E. coli* isolates were classified as sensitive or resistant to antibiotics tested using the disc diffusion assay (BSAC, 2002). All samples are listed in appendix A3.

There was no significant difference in the prevalence of antibiotic resistant (to one or more antibiotics) *E. coli* isolates between dogs with acute or chronic diarrhoea and the control group (P=0.98, table 5.3).

Table 5.3.Number of sensitive and resistant *E. coli* isolates from the 3 groups

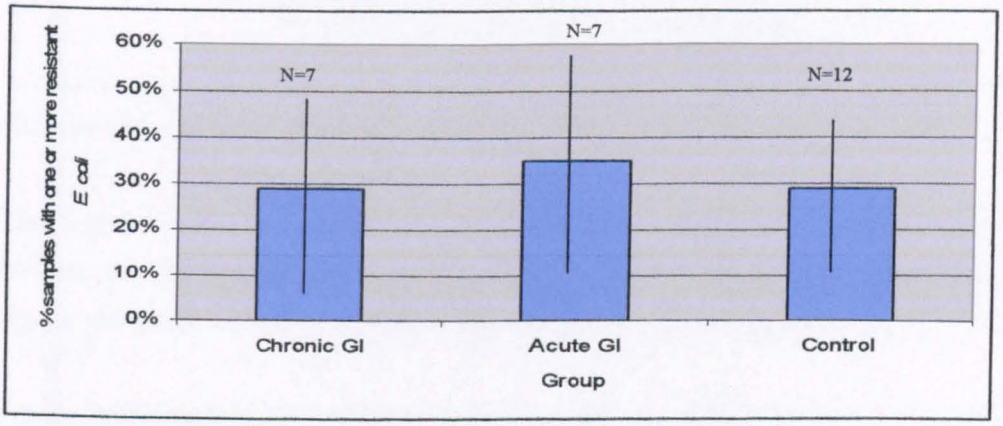
Group	Resistant *E. coli* isolates	Sensitive *E. coli* isolates
Chronic GI	18	50
Acute GI	15	45
Control	31	90

There was also no significant difference between the prevalence of antibiotic resistant *E. coli* isolated from dogs that were receiving antibiotic therapy and those that were not.

Therefore, these isolates were included into the GI and control groups rather than grouped separately.

There appeared to be a similar prevalence of antibiotic resistant *E. coli* isolated from all 3 groups. The highest number of *E. coli* isolates were resistant to ampicillin, a similar prevalence being isolated from all 3 groups (chronic GI n=7/28, acute GI n=7/22, control n=12/45, figure 5.4).

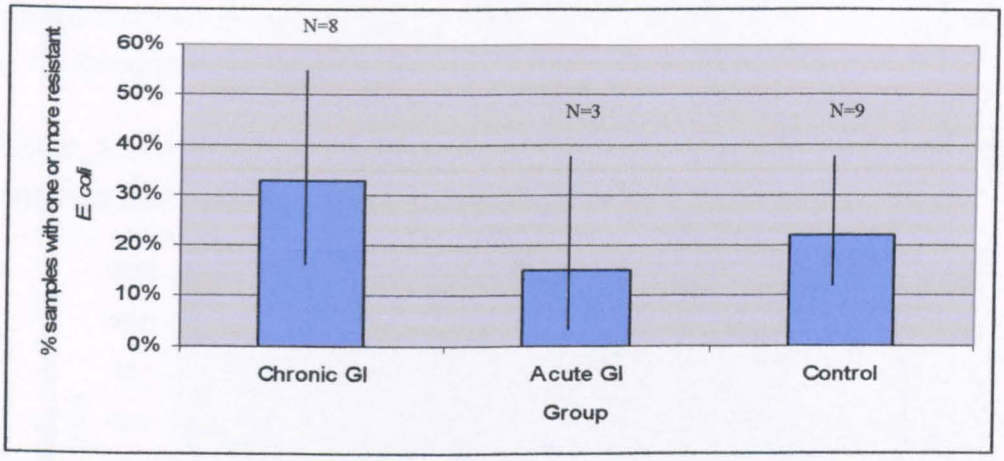
Figure 5.4.Percentage of samples from which one or more ampicillin resistant *E. coli* were isolated



(Bars plot 95% confidence interval levels, N = number of samples)

A similar prevalence of tetracycline resistant *E. coli* was also observed. Tetracycline resistant *E. coli* were isolated from a third of samples (n=8/28) from the chronic diarrhoea group (figure 5.5) but a lower prevalence was found in samples from the acute diarrhoea group (n=3/22) and samples from the control group (n=9/45).

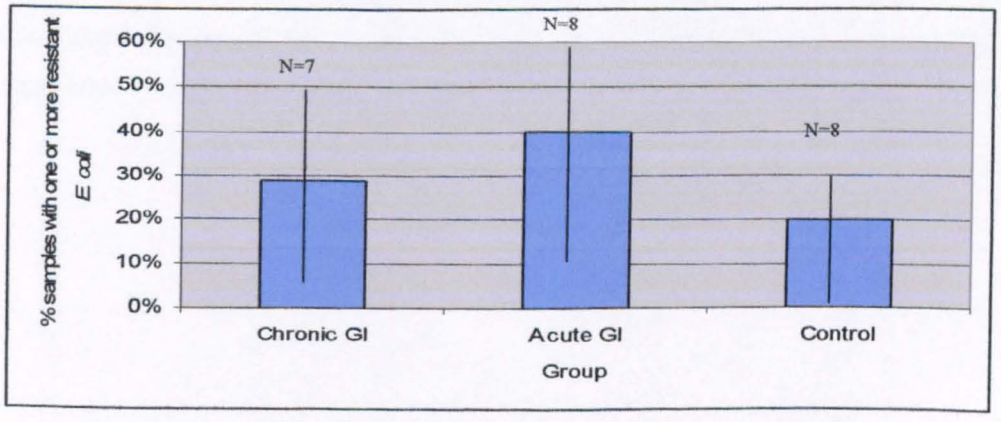
Figure 5.5.Percentage of samples from which one or more tetracycline resistant *E. coli* were isolated



(Bars plot 95% confidence interval levels, N = number of samples)

The highest prevalence of samples from which trimethoprim resistant *E. coli* were isolated was from dogs with acute diarrhoea (40%, n=8/22, figure 5.6). There was a similar prevalences in the control (n=8/45) and chronic GI groups (n=7/28).

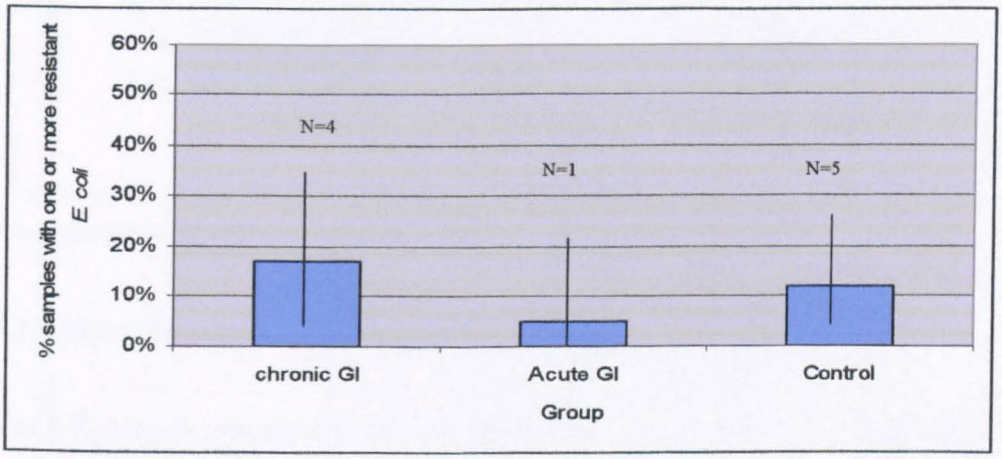
Figure 5.6.Percentage of samples from which one or more trimethoprim resistant *E. coli* were isolated



(Bars plot 95% confidence interval levels, N = number of samples)

Lower prevalences of nalidixic acid resistant *E. coli* were obtained from all three groups when compared to the previous antibiotics (figure 5.7). The prevalence was highest in the chronic diarrhoea group (n=4/28). All nalidixic acid resistant isolates were also resistant to the fluroquinolone, ciprofloxacin.

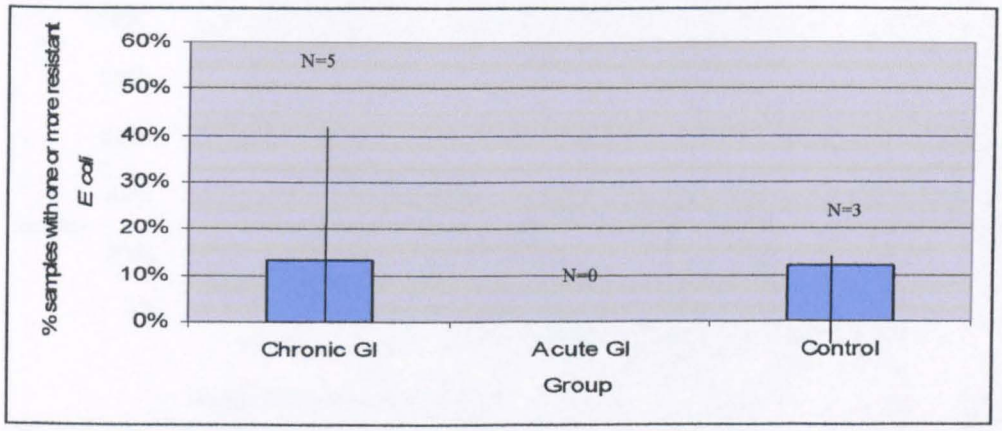
Figure 5.7.Percentage of samples from which one or more nalidixic acid and ciprofloxacin resistant *E. coli* were isolated



(Bars plot 95% confidence interval levels, N = number of samples)

The lowest prevalence of resistant *E. coli* was seen against chloramphenicol. A low prevalence was seen in the chronic diarrhoea (n=5/28) and control groups (n=3/45) and there were no resistant isolates from dogs with acute diarrhoea (figure 5.8).

Figure 5.8.Percentage of samples from which one or more chloramphenicol resistant *E. coli* were isolated



(Bars plot 95% confidence interval levels, N = number of samples)

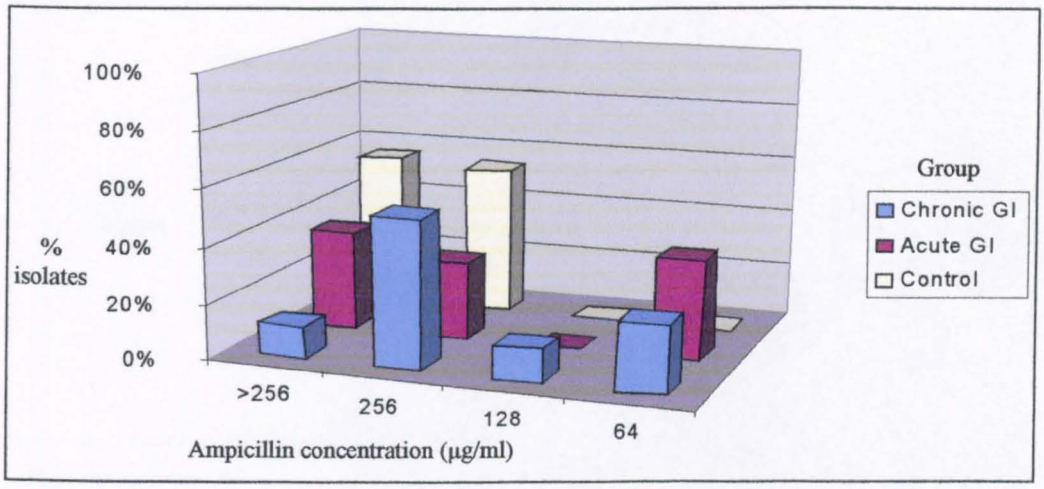
5.3.6 Molecular characterisation of antimicrobial resistance in *E. coli*

The following figures are expressed as the percentage of isolates rather than percentages of samples.

Ampicillin resistance

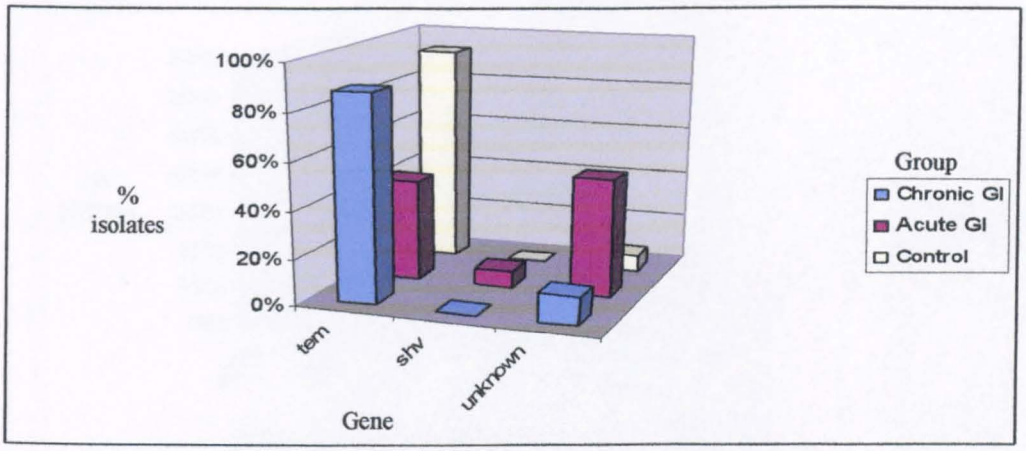
The breakpoint concentration of ampicillin indicative of resistance in *E. coli* is 16μg/ml (BSAC), or 32μg/ml (NCCLS). The MIC of ampicillin for the majority of isolates deemed resistant in disc assays was >256g/ml (figure 5.9). MICs of ampicillin of 64g/ml were found mainly for isolates from the acute (n=5/14) and chronic diarrhoea (n=4/17) groups.

Figure 5.9.MICs of ampicillin for *E. coli* isolates



The majority of ampicillin resistant *E. coli* isolates from the chronic GI (n=15/17) and control groups (n=25/27) possessed the *tem* gene (figure 5.10). Forty-three percent (n=6/14) of isolates from the acute GI group possessed the *tem* gene, and one isolate possessed the *shv* gene, but the mechanism of ampicillin resistance was undetermined in half of the isolates (n=7/14) from this group. There did not appear to be an association between non *tem/shv* carrying isolates and lower ampicillin MICs as 3 isolates had ampicillin MICs of 256µg/ml and 4 isolates had ampicillin MICs of 64µg/ml. In the chronic GI group, non *tem/shv* carrying isolates had ampicillin MICs of >256µg/ml (n=1) and 128µg/ml (n=1). In the control group the single non *tem/shv* carrying isolate had an MIC of ampicillin of 256µg/ml. All isolates produced β-lactamase as determined by the nitrocefin assay

Figure 5.10.Ampicillin resistance genes in *E. coli* from dogs



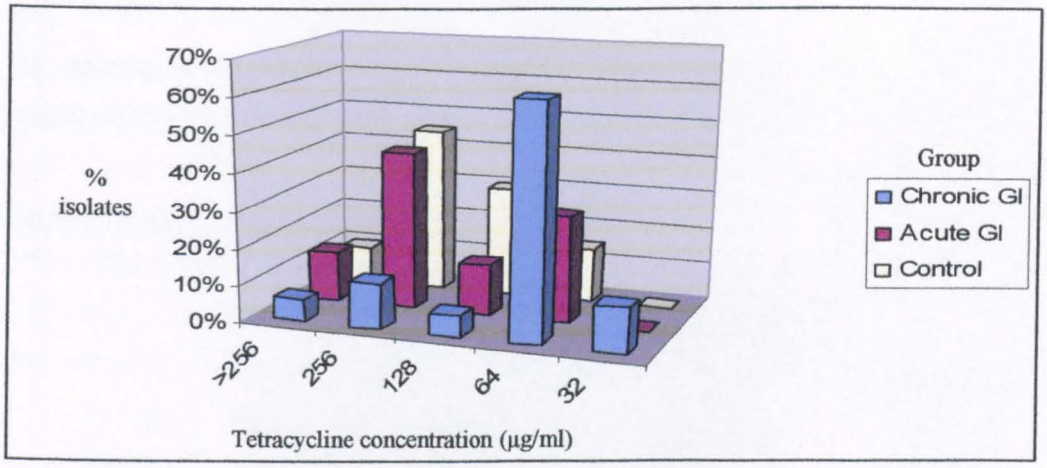
Three *E. coli* isolates from the control group and four isolates from the acute GI group were resistant to additional β-lactam antibiotics. From the control group, two isolates from the same sample were resistant to different antibiotics, one resistant to co-amoxyclav, aztreonam and ceftazidime and the other to aztreonam and cefuroxime. Another isolate from the control group from a different sample was resistant to ceftazidime. All isolates contained the *tem* gene and had ampicillin MICs of 256μg/ml or above.

In the acute GI group, one isolate was resistant to cefoxitin and co-amoxyclav, and three isolates were resistant to only cefoxitin. None of the isolates contained the *tem* or *shv* gene and all had ampicillin MIC's of 64μg/ml. The latter three isolates were from the same faecal sample.

Tetracycline resistance

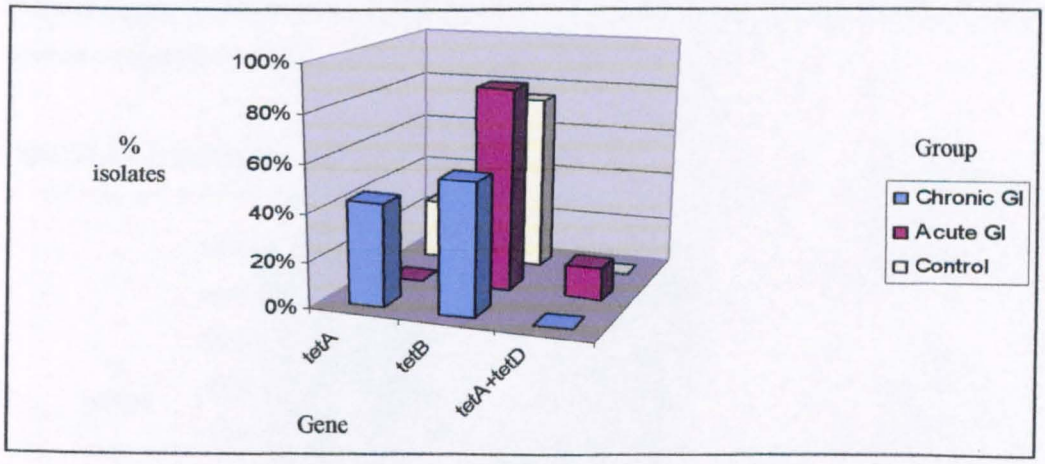
The breakpoint concentration of tetracycline in accordance to BSAC guidelines is 2μg/ml, and for NCCLS guidelines it is 16μg/ml. Tetracycline MICs in both the acute GI and control group were similar, the overall majority of isolates having tetracycline MICs of 256μg/ml (n=3/7, n=9/20, respectively, figure 5.11). The majority of isolates from the chronic diarrhoea group had lower tetracycline MICs of 64μg/ml (n=10/16).

Figure 5.11.MICs of tetracycline for *E. coli* isolates



The majority of isolates from all groups were positive for the *tetB* gene by PCR (chronic GI n=9/16, acute GI n=6/7, control n=15/20, figure 5.12). The *tetA* gene was carried by a minority of isolates in the chronic GI (n=7/16) and control groups (n=5/20). The *tetA* was only found, together with *tetD* in one isolate from a dog with acute diarrhoea.

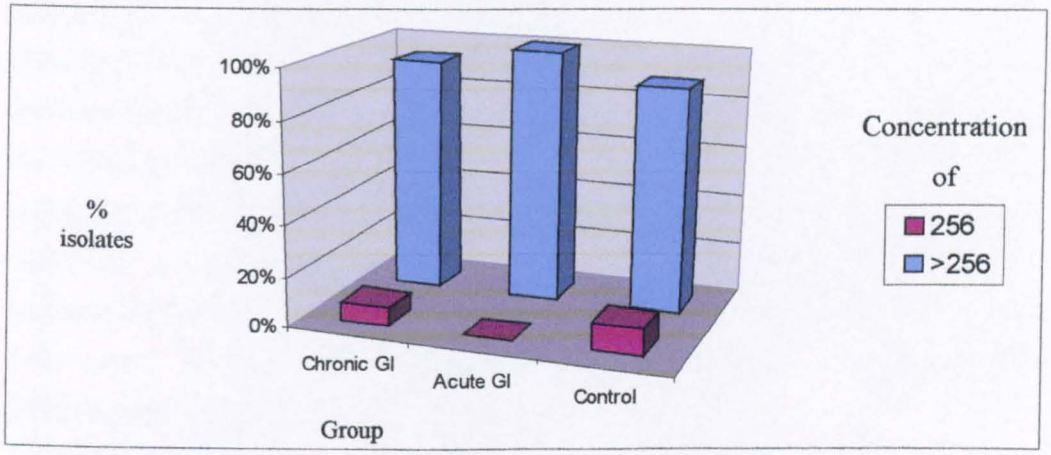
Figure 5.12.Tetracycline resistance genes



Trimethoprim resistance

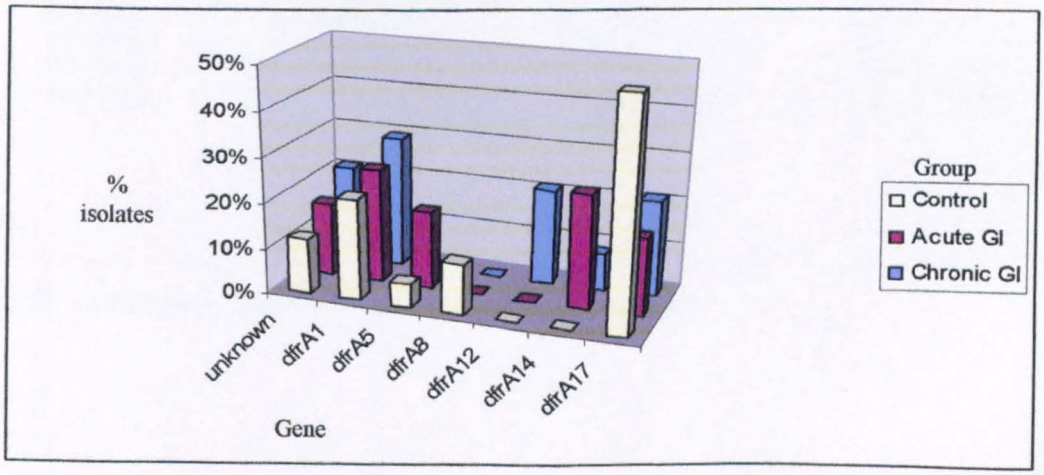
The majority of isolates from all three groups had trimethoprim MICs of >256μg/ml (chronic GI n=13/14, acute GI n=12/12, control n=16/18, figure 5.13).

Figure 5.13.MICs of trimethoprim for *E. coli* isolates



Trimethoprim resistant isolates contained a variety of resistance genes (figure 5.14). The majority of isolates in the control group carried *dfrA17* (n=9/18), whereas isolates in the acute and chronic GI groups carried a wider range of genes, including all *dfr* genes that were investigated by PCR.

Figure 5.14.Trimethoprim resistance genes

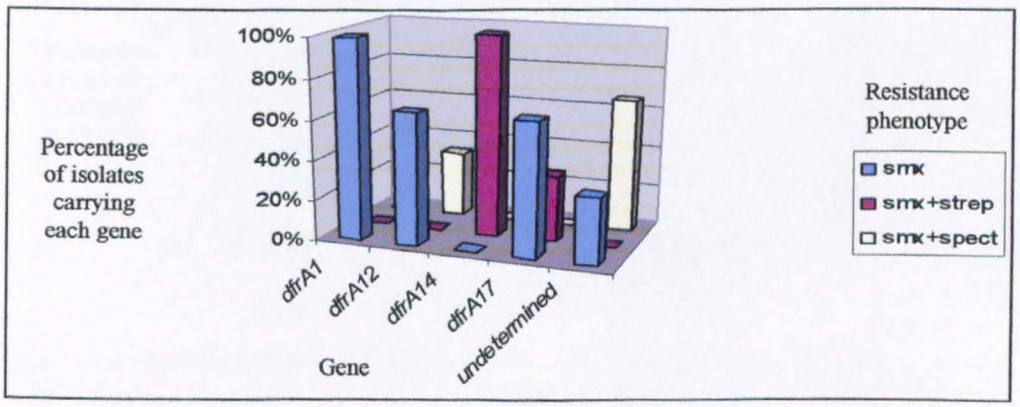


Streptomycin, Spectinomycin and sulphamethoxazole resistance.

All trimethoprim resistant isolates were tested for susceptibility to a sulphonamide (sulphamethoxazole) and aminogylcosides (streptomycin and spectinomycin).

Overall, 95% of trimethoprim resistant isolates were resistant to one or more of the above antibiotics. All of isolates possessing the *dfrA1* gene, from both the chronic and acute diarrhoea groups, were co-resistant only to sulphamethoxazole (chronic GI n=4/4, acute GI n=3/3; figure 5.15 and 5.16). In the control group (figure 5.17) the majority of isolates carrying *dfr1* (n=3/4) and also *dfr5* (n=1/1), *dfr8* (n=2/2) and had an undetermined gene responsible for resistance and were co-resistant to both sulphamethoxazole and streptomycin. Only a small number of isolates from the control (n=2) and acute GI group (n=5) were resistant to trimethoprim, sulphamethoxazole, streptomycin and spectinomycin. All isolates in both groups were carrying *dfr17*.

Figure 5.15.Percentage of trimethoprim resistant *E. coli* isolates carrying each gene from the chronic diarrhoea group that were resistant to streptomycin, spectinomycin and/or sulphamethoxazole



(SMX – sulpmethoxazole, strep – streptomycin, spect – spectinomycin)

Figure 5.16.Percenatge of trimethoprim resistant *E. coli* isolates carrying each gene from the acute diarrhoea group that were resistant to streptomycin spectinomycin and/or sulphamethoxazole

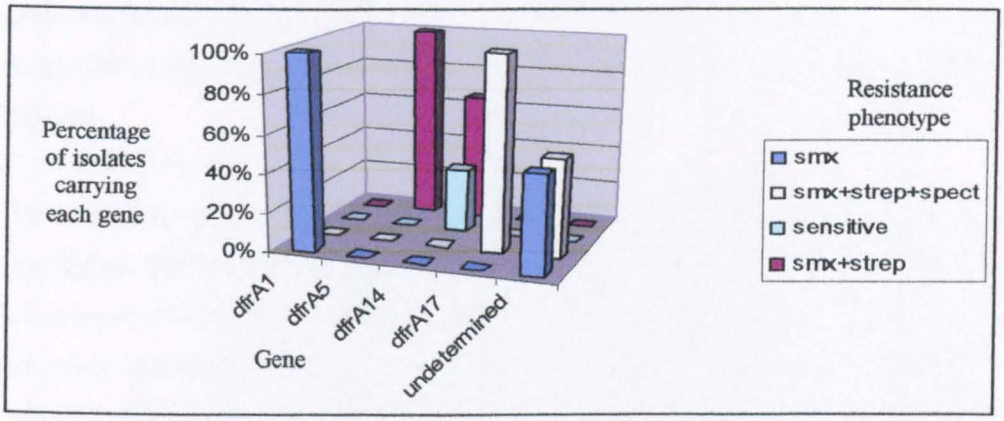
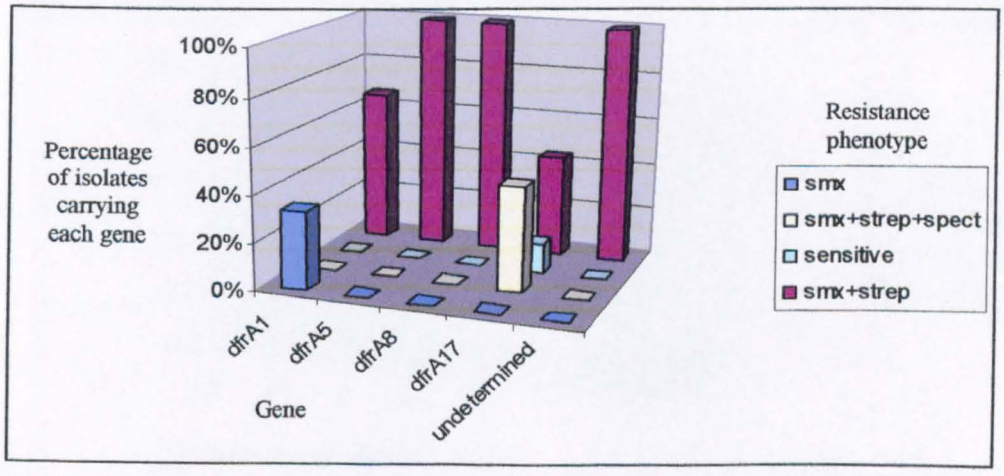


Figure 5.17.Percenatge of trimethoprim resistant *E. coli* isolates carrying each gene from the control group that were resistant to streptomycin, spectinomycin and/or sulphamethoxazole

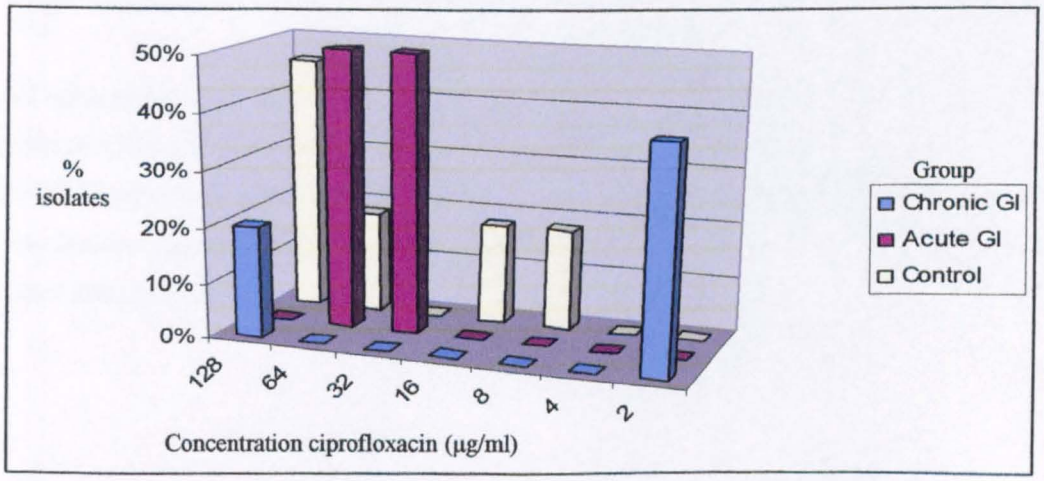


Nalidixic acid and ciprofloxacin MIC's

The NCCLS guidelines were followed which gave a breakpoint concentration for nalidixic acid of 16μg/ml. BSAC does not have a breakpoint concentration for this antibiotic. Nearly all of the isolates had a nalidixic acid MIC of >256μg/ml, the exception being one isolate from a dog with chronic diarrhoea that had a nalidixic acid MIC of 64μg/ml.

The breakpoint concentration for ciprofloxacin is 1μg/ml (BSAC) or 4μg/ml (NCCLS). The highest MICs of ciprofloxacin were 128μg/ml and were from isolates from dogs in the control (n=5/11, figure 5.18) and chronic diarrhoea (n=1/5) groups. Two isolates from the acute diarrhoea group had MICs of ciprofloxacin of 64μg/ml and 32μg/ml. Two isolates in the control group had MICs of 2μg/ml, rendering them as resistant by BSAC guidelines but not by NCCLS guidelines.

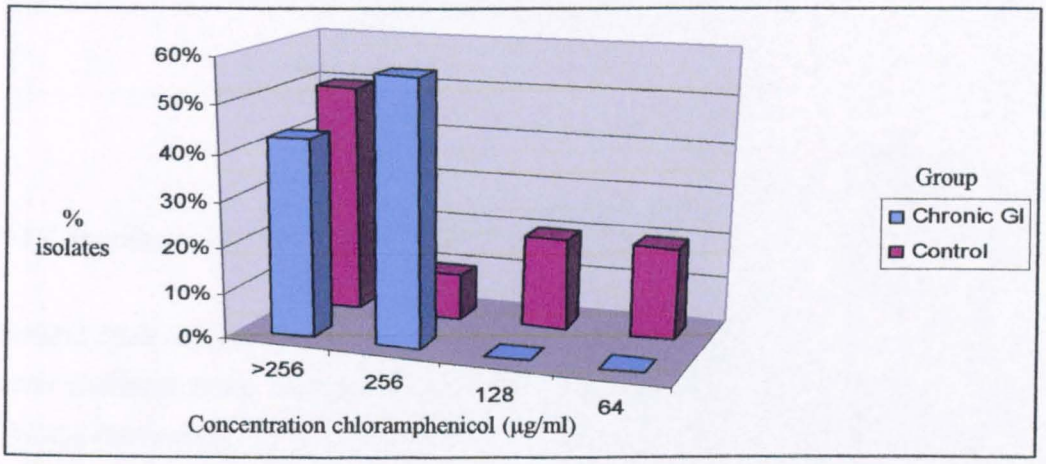
Figure 5.18.MICs of ciprofloxacin *E. coli* isolates



Chloramphenicol resistance

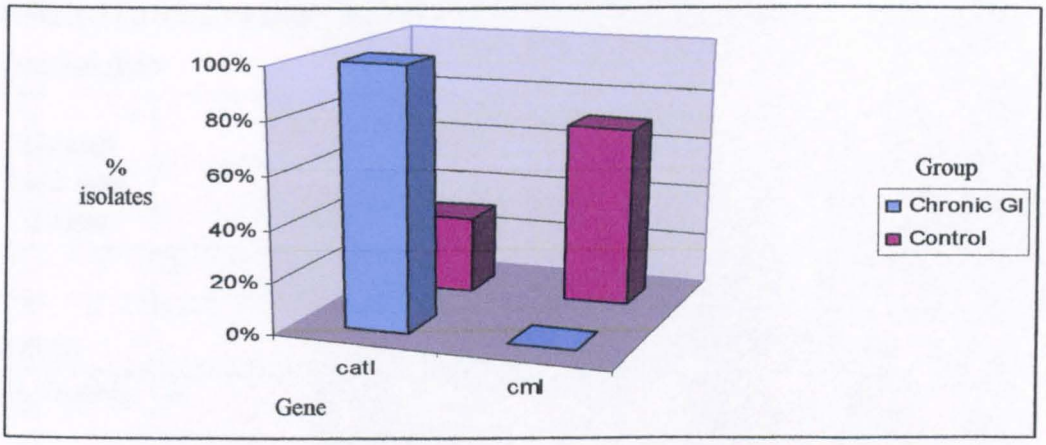
All resistant *E. coli* isolates from the chronic diarrhoea group had MICs of chloramphenicol of above 256μg/ml (figure 5.19). Half of the isolates from the control group had an MIC of chloramphenicol of >256μg/ml (n=5/10), other isolates were fairly evenly distributed over 256μg/ml (n=1/10), 128μg/ml (n=2/10) and 64μg/ml (n=2/10).

Figure 5.19.MICs of chloramphenicol in resistant isolates



All chloramphenicol resistant *E. coli* isolates (n=7) from the chronic diarrhoea group possessed the *catI* gene by PCR, whereas the majority of samples from the control group (n=9/10) possessed the *cmlA* gene (figure 5.20). There was a significant association between the presence of the *catI* gene and an MIC of 256μg/ml or above using Fisher's Exact test (P=0.023).

Figure 5.20.Choramphenicol resistance genes



5.3.7 Prevalence of antibiotic resistant *E. coli* isolated from repeated samples

Isolates from repeated samples collected from the same dogs in both the chronic and acute diarrhoea group, were not consistently resistant to antibiotics (see table 5.4). In the chronic diarrhoea group all isolates from one dog were MDR, whereas isolates from the other 2 dogs were sensitive to all antibiotics tested. In the acute diarrhoea group, isolates obtained from the first samples from two dogs were sensitive to all antibiotics. Isolates obtained from the second sample from one dog were MDR. One isolate from the other dog faecal sample was resistant to one antibiotic.

Table 5.4.Antibiotic susceptibility of *E. coli* isolates from repeated faecal samples from the same dogs

Group and dog name	Sample no						
	1	2	3	4	5	6	7
Acute							
No Trouble	S	**1 tet resistant isolate**	S	S	S	S	S
Cooke	S	**1 amp, apra, tm resistant isolate**	N/A	N/A	N/A	N/A	N/A
Chronic							
Marty	**3 tet, nal, chlor, tm resistant isolates**	S	S	N/A	N/A	N/A	N/A
Simba	S	S	S	N/A	N/A	N/A	N/A
Half pint	S	S	N/A	N/A	N/A	N/A	N/A

(S – sensitive, N/A – no samples, amp – ampicillin, apra – apramycin, nal – nalidixic acid, chlor – chloramphenicol, tet – tetracycline, tm – trimethoprim)

5.4 Discussion

The results of this study suggest that the presence of *Campylobacter* spp. is not significantly associated with diarrhoea in dogs, which agrees with the findings of a number of other studies (Holt 1980, Olsen and Sandstedt, 1987, Malik and Love, 1989, Figura 1991, Burnens *et al*, 1992, Adesiyun *et al*, 1997, Baker *et al*, 1999, Moser *et al*, 2001, Sandberg *et al*, 2002). However, the sample size in this study is very low, therefore, with such small numbers; it would be less likely to detect a significant association with disease.

There are many factors that can influence the isolation rate of *Campylobacter* spp. in dogs. The media used for isolation can affect the prevalence of individual species isolated and until recently, *C. jejuni* was considered to be the most common species isolated from dogs. However, in this and previous studies, when selective media have not been used, a higher prevalence of *C. upsaliensis* isolations has been noted (Burnens, 1992, Baker *et al.*, 1999, Moser *et al.*, 2001). Also in this study, an additional enrichment step was introduced, before streaking onto CA (cefoperazone and amphotericin) agar. CA agar has been shown to be comparable to the filtration method for the isolation of *C. upsaliensis* (Asphill *et al.* 2004) and an enrichment step would improve the isolation rate further. CA agar plates were also incubated for up to 5 days; a longer incubation period has been shown to be beneficial for *C. upsaliensis* detection (Moreno *et al.*, 1993, Hald *et al.*, 2004).

C. jejuni was only isolated from dogs with diarrhoea. A recent longitudinal study by Hald *et al.*, (2004) observed long term colonisation with *C. upsaliensis* in dogs but less frequent, and only shorter-term colonisation by *C. jejuni*. The study also observed a higher prevalence of *C. jejuni* from dogs under one year of age and this has been observed in similar studies (Burnens *et al.*, 1992, Moser *et al.*, 2001). In the present study *C. jejuni* was only isolated from dogs under one year of age and also with diarrhoea. It is also suggested that *C. jejuni* may have seasonality in its infection of dogs, being more

commonly isolated from dogs below one year of age in the summer (Lopez *et al*, 2002). The *C. jejuni* isolate from this study was obtained in the summer although no conclusions can be made, this being the only isolate.

Interestingly, although all *Campylobacter* spp. isolates were isolated from the vet-visiting population and thus resistance against antibiotics may be expected to be observed, all isolates were sensitive to all antibiotics tested. PFGE banding patterns showed that strains were not related following digestion with two separate enzymes. This suggests that isolates were not obtained from the same source i.e. the veterinary hospital environment, but since the dogs from which *Campylobacter* spp were isolated, were only visiting the veterinary hospital, this is not surprising. There are few studies that have investigated antibiotic resistance in *Campylobacter* spp isolated from dogs, although a study by Preston *et al.* (1990) reported isolates of *C. upsaleinsis* from dogs that were resistant to trimethoprim and teicoplanin.

The results of this limited study suggest that *E. coli* carrying the *eaeA* gene alone or the *eaeA* and the *bfpA* gene together (indicative of EPEC) are not sufficient cause diarrhoea in dogs, although, yet again the sample size is small so definite conclusions can not be made. *E. coli* carrying the *eaeA* gene have been associated with dogs with diarrhoea (Turk *et al.*, 1998, Nakazato *et al.*, 2004), although studies have also found no significance difference between the presence of the *eaeA* gene and diarrhoea in dogs (Holland *et al.*, 1999). The prevalence of *E. coli* carrying virulence determinants from both control dogs and dogs with GI signs from this study is lower than found in other studies (Neiger *et al.*, 2002). This may be because we randomly picked three single colonies for further investigation, whereas if more had been selected then the prevalence of *E. coli* carrying virulence determinants may have increased. Previous reports have suggested that canine variants of the *bfpA* gene may exist, due to *E. coli* isolates being *bfpA* negative by PCR, but positive using plasmid hybridisation (Goffaux *et al.*, 2000). Therefore, in this study there is the possibility that *bfpA* positive isolates may have also been missed.

For the majority of antibiotics tested, there was no difference in prevalence between resistant *E. coli* isolated in this, and the cross sectional study described in chapter 4. In this study the number of dogs on antibiotic treatment was very low and therefore no distinction could really be made between resistant *E. coli* isolated from dogs receiving antibiotic treatment and those that were not. It could be expected that a higher prevalence of antibiotic resistant *E. coli* be isolated from the vet-visiting population, since these dogs were exposed to an environment where there is much higher levels of antibiotic usage. Since many of the dogs and other animals in the vet-visiting population may be on antibiotic treatment, this increases the opportunity to acquire resistant *E. coli* when coming into contact with other dogs within the veterinary hospital environment.

A study by Minton *et al.*, (1983) found a high prevalence of antibiotic resistant *E. coli* in dogs with acute enteric infection. Resistance genes were found on plasmids in this study and were shown to be transferable. This would facilitate the spread of resistance determinants to other bacteria and also throughout the hospital environment. Sanchez *et al.* (2002) observed the same strain of resistant *E. coli* from dogs housed in the hospital intensive care unit and also from the hospital environment, suggesting spread of *E. coli* throughout veterinary hospitals and that being housed in the veterinary hospital may be a risk factor for the infection with, or carriage of antibiotic resistant *E. coli*. In this study the majority of dogs were visiting and were not actually kept in the hospital environment overnight. This may explain why the prevalence of antibiotic resistant *E. coli* is not as high as found in other studies of vet-visiting dog populations (Hirsh *et al.*, 1980, Monaghan *et al.*, 1981).

The majority of isolates in this study were multi-drug resistant (resistant to 2 or more antibiotics). It has been suggested that the emergence of multi-drug nosocomial pathogens in the veterinary community is from the widespread use of broad-spectrum antimicrobials in veterinary hospitals and this may create a community reservoir (Guardabassi *et al*, 2004). MDR isolates or isolates resistant to a single antibiotic were not consistently isolated from different samples obtained from the same dogs. This,

again, could be due to the method of selecting three random colonies so resistant colonies may have been missed.

There have been few studies investigating the genes responsible for antibiotic resistance in bacterial isolates from the vet visiting dog population. It is therefore difficult to compare our findings to previous studies. The majority of ampicillin resistant *E. coli* isolates in this study carried the *tem* gene. This gene encodes for a β-lactamase, an enzyme that breaks open the β-lactam ring of the antibiotic and is the most common gene isolated from ampicillin resistant *E. coli* (Baker, 1999). The *tem* gene has been observed in dog *E. coli* isolates from UTI infections (Teshager *et al.*, 2000, Féria *et al.*, 2002), but has not been reported from faecal samples from healthy dogs. A small proportion of ampicillin resistant isolates expressed activity against cephalosporins, co-amoxyclav and aztreonam. In isolates that carried the *tem* gene, this suggests many genes have acquired mutations (one or more amino acid substitutions) from the classic TEM enzyme, and may be extended spectrum β-lactamase (ESBLs) producers. Two isolates were resistant to ceftazidime, a third generation, broad-spectrum cephalosporin suggesting ESBL activity. However, one of the isolates was also resistant to co-amoxyclav, to which ESBLs are usually sensitive, although there are exceptions to the rule (Alvarez *et al.*, 2004).

Non *tem* and *shv* encoded β-lactamase's include AmpC type β-lactamase. AmpC type β-lactamases may have been responsible for resistance in the *E. coli* isolates that did not carry the *tem* or *shv* gene but were resistant to cefoxitin. High-level AmpC production is associated with resistance to many β-lactam antibiotics, including the cephamycins (cefoxitin). Unfortunately, due to time constraints, these genes were not investigated further and no definite conclusions about the genes responsible for resistance to additional β-lactam antibiotics can be made.

The majority of tetracycline resistant isolates expressed the *tetB* gene, the majority of the rest harbouring *tetA*, although one isolate did harbour *tetD*. Each three of these genes encode for efflux pumps, this being the most common mechanism of resistance in gram-negative bacteria (Chopra and Roberts, 2001). The *tetA*, *tetB* and *tetC* genes have been

found in *E. coli* isolates from dog faecal samples in a previous study (Bryan *et al.*, 2004). *tetA* and *tetB* have been reported to be prevalent in tetracycline resistant *E. coli* from pigs, cattle and chickens (Sengelov *et al.*, 2003).

The majority of chloramphenicol resistant *E. coli* isolates in the chronic GI group possessed the *catI* gene. This gene encodes for an acetyltransferase, an enzyme that acetylates chloramphenicol, thus preventing the drug from binding to its target on the ribosome. The majority of isolates from the control group carried the *cmlA* gene. This gene is thought to encode for a non-enzymatic efflux pump in *E. coli* (Keyes *et al.*, 2000). There was a significant association between the presence of the *catI* gene and an MIC of 256μg/ml and above using Fisher's Exact Test. This suggests that enzymic resistance encoded by the *catI* gene is more effective than non-enzymic resistance encoded by the *cmlA* gene. Chloramphenicol resistant *E. coli* have been isolated from wound and UTI infections in veterinary isolates from dogs and the *cmlA* homologue *flo* was found to be responsible (Sanchez *et al.*, 2002).

There have been few previous reports of *dfr* genes in *E. coli* from dog faecal samples. *Dfr17* has reported to be contained on a gene cassette from UTI and wound infections from dogs (Sanchez *et al.*, 2002). The majority of published gene cassettes contain resistance against trimethoprim and also aminoglycosides and/or sulphonamides.
In the control group the majority of isolates were resistant to sulphamethoxazole and streptomycin. These findings are similar to those from the cross sectional study in chapter 4. In both the acute and chronic GI group, many isolates were resistant to sulphmethoxazole (sulphonamide) alone. Although no further characterisation was carried out on these isolates, this may suggest that genes encoding for sulphonamide resistance are fairly widespread in trimethoprim resistant *E. coli* isolates from dogs. Genes encoding for trimethoprim resistance have been described on class 1 integrons, together with genes (*aadA4*) encoding for spectinomycin /streptomycin resistance (Chang *et al.*, 2000, Adrain *et al.*, 2000). In the control and acute diarrhoea groups, isolates that expressed *dfr17* were also resistant to all three further antibiotics tested against

suggesting the presence of integrons. Unfortunately, due to time constraints, trimethoprim resistance was not characterised further.

Also in this study, quinolone and fluoroquinlone, and apramycin resistant *E. coli* were not characterised further due to time constraints. The aminoglycoside-modifying enzyme AAC(3)IV, which also inactivates tobramycin and gentamicin is usually responsible for apramycin resistance in *E. coli* (Yates *et al.*, 2004)).

In conclusion, the results of this study suggest that *Campylobacter* spp. and *E. coli* carrying virulence determinants do not necessarily cause diarrhoea in dogs, although no definite conclusions can be made as the sample size was small. However, there have been very few studies that have characterised the genes responsible for antibiotic resistance from commensal *E. coli* in the canine veterinary visiting population, especially in the UK (Normand *et al.*, 2000a, Normand *et al.*, 2000b). The findings from this study show that dogs do carry *E. coli* carrying virulence determinants and antibiotic resistant *E. coli* that may pose a zoonotic risk for humans.

Chapter 6

Collection of faecal samples from healthy humans

6.1 Introduction

It has always been a challenge to obtain a faecal sample from healthy human subjects. The subject of excrement, both human and animal, is seen as remarkably unpleasant and embarrassing, even as a taboo. Few people are willing to provide faecal samples unless it is to determine a cause of illness. This creates a problem for studies in both human and veterinary research where volunteers are needed to participate as a control group.

The aim of this study was to find a method of collecting faecal samples from individuals, that would encourage compliance among healthy people and increase their participation in studies involving faecal collection. There are no published papers or guidelines on the collection of faecal samples from humans, ill or healthy. Methods that are currently used are not particularly pleasant and can be quite stressful for the individual. People are often embarrassed about providing faecal samples, and discouraged from taking part in studies where they are needed to provide them. Financial incentives may encourage healthy people to provide samples, however, many organisations are not in a position to provide the (most probably large!) incentives that would be needed, and many Ethel organisations disapprove of payment for taking part in medical trials.

It would have to be certain that the method used would collect enough faecal material to enable bacterial isolation. Also, that bacterial isolation would still be possible if the sample did not arrive back in the laboratory without delay.

With these aspects in mind, a questionnaire was distributed to investigate how people from a variety of occupations would prefer to give faecal samples and how they could be encouraged to do so. These methods were then tested for microbiological suitability.

6.2 Methods

6.2.1 Human faecal sample questionnaire

A questionnaire was devised (Human faecal Sample questionnaire, Appendix A4) asking individuals to put in preference order how they would prefer to collect faecal samples from themselves. Three options were given, 1) putting used toilet paper put into a pot of sterile diluent, 2) using a sponge soaked in a buffer in place of toilet paper after defecation, and putting the sponge into a sterile container, or 3) using a scoop attached to the lid of a sterile universal container to collect faeces. Individuals were also asked how often they would be prepared to provide samples, how they would prefer to return the samples, if partners would be willing to collect samples and if they would be prepared to collect samples from their children.

The chi-squared test was used to determine if there were any significance difference between answers given in the science and non-science occupational groups.

6.2.2 Compliance questionnaire

A second questionnaire (Compliance questionnaire, Appendix A4) to be completed by dog owners was also attached to the human faecal sample questionnaire. Individuals were asked how they would be prefer to be approached about a study involving the collection of faecal samples from both themselves and their dog and what incentives would persuade them to take part in such a study. Individuals were also asked if the institution carrying out the study would affect their decision to take part and how they would prefer to fill in questionnaires to provide information about themselves and their dog, should they be given them.

6.2.3 Human faecal sample trial

Collection of human faecal samples

Volunteers from the University of Liverpool Veterinary School were asked to provide faecal samples to test the validity of the methods. Packs were handed out that contained either a sterile pot with a sponge soaked in 10 ml of buffer (Polywipe, Medical Wire and Equipment Co.), the sponge to be used in place of toilet paper, or a pot of 10 ml diluent (2 different types were used) in which to place used toilet paper. Both packs also contained gloves to wear during faecal sample collection and a pot in which to collect a scoop of faeces from the same toilet visit. All packs contained instructions on how to collect the faecal sample and how to store packs before and after the sample was taken, so they reached the laboratory in the best possible condition. Controls were run for each method by putting a colony of *E. coli* and *Campylobacter* spp. separately onto either a sponge or a sheet of toilet paper and incubating at room temperature for 24 hours to simulate conditions in which the samples were most likely to be kept before arrival at the laboratory.

Preparation of faecal samples

Scoop faecal samples were made into a faecal emulsion using brain and heart infusion broth (LABM) containing 5% glycerol. Toilet paper samples and sponge faecal samples were vortexed and the diluent extracted. All samples were stored at 4°C until processed and the remainder of the samples were stored at -80°C

E. coli and Campylobacter spp. isolation

Isolation of *E. coli* and *Campylobacter* spp. isolation was carried out as described in chapter 2 (2.1.1 and 2.2.2 respectively)

6.3 Results

6.3.1 Human faecal sampling questionnaire

From the 343 questionnaires handed out, 66% (n=228) were returned suitably completed. Of these, 55% (n=125) were from individuals working in science related subjects (medics, vets, nurses, science technicians, university science students) and 45% (n=103) in non-science related subjects (stockbrokers, 6th form college students, engineering company, craft centre, call centre).

The preferred method of faecal collection was putting used toilet paper into a pot of sterile diluent (52%). Using a sponge in place of toilet paper was the next preferred method (34%), with using a scoop being the least preferred method (14%). Over half of respondents in the science group preferred toilet paper as a method of faecal collection (57%), whereas in the non-science group using the sponge or toilet paper methods were similarly preferred (43%, 47% respectively). There were significant differences (P=0.002) between the science and non-science groups for preferred method of collection.

Overall, respondents preferred to provide faecal samples once a week, once a month or only once. From the returned questionnaires, the non-science group had the highest percentage of people who were prepared only to give a single sample (35%), whereas in the science group, the highest percentage of people were prepared to give samples once a week (37%). Despite this difference in percentages, there were no significant differences between science and non-science groups and how often they would be prepared to give samples (P=0.133).

Most individuals in both the science and non-science groups did not have children (64% and 58% respectively). In the science group, the majority of individuals who did have children would be prepared to collect samples from both themselves, and their children,

rather than just themselves (20%, 10% respectively), whereas in the non-science group it was the reverse (21%, 16%, respectively). There was a significant difference between science and non-science groups concerning whether individuals would collect faecal samples from their children using the chi-squared test (P=0.010). Due to small numbers, Fisher's exact test was also used and found a borderline difference in significant levels (P=0.072).

Less than one sixth of individuals (15%) believed that their partner would be willing to provide a faecal sample; 59% of individuals said their partner would not, or they were unsure that their partner would, provide a faecal sample. There was little difference between the science and non-science group for all options and there was no significant difference between them (P=0.573).

The majority of individuals in the both the science and non-science groups said they would prefer to return samples by post (56%). Collection of samples from home and returning to their veterinary surgery were not well supported (22%, 17% respectively). Again, there was little difference between science and non-science groups and no significant difference between them (P=0.768).

The percentages in table 6.1 below are representative of the science and non-science groups. Significance was measured using the Chi-squared test.

Table 6.1. Questionnaire responses from science and non-science groups

Questions and options	Science n=125	Non-science n=103	Overall n=228	Significance	
Which method respondents preferred to collect faecal samples from themselves					
Toilet paper	**57% (n=71)**	**47% (n=48)**	**52% (n=119)**	χ_2^2	**P=**
Sponge	26% (n=33)	43% (n=44)	34% (n=77)		
Scoop	17% (n=21)	9% (n=10)	14% (n=31)	**7.67**	**0.022**
No answer	0% (n=0)	1% (n=1)	0% (n=1)		
How often respondents would be prepared to provide sample					
Once week	**37% (n=46)**	**25% (n=26)**	**32% (n=72)**	χ_4^2	P =
Once 2 weeks	7% (n=9)	9% (n=9)	8% (n=17)		
Once month	**27% (n=34)**	**23% (n=24)**	**25% (n=58)**	7.05	0.133
Once 6 weeks	6% (n=8)	8% (n=8)	7% (n=16)		
Only once	**21% (n=26)**	**35% (n=36)**	**27% (n=62)**		
No answer	2% (n=2)	0% (n=0)	1% (n=2)		
If respondents would collect samples from their children					
Children only	1% (n=1)	2% (n=2)	1% (n=3)	χ_2^2	**P =**
Both themselves and children	20% (n=25)	16% (n=16)	18% (n=41)	4.61	**0.010**
Themselves only	10% (n=13)	21% (n=22)	16% (n=35)		
No children	**64% (n=80)**	**58% (n=60)**	**61% (n=140)**		
No answer	5% (n=6)	3% (n=3)	4% (n=9)		

Table 6.1. Cont. Questionnaire responses from science and non-science groups

If respondents partner would be willing to provide sample					
Yes	16% (n=20)	15% (n=15)	15% (n=35)	χ_3^2	P =
No	19% (n=24)	21% (n=22)	20% (n=46)		
Unsure	**42% (n=53)**	**35% (n=36)**	**39% (n=89)**	2.00	0.573
Non-applicable	23% (n=28)	29% (n=30)	29% (n=58)		
How respondents would prefer to return samples					
Post	**54% (n=67)**	**60% (n=61)**	**56% (n=128)**	χ_2^2	P =
Handing to vet	18% (n=23)	16% (n=16)	17% (n=39)		
Collected from house	22% (n=27)	21% (n=22)	22% (n=49)	0.53	0.768
No answer	6% (n=8)	3% (n=3)	5% (n=11)		

Over half of questionnaires were returned by female, rather than male respondents (58%, female, 40% male and 2% respondents did not state gender, see table 6.2). Logistic regression was used to test if gender had an affect on the association between the preferred method of collecting faecal samples in the science and non-science groups. There was no significant association (P=0.203). Overall, 30% of male respondents stated that they would prefer to provide a sample only once whereas 24% of females respondents stated this. This was not significant between the science and non-science groups using the chi-squared test (P=0.41).

Table 6.2. Preferred method of faecal sample collection between genders

Preferred method	Female	Male
Toilet paper	48% (n=64)	24% (n=54)
Sponge	39% (n=51)	11% (n=25)
Scoop	13% (n=17)	6% (n=13)
Total % gender	**58% (n=132)**	**40% (n=92)**

Over one third of questionnaires were returned from people aged 21-31. The lowest number of questionnaires were returned from the below 21 and above 52 year age groups.

Table 6.3.Number of questionnaires returned from each age group

Age	<21	21-31	32-41	42-51	>51
% questionnaires returned	8%	41%	29%	14%	7%
	S=18	S=57	S= 44	S=18	S=5
	NS=1	NS=36	NS=22	NS=15	NS=10

(NS – non science group, S – science group)

6.3.2 Compliance questionnaire

From the 228 questionnaires that were returned, 113 compliance questionnaires were completed by dog owners (table 6.4).

The majority of respondents stated they would prefer to be approached about a study by letter (79%). Personal interview (14%) was the second most preferred option with telephone interview (5%) being the least favoured option. A large percentage of individuals stated they would prefer to give information about themselves and their dog by questionnaire (65%), rather than personal interview or telephone (5% and 8% respectively).

Thirty five percent of individuals said they would be encouraged to participate in the study if they already knew someone taking part, whereas 13% of individuals said it would not affect their decision. Fifty percent of individuals had no preference either way.

Over half of the respondents (58%) stated that the organisation conducting the study would influence their decision to participate, although 60% of these individuals had no preference as to which organisation it may be. Virtually the same percentage of individuals preferred a University or their Public Health Authority (PHA) to conduct the study (15% and 16% respectively), these organisations being preferred to a charity (6%).

Half of the subjects (51%) said an incentive would encourage them to participate in the study, with the remainder of individuals stating that incentives would not encourage them to participate (21%) or that they had no preference (27%).

Over two thirds of individuals would participate in consideration of both their dogs and their family's health (71%). Twenty-five percent of individuals said their family's health would be the major concern. The majority of individuals wanted to be informed of the results from both their dog and of the whole study (76% and 75% respectively). Only a small percentage of individuals said they did not want to be informed of the results from either their dog or from the study (7% and 10% respectively) with the remainder having no preference.

Table 6.4 Results of returned compliance questionnaires

Questions and options	**% Returned questionnaires**
How people would be preferred to be approached about a study where they would provide faecal samples from themselves and their dog	
Letter	**79% (n=89)**
Telephone	5% (n=6)
Personal interview	14% (n=16)
No answer	2% (n=2)
How people would prefer to provide information about themselves and their dog	
Personal interview	5% (n=6)
Brief questionnaire	**65% (n=73)**
Telephone	8% (n=9)
No preference	22% (n=25)
If individuals would take part in the study if they already knew people taking part	
Yes	**35% (n=40)**
No	13% (n=15)
No preference	13% (n=15)
No answer	2% (n=2)

Table 6.4.Cont.Results of returned compliance questionnaires

If the organisation conducting the study would affect the individuals decision to participate	
Yes	**58% (n=65)**
No	42% (n=48)
If yes, which organisation they prefer to be involved with.	
PHLA	16% (n=18)
University	15% (n=17)
Charity	6% (n=8)
No preference	**62% (n=70)**
Would incentives encourage the individual to participate in such a study	
Yes	**51% (n=58)**
No	21% (n=24)
No preference	27% (n=30)
No answer	1% (n=1)
What the individual's decision is to participate in the study is aimed towards	
Your dog and his health	4% (n=5)
Your family an your families health	25% (n=28)
Both	**71% (n=80)**
If the individual would like to informed about the results from their dog	
Yes	**76% (n=86)**
No	7% (n=8)
No preference	17% (n=19)
If the individual would like to informed about the results of the whole study	
Yes	**75% (n=85)**
No	10% (n=11)
No preference	15% (n=17)

6.3.3 Human sample collection

All human faecal samples collected are listed in appendix A5.

Sponge and scoop

Fourteen different human faecal samples were obtained as both sponge and scoop samples. *E. coli* was isolated from both the sponge and scoop sample from 11 samples. Two samples did not have *E. coli* isolated from either the sponge or scoop sample and one sample had *E. coli* isolated from the scoop but not the sponge sample.
No *Campylobacter* spp. were isolated from any of the samples.

Toilet paper and scoop

Fifteen different human faecal samples (both toilet paper and scoop) were obtained. Ten samples were returned with used toilet paper in Maximum Recovery Diluent (MRD), and 5 samples were returned with toilet paper in Cary-Blair (CB) diluent.

E. coli was isolated from both the toilet paper and scoop samples from all samples collected in MRD and three of the five samples collected in CB diluent. *E. coli* was not isolated from either the toilet paper or the scoop sample from either of the remaining two samples.

Again, no *Campylobacter* spp. were isolated from any of the samples.

6.4 Discussion

6.4.1 Human faecal questionnaire

The results from the distribution of these questionnaires may be biased slightly as they were not randomly distributed. Various University departments would not allow the distribution of the questionnaires as they believed that staff might find the subject matter offensive. Questionnaire respondents were separated into science and non-science groups in order to establish if there would be any significant difference between the two. Theoretically people who work in science-related subjects might have had more dealings with the subject of faeces than people who are in office based occupations.

Overall, the use of toilet paper was the preferred method of faecal collection with the sponge being the next preferred method. The use of toilet paper is typical behavior when individuals go to the toilet, and a sponge in place of toilet paper is also not much different. Using a scoop however is not as natural and furthermore, individuals would have to think about how to collect the sample. It was suggested on returned questionnaires to use an opaque, rather than a clear plastic container as individuals will not have to see sample once it is collected. There was a significant difference between the two groups for the preferred method of faecal collection. This suggests that when recruiting for a study, the occupation of potential volunteers should be taken into consideration.

Gender did not have an association between the preferred method of collection within the science and non-science groups (P=0.203), even though more questionnaires were returned by females. Females may feel more comfortable with the subject of faeces so would be more likely to return questionnaires. Questionnaires may also have been distributed in workplaces were there was an unequal distribution of males and females.

The majority of questionnaires were returned from the age groups 21-41. This may also be accounted for by the way in which the questionnaires were distributed throughout the

different workplaces. Nearly all the respondents under the age of twenty one were in the science group as they were mostly returned from university science students and nurses. Fewer questionnaires were returned from the age of 42 and above. People above this age may find the subject of faeces more embarrassing than younger respondents.

There was a significant difference between the science and non-science groups and when asked if they would be prepared to collect samples from their children. A larger proportion of individuals in the science group would be prepared to collect samples from their children, suggesting that respondents in this group are more comfortable with the collection of faeces. Individuals may be more inclined to collect samples from younger children who are taken to the toilet rather than asking older children to collect their own sample.

In the science group more respondents preferred to provide a sample once a week, in contrast to the non-science group where the majority of respondents preferred to provide a sample only once, although there was no significant difference between the two groups. Collection of samples once a week or once a month was most popular for both groups (with the exception of only once), possibly for the reason that they are easier dates to remember than once every 2 or 6 weeks. The preferred way of returning samples was through the post, which is the easiest and least embarrassing of options for individuals, although for the organisation conducting the study there is the issue of safe passage of samples through the post and also how quickly the sample will arrive and what sort of conditions they have been kept.

6.4.2 Collection of human faecal samples

The results from the human faecal collection preliminary trials suggest that putting used toilet paper into diluent is microbiologically possible, although we only managed to recruit a small number of people to provide samples. The 2 different diluents used both appeared to be feasible. Only 4 samples were collected in Cary-Blair diluent so this

media may require more investigation, nevertheless MRD performed well and is relatively cheap and easy to use. A problem with this method was people using too many sheets of toilet paper so not enough diluent was available to soak all the paper. This has 2 consequences, 1) it leaves some faecal material uncovered and dried out, and 2) it makes it difficult to extract enough diluent for bacterial enrichment. Both of these problems make it difficult for bacterial recovery. This was over come by providing a known amount of toilet paper (2 sheets) where all the paper would be covered and also be left with a small excess of diluent. The excess is useful, not only to break up the toilet paper, but also if the sample leaks. This would then ensure there is enough remaining sample for bacterial recovery.

Using a sponge in place of toilet paper also appeared micribiologically feasible; furthermore, we were able to isolate *E. coli* from a sponge sample when it was not possible from the scoop sample. Feedback from the trial suggested that although this method worked well, the sponge was very wet and uncomfortable to use in place of toilet paper.

A problem with both of these methods was people taking sample packs and not returning them. This suggests that when carrying out studies that involve fecal sample collection, more volunteers than necessary should be recruited to allow for people not returning samples. This should guarantee that the required number of samples that are needed for the study are returned.

This study shows that toilet paper is a viable option for human faecal sample collection, although we accept that this method would not be suitable for all situations. The importance of keeping samples cool and getting them back to the laboratory quickly as possible does need to be stressed to individuals participating. However, it does provide an alternative method for studies so people would be more likely to participate.

6.4.3 Compliance questionnaires

The majority of individuals stated that would prefer to be approached about a study concerning themselves and their dog by letter. This could be due to the fact that people would feel less pressure than if there is someone is actually present or on the end of the phone persuading them, and have an opportunity to read through and consider the literature in their own time. The disadvantage of this would be that people are more likely to forget, and they have to initiate contact with the organisation conducting the study themselves. Over half of individuals stated that the organisation conducting the study would affect their decision to take part, although two thirds of these individuals seemed to have no preference between a University group, a charity or the Public Health Authority.

More people said they would be likely to participate if there were incentives for doing so, although it was never stated what incentives they were, and how significant they would be. Most individuals also said that they would consider taking part in this study if it was aimed towards the wellbeing of both the dog and the family, which could in itself, be classed as an incentive.

The results of the questionnaires suggest that people that people would be prepared to participate in a study involving proving faecal samples from both themselves and their dog, if the organisation stressed the importance of the study to the health of the family and the dog and also, if possible, to provide incentives for taking part.

Chapter 7

Longitudinal study investigating transmission of *Campylobacter* spp. and pathogenic and commensal *E. coli* between humans and dogs

7.1 Introduction

The aim of this study was to investigate transmission of *Campylobacter* spp. and commensal and pathogenic *E. coli* between dogs and humans. Epidemiological studies have identified pet ownership as a risk factor for human *Campylobacter* spp. infection (Blaser *et al.*, 1978, Goossens *et al.*, 1990, Goossens *et al.*, 1991, Adak *et al.*, 1995, Neimann *et al.*, 2003), and a case-control study in the USA found an increased risk of human infection with *C. jejuni* and *C. coli* after contact with diarrhoeic animals, including cats and dogs (Saeed *et al.*, 1993). Two further studies by Wolfs *et al.*, (2001) and Damborg *et al.* (2004), isolated what appeared to be the same strains of *Campylobacter* spp. (based on PFGE or AFLP) from humans and pets, including dogs living in the same household. Furthermore, dog breeders recognise a dog 'diarrhoea season' (Spring to Autumn) when both owners and their dogs are prone to bouts of diarrhoea, the owner often getting ill after the dog. Dog breeders who own puppies with diarrhoea also sometimes report suffering diarrhoea at the same time as their puppies (M. Ursell, and J. Twinberrow, pers comms).

Cats and dogs have also been suggested as sources of pathogenic *E. coli* for humans. Johnson *et al.* (2001) showed clonality of *E. coli* strains causing urinary tract infections in humans and dogs by examining virulence-associated genes. Other studies have also found strains of *E. coli* in dog and cat faeces carrying human pathogenicity factors, and have suggested that pets might be a source of human infection (Synge *et al.*, 1996, Johnson *et al.* 2001, Johnson *et al.*, 2002b). Trevena *et al.* (1996) isolated an identical strain of verotoxigenic *E. coli* from a dog, human and horse, and Münich and Lübke-Becker (2004) demonstrated identical strains of *E. coli* in a dog breeder with chronic diarrhoea and two of their dogs, although the sources of infection could not be established in either case. No work appears to have been published on the transmission of commensal *E. coli* between pets and humans.

Although perhaps not as important as the transmission of pathogenic bacteria, commensal *E. coli* may serve as reservoirs of antibiotic resistance and represent a model for study of the transmission of pathogens.

7.2 Methods

7.2.1 Study design

Faecal samples were collected using sample packs (as described below) from dogs and their owners every week for 8 weeks, and then monthly for a further 4 months, giving an overall collection period of 6 months. The sampling regimen for this study was devised as a result of the questionnaire study and human faecal sample trial described in chapter 6.

7.2.2 Sample packs

Sample packs were provided to dog owners that contained separate kits for faecal collection from both the owner and their dog. Human faecal sample kits contained latex gloves, two sheets of toilet paper in a sterile bag and a sterile universal pot containing 10mls diluent (MRD). Dog faecal sample kits contained a sterile universal pot with a scoop for faecal sampling. Both packs were placed in a single bag containing two transport containers, a jiffy bag and a prepaid envelope in order to send the samples back to the laboratory. Extra dog faecal sample pots (including extra transport containers, jiffy bags and pre-paid envelopes) were provided if there were several dogs in the household. Instructions on how to collect samples and how to send them back correctly were also provided, with a brief questionnaire asking for information on where they acquired the dog, where the dog slept, the dogs diet, other pets in the household and how many members of the household there were to be filled in by the owner (see appendix A5). When sample kits were returned by owners, another kit was sent approximately 3 days before the next samples were due.

7.2.3 Recruitment of dog owners

Dog owners were recruited in three main ways.

Veterinary practice

A local veterinary practice recruited dog owners when they visited with puppies for first vaccinations. The owners were presented with an information sheet (appendix A5) that provided details of the purpose of the study and what it would entail. If they agreed to taking part or wished to know more about the study, the owners filled in a information sheet with their address, phone number and convenient contact times (see appendix A5), which was posted back to us by the veterinary practice. A sample pack was also given to them if they were happy to be included in the study before we had contacted them.

Puppy training classes

A brief talk was given at the beginning of local puppy training classes. If owners were interested in participating in the study, they were invited to take a sample pack. An information sheet with contact details was also handed out to owners during the talk in case they later decided to participate.

Magazine advert

An advertisement calling for volunteers to participate in the study was placed in the November 2003 issue of the magazine *Dog World*. It contained information on why we were doing the study and what it involved (see appendix A5).

In addition, some households were recruited through colleagues in the Department of Veterinary Pathology and Department of Medical Microbiology, or among friends who had recently obtained puppies.

7.2.4 Processing of samples

When samples were returned to the laboratory they were either processed the same day or left at 4°C for a maximum of 2 days before processing. Samples were tested for *E. coli* and *Campylobacter* spp. as described in chapter 2 (2.2.1 and 2.2.2).

7.2.5 Characterisation of bacterial isolates

E. coli isolates were subjected to PCR to test for the presence of virulence genes as described in chapter 2 (2.6.4). At the beginning of the study, 3 isolates of *E. coli* from each sample were investigated. From January 2004, due to time constraints, only one isolate of *E. coli* from each sample was subjected to PCR for virulence genes.

A selection of *E. coli* and *Campylobacter* spp. isolates were characterised by restriction fragment length polymorphism (RFLP) analysis and pulsed-field gel electrophoresis (PFGE) as described in chapter 2 (2.7). All *E. coli* isolates from a single dog were also subjected to PFGE. All dendrograms were constructed using BioRad Molecular analyst.

7.3 Results

7.3.1 Households

Overall, 20 human volunteers and 51 dogs from 19 households participated in the study (table 7.1). All samples are listed in appendix A6.

Nine households were recruited as a result of the advertisement in *Dog World* magazine. Four households were recruited using local veterinary surgeries and 2 households each from dog-training classes and friends who had recently acquired puppies. One household each were recruited from Liverpool University Department of Veterinary Pathology and Department of Medical Microbiology. Sixteen volunteers were female and 4 were male.

Eleven households provided samples for 6 months. Seven of these were recruited from the magazine advert, 1 from the Department of Medical Microbiology, 2 from dog-training classes and 1 household from Department of Veterinary Pathology. All the volunteers recruited from veterinary surgeries stopped providing samples after 4 months or less. Three of the four male volunteers were recruited from veterinary practice and all stopped providing samples after 3 months. The other male volunteer was from the Department of Medical Microbiology and participated for the full 6 months. All households were informed of both overall and individual results from the study.

Table 7.1 Questionnaire information from participants

Household	Members of household	Dogs participating in study	Dogs diet	Other pets	Dog Diarrhoea Before/ during		Human diarrhea before/ during		Where does dog sleep
A Fisher	2x adults	1 x Cocker spaniel	T, KS	2x guinea pigs	Y	Y	Y	Y	Kitchen
B Bascombe	2x adults 2x children	1x Welsh Springer Spaniel	D, C	2x dogs 2x cats	N	N	N	Y	Kitchen
C Gardiner	2x adults 2x children	1x Belgium Shepperd dog	D, T, C	None	N	Y	N	N	Dinning room
D Griffiths	1x adult 1x children	1x Staffordshire Bull terrier	D, C	2x dogs	N	N	N	N	Kitchen
E Burgess	2x adults 1x children	1x Bedlington terrier	D, T,	2x dogs	N	Y	N	N	Kitchen
F T Williams	1x adult	1x King Charles Caveliar	D, T, C	None	Y	Y	N	Y	Bedroom
G Hart	2x adults 1x children	1x Border Collie	D, C	None	Y	N	N	N	In House
H Reed	1x adult	1x W H White	D, C	4x cats	Y	Y	Y	Y	Bedroom
I Gray	1x adult	1x greyhound	D, T,	1x cat	Y	Y	N	N	Bedroom
J Mackie	3x adults	3x Labrador retriever	RMB	2x rabbits 2x goldfish	N	Y	N	Y	Utility room
K Sheppard	2x adults 2x children	1x Stafford bull terrier	D, T, KS, C	2x gerbils 1x rabbit 1x guinea pig	Y	N	N	N	Kitchen

Table 7.1.Cont.Questionnaire information from participants

L Baldwin	3x adults	1x Belgium sheppard	Natural	1x cat sheep/foxes/ rabbits	N	N	Y	N	Kitchen
M Tomlinson	1x adult	1x English Springer spaniel	D, T, KC, C	9x goldfish	Y	Y	N	N	Kitchen
N J Williams	2x adults 1x children	2x Jack russell 1x Pomeranian 1x GSD 1x Rottweiler 2x CRH	Natural KC	3x cats 1x guinea pig Horses	Y – 5 N - 2	Y	N	N	Bedrooms
O Twinberrow	1x adult 1x children	2x Flatcoated retrevier 1x Shetland sheep dog	D, KC, C	1x cat 1x pony 7x guinea pig 7x fish	Y	N	N	Y	Kitchen
P Ursell	2x adults	11x Borzoi	Natural, D, C	None	Y – 10 N - 1	Y	Y	Y	Outside kennels
Q Robertshaw	2x adults	6x Pointer 1x Crossbreed 1x Bull Mastiff	D, tripe	None	N	N	N	N	6x outside kennel 2x porch
R Povall	3x adults	1x Border terrior	D, C	None	Y	N	N	N	Kitchen
S Fletcher	?	?	?	1x dog	N	N	N	N	?

(CHR – Chinese Crested Hairless)

(D – dry food, C – chews/treats, KC – kitchen scraps, T – tinned food, Y – yes, N - no)

7.3.2 Prevalence of *Campylobacter* spp.

Overall *Campylobacter* spp. were isolated from 2% (n=9/433) dog faecal samples collected, these being from 3 (16%) different households (table 7.2). All isolates were *C. upsaliensis* and isolated between the months of November and December 2003. There were no *Campylobacter* spp. isolates from humans.

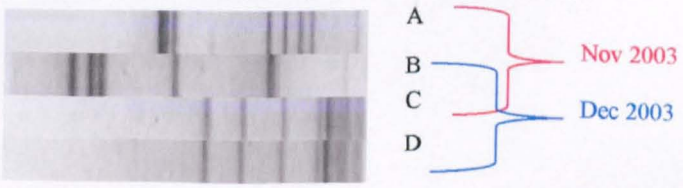
Table 7.2.*Campylobacter upsaliensis* isolation from different households

Household	No. *Campylobacter* spp. isolates	No. dogs in household	No. dogs affected	How many sampling occasions
E	1	1	1	1
N	4	7	4	2
Q	4	11	4	2

7.3.3 *Campylobacter upsaliensis* PFGE

There did not appear to be any similar isolates following PFGE analysis between the different households. Figure 7.1 shows an example of *C. upsaliensis* isolates obtained from different dogs in a single household (Q) after digestion with *SmaI*. Isolates A and C were isolated from faecal samples obtained from separate dogs in November 2003 (first time *C. upsaliensis* isolated). Isolates B and D were obtained from faecal samples from the same dog in December 2003 (second time *C. upsaliensis* isolated). Unfortunately the majority of isolates from household N were unable to be resuscitated from the freezer and were unable to be characterised. The *C. upsaliensis* isolate from household E was unique from the others characterised.

Figure 7.1.PFGE of *C. upsaliensis* isolates from household Q, following digestion with *smaI*



7.3.4 Commensal *E. coli*

The overall prevalence of *E. coli* from dog faecal samples was 91% (n=392/433). From human samples the prevalence was 79% (n=113/143).

E. coli isolates from 16 households were subjected to pulsed field gel electrophoresis following digestion with *XbaI*. In two households (K and P), *E. coli* isolates with identical PFGE patterns were obtained from both the dog and the owner: an example, household K, is shown in figure 7.2. There was no similarity seen between dog and human *E. coli* isolates from 14 households. In two households (O and Q) more than one dog appeared to be excreting the same PFGE type of *E. coli* an example of household Q is shown in figure 7.3. Indistinguishable isolates were obtained on two separate sampling occasions from household O, but on only one sampling occasion from household Q.

Figure 7.2. *Xba*I digest of *E. coli* isolates obtained from dog and human samples in Household K

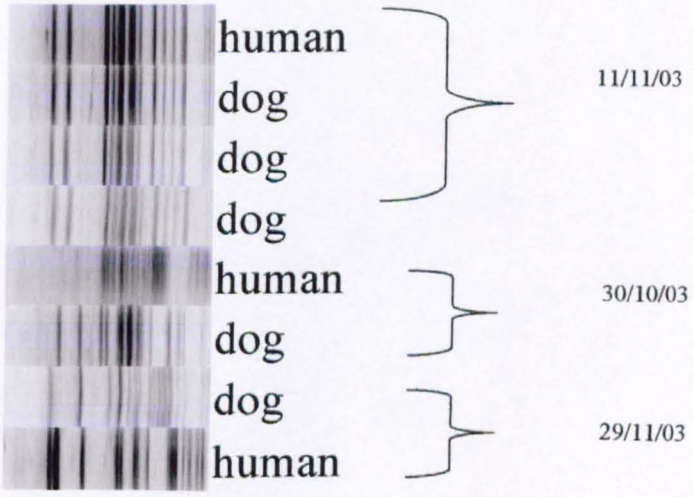
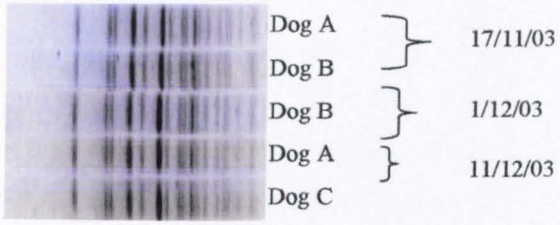


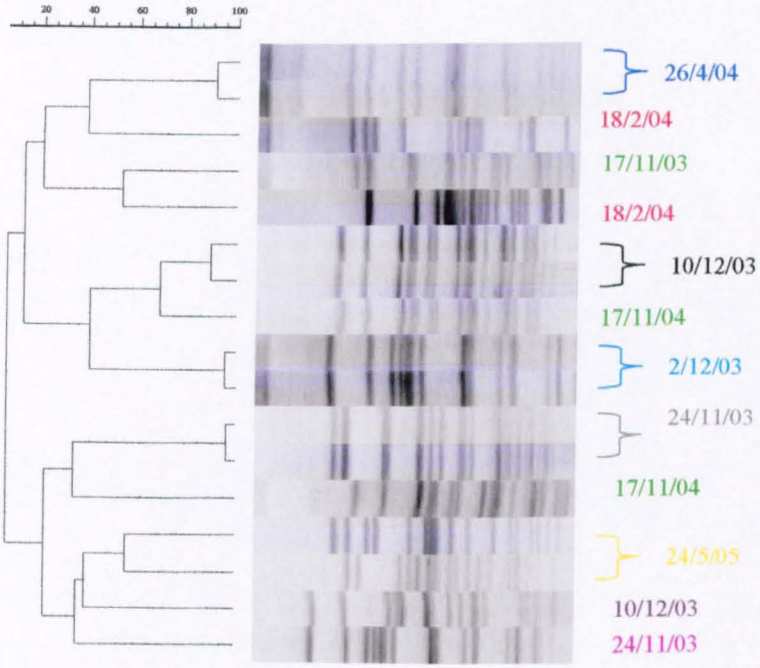
Figure 7.3. *Xba*I digest of *E. coli* isolates obtained from dogs in household Q



7.3.5 PFGE of commensal *E. coli* isolated from a single dog

The majority of *E. coli* isolates from a single dog were subjected to PFGE following digestion with *Xba*I (see figure 7.4). This was carried out to investigate variation in *E. coli* strains excreted from one dog. The dog intermittently suffered from diarrhoea and *E. coli* strains were found to vary. Strains isolated from each individual sample were usually identical, however, two different strains were isolated from the same faecal sample (isolates 1435a and 1435b).

Figure 7.4. *Xba*I digest of *E. coli* strains obtained from Dylan over a period of 6 months



7.3.6 *E. coli* carrying virulence determinants

The overall prevalence of *E. coli* carrying the *eaeA* gene from dog faecal samples was 4% (n=18/433). The prevalences of dog faecal samples carrying the *sta* gene or the *eaeA* and *bfpA* genes together were each 0.5% (n=2/433 each). *E. coli* carrying virulence genes were not isolated from any of the human samples. There were no *E. coli* isolates that exhibited the same PFGE banding pattern but carried different virulence determinants.

E. coli carrying one or more virulence genes were isolated from 69% (N=11/16) of households throughout the study. In 43% (N=7/16) of households, *E. coli* carrying virulence genes were isolated on more than one sampling occasion (table 7.3). Of the 5 households with more than one dog, only in one household (household N), were *E. coli* possessing virulence genes isolated from more than one dog. *E. coli* carrying virulence genes were not isolated from any of the dogs in other multi-dog households.

Table 7.3.Number of dog faecal samples from which *E. coli* carrying virulence genes were isolated from each household

Household	No. dogs in household	No. dogs affected (if applicable)	No. sampling occasions	Genes involved
A	1	1	4	*eaeA*
B	1	1	3	*eaeA* alone *eaeA* + *bfpA* (EPEC)
C	1	0	0	N/A
D	1	1	2	*eaeA* alone *sta* alone
E	1	1	2	*eaeA* alone *sta* alone
F	1	1	2	*eaeA*
G	1	1	1	*eaeA*
H	1	1	5	*eaeA* alone *eaeA* + *bfpA* (EPEC)
I	1	0	0	N/A
J	3	0	0	N/A
K	1	1	2	*eaeA*
L	1	1	1	*eaeA*
M	1	1	1	*eaeA*
N	7	2	1	*eaeA* *eaeA* + *bfpA* (EPEC)
O	3	0	0	N/A
P	11	0	0	N/A
Q	8	0	0	N/A
R	1	0	0	N/A
S	1	0	0	N/A

E. coli carrying virulence determinants and diarrhoea

In the eleven households where *E. coli* carrying virulence genes were isolated from dogs, eight of the dogs experienced diarrhoea before the study (it was not known how long before the study) and six dogs while they were participating in the study (table 7.4). Four owners also had diarrhoea during the study, although in only three households was diarrhoea reported when *E. coli* carrying virulence genes were isolated (table 7.5).

Table 7.4 *E. coli* carrying virulence genes isolated when owners or dogs reported diarrhoea

Household	Human diarrhoea		Dog diarrhoea		Diarrhoea reported when pathogenic *E. coli* isolated?
	Before study	During study	Before study	During study	
A	Y	Y	Y	Y	N
B		Y			Human diarrhoea
D					N
E				Y	N
F		Y	Y	Y	Human and dog diarrhoea
G			Y		N
H	Y	Y	Y	Y	Human and dog diarrhoea
K			Y		N
L			Y		N
M			Y	Y	Dog diarrhoea
N			Y	Y	N

(Y – diarrhoea reported, N – no diarrhoea reported, Spaces indicate no diarrhoea present. Last column indicates human/dog diarrhoea reported when *E. coli* carrying virulence determinants was also isolated from dogs)

Owners from three of the four households that experienced diarrhoea during the study, had clinical signs when *E. coli* carrying virulence determinants were also isolated from dog faecal samples. As seen in table 7.5, when *E. coli* carrying virulence

determinants are isolated from dog faecal samples, human diarrhoea is often reported. In household B, a child from the household was also reported to have diarrhoea (no sample given). *E. coli* carrying the *eaeA* gene were isolated again from this household 4 months later from the dog but diarrhoea was not reported in humans or the dog (not shown in table).

Table 7.5. *E. coli* carrying virulence genes isolated from the first 6 dog samples obtained and human and dog diarrhoea reported at the same time

Household	Sample no				
	1	2	3	4	5
B	*eaeA* No diarrhoea present	*eaeA* + *bfpA* **Human and dog diarrhoea**	N/A	N/A	N/A
F	N/A	*eaeA* No diarrhoea present	*eaeA* **Human and dog diarrhoea**	N/A	N/A
H	*eaeA* **Human and dog diarrhoea**	*eaeA* **Human and dog diarrhoea**	*eaeA* **Human and dog diarrhoea**	N/A **Human and dog diarrhoea**	*eaeA* + *bfpA* **Human and dog diarrhoea**
M	N/A	*eaeA* **Dog diarrhoea**	N/A	N/A **Dog diarrhoea**	N/A

(N/A – no pathogenic *E. coli* isolated from human or dog samples)

7.4 Discussion

The original plan for this study was to recruit owners of recently-acquired puppies from a local veterinary practice. However, only small numbers of volunteers were recruited, so we also recruited owners that already had puppies or dogs using additional recruiting methods. The problem with recruiting sufficient volunteers was most likely due to the nature of the samples which they were required to collect, and the time scale over which they were asked to participate. The majority of volunteers used in this study were recruited from the advert in Dog World magazine. Participants recruited in this way would obviously have a big interest in dogs, and were extremely interested in the results of the study. Also, the majority of participants provided samples for whole study period whereas volunteers recruited from other sources often stopped providing samples before the end of the 6 month period.

Throughout the study, better compliance was achieved when it was possible to speak to people directly rather than just sending out letters. This may be because, through direct contact with owners, it could be explained why the study was important to them directly, and readily discuss any reservations that they have about taking part in the study. Furthermore, direct contact gave owners confidence that they knew who they were dealing with and that all their information would be kept confidential.

Interestingly, the majority of volunteers were female, and also, 75% of the male volunteers stopped providing faecal samples after 3 months, if not before. This may be because females are less bothered about dealing with the subject of, and handling of faecal samples than males, or it may represent a gender difference in commitment to the study. In the questionnaire survey (chapter 6) there were more female, than male respondents and also a greater percentage of male respondents stated they would prefer to provide a sample only once (section 6.3.1).

At the beginning of the study, packs were given out for 4 weeks of sample collection. This was then stopped due to people dropping out of the study and not returning the packs, and also owners forgetting to send them back on time. Being sent a pack every week helped reminded owners to collect the samples when the packs came through the door.

There was a low prevalence of isolation of *Campylobacter* spp. from dog faecal samples and no *Campylobacter* spp. was isolated from any human samples. The delay in samples being returned to the laboratory by being sent through the post may have had an effect on the isolation of *Campylobacter* spp. Samples did not often arrive at the laboratory until 2-3 days after collection and the conditions in which they may have been stored throughout that time period may not have been favourable. A recent study by Koane *et al.* (2004) has highlighted the importance of processing samples as quickly as possible in order to isolate *Campylobacter* spp. *Campylobacter* spp. may have been present in human faecal samples but may not have been isolated due to a small number of bacteria being present or not enough faecal material in the sample due to the nature of collection with toilet paper. The lower prevalence of *E. coli* isolated from human samples in comparison to dog samples may also result from the sampling method.

Dog breeders recognise a 'diarrhoea season', when both themselves and their dogs suffer from diarrhoea, this being during the spring and summer months. Unfortunately this study was not carried out over the months when owners normally report this and all *Campylobacter* spp. isolates from this study were from samples provided in November and December. A seasonal pattern of *Campylobacter* spp. prevalence has been observed in previous studies (Wright, 1982, Torre and Tello, 1993, Lopez *et al*, 2002, Sandberg *et al*, 2002), as also being over the spring, summer and autumn months. All isolates in this study were *C. upsaliensis*, which agrees with findings from other studies that have found this species of *Campylobacter* to be the most common isolated from cats and dogs when selective media has not been used (Koene *et al*, 2004, Hald *et al.*, 2004).

The same *C. upsaliensis* PFGE strain was shown to be present in dogs from the same household but on different sampling occasions. This suggests that dogs may have acquired *C. upsaliensis* from the same source on different occasions, or that it may have persisted in the dog or kennel environment, thus, infecting the other dog at a later date. Also, individual dogs may have acquired the same strain at the same time but we were unable to isolate it from faecal samples for the reasons mentioned above. A recent study by Hald *et al.*, (2004) isolated different strains of *C. upsaliensis* from

dogs over a period of two years, sometimes from the same sample indicating that dogs are able to be colonised with and excrete different strains. *C. upsaliensis* may also have been present in faecal samples from other dogs in the same household but we may not have isolated it, again for the reasons mentioned above.

PFGE banding patterns of commensal *E. coli* isolates were found to be almost identical between dogs and owners in 2 different households and between different dogs resident in 2 different households. In household Q, the same strain was found from different dogs on separate sampling occasions, indicating dogs are colonised with the same strain. These findings may suggest that there may be transmission of *E. coli* between dogs in the same household and also between dogs and their owners. However, in this study the source of infection or possible route of transmission could not be determined. Dogs and humans may have acquired the same strain from the same source. In household K the dog was given kitchen scraps and therefore the dog and owner could have acquired the same *E. coli* strain via contaminated food. In this household the dog also slept in the kitchen. In household P, the dogs were not given kitchen scraps and they were also kept in outside kennels, thus, a source of infection could not be suggested. There were many limitations in this study; only 3 single colonies of *E. coli* were selected from each human and dog sample, therefore many similar strains could have been missed and humans may have carried similar commensal *E. coli* as dogs. However, due to time constraints it would have been difficult to investigate other strains. Unfortunately, due to the small numbers in this study, no statistical analysis could be carried out.

Overall, there was a low prevalence of *E. coli* carrying known virulence determinants when compared to the cross sectional survey in chapter 4 and also other previous studies (Neiger *et al.*, 2002, Sancak *et al.*, 2004). However, *E. coli* carrying virulence determinants were isolated from at least one dog sample in 58% of the households suggesting dogs may pose a risk for possible human infection when coming into contact with dog faeces. There were not any *E. coli* isolates that exhibited the same PFGE banding patterns and carried different virulence determinants, however, there was a small number of isolates subjected to PFGE so similar isolates may have been missed. The extent to which *E. coli* virulence determinants are associated with GI disease in dogs is not really known and pathogenic *E. coli* can be isolated from both

healthy dogs and dogs with diarrhoea (Beutin *et al.*, 1993, Beaudry *et al.*, 1996, Beautin, 1999, Goffaux *et al.*, 2000, Neiger *et al.*, 2002, Holland *et al.*, 1999). Interestingly, *E. coli*, (*eaeA* positive or *eaeA* and *bfpA* positive, indicative of EPEC) known to be pathogenic for humans was isolated from 75% of households where owners reported diarrhoea during the study. These were however, different households from those in which the same strain of commensal *E. coli* was isolated from both the owner and the dog. The findings from this study suggest that healthy dogs do carry *E. coli* possessing virulence determinants, which may be transmitted to humans, although no evidence of transmission was observed during this study. *E. coli* possessing virulence determinants may also have been present in human samples but not isolated (also possible for dog faecal samples), due to the reasons mentioned above.

To our knowledge this is the first study of its kind in the UK. Due to the nature of such a study and the expense, time and effort it needed to recruit volunteers to participate and to encourage them to continue participating; an insufficient number of samples were obtained to draw definite conclusions. The number of participants in the study was small, the presence of only two different types of bacteria were investigated and an even smaller number of bacterial isolates were subjected to further characterisation. Therefore, the true extent of transmission between dogs and owners may be underestimated. However, humans and dogs are able to carry the same strain of *E. coli* which requires further investigation to try and understand the source and mechanisms of transmission involved between dogs and owners. This study has shown that larger-scale studies are not only feasible, but likely to yield very interesting results.

Chapter 8

General discussion

The aims of this study were to investigate the prevalence of antibiotic resistant and potentially enteropathogenic bacteria in dog faecal samples, and the potential for transmission of these bacteria to humans. This was carried out to assess healthy dogs in the UK as a potential zoonotic risk for humans. The general prevalence of *E. coli*, *Salmonella* spp., *Campylobacter* spp., VRE and MRSA, and their antimicrobial susceptibilities were primarily investigated by conducting a cross-sectional survey, obtaining dog faecal samples from parks, boarding and rescue kennels and households. This revealed that there was a high prevalence of dogs excreting antibiotic resistant *E. coli* and *E. coli* carrying virulence determinants.

There was generally a higher prevalence of antibiotic resistant and pathogenic *E. coli* in faecal samples from dogs in boarding and rescue kennels in comparison to samples obtained from dogs in households and parks. De Graef *et al.* (2004) reported similar findings; a higher prevalence of MDR *E. coli* being isolated from kennel dogs than privately owned dogs. Dogs in kennels have contact with a greater number of dogs than they would normally, the dogs being from varied backgrounds and thus, this might increase the potential reservoir of different antibiotic resistant *E. coli*. Many of the *E. coli* antibiotic resistance genes in this study could not be determined due to time constraints. It would be interesting and useful to take these studies further to determine if they are novel genes, found mainly in dogs, or rarer genes, at a higher prevalence in dogs than other species. Many of the identified genes carried by *E. coli* isolates in this study were found to be those observed in both human and veterinary isolates (Baker *et al.*, 1999, Chopra and Roberts, 2001, Lee *et al.*, 2001, Everett *et al.*, 1996, Vila *et al.*, 1996, Sáenz *et al.*, 2003, Chaniotaki *et al.*, 2004). Several genes found in this study have also been observed in dog faecal samples previously (Sanchez *et al.*, 2002, Bryan *et al.*, 2004).

The high prevalence of the known virulence determinants from healthy dogs in this study suggests that the presence of these genes is not necessarily associated with diarrhoea in dogs. Virulence determinants attributed to pathogenic strains of *E. coli*,

usually associated with diarrhoea in dogs have also been found in healthy dogs in previous studies (Holland *et al.*, 1999). This does not mean that these genes are not involved in diarrhoeal disease in dogs, but shows that they are not sufficient to cause disease, and that other factors must be involved.

There have been few previous studies investigating *E. coli* carrying known virulence determinants, and also antibiotic resistant *E. coli* and the resistance genes responsible from healthy dogs, and to our knowledge this is the first study in the UK. This also makes it difficult to compare these findings with others. This study could be taken further to investigate how similar the resistance genes are to those in isolates found in human infections by PCR and DNA sequencing.

A higher prevalence of *Campylobacter* spp. was observed in household dogs (13%) than in boarding kennel dogs (10%) and rescue homes (3%), in contrast to other studies that have identified communal settings as a risk for factor for *Campylobacter* isolation in dogs (Malik and Love, 1989, Bruce *et al.*, 1993, Cantor *et al.*, 1997, Baker *et al.*, 1999). There was no significant association between *Campylobacter* spp. isolation and the presence of diarrhoea in dogs referred to the Small Animal Hospital for GI disease, suggesting that this bacterium is not a cause of diarrhoea. This finding agrees with other studies that have observed *Campylobacter* spp. in healthy dogs (Olsen and Sandstedt, 1987, Figura 1991, Burnens *et al*, 1992, Adesiyun *et* al, 1997, Baker *et* al, 1999). The majority of *Campylobacter* spp, isolated in this study were *C. upsaliensis* this being consistent with other studies that have not used inhibitory media (Burnens *et al.*, 1992, Engvall *et al.*, 2003, Hald *et al.*, 2004). All isolates were sensitive to the antibiotics tested. The results from this study demonstrate that *C. upsaliensis* is very common in dogs and at present, no other significant reservoir of this strain is known, other than in cats.

Salmonella spp. were observed only in an outbreak at a dog re-homing centre and from dogs that were excreting asymptomatically. Interestingly, identical strains (PFGE) were isolated over a 5 month period suggesting either persistence within the environment or transmission between dogs. Unfortunately, once the staff were informed, faecal samples were not provided from dogs that *Salmonella* spp. were previously isolated from, or from the housing block where the outbreak appeared

centred. It was also unknown if staff suffered from any GI symptoms. Previous studies have also observed *Salmonella* spp. from healthy dogs (Kwaga, 1989, Hackett, 2003, Kozak *et al.*, 2003), although the organism has been implicated in noscomial infections also suggesting transmission between dogs or persistence in the environment (Uhaa *et al.*, 1988).

There was a low prevalence of *C. perfringens* and VRE isolated from dog faecal samples. It is unclear whether *C. perfringens* is involved as a cause of diarrhoea in dogs due to its presence as part of the normal gut flora. Studies have suggested that *C. perfringens* is an opportunistic pathogen, only causing disease secondary to intestinal disruption by another pathogen (Turk *et al.* 1992). There have been limited reports of VRE isolation from dogs and the majority of studies have also not isolated this organism (Harwood *et al.*, 2001, Wagenvoort *et al.*, 2003). MRSA was not isolated from any faecal samples in this study. To my knowledge there have not been any studies investigating the presence of MRSA from dog faecal samples as the organism is not a usual GI inhabitant or a cause of diarrhoea in dogs. The prevalence of *Salmonella* spp., *C. perfringens* and VRE was low, but their presence in dogs may be a source of zoonotic infection for humans, and should be investigated further.

A longitudinal study was carried out to investigate the transmission of *Campylobacter* spp., commensal *E. coli* and *E. coli* carrying virulence determinants between dogs and their owners. Primarily a questionnaire was designed and distributed to obtain information on how healthy people would prefer to collect faecal samples if given a choice. Several University departments would not allow the questionnaire to be distributed to staff as they may have found the subject matter offensive: in itself this is interesting since such lack of support even within an academic institution demonstrates the difficulties faced in undertaking research of this kind. Therefore questionnaires were not randomly distributed and were sent to departments in which contacts were already established limiting the value of statistical analysis of the data collected.

However, the results gave a good indication of how people would prefer to collect faecal samples, thus enabling a trial to be carried out in which the two preferred methods could be tested. Faecal samples were collected from volunteers in the

Department of Veterinary Pathology (Liverpool University Veterinary School) and it was established that the preferred method of putting used toilet paper into a pot of sterile diluent was viable. This study therefore, devised a novel method of collecting faecal samples that may encourage a larger number of healthy individuals to participate in future similar studies. However, the method may not be the most sensitive way of collecting faecal samples from humans and the advantage and disadvantage of increased participant numbers, but possible reduced sensitivity would have to be assessed for individual studies using this method of human faecal sampling.

During the longitudinal study, the same strain of commensal *E. coli* was found in dogs and their owners, and also in different dogs in the same household. There may have been further strains of *E. coli* that had similar or identical banding patterns but unfortunately due to time constraints only three colonies were investigated per faecal sample. *E. coli* carrying known virulence determinants were only isolated from dog faecal samples. The virulence genes investigated in *E. coli* isolates are known to be pathogenic in humans, but the extent to which they cause disease in dogs is unclear. The majority of human participants did not experience diarrhoea during in the study period so it is not surprising that *E. coli* carrying virulence determinants were not isolated from dog owners. However, it is interesting that when humans did have diarrhoea in this study, on the majority of occasions pathogenic *E. coli* were isolated from dog faecal samples. Of course, this may represent human to dog transmission rather than the reverse.

As previously discussed, the way of collecting human faecal samples in this study is probably not as sensitive as collecting an actual stool specimen, therefore there may have been decreased faecal material from which to isolate *E. coli*. This combined with investigating just three colonies from each sample would decrease the probability of finding virulence determinants in *E. coli*. Towards the end of the study, due to time constraints, only one isolate was subjected to PCR for virulence determinants, thus decreasing the chances furthermore and it was interesting that *E. coli* carrying virulence determinants were not found after this date. Ideally, more *E. coli* isolates would have been investigated for both virulence determinants and strain differentiation.

Campylobacter spp. were not isolated from any human samples. As samples were sent to the laboratory through the post, the conditions in which they were kept may not have been particularly favourable and this may have had an effect on the survival of *Campylobacter* spp. Also, because of the manner in which human samples were collected, there may not have been enough faecal material present to isolate *Campylobacter* spp. as mentioned above. A study in Los Angeles, USA found that *C. upsaliensis* was the second most frequently isolated species from patients with Campylobacteriosis. Eighty-three percent of the patients had pets at home and 33% had dogs from which *C. upsaliensis* was isolated. A study from Denmark reported that the same quinolone-resistant *C. jejuni* strain, was isolated from both a dog and a 2 year old member of the household (Damborg *et al.*, 2004). Both studies suggest transmission of the organism between dog and humans in the same household.

All isolates obtained in the longitudinal study were *C. upsaliensis*, indicating again that this species is common in dogs. Although it is not isolated very frequently from human infection, dogs may be a source for a significant number of human cases. The prevalence of *C. upsaliensis* may be under estimated in human infections due to due the nature of media used which is inhibitory to this species of *Campylobacter*.

The range of volunteers participating in the longitudinal study was very limited and it appeared that only people who were interested in dogs appeared to take part, the majority of participants being dog breeders and/or trainers. Due to the limitations and bias of the longitudinal study, firm conclusions can not be drawn from its findings, however, as a pilot study, the results were very interesting and further work should be carried out to assess fully the transmission of pathogenic bacteria to humans from dogs. This would ideally involve a greater number of participants from a more varied background, getting samples back to the laboratory within 24 hours under favourable conditions and investigation and characterisation of more isolates.

In conclusion, the aims of this study were to investigate the presence of pathogenic and antibiotic resistant bacteria in dog faecal samples and the possibility of transmission to humans. The results from this study have demonstrated that a high prevalence of healthy dogs in the UK excrete antibiotic resistant *E. coli*, *E. coli*

carrying virulence determinants and *C. upsaliensis*, suggesting that dogs could be a possible zoonotic risk for humans. Isolation of identical strains of commensal *E. coli* from dogs and their owners further supports this possibility and further work should be carried out to determine this further.

Further work

- Longitudinal study
    - A larger study involving more people from varied backgrounds
    - Further investigation into isolates obtained by PFGE and MLST
- Cross sectional study
    - Further investigation into genes responsible for antibiotic resistance and compare against genes found in human isolates
    - Investigate presence of mobile genetic elements

References.

Aarestrup, F.M., Agerso, Y., Gerner-Smidt, P., Madsen, M., Jensen, L.B. (2000) Comparison of antimicrobial resistance phenotypes and resistance genes in *Enterococcus faecalis* and *Enterococcus faecium* from humans in the community, broilers and pigs in Denmark. *Diag microb infect dis* **37** 127-137

Aarestrup F.M., Seyfarth A.M., Emborg H.D., Pedersen K., Hendriksen R.S., Bager F. (2001) Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in faecal enterococci from food animals in Denmark. *Antimicrob Agents Chemother* **45** 2054-2059

Abass S., Franklin A., Kuehn I., Orskov F., Orskov I. (1989) Cytotoxin activity on Vero cells among *Escherichia coli* strains associated with diarrhoea in cats. *Am J Vet Res* **50** 1294-1296

Adak G.K., Cowden J.M., Nicholas S. and Evans H.S. (1995) The Public Health Laboratory Service national case control study of primary indigenous sporadic cases of Campylobacter infection. *Epidemiol infect* **115** 15-22

Adak G.K., Long S.M., O'Brien S.J. (2002) Trends in indigenous foodborne disease and deaths, England and Wales: 1992 to 2000. *Gut* **51** 832-41

Adesiyun A.A., Campbell M., Kaminjolo J.S. (1997) Prevalence of bacterial enteropathogens in pet dogs in Trinidad. *J Vet Med* **44** 19-27

Adesiyun A.A. (1999) Antibiograms of thermophilic *Campylobacter* and *Salmonella* species isolated from stray and pet animals and wildlife on Trinidad. *Veterinarski Arhiv* **69** 229-237

Adrian P.V., Klugman K.P., Amyes S.G. (1995) Prevalence of trimethoprim resistant dihydrofolate reductase genes identified with oligonucleotide probes in plasmids from isolates of commensal faecal flora. *J Med Micro* **35** 497-508

Adu-Bobie J., Frankel G., Bain C., Goncalves A.G., Trabulsi L.R., Douce G. (1998) Detection of intimins alpha, beta, gamma and delta, four intimin derivatives expressed by attaching and effacing microbial pathogens. *Clin Microbiol* **36** 662-668

Akkina J.E., Hogue A.T., Angulo F.J. (1999) Epidemiological aspects, control and importance of multiple-drug resistant *Salmonella* Typhimurium DT104 in the United States *J Am Med Assoc* **111** 189-197

Alekshun, M. N. and S. B. Levy (1997) Regulation of chromosomally mediated multiple antibiotic resistance: the mar regulon. *Antimicrob Agents Chemother* **41** 2067-75.

Alexander C. J., Citron D.M., Brazier J.S., Goldstein E.J.C. (1995) Identification and antimicrobial resistance patterns of clinical isolates of *Clostridium clostridioforme, Clostridium innocuum*, and *Clostridium ramosum* compared with those of clinical isolates of *Clostridium perfringens*. *J Clin Microbiol* **33** 3209-15.

Allos B.M. (2001) *Campylobacter jejuni* infections. Updates on emerging issues and trends. *Clin Infect Dis* **32** 1201-1206

Alonso A, Sánchez P, and Martínez JL (2001) Environmental selection of antibiotic resistance genes. *Environ Microbiol* **3** 1-9.

Altekruse S.F., Hunt J.M., Tollefson L.K., Madden J.M. (1994) Food and animal sources of *Campylobacter jejuni* infection. *JAVMA* **204** 57-61

Altekruse S.F., Stern N.J., Fields P.I., Swerdlow D.L. (1999) *Campylobacter jejuni* - an emerging foodborne pathogen. *Emerg Infect Dis* **5** 28-35

Alton N.K., Vapnek K. (1979) Nucleotide sequence analysis of the chloramphenicol resistance transposon Tn9. *Nature* **282** 864-869

Alvarez M., Tran J.H., Chow N., Jacoby G.A. (2004) Epidemiology of conjugative plasmid mediated AmpC β-lactamases in the United States. *Antimicrob Agents Chemother* **48** 533-537

An H., Fairbrother J.M., Dubreuil J.D., Harel J. (1997) Cloning and characterisation of the *eae* gene from dog attaching and effacing *Escherichia coli* strain 4221. *FEMS Microbiol Lett* **148** 239-245

Armitage P., Berry G., Mattheux J.N.S. (2002) Statistical methods in Medical Research. 4th Ed. Blackwell Science 504-506

Avrain L., Vernozy-rozand L., Kempf I. (2004) Evidence for natural horizontal transfer of *tetO* between *Campylobacter jejuni* in chickens. *J Appl Microbiol* **97** 134-140

Badger F. (2000) *Salmonella*, Dogs treats – Denmark.http//www.promed-mail.org.

Bager F. Ed (1997) Consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. 1 Copenhagen, Denmark

Baker J., Barton M.D., Lanser J. (1999). *Campylobacter* species in cats and dogs in South Australia. *Aust Vet J* 77 662-666

Barker, K. F. (1999) Antibiotic resistance: a current perspective. *Br J Clin Pharmacol* **48** 109-24

Ball M.D. (1951) Salmonella in dogs and cats of the Los Angeles, Honolula and Bermuda areas *JAVMA* **888** 164-166

Balucinska B., Vasicek L., BreuerStrosberg R., AwadMasalmeh M. (1997) Occurrence of *Campylobacter* in dogs. *Wiener Tierarztliche Monatsschrift* **84** 322-324

Bates. J., Jordens, J.Z., Griffiths, D.T., (1993) Evidence for animal origin of VRE (Letter) *Lancet* **342** 490-491

Beaudry M., Zhu C., Fairbrother J.M., Harel J. (1996) Genotypic and phenotypic characterisation of *Escherichia coli* isolates from dogs manifesting attaching and effacing lesions. *J Clin Microbiol* **34** 144-148

Benveniste R and Davies J (1973) Aminoglycoside antibiotic inactivating enzymes in actionomycetes similar to those present in clinical isolates of antibiotic resistant bacteria. *Proc Natl Acad Sci USA*, **70** 2276-2280.

Bertrand X., Mulin B., Viel J.F., Thouverez M., Talon D. (2000) Common PFGE patterns in antibiotic resistant *Enterococcus faecalis* from humans and cheeses. *Food Microbiol* **17** 543-551

Beutin L., Geier D., Steinrueck H., Zimmermann S., Scheutz F. (1993) Prevalence and some properties of verotoxin (shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. *J Clin Microbiol* **31** 2483-2488

Beutin L., Aleksic S., Zimmermann S., Gleier K. (1994) Virulence factors and phenotypical traits of verotoxigenic strains of *Escherichia coli* isolated from human patients in Germany. *Med Microbiol* **183** 13-21

Beutin L., Geiger D., Zimmermann S., Karch H. (1995) Virulence markers of Shiga-like toxin-producing *Escherichia coli* strains originating from healthy domestic animals of different species. *J Clin Microbiol* **33** 631-635

Beutin L., Geier D., Zimmermann S., Aleksic S., Gillespie H.A., Whittam T.S. (1997) Epidemiological relatedness and clonal types of natural populations of *Escherichia coli* strains producing Shiga toxins in separate populations of cattle and sheep. *App Environ Microbiol* **63** 2175-2180

Beautin L. (1999) *Escherichia coli* as a pathogen in cats and dogs. *Vet Res* **30** 285-298

Billington, S. J., Wieckowski E. U., Sarker M.R., Bueschel D., Songer J.G., McClane B.A. (1998) *Clostridium perfringens* type E animal enteritis isolates with highly conserved, silent enterotoxin gene sequences. *Infect Immun* **66** 4531-6

Bissonnette L., Champetier S., Buisson J.-P., Roy P.H. (1991) Characterisation of the non-enzymatic chloramphenicol resistance (*cmlA*) gene of the In4 integron of Tn1696: similarity of the product to transmembrane transport proteins. *J Bacteriol* **173** 4493-4502

Blake D.P., Humphry R.W., Scott K.P., Hillman K., Fenlon D.R., Low J.C. (2003) Influence of tetracycline exposure on tetracycline resistance and the carriage of tetracycline resistance genes within the commensal *Escherichia coli* populations. *J Appl Microbiol* **94** 1087-1097

Blaser MJ, Berkowitz DI, LaForce FM, Cravens R, Reller LB, Wang WLL (1979) *Campylobacter enteritis*: clinical and epidemiologic features. *Ann Inter Medicine* **91** 179-185

Blaser M.J., Laforce F.M., Wilson N.A., Wang W.L.L. (1980) Reservoirs for human campylobacteriosis. *J Infect Dis* **141** 665-669

Blazquez J., Morosini M.I., Negri M.C., Baquero F. (2000) Selection of naturally occurring extended spectrum TEM β-lactamase variants by fluctuating β-lactam pressure. *Antimicro Agents Chemother* **44** 2182-2184

Borgen K., Sorum M., Wasteson Y., Kruse H (2001) VanA-type vancomycin resistant enterococci remain prevalent in poultry carcasses 3 years after avoparcin was banned. *Inter J Food Microbiol* **64** 89-94

Briggs C.E., Fratamico P.M. (1999) Molecular characterisation of an antibiotic resistance gene cluster of *Salmonella* Typhimurium DT104. *Antimicro Agents Chemother* **43** 846-849

Briñas L., Zarazaga M., Sáenz Y., Ruiz-Larrea F., Torres C. (2002) β-lactamases in ampicillin-resistant *Escherichia coli* isolates from foods, humans and healthy animals. *Antimicro Agents Chemother* **46** 3156-3163

Broes A., Fairbrother J.M., Mainil J., Harel J., Lariviere S (1988) Natural infection with an attaching and effacing *Escherichia coli* in a diarrheic puppy. *Can J Vet Res.* **52** 280-282

Bruce D., Zochowski W., Fleming G.A. (1980) *Campylobacter* infections in cats and dogs. *Vet Rec* **107** 200

Bruce D., Fleming G.A. (1983) *Campylobacter* isolations from household dogs. *Vet Rec* **112** 16

Bryan L.E., Kowand S.K., van den Elzen H.M. (1979) Mechanism of aminogylcoside antibiotic resistance in anaerobic bacteria. *Clostridium perfringens* and *Bacteroides fragilis*. *Antimicro Agents Chemother* **15** 7-13

Bryan A., Shapir N., Sadowsky M.J. (2004). Frequency and distribution of tetracycline resistance genes in genetically diverse, nonselected and nonclinical *Escherichia coli* strains from diverse human and animal sources. *Appl Environ Biol* **70** 2503-2507

Brynestad S., Granum P.E. (2002) *Clostridium perfringens* and foodborne infections. *Int J Food Microbiol* **72** 195-202

BSAVA news (1998) *Campylobacter* infection. *JSAP* **39** 99-100

Burchall J.J. and Hitchings G.H. (1965) Inhibitor binding analysis of dihydrofolate reductases from various species. *Mol Pharma* **1** 126-136

Burdett V. (1993) tRNA modification activity is necessary for tet(M)-mediated tetracycline resistance. *J bacteriol* **175** 2709-2715

Burnens A.P., Angéloz-Wick B., Nicolet J. (1992) Comparison of *Campylobacter* carriage rates in diarrheic and healthy pet animals. *J Vet Med* **39** 175-180

Burnens A.P., Nicolet J. (1992) Detection of *Campylobacter upsaliensis* in diarrheic dogs and cats, using a selective medium with cefoperazone. *Am J Vet Res* **53** 48-51

Busani L., Graziani C., Battisti A., Franco A., Ricci A., Vio D., Digiannan E., Paterlini F., D'Incau M.,m Owczarek S., Caprioli A., Luzzi I. (2004) Antibiotic resistance in *Salmonella* enteric serotypes Typhimurium, Enteritidis and Infantis from human infection, foodstuffs and farm animals in Italy. *Epidemiol Infect* **132** 245-251

Butler C.E., Herd B.R., (1965) Human enteric pathogens in dogs in central Alaska. *J Infect Dis.* **115** 233-236

Bush K., Jacoby G.A., Medeiros A.A. (1995) A functional classification scheme for β-lactamases and its correlation with molecular structure. *Antimicro Agents Chemother* **39** 1211-1233

Bush K., Miller G.H. (1995) Bacterial resistance: β-lactamases and amnioglycoside modifying enzymes *Curr Opin Microbiol* **1** 509-515

Byrne C., Doherty D., Moonely A., Byrne M., Woodward D., Johnson W., Rodgers F., Bourke B. (2000) Basis of the superiority of cefoperazone amphotericin teicoplanin for isolating *Campylobacter upsaliensis* from stools. *J Clin Microbiol* **39** 2713-2716

Cabral J.H.M., Jackson A.P., Smith C.V., Shikotra N., Maxwell A., Liddington R.C. (1997) Crystal structure of the breakage-reunion domain of DNA gyrase *Nat* **388** 903-906

Cannon M., Harford S., Davies J. (1990). A comparative study on the inhibitory actions of chloramphenicol, thiamphenicol, and some fluorinated derivatives. *J Antimicro Chemother* **26** 307-317

Cantor G.H., Nelson S., Vanek J.A., Evermann J.F., Eriks I.S., Basaraba R.J., Besser T.E. (1997) *Salmonella* shedding in racing sled dogs. *J Vet Diagn Invest* **9** 447-448

Caroff N., Espaze E., Berard I., Richet H., Reynaud A. (1999) Mutations in the ampC promoter of *Escherichia coli* isolates resistant to oxyiminocephalosporins with out extended spectrum β-lactamase production. *FEMS Mcriobiol Lett* **173** 459-465

Carmen R.J., Lewis J.C. (1983) Recurrent diarrhoea in a dog associated with *Clostridium perfringens* type A. *Vet Rec* **112** 342-343

Cassutto B.H., Cook L.C. (2002) An epidemiological survey of *Clostridium perfringens*-associated enterotoxemia at an army veterinary treatment facility. *Mil Med* **167** 219-222

Cave N.J., Marks S.L., Kass P.H., Melli A.C., Brophy M.A. (2002) Evaluation of a routine diagnostic faecal panel in dogs with diarrhoea. *J Am Vet Med Assoc* **221** 52-59

Centres for Disease Control and Prevention (2001) Outbreaks of multi-drug resistant *Salmonella* Typhimurium associated with veterinary facilities – Idaho, Minnesota and Washington. *MMWR* **50**, 701-704

Centres for Disease Control and prevention (2002) *S. aureus* resistant to vancomycin – United States. *MMWR* **51** 565-567

Cetinkaya Y, Falk P and Mayhall CG (2000) Vancomycin-Resistant Enterococci. *Clin Microbiol Rev* **13** 686-707.

Chambers H.F. (2001) The changing epidemiology of *Staphylococcus aureus*? *Emerg Infect Dis* 7 178-182

Chang, C. Y., Chang L.L., Chang Y.H., Lee T.M., Li Y.H., Chang S.F. (2000) Two new gene cassettes, *dfr17* (for trimethoprim resistance) and *aadA4* (for spectinomycin/streptomycin resistance), inserted in an *Escherichia coli* class 1 integron. *J Antimicro Chemother* **46** 87-9.

Chaniotaki S., Giakouppi P., Tzouvelekis L.S., Panagiotakos D., Kozanitou M., Petrikkos G., Avlani A. (2004) Quinolone resistance among *Escherichia coli* strains from community-acquired urinary tract infections from Greece. *Clin Microbiol Infect Dis* **10** 70-83

Chattopadhyay U.K., Rashid M., Sur S.K., Pal D. (2001) The occurrence of Campylobacteriosis in domestic animals and their handlers in and around Calcutta. *J Med Micro* **50** 933-934

Chiou C.S., Hsu S.Y., Chiu S.I. (2000) *Vibrio parahaemolyticus* serovar O3:K6 as the cause of the unusually high incidence of food borne disease outbreaks in Taiwan from 1996 to 1999. *J Clin Microbiol* **38** 4621-4625

Christensen E., Torp M., Nygård K., Kruse H. (1999) Tyggeben til hund og risiko for salmonellose hos mennesker. *Norwegian MSIS Bulletin* **48** 1

Chopra, I., and M. Roberts. 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol Mol Biol Rev* **65** 232-260

Clark N.C., Teixeira L.M., Facklam R.R., Tenover F.C. (1998) Detection and differentiation of *vanC-1, vanC-2* and *vanC-3* glycopeptide resistance genes in enterococci. *J Clin Micro* **37** 2294-2297

Cloeckaert A., and S. Schwarz 2001 Molecular characterization, spread and evolution of multidrug resistance in *Salmonella enterica* Typhimurium DT104. *Vet Res* **32** 301-310

Cohen S.P., MuMurry L.M., Levy S.B. (1988) *marA* locus causes decreased exdpression of the OmpF porin in multiple antibiotic resistant (MAR) mutants of *Escherichia coli. J bacteriol* **170** 5416-5422

Cooke C.L., Singer R.S., Jang S.S., Hirsh D.C. (2002) Enrofloxacin resistance in *Escherichia coli* isolated from dogs with urinary tract infections. *JAVMA* **220** 190-192

Coque, TM, Tomayko, JF, Ricke, SC, Okhyusen, PC, Murray BE (1996) Vancomycin resistant Enterococci from nosocomial, community and animal sources in the United States. *Antimicro Agents Chemother* **40** 2605-2609

Cox L.A., Jr (2001) Risk analysis: Foundations, models and methods. Kluwer, Boston, MA, USA

Craig N.L. (1991) Tn7: A target site-specific transposon. *Mol Microbiol* **5** 2569-2573

Cristino J.M. (1999) Correlation between consumption of antimicrobials in humans and development of resistance in bacteria. *Int J Antimicrob Agents* **12** 199-202

Damborg P., Olsen K.E.P., Nielsen E.M., Guardabassi L. (2004) Occurrence of *Campylobacter jejuni* in pets living with human patients. *J Clin Microbiol* **42** 1363-1364

DANMAP 2002. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. In: Bager, F., Emborg, H.D., Heuer, O.E. (Eds.). Danish Veterinary Institute, Copenhagen, Denmark, 2003. ISSN 1600–2032 (also available on http://www.vetinst.dk; 2003-06-20)

Danner S.J., Shears P., Platt D.J. (1997) Isolation and characterization of coliforms from glacial ice and water in Canada's High Artic. *J Appl Bacteriol* **82** 597-609

De Graef E.M., Decostere A., Devriese L.A., Haesebrouck F. (2004) Antibiotic resistance among faecal indicator bacteria from healthy individually owned and kennel dogs. *Micro Drug Resist* **10** 65-69

De Groot R., Sluijter M., de Bruyn A., Camps J., Goessens W.H.F., Smith A. L., Hermans P.W.M. (1996) Genetic characterisation of trimethoprim resistance in *Haemophilus influenzae*. *Antimicro Agents Chemother* **40** 2131-2136

Del Grosso M., Capriol A., Chinzari P., Fantana M.C., Pezzotti G., Manfria A., Giannatale E.D., Goffredo E., Pantosti A. (2000) Detection and characterisation and vancomycin-resistant enterococci in farm animals and raw meat products in Italy. *Micro Drug Resist* **6** 313-318

Devriese L.A., Ieven M., Goossens H., Vandamme P., Pot B., Hommez J., Haesebrouck F. (1996) Presence of vancomycin-resistant enterococci in farm and pet animals. *Antimicro Agents Chemother* **40** 2285-2287

Donnenberg M.S., Kaper J.B. (1992) Enteropathogenic *Escherichia coli. Infec Immun* **60** 3953-3961

Drazenovich N., Ling G.V., Foley J. (2004) Molecular characterisation of *Escherichia coli* strains associated with apparently persistent urinary tract infection in dogs. *Vet Intern Med* **18** 301-306

Drolet R., Fairbrother J.M., Harel J., Hélie P. (1994) Attaching and effacing enterotoxigenic *Escherichia coli* associated with enteric colibacillosis in the dog. *Can Vet J Res* **58** 87-92

Dunne W.M., Dunne B.S., Smith D. (1996) Watch out where the huskies go. *Am Soc for Microbiol News* **62** 283

Dutka-Malen S., Evers S., Courvalin P. (1995) Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. *J Clin Microbiol* **33** 24-27

Echeverria P., Serichantalerg O., Changchawalit S., *et al.*, (1992) Tissue-culture adherent *Escherichia coli* in infantile diarrhoea. *J Infect Dis* **165** 141-143

Eickhoff, TC (1992) Antibiotics and nosocomial infections. In Bennett JV, Brachman PS (Eds), Hospital infections, 3rd edition. Little, Brown and Company, Boston, MA, pp. 245-264.

Endtz H.P., van den Braak N., van Belkum A. High prevalence and clonal spread of T-n1546, a transposon conferring resistance to vancomycin in enterococci from humans and consumer poultry. Proceedings of 37th ICAAC conference, Toronto. C-138

Engstrom B.E., Fermer C., Lindberg A., Saarinen E., Baverud V., Gunnarsson A. (2003) Molecular typing of isolates of *Clostridium perfringens* from healthy and diseased poultry. *Vet Microbiol* **94** 225-235

Engvall E.O., Brandstrom B., Andersson L., Baverud V., Trowald-Wigh G., Englund L. (2003) Isolation and identification of thermophilic *Campylobacter* species in faecal samples from Swedish dogs. *Scand J Infect Dis* **35** 713-8

Enne V.L., Livermore D.M., Stephens P., Hall L.M.C. (2001) Persistence of sulphonamide resistance in *Escherichia coli* in the UK despite national prescribing restriction. *Lancet* **357** 1325-1328

Essack S.Y. (2000) Treatment options for extended spectrum β-lactamase-producers *FEMS* **190** 181-184

Evans S.J. (1993) The seasonality of canine births and human campylobacteriosis: a hypothesis. *Epidemiol Infect* **110** 267-272

Farrow K.A., Lyras D., Polekhina D., Koutsis K., Parker M.W., Rood J.I. (2002). Identification of Essential Residues in the Erm(B) rRNA Methyltransferase of *Clostridium perfringens*. *Antimicro Agents Chemother* **46** 1253-1261

Fang F.F., Fierer J. (1991) Human infections with *Salmonella Dublin Medicine* **70** 198-207

Férina C, Ferreira E., Correia J.D., Gonçalves J., Caniça M. (2002) Patterns and mechanisms of resistance to β-lactams and β-lactamase inhibitors in uropathogenic *E. coli* isolated from dogs in Portugal. *J Antimicro Chemo* **49** 77-85

Fey P.D., Safranek T.J., Rupp M.E., Dunne E.F., Ribot E., Iwen P.C., Bradford P.A., Angulo F.J., Hinrichs S.H. (2000) Ceftriaxone-resistant *Salmonella* infection acquired by a child from cattle. *N Engl J Med* **342** 1242-1249

Fey P.D., Saïd-Salim B., Rupp M.E., Hinrichs S.H., Boxrud D.J., Davis C.C., Kreiswirth B.N., Schlievert P.M. (2003) Comparative molecular analysis of community- or hospital-acquired methicillin resistant *Staphylococcus aureus*. **47** 196-203

Figura N. (1991) *Campylobacter* spp. isolated from dog faeces. *Lancet* **338** 1403

Fish N.A., Finlayson M.C., Carere R.P., (1967) Salmonellosis: report of a human case following direct contact with infected cattle. *Can Med Assoc J* **96** 1163-1165.

Flemming M.P. (1980) Incidence of *Campylobacter* infection in dogs. *Vet Rec* **107** 202

Flensburg J., Sköld O. (1987) Massive overproduction of dihydrofolate reductase as a response to the use of trimethoprim. *Eur J Biochem* **162** 473-476

Fluit, A. C., Visser M.R., Schmitz F.J. (2001) Molecular detection of antimicrobial resistance. *Clin Microbiol Rev* **14** 836-71

Fox J.G., Moore R., Ackerman J.I. (1983) *Campylobacter jejuni*-associated diarrhoea in dogs. *JAVMA* **183** 1430-1433

Franz C.M., Holzapfel W.H., Stiles M.E. (1999) Entercocci at the crossroads of safety? *Int Food Microbiol* **47** 1-24

Frech G., Schwarz S. (1998) Tetracycline resistance in *Salmonella* enterica subsp. Enterica Serovar Dublin. *Antimicro Agents Chemo* **42** 1288-1289

Frost J.A., Kelleher A., Rowe B. (1996) Increasing ciprofloxacin resistance in Salmonellas in England and Wales 1991-1994. *J Antimicro Chemo* **37** 85-91

Frost, J.A., Oza, A.N., Thwaites, R.T., Rowe, B. (1998) Serotyping scheme for *Campylobacter jejuni* and *Campylobacter coli* based on direct agglutination of heat stable antigens. *Journal of Clin Microbiol* **36** 335-339.

Frost J.A. (2001) Current epidemiological issues in human campylobacteriosis. *J Appl Microbiol* **90** 85S-96S

Frost J.A., Gillespie I.A., O'Brien S.J. (2002) Public health implications of *Campylobacter* outbreaks in England and Wales, 1995-9: epidemiological and microbiological outbreaks. *Epidemiol Infect* **128** 111-118

Friis C (1999) Consumption of antimicrobials by food animals and the resistance of pathogens in food animals. *J Hosp Infect* **14** 223-245

Fukata T., Naito F., Yoshida N., Yamaguchi T., Mizumura Y., Hirai K. (2002) Incidence of *Salmonella* infection in healthy dogs in Gifu Prefecture, Japan. *J Vet Med Sci* **64** 1079-1080

Gaffney D.F., Cundliffe E., Foster T.J. (1981) Chloramphenicol resistance that does not involve chloramphenicol acetyltransferase encoded by plasmids from gram-negative bacteria. *J Gen Microbiol* **125** 113-121

Gallardo, F., Ruiz J., Marco F., Towner K.J., Vila J. (1999) Increase in incidence of resistance to ampicillin, chloramphenicol and trimethoprim in clinical isolates of *Salmonella* serotype Typhimurium with investigation of molecular epidemiology and mechanisms of resistance. *J Med Microbiol* **48** 367-74.

Galton M.M., Scatterday J.E., Hardy A.V. (1952) Salmonellosis in dogs. Bacteriological, epidemiological and clinical considerations. *J infect Dis* **91** 1-5

Gaskins H.R., Collier C.C., Anderson D.B. (2002) Antibiotics as growth promoters; mode of action. *Ani biotech* **13** 29-42

Gaudrean C., Gilbert, H. (1993) Antimicrobial resistance of clinical strains of *Campylobacter jejuni* subsp. *jejuni* isolated from 1985 to 1997 in Quebec, Canada. *Antimicro Agents Chemother* **42** 2106-2108

Gaynes R. (1997) The impact of antimicrobial use the emergence of antimicrobial-resistant bacteria in hospitals. *Infect Dis Clin North Am* **11** 757-765

Gibreel A., Sköld O. (1998) High-level resistance to trimethoprim in clinical isolates of *Campylobacter jejuni* by acquisition of foreign genes (*dfr1* and *dfr9*) expressing drug-insensitive dihydrofolate reductases. *Antimicrob Agents Chemother* **42** 3059-3064

Gilardoni L., Maubecin E., Carloni G., Mas J. (1999) *Clostridium perfringens* isolated from a urinary tract infection in a canine. *Anaerobe* **5** 397-398

Gillespie I.A., O'Brien S.J., Adak G.K., Tam C.C., Frost J.A., Bolton F.J., Tompkins D.S. (2003) Point source outbreaks of *Campylobacter jejuni* infection - are they more common than we think and what might cause them? *Epidemiol Infect* **130** 367-375

Gillivor M.A., Bennett M., Began M., Hazel S.M., Hart C.A. (1999) Antibiotic resistance found in wild rodents. *Nature* **401** 233-234

Giraffa G. (2002) Enterococci from foods. *FEMS* **26** 163-171

Giraffa G., Olivari A.M., Neviani E. (2000) Isolation of vancomycin resistant *Enterococcus faecium* from Italian cheeses. *Food Microbiol* **17** 671-677.

Gniadkowski, M. (2001) Evolution and epidemiology of extended spectrum β-lactamases and ESBL producing organisms. *Clin Microbiol infect* **7** 597-608

Goffaux F., Janssen L., Jacquemin E., China B., Mainil J. (2000) Genotypic characterisation of enteropathogenic *Escherichia coli* (EPEC) isolated in Belgium from dogs and cats. *Res Microbiol*

Gordon C.L., Ahmad M.H. (1991) Thermal susceptibility of *Streptococcus faecium* strains isolated from frankfurters. *Can J Microbiol* **37** 609-612

Gorham J.R., Garner F.M., (1951) The incidence of *Salmonella* infections in dogs and cats in a non-urban area. *Am J Vet Res* **12** 35-37

Gortel K., Campbell K.L., Kakoma I., Whittem T., Schaeffer D.J., Weisiger R.M. (1999) Methicillin resistance amomg Staphylococci isolated from dogs. *AJVR* **60** 1526-1527

Gondrosen B., Knævel T., Dommarsnes K. (1985) Isolation of theromphilic campylobacters from Norwegian dogs and cats. *Acta Vet Scand* **26** 81-90

Goosens H., Vlaes L., DeBoeck M., Pot B., Kersters K., Levy J., De Mol P., Butzler J.P., VanDamme P. (1990) Is *Campylobacter upsalinesis* an unrecognised cause of diarrhoea? *Lancet* **335** 584-586

Goossens H., Vlaes L., Butzler J.P., Adnet A., Hanicq P., N'Jufom S., Massart D., De Schrijver G., Blomme W (1991) *Campylobacter upsaliensis* associated with canine infections. *Lancet* **337** 1486-1487

Graham S.M., Molyneux E.M., Walsh A.L, Cheesbrough J.S., Molyneux M.E., Hart C.A. (2000) Nontyphoidal Salmonella infections of children in Southern Africa. *Pediatr Infect Dis J* **9** 1189-96

Griffiths N.J. Studies on *Clostridium perfringens* in the horse. Ph.D. Thesis. University of Liverpool, 1998

Groom A.V., Wolsey D.H., Naimi T.S., Smith K., Boxrud D., Moore K.A., Cheek J.E., (2001) Community acquired MRSA in a rural American Indian Community. *JAMA* **286** 1201-1204

Gross R.J., Rowe B. (1985) *Escherichia coli* diarrhoea. *J Hyg* **95** 531-550

Grumbles L.C., Maciolek J.O. (1955) Isolation of enteric pathogens from humans and dogs in Barrow, Alaska. *Sci in Alaska 6th Alask Sci Conf*, pp 132-133

Guardabassi L., Loeber M.E., Jacobson A. (2004) Transmission of multiple antimicrobial-resistant *Staphylococcus intermedius* between dogs affected by pyoderma and their owners. **98** 23-27

Hackett T. (2003) Prevalence of Enteric Pathogens in dogs of North-Central Colorado. *J Amer Animal Hosp Assoc* **39** 52-56

Hald B., Madsen M. (1997) Healthy puppies and kittens as carriers of Campylobacter spp., with special reference to *Campylobacter upsaliensis*. *J Clin Micro* **35** 2251-3352

Hald B., Pedersen K., Wainø M., Jørgensen J.C., Madsen M. (2004) Longitudinal study of the excretion patterns of thermophilic *Campylobacter* spp. in young pet dogs in Denmark. *J Clin Microbiol* **42** 2003-2012

Hamilton-Miller J.M.T. (1984) Resistance to antibacterial agents acting onfolate metabolism, in Bryan L.E. (Ed), Antimicrobial drug resistance, Academic Press, Inc., New York. 173-190

Hammermueller J., Kruth S., Prescott J., Gyles C. (1995) Detection of toxin genes in *Escherichia coli* oslated from normal dogs and dogs with diarrhea. *Can J Vet Res* **59** 265-270

Hancock P. (1997) Antibiotic-associated diarrhoea. *Clostridium difficile* or *Clostridium perfringens*. *Rev Med Micro* **8 (S1)** S66-S67

Hart C.A., Percival A. (1982) Resistance to cephalosporins among gentamicin-resistant *Klebsiellae*. *JAC* **9** 275-286

Hart C.A., Batt R.M., Saunders J.R. (1993) Diarrhoea caused by *Escherichia coli*. *Ann Trop Ped* **13** 121-131

Harwood V.J., Brownell M., Perusek W., Whitlock J.E. (2001) Vancomycin-resistant enterococcus spp. isolated from wastewater and chicken faeces in the United States. *Appl Environ Micrbiol* **67** 4930-4933

Heisig P. (1993) High level fluoroquinolone resistance in *Salmonella* Typhimurium isolate due to alterations in both *gyrA* and *gyrB* genes. *J Antimicrob Chemother* **32** 367-377

Hill S.L., Cheney J.M., Taton-Allen G.F., Reif J.S., Bruns C., Lappin M.R. (2000) Prevalence of enteric zoonotic organisms in cats. *J Vet Med Assoc* **216** 687-692

Hiramatsu K., Hanaki H., Ino T., Yahuta K., Oguri T., Tenover F.C. (1996) Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin activity. *Lancet* **40** 135-136

Hoise B.D., Nicolson T.B., Henderson D.B. (1979) *Campylobacter* infections in normal and diarrhoeic dogs. *Vet Rec* **105** 80

Holland R.E., Walker R.D., Sriranganathan N., Wilson R.A., Ruhl D.C. (1999) Characterisation of *Escherichia coli* from healthy dogs. *Vet Microbiol* **70** 261-268

Holmberg S.D., Blake P.A. (1984) Staphylococcal food poisoning in the United States: New facts and old misconceptions. *JAMA* **251** 487-489

Holmes D.S., Quigley M., (1981) A rapid boiling method for the preparation of bacterial plasmids. *Analytical Chem* **114** 193-197

Hooper, D. C. (1999) Mechanisms of fluoroquinolone resistance. *Drug Resist Updat* **2** 38-55

Holt (1980) Incidence of *Campylobacter, Salmonella* and *Shigella* infections in dogs in an industrial town. *Vet Rec* **107** 254

Houng H.H., Sethabutr O., Nirdnoy W., Katz D.E., Pang L.W. (2001) Development of a *ceu*E-based multiplex polymerase chain reaction (PCR) assay for direct detection and differentiation of *Campylobacter jejuni* and *Campylobacter coli* in Thailand. *Diag Microbiol Infect Dis* **40** 11-19

Huovvinen P. (1987) Trimethoprim resistance. *Antimicro Agents Chemo* **31** 1451-1465

Ida T., Okamoto R., Shimauchi C., Okubo T., Kuga A., Inoue M. (2001) Identification of aminoglycoside-modifying enzymes by susceptibility testing: epidemiology of methicillin-resistant *Staphylococcus aureus* in Japan. *J Clin Microbiol* **39** 3115-21

Jansson C., Sköld O. (1991) The appearance of a new trimethoprim resistance gene *dhfr*IX, in *E. coli* from swine. *Antimicrob Agents Chemother* **35** 1891-1899

Jansson C., Franklin A., Sköld O. (1992) Spread of a new trimethoprim resistance gene *dhfr*IX, among porcine isolates and human pathogens. *Antimicrob Agents Chemother* **36** 2704-2708

Jerse A.E., Yu J., Tall B.D., Kaper J. B. (1990) A genetic locus of Enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. *Proc Natl Acad Sci* **87** 7839-7843

Janke B.H., Francis D.H., Collins J.E., Libal M.C., Zemen D.H., Johnson D.D. (1989) Attaching and effacing *Escherichia coli* infections in calves, pigs, lambs and dogs. *J Vet Diagn* **1** 6-11

Jann K., Hoschutsky (1991) Nature and organisation of adhesions. *Curr Top Microbiol Immunol* **151** 55-85

Jenkin G.A., Tee W., (1998) *Campylobacter upsaliensis* – Associated diarrhoea in HIV infected patients. *Clin Infect Dis* **27** 816-821

Joffe D.J., Schlesinger D.P. (2002). Preliminary assessment of the risk of *Salmonella* infection in dogs fed raw chicken. *Can Vet J* **43** 441-442

Johansson A, Greko C, Engstrom BE, Karlsson M. (2004) Antimicrobial susceptibility of Swedish, Norwegian and Danish isolates of *Clostridium perfringens* from poultry, and distribution of tetracycline resistance genes. *Vet Microbiol* **19** 251-257

Johnson J.R., O'Bryan T.T., Low D.A., Ling G., Delavari P., Fasching C., Russo T.A., Carlino U., Stell A.L. (2000) Evidence of commonality between canine and human extraintestinal pathogenic *Escherichia coli* that express *papG* allele III. *Infect Immune* **68** 3327-3336

Johnson J.R., Stell A.L., Delavari P. (2001) Canine faeces as a reservoir of extraintestinal pathogenic *Escherichia coli. Infect Immunity* **69** 1306-1314

Jonas D., Biehler K., Hartung D., Spitzmüller B., Daschner F.D. (2005) Plasmid-Mediated quinolone resistance in isolates obtained in German intensive care units. *Antimicrob Agents Chemo* **49** 773-775

Jones Y.E., Chappell S., McLaren I.M., Davies R.H., Wray C. (2002) Antimicrobial resistance in *Salmonella* isolated from animals and their environment in England and Wales from 1988 to 1999. *Vet Rec* **150** 649-654

Jorgensen J.H. (1986) Laboratory and epidemiological experience with methicillin resistrant *Staphylococcus aureus* in the USA. *Eur J Clin Microbiol* **5** 693-696.

Jores J., Rumer L., Wieler L.H. (2004) Impact of the locus of enterocyte effacement pathogenicity island on the evolution of pathogenic *Escherichia coli. Int J Med Microbiol* **294** 103-113

Karmali M.A., Petric M., Lim C., Fleming P.C., Steele E.T. (1983) *Escherichia coli* cytotoxin, haemolytic-uraemic syndrome and haemorrhagic colitis. *Lancet* **2** 1299-1230

Kawaga J.K., Adesiyun A.A., Abdullahi S.U., Bello C.S. (1989) Prevalence of Salmonellae, shigellae and Plesiomonas shigelloides in dogs in Zaria, Nigeria. *Br Vet J* **145** 174-177

Keyes K., Hudson C., Maurer J.J., Thayers S., White D.J., Lee M.D. (2000) Detection of florfenicol resistance genes in *Escherichia coli* isolates from sick chickens. *Antimicrob Agents Chemother* **44** 421-424

Kim, M. N., Pai C.H., Woo J.H., Ryu J.S., Hiramatsuk K. (2000) Vancomycin-intermediate *Staphylococcus aureus* in Korea. *J Clin Microbiol* **38** 3879-81

King C.H., Shlaes D.M., Dul M.J. (1983) Infection caused by a thymidine-requiring, trimethoprim resistant bacteria. *J Clin Microbiol* **18** 79-83

Kliebe, C., Nies, B.A., Meyer, J.F., Tolxdorff-Neutzling, R.M., Wiedemann, B. (1985) Evolution of plasmid-coded resistance to broad spectrum cephalosporins. *Antimicrob Agents Chemo* **28** 302-307

Knutton S., Lloyd D.R., McNeish A.S. (1987) Plasmid-mediated factors conferring diffuse and localised adherence of enteropathogenic *Escherichia coli*. *Infect Immun* **55** 69-77

Koene M.G.J., Houwers D.J., Dijkstra J.R., Duim B., Wagenaar J.A. (2004) Simultaneous presence of multiple *Campylobacter* species in dogs. *J Clin Microbiol* **42** 819-821

Konowalchuk J., Speirs J.I., Stavric S. (1977) Vero response to a cytotoxin of *Escherichia coli Infect Immun* **18** 775-779

Kozak M., Horosova K., Lasanda V., Bilek J., Kyselova J. (2003) Do dogs and cats present a risk of transmission of Salmonella to humans? *Bratisl Lek Listy* **104** 323-328

Kreiswirth B., Kornblum J., Arbeit R.D., Eisner W., Maslow J.N., McGeer A (1993) Evidence for a clonal origin of methicillin resistance in *Staphylococcus aureus*. *Sci* **259** 227-230

Kruse H., Johansen B.K., Rorvik L.M., Schaller G. (1999) The use of avoparcin as a growth promoter and the occurrence of vancomycin-resistant enterococcus species in Norwegian poultry and swine production. *Microb Drug Resist* **5** 135-9

Kruth S.A., Prescott J.F., Welch M.K., Brodsky M.H. (1989) Nosocomial diarrhea associated with enterotoxigenic *Clostridium perfringens* infection in dogs. *J Am Vet Med Assoc* **195** 331-334

Labarca J.A., Sturgeon J., Borenstein L., Salem N., Harvey S.M., Lehnkering E., Reporter R., Mascola L. (2002) *Campylobacter upsaliensis*: Another pathogen for consideration in the United States. *CID* **34** 59-60

Labbé R.G. (2000) *Clostridium perfringens.* In Lund B., Baird-Parker T., Gould G. The Microbiological Safety and Quality of Food. Aspen Publishers MD, USA, 1110-1135.

Lance S.E., Miller G.Y., Hancock D.D., Bartlett P.C., Heider L.E. (1992) *Salmonella* infection in neonatal dairy calves. *JAVMA* **201** 864-868

Lanz R., Kuhnert P., Boerlin P. (2003) Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animal species in Spain. *Vet Microbiol* **91** 73-84

Layton M.C., Hierholzer W.J., Patterson J.E. (1995) The evolving epidemiology of MRSA at a university hospital. *Infect Cont Hosp Epidemiol* **16** 12-17

Leclercq R. (2002) Mechanisms of resistance to macrolides and lincosamides: Nature of the resistance elements and their clinical implications. *CID* **34** 482-492

Leclercq R., Derlot E., Duval J., Courvalin P. (1988). Plasmid mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. *N Engl JMed* **319** 157-161

Lee C., Langlois B.E., Dawson K.A. (1993) Detection of tetracycline resistance determinants in pig isolates from three herds with different histories of antimicrobial exposure. *Appl Environ Microbiol* **59** 1467-1472

Lee C.Y., Tai C.L., Lin S.C., Chen Y.T. (1994). Occurrence of plasmids and tetracycline resistance among *Campylobacter jejuni* and *Campylobacter coli* isolated from whole market chickens and clinical samples. *Int J Food Microbiol* **24** 161-70

Lee J.C., Oh J.Y., Cho J.W., Park J.C., Kim J.M., Seol S.Y., Cho DT. (2001) The prevalence of trimethoprim-resistance-conferring dihydrofolate reductase genes in urinary isolates of *Escherichia coli* in Korea. *Antimicro Chemother* **47** 599-604

Lee J.H. (2003) Methicillin (oxacillin)-resistance *Staphylococcus aureus* isolated from major food animals and their potential transmission to human. *Appl Environ Microbiol* **69** 6489-6494

Leflon-Guibout, V., Speldooren V., Heym B., Nicolas-Chanoine M. (2000) Epidemiological survey of amoxicillin-clavulanate resistance and corresponding molecular mechanisms in *Escherichia coli* isolates in France: new genetic features of bla(TEM) genes. *Antimicrob Agents Chemother* **44** 2709-14

Lemcke R., Bülte M. (2000) Occurrence of the vancomycin-resistance genes *vanA, vanB, vanC1, vanC2* and *vanC3* in *Enterococcus* strains isolated from poultry and pork. *Inter J Food Microbiol* **60** 185-194

Leung P.H., Yam W.C., Ng W.W., Peiris J.S. (2001) The prevalence and characteristaion of verotoxin-producing *Escherichia coli* isolated from cattle and pigs in an abattoir in Hong Kong *Epidemiol Infect* **126** 173-179

Levine M.M., Edelman R. (1984) Enteropathogenic *Escherichia coli* of classic serotypes associated with diarrhea: epidemiology and pathogenesis *Epidemiol Rev* **6** 31-51

Levine M.M. (1987) *Escherichia coli* that cause diarrhoea: Enterotoxigenic, Enteropathogenic, Enteroinvasive, Enterohemorrhagic and Enteroadherent. *J Infect Diagn* **155** 377-389

Levy S.B., McMurray L.M., Barbosa T.M., Burdett V., Courvalin P., Hillen W. (1999) Nomenclature for new tetracycline resistance determinants. *Antimicrob Agents Chemother* **43** 1523-4

Lindblom G.B., Sjogren E., Hansson-Westernberg J., Kaijser B. (1995) *Campylobacter upsaliensis, C. sputorum* and *C. concisus* as common causes of diarrhoe in Swedish Children [letter]. *Scand J infect Dis* **27** 187-188

Linden P., Pasculle A.W., Kramer D.J., Jusne S., Manez R. (1994). The duration of faecal carriage of vancomycin-resistant *Enterococcus faecium* (VREF). Abstract, 34th Interscience Conference on Antimicro Agents Chemother, Orlanda, Florida

Ling M.L., Goh K.T., Wang G.C.Y. Neo K.S., Chua T. (2002) An outbreak of multidrug resistant *Salmonella enterica* subsp. *Enterica* serotype Typhimurium DT104 linked to dried anchovy in Singapore. *Epidemiol Infect* **128** 1-5

Ling J.M., Koo I.C., Kam K.M., Cheng A.F. (1998) Antimicrobial susceptibilities and molecular epidemiology and *Salmonella enterica* serotype Enteritidis strains isolated in Hong Kong form 1986 to 1996. *J Clin Microbiol* **36** 1693-1699

Linton D., Owen R.J., Stanley J. (1996) Rapid identification by PCR of the genus *Campylobacter* and of five *Campylobacter* species enteropathogenic for man and animals. *Res Microbiol* **147** 707-718

Livermore D.M. (1995) β-lactamases in Laboratory and Clinical resistance. *Clin Micobiol Rev* **8** 557-584

Livermore DM, Stephens P, Weinberg J, Johnson AP, Gifford T, Northcott D, James D, George RC, Speller DC. (2000) Regional variation in ampicillin and trimethoprim resistance in *Escherichia coli* in England from 1990 to 1997, in relation to antibacterial prescribing. *J Antimicro Chemother* **46** 411-422

Lloyd D.H., Lamport A.I., Feeney D.J. (1996) Sensitivity to antibiotics amongst cutaneous and mucosal isolates of canine pathogenic *Staphylococci* in the UK, 1980-1996. *Vet Dermatology* 7 171-175

Lopez L., Castillo F.J., Clavel A., Rubio M.C. (1998) Use of selective a selective medium and a membrane filter method for isolation of campylobacter species from Spanish paediatric patients. *Eur J Clin Micro Infect Dis* **17** 489-492

López C.M., Giacoboni G., Agostini A., Cornero F.J., Tellechea D.M., Trinidad J.J. (2002) Thermotolerant Campylobacters in domestic animals in a defined population in Buenos Aires, Argentina. *Prev Vet Med* **55** 193-200

Lovseth, A., Loncarevic S., Berdal K.G. (2004) Modified multiplex PCR method for detection of pyrogenic exotoxin genes in *staphylococcal* isolates. *J Clin Microbiol* **42** 3869-72

Lyras D. and Rood, J.I., (1996). Genetic organization and distribution of tetracycline resistance determinants in *Clostridium perfringens*. *Antimicrob Agents Chemother* **40** 2500–2504

Ma D. Cook D.N., Hearst J.E., Nikaido H. (1994) Efflux pumps and drug resistance in gram negative bacteria *Trends Microbiol* **2** 489-493

Mackel D.C., Galton M.M., Gray H., Hardy A.V. (1952) Salmonellosis in dogs. Prevalence in normal dogs and their contacts. *J Infect Dis* **91** 15-18

Madigan M.T., Martinko J.M., Parker J. (1997) Brock Biology of Organisms. Eight Ed, Prentice Hall Inc.

Magnus C.A., Ingledew W.M., McCurdy A.R. (1986) Thermal resistance of Streptococcus isolated from pasteurised ham. *J Can Inst Food Sci Tech* **19** 62-67.

Mainil J.J., Janssen L., Charlier G., Jacquemin E., China B., Goffaux F. (2000) Pathogenic *Escherichia coli* strains from dogs and cats. Clinical and bacteriological data on enteropathogenic *Escherichia coli* strains. *Ann Méd Vét* **144** 335-343

Malik R., Love D.N. (1989) The isolation of *Campylobacter jejuni/coli* from pound dogs and canine patients in a veterinary hospital. *Aust Vet Practit* **19** 16-18

Mallon D.J.P., Corkill J.E., Hazel S.M., Wilson J.S., Hart C.A. (2002) Excretion of vancomycin-resistant enterococci by wild mammals. *Emerg Infect Dis* **8** 636-638

Manian F.A. (2003) Asymptomatic nasal carriage of Mupirocin-resistant methicillin-resistant *Staphylococcus aureus* (MRSA) in a pet dog associated with MRSA infection in household contacts. *Clin Infect Dis* **36** e26-e28

Marks M.L., Kather E.J., Kass P.H., Melli A.C. (2002) Genotypic and phenotypic characterisation of *Clostridium perfringens* and *Clostridium difficile* in diarrheic and healthy dogs. *J Vet Inter Med* **16** 533-540

Marks S.L., Kather E.J. (2003) antimicrobial susceptibilities of canine *Clostridium difficile* and *Clostridium perfringens* isolates to commonly ulitised antimicrobial drugs. *Vet Microbiol* **94** 39-45

Martinez-Martinez L., Pascual A., Jacoby G.A. (1998) Quinolone resistance from a transferable plasmid. *Lancet* **351** 208-215

Matthews D.A., Bolin J.T., Burridge J.M., Filman D.J., Volz K.W., Kraut J. (1987) Dihydrofolate reductase. The stereochemistry of inhibitor selectivity. *J Biol Chem* **260** 392-399

Mathewson J.J., Johnson P.C., DuPont H.L. (1986) Pathogenicity of enteroadherent *Escherichia coli* in adult volunteers. *J infect Dis* **154** 524-527

McClane B.A. (2001) The complex interactions between *Clostridium perfringens* enterotoxin and epithelial tight junctions. *Toxicon* **39** 1781-1792

McClelland RG, Pinder AC (1994) Detection of *Salmonella* Typhimurium in dairy products with flow cytometry and monoclonal antibodies. *Appl Environ Microbiol* **60** 4255-4262

McDonald C.F., Kuehnert M.J., Tenover F.J., Jarvis W.R. (1997) Vancomycin-resistant enterococci from nosocomial, community, and animal sources in the United States. *Emerg Infect Dis* **40** 2605-2609

McOrist S. and J.W. Browning (1982) Carriage of *Campylobacter jejuni* in healthy and diarrhoeic dogs and cats. *Aust Vet J* **58** 33-34

Mead P., Slutsker S.L., Dietz V., McCraig L.F. Bresee J.S., Shapiro C., Griffin P.M., Tauxe R.V. (1999) Food-related illness and death in the United States. *Emerg Infect Dis* **5** 607-625

Mermin J., How B., Angulo F.J. (1997) Iguanas and *Salmonella marina* infection in children: a reflection on increasing incidence of reptile associated salmonellosis in the United States. *Ped* **99** 399-402

Miller G.H., Sabatelli F.J., Naples L., Hare R.S., Shaw K.J. (1997) The most frequently occurring aminoglycoside resistance mechanisms - Combined results of surveys in eight regions of the world. *J Chemother* **7(supp 2)** 17-30

Mingeot-Leclercq M.P., Glupczynski Y., Tulkens P.M. (1999) Aminoglycosides: Activity and Resistance. *Antimicrob Agents Chemother* **43** 727-737

Minton N.P., Marsh J., Atkison T. (1983) The R-factors of multiple antibiotic resistant faecal coliforms isolated from a domestic dog. *J Appl Microbiol* **55** 445-452

Modolo J.R. and Giuffrida R. (2004) *Campylobacter upsaliensis* isolated from young dogs with and without diarrhoea. *Rev Soc Bras maed Trop* **37** 72-73

Moken, M. C., McMurry L.M., Levy S.B. (1997) Selection of multiple-antibiotic-resistant (mar) mutants of *Escherichia coli* by using the disinfectant pine oil: roles of the mar and acrAB loci. *Antimicrob Agents Chemother* **41** 2770-2

Monaghan C., Tierney U., Colleran E. (1981) Antibiotic resistance and R-factors in the faecal coliform flora of urban and rural dogs. *Antimicrob Agents Chemo* **19** 266-270

Montenegro M.A., Bulte M., Trumpf T., Aleksic A., Reuter G., Bulling E., Helmuth (1990) Detection and characterisation of faecal verotoxin-producing *Escherichia coli* from healthy cattle. *J Clin Microbiol* **31** 1531-1536

Moore J.E., McLernon P., Wareing D., Xu J., Murphy P.G. (2002) Characterisation of Fluoroquinolone resistant *Campylobacter* species isolated from human beings and chickens *Vet Rec* **150** 518-520

Moore P.C.L., Lindsay J.A. (2001) Genetic variation among hospital isolates of methicillin sensitive *Staphylococcus aureus*: Evidence for horizontal transfer of virulence genes. *J Clin Microbiol* **39** 2760-2767

Moreno G.S., Griffiths P.L., Connerton I.F., Park R.W.A. (1993) Occurrence of Campylobacters in small domestic and laboratory animals. *J App Bacteriol* **75** 49-54

Morosini M.I., Ayala J.A., Baquero F., Martinez J.L., Blazquez J. (2000) Biological cost of AmpC production for *Salmonella enterica* serotype Typhimurium. *Antimicrob Agents Chemother* **44** 3137-3143

Morrison D., Woodford N., Cookson B. (1997) Enterococci as emerging pathogens of humans. *J Appl Micro* **83** 89-99.

Moser I., Rieksneuwöhner Lentzsch P., Schwerk P., Wieler L.H. (2001) Genomic heterogeneity and O-antigen diversity of *Campylobacter upsaliensis* and *Campylobacter helveticus* strains isolated from dogs and cats in Germany. *J Clin Microbiol* **39** 2548-2557

Moss S., Frost A.J. (1984) The resistance to chemotherapeutic agents of *Escherichia coli* from domestic dogs and cats. *Aust Vet J* **61** 82-84

Moxely R.A., Francis D.H. (1986) Natural and experimental infection with an attaching and effacing strain of *Escherichia coli* in calves. *Infect Immun.* **53** 339-346

Nachamkin I. (2002) Chronic effects of *Campylobacter* infection. *Microb Infect* **4** 399-403

Nagai Y., Mitsuhashi S. (1972) New type of R factors incapable of inactivating chloramphenicol. *J Bacteriol* **109** 1-7

Nagy B., Fekete P.Z. (1999) Enterotoxigenic *Escherichia coli* (ETEC) in farmyard animals. *Vet Res* **30** 259-284

Nair G.B., Sarker R.K., Chowdhury S., Pal S.C. (1985) *Campylobacter* infection in the domestic dogs. *Vet Rec* **116** 237-238

Nakamura S. (1997) Mechanisms of quinolone resistance. *J Infect Chemother* **3** 128-138

Nakumura S.M., Nakumura T., Kojima T., Yoshida H. (1989) *GyrA* and *gyrB* mutations in quinolone resistant strains of *Escherichia coli*. *Antimicro Agents chemother* **33** 254-255

Nakazato G., Gyles C., Zieball K., Keller R., Trabulsi L.R., Gomes T.A.T., Irino K., Dias Da Silveira W., Prestana De Castro A.F. (2004). Attaching and effacing *Escherichia coli* isolated from dogs in Brazil: characteristic and serotypic relationship to human enteropathogenic *Escherichia coli* (EPEC). *Vet Microbiol* **101** 269-277

Nastasi A., Massenti M.F., Scarlata G., Mammina C., Calco C., Villafrate M.R. (1986). *Salmonella* and *Yersinia entercolitica* in soil and dog faeces. *Boll Ist Sieroter Milan* **65** 150-151

Nataro J.P., Baldini M.M., Kaper J.B., Black R.E., Bravo N., Levine M.M. (1985). Detection of an adherence factor of Enteropathogenic *Escherichia coli* with a DNA probe. *Infect Immun* **152** 560-565.

Nataro J.P., Kaper J.B. (1998). Diarrheogenic *Escherichia coli*. *Clin Microbiol Rev* **11** 142-201

National committee for Clinical Laboratory Standards (2000) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. NCCLS document M7-A5. National Committee for Clinical Laboratory Standards, Wayne P.A.

Neal K., Hebden R.J., Spiller R. (1997) Prevalence of gastrointestinal symptoms six months after bacterial gastroenteritis and risk factors for development of the irritable bowel syndrome: postal survey of patients. *BMJ* **314** 779-82

Neiger R., Lane E., Sutton K. (2002) The role of enteropathogenic *Escherichia coli* in dogs as a causative agent of gastrointestinal symptoms. Proceedings of 45th BSAVA conference, Birmingham.

Neimann J., Engberg J., Mølbak K., Wegener H.C. (2003) A case control study of risk factors for sporadic Campylobacter infections in Denmark. *Epidemiol Infect* **130** 353-336

Newton C.M., Newell D.G., Wood M., Baskerville M. (1988) *Campylobacter* infection in a closed breeding colony. *Vet Rec* **123** 152-154

Ng L.-K., Martin I., Alfa M., Mulvey M. (2001) Multiplex PCR for the detection of tetracycline resistance genes. *Mol Cell Probes* **15** 209-215

Noble, W.C., Virani, Z., Cree, R.G.A. (1992) Co-transfer of vancomycin to and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphlococcus aureus*. *FEMS Microbiol lett* **93** 195 – 198

Normand E.H.Gibson N.R., Taylor D.J., Carmicheal S., Reid S.W.J. (2000a) Trends of antimicrobial resistance in bacterial isolates from a small animal referral hospital. *Vet Rec* **146** 151-155

Normand[b] E.H., Gibson N.R., Reid S.W.J., Carmichael S., D.J.Taylor. (2000) Antimicrobial-resistance trends in bacterial isolates from companion-animal community practice in the UK. *Prev Vet Med* **46** 267-278

Normark S.T., Grundstrom S.T., Bergstrom S. (1980) Susceptibility to penicillins and cephalosporins in β-lactamase producing strains of *Escherichia coli* and relative amount of β-lactamse produced by these strains. *Scand J infect Dis* **25** 23-29

Okeke I.N., Nataro J.P. (2001) Enteroaggregative *Escherichia coli*. *Lancet Infect Dis* **1** 304-313

Okusu H., Ma D., Nikaido H. (1996) AcrAB efflux pumps plays a major role in the antibiotic resistance phenotype of *Escherichia coli* multiple antibiotic resistant (Mar) mutants. *J Bacteriol* **178** 306-308

Olson P., Hedhammer A., Faris A., Krovacek K., Wadstrom T. (1985) Enterotoxigenic *Escherichia coli* (ETEC) and *Klebsiella pneumoniae* isolated from dogs with diarrhoea. *Vet microbiol* **10** 577-589

Olson P., Sandstedt K. (1987) *Campylobacter* in the dog: a clinical and experimental study. *Vet Rec* **121** 99-101

Oram M., Fisher M. (1991) 4-quinolone resistant mutations in DNA gyrase of *Escherichia coli* clinical isolates identified by using PCR. *Antimicrob Agents Chemother* **35** 387-389

O'Rourke (2002) Methicillin-resistant infections worry researchers. *J Amer Vet Med Ass*

Orden J.A., Ruiz-Santa-Quiteria J.A., Cid D., Garcia S., Sanz R., de la Fuente (1998) Verotoxin-producing *E. coli* (VTEC) and *eae*-positive non-VTEC in 1-30-days-old diarrhoea calves. *Vet Microbiol* **63** 239-248

Orden, J.A., Ruiz-Santa-Quiteria, J.A., Cid, D., de la Fuente, R. (2000) Quinolone resistance found in bacteria of animal origin and implications on human health. *Res Ad in Antimicro Agents and Chemother* **1** 35 – 48

Pak S.I., Han H.R., Shimizu A. (1999) Characterisation of methicillin-resistant *Staphylococcus aureus* isolated from dogs on Korea. *J Vet Med Sci* **61** 1013-1018

Pan T.M., Wang T.K., Lee C.L. (1997) Food-borne disease outbreaks due to bacteria in Taiwan 1986-1995. *J Clin Micro Biol* **35** 1260-1262

Parada J., Baljer G., de Rycke J., Steinruck H., Zimmerman S., Stephan R., Beutin L. (1991) Characteristics of α-hemolytic strains of *Escherichia coli* isolated from dogs with gastroenteristis. *Vet Microbiol* **29** 59-73

Pasha, A.S., Tailor, P., Boyce, J., Cohen, M., Vender, R. (2001) MRSA diarrhoea: an important cause of diarrhoea in hospitalised patients. *Am J Gastro* **96** S161.

Paulsen I.T., Littlejohn T.G., Rådström P., Sundström L., Sköld O., Swedberg G., Skurray R.A. (1993) The 3' conserved segment of integrons contains a gene associated with multi-drug resistance to antiseptics and disinfectants. *Antimicrob Agents Chemother* **37** 761-768

Payne D.J., Woodford N., Amyes S.G.B. (1992) Characterisation of the plasmid mediated β-lactamase BIL-1 *J Antimicrob Chemother* **21** 119-127

Pellerin J.L., Bourdeau P., Sebbag H. (1998) Epidemiosurveillance of antimicrobial compound resistance of *Staphylococcus intermedius* clinical isolates from canine pyodermas. *Comp Immunol Micro Infect Dis* **21** 115-133

Perichon, B., Reynolds P., Courvalin P. (1997) VanD type glycopeptide resistant *Enterococcus faecium* BM4339. *Antimicrob Agents Chemother* **38** 163-169

Petit L., Gilbert M., Popoff M.R. (1999) *Clostridium perfringens*: toxinotype and genotype. *Trends Microbiol* **7** 104-110

Petrosino, J.F. and Palzkill, T. (1996) Systematic mutagenesis of the active site Omega Loop of the TEM – 1 β-lactamase. *J bacteriology* **178** 1821-1828

Philippon A., Arlet G., Jacoby G. (2002) Plasmid-determined AmpC-Type β-lactamases. **46** 1-11

Piddock L.J.V., Whale K., Wise R. (1990) Quinolone resistance in *Salmonella*: clinical experience. *Lancet* **335** 1459

Piddock L.J.V. (2002) Flouroquinolone resistance in *Salmonella* serovars isolated from humans and food animals. *FEMS Microbiol Rev* **26** 3-16

Pitout J.D.D., Thomson K.S., Hanson N.D., Ehrhardt A.F., Moland E.S., Sanders C.C. (1998) β-lactamases responsible for resistance to extended-spectrum cephalosprins in *Klebsiella pneumoniae, Escherichia coli* and *Proteus mirabilis* isolated recovered in South Africa. *Antimicrob Agents Chemother* **42**1350-1354

Pitout J.D., Reisbig M.D., Mulvey M., Chui L., Louie M., Crowe L., Church D.L., Elsayed S., Gregson D., Ahmed R., Tilley P., Hanson N.D. (2003) Association between handling of pet treats and infection with *Salmonella enterica* serotype Newport expressing the AmpC β-lactamase, CMY-2. *J Clin Microbiol* **41** 4578-4582

Poirel L., Le Thomas I., Naas T., Karim A., Nordmann P. (2000). Biochemical sequence analyses of GES-1, a novel class A extended-spectrum β-lactamase, and class 1 integron In52 from *Klebsiella pneumoniae. Antimicrob Agents Chemother* **44** 622-632

Ploy C.M., Courvalin P., Lambert T. (1998) Characterisation of In40 of *Enterobacter aerogenes* BM2688, a class 1 integron with two novel gene cassettes *cmlA2* and *qacF*. *Antimicrob Agents Chemother* **42** 2557-2563

Pomposiello P.J., Demple B. (2000) Indentification of SoxS-regulated genes in *Salmonella* enterica serovar Typhimurium. *J Bacteriol* **179** 1857-1866

Power E.G., Abdulla Y.H., Talsania H.G., Spice W., Aathithan S., French G.L. (1995) VanA genes in vancomycin-resistant clinical isolates of *Oerskovia turbata* and *Arcanbacterium (Corynebacterium) haemolyticum*. *J Antimicrob Chemother* **36** 595-606

Prestcott J.F., Barker I.R., Manninen K.I., Miniats O.P. (1981) *Campylobacter jejuni* colitis in gnotobiotic dogs. *Can J Camp Med* **45** 377-383

Preston M.A., Simor A.E., Walmsley S.L. (1990) In vitro susceptibility of *Campylobacter upsaliensis* to twenty-four antimicrobial agents. *Eur J Clin Microbiol Infect Dis* **9** 822-824

Pritchett S. J. (1991) Enterotoxaemia in dogs. *Vet Rec* **129** 391

Quabdesselam S.P., Hooper D.C., Tankovic J., Soussy C.J. (1995) Detection of *gyrA* and *gyrB* mutations in quinolone resistant isolates of *Escherichia coli* by single strand conformation polymorphism analysis and determination of levels of resistance conferred by two different single *gyrA* mutations. *Antimicrob Agents Chemother* **39** 1667-1670

Randall L.P. Woodward M.J. (2002) The multiple antibiotic resistance (mar) locus and its significance. *Res Vet Sci* **72** 87-93

Rappelli P., Maddau G., Mannu F., Colombo M.M., Fiori P.L., Cappuccinelli P. (2001) Development of a set of multiplex PCR assays for the simultaneous identification of Enterotoxigenic, Enteropathogenic, Enterohemorrhagic and Enteroinvasive *Escherichia coli. Microbiologica* **24** 77-83

Reacher M., ShahA., Livermore D.M. (2000) Determination of trends in bacteraemia and antibiotic resistance of its pathogens reported in England and Wales between 1990 and 1998. *Br Med J* **320** 213-216

Ribot E., Fitzgerald C., Kubota K., Swaminathan B., Barrett T.J. (2001) Rapid Pulsed-Field Gel Electrophoresis protocol for subtyping of *Campylobacter jejuni. J Clin Microbiol* **39** 1889-1894

Rice, L.B. and Carias, L.L. (1998) Transfer of Tn5385, a composite multi-resistance chromosomal element from *Enterococcus faecalis. J Bacteriol* **180** 714-721

Rich M., and Roberts L. (2004) Methicillin-resistant *Staphylococcus aureus* isolates from companion animals. *Vet Rec* **6** 2004

Ridley A., Threlfall E.J. (1998) Molecular epidemiology of antibiotic resistance genes in multiresistant epidemic *Salmonella* Typhimurium DT104. *Microb Drug Resist* **4** 113-8

Roberts M.C. (1996) Tetracycline resistance determinants: mechanisms, transfer and clinical significance. *Clinical Microbiology Reviews* **5** 387-99

Roberts M.C., Stutcliffe J., Courualin P., Jensen L.B., Rood J., Seppala H. (1999) Nomenclature for macrolide-lincosamide-strepogramin B resistance determinants. *Antimicro Agents Chemother* **43** 2823-2830

Robredo B., Singh K.V., Baquero F., Murray B.E., Torres C. (2000) Vancomycin resistant enterococci isolated from animals and food. *Inter J food Microbiol* **54** 197-204

Rood J.I., Cole S.T. (1991) Molecular genetics and pathogenesis of *Clostridium perfringens. Microbiol Rev* **55** 621-648

Rubens C.E., Ncneill W.F., Farrar W.E. (1979) Transposable plasmid deoxyribonucleic acid sequence in *Pseudomonas aeruginosa* which mediates resistance to gentamicin and four other antimicrobial agents. *J Bacteriol* **139** 877-882

Sackey B.A., Mensah P., Collison E., Sakyi-Dawson (2001) *Campylobacter, Salmonella, Shigella* and *Escherichia coli* in live and dressed poultry from the metropolitan area. *Inter J Food Microbiol* **71** 21-28

Saenz Y., Zarazaga M., Lantero M., Gastanares M.J., Baquero F., Torres C. (2000) Antibiotic resistance in *Campylobacter* strains isolated from animals, foods, and humans in Spain in 1997-1998. *Antimicrob Agents Chemother* **44** 267-71

Saenz Y., Zarazaga M., Brinas L., Ruiz-Larrea F., Torres C. (2003) Mutations in *gyrA* and *parC* genes in nalidixic acid-resistant *Escherichia coli* strains from food products, humans and animals. *J Antimicrob Chemother* **51** 1001-1004

Sahm D.F., Free L., Handwerger S. (1995) Inducible and constitutive expression of *vanC-1* encoded resistance in *Enterococcus gallinarum*. *Antimicob Agents Chemother* **39** 1480 – 1484.

Salfield N.J., Pugh E.J., (1987) *Campylobacter* enteritis in young children living in households with puppies. *Br Med J* **294** 21-22

Sancak A.A, Rutgers H.C., Hart C.A., Batt R.M. (2004) Prevalence of enteropathogenic *Escherichia coli* in dogs with acute and chronic diarrhoea. *Vet Rec* **154** 101-106

Sanchez S., McCrackin Steveson M.A., Hudson C.R., Maier M., Buffington T., Dam Q., Maurer J.J. (2002) Characterisation of multi-drug resistant *Escherichia coli* isolates associated with nosocomial infections in dogs. *J Clin Microbiol* **40** 3586-3595

Sandberg M., Bergsjø B., Hofshagen M., Skjerve E., Kruse H. (2002) Risk factors for *Campylobacter* infection in Norwegian dogs *Prev Vet Med* **55** 241-253

Sandstedt K., Ursing J., Walder M. (1983) Thermotolerant *Campylobacter* with no or weak catalase activity isolated from dogs. *Curr Microbiol* **4** 209-213

Sanders C.C. Sanders W.E. Jr. (1992) β-lactam resistance in gram-negative bacteria: global trends and clinical impact. *Clin Infect Dis* **15** 824-39

Saravolatz L.D., Pohlod D.J., Arking L.M. (1982) Community-acquired methicillin resistant *Staphylococcus aureus* infections: a new source for nosocomial outbreaks. *Ann Intern Med* **97** 325-329

Sarker M.R., Shivers R.P., Sparks S.G., Juneja V.K., McClane B.A. (2000) Comparative experiments to examine the effects of heating on vegetative cells and spores of *Clostridium perfringens* isolates carrying plasmid genes versus chromosomal enterotoxin genes. *Appl Environ Microbiol* **66** 3234-40

Saski J., Goryo M., Asahina M., Makara M., Shishido S., Okada K. (1999) Hemorrhagic enteritis associated with *Clostridium perfringens* in a dog. *J Vet Med Sci* **61** 175-177

Sasaki Y., Yamamoto, K., Tamura, Y. and Takahashi, T., 2001. Tetracycline-resistance genes of *Clostridium perfringens*, *Clostridium septicum* and *Clostridium sordellii* isolated from cattle affected with malignant edema. *Vet Microbiol* **83** 61–69

Sato Y., Kuwamoto R. (1992) A case of Salmonellosis due to *Salmonella* infantis. *Epidemiol Infect* **108** 59-65

Sato Y., Mori T., Koyama T., Nagase H. (2000) *Salmonella* Virchow infection in an infant transmitted by household dogs. *J Vet Med Sci* **62** 767-769

Schaberg D.R., Culver D.H., Gaynes R.P. (1991) Major trends in the microbial etiology of nosocomial infection. *Am J Med* **91** 72S-75S

Schouten M.A., Voss A., Hoogkamp-Korstanje J.A. (1999) Antimicrobial susceptibility patterns of enterococci causing infections in Europe. The European VRE Study group. *Antimicrob Agents Chemo* **43** 2542-2546

Schmidt H. Knop C., Franke S., Aleksic S., heesemann J., Karch H. (1995) Development of PCR for screening of enteroaggregative *Escherichia coli*. *J Clin Microbiol* **33** 701-705

Schnapping D. and Hillen W. (1996) Tetracyclines: antibiotic action, uptake and resistance mechanisms. *Arch Microbiol* **165** 359-369

Scotland S.M., Smith H.R., Willshaw G.A. and Rowe B. (1983) Vero-cytotoxin production in strains of Escherichia coli is determined by genes carried on bacteriophage. *Lancet* **ii** 885-886

Seepersadsingh N., Adesiyun A.A., Seebaransingh R. (2004) Prevalence and antimicrobial resisatnce of *Salmonella* spp. in non-diarrhoeic dogs in Trinidad. *J Vet Med* **B51** 337-342

Seguin J.C., Wallon R.D., Caron J.P., Kloos W.E., George C.G., Hollis R.J., Jones R.W., Pfaller M.A. (1999) Methicillin-resistant *Staphylococcus aureus* outbreak in a veterinary teaching hospital: potential human-animal transmission. *J Clin Microbiol* **37** 1459-1463

Seone A.S., Levy B. (1995) Characterisation of marA, the repressor of the multiple antibiotic resistance (mar) operon in *Escherichia coli*. *J Bacteriol* **177** 3414-3417

Service, R.F. (1995) Antibiotics that resist resistance. *Sci* **270** 724-727

Shimizu A., Fujita M., Igarashi H., Takagi M., Nagase N., Sasaki A., Kawano J. (2000) Characterisation of *Staphylococcus aureus* Coagulase Type VII isolates from Staphylococcal food poisoning outbreaks (1980-1995) in Tokyo, Japan, by PFGL. *J Clin Microbiol* **38** 3746-3749

Sethi, D., Wheeler J.G., Cowden J.M., Rodrignes L.G., Sockett P.W., Roberts J.A., Cumberland P., Tompkins D.S., Wall P.G., Hudson M.J., Roderick P.J. (1999) A study of infectious intestinal disease in England: plan and methods of data collection. *Commun Dis Pub Health* **2** 101-7

Shaw K.J., Rather P.N., Hare R.S., Miller G.H. (1993) Molecular genetics of aminoglycoside resistance genes and the familial relationships of the aminoglycoside-modifying enzymes. *Microbiol Rev* **57** 138-163

Shimi A., Barin A. (1977) Salmonella infection in dogs, cats, tortoises and terrapins *J Comp Pathol* **87** 315-318

Shimizu A.Y., Wakita Y., Nagase S., Okake M., Koji T., Hayashi T., Nagase N., Sasaki A., Kawano J., Yamashita K., Takagi M. (2001) Antimicrobial susceptibility of *Staphylococcus intermedius* isolated from healthy and diseased dogs. *J Vet Med Sci* **63** 357-60

Silverman J., Thal L.A., Perri M.B., Bostic G., Zervos M.J. (1998) Epidemiological evaluation of antimicrobial resistance in community-acquired enterococci. *J Clin Microbiol* **36** 830-831

Sjögren E.E., Lindblom G.B., Kaijser B. (1993) Rapid development of resistance to quinolones in *Campylobacter* in Sweden. *Acta Gastro-Enterologica Belgica* **46** supp 10

Skirrow MB (1977) Campylobacterenteritis: A new disease. *BMJ* **I** 9-11

Skirrow M.B. (1994) Diseases due to *Campylobacter, Helicobacter* and other related bacteria. *J Comp Pathol* **111** 113-149

Sköld O. (2002) Resistance to trimethoprim and sulfonamides. *Vet Res* **32** 261-273

Sloan J., McMurry L.M., Lyras D., Levy S.B., Rood J.I. (1994). The *Clostridium perfringens* Tet P determinant comprises two overlapping genes: *tetA*(P), which mediates active tetracycline efflux, and *tetB*(P), which is related to the ribosomal protection family of tetracycline-resistance determinants. *Mol Microbiol* **11** 403-415

Smith H.R., Cheasty T., Rowe B. (1997) Enteroaggregative *Escherichia coli* and outbreaks of gastroenteritis in the UK. *Lancet* **350** 814-815

Smith, K. A., Kruth S., Hammermueller J., Gyles C., Wilson J.B. (1998) A case-control study of verocytotoxigenic *Escherichia coli* infection in cats with diarrhoea. *Can J Vet Res* **62** 87-92

Spain C.V. Scarlett J.M., Wade S.E., McDonough P. Prevalence of enteric zoonotic agents in cats less than one year old in central New York State. *J Intern Med* **15** 33-38

Soussy C.J., Wolfson J.S., Ng E.Y., Cooper D.C. (1993) Limitations of plasmid complementation test for the determination of quinolone resistance due to changes in the gyrase A protein and identification of conditional quinolone resistance locus. *Anti Microbiol Agents Chemother* **37** 2588-2592

Stark R.L., Duncan C.L. (1971) Biological characteristics of *Clostridium perfringens* type A enterotoxin. *Infect Immun* **4** 89-96

Starčič M., Johnson J.R., Stell A.L., van der Goot J., Hendriks H., van Vorstenbosch C., van Dijk L., Gaastra W. (2002) Haemolytic *Escherichia coli* isolated from dogs with diarrhoea have characteristics of both uropathogenic and necrotoxigenic strains. *Vet Microbiol* **85** 361-377

Steinhauserova I., Fojitkova K., Klimes J. (2000) The incidence and PCR detection of *Campylobacter upsaliensis* in cats and dogs. *Lett Appl Micro* **31** 209-212

Struble A.L., Tang Y.J., Kass P.H., Gumerlock P.H., Madewel B.R., Silva J.J.R. (1994) Feacal shedding of *Clostridium difficile* in dogs: a period prevalence survey in a veterinary medical teaching hospital. *J Vet Diagn Invest* **6** 342-347

Sundström L., Jansson C., Bremer K., Heikkilä E., Olsson-Liljequist B., Sköld O. (1995) A new *dhfr*VIII trimethoprim resistance gene, flanked by IS*26* whose product is remote from any other dihydrofolate reductases in parsimony analysis. *Gene* **154** 7-14

Swartz M.N. (2002) human diseases caused by foodborne pathogens of animal origin. *Clin Infect dis* **34** S111-122

Tan J. (1997) Human zoonotic infections transmitted by cats and dogs. *Arch Inter Med* **157** 1933-1943

Taylor D.E., Chang R.S., Sherburne R., Mueller L. (1986). Incidence of antibiotic resistance and characterisation of plasmids in *Campylobacter jejuni* strains isolated from clinical sources in Alberto, Canada. *Can J Microbiol* **32** 28-32

Taylor D.E., Hiratsuka K., Ray H., Manavathu K.E., (1987) Characterisation and expression of a cloned tetracycline resistance determinant in *Campylobacter jejuni* plasmid pUA466. *J Bacteriol* **20** 391-407

Tenkate T.D., Stafford R.J. (2001) Risk factors for Campylobacter infection in infants and young children: a match case control study. *Epidemiol Infect* **127** 399-404

Tenover F.C., Arbiet R.D., Goering R.V., Mickelsen P.A., Murray B.E., Persing D.H., Swaminathan B. (1995) Interpreting chromosomal DNA restriction patterns produced by Pulsed Field Gel Electrophoresis. *J Clin Microbiol* **33** 2233-2239

Teshager T., Domínguez L., Moreno M.A., Saénz Y., Zarazaga M., Torres C. (2000) Isolation of an SHV-12 β-lactamase-producing *Escherichia coli* strain from a dog with recurrent urinary tract infections. *Antimicrob Agents Chemother* **44** 3483-4

Teuber M. (2001) Veterinary use and antibiotic resistance. *Curr Opin Microbiol* **4** 493-499

Teuber M., Meile L., Schwarz F. (1999) Acquired antibiotic resistance in lactic acid bacteria from food. *Antonie van Leeuwenhoek* **76** 115-137

Threlfall E.J., Rowe B., Ward L.R. (1993) A comparison of multiple drug resistance in Salmonellas from humans and food animals in England and Wales, 1981 and 1990. *Epidemiol infect* **111** 189-197

Threlfall, E.J., Frost, J.A., Ward, L.R., Rowe, B. (1994) Epidemic in cattle and humans of *Salmonella* Typhimurium DT104 with chromosomally integrated multiple drug resistance. *Vet Rec* 134 557

Threlfall E.J., Frost J.A., Rowe B. (1999) Fluoroquinolone resistance in Salmonellas and Campylobacters from humans. *BMJ* **318** 943-4

Threlfall E. J., (2000) Epidemic *Salmonella* Typhimurium DT 104 – a truly international multi-resistant clone. *J Antimicrob Chemother* **46** 7-10

Threlfall E.J., Ward L.R., Skinner J.A., Graham A. (2000) Antimicrobial drug resistance in non-typhodial salmonellas from humans in England and Wales in 1999: decrease in multiple resistance in *Salmonella enterica* serotypes Typimurium, Virchow and Hadar *Microb Drug Resist* **6** 319-325

Threlfall E.J., Ward L.R., Frost J.A., Willshaw G.A. (2000) Spread of resistance from food animals to man- the UK experience. *Acta Vet Scand Suppl* **93** 63-68

Threlfall E.J (2002) Antimicrobial drug resistance in zoonotic Salmonella from cases of human infection: current trends. Surveillance for antimicrobial resistance in domestic livestock and the risk to Public Health, Meeting, Warwickshire.

Thwaites R. T., Frost J.A. (1999) Drug resistance in *Campylobacter jejuni, Campylobacter coli,* and *Campylobacter lari* isolated from humans in north-west England and Wales, 1997. *J Clin Pathol* **52** 812-4

Tiemersma W., Bronzwaer S.L.A.M., Lyytikäinen O., Degener J.E., Schrijnemakers P., Bruinsma N., Monen J., Witte W., Grundmann H. and European Antimicrobial Resistance Surveillance System Participants (2004) Methicillin-resistant *Staphylococcus aureus* in Europe, 1999–2002. *Emerg Infect Dis* **10** 1627-1634

Trabulsi L.R., Keller R., Tradelli Gomes T.A. (2002) Typical and Atypical Enteropathogenic *Escherichia coli. Emerg Infect Dis* **8** 508-513

Trakulsomboon S., Danchaivijitr S., Rongrungruany Y., Dhiraputra C., Susaemgrat W., Ito T., Hiramatsu K. (2001) First report of methicillin-resistant *Staphylococcus aureus* with reduced susceptibility to vancomycin in Thailand. *J Clin Microbiol* **39** 591-5

Tran J.H., Jacoby G.A. (2002) Mechanism of plasmid-mediated quinolone resistance. *Proc Natl Acad Sci, USA*. **99** 5638-5642

Trevena W.B., Hooper R.S., Wray C., Willshaw G.A., Cheasty T., Domingue G. (1996) Vero cytotoxin-producing *Escherichia coli* O157 associated with companion animals. *Vet Rec (Letter)* 400

Trevena W.B., Willshaw G.A., Cheasty T., Domingue G., Wray C. (1999) Transmission of Verocyctotoxin producing *Escherichia coli* O157 infection from farm animals to humans in Cornwall and West Devon. *Comm Dis Pub Health* **2** 263-268

Trieber C.A., Taylor D.E. (2001) 23S rRNA mutations and macrolide resistance in *Campylobacter*. *Int J Med Microbiol* **291** 295.

Tritton, T.R. (1977) Ribosome-tetracycline interactions. *Biochemistry* **16** 4133-4138

Trott D.J., Filippich L.J., Bensink J.C., Downs M.T., McKenzie S.E., Townsend K.M., Moss SmM., Chin J.J.-C. (2004) Canine model for investigating the impact of oral enrofloxacin on commensal coliforms and colonisation with multi-drug resistant *Escherichia coli*. *J Med Micro* **53** 439-443

Trzcinski K., Cooper B.S., Hryniewicz W., Dowson C.G. (1995). Expression of resistance to tetracyclines in strains of methicillin-resistant *Staphylococcus aureus*. *J Antimicro Chemo* **45** 763-770

Tschirdewahn B., Notermans S., Wernars K. (1991) The presence of enterotoxigenic *Clostridium perfringens* strains in faeces of various animals. *Int J Food Microbiol* **14** 175-178

Turk J., Fales W., Miller M., Pace L., Fischer J., Johnson G., Kreeger J., *Turnquist S., Pittman L., Rottinghaus A., Gosser H. (1992)* Enteric *Clostridium perfringens* in infection associated with parvoviral enteritis in dogs: 74 cases (1987-1990) *JAVMA* **200** 991-994

Turk J., Maddox C., Fales W., Ostlund E., Miller M., Johnson G., Pace L., Turnquist S., Kreeger J. (1998) Examination for heat-labile, heat stable and shiga-like toxins and for the *eaeA* gene in *Escherichia coli* isolates obtained from dogs dying with diarrhoea: 122 cases (1992-1996). *J Am Med Assoc* **212** 1735-1736

Twedt D.C. (1993) *Clostridium perfringens* associated diarrhoea in dogs. *Amer Coll Vet Sci 11th Annu Forum, Washington DC* 121-125

Ubukata K., Yamashita N., Gotoh A., Konno M. (1984) Purification and characterization of aminoglycoside-modifying enzymes from *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Antimicrob Agents Chemother* **25** 754-759

Uhaa I.J., Hird D.W., Hirsh D.C., Jang S.S., (1998) Case-control study of risk factors associated with nosocomial *Salmonella* Krefeld infection in dogs. *Am J Vet Res* **49** 1501-1505

Urban J.E., Broce A., (1998) Flies and their bacterial loads in Greyhound dog kennels in Kansas. *Curr Microbiol* **36** 164-170

Uttley A.H.C., Collins C.H., Naidoo J., George R.C. (1988) Vancomycin resistant enterococci. *Lancet* **I** 57-58

Vacher S.; Ménard A.; Bernard E.; Mégraud F. (2003) PCR-restriction fragment length polymorphism analysis for detection of point mutations associated with macrolide resistance in *Campylobacter* spp. *Antimicrob Agents Chemo* **47** 1125-1128

Van Belkum A., van den Braak N., Thomassen R. (1996) Vancomycin-resistant Enterococci in cats and dogs. *Lancet* **350** 1038-1039

Van den Bogaard A.E., Stobberingh E.E. (1999) Antibiotic use in animals: impact on bacterial resistance and public health. *Drugs* **58** 589-607

Van den Bogaard A.E., Stobberingh E.E. (2000) Epidemiology of resistance to antibiotics. Links between humans and animals. *Inter J Antimicrob Agents* **14** 327-335

Van der Auwera P., Pensart N., Korten V. (1996) Influence of oral glycopeptides on the faecal flora of human volunteers: selection of highly glycopeptide-resistant enterococci. *J Infect Dis* **173** 1129-1136

Van Duijkeren E., Box A.T.A., Mulder J. (2003) Methicillin resistant *Staphylococcus aureus* (MRSA) infection in a dog in the Neatherlands. *Tijdschrift voor Diergeneeskunde* **128** 314-315

Van Immerseel F., Pasmans F., De Buck J., Rychilk I., Hrsdecka H., Collard J.-M., Wildemauwe C., Heyndrickx M., Ducatelle R., Haesebrouck F. (2004) Cats as a risk for transmission of antimicrobial drug-resistant *Salmonella*. *Emerg Infect Dis* **10** 2169-2174

Van Looveren M., Daube G., De Zutter L., Dumont J.M., Lammens C., Wijdooghe M., Vandamme D., Jouret M., Cornelis M., Goosens H. (2001) Antimicrobial susceptibilities of *Campylobacter* strains isolated from food animals in Belgium. *J Antimicro Chemo* **48** 235-240

Vassort-Bruneau C., Lesage-Descauses M.C., Martel J.L., Lafont J.P., Chaslus-Dancla E. (1996) CAT III chloramphenicol resistance in *Pasteurella haemolytica* and *Pasteurella multocida* isolated from claves. *J Antimicrob Chemother* **38** 205-213

Vial P.A., Robins-Browne R., Loir H. *et al.* (1999) Characterisation of enteroadherent-aggregative *Escherichia coli*, a putative agent of diarrhoeal disease. *J Infect Dis* **158** 70-79

Vila J., Ruiz J., Goni P., Jimenez de Anta M.T., (1996) Detection of mutations in *parC* in quinolone resistant clinical isolates of *Escherichia coli*. *Antimicrob Agents Chemother* **40** 491-493

Wagenvoort J.H., Burgers D.M., Wagenvoort T.H., Burgers E. (2003) Absence of vancomycin-resistant enterococci (VRE) in companion dogs in the conurbation of Parkstad Limburg, The Netherlands. *J Antimicrob Chemother* **52** 532

Walker R.A., Lawson A.J., Lindsay E.A., Ward L.R., Wright P.A., Bolton F.J., Wareing D.R., Corkish J.D., Davies R.H., Threlfall E.J. (2000) Decreased susceptibility to ciprofloxacin in out-break multi-resistant *Salmonella* Typimurium DT104 *Vet Rec* **147** 395-396

Wall P.G., Morgan D., Lamden K., Driffen M., Threlfall L.R., Ward L.R., Rowe B. (1995) Transmission of multi-drug resistant strains of *Salmonella* Typhimurium from cattle to man. *Vet Rec* **136** 591-592

Wareham D.W. and Wilson P. (2002) Chloramphenicol in the 21st century. *Hosp Med* **63** 157-161

Wasteson Y., Olsvik O., Skancke E., Bopp C.A., Fossum K. (1988) Heat-stable-enterotoxin- producing *Escherichia coli* strains isolated from dogs. *J Clin Microbiol* **26** 2564-2566

Webb V, and Davies J (1993) Antibiotic preparations contain DNA: a source of drug resistance genes? *Antimicrob Agents Chemo* **37** 2379-2384.

Webber A., Wachowitz R., Weigle U., Schafer-Schmidt R. (1995) Occurrence of *Salmonella* in faecal samples of dogs and cats in Northern Bavaria from 1975-1994. *Berl Munch Tierarztl Wochenschr* **108** 401-405

Wegener H.C., Madsen M., Nielsen N., Aarestrup F.M. (1997) Isolation of vancomycin resistant Enterococcus faecium from food. *Inter J Food Microbiol* **35** 57-66

Weese[a] J.S., Greenwood S.J., Staempfli H.R., (2001) Recurrent diarrhoea associated with enterotoxigenic *Clostridium pefringens* in 2 dogs. *Can Vet J* **42** 292-294

Weese[b] J.S., Staempfli H.R., Prescott J.F., Kruth S.A., Greenwood S.J., Weese H.E. (2001) The roles of *Clostridium difficile* and enterotoxigenic *Clostridium perfringens* in diarrhoea in dogs. *J Vet Intern Med* **15** 374-378

Weill F-X., Demartin M., Fabre L., Grimont A.D. (2004) Extended-spectrum-β-lactamse (TEM-52) producing strains of *Salmonella* isolated in France. *J Clin Microbiol* **42** 3359-3362

Weisblum B. (1995) Erythromycin resistance by ribosomal modification. *Anti Micro Agents Chemo* **39** 577-585

Werber D., Fruth A., Buchholz U., Prager R., Krammer M.H., Ammon A., Tschape H. (2003) Strong association between Shiga toxin-producing *Escherichia coli* O157 and virulence genes *stx2* and *eae* as possible explanation for predominance of serogroup O157 in patients with haemolytic uraemic syndrome. *Eur J Clin Microbiol Infect Dis* **22** 726-730

White D.G., Goldman J.D., Demple B., Levy S.B. (1997) Role of the *acrAB* locus in organic solvent tolerance mediated by expression of *MarA, Sox** or *robA* in *Escherichia coli. J Bacteriol* **179** 6122-6126

White D.G., Zhao S., Simjee S., Wagner D.D., McDermott P.F. (2002) Antimicrobial resistance of foodborne pathogens. *Micro infect.* **4** 405-412

Wielders C.L.C., Vriens M.R., Brisse S., de Graaf-Miltenburg L.A.M., Troelstra A., Fleer A., Schmitz F.J., Verhoef J., Fluit A.C. (2001) Evidence for in-vivo transfer of *mecA* DNA between strains of *Staphylococcus aureus. Lancet* **357** 1674-1675

Willems R.J.L., Top J., van den Braak N. et al., (2000). Host specificity of vancomycin-resistant *Enterococcus faecium. J Infect Dis* **182** 816-823

Williams S., Forbes J.F., McIlwaine and Rosenberg K. (1987) *Campylobacter* enteritis in young children living in households with puppies. *BMJ* **294** 21-22

Williams R.A.D., Lambert P.A., Singleton P. (1996) Antimicrobial drug action. Bios Scientific Publishing ISNB 1 872748 81 3

Willis C. (2001) Isolation of *Salmonella* species from imported dog chews. *Vet Rec*

Winokur P.L., Vonstein D.L., Hoffman L.J., Uhlenhopp E.K., Doern G.V. (2001) Evidence for transfer of CMY-2 AmpC beta-lactamase plasmids between *Escherichia coli* and *Salmonella* isolates from food animals and humans. *Antimicrob Agents Chemother* **45** 2716-2722

Wissing A., Nicolet J., Boerlin P. (2001) Antimicrobial resistance situation in Swiss veterinary medicine. *Schweizer Archiv fur Tierheilkunde* **143** 503-510

Woldehiwet Z., Jones J.J., Tennant B.J., Jones D.M. (1990) Serotypes of *Campylobacter jejuni* and *Campylobacter coli* isolates from dogs. *JSAP* **31** 382-384

Wolf K.W. (1997) Occurrence, distribution and associations of O and H-serogroups, colonisation factor antigens, and toxins of enterotoxigenic *Escherichia coli. Clin Microbiol Rev* **10** 569-584

Wittle W. (1999) Antibiotic resistance in gram-positive bacteria: epidemiological aspects. *J Antimicrob Chemother* **44** Suppl A, 1-9

Yang H., Chen S., White D.G., Zhao S., McDermott P., Walker R., Meng J. (2004) Characterization of multiple-antimicrobial-resistant *Escherichia coli* isolates from diseased chickens and swine in China. *J Clin Microbiol* **42** 3483-3489

Yomagishi J.H., Yoshida M., Nakamura S. (1986) Nalidixic acid resistant mutations of the *gyrB* gene of *Escherichia coli. Mol Gen Genet* **204** 367-373

Yu H.S., Lee J.C., Kang H.Y., Ro D.W., Chung J.Y., Jeong Y.S., Tae S.H., Choi C.H., Lee E.Y., Seol S.Y., Lee Y.C., Cho D.T. (2003) Changes in gene cassettes of class 1 integrons among *Escherichia coli* isolates from urine specimens collected in Korea during the last two decades. *J Clin Microbiol* **41** 5429-33

Zentek J., Marquart B., Pietrzak T., Ballevre O., Rochat F. (2003) Dietary effects on *Bifidobacteria* and *Clostridium perfringens* in the canine intestinal tract. *J Anim Physiol Anim Nutr (Berl)* **87** 397-407

Zhang, X.H., Wang Y., Hu X., Xie Y., Chen G., Zhou J., Wu S., Xu S., Wang Q., Du X (1997) Susceptibility of 5374 isolates to 18 antibiotics from 5 hospitals in Beijing. *Natl Med J China* 77 543-544

Abbreviations

BK	boarding kennel
SAH	small animal hospital
AB	antibiotics
MDR	multi-drug resistant
MRSA	methicillin-resistant *Staphylococcus aureus*
VRE	vancomycin resistant enterococci
DHFR	dihydrofolate reductase
EHEC	enterohaemorrhagic *E. coli*
VTEC	verocytotoxic *E. coli*
EPEC	enteropathogenic *E. coli*
ETEC	enterotoxigenic *E. coli*
EAEC	enteroaggregative *E. coli*
EMBA	eosin Methylene Blue agar
CA	cefoperazone, amphotericin
MIC	minimum inhibitory concentration
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism
PFGE	pulsed field gel electrophoresis
MLST	multi locus sequence typing
RNA	ribo-nucleic acid
DNA	deoxyribo-nucleic acid
Amp	ampicillin
Apra	apramycin
Nal	nalidixic acid
Tet	tetracycline
Trim	trimethoprim
DT	definitive phage type

A.1.1 PCR primers

***Campylobacter* spp.**

Linton *et al.* 1996

Assay no	Assay	Oligonucleotide sequence (5'-3')	Amplicon size (base pairs)
1	16S rRNA	GGATGACACTTTTCGGAGC CATTGTAGCACGTGTGTC	816
2	*C.helveticus*	GGGACAACACTTAGAAATGAG CCGTGACATGGCTGATTCAC	1225
	C. upsaliensis	GGGACAACACTTAGAAATGAG CACTTCCGTATCTCTACAGA	878
3	*C. fetus*	GCAAGTCGAACGGAGTATTA GCAGCACCTGTCTCAACT	997
	C. hyointestinalis	GCAAGTCGAACGGAGTATTA GCGATTCCGGCTTCATGCTC	1287
4	*C. lari*	CAAGTCTCTTGTGAAATCCAAC ATTTAGAGTGCTCACCCGAAG	561

Houng *et al.*, 2001

Assay	Oligonucleotide sequences (5'-3')	Amplicon size (base pairs)
C. jejuni	CTGCTACGGTGAAAGTTTTGC GATCTTTTTGTTTTGTGC	645 bp
C. coli	GATTTTATTATTTGTAGCAGCG TCCATGCCCTAAGACTTAACG	783 bp

E. coli virulence genes

Rappelli *et al.*, 2001

Assay no	Target gene	Oligonucleotide sequence (5'-3')	Amplicon size (bp)
Assay no 1	*Elt*	TCTCTATGTGCACACGGAGC CCATACTGATTGCCGCAAT	322
	Sta	TCTTTCCCCTCTTTTAGTCAGTC CCAGCACAGGCAGGATTAC	170
	uidA	CCAAAAGCCAGACAGAGT GCACAGCACATCAAAGAG	623
Assay no 2	*eae*	TGATAAGCTGCAGTCGAATCC CTGAACCAGATCGTAACGGC	229
	bfpA	CACCGTTACCGCAGGTGTGA GTTGCCGCTTCAGCAGGAGT	450
Assay no 3	*Stx1*	GAAGAGTCCGTGGGATTACG AGCGATGCAGCTATTAATAA	130
	Stx2	GGGTACTGTGTGCCTGTTACTGG GCTCTGGATGCATCTCTGGT	510
	ial	CTGGTAGGTATGGTGAGG CCAGGCCAACAATTATTTCC	320

E. coli antibiotic resistance genes

Ampicillin resistance

Pitout *et al.*,

Target gene	Oligonucleotide sequence (5'-3')	Amplicon size (bp)
Shv	CACTCAAGGATGTATTGTG TTAGCGTTGCCAGTGC	885

Tem	TCGGGGAAATGTGCGCG TGCTTAATCGTGAGGCACC	971

Tetracycline resistance

Ng et al, 2001

Assay	Tetracycline resistance gene	Oligonucleotide sequence (5'-3')	Amplicon size (base pairs)
1	*tet*B	TTGGTTAGGGGCAAGTTTTG GTAATGGGCCAATAACACCG	659
	*tet*C	CTTGAGAGCCTTCAACCCAG ATGGTCCTCATCTACCTGCC	418
	*tet*D	AAACCATTACGGCATTCTGC GACCGGATACACCATCCATC	787
2	*tet*A	GCTACATCCTGCTTGCCTTC CATAGATCGCCGTGAAGAGG	210
	*tet*E	AAACCACATCCTCCATACGC AAATAGGCCACAACCGTCAG	278
	tetG	GCTCGGTGGTATCTCTGCTC AGCAACAGAATCGGGAACAC	210

Trimethoprim resistance

Gibreel and SkÖld, 1998

Target gene	Oligonucleotide sequence (5'-3')	Amplicon size (base pairs)
Dfr1	ACGGATCCTGGCTGTTGGTTGGACGC CGGAATTCACCTTCCGGCTCGATGTC	254
Dfr9	ATGAATTCCCGTGGCATGAACCAGAAGAT	399

	ATGGATCCTTCAGTAATGGTCGGGACCTC	

Lee *at al.*, 2001

Assay	Target genes	Oligionucleotide sequence 3'-5'	Amplicon size
1	*dfrA12, dfrA13*	CCGTGGGTCGATGTTTGATG GCATTGGGAAGAAGGCGTTCAC	485
2	*dfrA7, dfrA17*	GTCGCCCTAAAACAAAGTTA CGCCCATAGAGTCAAATGT	195
3	*dfrA5, dfrA14*	GTTGCGGTCCAGACATAC CCGCCACCAGACACTA	252
4	*dfrA8*	TCGAGCTTCATGCCATTT TCTTCCATGCCATTCTGC	463

Chloramphenicol resistance

Vassort-Bruneau *et al.*, 1996

Target gene	Sequence 3'-5'	Ampicon size (bp)
Cat I	AGTTGCTCAATGTACCTATAACC TTGTAATTCATTAAGCATTCTGCC	585
Cat II	ACACTTTGCCCTTTATCGTC TGAAAGCCATCACATACTGC	495
Cat III	TTCGCCGTGAGCATTTTG TCGGATGAGTATGGGCAAC	508

Keyes et al., 2000

Target gene	Sequence 3'-5'	Amplicon size
cml	CCGCCACGGTGTTGTTGTTATC CACCTTGCCTGCCCATCATTAG	698

Nalidixic acid resistance

Target gene	Sequence 3'-5'	Amplicon size (bp)
GyrA	TACACCGGTCAACATTGAGG TTAATGATTGCCGCCGTCGG	648
parC	AAACCTGTTCAGCGCCGCATT GTGGTGCCGTTAAGCAAA	395

Clostridium perfringens toxin genes

Primer	Toxin gene	Sequence 5'-3'	Amplicon size bp
CPa	Alpha	CCTGCTAATGTTACTGCCG CTCATGCTAGCATGAC	226
CPent	Enterotoxin	GATCTGTATCTACAACTG GAGTCCAAGGGTATGAGTTAG	362
CPb	Beta	CGGATGCCTATTATCACCAAC GGTTGAATGATCTGTCTGTATAG	566
CPep	Epsilon	CCAACTAATGTAATAGCTAAGG GTAGTTGCAGTTACTGTATC	370

Vancomycin resistant Enterococci species and resistance genes

Dutka-Malen *et al.*, 1995

Target gene	Oligionucleotide sequence 3'-5'	Amplicon size

vanA	GGGAAAACGACAATTGC GTACAATGCGGCCGTTA	732
vanB	ATGGGAAGCCGATAGTC GATTTCGTTCCTCGACC	635
E. gallinarium	GGTATCAAGTGAAACCTC CTTCCGCCATCATAGCT	822
E. casseliflavus	CTCCTACGATTCTCTTG GCAGCAAGACCTTTAAG	439
vanD	TTAGGCGCTTGCATATACCG TGCAGCCAAGTATCCGGTAA	461
E. faecalis	ATCAAGTACAGTTAGTCTT ACGATTCAAAGCTAACTG	941
E. favium	GCAAGGCTTCTTAGAGA CATCGTGTAAGCTAACTTC	550

A1.2 Reagents used in pulsed-field gel electrophoresis

Cell lysis buffer (CLB)

50mM Tris, 50mM EDTA, pH8.0, + 1% Sarcosyl

Tris-base	6.055g
EDTA	14.6g
N-lauroyl sarcosine	10g

Mix all reagents in 900ml water and dissolve on a heated stirring plate. Adjust to pH8, then make up to 1l and autoclave.

Lysozyme (Sigma)

20mg/ml

Makeup in 1ml aliquots with molecular grade water (Sigma) and store at -20C

SDS

2g of SDS was added to 10ml 1XTE buffer (sterile) in a sterile bottle and mixed.

Proteinase K (Sigma)

25mg/ml

Make up in 1ml aliquots with molecular grade water (Sigma) and store at -20°C

E. coli pulsed field – agarose mixture

	Agarose	20% SDS	1XTE
Small gel (15 well)	0.04g	200µl	3.8ml
Large gel (27 well)	0.09g	300µl	5.7ml

A1.3 MIC

Preparation of antimicrobial agents

Stock solutions for each antimicrobial are prepared by weighing the test substance and dissolving to produce solution A as follows,

Ampicillin: 32.3mg ampicillin (A9393) + 7ml phosphate buffer

Apramycin: 32.3mg apramycin +7ml sterile water

Chloramphenicol: 32 mg chloramphenicol (C0378) + 7ml 95% ethanol

Nalidixic acid: 32mg nalidixic acid + 7ml sterile water

Ciprofloxacin: 32mg ciprofloxacin +7ml sterile water

Tetracycline: 33.2mg tetracycline (T4062) + plus 7ml sterile water

Trimethoprim: 32mg trimethoprim (T7883) + 1 ml 0.05m/l HCL + 6 ml sterile water

Further dilution to produce less concentrated stock solutions is in sterile water, with the exception of ampicillin which is further diluted in phosphate buffer.

Solution B - 1.0 ml solution A + 7 ml diluent

Solution C – 1.0 ml solution B + 7 ml diluent

Solution D – 1.0 ml solution C + 7 ml diluent

Preparation of plates

Agar plates are prepared from 20ml bottles of iso-sensitest agar (Lab M) which are autoclaved at 121°C for 15 minutes and kept molten at 50°C until required.
The volumes of each test antimicrobial are added to the bottles of iso-sensitest agar as to the specifications below and should be mixed and poured immediately.

			Nominal concentration
Solution A:	5330µg/ml	- 1 ml into 20 ml agar	= 256µg/ml
		- 0.5 ml into 20 ml agar	= 128µg/ml
		- 0.25 ml into 20 ml agar	= 64µg/ml
Solution B:	666µg/ml	- 1 ml into 20 ml agar	= 32µg/ml
		- 0.5 ml into 20 ml agar	= 16µg /ml
		- 0.25 ml into 20 ml agar	= 8 µg/ml
Solution C:	83µg/ml	- 1 ml into 20 ml agar	= 4µg/ml
		- 0.5 ml into 20 ml agar	= 2µg/ml
		- 0.25 ml into 20 ml agar	= 1µg/ml
Solution D:	10.4µg/ml	- 1 ml into 20 ml agar	= 0.5µg/ml
		- 0.5 ml into 20 ml agar	= 0.25µg/ml
		- 0.25 ml into 20 ml agar	= 0.125µg/ml

All plates should be dried before inoculation

The WALTHAM cut-out-and-keep guide

Grade 1
Hard, dry and crumbly; 'bullet-like'

Grade 1.5
Hard and dry

Grade 2
Well formed; does not leave a mark when picked up; 'kickable'

Grade 2.5
Well formed with a slightly moist surface, which leaves a mark when picked up; 'almost sticky to touch'

Grade 3
Moist, beginning to lose form, leaving a definite mark when picked up

Grade 3.5
Very moist, but still has some definite form

Grade 4
The majority, if not all the form is lost, poor consistency, viscous

Grade 4.5
Diarrhea, with some areas of consistency

Grade 5
Watery diarrhea

Figure 1 *A reference chart – the Waltham Feces Scoring Chart – illustrates the different standards, and is designed to be used for both cat and dog feces.*

A 2

THE UNIVERSITY
of LIVERPOOL

Dog owner questionnaire

Case / Sample number: _____/_____

Dog owner	**Dog name** .....
Address	**Dog Breed** .....
.....	**Age** .....
.....	**Sex:** F / M **Neutered:** Yes / No

How many members of your household are there?

Adults ☐ Children ☐

If you have children, how old are they? .....

Do you have any other pets in the household? (Please tick)

No ☐

Yes ☐ **- If yes, what species and how many?** .....

.....

What do you feed your dog? (Please tick more than one if applicable)

Dry food / biscuits ☐ Tinned food ☐

Kitchen scraps ☐ Dog chews / treats ☐

Other .....

Have you had your dog wormed recently?

No ☐

Yes ☐ **- If yes, please indicate approximate date of treatment.**

.....

Has your dog suffered diarrhoea in the last - (Please tick)

2 months ☐

2 weeks ☐

- **If yes, how many times?** .....

- **How long does it usually last?** .....

.....

Where does your dog sleep? .....

If you have other pets in the household, do they sleep in the same place as the dog? (Please tick)

N/A ☐ No ☐ Yes ☐

If you have other pets, have any of them had diarrhoea recently? (Please tick)

No ☐

Yes ☐ - **If yes, which species?** .....

- **How long ago was it?** .....

- **How long did it last?** .....

Have you, or any members of your family had diarrhoea recently? (Please tick)

No ☐

Yes ☐ - **If yes, how long ago was it?** .....

- **How many members of the household did it affect?**

Adults ☐ Children ☐

- **Approximately how many days did the illness last? (Please tick)**

1 day 2 days 3 days longer than 3 days

Thank you for taking the time to fill in this questionnaire

Boarding kennel samples												
SampleNumber	Origin	F/C	*E.coli* culture no	Amp	Apra	Chlor	Nal	Tet	Trim	*E.coli* virulenc	*Campylobacter* spp.	
68	GB	3	201	S	S	S	S	S	S			
			202	S	S	S	S	S	S			
69	GB	1.5	203	S	S	S	S	S	S			
70	GB	3	negative									
71	GB	3	205	S	S	S	S	S	S			
			206	S	S	S	S	S	S	*eaeA*		
			430	S	S	S	S	S	dfr5 >256µg/ml	*eaeA*		
72	GB	2	207	S	S	S	S	S	S		*C.upsaliensis*	
73	GB	1.5	208	S	S	S	S	S	S		*C.upsaliensis*	
			209	S	S	S	S	S	S			
			210	S	S	S	S	S	S			
74	GB	3.5	211	S	S	S	S	S	S			
			212	tem 256µg/ml	S	S	S	S	S			
			213	tem 256µg/ml	S	catI >256µg/ml	>4µg/ml	tetA/tetB 128µg/ml	dfr1 >256µg/ml			
			431	tem >256µg/ml	S	S	S	S	S			
75	GB	3.5	214	tem >256µg/ml	S	S	S	tetA 256µg/ml	S			
			215	S	S	S	S	S	dfr1 >256µg/ml			
			216	tem 256µg/ml	S	S	S	tetA 256µg/ml	dfr1 >256µg/ml			
76	GB	2	217	tem 256µg/ml	S	catI >256µg/ml	>256µg/ml	tetB 1µg/ml	dfr1 >256µg/ml			
			218	S	S	S	S	S	S			
			219	tem 256µg/ml	S	catI >256µg/ml	>256µg/ml	tetB 128µg/ml	dfr1 >256µg/ml			
77	GB	2	220	S	S	S	S	S	S			
			221	S	S	S	S	S	S			
			222	S	S	S	S	S	S	eaeA		
78	GB	3.5	negative									
79	GB	2.5	223	S	S	S	S	S	S	eaeA		
			224	S	S	S	S	S	S	eaeA		
80	GB	2.5	225	S	S	S	S	S	S			
			226	S	S	S	S	S	S			
81	GB	2.5	227	S	S	S	S	S	S	eaeA		
			228	S	S	S	S	S	S			
82	GB	3	negative									
83	GB	3	229	S	S	S	S	tetA 128µg/ml	S			
			230	S	S	S	S	tetA 64µg/ml	S			
			231	S	S	S	S	tetA 128µg/ml	S			
84	GB	3	232	S	S	S	S	S	S			
			233	S	S	S	S	S	S			
85	GB	2.5	234	S	S	S	S	S	S			
86	GB	1.5	235	S	S	S	S	S	S			
			236	S	S	S	S	S	S			
			237	S	S	S	S	S	S			
87	GB	2.5	238	S	S	S	S	S	S			
88	GB	3	239	S	S	S	S	S	S	eaeA		
			240	S	S	S	S	S	S	eaeA		
			241	S	S	S	S	S	S	eaeA		
89	GB	3	242	S	S	S	S	S	S			
			243	tem >256µg/ml	S	catI >256µg/ml	>256µg/ml	tetB 128µg/ml	dfr1 >256µg/ml			
			244	S	S	S	S	S	S			
473	GB	2.5	1533	S	S	S	S	S	S	eaeA		
			1534	S	S	S	S	S	S	eaeA		

474	GB	3	1535	S	S	S	S	S	S	eaeA		
			1536	S	S	S	S	S	S	eaeA		
			1537	S	S	S	S	S	S			
			1538	S	S	S	S	S	S			
475	GB		1539	S	S	S	S	S	S			
		2	1540	S	S	S	S	S	S			
			1541	S	S	S	S	S	S			
476	GB	3	1542	S	S	S	S	S	S			
			1543	S	S	S	S	S	S			
			1544	S	S	S	S	S	S			
477	GB	4	1545	S	S	S	S	S	S			
			1546	S	S	S	S	S	S			
			1547	S	S	S	S	S	S	eaeA		
482	BK - Wynnestay	3	1596	tem >256µg/ml	S	S	S	tetB 256µg/ml	S			
			1597	>256µg/ml	S	S	S	tetB 256µg/ml	S			
			1598	tem 128µg/ml	S	S	S	tetB 64µg/ml	>256µg/ml			
483	BK - Wynnestay	2.5	1599	tem >256µg/ml	S	S	S	tetB 128µg/ml	S			
			1600	S	S	S	S	S	S			
			1601	S	S	S	S	S	S			
484	BK - Wynnestay	2.5	1602	tem >256µg/ml	S	S	S	tetA/tetB 256µg/ml	S			
			1603	S	S	S	S	S	S			
			1604	S	S	S	S	S	S			
485	BK - Wynnestay	3	1605	S	S	S	S	S	S			
			1606	S	S	S	S	S	S			
			1607	S	S	S	S	S	S			
486	BK - Wynnestay	2.5	1608	S	S	S	S	S	S			
			1609	S	S	S	S	S	S			
			1610	S	S	S	S	S	S			*C.upsaliensis*
487	BK - Wynnestay	2	1611	S	S	S	S	S	S			
			1612	S	S	S	S	S	S			
			1613	S	S	S	S	tetA 64µg/ml	S			
488	BK - Wynnestay	3	1614	S	S	S	S	S	S			
			1615	S	S	S	S	S	S			
			1616	8µg/ml	S	S	S	S	dfr1 256µg/ml			
489	BK - Wynnestay	2	1617	tem >256µg/ml	S	S	S	tetB 128µg/ml	S			*C. upsaliensis*
			1618	tem >256µg/ml	S	S	S	tetA 32µg/ml	256µg/ml			
			1619	S	S	S	S	tetB 32µg/ml	S			
490	BK - Wynnestay	2	1620	S	S	S	S	S	S			
			1621	S	S	S	S	S	S			
			1622	>256µg/ml	S	S	S	tetA/tetB 256µg/ml	S			
491	BK - Wynnestay	2	1623	S	S	S	S	tetA 16µg/ml	S			
			1624	tem 256µg/ml	S	S	S	tetB 128µg/ml	S			
			1625	S	S	S	S	S	S			
492	BK - Wynnestay	2.5	1626	256µg/ml	S	S	S	tetA/tetB 128µg/ml	S			
			1627	S	S	S	S	S	S			
			1628	S	S	S	S	tetA 32µg/ml	S			
493	BK - Wynnestay	2	1629	S	S	S	S	S	S			
			1630	S	S	S	S	S	S			
			1631	tem 128µg/ml	S	S	S	tetB 128µg/ml	>256µg/ml			
			1848	tem 256µg/ml	S	S	S	tetB 128µg/ml	S			
494	BK - Wynnestay	3.5	1632	S	S	S	S	S	S			
			1633	S	S	S	S	S	S			

			1634	S	S	S	S	S	S				
501	GB	3	1703	S	S	S	S	S	S				
			1704	S	S	S	S	S	S				
			1705	S	S	S	S	S	S				
502	GB	3	1706	S	S	S	S	S	S	*eaeA*			
			1707	tem >256µg/ml	S	S	>256µg/ml	tetA 256µg/ml	dfr1 >256µg/ml				
			1708	tem >256µg/ml	S	S	>256µg/ml	tetA 128µg/ml	dfr1 >256µg/ml				
503	GB	3	1709	S	S	S	S	S	S				
			1710	S	S	S	S	S	S				
			1711	S	S	S	S	S	S	*sta*			
504	GB	4	1712	S	S	S	S	S	S				
			1713	S	S	S	S	S	S				
			1714	S	S	S	S	S	S				
506	Widnes - Rose Tree	3	1718	S	S	S	S	S	S				
			1719	S	S	S	S	S	S	*eaeA*			
			1720	S	S	S	S	S	S	*eaeA*			
507	Widnes - Rose Tree	2.5	1721	S	S	S	S	S	dfr8 >256µg/ml				
			1722	S	S	S	S	S	S				
			1723	S	S	S	S	S	S				
508	Widnes - Rose tree	2	1724	tem >256µg/ml	S	S	S	S	dfr8 >256µg/ml				
509	Widnes - Rose tree	2	1727	S	S	S	S	S	S				
			1728	S	S	S	S	S	S				
			1729	S	S	S	S	S	S				
510	Widnes - Rose tree	2.5	1730	S	S	S	S	S	S				
			1731	S	S	S	S	S	S				
			1732	S	S	S	S	S	S				
511	Widnes - Rose tree	2	1733	S	S	S	S	S	S				
			1735	S	S	S	S	S	S				
512	Widnes - Rose tree	1.5	1736	tem >256µg/ml	S	S	S	S	>256µg/ml				
			1737	tem 64µg/ml	S	S	S	S	>4µg/ml				
			1738	S	S	S	S	S	S				
513	Widnes - Rose tree	2	1741	S	S	S	S	S	256µg/ml				
514	Widnes - Rose tree	2.5	1742	S	S	S	S	S	S				
			1743	S	S	S	S	S	S				
			1744	S	S	S	S	S	S				
515	Widnes - Rose tree	3.5	1745	S	S	S	S	S	S				
			1746	S	S	S	S	S	S				
			1747	S	S	S	S	S	S				
516	Widnes - Rose tree	3.5	1748	S	S	S	S	S	S				
			1749	S	S	S	S	S	S				
			1750	S	S	S	S	S	S				
517	Widnes - Rose tree	2.5	1751	S	S	S	S	S	S				
			1752	S	S	S	S	S	S				
			1753	S	S	S	S	S	S				
520	BK - Raby rd	2	1760	S	S	S	S	S	S				
			1761	S	S	S	S	S	S				
			1762	S	S	S	S	S	S				
521	BK - Raby rd	2.5	1763	S	S	S	S	S	S				
			1764	S	S	S	S	S	S				
			1765	S	S	S	S	S	S				
522	BK - Raby rd	3	1766	tem 256µg/ml	S	S	S	S	>256µg/ml				
			1767	tem >256µg/ml	S	S	S	S	dfr5 >256µg/ml				

			1768	tem >256µg/ml	S	S	S	S	dfr1 >256µg/ml			
523	BK - Raby rd	3	1769	S	S	S	S	S	S			
			1770	S	S	S	S	S	S			
			1771	S	S	S	S	S	S			
536	GB	2	1805	S	S	S	S	S	dfr14 >256µg/ml			
			1806	S	S	S	S	S	dfr14 >256µg/ml			
			1807	tem 64µg/ml	S	S	S	S	S			
537	GB	4	1808	S	S	S	S	S	S			
			1809	S	S	S	S	S	S			
			1810	S	S	S	S	S	S			
538	GB	4	1811	S	S	S	S	S	S			
			1812	S	S	S	S	S	S			
			1813	S	S	S	S	S	S			
539	GB	4.5	1814	S	S	S	S	S	S	*eaeA*		
			1815	S	S	S	S	S	S	*eaeA*		
			1816	S	S	S	S	S	S	*eaeA*		
540	GB	3	1817	S	S	S	S	S	S			
			1818	S	S	S	S	S	S			
			1819	S	S	S	S	S	S			
542	BK - 5 oaks	0.1/1	negative									
543	BK - 5 oaks	3	1823	S	S	S	S	S	S			
			1824	S	S	S	S	S	S			
			1825	S	S	S	S	S	S			
544	BK - 5 oaks	2	1826	S	S	S	S	S	S			
			1827	S	S	S	S	S	S			
			1828	S	S	S	S	S	S			
545	BK - 5 oaks	2.5	1829	S	S	S	S	S	S			
			1830	S	S	S	S	S	S			
			1831	S	S	S	S	S	S			
546	BK - 5 oaks	2	1832	S	S	S	S	S	S		*C.upsaliensis*	
			1833	S	S	S	S	S	S			
			1834	S	S	S	S	S	S			
547	BK - 5 oaks	2.5	1835	S	S	S	S	S	S			
			1836	S	S	S	S	S	S			
			1837	S	S	S	S	S	S			
548	BK - 5 oaks	3	1838	S	S	S	S	S	S			
			1839	S	S	S	S	S	S			
			1840	S	S	S	S	S	S			
549	BK - 5 oaks	2.5	1841	S	S	S	S	S	S			
			1842	S	S	S	S	S	S			
			1843	S	S	S	S	S	S			
550	BK - 5 oaks	3	1844	S	S	S	S	S	S		*Campylobacter* spp.	
			1845	S	S	S	S	S	S			
			1846	S	S	S	S	S	S			
553	BK - Raby rd	0.3/1	1856	S	S	S	S	S	S			
			1857	S	S	S	S	S	S			
			1858	S	S	S	S	S	S			
554	BK - Raby rd	5	1859	S	S	S	S	S	S			
			1860	S	S	S	S	S	S			
			1861	S	S	S	S	S	S			
555	BK - Raby rd	2.5	1862	S	S	S	S	S	S			
			1863	S	S	S	S	S	S			

			1864	S	S	S	S	S	S			
556	BK - Raby rd	1.5	1865	S	S	S	S	S	S		*C. upsaliensis*	
			1866	S	S	S	S	S	S			
			1867	S	S	S	S	S	S			
557	BK - Raby rd	2.5	1868	S	S	S	S	S	S		*C. upsaliensis*	
			1869	S	S	S	S	S	S			
			1870	S	S	S	S	S	S			
558	BK - Raby rd	2.5	1871	S	S	S	S	S	S			
			1872	S	S	S	S	S	S			
			1873	S	S	S	S	S	S			

Trimethoprim resistant isolates

f/c - faecal consistency

Sample no	E. coli culture no.	Streptomycin	Spectinomycin	SMX
71	430	S	S	S
74	213	S	S	R
75	215	S	S	R
	216	S	S	R
76	217	S	S	R
	219	S	S	R
89	243	S	S	R
482	1598	R	S	R
488	1616	S	S	R
489	1618	S	S	S
493	1631	R	S	R
502	1707	R	S	R
	1708	R	S	R
507	1721	R	S	R
508	1724	R	S	R
	1725	R	S	R
	1726	R	S	R
512	1736	S	S	R
	1737	S	S	R
	1741	R	S	R
522	1766	R	S	R
	1767	R	S	R
	1768	R	S	R
536	1805	R	R	R
	1806	R	R	R

R - resistant
S - sensitive

Samples from rescue homes											
Sample no	Origin	F/C	CultureNumber	Amp	Apra	Chlor	Nal	Tet	Trim	*E. coli* virulence	*Campylobacter* spp.
136	RSPCA - Warrington	2	395 S	S	S	S	S	S			
			396 S	S	S	S	S	S		*eaeA*	
			397 S	S	S	S	S	S		*eaeA*	
137	RSPCA - Warrington	3.5	398 S	S	S	S	S	S			
			399 S	S	S	S	S	S			
			400 S	S	S	S	S	S			
138	RSPCA - Warrington	4	401 S	S	S	S	S	S			
			402 tem 64µg/ml	S	S	S	tetB 256µg/ml	dfr14 >256µg/ml			
			403 S	S	S	S	S	S			
139	RSPCA - Warrington	3.5	404 S	S	S	S	S	S			
			405 S	S	S	S	S	S			
			406 S	S	S	S	S	S			
140	RSPCA - Warrington	2.5	407 S	S	S	S	S	S			
			408 S	S	S	S	S	S			
			409 S	S	S	S	S	S			
141	RSPCA - Warrington	3	410 S	S	S	S	S	S			
			411 S	S	S	S	S	S			
			412 S	S	S	S	S	S			
142	RSPCA - Warrington	2.5	413 S	S	S	S	S	S			
			414 tem >256µg/ml	S	S	S	tetB 64µg/ml	S		*eaeA*	
			415 S	S	S	S	S	S			
143	RSPCA - Warrington	4	416 tem 128µg/ml	S	S	S	S	S			
			417 tem >256µg/ml	S	S	S	S	S			
			418 tem >256µg/ml	S	S	S	S	S		*sta*	
144	RSPCA - Warrington	3	459 S	S	S	S	S	S			
			460 S	S	S	S	S	S			
			461 S	S	S	S	S	S			
145	RSPCA - Warrington	4	419 S	S	S	S	S	S			
			420 S	S	S	S	S	S			
			421 S	S	S	S	S	S			
146	RSPCA - Warrington	2.5	negative								
147	RSPCA - Warrington	4	432 S	S	S	S	S	S			
			433 S	S	S	S	S	S			
			434 S	S	S	S	S	S			
148	RSPCA - Warrington	3.5	422 S	S	S	S	S	S			
			423 S	S	S	S	S	S			

			424 S	S	S	S	S	S		
149	RSPCA - Warrington	4	425 tem 256µg/ml	S	S	S	S	S		
			426 tem 256µg/ml	S	S	S	S	S		
			427 S	S	S	S	S	S		
99	RSPCA - Halewood	3	285 S	S	S	S	S	S		
			286 S	S	S	S	S	S		
			287 S	S	S	S	S	S		
100	RSPCA - Halewood	4	288 S	S	S	S	S	S		
			289 S	S	S	S	S	S		
			290 S	S	S	S	S	S		
101	RSPCA - Halewood	3.5	291 S	S	S	S	S	S	*eaeA*	
			292 S	S	S	S	S	dfr1 >256µg/ml	*eaeA*	
			293 tem 128µg/ml	S	S	S	S	dfr1 >256µg/ml	*eaeA*	
102	RSPCA - Halewood	3	294 tem >256µg/ml	S	S	S	tetB 64µg/ml	>256µg/ml		
			295 tem >256µg/ml	S	S	S	tetB 64µg/ml	>256µg/ml		
			296 tem >256µg/ml	S	S	S	tetB 256µg/ml	>256µg/ml		
103	RSPCA - Halewood	2.5	negative							
104	RSPCA - Halewood	4	297 S	S	S	S	S	S		
			298 S	S	S	S	S	S		
			299 S	S	S	S	S	S		
105	RSPCA - Halewood	5	300 S	S	S	S	S	S		*C.upsaliensis*
			301 S	S	S	S	S	S		
			302 S	S	S	S	S	S		
106	RSPCA - Halewood	2	negative							
107	RSPCA - Halewood	4	303 S	S	S	S	S	S		
			304 S	S	S	S	S	S		
108	RSPCA - Halewood	2	305 tem >256µg/ml	S	catI 256µg/ml	S	tetB 64µg/ml	dfr5 >256µg/ml		
			306 tem >256µg/ml	S	catI >256µg/ml	S	tetB 256µg/ml	dfr5 >256µg/ml		
			307 tem >256µg/ml	S	S	S	tetA 64µg/ml	dfr14 >256µg/ml		
109	RSPCA - Halewood	2	308 S	S	S	S	S	S		
			309 S	S	S	S	S	S		
			310 S	S	S	S	S	S	*eaeA*	
110	RSPCA - Halewood	3	311 tem >256µg/ml	S	catI 128µg/ml	S	tetB 256µg/ml	S		
111	RSPCA - Halewood	4	312 S	S	S	S	S	S		
			313 S	S	S	S	S	S		
			314 S	S	S	S	S	S		

112	RSPCA - Halewood	4	315 tetm >256µg/ml	S	S	S	tetA/tetB 128µg/ml	dfr1 >256µg/ml		
			316 tetm >256µg/ml	S	S	S	tetA 128µg/ml	dfr1 >256µg/ml		
113	RSPCA - Halewood	3	317 S	S	S	S	S	S		
			318 S	S	S	S	S	S		
114	RSPCA - Halewood	3	319 tetm >256µg/ml	S	catI >256µg/ml	S	tetB 128µg/ml	dfr1 >256µg/ml		
			320 tetm >256µg/ml	S	catI >256µg/ml	S	tetB 128µg/ml	dfr1 >256µg/ml		
			321 tetm 128µg/ml	S	S	S	S	S	*cadA*	
115	RSPCA - Halewood	3.5	322 tetm >256µg/ml	S	catI >256µg/ml	S	tetB >256µg/ml	dfr14 8µg/ml		
			323 S	S	S	S	S	S		
			324 S	S	S	S	S	S		
116	RSPCA - Halewood	2.5	325 tetm 256µg/ml	S	S	S	S	S		
			326 tetm 256µg/ml	S	S	S	S	S		
			327 S	S	S	S	S	S		
117	RSPCA - Halewood	2	328 S	S	S	S	S	S		
			329 tetm 256µg/ml	S	S	S	tetA 128µg/ml	dfr5 >256µg/ml		
118	RSPCA - Halewood	2	330 tetm >256µg/ml	S	catI >256µg/ml	S	tetB 256µg/ml	>256µg/ml		*Campylobacter* spp.
			331 S	S	S	S	S	S		
			332 S	S	S	S	S	S		
119	RSPCA - Halewood	3	333 S	S	S	S	S	S		
			334 S	S	S	S	S	S		
			335 S	S	S	S	S	S		
120	RSPCA - Halewood	3.5	336 S	S	S	S	64µg/ml	S		
			337 S	S	S	S	S	S		
			338 S	S	S	S	S	S		
202	CHD	3.5	624 S	S	S	S	S	S		
203	CHD	3	625 S	S	S	S	S	S		
			626 S	S	S	S	S	S		
			627 S	S	S	S	S	S		
204	CHD	2.5	628 S	S	S	S	S	S		
			629 S	S	S	S	S	S		
			630 S	S	S	S	S	S		
205	CHD	2	631 S	S	S	S	S	S		
			632 S	S	S	S	S	S		
			633 S	S	S	S	S	S		
206	CHD	2	634 S	S	S	S	S	S		
			635 S	S	S	S	S	S		
			636 S	S	S	S	S	S		
207	CHD	2.5	637 S	S	S	S	S	S		
			638 S	S	S	S	S	S		
			639 S	S	S	S	S	S		
208	CHD	3.5	640 S	S	S	S	S	S		
			641 S	S	S	S	S	S		

			642 S	S	S	S	S	S		
209	CHD	3	643 S	S	S	S	S	S		
			644 S	S	S	S	S	S		
			645 S	S	S	S	S	S		
210	CHD	3	646 S	S	S	S	S	S		
			647 S	S	S	S	S	S		
			648 S	S	S	S	S	S		
211	CHD	2	649 S	S	S	S	S	S		
			650 S	S	S	S	S	S	*eaeA*	
			651 S	S	S	S	S	S		
212	CHD	2	652 S	S	S	S	tetA 128µg/ml	S		
			653 S	S	S	S	S	S		
	RSPCA -									
742	Halewood	2.5	2490 tem >256µg/ml	R	S	S	tetA 256µg/ml	S		
			2491 tem >256µg/ml	S	S	S	tetA 64µg/ml	S		
			2492 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
	RSPCA -									
743	Halewood	4	2496 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
			2497 tem >256µg/ml	S	S	S	tetB >256µg/ml	dfr5 >256µg/ml		
			2498 S	S	S	S	S	S		
	RSPCA -									
744	Halewood	3.5	2493 tem >256µg/ml	S	S	S	S	S		
			2494 tem >256µg/ml	S	S	S	S	S		
			2495 S	S	S	S	S	S		
	RSPCA -									
745	Halewood	2.5	2499 S	S	S	S	S	S		
			2500 S	S	S	S	S	S		
			2501 tem 256µg/ml	S	catI >256µg/ml	S	tetB 256µg/ml	dfr17 >256µg/ml		
	RSPCA -									
746	Halewood	2	2502 tem >256µg/ml	S	S	S	S	S		
			2503 tem >256µg/ml	S	S	S	S	S		
			2504 S	S	S	S	S	S		
	RSPCA -									
747	Halewood	2	2506 >256µg/ml	S	S	S	tetB 256µg/ml	S		
			2507 >256µg/ml	S	S	S	tetB >256µg/ml	S		
	RSPCA -									
748	Halewood	2.5	2508 tem >256µg/ml	S	S	S	tetB 256µg/ml	>256µg/ml		
			2509 S	S	S	S	S	S		
			2510 S	S	S	S	S	S		
	RSPCA -									
749	Halewood	2	2511 S	S	S	S	S	S		
			2512 S	S	S	S	S	S		
			2513 S	S	S	S	S	S		
	RSPCA -									
750	Halewood	2.5	2514 tem >256µg/ml	S	S	S	tetB 256µg/ml	>256µg/ml		
			2515 tem >256µg/ml	S	S	S	tetA 256µg/ml	S		
			2516 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
751	RSPCA - Hale	3	2517 >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
			2518 >256µg/ml	S	S	S	tetB >256µg/ml	S		
			2519 >256µg/ml	S	S	S	tetB >256µg/ml	S	*sta*	
752	RSPCA - Hale	2.5	2520 S	S	S	S	S	S		

			2521	S	S	S	S	S	S		
			2522	S	S	S	S	S	S		
753	RSPCA - Hale	2.5	2523	S	S	S	S	tetB 128µg/ml	S		
			2524	tem 128µg/ml	S	S	S	S	S		
			2525	S	S	S	S	tetB 256µg/ml	S		
754	RSPCA - Hale	2.5	2526	S	S	S	S	S	S		
			2527	S	S	S	S	S	S		
			2528	S	S	S	S	S	S		
755	RSPCA - Hale	1.5	2529	tem 64µg/ml	S	S	S	S	S		
			2530	tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
			2531	S	S	S	S	S	S		
756	RSPCA - Hale	2	2532	tem 256µg/ml	S	S	128µg/ml	tetA 128µg/ml	dfr1 >256µg/ml		
			2533	S	S	S	S	S	S		
			2534	S	S	S	S	S	S		
757	RSPCA - Hale	3.5	2535	tem >256µg/ml	S	S	S	S	S		
			2536	S	S	S	S	S	S		
			2537	tem >256µg/ml	S	S	S	tetA 128µg/ml	S		
758	RSPCA - Hale	3.5	2538	tem 1µg/ml	S	S	S	S	S		
			2539	S	S	S	S	S	S		
			2540	tem >256µg/ml	S	S	S	S	S		
759	RSPCA - Hale	3.5	2541	tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
			2542	tem >256µg/ml	S	S	S	tetB 256µg/ml	>256µg/ml		
			2543	tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
760	RSPCA - Hale	3	2544	S	S	S	S	S	S		
			2545	tem >256µg/ml	S	S	S	tetA 256µg/ml	S		
			2546	tem >256µg/ml	S	S	S	tetA 256µg/ml	S		
761	RSPCA - Hale	3.5	2547	tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
			2548	>256µg/ml	S	S	S	tetA 128µg/ml	>256µg/ml		
			2549	>128µg/ml	S	S	S	tetA 128µg/ml	dfr5 >256µg/ml		
956	Blue cross	2	3062	S	S	S	S	tetB 128µg/ml	S		
			3063	S	S	S	S	tetB 128µg/ml	S		
			3064	S	S	S	S	tetB 64µg/ml	S		
957	Blue cross	3	3065	S	S	S	S	S	S		
			3066	S	S	S	S	S	S		
			3067	S	S	S	S	S	S		
958	Blue cross	2.5	3068	S	S	S	S	S	S		
			3069	S	S	S	S	S	S		
			3070	S	S	S	S	S	S		
959	Blue cross	3	3071	S	S	S	S	S	S		
			3072	S	S	S	S	S	S		
			3073	S	S	S	S	S	S		
960	Blue cross	3.5	3074	S	S	S	S	S	S		
			3075	S	S	S	S	S	S		
			3076	S	S	S	S	S	S		
961	Blue cross	3	3077	S	S	S	S	S	S		
			3078	S	S	S	S	S	S		
			3079	S	S	S	S	S	S		
962	Blue cross	3.5	3080	S	S	S	S	S	S		
			3081	S	S	S	S	S	S		
			3082	S	S	S	S	S	R		
963	Blue cross	3									
964	Blue cross	2.5	3083	S	S	S	S	S	S		

			3084	S	S	S	S	S	S			
			3085	S	S	S	S	S	S			
965	Blue cross	3.5	3116	tem 2µg/ml	S	S	S	S	S			
			3117	tem 64µg/ml	S	catl 256µg/ml	S	tetB 64µg/ml	R			
			3118	tem 64µg/ml	S	S	S	S	S			
966	Blue cross	2.5	3119	S	S	S	S	S	S			
			3120	S	S	S	S	S	S			
			3121	tem 256µg/ml	R	S	S	tetB 128µg/ml	S			
Trimethoprim resistant E. coli isolates												
Sample no	Culture no	Streptomycin	Spectinomycin	SMX		F/C - faecal consistency						
138	402	R	S	R								
101	292	R	S	R								
	293	R	S	R								
102	294	S	S	R								
	295	S	S	R								
	296	S	S	R								
108	305	R	S	R								
	306	R	S	R								
	307	S	S	R								
112	315	R	S	R								
	316	R	S	R								
114	319	R	S	R								
	320	R	S	R								
115	322	S	S	S								
117	329	R	S	R								
118	330	R	S	R								
742	2492	S	S	R								
743	2496	S	S	R								
	2497	S	S	R								
745	2501	R	S	R								
746	2504	S	S	R								
748	2508	S	S	R								
750	2514	S	S	R								
	2516	S	S	R								
751	2517	R	R	R								
755	2530	S	S	R								
756	2532	R	S	R								
759	2541	S	S	R								
	2542	S	S	R								
	2543	S	S	R								
761	2547	S	S	R								
	2548	R	S	R								
	2549	S	S	R								

Samples from parks												
SampleNumber	Origin	F/C	E. coli culture no	Amp	Apra	Chlor	Nal	Tet	Trim	*E. coli* virueince	*Campylobacter* spp.
447	Marshes	2	1457	S	S	S	S	S	S		
			1459	S	S	S	S	S	S		
448	Marshes	2.5	1460	tem >256µg/ml	S	S	S	tetB 256µg/ml	S		
			1461	tem 2µg/ml	S	S	S	tetB 256µg/ml	S		
			1462	>256µg/ml	S	S	S	tetB 256µg/ml	S		
449	Marshes	2	1464	S	S	S	S	S	S		
			1465	S	S	S	S	S	S		
450	Marshes	2.5	1466	S	S	S	S	S	S	*eaeA*	
			1467	S	S	S	S	S	S	*eaeA*	
			1468	S	S	S	S	S	S	*eaeA*	
451	Marshes	2.5	1469	S	S	S	S	32µg/ml	S		
			1470	S	S	S	S	32µg/ml	S		
452	Marshes	2.5	1472	S	S	S	S	S	S		
			1473	tem 128µg/ml	S	S	S	S	S	*eaeA*	
			1474	S	S	S	S	S	S		
453	Marshes	2	1475	S	S	S	S	S	>256µg/ml		
			1476	S	S	S	S	S	S		
			1477	S	S	S	S	S	>256µg/ml		
454	Marshes	3	negative								
455	Marshes	2.5	1478	S	S	S	S	S	S		
			1479	S	S	S	S	S	S		
			1480	S	S	S	S	S	S		
456	Marshes	2.5	1481	S	S	S	S	S	S		
			1483	S	S	S	S	S	S		
457	Marshes	2.5	1484	S	S	S	S	S	S		
			1485	S	S	S	S	S	S		
			1486	S	S	S	S	S	S		
458	Marshes	3.5	1487	S	S	S	S	S	S		
			1488	S	S	S	S	S	S		
			1489	S	S	S	S	S	S		
459	Marshes	2.5	1490	tem 256µg/ml	S	S	S	tetB 128µg/ml	dfr1 >256µg/ml		
			1491	tem 256µg/ml	S	S	S	tetB 128µg/ml	dfr1 >256µg/ml		
			1492	tem 256µg/ml	S	S	S	tetB 256µg/ml	dfr1 >256µg/ml		
460	Marshes	2.5	1493	S	S	S	S	S	S		
			1495	S	S	S	S	S	S		
461	Marshes	3	1497	S	S	S	S	S	S		
			1498	S	S	S	S	S	S		
			1499	S	S	S	S	S	S		
462	Marshes	3	1500	tem 256µg/ml	S	S	S	tetA 256µg/ml	S		
			1501	tem 256µg/ml	S	S	S	S	dfr5 >256µg/ml		
			1502	tem 256µg/ml	S	S	S	S	dfr5 >256µg/ml		
16	Marshes	4	42	tem 256µg/ml	S	S	S	tetB 256µg/ml	dfr1 >256µg/ml		

			43	S	S	S	S	S	S	*eaeA+bfpA*	*C. upsaliensis*
			44	S	S	S	S	S	**dfr5 >256µg/ml**		
17	Marshes	3	45	S	S	S	S	S	S		
			46	S	S	S	S	S	S		
			47	S	S	S	S	S	S		
18	Marshes	3	48	S	S	S	S	S	S		
			49	S	S	S	S	S	S		
			50	S	S	S	S	S	S		
19	Marshes	2.5	51	S	S	S	S	S	S		
			52	S	S	S	S	S	S		
20	Marshes	3.5	53	S	S	S	S	S	**dfr1 >256µg/ml**		
			54	S	S	S	S	S	S		
			55	S	S	S	S	S	**dfr1 >256µg/ml**		
21	Marshes	2	negative								
22	Marshes	3	56	S	S	S	S	S	S		*C. upsaliensis*
			57	S	S	S	S	S	S	*eaeA*	
			58	S	S	S	S	S	S	*eaeA*	
23	Marshes	3	59	S	S	S	S	S	S		
			60	S	S	S	S	S	S		
			61	S	S	S	S	S	S		
24	Marshes	2.5	62	**tem 128µg/ml**	S	S	S	S	S		
			63	**tem 128µg/ml**	S	S	S	S	S		
			64	**tem 128µg/ml**	S	S	S	**tetA 128µg/ml**	S		
25	Marshes	4	65	S	S	S	S	S	S		
			66	S	S	S	S	S	S		
			67	S	S	S	S	S	S		
26	Marshes	2	68	S	S	S	S	S	S		
			69	S	S	S	S	S	S		
			70	S	S	S	S	S	S		
27	Marshes	1.5	73	S	S	S	S	S	S		
28	Marshes	2	74	**tem 64µg/ml**	S	S	S	**tetA 16µg/ml**	S		
			75	S	S	S	S	S	S	*eaeA*	
			76	S	S	S	S	S	S		
29	Marshes	4	77	S	S	S	S	S	S		
			78	S	S	S	S	S	S		
			79	S	S	S	S	S	S		
30	Marshes	3.5	80	S	S	S	S	S	S	*eaeA*	
			81	S	S	S	S	S	S		
			82	**tem 64µg/ml**	S	S	S	**tetA/tetB 64µg/ml**	S	*eaeA*	
586	Marshes	2.5	2002	**tem 256µg/ml**	S	S	S	S	S		
587	Marshes	3	2005	S	S	S	S	S	S		
			2006	S	S	S	S	S	S		
			2007	S	S	S	S	S	S		
588	Marshes	3	2008	**tem >256µg/ml**	S	S	S	**tetB 64µg/ml**	**dfr14 >256µg/ml**		

			2009	S	S	S	S	S	S			
			2010	tem >256µg/ml	S	S	S	tetB 64µg/ml	>256µg/ml			
589	Marshes	4	2011	S	S	S	S	S	S			
			2012	S	S	S	S	S	S			
			2013	S	S	S	S	S	S			
590	Marshes	2	2014	S	S	S	S	S	S			
			2015	S	S	S	S	S	S			
			2016	S	S	S	S	S	S			
591	Marshes	2	2017	S	S	S	S	S	S			
			2018	S	S	S	S	S	S			
			2019	S	S	S	S	S	S			
592	Marshes	4	negative									
593	Marshes	3.5	2020	S	S	S	S	S	S			
			2021	S	S	S	S	S	S			
			2022	S	S	S	S	S	S			
594	Marshes	3.5	2023	S	S	S	S	S	S			
			2024	S	S	S	S	S	S			
			2025	S	S	S	S	S	S			
595	Marshes	2.5	2026	S	S	S	S	S	S			
			2027	S	S	S	S	S	S			
			2028	S	S	S	S	S	S			
596	Marshes	4	2029	S	S	S	S	S	S			
			2030	S	S	S	S	S	S			
			2031	S	S	S	S	S	S			
597	Marshes	4	negative									
618	Sussex	3	2086	S	S	S	S	S	S			
			2087	S	S	S	S	S	S			
			2088	S	S	S	S	S	S			
619	Sussex	2.5	2089	S	S	S	S	S	S			
			2090	S	S	S	S	S	S			
			2091	S	S	S	S	S	S			
620	Sussex	2.5	2092	S	S	S	S	S	S			
			2093	S	S	S	S	S	S			
			2094	S	S	S	S	S	S			
621	Sussex	3	2095	S	S	S	S	S	S			
			2096	S	S	S	S	S	S			
			2097	S	S	S	S	S	S			
622	Sussex	3.5	2098	tem 64µg/ml	S	S	S	tetB 128µg/ml	S			
			2099	tem 64µg/ml	S	S	S	tetB 128µg/ml	S			
			2100	tem 64µg/ml	S	S	S	tetB 128µg/ml	S			
623	Sussex	4	2101	S	S	S	S	S	S			
			2102	S	S	S	S	S	S			
			2103	S	S	S	S	S	S			
624	Sussex	4.5	2104	S	S	S	S	S	S			

			2105	S	S	S	S	S	S			
			2106	S	S	S	S	S	S			
664	Widnes	3	2206	tem 64µg/ml	S	S	S	tetA 128µg/ml	S			
			2207	tem 64µg/ml	S	S	S	tetA 128µg/ml	S			
			2208	tem 128µg/ml	S	S	S	tetA 128µg/ml	S			
665	Widnes	3.5	2209	S	S	S	S	tetB 64µg/ml	>256µg/ml			
			2210	S	S	S	S	tetB 128µg/ml	>256µg/ml			
666	Widnes	3	2211	S	S	S	S	S	S			*Campylobacter* spp.
			2212	S	S	S	S	S	S			
			2213	S	S	S	S	S	S			
667	Widnes	2.5	2215	S	S	S	S	S	S			*C. upsaliensis*
769	Marshes	2.5	2571	S	S	S	S	S	S			
			2572	S	S	S	S	S	S	*eaeA*		
			2573	S	S	S	S	S	S	*eaeA*		
770	Marshes	3	2574	S	S	S	S	S	S			
			2575	S	S	S	S	S	S			
			2576	S	S	S	S	S	S			
771	Marshes	2	2577	S	S	S	S	S	S			
			2578	S	S	S	S	S	S			
			2579	S	S	S	S	S	S			
772	Marshes	2.5	2580	S	S	S	S	S	S			
			2581	tem >256µg/ml	S	S	S	tetA/tetB 256µg/ml	dfr1 >256µg/ml			
			2582	S	S	S	S	S	S			
773	Marshes	3	2583	tem 256µg/ml	S	S	S	S	256µg/ml	*eaeA*		
			2584	S	S	S	S	S	S	*eaeA*		
			2585	S	S	S	S	S	S	*eaeA*		
774	Marshes	3	2586	S	S	S	S	S	S			
			2587	S	S	S	S	S	S			
			2588	S	S	S	S	S	S			
775	Marshes	2.5	2589	S	S	S	S	S	S			
			2590	S	S	S	S	S	S	*eaeA*		
			2591	S	S	S	S	tetB 128µg/ml	S			
776	Marshes	2.5	2592	S	S	S	S	S	S	*eaeA*		
			2593	S	S	S	S	S	S	*eaeA*		
			2594	S	S	S	S	S	S			
777	Marshes	2.5	2595	S	S	S	S	S	S			
			2596	S	S	S	S	S	S			
			2597	tem >256µg/ml	S	S	>256µg/ml	tetB 128µg/ml	dfr14 >256µg/ml			
778	Marshes	2	2598	S	S	S	S	S	S			
			2599	S	S	S	S	S	S			
			2600	S	S	S	S	S	S			
779	Marshes	2.5	2601	S	S	S	S	tetB 32µg/ml	S			
			2602	S	S	S	S	tetA 64µg/ml	S			
			2603	S	S	S	S	S	S			

780	Marshes	2.5	2604	S	S	S	S	S	S		
			2605	S	S	S	S	S	S		
			2606	S	S	S	S	S	S	*eaeA*	
781	Marshes	3	2607	S	S	S	S	S	S	*eaeA*	
			2608	S	S	S	S	S	S	*eaeA*	
			2609	S	S	S	S	S	S	*eaeA*	

Trimethoprim resistant E. coli isolates

Sample no	Culture no	Streptomycin	Spectinomycin	SMX		F/C - faecal consistency					
453	1475	S	S	R							
	1477	S	S	R							
459	1490	S	S	R							
	1491	S	S	R							
	1492	S	S	R							
462	1501	R	S	R							
	1502	R	S	R							
16	42	S	S	R							
	44	R	S	R							
20	53	S	S	R							
	55	S	S	R							
588	2008	R	S	R							
	2010	R	S	R							
665	2209	S	S	R							
	2210	S	S	R							
772	2581	S	S	R							
773	2583	S	S	R							
777	2597	R	S	R							

Cat samples												
SampleNumber	F/C	Origin	E. coli culture no	Amp	Apra	Chlor	Nal Acid	Tet	Tm	*E. coli* virulence	*Campylobacter* spp.	
64	4	BK - GB	190	S	S	S	S	S	S			
			191	S	S	S	S	tetA 64µg/ml	S			
65	1.5	BK - GB	192	tem >256µg/ml	S	S	S	S	S			
			194	tem >256µg/ml	S	S	S	S	S			
66	1.5	BK - GB	195	S	S	S	S	S	S			
			196	S	S	S	S	S	S			
			197	S	S	S	S	S	S			
67	1	BK - GB	198	S	S	S	S	S	S			
			199	S	S	S	S	S	S			
			200	S	S	S	S	S	S			
135	2	Prof. Hart	380	S	S	S	S	S	S			
			381	S	S	S	S	S	S	*sta*		
			382	S	S	S	S	S	S			
174	2	Prof. Hart	518	S	S	S	S	S	S			
			519	S	S	S	S	S	S			
			520	S	S	S	S	S	S			
176	2	Prof. Hart	524	S	S	S	S	S	S	*eaeA*		
			525	S	S	S	S	S	S	*eaeA*		
			526	S	S	S	S	S	S	*eaeA*		
469	4	BK - GB	1521	S	S	S	S	S	S			
			1522	S	S	S	S	S	S			
			1523	S	S	S	S	S	S			
470	3	BK - GB	1524	S	S	S	S	S	S			
			1525	S	S	S	S	S	S			
			1526	S	S	S	S	S	S			
471	2.5	BK - GB	1527	tem 64µg/ml	S	S	S	S	S		*C. jejuni*	
			1528	tem 64µg/ml	S	S	S	S	S			
			1529	tem 64µg/ml	S	S	S	S	S			
472	3	BK - GB	1530	S	S	S	S	S	S			
			1531	S	S	S	S	S	S			
			1532	S	S	S	S	S	S			
529	4.5	SAH	1784	tem >256µg/ml	S	S	S	S	S			
			1785	tem 128µg/ml	S	S	S	S	S			
			1786	tem 64µg/ml	S	S	S	S	S			
640	3	SAH	2149	tem 128µg/ml	S	S	S	S	>256µg/ml			
			2150	S	S	S	S	S	S			
			2151	S	S	S	S	S	S			
674	2	SAH	2236	tem 128µg/ml	S	S	S	S	S			
			2237	tem 128µg/ml	S	S	S	S	S			
			2238	tem 64µg/ml	S	S	S	S	S			
690	4	SAH	2298	tem 64µg/ml	S	S	S	S	tetB 32µg/ml	S		
			2299	S	S	S	S	S	S			
			2300	tem 64µg/ml	S	S	S	S	tetB 32µg/ml	S		
947	2.5	Blue cross	3041	S	S	S	S	S	S			
			3042	S	S	S	S	S	S			
			3043	S	S	S	S	S	S			
948	4	Blue cross	negative									
949	2.5	Blue cross	3044	S	S	S	S	S	S			
			3045	S	S	S	S	S	S			
			3046	S	S	S	S	S	S			

950	2.5	Blue cross		3047	S	S	S	S	S	S			
				3048	S	S	S	S	S	S			
				3049	S	S	S	S	S	S			
951	3	Blue cross		3050	S	S	S	S	tetB 128µg/ml	S			
				3051	tem >256µg/ml	S	S	S	tetA 64µg/ml	>256µg/ml			
				3052	S	S	S	S	tetB 128µg/ml	S			
952	2.5	Blue cross	negative										
953	4	Blue cross		3053	S	S	S	S	S	S			
				3054	S	S	S	S	S	S			
				3055	S	S	S	S	S	S			
954	3	Blue cross		3056	tem >256µg/ml	S	catI >256µg/ml	S	tetA 128µg/ml	S			
				3057	tem >256µg/ml	S	catI >256µg/ml	S	tetA 64µg/ml	S			
				3058	S	S	S	S	S	S			
955	2	Blue cross		3059	S	S	S	S	S	S	*eaeA*		
				3060	S	S	S	S	S	S	*eaeA*		
				3061	S	S	S	S	S	S	*eaeA*		
Trimethoprim resistant isolates													
Culture no	Streptomycin	Spectinomycin	SMX										
2149	R	S	R										
3051	R	S	R										

Farm dog samples											
Sample no	F/C	*E. coli* culture no	amp	apra	chlor	nal	tet	trim	*E. coli* vir	*Campylobacter* spp.	
495	2.5	1635	S	S	S	S	S	S			
		1637	S	S	S	S	S	S			
496	2.5	1638	S	S	S	S	256µg/ml	S			
		1639	S	S	S	S	256µg/ml	S			
		1640	S	S	S	S	tetA 256µg/ml	S			
497	3	1641	S	S	S	S	S	S			
		1642	S	S	S	S	S	S			
		1643	S	S	S	S	S	S			
498	3	1644	S	S	S	S	S	S			
499	2	1647	S	S	S	S	tetA 64µg/ml	S			
		1648	S	S	S	S	tetA 128µg/ml	S			
		1649	S	S	S	S	tetA >256µg/ml	S			
518	2	1754	S	S	S	S	S	S			
		1755	S	S	S	S	S	S			
		1756	S	S	S	S	S	S			
519	2	1757	S	S	S	S	S	S			
		1758	S	S	S	S	S	S			
		1759	S	S	S	S	S	S			
156	3	469	S	S	S	S	S	S			
		470	S	S	S	S	S	S			
		471	S	S	S	S	S	S			
157	2.5	472	S	S	S	S	S	S			
		473	S	S	S	S	S	S			
		474	S	S	S	S	S	S			
158	3	475	tem 128µg/ml	S	S	S	tetA/tetB 64µg/ml	S			
		477	S	S	S	S	S	S			
159	3	478	S	S	S	S	S	S			
		479	S	S	S	S	S	S			
		480	S	S	S	S	tetA 32µg/ml	S			
160	3	481	S	S	S	S	S	S			
		482	S	S	S	S	S	S			
		483	S	S	S	S	S	S			
150	3.5	462	S	S	S	S	S	S			
		463	S	S	S	S	S	S			
		464	S	S	S	S	S	S			
151	2	465	tem 256µg/ml	S	S	S	tetA 128µg/ml	S			
152	2	466	S	S	cml 128µg/ml	S	tetA/tetB 128µg/ml	dfr1 >256µg/ml			
		467	S	S	S	S	S	S			
		468	tem >256µg/ml	S	S	S	tetA/tetB 256µg/ml	dfr8 >256µg/ml			
35	4	133	S	S	S	S	S	S			
		134	S	S	S	S	S	S			
		135	S	S	S	S	S	S			

36	2.5	136	tem 128µg/ml	S	S	S	S	dfr5 >256µg/ml			
		137	tem 128µg/ml	S	S	S	S	dfr5 >256µg/ml			
		138	tem 128µg/ml	S	S	S	S	dfr5 >256µg/ml			
37	2.5	127	tem 256µg/ml	S	S	S	tetA 64µg/ml	dfr5 >256µg/ml			
		128	tem >256µg/ml	S	S	S	tetA 64µg/ml	dfr5 >256µg/ml			
		129	S	S	S	S	S	S			
38	4	150	S	S	S	S	S	S			
		151	S	S	S	S	S	S			
		152	tem 256µg/ml	S	S	S	S	S			
39	4	124	tem 256µg/ml	S	S	S	S	dfr5 >256µg/ml			
		125	tem 256µg/ml	S	S	S	S	dfr5 >256µg/ml			
		126	tem 128µg/ml	S	S	S	S	dfr5 >256µg/ml			
721	3	negative									
						f/c - faecal consistency					

Trimethoprim resistant isolates				
sample no	E. coli culture	Streptomycin	Spectinomycin	SMX
152	466	S	R	S
	468	R	S	R
36	136	R	S	R
	137	R	S	R
	138	R	S	R
37	127	R	S	R
	128	R	S	R
39	124	R	S	R
	125	R	S	R
	126	R	S	R

Dogs on antibiotics for kennel cough

Sample no	Origin	F/C	*E. coli* culture no	Amp	Apra	Chlor	Nal	Tet	Trim	*E. coli* virulence	*Campylobacter* spp.
121	RSPCA - Halewood	4		339 tem >256µg/ml	S	catI >256µg/ml	S	tetB >256µg/ml	>256µg/ml		
				340 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
				341 tem >256µg/ml	S	catI >256µg/ml	S	tetB 256µg/ml	>256µg/ml		
122	RSPCA - Halewood	3.5		342 tem >256µg/ml	S	S	S	tetB 128µg/ml	>256µg/ml		
				343 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
123	RSPCA - Halewood	3		344 tem 256µg/ml	S	S	S	tetB >256µg/ml	S		*C. upsaliensis*
				345 tem 128µg/ml	S	catI >256µg/ml	>256µg/ml	tetB >256µg/ml	dfr17 128µg/ml		
				346 tem 128µg/ml	S	catI >256µg/ml	>256µg/ml	tetB 128µg/ml	dfr17 >256µg/ml		
124	RSPCA - Halewood	2.5		347 tem >256µg/ml	S	128µg/ml	>256µg/ml	tetB 256µg/ml	dfr1 >256µg/ml		
				348 tem >256µg/ml	S	catI >256µg/ml	>256µg/ml	tetB >256µg/ml	dfr7 >256µg/ml		
				349 tem >256µg/ml	S	S	S	tetB >256µg/ml	dfr7 >256µg/ml		
125	RSPCA - Halewood	3		350 tem >256µg/ml	S	S	S	tetB >256µg/ml	dfr1 >256µg/ml		
				351 tem >256µg/ml	S	S	S	tetB 256µg/ml	>256µg/ml		
				352 tem >256µg/ml	S	catI >256µg/ml	S	tetB >256µg/ml	>256µg/ml		
126	RSPCA - Halewood	4		353 S	S	S	S	tetA 128µg/ml	S		
				354 S	S	S	S	tetA 256µg/ml	S	*sta*	
				355 S	S	S	S	tetA 128µg/ml	S		
127	RSPCA - Halewood	3		356 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
				357 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
				358 tem >256µg/ml	S	S	S	tetB >256µg/ml	dfr1 >256µg/ml		
128	RSPCA - Halewood	2.5		359 >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
				360 >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
				361 >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
129	RSPCA - Halewood	4		362 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
				363 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
				364 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
130	RSPCA - Halewood	2.5		365 tem >256µg/ml	S	catI >256µg/ml	S	tetB >256µg/ml	>256µg/ml		
				366 tem >256µg/ml	S	catI >256µg/ml	S	tetB >256µg/ml	>256µg/ml		
				367 tem >256µg/ml	S	catI >256µg/ml	S	tetB 256µg/ml	dfr14 >256µg/ml		
131	RSPCA - Halewood	2.5		368 tem >256µg/ml	S	catI >256µg/ml	S	tetB >256µg/ml	dfr14 >256µg/ml		*C. upsaliensis*
				369 tem >256µg/ml	S	catI >256µg/ml	S	tetB 256µg/ml	dfr14 >256µg/ml		
				370 tem >256µg/ml	S	catI >256µg/ml	S	tetB >256µg/ml	dfr14 >256µg/ml		
132	RSPCA - Halewood	4.5		371 tem 256µg/ml	S	catI 128µg/ml	>256µg/ml	tetB 256µg/ml	dfr7 >256µg/ml		
				372 tem 256µg/ml	S	catI 32µg/ml	>256µg/ml	tetB 256µg/ml	dfr7 >256µg/ml		
				373 tem 256µg/ml	S	catI 256µg/ml	>256µg/ml	tetB 256µg/ml	dfr7 >256µg/ml		
133	RSPCA - Halewood	4		374 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
				375 tem >256µg/ml	S	S	S	tetB 256µg/ml	>256µg/ml		
				376 tem >256µg/ml	S	S	S	tetB 256µg/ml	>256µg/ml		

134	RSPCA - Halewood	4		377 >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		C. jejuni
				378 tem >256µg/ml	S	catI 256µg/ml	>256µg/ml	tetB 256µg/ml	dfr17 >256µg/ml		
				379 tem 256µg/ml	S	catI 256µg/ml	>256µg/ml	tetB 256µg/ml	dfr17 >256µg/ml		
727	RSPCA - Halewood		3	2447 tem >256µg/ml	S	S	S	tetA 256µg/ml	dfr14 >256µg/ml		
				2448 tem >256µg/ml	S	S	S	S	S		
				2449 tem >256µg/ml	S	S	S	tetA >256µg/ml	>256µg/ml		
728	RSPCA - Halewood	2		2450 S	S	S	S	S	S	cseA	
				2451 S	S	S	S	S	S		
				2452 S	S	S	S	S	S		
729	RSPCA - Halewood	3		2453 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
				2454 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
				2455 >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
730	RSPCA - Halewood	2.5		2456 S	S	S	S	S	S		
				2457 S	S	S	S	S	S		
				2458 S	S	S	S	S	S		
731	RSPCA - Halewood	3.5		2459 >256µg/ml	S	S	S	tetB >256µg/ml	S		
				2460 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
				2461 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
732	RSPCA - Halewood	2.5									
733	RSPCA - Halewood	3		2462 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
				2463 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
				2464 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
734	RSPCA - Halewood	2		2465 tem >256µg/ml	S	S	S	tetB 256µg/ml	256µg/ml		
				2466 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
				2467 tem >256µg/ml	S	S	S	tetB 256µg/ml	dfr1 >256µg/ml		
735	RSPCA - Halewood	2		2468 tem 64µg/ml	S	S	S	S	S	cseA	
				2469 tem >256µg/ml	S	S	S	tetB >256µg/ml	dfr1 >256µg/ml		
				2470 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
736	RSPCA - Halewood	2		2471 tem >256µg/ml	S	S	S	tetA >256µg/ml	S		
				2472 S	S	S	S	tetA 256µg/ml	dfr1 256µg/ml		
				2473 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
737	RSPCA - Halewood	2		2474 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
				2475 tem >256µg/ml	S	S	S	S	S		
				2476 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
738	RSPCA - Halewood	3.5		2477 tem >256µg/ml	S	S	S	S	S		
				2478 tem >256µg/ml	S	S	S	S	S		
				2479 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
739	RSPCA - Halewood	2		2480 S	S	S	S	S	>256µg/ml	cseA	
				2481 S	S	S	S	S	S		
				2482 S	S	S	S	S	S	cseA	

740	RSPCA - Halewood	2	2483	tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml
			2485	tem >256µg/ml	S	S	S	tetB 256µg/ml	>256µg/ml
			2486	>256µg/ml	S	S	S	S	>256µg/ml
741	RSPCA - Halewood	4	2487	tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml
			2488	tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml
			2489	tem >256µg/ml	S	S	S	tetB 256µg/ml	>256µg/ml

Trimethoprim resistant E. coli isolates

Sample no	Culture no	Streptomyc	Spectinomycin	SMX
121	339	S	S	R
	340	S	S	R
	341	S	S	R
122	342	R	S	R
	343	R	S	R
123	345	S	S	R
	346	R	S	R
124	347	R	S	R
	348	R	S	R
	349	S	S	R
125	350	S	S	R
	351	R	S	R
	352	R	S	R
127	356	R	R	R
	357	R	R	R
	358	S	R	R
128	359	R	R	R
	360	R	R	R
	361	R	R	R
129	362	S	R	R
	363	S	S	R
	364	S	S	R
130	365	S	S	R
	366	S	S	R
	367	R	R	R
131	368	R	R	R
	369	S	S	R
	370	S	S	R
132	371	R	S	R
	372	S	S	R
	373	R	S	R
133	374	S	S	R
	375	S	S	R
	376	S	S	R
134	377	R	R	R
	378	R	S	R
	379	R	S	R
727	2447	S	S	R
	2449	S	S	R
729	2453	S	S	R

	2454 S	S	R
	2455 S	S	R
731	2460 S	S	R
	2461 S	S	R
733	2462 S	S	R
	2463 S	S	R
	2464 S	S	R
734	2465 S	S	R
	2466 S	R	R
	2467 S	S	R
735	2469 S	S	R
	2470 S	S	R
736	2472 S	S	R
	2473 S	S	R
737	2474 S	S	R
	2476 S	S	R
738	2479 S	S	R
740	2483 S	S	R
	2484 S	S	R
	2485 S	S	R
741	2486 R	S	R
	2487 S	S	R
	2488 S	R	R

Household dog samples											
SampleNumber	Origin	F/C	CultureNumber	Amp	Apra	Chlor	Nal Acid	Tet	Trim	*E. coli* virulence	*Campylobacter* spp.
1	Leahurst	3	1	S	S	S	S	S	S		
			2	S	S	S	S	S	S		
			3	S	S	S	S	S	S		
2	Leahurst	2.5	4	S	S	S	S	S	S		*C. upsaliensis*
			5	S	S	S	S	S	S		
			6	S	S	S	S	S	S		
3	Leahurst	2	7	S	S	S	S	S	S		
			8	S	S	S	S	S	S		
			9	S	S	S	S	S	S		
5	Leahurst	2	10	tem >256µg/ml	S	S	S	tetB 256µg/ml	S		*C. upsaliensis*
			11	S	S	S	S	S	S		
			12	S	S	S	S	S	S		
6	Leahurst	4	negative								
7	Leahurst	2.5	147	S	S	S	S	S	S	*eaeA*	*C. upsaliensis*
			148	S	S	S	S	S	S	*eaeA*	
			149	S	S	S	S	S	S		
10	Leahurst	3.5	13	tem >256µg/ml	S	S	S	S	S		*C. upsaliensis*
			14	tem >256µg/ml	S	S	S	S	S		
			15	tem 128µg/ml	S	S	S	S	S		
11	Leahurst	3	16	S	S	S	S	S	S		
			17	S	S	S	S	S	S		
			18	S	S	S	S	S	S		
12	Leahurst	3	19	S	S	S	S	S	S		
13	Leahurst	2.5	20	S	S	S	S	S	S		
			21	S	S	S	S	S	S		
			22	S	S	S	S	S	S		
14	Leahurst	3.5	39	S	S	S	S	S	S	*eaeA*	*C. upsaliensis*
			40	S	S	S	S	S	S		
			41	S	S	S	S	S	S		
15	Leahurst	2	89	S	S	S	S	S	S		*C. upsaliensis*
			90	S	S	S	S	S	S		
			91	S	S	S	S	S	S		
31	Leahurst	4	92	S	S	S	S	S	S		
			93	tem >256µg/ml	S	catI >256µg/ml	>256µg/ml	tetB 256µg/ml	dfr1>256µg/ml		
			94	tem >256µg/ml	S	catI >256µg/ml	>256µg/ml	tetA 128µg/ml	dfr1>256µg/ml		
32	Leahurst	3.5	95	tem 128µg/ml	S	catI >256µg/ml	>256µg/ml	tetA/tetB >256µg/ml	>256µg/ml		
			96	S	S	S	S	S	S		
			97	tem 128µg/ml	S	catI >256µg/ml	>256µg/ml	tetB 256µg/ml	dfr7 >256µg/ml		
33	Leahurst	3.5	98	S	S	S	S	S		*eaeA*	
			99	S	S	S	S	S	S		
			100	S	S	S	S	S	S		
34	Leahurst	2	130	S	S	S	S	S	dfr1>256µg/ml		*C. upsaliensis*
			131	S	S	S	S	S	dfr1>256µg/ml		
			132	S	S	S	S	S	dfr1>256µg/ml		
40	Leahurst	3	139	S	S	S	S	S	S		

			140	tem 128µg/ml	S	S	S	S	S	
			141	S	S	S	S	S	S	
41	Leahurst	3	142	S	S	S	S	S	S	*eaeA*
			143	S	S	S	S	S	S	
			144	S	S	S	S	S	S	*eaeA*
42	Leahurst	2.5	121	S	S	S	S	S	S	*eaeA*
			122	S	S	S	S	S	S	
			123	S	S	S	S	S	S	
43	Leahurst	2	negative							
44	Leahurst	4	118	S	S	S	S	S	S	*Campylobacter spp.*
			119	S	S	S	S	S	S	
			120	S	S	S	S	S	S	
45	Leahurst	2.5	negative							
46	Leahurst	3.5	157	S	S	S	S	S	S	
			158	S	S	S	S	S	S	
47	Leahurst	2	160	S	S	S	S	tetB 64µg/ml	S	*C. upsaliensis*
48	Leahurst	4	negative							
49	Leahurst	3.5	153	tem >256µg/ml	S	S	S	tetA/tetB 256µg/ml	>256µg/ml	
			154	tem >256µg/ml	S	S	S	tetA/tetB 64µg/ml	dfr7 >256µg/ml	
			155	tem >256µg/ml	S	S	S	tetA/tetB 128µg/ml	dfr7 >256µg/ml	
52	Leahurst	2.5	173	S	S	S	S	S	S	
55	Dog Club	4	174	S	S	S	S	S	S	
			175	shv 128µg/ml	S	S	S	tetB >256µg/ml	dfr1>256µg/ml	
			176	S	S	S	S	S	S	
56	Dog Club	3.5	negative							
58	Dog Club	3	177	S	S	S	S	S	S	
			178	S	S	S	S	S	S	
			179	S	S	S	S	S	S	
59	Prof. Hart	3	181	S	S	S	S	S	S	
			182	S	S	S	S	S	S	
			183	S	S	S	S	S	S	
60	Prof. Hart	3	255	S	S	S	S	S	S	
			256	S	S	S	S	S	S	
			257	S	S	S	S	S	S	
61	Prof. Hart	4	184	S	S	S	S	S	S	
			185	S	S	S	S	S	S	
62	Prof. Hart	3.5	186	S	S	S	S	S	S	
			187	S	S	S	S	S	S	
63	Leahurst	2	188	tem 128µg/ml	S	catI 256µg/ml	S	S	dfr1>256µg/ml	
			189	256µg/ml	S	catI 256µg/ml	S	S	dfr1>256µg/ml	
90	Prof. Hart	2	258	S	S	S	S	S	S	
			259	S	S	S	S	S	S	
			260	S	S	S	S	S	S	
91	Prof. Hart	2.5	negative							
92	Leahurst	3.5	279	S	S	S	S	S	S	
			280	S	S	S	S	S	S	
			281	S	S	S	S	S	S	

93	Leahurst	4	282 S	S	S	S	S	S		
			283 S	S	S	S	S	S		
			284 S	S	S	4µg/ml	S	S		
94	Leahurst	3.5	450 tem >256µg/ml	S	S	S	S	S		
			451 tem >256µg/ml	S	S	S	tetB >256µg/ml	>256µg/ml		
			452 tem >256µg/ml	S	S	S	S	S		
95	Leahurst	2.5	539 S	S	S	S	S	S		
			540 S	S	S	S	S	S		
			541 S	S	S	S	S	S		
96	Leahurst	2	453 S	S	S	S	S	S		
			454 S	S	S	S	S	S		
			455 S	S	S	S	S	S		
97	Leahurst	2.5	456 S	S	S	S	S	S	*eaeA*	
			457 S	S	S	S	S	S	*eaeA*	
			458 S	S	S	S	S	S		
153	Dog Club	4	negative							
154	Dog Club	?	439 tem >256µg/ml	S	catI >256µg/ml	S	tetA 64µg/ml	>256µg/ml		
			440 tem >256µg/ml	S	catI >256µg/ml	S	tetA 64µg/ml	dfr5 >256µg/ml		
155	Dog Club	2.5	442 S	S	S	S	S	S		
			443 S	S	S	S	S	S		
161	Leahurst	3	484 S	S	S	S	S	S		
			485 S	S	S	S	S	S		
162	Dog Club	4	488 S	S	S	S	S	S		
			489 S	S	S	S	S	S		
			490 tem >256µg/ml	S	S	S	S	S		
163	Dog Club	3	491 S	S	S	S	S	S	*Campylobacter* spp.	
			492 S	S	S	S	S	S		
			493 S	S	S	S	S	S		
164	Dog Club	2.5	494 S	S	S	S	S	S		
			495 S	S	S	S	S	S		
			496 S	S	S	S	S	S		
165	Dog Club	3	497 S	S	S	S	S	>256µg/ml		
			498 S	S	S	S	S	S		
			499 S	S	S	S	S	S		
166	Dog Club	4	500 S	S	S	S	S	S		
			501 S	S	S	S	S	S		
			502 S	S	S	S	S	S		
167	Dog Club	3	504 S	S	S	S	S	S		
			505 S	S	S	S	S	dfr1>258µg/ml		
168	Dog Club	3	negative							
169	Dog Club	3.5	507 S	S	S	S	S	S	*Campylobacter* spp.	
170	Dog Club	4	509 S	S	S	S	S	S		
			510 S	S	S	S	S	S		
			511 S	S	S	S	S	S		
171	Dog Club	2.5	512 S	S	S	S	tetA 64µg/ml	S		
			513 S	S	S	S	tetA 64µg/ml	S		

		514 S	S	S	S	tetA 64µg/ml	S		
172	Dog Club 4	548 S	S	S	S	S	S		
		549 S	S	S	S	S	S		
		550 S	S	S	S	S	S		
173	Dog Club 4	515 S	S	S	S	S	S		
		516 S	S	S	S	S	S		
		517 S	S	S	S	S	S		
175	Prof. Hart 3	521 S	S	S	S	S	S		
		522 S	S	S	S	S	S		
		523 S	S	S	S	S	S		
176	Prof. Hart 4	524 S	S	S	S	S	S	*eaeA*	
		525 S	S	S	S	S	S	*eaeA*	
		526 S	S	S	S	S	S	*eaeA*	
180	Dog Club 3	536 S	S	S	S	S	S		
		537 tem 128µg/ml	S	S	S	S	dfr5 >256µg/ml		
		538 tem 256µg/ml	S	S	S	S	dfr5 >256µg/ml		
192	Leahurst 3	590 S	S	S	S	S	>256µg/ml		
		591 S	S	S	S	S	>256µg/ml		
		592 S	S	S	S	S	S		
193	Leahurst 3	593 S	S	S	S	S	S		
		594 S	S	S	S	S	>256µg/ml		
		595 S	S	S	S	S	S		
282	Dog Club 2.5	979 S	S	S	S	S	S		
		980 S	S	S	S	S	S		
		981 S	S	S	S	S	S		
293	Dog Club 2	982 S	S	S	S	S	S	*eaeA*	
		983 S	S	S	S	S	S	*eaeA*	
		984 S	S	S	S	S	S	*eaeA*	
294	Leahurst 2.5	985 S	S	S	S	S	S		
		986 S	S	S	S	S	S		
		987 S	S	S	S	S	S		
295	Leahurst 2.5	988 S	S	S	S	S	S		
		989 S	S	S	S	S	S		
		990 S	S	S	S	S	S		
296	Dog Club 2.5	991 S	S	S	S	S	S		
		992 S	S	S	S	S	S		
		993 S	S	S	S	S	S		
304	Dog Club 3	1012 S	S	S	S	S	S		
		1013 S	S	S	S	S	S		
		1014 S	S	S	S	S	S		
305	Dog Club 2.5	1015 S	S	S	S	S	S		
		1016 S	S	S	S	S	S		
		1017 S	S	S	S	S	S		
306	Dog Club 2.5	1018 S	S	S	S	S	S		
		1019 S	S	S	S	S	S		
		1020 S	S	S	S	S	S		
438	Leahurst 2	1389 S	S	S	S	S	S		
		1390 S	S	S	S	S	S	*eaeA*	

			1391	S		S	S	S	S	S	*eseA*		
468	Leahurst	3	1518	S		S	S	S	S	S			
			1519	S		S	S	S	S	S			
			1520	S		S	256µg/ml	S	S	S			
478	Leahurst	2.5	1587	S		S	S	S	S	S			
			1588	S		S	S	S	S	S			
			1589	S		S	S	S	S	S			
479	Leahurst	2.5	1590	S		S	S	S	S	S			
			1591	S		S	S	S	S	S			
			1592	S		S	S	S	S	S	*eseA*		
480	Leahurst	7	negative										
524	Leahurst	2.5	1772	S		S	S	S	S	S			
			1773	S		S	S	S	S	S			
			1774	S		S	S	S	S	S			
525	Leahurst	2	1775	S		S	S	S	S	S			*C. upsaliensis*
			1776	S		S	S	S	S	S			
			1777	S		S	S	S	S	S			
526	Leahurst	2	1778	S		S	S	S	S	S			*C. upsaliensis*
			1779	S		S	S	S	S	S			
			1780	S		S	S	S	S	S			
527	Leahurst	3	negative										
541	Leahurst	2.5	1820	tem 128µg/ml		S	S	S	tetB 256µg/ml	dfr14 >256µg/ml			
			1821	tem 256µg/ml		S	S	S	tetB 128µg/ml	dfr14 >256µg/ml			
			1822	tem 256µg/ml		S	S	S	tetB 256µg/ml	dfr14 >256µg/ml			
617	Sussex	2.5	2083	S		S	S	S	S	S			
			2084	S		S	S	S	S	S			
			2085	S		S	S	S	S	S			
625	Leahurst	3	2107	S		S	S	S	S	S			
			2108	S		S	S	S	S	S			
			2109	S		S	S	S	S	S			

Trimethoprim resistant E. coli isolates						
Sample no	Culture no	Streptomycin	Spectinomycin	SMX		
31	93	S	S	R	F/C - faecal consistency	
	94	S	S	R		
32	95	R	S	R		
	97	R	S	R		
34	130	S	S	S		
	131	S	S	S		
	132	S	S	S		
49	153	R	S	R		
	154	R	S	R		
	155	R	S	R		
55	175	R	R	R		
83	188	R	R	R		
	189	R	R	R		

94	451	S	R	R							
154	439	R	S	R							
	440	R	S	R							
185	497	R	S	R							
187	505	S	R	R							
180	537	R	S	R							
	538	R	S	R							
192	590	S	S	R							
	591	S	S	S							
193	594	S	S	S							
528	1781	S	S	R							
541	1820	R	R	R							
	1821	R	S	R							
	1822	R	R	R							

					Household data from questionnaires								
Sample no	Area	Breed	Age	Sex	AB-mth?	AB - yr?	AB - ever?	AB - time	D - 2 wks?	D - 2mths?	Other pets	food	Exercise
1	Leahurst	Dachshund	8yrs	F		N	Y		N		2xdog	T,D,S	park
3	Leahurst	Gorden setter	1yr	M		Y	Y		Y		2xdog	D,S	park
6	Leahurst	Boxer	1yr3mths	M		Y	Y		Y		1xdog	T	everywhere
11	Leahurst	Paterdale terrior	3yrs	M		N	Y		Y		1xhamster	D,S	everywhere
12	Leahurst	Border collie	3yrs	M		N	Y		Y		1xdog	D,S	park
13	Leahurst	Border collie	3yrs	M		N	N		Y		1xdog	D,S,	park
31	Leahurst	Hungarian puli	2yrs5mths	M		N	N		Y		1xdog, 1xgerbil	T,C	everywhere
32	Leahurst	Hungarian puli	3mths	M		N	N		N		1xdog, 1xgerbil	D,C	none
33	Leahurst	X-Breed	2yrs	M		N	N		Y		3xcats	T	
40	Leahurst	Lab	9yrs	F		N	N		N		None	T	park
41	Leahurst	Lab	12yrs	F		N	Y		N		2xdogs,3xpigs	T,D	garden
42	Leahurst	Lab	10yrs	F		N	N		N		2xdogs,3xpigs	T,D	park
43	Leahurst	Mongrel	9yrs	F		Y	N		N		2xdogs,3xpigs	T,D,	park
45	Leahurst	X-Breed	8mths	F		N	N		Y		1xdog,1xhamster,1xcat	D,S	beach
46	Leahurst	Wireherd terrior	4yrs	M		N	N		N		1xbudgie	D,S	park
48	Leahurst	Poodle	5yrs	M		Y	Y		Y		None	T,D,S	everywhere
49	Leahurst	Golden ret	11yrs6mth	M		Y	Y		Y		1xcockatiel	T,D,S	everywhere
52	Leahurst	X-Breed	8yrs	F		N	Y		Y		1xdog,1xcat	D,S,C	field
55	Casterton	Border collie	11yrs	F		N	N		N		1xdog	T,D	everywhere
56	Casterton	X-Breed	11yrs	M		N	N		N		1xdog	T,D	everywhere
58	South Lake	Retrever	5yrs	F		N	N		Y		None	D	Agriculture land
59	Med micro	Border collie	8yrs	F		N	Y		Y		1xcat	D	everywhere
60	Chorley	Border collie	6wks	F		N	N		N		None		garden
61	Crosby	Staff terrior	1yr7mths	F		N	N		Y		None	D	?
62	Formby	Mongrel	13yrs	M		N	N		N		None	D	everywhere
90	Med micro	X-Breed	8yrs	M		Y	Y		N		None	D	?
91	Med micro	Fox terrior	12yrs	F		Y	Y		N		None	D	?
94	Walton	X-Breed	8yrs	M	N	N	Ampicillin	7 days	N	N	2xdogs	D,S,C	beach
95	Burscough	X-Breed	?	F	N	Y	Y	2 weeks	N	N	None	D,C	field
96	Walton	X-Breed	10yrs	F	N	N	Ampicillin/ceporex	6wks/2wks	N	N	2xdogs	D,C	beach
97	Walton	X-Breed	7yrs	F	N	N	N		N	N	2xdogs	D,C	beach
153	Flint	X-Breed	9yrs	F	N	Y	Synulox	1wk	Y	Y	1xdog	S,C	park
154	Flint	X-Breed	16yrs	F	N	Y	Synulox	1wk	Y	Y	1xdog	S,C	park
155	West Derb	Cocker spaniel	1yr2mths	F	N	N	?		Y	N	1xdog	T,D,S	grassland
161	Neston	Jack russell	12yrs	F	Y	Y	Clamoxyl/acdin/antirobe	5days	N	Y	1xguinea pig	T,D,S	park
162	Moreton	GSD	5mths	M	N	N	N		N	Y	1xguinea pig, 2xtortoises	T,D,S	everywhere
164	Mancot	Bullmastiff	4mths	M	N	N	N		N	N	1xdog,3xguinea pigs, 1xhamster	D,C	none
165	Wallasey	X-Breed	8mths	F	Y	Y	ampicillin/duphatrim	5days	N	Y	2xcats	D,S,C	beach
166	Wirral	Kingcharles	3yrs	M	Y	Y	Y	1mth	N	Y	1xcat	D,C	beach
167	Chester	Pembroke corgi	11yrs	F	Y	Y	Amoxil	7days	N	N	1xdog,1xbudgie	T,D,C	beach
168	Chester	Pembroke corgi	8yrs	F	N	N	Y	?	N	N	1xdog,1xbudgie	T,D,C	beach
170	Wirral	Cocker spaniel	2yrs5mths	F	N	N	N		N	N	1xdog,2xcats,3guinea pigs	D,S	everywhere
171	Wirral	Mongrel	9yrs	M	N	N	N		N	N	1xdod,2xcats,3xguinea pigs	D,S	everywhere
172	Wallasey	Hungarian puli	3yrs10mths	M	Y	Y	rimadyl/tribrissali	7days	Y	Y	1xdog	T,D	beach
173	Wallasey	Hungarian puli	2yrs7mths	M	N	N	clamoxyl/zenecarp	7days	N	Y	1xdog	T,D	beach
175	St Helens	Golden ret	2yrs	M	Y	Y	Y	2wks	N	Y	cat, hamster	D,S	woods
180	West Kirby	X-Breed	10yrs	M	N	N	Y	?	N	N	None	D,S	beach
292	Meols	Golden ret	4mths	F	N	N	N		N	N	1xcat,1xguinea pig	T,D,C	?
293	Wirral	Shetland sheepdo	8mths	M	N	N	N		N	Y	1xcat	T,D,S	beach
296	Mancot	Golden ret	4yrs6mths	F	N	N	N		N	N	1xdog,2xguinea pigs,1xhamster	T,D,C	everywhere

05/11/2001	178	Dalmation (Max)	530	S	S	S	S	S	S						
			531	S	S	S	S	S	S						
			532	S	S	S	S	S	S						
16/11/2001	196		604	S	S	S	S	S	S						
			605	S	S	S	S	S	S						
			606	S	S	S	S	S	S						
29/01/2002	255	Griffin (Mickey)	852	S	S	R	S	S	S						
			853	R	S	R	S	S	R						
			854	R	S	R	S	S	R						
03/02/2002	259		867	R	S	R	S	S	R						
			868	R	S	R	S	S	R						
			869	R	S	R	S	S	R						
09/02/2002	263	GSD (Holly)	874	S	S	S	S	S	S						
			875	S	S	S	S	S	S						
			876	S	S	S	S	S	S						
17/03/2002	270		915	S	S	S	S	S	S						
			1174	R	S	S	S	S	S						
			916	S	S	S	S	S	S						
			917	S	S	S	S	S	S						
29/11/2001	216	Skarrel (Destiny)	723	R	S	S	S	S	S						
			724	S	S	S	S	S	S						
09/12/2001	219		1439	R	S	S	S	S	R						
			1440	R	S	S	S	S	R						
			1441	R	S	S	S	S	R						
13/05/2002	363	Spaniel (Speedy)	1152	S	S	S	S	S	S						
			1153	S	S	S	S	S	S						
			1154	R	S	S	S	R	R						
26/05/2002	387		1237	S	S	S	S	S	S						
			1238	S	S	S	S	S	S						
			1239	S	S	S	S	S	S						
13/03/2002	314	Spaniel X (Branston)	1033	S	S	S	S	S	S						
			1034	S	S	S	S	S	S						
			1035	S	S	S	S	S	S						
24/06/2002	409		1297	S	S	S	S	S	S						
			1298	S	S	S	S	S	S						
			1299	S	S	S	S	S	S						
24/02/2002	282	Staff (Axel)	949	S	S	S	S	S	S						
			950	S	S	S	S	S	S						
			951	R	S	S	S	S	S						
04/03/2002	289		970	S	S	S	R	S	S						
			971	S	S	S	R	S	S						
			972	S	S	S	R	S	S						
14/01/2002	231	Staff (Samson)	754	S	S	S	S	S	S						
			755	S	S	S	S	S	S						
			756	S	S	S	S	S	S						
06/05/2002	335		1087	R	S	R	S	R	S						
			1088	S	S	S	S	S	S						
			1089	R	S	R	S	R	S						
13/05/2002	368	Westie (Toby)	1168	S	S	S	S	S	S						
			1169	S	S	S	S	S	S						
			1170	S	S	S	S	S	R						
20/05/2002	381		1219	S	S	S	S	S	S						
			1220	S	S	S	S	S	S						
			1221	S	S	S	S	S	S						
07/01/2002	224	Whippet (Gabo)	736	S	S	S	S	S	S						
			737	S	S	S	S	S	S						
			738	S	S	S	S	S	S						
07/01/2002	227		745	S	S	S	S	S	S						
			746	S	S	S	S	S	S						
			747	S	S	S	S	S	S						
18/04/2002	321	X -breed (Benji)	1054	S	S	S	S	S	S						
			1055	S	S	S	S	S	S						
			1056	S	S	S	S	S	S						
14/04/2002	328		1072	S	S	S	S	S	S						
			1073	S	S	S	S	S	S						
			1074	S	S	S	S	S	S						
07/01/2002	225	X breed (Emma)	739	S	S	S	S	S	S						
			740	S	S	S	S	S	S						
			741	S	S	S	S	S	S						
14/01/2002	237		769	S	S	S	S	S	S						
			770	S	S	S	S	S	S						
			771	S	S	S	S	S	S						
09/12/2001	222	X breed (Molly)	1448	S	S	S	S	S	S						
			1449	S	S	S	S	S	S						
			1450	S	S	S	S	S	S						
28/01/2002	252		846	S	S	S	S	S	S						
			847	R	S	S	S	R	S						
			848	R	S	S	S	R	S						
13/05/2002	370	X breed (Pumpkin)	1195	S	S	S	S	S	S						
			1196	S	S	S	S	S	S						

Genomic DNA sequences for quinolone resistant *E. coli* strains

QyrA

E. coli strain and origin	Sequence	
	Ser-83	Asp-87
E. coli 0157 (accession number AP002560)	GAC TCG GCA GTT TAT GAC ACG	
BK – 213	GAC TTG GCG GTT TAT rAC ACG	
BK – 243	GAC TTG GCG GTT TAT AAC ACG	
T – 371	GAC TTG GCG GTC TAT AAC ACG	
R – 2532	GAC TCG GCG GTT TAT GAC ACG	
P – 2597	GAC TTG GCG GTC TAT AAC ACG	

ParC

E. coli strain and origin	Sequence	
	Ser-80	Glu-84
E. coli K12 (accession number U0096)	GAT AGT GCC TGT TAT GAA GCG	
BK – 213	GAT ATC GCC TGT TAT GrA GCG	
BK – 243	GAT ATC GCC TGT TAT GGA GCG	
T – 371	GAT ATC GCC TGT TAT GAA GCG	
R – 2532	GAT AGC GCC TGT TAT GAA GCG	
H – 94	GAT AkC GCC TGT TAT GAA GCG	
H – 284	GAT AGC GCC TGT TAT GAG GCG	

Sequencing unsure – k = G or T, r = A or G

Feacal samples from the Small Animal hospital - dogs with GI disease

Dog name	Sample no	symptoms	F/C	Breed	age	sex	*E. coli* culture no	Amp	Apra	Chlor	Nal	Tet	Trim	*E. coli* virulence	*Campylobacter* spp.
Robbie Burns	463	Chronic GI	5	YORKIE	1y2m	M	1503	tem 64µg/ml	S	S	S	tetB 32µg/ml	S		
							1504	tem 128µg/ml	S	S	S	tetB 32µg/ml	S		
							1505	tem >256µg/ml	S	S	>256µg/ml	tetB 256µg/ml	dfr17 >256µg/ml		
Poppy Bolton	464	Chronic GI	3	SHITZU	6y	F	1507	S	S	S	S	S	S		
							1508	S	S	S	S	S	S		
Lucy davies	465	Acute GI	5	COLLIE	0y2m	F	1509	S	S	S	S	S	S		*C. jejuni*
							1510	S	S	S	S	S	S		
							1511	S	S	S	S	S	S		
Scooby Marsden	466	Chronic GI	3	Cocker spaniel	0y11m	M	1512	S	S	S	S	S	S		
							1513	S	S	S	S	S	S	*eaeA*	
							1514	S	S	S	S	S	S	*eaeA*	
???	467	Acute GI	3			M	1515	S	S	S	S	S	S		*C. jejuni*
							1516	S	S	S	S	S	S		
							1517	S	S	S	S	S	S		
Sapphire Williams	481	Acute GI	4	Great Dane	2y	F	FALSE								
Sheba Taylor	500	Chronic GI	4.5	X - Breed	1y7m	F	1700	tem 256µg/ml	S	S	S	tetA 64µg/ml	dfr1 >256µg/ml		
							1701	tem 256µg/ml	S	S	S	tetA 64µg/ml	dfr1 >256µg/ml		
							1702	tem 256µg/ml	S	S	S	tetA 64µg/ml	dfr1 >256µg/ml		
	505	Acute GI	3	Bull mastiff	7y	F	1715	tem >256µg/ml	S	S	S	tetB 128µg/ml	dfr1 >256µg/ml		
							1716	tem >256µg/ml	S	S	S	tetB 256µg/ml	dfr1 >256µg/ml		
							1717	tem >256µg/ml	S	S	S	tetB 256µg/ml	dfr1 >256µg/ml		
Sandy Mooney	528	Acute GI	4	Staff bull terrior	2Y	m	1781	S	S	S	S	S	>256µg/ml		
							1782	S	S	S	S	S	S		
							1783	S	S	S	S	S	S		
Marty Hinden	551	Chronic GI	4.5	X - breed	8y8m	M	1850	S	S	S	S	S	S		
(repeat)							1851	tem 64µg/ml	S	catl 256µg/ml	>256µg/ml	tetB 128µg/ml	S		
							1852	S	S	S	S	tetB 256µg/ml	S		
Crunchie Dean	559	Chronic GI	4.5	Poodle	8y	M	FALSE								
Simba Goldring	564	Chronic GI	4	GSD	3y	M	1951	S	S	S	S	S	S		
							1952	S	S	S	S	S	S		
							1953	S	S	S	S	S	S		
Lassie Neary	567	Chronic GI	3	Border collie	8y2m	Female	1960	S	S	S	S	tetA 128µg/ml	S		
		antibiotics					1961	S	S	S	S	tetA 64µg/ml	S		
							1962	S	S	S	S	tetA 64µg/ml	S		
Tanner Walker	569	Chronic GI	3	SBT	8y	F	FALSE								
Coco Bisset	570	Chronic GI	4	X - breed	11y	F	1963	tem 64µg/ml	S	catl >256µg/ml	>256µg/ml	tetB 64µg/ml	dfr17 256µg/ml		
							1964	tem 256µg/ml	S	catl >256µg/ml	64µg/ml	tetB 64µg/ml	dfr17 >256µg/ml		
							1965	tem 256µg/ml	S	catl >256µg/ml	S	tetB 64µg/ml	S		
Sprockett	571	Acute GI	3.5	Border collie	4y	M	1966	S	S	S	S	S	S		
							1967	S	S	S	S	S	S		
							1968	S	S	S	S	S	S		
Rupert Downs	576	Acute GI	3	shih tzu	0y9m		FALSE								
Scamp Robinson	579	Chronic GI	2.5	X - breed	10yrs	M	1984	tem 256µg/ml	S	S	S	S	S		
							1985	256µg/ml	S	S	S	S	S		
							1986	tem 256µg/ml	S	S	S	tetA 64µg/ml	S		
Simba Goldring	583	Chronic GI	5	GSD	3y3m	M	1993	S	S	S	S	S	S		*Campylobacter* spp.
(repeat)							1994	S	S	S	S	S	S		
							1995	S	S	S	S	S	S		
Jess Hitch	584	Acute GI	3.5	X - terrier	1yrs	F	1996	S	S	S	S	S	S		
							1997	S	S	S	S	S	S		
							1998	S	S	S	S	S	S		
Scamp O'Neil	600	Chronic GI	3.5	Dalmation	1yrs8mth	M	2038	S	S	S	S	S	S		
							2039	S	S	S	S	S	S		
							2040	S	S	S	S	S	S		
Marty Hinden	608	Chronic GI	4	X - breed	8y7m	Male	2059	S	S	S	S	S	S	*eaeA, bfpA*	
(repeat)		antibiotics					2060	S	S	S	S	S	S	*eaeA, bfpA*	
							2061	S	S	S	S	S	S	*eaeA, bfpA*	
Barney Lloyd	609	Chronic GI	3	GSD	8y2m	Male	2062	S	S	S	S	S	S		
							2063	S	S	S	S	S	S		
							2064	S	S	S	S	S	S		
Badger Brown	610	Chronic GI	3.5	Australian shee	1y3m	Male	2065	S	S	S	S	S	S		
							2066	S	S	S	S	S	S		
							2067	S	S	S	S	S	S		

Oscar Bown	612	Chronic GI	4	Boxer	2yrs	Male	2068	S	S	S	S	S	S			
							2069	S	S	S	S	S	S			
							2070	S	S	S	S	S	S			
Chester Spanton	613	Acute GI	3	Golden retrieve	1y3m	Male	2071	tem >256µg/ml	S	S	S	tetA/tetD 64µg/ml	dfr5 >256µg/ml			
							2072	S	S	S	S	tetB 64µg/ml	S			
							2073	S	S	S	S	S	S			
Isaac Jones	626	Acute GI	3	Bearded collie	1y10m	Male	2110	S	S	S	S	S	S			
							2111	S	S	S	S	S	S			
							2112	S	S	S	S	S	S			
Murphy Kendall	627	Chronic GI	3.5	X - breed	12y	Male	2113	S	S	S	S	S	S			C. upsaliensis
							2114	S	S	S	S	S	S			
							2115	S	S	S	S	S	S			
Boozer Jones	628	Chronic GI	4	Dalmation	4y	Male	2116	tem 64µg/ml	S	S	S	tetB 256µg/ml	dfr14 >256µg/ml			
							2117	tem 256µg/ml	S	S	S	S	S			
							2118	S	S	S	S	tetB 128µg/ml	S			
Elton Roberts	633	Chronic GI	4.5	Bernesse moutain dog		Male	2131	S	S	S	S	S	S			
							2132	S	S	S	S	S	S			
							2133	S	S	S	S	S	S			
Half pint	635	Chronic GI	3.5	JRT	4y	Female	2134	S	S	S	S	S	S			C. upsaliensis
							2135	S	S	S	S	S	S			
							2136	S	S	S	S	S	S			
Branwen Jameson	641	Chronic vomiting	3.5	Leonberg	4y	Female	2155	S	S	S	S	S	S			
							2156	128µg/ml	S	S	S	tetB 64µg/ml	S			
							2157	S	S	S	S	S	S			
Marty Hinden	643	Chronic GI	3	X - breed	8y7m	Male	2161	S	S	S	S	S	S			
							2162	S	S	S	S	S	S			
							2163	S	S	S	S	S	S			
Shadow	650	Chronic GI		GSD	6yrs7mths	Male	FALSE									
No trouble	651	Acute GI		X - breed	5mth	Male	2173	S	S	S	S	S	S			
							2174	S	S	S	S	S	S			
							2175	S	S	S	S	S	S			
Half pint	655	Acute GI	3	JRT	4y	Female	2182	S	S	S	S	S	S			
(repeat)							2183	S	S	S	S	S	S			
							2184	S	S	S	S	S	S			
Murphy Kendall	656	Acute GI	2.5	X - breed	12y	Male	2185	tem >256µg/ml	S	cml >256µg/ml	256µg/ml	tetA 64µg/ml	dfr1 >256µg/ml			
(repeat)							2186	tem >256µg/ml	S	cml >256µg/ml	256µg/ml	tetA 64µg/ml	dfr1 >256µg/ml			
							2187	tem >256µg/ml	S	cml >256µg/ml	256µg/ml	tetA 64µg/ml	dfr1 >256µg/ml			
Marty Cross	657	Chronic GI	3.5	X - breed	8y7mths	Male	FALSE									
(repeat)																
No trouble	658	Acute GI	2	X - breed	5mths		2188	S	S	S	S	tetB 128µg/ml	S			
(repeat)		antibiotics					2189	S	S	S	S	S	S			
							2190	S	S	S	S	S	S			
No trouble	659	Acute GI	2.5	X - breed	5mths	Male	2191	S	S	S	S	S	S			
(repeat)							2192	S	S	S	S	S	S			
							2193	S	S	S	S	S	S			
No trouble	660	Acute GI	2	X - breed	5mths	Male	2194	S	S	S	S	tetB 128µg/ml	S			
(repeat)							2195	S	S	S	S	S	S			
							2196	S	S	S	S	S	S			
No trouble	661	Acute GI	2	X - breed	5mths	Male	2197	S	S	S	S	S	S			
(repeat)							2198	S	S	S	S	S	S			
							2199	S	S	S	S	S	S			
No trouble	662	Acute GI	2.5	X - breed	5mths	Male	2200	S	S	S	S	S	S			
(repeat)							2201	S	S	S	S	S	S			
							2202	S	S	S	S	S	S			
No trouble	663	Acute GI	3	X - breed	5mths	Male	2203	S	S	S	S	S	S			
(repeat)							2204	S	S	S	S	S	S			
							2205	S	S	S	S	S	S			
Toyah Mercer	669	Chronic GI		Great Dane			FALSE									
Hyatt	672	Chronic GI	3	Bull Mastiff	5yrs	Female	2230	S	S	S	S	S	S	eaeA		
							2231	S	S	S	S	S	S			
							2232	S	S	S	S	S	S			
??	673	Acute GI	2	Yorkshire terrie	6mths	Male	2233	S	S	S	S	S	S	eaeA		
							2234	S	S	S	S	S	S	eaeA		
							2235	S	S	S	S	S	S	eaeA		
????	675	Acute GI	3.5		5yrs	Male	2239	shv 64µg/ml	S	S	S	S	S			

								2240	tem 256µg/ml	S	S	256µg/ml	tetB 256µg/ml	dfr17 >256µg/ml		
								2241	tem >256µg/ml	S	S	256µg/ml	tetB >256µg/ml	dfr17 >256µg/ml		
	676	Acute GI	4	Shitzo	9mths	Male	FALSE									
Zak Ford	680	Chronic GI	4	GSD	6yrs7mths	Male		2250	tem >256µg/ml	S	S	256µg/ml	tetB >256µg/ml	dfr5 >256µg/ml		
		antibiotics						2251	S	S	S	S	S	dfr1 >256µg/ml	eaeA	
								2252	S	S	S	S	S	S		
Bertha Tierney	681	Chronic GI	4.5	Weim	7yrs	Female		2253	S	S	S	S	S	S		
								2254	S	S	S	S	S	S		
								2255	S	S	S	S	S	S		
Max Lester	685	Chronic GI	2	Golden retrever	10yrs	Male		2283	S	S	S	S	S	S		
								2284	S	S	S	S	S	S		
								2285	S	S	S	S	S	S		
Fudge Boyd	686	Acute GI	5	CKS	11yrs	Male		2286	S	S	S	S	S	S		
								2287	64µg/ml	S	S	S	S	S	eaeA	
								2288	S	S	S	S	S	S		
Jess Smith	687	Acute GI	3	SBT	6yrs	Female		2289	64µg/ml	S	S	S	R	S		
								2290	64µg/ml	S	S	S	R	S		
								2291	64µg/ml	S	S	S	R	S		
Cooke	698	Acute GI	3.5	Pointer	7yrs	Male		2322	S	S	S	S	S	S		
								2324	S	S	S	S	S	S		
								2325	S	S	S	S	S	S		
	699	Acute GI	2.5	Pointer	5yrs	Male		2326	S	S	S	S	S	S		
								2327	S	S	S	S	S	S		
								2328	S	S	S	S	S	S		
Cooke	700	Acute GI	3	Pointer	7yrs	Male		2329	S	S	S	S	S	S		
(repeat)								2330	S	S	S	S	S	S		
								2331	>256µg/ml	>256µg/ml	R	S	S	dfr14 >256µg/ml		
Cleo Pritchard	703	Acute GI	4	Weim	4yrs	Male		2338	S	S	S	S	S	S		
								2339	S	S	S	S	S	S		
								2340	S	S	S	S	S	S		
Olaf Lindfield	716	Acute GI	3	Lab	4yrs1mth	Male		2418	>256µg/ml	S	S	S	S	dfr14 >256µg/ml		
								2419	S	S	S	S	S	dfr5 >256µg/ml		
								2420	>256µg/ml	S	S	S	S	dfr14 >256µg/ml		
Zoe Highton	724	Acute GI	4	Boxer	4yrs2mths	Female		2436	S	S	S	S	S	S		
								2437	S	S	S	S	S	S		
								2438	S	S	S	S	S	S		
Bruno Hughes	784	Chronic GI	2.5	Boxer	7mths	Male		2615	S	S	S	S	S	S		
								2617	S	S	S	S	S	S		
								2618	S	S	S	S	S	S		
Bonnie Morrison	785	Chronic GI		X - breed	1yr	Female		2619	S	S	cati 256µg/ml	S	S	dfr12 >256µg/ml		
								2620	S	S	cati 256µg/ml	S	S	dfr12 >256µg/ml		
								2621	S	S	cati 256µg/ml	S	S	dfr12 >256µg/ml		
Champ Beesley	792	Acute GI		Boxer	5yrs10mth	Male		2640	S	S	S	S	S	S		
								2641	S	S	S	S	S	S		
								2642	S	S	S	S	S	S		

Feacal samples from the Small Animal Hospital - control dogs																
DOG NAME	SAMPLE NO	Group	GROUP	CONSISTENCY	BREED	AGE	SEX	E.coli cul no	AMP	APRA	CHLOR	NAL	TET	TRIM	E. coli virulenc	Campylobacter spp.
Meg Hope	530	Antibiotics		3	Boxer	4m	Female	1787	S	S	S	S	S	S		
		synulox						1788	S	S	S	S	S	S		
								1789	S	S	S	S	S	S		
Paddy Roche	531	Control	orthopaedic	3	Bullmastiff	4m	Male	1790	S	S	S	S	S	S		
								1791	S	S	S	S	S	S		
								1792	S	S	S	S	S	S		
Sally Greaves	532	Control	cardiovascular	3.5	Miniature schnauzer	12y	Female	1793	tem >256µg/ml	>256µg/ml	S	S	tetB 256µg/ml	dfrB 256µg/ml		
								1794	tem >256µg/ml	16µg/ml	S	S	tetB 256µg/ml	dfrB >256µg/ml		
								1795	tem 256µg/ml	16µg/ml	S	S	tetB 256µg/ml	dfrA1 256µg/ml		
Sode Bebb	533	Control	orthopaedic	2	Neopolitan	3y	Female	1796	256µg/ml	S	cefl >256µg/ml	S	S	>256µg/ml		
								1797	tem >256µg/ml	S	cefl >256µg/ml	S	S	>256µg/ml		
Menna Lloyd	534	Control	urogenital	4.5	Border collie	5m	Female	1799	S	S	S	S	S	S		
								1800	S	S	S	S	S	S		
Rufus McColl	535	Control	staff	3	Bernesse Mountain dog	6y6m	Male	1802	S	S	S	S	S	S		
								1803	S	S	S	S	S	S		
								1804	S	S	S	S	S	S		
Fred Edwards	552	Control		4	Basselt Hound	5y	Male	1853	S	S	S	S	S	S		
								1854	S	S	S	S	S	S		
								1855	S	S	S	S	S	S		
Jet Clarkeson	560	Control	staff	2	Border collie	5y	Male	1939	S	S	S	S	S	S		
								1940	S	S	S	S	S	S		
								1941	S	S	S	S	S	S		
Murphy Senior	561	Control	staff	2	X - breed	8y	Male	1942	S	S	S	S	S	S		
								1943	S	S	S	S	S	S		
								1944	S	S	S	S	S	S		
Arthur Innes	562	Control	staff	2.5	JRT	1y	Male	1945	S	S	S	S	S	S		
								1946	S	S	S	S	S	S		
								1947	S	S	S	S	S	S		
Tilly Senior	563	Control	staff	2.5	X - breed	5y	Female	1948	S	S	S	S	S	S		
								1949	S	S	S	S	S	S		
								1950	S	S	S	S	S	S		
Nancy Roberts	565	Control	orthopaedic	4	Bernesse Mountain dog	5y	Female	1954	tem >256µg/ml	>256µg/ml	S	S	tetA 256µg/ml	S		
								1955	tem >256µg/ml	>256µg/ml	S	S	tetA 256µg/ml	S		
								1956	S	S	S	S	S	S		
Manny Hanratty	566	Control	cardiovascular	2	Grt Dane	7m	Male	1957	S	S	S	S	S	S		
								1958	S	S	S	S	S	S		
								1959	S	S	S	S	S	S		
Dusty Hilton	568	Control	oncology	3	X - breed	8y	Female	FALSE								
Blaid Dean	572	Control	staff	2	Border collie	9y	NM	1969	S	S	S	S	S	S		
								1970	S	S	S	S	S	S		
								1971	S	S	S	S	S	S		
Benny Hardy	573	Control	staff	2	JRT	1y3m	Male	1972	S	S	S	S	S	S		
								1973	S	S	S	S	S	S		
								1974	S	S	S	S	S	S		
Gromit Dean	574	Control	staff	2.5	JRT	9y	NM	1975	S	S	S	S	S	S		
								1976	S	S	S	S	S	S		
								1977	S	S	S	S	S	S		
Jess Smith	575	Control	staff	2	SBTx	6y	NF	1978	S	S	S	S	S	S		
								1979	S	S	S	S	S	S		
								1980	S	S	S	S	S	S		
Roxy McDonough	577	Control		2	X - breed	1y	Female	1981	S	S	S	S	S	S		
								1982	S	S	S	S	S	S		
								1983	S	S	S	S	S	S		
Jasmin Carline	578	Control	endocrine	2	Border collie	3y7m	Female	FALSE								
Jeep Cobley	582	Control	respiratory	3	X - breed	5y1m	Male	1990	S	S	S	S	S	S		
								1991	S	S	S	S	S	S		
								1992	S	S	S	S	S	S		
Merlin Osbiston	585	Control	neurology	2.5	Pomeranian	5y6m	Male	1999	S	S	S	S	S	S		
								2000	S	S	S	S	S	S		
								2001	S	S	S	S	S	S		
Amber McGuinness	598	Control	oncology	3	SBT	11y5m	Female	2032	S	S	S	S	S	S		
								2033	S	S	S	S	S	S		
								2034	S	S	S	S	S	S		
Casper Smith	599	Control	respiratory	2.5	GSD	5y3m	Male	2035	tem 256µg/ml	S	S	S	tetB 128µg/ml	S		
								2036	S	S	S	S	S	S		
								2037	tem 256µg/ml	S	S	S	tetB 128µg/ml	S		
Polly Mills	601	Control	urogenital	2.5	Labrador	4y6m	Female	2041	S	S	S	S	tetA 256µg/ml	S		
								2042	S	S	S	S	tetA 128µg/ml	S		
								2043	S	S	S	S	tetA 64µg/ml	S		
Angel Cartwright	602	Control	urogenital	2	Boxer	4m	Male	2044	S	S	S	S	S	S		

								2045	S	S	S	S	S	S			
								2046	S	S	S	S	S	S			
Holly Carrey	603	Control	oesophageal	2	X - breed	5y7m	Female	2047	tem 256µg/ml	S	cmi 128µg/ml	>256µg/ml	tetB 64µg/ml	dfr17 >256µg/ml			
								2048	tem 256µg/ml	S	cmi 128µg/ml	>256µg/ml	tetB 64µg/ml	dfr17 >256µg/ml			
								2049	tem >256µg/ml	S	cmi 256µg/ml	>256µg/ml	tetB 256µg/ml	dfr17 >256µg/ml			
Clarence Cameron	604	Control	oncology	2	Norfolk terrier	10y3m	Male	2050	S	S	S	S	S	S	*eee, bfpe*		
								2051	S	S	S	S	S	S	*ste*		
								2052	S	S	S	S	S	S	*eee, bfpe*		
Benson Brown	605	Control	endocrine	2	Shar-pei	5m	Male	2053	S	S	S	S	S	S			
								2054	S	S	S	S	S	S			
								2055	S	S	S	S	S	S			
Henry Horrox	606	Control	orthopedics	3	WHWT	7y	Male	2056	S	S	S	S	S	S			
								2057	S	S	S	S	S	S			
								2058	S	S	S	S	S	S			
Jake Gerram	607	Control	oncology	3	Rhodesian ridgeback	5y1m	Male	FALSE									
Louie Wenham	611	Control		2.5	Boxer	2y	Male	FALSE									
Muffin Minto	616	Control	respiratory	3	Dalmation	4y3m	Female	2080	S	S	S	S	S	S			
								2081	S	S	S	S	S	S			
								2982	S	S	S	S	S	S			
Benji Schofield	629	Control		1.5	X - breed	10y	Male	2119	tem >256µg/ml	S	cmi 64µg/ml	>256µg/ml	tetB 128µg/ml	dfr17 >256µg/ml			
								2120	tem >256µg/ml	S	S	R	S	dfr1 >256µg/ml			
								2121	tem >256µg/ml	S	cmi 64µg/ml	>256µg/ml	tetB 128µg/ml	dfr17 >256µg/ml			
Charlie Partington	630	Control		2.5	Bulldog	5y	Male	2123	S	S	S	S	S	S			
								2124	S	S	S	S	S	S			
								2125	S	S	S	S	S	S			
Max Good	642	Antibiotics		3	X - breed	7y	Male	2158	tem 256µg/ml	S	S	S	S	S			
								2159	tem >256µg/ml	16µg/ml	cmi >256µg/ml	>256µg/ml	tetB 128µg/ml	dfr17 >256µg/ml			
								2160	tem >256µg/ml	>256µg/ml	cmi >256µg/ml	>256µg/ml	tetB 128µg/ml	dfr17 >256µg/ml			
Jody Harris	644	Control		2.5	X - breed	13y	Female	2164	tem >256µg/ml	S	S	S	S	S			
								2165	tem 256µg/ml	S	S	S	S	S			
								2166	tem 256µg/ml	S	S	S	S	S			
Max Ulsten	652	control		2.5			Male	2176	S	S	S	S	S	S			
								2177	S	S	S	S	S	>256µg/ml			
								2178	tem >256µg/ml	S	S	S	S	dfr3 >256µg/ml			
Kylie Ulsten	653	Control		2.5			Female	2215	S	S	S	S	S	S			
								2216	S	S	S	S	S	S			
								2217	S	S	S	S	S	S			
Busa Ulsten	654	Control		2.5				2179	S	S	S	S	S	S			
								2180	S	S	S	S	S	S			
								2181	S	S	S	S	S	S	*eee, bfpe*		
Buster Gresham	668	Control		3	SBT	4yrs	Male	2218	tem 256µg/ml	>256µg/ml	S	>256µg/ml	S	>256µg/ml			*C. upsaliensis*
								2219	S	S	S	S	S	S	*eee*		
								2220	S	S	S	S	S	S			
Bracken Smith	679	Control		3	Patterdale	3yrs7mths	Female	2247	S	S	S	S	S	S	*eee*		
								2248	S	S	S	S	S	S	*eee*		
								2249	S	S	S	S	S	S	*eee*		
Thames	701	Control		3	Welm	4yrs	Female	2332	S	S	S	S	S	S			
								2333	S	S	S	S	S	S	*eee*		
								2334	S	S	S	S	S	S			
Barney	702	Control		2.5	Boxer	6yrs4mths	Male	2335	S	S	S	S	S	S	*eee*		*C. upsaliensis*
								2336	tem >256µg/ml	S	S	S	S	dfr17 >256µg/ml	*eee*		
								2337	S	S	S	S	S	S			
Zoe Highton	766	Antibiotics			Boxer	4yrs2mths	Female	2562	tem >256µg/ml	S	S	>256µg/ml	S	dfr1 >256µg/ml			
		metacam						2563	tem 256µg/ml	S	ceti >256µg/ml	>256µg/ml	tetB >256µg/ml	dfr17 >256µg/ml			
		fentanyl						2564	tem >256µg/ml	S	S	>256µg/ml	tetB 256µg/ml	dfr1 >256µg/ml			

A4

Human faecal sample questionnaire.

The purpose of this questionnaire is to find out the preferred method of collecting faecal samples from healthy people. It is completely anonymous and will be used to give an idea of the preferred methods of collection.

Age group: (Please circle) <21 21-31 32-41 42-51 >52

Gender: Female Male

Occupation:_____

Which of the methods below would you prefer to collect the sample?

(1 most preferred method, 3 least preferred method, please circle)

Method 1 - Toilet paper – after defecation, putting the used toilet paper into a sterile container.

1 2 3

Method 2 – Moist sponge – (use instead of toilet paper) after defecation, putting the used sponge into a sterile container.

1 2 3

Method 3 – Lining the toilet with toilet paper and using a swap or spatula to take a sample of faeces.

1 2 3

How often would you be prepared to give this sample? (please circle)

1. Once a week
2. Once every two weeks
3. Once a month
4. Once every six weeks
5. Only once

If you have children would you be willing to collect samples from them? (please circle)

1. Would collect samples only from my children
2. Would collect samples from both children and myself
3. Would just collect samples from myself
4. Have no children

If you have a partner would they be willing to provide a sample? (Please circle)

1. Yes
2. No
3. Unsure
4. Not applicable

How you would prefer the samples to be returned? (Please circle)

1. By post
2. By handing to your veterinary surgeon
3. Somebody to collect the sample from your house

Is there anything else that you think would make collecting faecal samples more appealing?

_____

_____

_____

_____

Compliance questionnaire.

We wish to identify the most appropriate way of collecting faecal samples from people and their pets to help aid research studies in human and veterinary medicine.
Please imagine that you have been asked to participate in a research study that would involve providing faecal samples from yourself, your dog and / or other members of your family. There would also be a short questionnaire to fill in.

1. How would you prefer to be approached about the study? (Please circle)

- Letter
- Telephone
- Personal interview

2. How would you prefer the questionnaire information to be collected? (Please circle)

- Personal interview
- Brief questionnaire
- Telephone
- No preference

3. Would you be more willing to take part in this study if you knew someone who was already taking part? (Please circle)

- Yes
- No
- No preference

4. Would the organisation conducting the study affect your decision to take part? (Please circle)

- No
- Yes

- If yes, please put in preference order (1 highest preference, 3 lowest preference) which organisations involvement would most influence you to take part in the study.

- Public health authority **1 2 3**
- University research group **1 2 3**
- Charity research group **1 2 3**
- No preference

5. If there was an incentive to take part in the study, e.g. free dog treats, would you be more likely to consider taking part? (Please circle)

- Yes
- No
- No preference

6. Would your decision to take part in this study be influenced by the fact that it is aimed more towards helping? (Please circle)

- your dog and his/her health
- you and your family's health
- both

7. Would you want to be informed of the results of the study carried out on your dog? (Please circle)

- Yes
- No
- No preference

8. Would you want to be informed on the results of the completed study as a whole? (Please circle)

- Yes
- No
- No preference

9. What advice would you give researchers to encourage other people to take part in the study?

_____

_____

_____

_____

Human samples									
Sample no	Subject	Type of sample	*E. coli* culture no			Sample no	Subject	Type of sample	*E. coli* culture no
339	1	Sponge	1096			359	11	Sponge	1143
			1097						1144
			1098						1145
340	1	Scoop	1099			360	11	Scoop	1146
			1100						1147
			1102						1148
341	2	Sponge	1102			361	12	Sponge	negative
			1103			362	12	Scoop	1149
342	2	Scoop	1104						1150
			1105						1151
343	3	Sponge	1106			371	13	Sponge	1198
			1107						1199
			1108						1200
344	3	Scoop	1109			372	13	Scoop	1201
			1110						1202
			1111						1203
345	4	Sponge	1112			373	14	Sponge	negative
			1113			372	14	Scoop	negative
			1114						
346	4	Scoop	1115						
			1116						
			1117			411	1	toilet paper - MR	1317
347	5	Sponge	1118						1318
348	5	Scoop	1119						1319
			1120			412	1	Scoop	1320
			1121						1321
349	6	Sponge	1122						1322
			1123			413	2	toilet paper - MR	1323
			1124						1324
350	6	Scoop	1125						1325
			1126			414	2	Scoop	1326
			1127						1327
351	7	Sponge	1128						1328
			1129			415	3	toilet paper - MR	1329
			1130						1330
352	7	Scoop	1131						1331
			1132			416	3	Scoop	1332
			1133						1333
353	8	Sponge	1134						1334
			1135			417	4	toilet paper - MR	1335
			1136						1336
354	8	Scoop	negative						1337
355	9	Sponge	1137			418	4	Scoop	1338
			1138						1339
			1139						1340
356	9	Scoop	1140			419	5	toilet paper - cb	1341
			1141						1342
			1142						1343
357	10	Sponge	negative			420	5	Scoop	1344
358	10	Scoop	negative						1345

Sample no	Subject	Type of sample	*E. coli* culture no
421	6	toilet paper - MRD	1347
			1348
			1349
422	6	Scoop	1350
			1351
			1352
423	7	toilet paper - cb	1353
			1354
			1355
424	7	Scoop	1356
			1357
			1358
425	8	toilet paper - cb	Negative
426	8	Scoop	Negative
427	9	toilet paper - MRD	1359
			1360
			1361
428	9	Scoop	1362
			1363
			1364
429	10	toilet paper - MRD	1365
			1366
			1367
430	10	Scoop	1368
			1369
			1370
431	11	toilet paper - cb	1371
			1372
			1373
432	11	Scoop	1374
			1375
			1376
433	12	toilet paper - MRD	1377
			1378
			1379
434	12	Scoop	1380
			1381
			1382
435	13	toilet paper - MRD	1383
			1384
			1385
436	13	Scoop	1386
			1387
			1388
443	14	toilet paper - cb	negative
444	14	Scoop	negative

A5

Sample information

Owner name .....

Dog name ..... **Date** .....

Has your dog suffered from diarrhoea since the last sample was taken?

No ☐ Yes ☐

Has your dog had any treatment from your vet since the last sample was taken?

No ☐ Yes ☐

If yes, what condition was it for?.....

Has your dog been wormed since that last sample was taken?

No ☐ Yes ☐

Has your dog had contact with other dogs?

Walks ☐ Dog club / obedience class ☐ Other .....

Have any members of your household suffered from diarrhoea since the last sample was taken?

No ☐ Yes ☐

- **If yes, how many members of your household did it affect?**

  Adults ☐ Children ☐

Are there any other comments you would like to make?

.....

.....

.....

.....

.....

.....

.....

THE UNIVERSITY
of LIVERPOOL

Longitudinal study information sheet

I would like to thank you for agreeing to take part in this study. I would like to stress that all information and results obtained are confidential.

I will contact you within the next week to answer any questions or queries that you may have on the study. I have provided contact details so please feel free to contact me with any problems or questions you have before then.

There is a questionnaire enclosed, which I would be grateful if you would fill in, and send back as soon as possible using the prepaid envelope. The brown envelope that you have been given contains pots for one week's samples (from yourself and your dog) and provisions for collection or posting. Samples will be collected once a week for 2 months and then once a month. Instructions for sample collection are with the pots provided. Please could you keep the contents of the envelope, both before and after sample collection in a cool place.

Any problems please do not hesitate to contact me.

Siân Wilson

Calling all puppy owners!!!

If you have recently adopted a puppy then we would like your help!

The veterinary pathology department at the University of Liverpool is conducting a study involving puppies and their owners.

We are interested in finding out about the types of bacteria that can affect dogs and humans alike. Both dogs and humans naturally carry lots of harmless bacteria, and many of them are actually good for us. When harmful bacteria cause infections they can be treated with antibiotics, however, bacteria are becoming increasingly resistant to many antibiotics and this is a great concern for both vets and doctors. This study would allow us to see just how similar the bacteria from your intestines are from those found in your dog. It would involve taking faecal samples from your dog over a period of six months and we would also like to take samples from yourself (used toilet paper is all we would need).

A study like this has never been done before and the results could be very interesting and useful to both humans and dogs alike.

If you feel that you could help us in our research, or wish to know more about the study then please contact me on the below details.

Sian Wilson
Department of Veterinary Pathology
University of Liverpool
Leahurst
Neston
CH64 7TE
Tel: 0151 794 6012
Email: J.S.Wilson@liverpool.ac.uk

After defecation

Using the white pot (human samples)

1. Using a gloved hand, take the toilet paper provided
2. Wipe as normal
3. Fold toilet paper and place into white pot containing liquid
4. Close lid tightly, shake pot so all the toilet paper is covered in liquid
5. Place the white pot into the white transport container and store in a cool place until returned.

Using the blue pot (dog samples)

1. Use the scoop attached inside the lid of the blue pot to collect a large, single scoop of dog faeces.
2. Put the scoop back into the pot and close lid tightly
3. Place the blue pot into white transport container and store in a cool place until returned.

Could you please write the date the samples were collected and your surname on the pots and fill in the small questionnaire provided. Please return the blue questionnaire with the samples.

Posting – please place the pots into separate transport containers and put these in jiffy bag provided. Place the jiffy bag into the self addressed, pre-paid envelope to post.

Collection – please place pots into the jiffy bag to be taken to the veterinary surgery or to be collected.

Household A														
Sample no	Origin	Date taken	Date processed	*E. coli* culture no	*E. coli* virulence	*Campylobacter* spp.	Sample no	Origin	Date taken	Date processed	*E. coli* culture no	*E. coli* virulence	*Campylobacter* spp.	
677	Dog	11/07/2003	14/07/2003	2242			1191	dog	13/01/2004	15/01/2004	3699			
				2243							3700			
				2244	*eaeA*						3701			
678	Human	11/07/2003	14/07/2003	2245			1192	human	13/01/2004	15/01/2004	3702			
				2246							3704			
682	Dog	17/07/2003	21/07/2003	2256	*eaeA*						3705			
				2257	*eaeA*		1358	human	07/04/2004	14/07/2004	4089			
				2258	*eaeA*						4090			
683	Human	17/07/2003	21/07/2003	2259							4091			
				2260			1357	dog	07/04/2004	14/04/2004	4086			
				2261							4087			
691	Dog	25/07/2003	28/07/2003	2301	*eaeA*						4088			
				2302	*eaeA*									
				2303										
692	Human	25/07/2003	28/07/2003	2304										
				2305										
				2306										
696	Dog	30/07/2003	31/07/2003	2316	*eaeA*									
				2317	*eaeA*									
				2318	*eaeA*									
697	Human	30/07/2003	31/07/2003	2319										
				2321										
708	Dog	14/08/2003	19/08/2003	2394										
				2395										
				2396										
709	Human	14/08/2003	19/08/2003	2397										
				2398										
717	Dog	10/09/2003	11/09/2003	2421										
				2422										
				2423										
718	Human	10/09/2003	11/09/2003	2424										
				2425										
				2426										
725	Dog	18/09/2003	22/09/2003	2441										
				2442										
				2443										
726	Human	18/09/2003	22/09/2003	2444										
				2445										
				2446										
851	Dog	01/11/2003	04/11/2003	2795										
				2796										
				2797										
852	Human	01/11/2003	04/11/2003	2798										
				2799										
1014	dog	28/11/2003	02/12/2003	3223										
				3224										
				3225										
1015	human	28/11/2003	02/12/2003	3226										
				3227										
				3228										

Household B							
Sample no	Origin	Date taken	Date processed	*E. coli* culture no	*E. coli* virulence	*Campylobacter* spp.	
762	Dog	19/09/2003	21/09/2003	2550			
				2551			
				2552			
763	Human	19/09/2003	21/09/2003	2553			
				2554			
				2555			
786	Dog	30/09/2003	06/09/2003	2622			
				2623			
				2624			
787	Human	30/09/2003	06/09/2003	2625			
				2626			
				2627			
801	Dog	13/10/2003	15/10/2003	2664			
				2665			
				2666			
802	Human	13/10/2003	15/10/2003	2667			
				2668			
				2669			
824	Dog	24/10/2003	27/10/2003	2724			
				2725			
				2726			
825	Human	24/10/2003	27/10/2003	negative			
864	Dog	05/11/2003	07/11/2003	2820			
				2821			
				2822			
865	human	05/11/2003	07/11/2003	2823			
				2824			
				2825			
1150	human	07/01/2004	08/01/2004	3589			
				3590			
				3591			
1149	dog	07/01/2004	08/01/2004	3586			
				3587			
				3588			
1264	dog	07/02/2004	10/02/2004	3893			
				3894			
				3895			
1265	human	07/02/2004	10/02/2004	3896			
				3897			
				3898			

Household C							
Sample no	Origin	Date taken	Date processed	*E. coli* culture no	*E. coli* virulence	*Campylobacter* spp.	
764	Dog	15/09/2003	17/09/2003	2556			
				2557			
				2558			
765	Human	15/09/2003	17/09/2003	2559			
				2560			
				2561			
788	Dog	02/10/2003	06/10/2003	2628			
				2629			
				2630			
789	Human	02/10/2003	06/10/2003	2631			
				2632			
				2633			
841	Dog	30/10/2003	31/10/2003	2770			
				2771			
				2772			
842	Human	30/10/2003	31/10/2003	negative			
891	Dog	12/11/2003	13/11/2003	2888			
				2889			
				2890			
892	Human	12/11/2003	13/11/2003	2891			
				2892			
				2893			
1160	human	08/01/2004	19/01/2004	negative			
1161	dog	08/01/2004	19/01/2004	3613			
				3614			
				3615			
1195	human	18/01/2004	19/01/2004	3712			
				3713			
				3714			
1196	dog	18/01/2004	19/01/2004	3715			
				3716			
				3717			
1231	human	27/01/2004	03/02/2004	3799			
				3780			
				3781			
1231	dog	27/01/2004	03/02/2004	3802			
				3803			
				3804			
1293	human	21/02/2004	24/02/2004	negative			
1294	dog	21/02/2004	25/02/2004	3966			
				3967			
				3968			
1369	dog	24/04/2004	25/04/2004	4131			
				4132			
				4133			
1370	human	24/04/2004	25/04/2004	4134			
				4135			
				4136			

Household D							
Sample no	Origin	Date taken	Date processed	*E. coli* culture no	*E. coli* virulence	*Campylobacter* spp.	
790	Dog	03/10/2003	06/10/2003	2634			
				2635			
				2636			
791	Human	03/10/2003	06/10/2003	2637			
				2638			
				2639			
816	Dog	12/10/2003	22/10/2003	2703	*eaeA*		
				2704	*eaeA*		
				2705	*eaeA*		
817	Human	12/10/2003	22/10/2003	2706			
				2707			
				2708			
818	Dog	22/10/2003	23/10/2003	2709			
				2710			
				2711	*sta*		
819	Human	22/10/2003	23/10/2003	2712			
				2713			
				2714			
860	Dog	05/11/2003	06/11/2003	2814			
				2815			
				2816			
861	Human	05/11/2003	06/11/2003	2817			
				2818			
				2819			

Household E							
Sample no	Origin	Date taken	Date processed	*E. coli* culture no	*E. coli* virulence	*Campylobacter* spp.	
793	Dog	06/10/2003	07/10/2003	negative			
794	Human	06/10/2003	07/10/2003	negative			
803	Dog	16/10/2003	17/10/2003	2670	*sta*		
				2671	*sta*		
				2672	*sta*		
804	Human	16/10/2003	17/10/2003	2673			
				2674			
				2675			
822	Dog	24/10/2003	27/10/2003	2718			
				2719			
				2720			
823	Human	24/10/2003	27/10/2003	2721			
				2722			
				2723			
843	Dog	30/10/2003	31/10/2003	2773			
				2774			
				2775			
844	Human	30/10/2003	31/10/2003	2776			
				2777			
				2778			
866	Dog	05/11/2003	07/10/2003	2826			
				2827			
				2828			
867	Human	05/11/2003	07/10/2003	2829			
				2830			
				2831			
878	Dog	11/11/2003	12/11/2003	negative			
879	Human	11/11/2003	12/11/2003	2857			
				2858			
				2859			
938	dog	18/11/2003	21/11/2003	3017	*eaeA*	*C. upsaliensis*	
				3018	*eaeA*		
				3019	*eaeA*		
939	human	18/11/2003	21/11/2003	3020			
				3021			
				3022			
1040	dog	01/12/2003	03/12/2003	3358			
				3359			
				3360			
1041	human	01/12/2003	03/12/2003	3361			
				3362			
				3363			
1153	human	07/01/2004	08/01/2004	3598			
				3599			
				3600			
1152	dog	07/01/2004	08/01/2004	3595			
				3596			
				3597			
1266	dog	12/02/2004	14/02/2004	3905			
				3906			
				3907			
1269	human	12/02/2004	14/02/2004	3908			
				3909			
				3910			

Household F							
Sample no	Origin	Date taken	Date processed	*E. coli* culture no	*E. coli* virulence	*Campylobacter* spp.	
795	Dog	04/10/2003	07/10/2003	2649			
				2650			
				2651			
796	Human	04/10/2003	07/10/2003	2652			
				2653			
				2654			
799	Dog	11/10/2003	15/10/2003	2661	*eaeA*		
				2662	*eaeA*		
				2663	*eaeA*		
800	Human	11/10/2003	15/10/2003	negative			
814	Dog	18/10/2003	22/10/2003	2697	*eaeA*		
				2698	*eaeA*		
				2699	*eaeA*		
815	Human	18/10/2003	22/10/2003	2700			
				2701			
				2702			
820	Dog	26/10/2003	27/10/2003	2717			
821	Human	26/10/2003	27/10/2003	negative			
856	Dog	04/11/2003	05/11/2003	2803			
				2804			
857	Human	04/11/2003	05/11/2003	2805			
				2806			
				2807			
899	dog	13/11/2003	17/11/2003	negative			
900	human	13/11/2003	17/11/2003	2912			
				2913			
				2914			
1223	dog	24/01/2004	26/01/2004	3790			
				3791			
				3792			
1224	human	24/01/2004	26/01/2004	3793			
				3794			
				3795			

Household G															
Sample no	Origin	Date taken	Date processed	*E. coli* culture no	*E. coli* virulence	*Campylobacter* spp.	Sample no	Origin	Date taken	Date processed	*E. coli* culture no	*E. coli* virulence	*Campylobacter* spp.		
807	Dog	14/10/2003	17/10/2003	2682			1245	dog	02/02/2004	05/02/2004	3841				
				2683							3842				
				2684							3843				
808	Human - CA	14/10/2003	17/10/2003	2685			1246	human-JA	02/02/2004	05/02/2004	negative				
				2686			1247	human-CA	02/02/2004	05/02/2004	3844				
				2687			1321	dog	20/03/2004	22/03/2004	negative				
809	Human - JA	14/10/2003	17/10/2003	negative			1322	human - C	20/03/2004	22/03/2004	4032				
810	Dog	20/10/2003	21/10/2003	2688	*eaeA*						4033				
				2689	*eaeA*						4034				
				2690			1323	human - JA	20/03/2004	22/03/2004	negative				
811	Human - JA	20/10/2003	21/10/2003	negative			1366	human-CA	20/04/2004	22/04/2004	4125				
812	Human - CA	20/10/2003	21/10/2003	2691							4126				
				2692							4127				
				2693			1367	human-JA	20/04/2004	22/04/2004	negative				
838	Dog	28/10/2003	30/10/2003	2764			1368	dog	20/04/2004	22/04/2004	4129				
				2765							4130				
				2766											
839	Human - JA	28/10/2003	30/10/2003	negative											
840	Human - CA	28/10/2003	30/10/2003	2767											
				2768											
				2769											
853	Dog	03/11/2003	04/11/2003	negative											
854	Human - CA	03/11/2003	04/11/2003	2800											
				2801											
				2802											
855	Human - JA	03/11/2003	04/11/2003	negative											
882	Dog	09/11/2003	12/11/2003	2866											
				2867											
				2868											
883	Human - CA	09/11/2003	12/11/2003	2869											
				2870											
				2871											
884	Human - JA	10/11/2003	12/11/2003	negative											
935	dog	18/11/2003	20/11/2003	3014											
				3015											
				3016											
936	human - JA	18/11/2003	20/11/2003	negative											
937	human - CA	18/11/2003	20/11/2003	negative											
1001	dog	24/11/2003	27/11/2003	3187											
				3188											
				3189											
1002	human - CA	24/11/2003	27/11/2003	3190											
				3191											
				3192											
1003	human - JA	24/11/2003	27/11/2003	negative											
1156	dog	05/01/2004	09/01/2004	3610											
				3611											
				3612											
1157	human-JA			negative											
1158	human-CA			negative											

Household H														
Sample no	**Origin**	**Date taken**	**Date processed**	***E. coli* culture no**	***E. coli* virulence**	***Campylobacter* spp.**	**Sample no**	**Origin**	**Date taken**	**Date processed**	***E. coli* culture no**	***E. coli* virulence**	***Campylobacter* spp.**	
826	Dog	23/10/2003	28/10/2003	2731	*eaeA*		1167	dog	12/01/2004	14/01/2004	3631			
				2732							3632			
				2733	*eaeA*						3633			
827	Human	23/10/2003	28/10/2003	2734			1168	human	12/01/2004	14/01/2004	3635			
				2735							3636			
				2736			1266	dog	08/02/2004	16/02/2004	3899	*eaeA*		
858	Dog	30/10/2003	05/11/2003	2808	*eaeA*						3900	*eaeA*		
				2809	*eaeA*						3901			
				2810	*eaeA*		1267	human	08/02/2004	16/02/2004	3902			
859	Human	30/10/2003	05/11/2003	2811							3903			
				2812							3904			
				2813			1319	dog	08/03/2004	10/03/2004				
889	Dog	09/11/2003	13/11/2003	2884	*eaeA*									
				2885	*eaeA*									
				2886	*eaeA*		1320	human	08/03/2004	10/03/2004				
890	Human	09/11/2003	13/11/2003	2887A										
				2887B										
				2887C			1359	dog	17/04/2004	20/04/2004				
901	dog	14/11/2003	18/11/2003	2915										
				2916										
				2917			1360	human	17/04/2004	20/04/2004				
902	human	14/11/2003	18/11/2003	2918										
				2919										
				2920										
943	dog	20/11/2003	21/11/2003	3029	*eaeA, bfpA*									
				3030	*eaeA, bfpA*									
				3031	*eaeA, bfpA*									
944	human	20/11/2003	21/11/2003	3032										
				3033										
				3034										
969	dog	23/11/2003	25/11/2003	3092										
				3093										
				3094										
970	human	23/11/2003	25/11/2003	3095										
				3096										
				3097										
1030	dog	30/11/2003	03/12/2003	3271										
				3272										
				3273										
1031	human	30/11/2003	03/12/2003	3295										
				3296										
				3297										
1084	dog	07/12/2003	11/12/2003	3310										
				3311										
				3312										
1085	human	07/12/2003	11/12/2003	3313										
				3314										
				3315										

Household I							
Sample no	Origin	Date taken	Date processed	*E. coli* culture no	*E. coli* virulence	Campylobacter spp.	
836	dog	28/10/2003	30/10/2003	2758			
				2759			
				2760			
837	human	28/10/2003	30/10/2003	2761			
				2762			
				2763			
1012	dog	30/11/2003	01/12/2003	3217			
				3218			
				3219			
1013	human	30/11/2003	01/12/2003	3220			
				3221			
				3222			
1154	dog	09/01/2004	09/01/2004	3394			
				3395			
				3396			
1155	human	09/01/2004	09/01/2004	3397			
				3398			
				3399			
1272	dog	19/02/2004	19/02/2004	negative			
1273	human	19/02/2004	19/02/2004	negative			

Household J													
Sample no	Origin	Date taken	Date processed	*E. coli* culture no	*E. coli* virulence	*Campylobacter* spp.	Sample no	Origin	Date taken	Date process	*E. coli* culture no	*E. coli* virulence	*Campylobacter* spp.
832	Bella			2749			1170	barbie	12/01/2004	14/01/2004	3640		
				2750							3641		
				2751							3642		
833	Human			2752			1165	bella	12/01/2004	14/01/2004	3625		
				2753							3626		
				2754							3627		
834	Barbie			negative			1207	human	19/01/2004	21/01/2004	3748		
835	Kiwi			2755							3749		
				2756							3750		
				2757			1206	kiwi	19/01/2004	21/01/2004	3751		
847	Barbie	03/11/2003	04/11/2003	2785							3752		
				2786							3753		
				2787			1220	barbie	19/01/2004	22/01/2004	negative		
848	Kiwi	03/11/2003	04/11/2003	2788			1225	human	26/01/2004	27/01/2004	negative		
				2789			1126	barbie	26/01/2004	27/01/2004	3796		
				2790			1127	kiwi	26/01/2004	27/01/2004	negative		
849	Bella	03/11/2003	04/11/2003	2791			1128	bella	26/01/2004	27/01/2004	negative		
				2792			1237	kiwi	02/02/2004	05/02/2004	3817		
850	human	03/11/2003	04/11/2003	2793							3818		
				2794							3819		
885	Kiwi	10/11/2003	12/11/2003	2872			1238	barbie	02/02/2004	05/02/2004	3820		
				2873							3821		
				2874							3822		
886	Barbie	10/11/2003	12/11/2003	2875			1239	human	02/02/2004	05/02/2004	3823		
				2876							3824		
				2877							3825		
887	Bella	10/11/2003	12/11/2003	2878			1240	bella	02/02/2004	05/02/2004	3826		
				2879							3827		
				2880							3828		
888	Human	10/11/2003	12/11/2003	2882			1313	bella	08/03/2004	10/03/2004	4011		
				2883							4012		
HOLIDAY											4013		
1083	human	09/12/2003	11/12/2003	3307			1314	barbie	08/03/2004	10/03/2004	4014		
				3308							4015		
				3309							4016		
1071	barbie	09/12/2003	10/12/2003	3424			1315	kiwi	08/03/2004	10/03/2004	4017		
				3425							4018		
				3426							4019		
1072	kiwi	09/12/2003	10/12/2003	3427			1316	human	08/03/2004	10/03/2004	negative		
				3428			1361	kiwi	17/04/2004	20/04/2004	negative		
				3429			1362	bella	17/04/2004	20/04/2004	4119		
1082	bella	09/12/2003	11/12/2003	3304							4120		
				3305							4121		
				3306			1363	barbie	17/04/2004	20/04/2004	negative		
1166	human	12/01/2004	14/01/2004	3628			1364	human	17/04/2004	20/04/2004	4122		
				3629							4123		
				3630							4124		
1169	kiwi	12/01/2004	14/01/2004	3637									
				3638									
				3639									

Household K							
Sample no	**Origin**	**Date taken**	**Date processed**	***E. coli* culture no**	***E. coli* virulence**	***Campylobacter* spp.**	
845	dog	30/10/2003	31/10/2003	2779	*eaeA*		
				2780			
				2781			
846	human	30/10/2003	31/10/2003	2782			
				2783			
				2784			
893	dog	11/11/2003	14/11/2003	2894	*eaeA*		
				2895	*eaeA*		
				2896	*eaeA*		
894	human	11/11/2003	11/11/2003	2897			
				2898			
				2899			
1038	dog	29/11/2003	01/12/2003	3292			
				3293			
				3294			
1039	human	29/11/2003	01/12/2003	3355			
				3356			
				3357			

Household L							
Sample no	Origin	Date taken	Date processed	*E. coli* culture no	*E. coli* virueInce	*Campylobacter spp.*	
862	human	05/11/2003	not processed				
863	dog	05/11/2003	not processed				
897	dog	13/11/2003	14/11/2003	2906			
				2907			
				2908			
898	human	13/11/2003	14/11/2003	2909			
				2910			
				2911			
945	dog	21/11/2003	24/11/2003	3035			
				3036			
				3037			
946	human	21/11/2003	24/11/2003	3038			
				3039			
				3040			
1010	dog	28/11/2003	01/12/2003	3211			
				3212			
				3213			
1011	human	28/11/2003	01/12/2003	3214			
				3215			
				3216			
1076	dog	09/12/2003	11/12/2003	3436	*eaeA*		
				3437	*eaeA*		
				3438			
1077	human	09/12/2003	11/12/2003	3439			
				3440			
				3441			

Household M							
Sample no	Origin	Date taken	Date processed	*E. coli* culture no	*E. coli* virulence	*Campylobacter* spp.	
868	dog	24/10/2003	not processed				
869	human	24/10/2003	not processed				
905	dog	11/11/2003	18/11/2003	2927	*eaeA*		
				2928	*eaeA*		
				2929	*eaeA*		
906	human	11/11/2003	18/11/2003	2930			
				2931			
				2932			
1008	dog	28/11/2003	01/12/2003	3205			
				3206			
				3207			
1009	human	28/11/2003	01/12/2003	3208			
				3209			
				3210			
1060	dog	02/12/2003	08/12/2003	negative			
1061	human	02/12/2003	08/12/2003	3412			
				3413			
				3414			
1104	dog	13/12/2003	16/12/2003	3460			
				3461			
				3462			
1105	human	13/12/2003	16/12/2003	3463			
				3464			
				3465			
1222	human	22/01/2004	26/01/2004	negative			
1221	dog			3787			
				3788			
				3789			
1129	dog	27/01/2004	03/02/2004	negative			
1130	human			negative			
1317	dog	29/02/2004	10/03/2004	negative			
1318	human			4020			
				4021			
				4022			

Household O														
Sample no	Origin	Date taken	Date processed	E. coli culture no	E. coli virulence	Campylobacter spp.	Sample no	Origin	Date taken	Date processed	E. coli culture no	E. coli virulence	Campylobacter spp.	
870	Human	10/11/2003	11/11/2003	2835			1088	wispa	11/12/2003	12/12/2003	3322			
				2836							3323			
				2837							3324			
871	Kizzie	10/11/2003	11/11/2003	2838			1089	kizzie	11/12/2003	12/12/2003	3325			
				2839							3326			
				2840							3327			
880	Bonnie	10/11/2003	12/11/2003	2860			1090	bonnie	11/12/2003	12/12/2003	3328			
				2861							3329			
				2862							3330			
881	Wispa	10/11/2003	12/11/2003	2863			1128	human	05/01/2004	06/01/2004	3524			
				2864							3525			
				2865							3526			
932	human	17/11/2003	20/11/2003	3005			1125	bonnie	05/01/2004	06/01/2004	3515			
				3006							3516			
				3007							3517			
903	kizzie	17/11/2003	19/11/2003	2921			1126	kizzie	05/01/2004	06/01/2004	3518			
				2922							3519			
				2923							3520			
904	wispa	17/11/2003	19/11/2003	2924			1127	wispa	05/01/2004	06/01/2004	3521			
				2925							3522			
				2926							3523			
931	bonnie	17/11/2003	20/11/2003	3002			1175	human	12/01/2004	14/01/2004	negative			
				3003			1176	wispa	12/01/2004	14/01/2004	3656			
				3004			1189	kizzie	12/01/2003	15/01/2003	3693			
1005	human	24/11/2003	27/11/2003	3196							3694			
				3197							3695			
				3198			1190	bonnie	12/01/2004	15/01/2004	3696			
986	wispa	24/11/2003	26/11/2003	3146							3697			
				3147							3698			
				3148			1201	human	19/01/2004	21/01/2004	3730			
987	kizzie	24/11/2003	26/11/2003	3149							3731			
				3150							3732			
				3151			1200	bonnie	19/01/2004	21/01/2004	3727			
1004	bonnie	24/11/2003	27/11/2003	3193							3728			
				3194							3729			
				3195			1204	kizzie	19/01/2004	21/01/2004	3739			
1042	human	01/12/2003		3364							3740			
				3365							3741			
				3366			1205	wispa	19/01/2004	21/01/2004	3742			
1032	wispa	01/12/2003		3274							3743			
				3275							3744			
				3276			1292	human	23/02/2004	24/02/2004	negative			
1033	kizzie	01/12/2003		3277			1289	kizzie	23/02/2004	24/02/2004	3957			
				3278							3958			
				3279							3959			
1034	bonnie	01/12/2003		3280			1290	wispa	23/02/2004	24/02/2004	3960			
				3281							3961			
				3282							3962			
1091	human	11/12/2003	12/12/2003	3331			1291	bonnie	23/02/2004	24/02/2004	3963			
				3332							3964			
				3333							3965			
							1324	human	22/03/2004	23/03/2004	negative			
							1325	wispa	22/03/2004	23/03/2004	negative			

Sample no	Origin	Date taken	Date processed	*E. coli* culture no	*E. coli* virulence	*Campylobacter* spp.									
1326	kizzie	22/03/2004	23/03/2004	negative											
1327	bonnie	22/03/2004	23/03/2004	negative											
1388	bonnie	27/04/2004	29/04/2004	4182											
				4183											
				4184											
1389	kizzie	27/04/2004	29/04/2004	4185											
				4186											
				4187											
1392	wispa	27/04/2004	29/04/2004	4191											
				4192											
				4193											
1393	human	27/04/2004	29/04/2004	4194											
				4195											
				4196											

Household P														
Sample no	Origin	Date taken	Date processed	E. coli culture no	E. coli virulence	Campylobacter spp.	Sample no	Origin	Date taken	Date processed	E. coli culture no	E. coli virulence	Campylobacter spp.	
926	human	17/11/2003	19/11/2003	2987			980	thomas	24/11/2003	26/11/2003	3131			
				2989							3132			
929	bertie	17/11/2003	10/11/2003	2996							3133			
				2997			981	harvey	24/11/2003	26/11/2003	3134			
				2998							3135			
930	polly	17/11/2003	10/11/2003	2999							3136			
				3000			982	bertie	24/11/2003	26/11/2003	negative			
				3001			984	barney	24/11/2003	26/11/2003	3140			
928	barney	17/11/2003	10/11/2003	2993							3141			
				2994							3142			
				2995			985	billy	24/11/2003	26/11/2003	3143			
927	pip	17/11/2003	10/11/2003	2990							3144			
				2991							3145			
				2992			1055	human	02/12/2003	08/12/2003	3397			
925	dotty	17/11/2003	10/11/2003	2984							3398			
				2985							3399			
				2986			1048	pip	02/12/2003	08/12/2003	3376			
924	dylan	17/11/2003	10/11/2003	2981							3377			
				2982							3378			
				2983			1049	ted	02/12/2003	08/12/2003	3379			
923	travis	17/11/2003	10/11/2003	2978							3380			
				2979							3381			
				2980			1050	billy	02/12/2003	08/12/2003	3382			
922	thomas	17/11/2003	10/11/2003	2975							3383			
				2976							3384			
				2977			1051	barney	02/12/2003	08/12/2003	3385			
921	harvey	17/11/2003	10/11/2003	2972							3386			
				2973							3387			
				2974			1052	thomas	02/12/2003	08/12/2003	3388			
920	ted	17/11/2003	10/11/2003	2969							3389			
				2970							3390			
				2971			1053	harvey	02/12/2003	08/12/2003	3391			
919	billy	17/11/2003	10/11/2003	negative							3392			
983	human	24/11/2003	26/11/2003	3137							3393			
				3138			1054	bertie	02/12/2003	08/12/2003	3394			
974	dylan	24/11/2003	26/11/2003	3107							3395			
				3108							3396			
				3109			1056	dotty	02/12/2003	08/12/2003	3400			
975	travis	24/11/2003	26/11/2003	3110							3401			
				3111							3402			
				3112			1057	polly	02/12/2003	08/12/2003	3403			
976	polly	24/11/2003	26/11/2003	3113							3404			
				3114							3405			
				3115			1058	dylan	02/12/2003	08/12/2003	3406			
977	dotty	24/11/2003	26/11/2003	3128							3407			
				3129							3408			
				3130			1059	travis	02/12/2003	08/12/2003	3409			
978	pip	24/11/2003	26/11/2003	3122							3410			
				3123							3411			
				3124			1103	human	10/12/2003	12/12/2003	3457			
979	ted	24/11/2003	26/11/2003	3125							3458			
				3126			1092	harvey	10/12/2003	12/12/2003	negative			
				3127										

Sample no	Origin	Date taken	Date processed	*E. coli* culture no	*E. coli* virulence	*Campylobacter* spp.	Sample no	Origin	Date taken	Date processed	*E. coli* culture no	*E. coli* virulence	*Campylobacter* spp.
1093	thomas	10/12/2003	12/12/2003	3334			1146	polly	07/01/2004	08/01/2004	3577		
				3335							3578		
				3336							3579		
1094	polly	10/12/2003	12/12/2003	negative			1147	dylan	07/01/2004	08/01/2004	3580		
1095	bertie	10/12/2003	12/12/2003	3337							3581		
				3338							3582		
				3339			1148	Barney	07/01/2004	08/01/2004	3583		
1096	barney	10/12/2003	12/12/2003	3340							3584		
				3341							3585		
				3342			1177	human	12/01/2004	15/01/2004	3658		
1097	ted	10/12/2003	12/12/2003	3343							3659		
				3344							3660		
				3345			1178	Harvey	12/01/2004	15/01/2004	3661		
1098	billy	10/12/2003	12/12/2003	3346							3662		
				3347							3663		
				3348			1179	Dylan	12/01/2004	15/01/2004	3664		
1099	pip	10/12/2003	12/12/2003	3349							3665		
				3350							3666		
				3351			1180	travis	12/01/2004	15/01/2004	3667		
1100	dylan	10/12/2003	12/12/2003	3448							3668		
				3449							3669		
				3450			1181	thomas	13/01/2004	14/01/2004	3670		
1101	travis	10/12/2003	12/12/2003	3451							3671		
				3452							3672		
				3453			1182	ted	13/01/2004	14/01/2004	3673		
1102	dotty	10/12/2003	12/12/2003	3454							3674		
				3455							3675		
				3456			1183	billy	13/01/2004	14/01/2004	3676a		
1151	human	07/01/2004	08/01/2004	3592							3676b		
				3593							3677		
				3594			1184	pip	13/01/2004	14/01/2004	3678		
1129	dotty	07/01/2004	08/01/2004	3527							3679		
				3528							3680		
				3529			1185	bertie	13/01/2004	14/01/2004	3681		
1130	bertie	07/01/2004	08/01/2004	3530							3682		
				3531							3683		
				3532			1186	polly	13/01/2004	14/01/2004	3684		
1131	pip	07/01/2004	08/01/2004	3533							3685		
				3534							3686		
				3536			1187	dotty	13/01/2004	14/01/2004	3687		
1132	travis	07/01/2004	08/01/2004	3537							3688		
				3538							3689		
				3539			1188	barnry	13/01/2004	14/01/2004	3690		
1133	ted	07/01/2004	08/01/2004	3540							3691		
				3541							3692		
				3542			1236	human	02/02/2004	05/02/2004	3814		
1134	billy	07/01/2004	08/01/2004	3543							3815		
				3544							3816		
				3546			1233	billy	02/02/2004	05/02/2004	3805		
1135	harvey	07/01/2004	08/01/2004	negative							3806		
1136	thomas	07/01/2004	08/01/2004	3547							3807		
				3548			1234	pip	02/02/2004	05/02/2004	3808		
				3549							3809		
											3810		

Sample no	Origin	Date taken	Date processed	*E. coli* culture no	*E. coli* virulence	*Campylobacter* spp.		Sample no	Origin	Date taken	Date processed	*E. coli* culture no	*E. coli* virulence	*Campylobacter* spp.	
1093	thomas	10/12/2003	12/12/2003	3334				1146	polly	07/01/2004	08/01/2004	3577			
				3335								3578			
				3336								3579			
1094	polly	10/12/2003	12/12/2003	negative				1147	dylan	07/01/2004	08/01/2004	3580			
1095	bertie	10/12/2003	12/12/2003	3337								3581			
				3338								3582			
				3339				1148	Barney	07/01/2004	08/01/2004	3583			
1096	barney	10/12/2003	12/12/2003	3340								3584			
				3341								3585			
				3342				1177	human	12/01/2004	15/01/2004	3658			
1097	ted	10/12/2003	12/12/2003	3343								3659			
				3344								3660			
				3345				1178	Harvey	12/01/2004	15/01/2004	3661			
1098	billy	10/12/2003	12/12/2003	3346								3662			
				3347								3663			
				3348				1179	Dylan	12/01/2004	15/01/2004	3664			
1099	pip	10/12/2003	12/12/2003	3349								3665			
				3350								3666			
				3351				1180	travis	12/01/2004	15/01/2004	3667			
1100	dylan	10/12/2003	12/12/2003	3448								3668			
				3449								3669			
				3450				1181	thomas	13/01/2004	14/01/2004	3670			
1101	travis	10/12/2003	12/12/2003	3451								3671			
				3452								3672			
				3453				1182	ted	13/01/2004	14/01/2004	3673			
1102	dotty	10/12/2003	12/12/2003	3454								3674			
				3455								3675			
				3456				1183	billy	13/01/2004	14/01/2004	3676a			
1151	human	07/01/2004	08/01/2004	3592								3676b			
				3593								3677			
				3594				1184	pip	13/01/2004	14/01/2004	3678			
1129	dotty	07/01/2004	08/01/2004	3527								3679			
				3528								3680			
				3529				1185	bertie	13/01/2004	14/01/2004	3681			
1130	bertie	07/01/2004	08/01/2004	3530								3682			
				3531								3683			
				3532				1186	polly	13/01/2004	14/01/2004	3684			
1131	pip	07/01/2004	08/01/2004	3533								3685			
				3534								3686			
				3536				1187	dotty	13/01/2004	14/01/2004	3687			
1132	travis	07/01/2004	08/01/2004	3537								3688			
				3538								3689			
				3539				1188	barnry	13/01/2004	14/01/2004	3690			
1133	ted	07/01/2004	08/01/2004	3540								3691			
				3541								3692			
				3542				1236	human	02/02/2004	05/02/2004	3814			
1134	billy	07/01/2004	08/01/2004	3543								3815			
				3544								3816			
				3546				1233	billy	02/02/2004	05/02/2004	3805			
1135	harvey	07/01/2004	08/01/2004	negative								3806			
1136	thomas	07/01/2004	08/01/2004	3547								3807			
				3548				1234	pip	02/02/2004	05/02/2004	3808			
				3549								3809			
												3810			

Sample no	Origin	Date taken	Date processed	*E. coli* culture no	*E. coli* virulence	*Campylobacter* spp.	Sample no	Origin	Date taken	Date processed	*E. coli* culture no	*E. coli* virulence	*Campylobacter* spp.
1235	travis	02/02/2004	05/02/2004	3812			1261	thomas	09/02/2004	11/02/2004	3884		
				3813							3885		
				3814							3886		
1241	dylan	02/02/2004	05/02/2004	3829			1262	barney	09/02/2004	11/02/2004	3887		
				3830							3888		
				3831							3889		
1242	polly	02/02/2004	05/02/2004	3832			1277	human	18/02/2004	23/02/2004	3921		
				3833							3922		
				3834							3923		
1243	bertie	02/02/2004	05/02/2004	3835			1278	billy	18/02/2004	23/02/2004	3924		
				3836							3925		
				3837							3926		
1244	dotty	02/02/2004	05/02/2004	3838			1279	harvey	18/02/2004	23/02/2004	3927		
				3839							3928		
				3840							3929		
1248	ted	02/02/2004	11/02/2004	3847			1280	dotty	18/02/2004	23/02/2004	3930		
				3848							3931		
				3849							3932		
1249	harvey	02/02/2004	11/02/2004	3850			1281	ted	18/02/2004	23/02/2004	3933		
				3851							3934		
				3852							3935		
1250	thomas	02/02/2004	11/02/2004	3853			1282	dylan	18/02/2004	23/02/2004	3936		
				3854							3937		
				3855							3938		
1251	barney	02/02/2004	11/02/2004	3856			1283	bertie	18/02/2004	23/02/2004	3939		
				3857							3940		
				3858							3941		
1263	human	09/02/2004	11/02/2004	3890			1284	polly	18/02/2004	23/02/2004	3942		
				3891							3943		
				3892							3944		
1252	bertie	09/02/2004	11/02/2004	3860			1285	thomas	18/02/2004	23/02/2004	3945		
				3861							3946		
				3862							3947		
1253	dylan	09/02/2002	11/02/2004	3863			1286	barney	18/02/2004	23/02/2004	3948		
				3864							3949		
				3865							3950		
1254	billy	09/02/2004	11/02/2004	negative			1287	pip	18/02/2004	23/02/2004	3951		
1255	ted	09/02/2004	11/02/2004	3866							3951		
				3867							3953		
				3868			1288	travis	18/02/2004	23/02/2004	3954		
1256	harvey	09/02/2004	11/02/2004	3869			1354	human	18/02/2004	23/02/2004	negative		
				3870			1343	pip	24/03/2004	29/03/2004	negative		
				3871			1344	harvey	24/03/2004	29/03/2004	4104		
1257	polly	09/02/2004	11/02/2004	negative							4105		
1258	dotty	09/02/2004	11/02/2004	3875							4106		
				3876			1345	dylan	24/03/2004	29/03/2004	4107		
				3877							4108		
1259	pip	09/02/2004	11/02/2004	3878							4109		
				3879			1346	bertie	24/03/2004	29/03/2004	4110		
				3880							4111		
1260	travis	09/02/2004	11/02/2004	3881							4112		
				3882			1347	dotty	24/03/2004	29/03/2004	4113		
				3883							4114		
											4115		

Sample no	Origin	Date taken	Date processed	*E. coli* culture no	*E. coli* virulence	*Campylobacter* spp.	Sample no	Origin	Date taken	Date processed	*E. coli* culture no	*E. coli* virulence	*Campylobacter* spp.
1348	polly	24/03/2004	29/03/2004	negative			1427	bertie	24/05/2004	26/05/2004	4326		
1349	travis	24/03/2004	29/03/2004	4116							4296		
				4117							4297		
				4118			1428	Polly	24/05/2004	26/05/2004	4298		
1350	billy	24/03/2004	29/03/2004	4069							4299		
				4070							4300		
1351	ted	24/03/2004	29/03/2004	4071			1429	billy	24/05/2004	26/05/2004	negative		
				4072			1430	Barney	24/05/2004	26/05/2004	negative		
				4073			1431	harvey	24/05/2004	26/05/2004	4305		
1352	barney	24/03/2004	29/03/2004	4074							4306		
				4075							4307		
				4076			1432	pip	24/05/2004	26/05/2004	4308		
1353	thomas	24/03/2004	29/03/2004	4077							4309		
				4078							4310		
				4079			1433	thomas	24/05/2004	26/05/2004	4311		
1402	human	26/04/2004	29/04/2004	4230							4312		
				4231							4313		
				4233			1434	travis	24/05/2004	26/05/2004	4314		
1374	billy	26/04/2004	29/04/2004	4143							4315		
				4144							4316		
				4145			1435	dylan	24/05/2004	26/05/2004	4317		
1375	ted	26/04/2004	29/04/2004	4146							4318		
				4147							4319		
				4148			1436	ted	24/05/2004	26/05/2004	4320		
1376	thomas	26/04/2004	29/04/2004	4149							4321		
				4150							4322		
				4151									
1377	travis	26/04/2004	29/04/2004	4152									
				4153									
				4154									
1380	barney	26/04/2004	29/04/2004	4161									
				4162									
				4163									
1381	jack	26/04/2004	29/04/2004	4164									
				4165									
				4166									
1395	pip	26/04/2004	29/04/2004	4200									
				4201									
				4202									
1396	dylan	26/04/2004	29/04/2004	4203									
				4204									
				4205									
1399	bertie	26/04/2004	29/04/2004	4212									
				4213									
				4214									
1400	polly	26/04/2004	29/04/2004	4215									
				4216									
				4217									
1401	harvey	26/04/2004	29/04/2004	4218									
				4219									
				4220									
1437	human	24/05/2004	29/04/2004	4323									
				4324									
				4325									