Antimicrobial Resistance and the Public Health Impact of Feeding Raw Meat Diets to Dogs

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

by

Genever Bethan Morgan

July 2023

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Acknowledgements

Firstly, thank you so very much to my supervisors Professor Nicola Williams, Professor Gina Pinchbeck and Dr Vanessa Schmidt. The constant unwavering support and encouragement from these three inspiring and brilliant people has meant I have been able to learn and achieve so much more in this PhD than I could ever have imagined. Thank you for taking so much time to share your knowledge, skill and experience with me, and for always having an open door- I am extremely grateful.

Another huge thank you must go to the laboratory technicians at Leahurst, in particular, Dave, Jenny, Shirley and Karen. I couldn't have got my laboratory work done without your help, particularly during those first weeks back in the lab after Covid. Thank you also to everyone in the Veterinary Microbiology Diagnostic Laboratory at Leahurst for your advice and support during my laboratory work, I have learned an awful lot from you all. I must also thank Ruth and Shannon for helping me with all of the 'poo post'! Thanks also to the veterinary students who have been involved with various studies in this PhD, their help has been invaluable. A special thank you goes to Dr Amy Wedley for your friendship, guidance and time in the lab, thanks for being so patient with me- I felt very out of place in the lab initially and you helped me feel at least vaguely competent!

I have been privileged to work within an exciting research community at Leahurst and have met some wonderful people. I have had my horizons expanded in ways I'd never have been able to if I hadn't undertaken this PhD, and I feel extremely lucky to have had this experience. Thank you to the 11am coffee club for keeping me sane, particularly on AST days…! In particular, thank you to Becky, Tamzin, Amyleigh, Louise, Nicola S, Heather, April and Khalid for the friendship, laughs and support.

Thank you to the Veterinary Medicines Directorate for providing funding for my PhD and in particular, thank you to Tamsin Dewe for being so friendly and engaged in our catch-up meetings. It has been great to work with you! Of course, I must extend my great appreciation to all of the dog owners who participated in my studies- none of this work would have been possible without their involvement and for this I am tremendously thankful.

And then to my family. Thank you to my wonderful Mum. We have had some really tough times as a family in recent years, and without your support I certainly could not have achieved this PhD. I am forever grateful for your unwavering belief in me, even when my career path has taken some unexpected turns! Thank you to Jon and Jess for the fun and laughs, it has been much needed at times. An enormous thank you must go to my partner, James. I can't

thank you enough for your patience, support and kindness throughout my PhD. I am particularly grateful for your tolerance when helping me with Excel, Word and Powerpoint…! You inspired me to do this in the first place, you have stuck with me through thick and thin, and you have believed in me when I didn't think I could do this. To James' parents, Jasmin and Colin- thank you for being my second home on my trips down South, for being interested in my work, and for coming up to visit James and I when we've not been able to make it down to you.

To my pets, past and present. Merlin, Ruby, Tilly and our new addition, Marnie. My love for animals was what made me want to be a vet to begin with, and your unconditional love has kept me going throughout this journey.

Finally, to my Dad. You aren't here anymore, but I hope I've made you proud.

Abstract

ANTIMICROBIAL RESISTANCE AND THE PUBLIC HEALTH IMPACT OF FEEDING RAW MEAT DIETS TO DOGS

Genever B. Morgan

There is an ever-increasing range of diets available for pets, and raw meat diets (RMD), comprised of non-heat-treated and non-processed animal tissues, are an increasingly popular alternative diet choice. However, RMD worldwide have been demonstrated to harbour zoonotic bacteria and there is growing evidence to suggest that RMD, and the dogs fed them, are at risk for antimicrobial-resistant (AMR) bacterial carriage, including with bacteria resistant to highest priority critically important antibiotics (HPCIAs). AMR is a global One Health concern, associated with increased morbidity and mortality of patients and reduced options for the treatment of bacterial disease in humans and animals. Whilst studies have investigated AMR associated with RMD for pets elsewhere, there is a lack of data from the UK.

The aims of this thesis were to investigate the reasons and beliefs behind dog owners' choice of diet for their pet, hygiene around preparation and the risk perception surrounding pet foods. Furthermore, it aimed to determine the presence of AMR of the *Enterobacterales* within RMD and conventional non-raw diets (NRMD), as well as investigating the faecal carriage of AMR-*Escherichia coli* by dogs fed these diets. Finally, it aimed to investigate the longitudinal carriage of AMR-*E. coli* by dogs fed RMD or NRMD and their owners, alongside environmental contamination within the home.

Four studies were undertaken; (1) an online survey of 1831 (915 RMD, 916 NRMD) dog owners within the UK; (2) a cross-sectional study of popular brands of RMD (110 samples) and NRMD (24 samples) used by dog owners reported from the online survey; (3) a cross-sectional study of 432 (193 RMD, 239 NRMD) UK dog faecal samples, and (4) a longitudinal study of 19 households (8 RMD, 9 NRMD, 2 of which fed both RMD and NRMD) conducted over 6 months. Questionnaires discussing dog and owner lifestyle factors were included in studies 3 and 4.

Food samples, canine and human faecal samples, and environmental swabs were collected and *Enterobacterales* spp were isolated. Enumeration was undertaken on *Escherichia coli* and other *Enterobacteriaceae* isolated from food samples, and *E. coli* isolated from food, faecal and environmental samples underwent antimicrobial susceptibility testing. Whole genome sequencing (WGS) was conducted on ESBL-producing *E. coli* isolates to identify significant resistance genes, plasmids and genotypes. Univariable and multivariable logistic regression analyses were conducted to determine dog and owner factors associated with raw feeding, and risk factors for shedding of third-generation cephalosporin-resistant (3GCR)-*E. coli*, extended-spectrum beta-lactamase (ESBL)-producing *E. coli* and multidrug-resistant (MDR) *E. coli*.

Differences were identified in the reasons for selecting a pet diet and sources of information regarding diet between RMD and NRMD-feeding owners. RMD-feeding owners were more likely to choose a diet based on it being 'more natural', and were more likely to consult pet food groups on social media, a breeder or a friend/family than a veterinary professional for dietary advice. RMD-feeding owners perceived RMD to provide a range of health benefits, and did not perceive a risk to their dog, themselves or to in-contact dogs or people. However, RMD food samples were found to be frequently contaminated with high numbers of *E. coli* and other *Enterobacteriaceae*. ESBL-producing *E. coli* was isolated from 13.6% of RMD samples, and MDR-ESBL-producing *E. coli* from 10% of samples. *Salmonella* spp. isolates were present in 4.5% of RMD samples. No *E. coli*, other *Enterobacteriaceae* or *Salmonella* spp. were isolated from any NRMD samples. Breaches in food packaging and limited product traceability were also observed.

RMD-fed dogs carried significantly more ESBL-producing, MDR-ESBL-producing and 3GCR-*E. coli* than dogs fed NRMD; 24% of RMD-fed dogs shed ESBL-producing *E. coli* and 17% shed MDR-ESBL-producing *E. coli*. Risk factors for ESBL-producing, 3GCR and MDR-*E. coli* were provision of RMD, visiting a vet and antibiotic treatment. Furthermore, more RMD-fed dogs shed AMR-*E. coli* over a prolonged time. Across studies, $bla_{\text{CTX-M-15}}$ predominated within the ESBL-producing *E. coli* isolates. Additional bla_{ESBL} genes of interest, including $bla_{\text{CTX-M-55}}$ and $bla_{\text{SHV-66}}$ were isolated from RMD-fed dogs only.

These findings indicate that RMD provided to UK dogs is associated with zoonotic AMR bacteria, both within the foods themselves and carried by the dogs fed it. Important resistance mechanisms were identified, and resistance to antibiotics crucial for treatment of bacterial disease in humans and veterinary species, including HPCIAs, was demonstrated. The findings of this thesis suggest that RMD may pose a significant One Health concern and a multifaceted approach is needed to manage the risks that such diets pose.

Abbreviations

3GCR	Third-generation cephalosporin resistant
95% CI	95% confidence intervals
ABP	Animal by-product
AMR	Antimicrobial resistant
APHA	Animal and Plant Health Agency
AST	Antimicrobial susceptibility testing
BPW	Buffered Peptone Water
CASE	Chromogenic Agar for Salmonella Esterase
CC	Clonal complex
CFU	Colony forming units
cgMLST	Core genome multilocus sequence type
CLSI	Clinical and Laboratory Standards Institute
Cx	Cefotaxime
ESBL	Extended-spectrum beta-lactamase
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
ExPEC	Extraintestinal Pathogenic *Escherichia coli*
FE	Female entire
FN	Female neutered
FQR	Fluoroquinolone resistant
HECA	Harlequin *E. coli*/Coliform Agar
HPCIA	Highest priority critically important antibiotic
ICU	Intensive Care Unit
MALDI-TOF	Matrix-assisted laser desorption/ionisation-time of flight mass spectrometry
MDR	Multidrug resistance
ME	Male entire
MN	Male neutered
MOST	Metric Orientated Sequence Type
NA	Nutrient Agar
NCBI	National Centre for Biotechnology Information
NRMD	Non-raw diet
pAmpC	Plasmid-mediated AmpC
PAT	Pets As Therapy
PAW	PDSA Animal Welfare report
PCR	Polymerase Chain Reaction
PDSA	People's Dispensary for Sick Animals
PFMA	Pet Food Manufacturer's Association
QC	Quality Control
RFVS	Raw Feeding Veterinary Society
RGI	Resistance Gene Identifier
RMD	Raw meat diet
RVB	Rappaport Vassiliadis Broth
ST	Sequence Type

STEC	Shiga-toxin producing *Escherichia coli*
UK	United Kingdom
UKHSA	United Kingdom Health Security Agency
USA	United States of America
WGS	Whole Genome Sequencing
WHO	World Health Organisation
ZOI	Zone of Inhibition

Chapter 1: Introduction

Prior to the discovery of antibiotics in the early part of the 20th Century, bacterial infectious diseases were a leading cause of mortality worldwide (Conly and Johnston, 2005; da Cunha, Fonseca and Calado, 2019). Following the introduction of antibiotics, and combined with increased public health efforts following World War II, deaths as a result of infectious disease in England fell from approximately 25% in 1900 to <1% in 1945 (Smith, Watkins and Hewlett, 2012). However, ever since the first introduction of antibiotics, the sulphonamides, in 1937, the development of antimicrobial resistance (AMR) mechanisms by target bacteria has been a problem (Davies and Davies, 2010). AMR has been defined by the World Health Organisation (WHO) as 'when bacteria, viruses, fungi, and parasites change over time and no longer respond to medicines, making infections harder to treat, and increasing the risk of disease spread, severe illness, and death' (WHO, 2023). AMR may be naturally occurring (which may be intrinsic, whereby the resistance occurs universally within a species of bacteria, independent of antibiotic exposure and not related to horizontal gene transfer; or induced, whereby resistance genes occur naturally within the bacteria, however are only expressed after exposure to an antibiotic), or acquired by horizontal gene transfer (Reygaert, 2018). Although penicillin was first discovered by Alexander Fleming in 1928, it wasn't available for mass therapeutic use until the mid-1940s (Gaynes, 2017); however, a naturally occurring bacterial penicillinase was first discovered in 1940 and resistant bacterial strains quickly became prevalent once penicillin became widely used (Davies and Davies, 2010).

The 'Golden Age' of antibiotic development was underway by the 1950s, and many new classes of antibiotics still in use today were discovered in the period up to the late 1960s. Antibiotics were so successful that bacterial infectious disease was thought to be largely controlled and would ultimately be conquered in the near future, with the US Surgeon General stating in 1970 'It's time to close the book on infectious diseases…and shift national resources to such chronic problems as cancer and heart disease' (da Cunha, Fonseca and Calado, 2019). However in the decades following, few new classes of antibiotics were developed (Figure 1.1) (ECDC/EMEA Joint Technical Report, 2009; Davies and Davies, 2010).

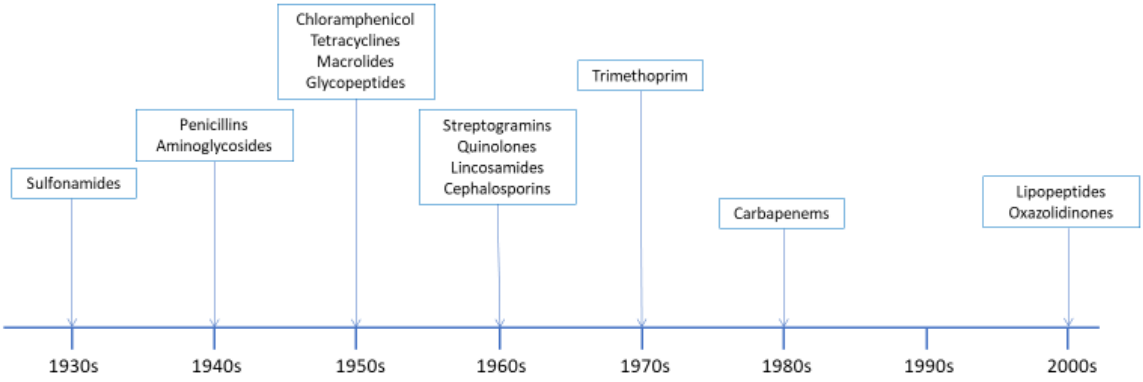


Figure 1.1: *Timeline of development of new antibiotic classes in the 20th and 21st Centuries*

However, mass production of antibiotics, easy availability and indiscriminate and inappropriate use within humans, animals, food production and the environment continued to lead to intensive selection pressure on bacteria, resulting in the evolution and global dissemination of a wide range of AMR mechanisms. In the 1990s and early 2000s, AMR-associated infections increased dramatically, becoming one of the most significant threats to human health (Conly and Johnston, 2005). In 2011, the director of the WHO stated that 'In the absence of urgent corrective and protective actions, the world is heading towards a post-antibiotic era, in which many common infections will no longer have a cure and, once again, kill unabated' (da Cunha, Fonseca and Calado, 2019).

Based on expert opinion, and to enable management of the risk of AMR to humans because of non-human antibiotic use, the WHO has ranked antibiotics on their importance to human medicine, with a list of 'critically important antibiotics' first created in 2005 and undergoing frequent revisions in subsequent years. Antibiotics are categorised based on specific criteria as 'important', 'highly important' and 'critically important', and within the 'critically important' category, antibiotics are further prioritised as 'high priority' and 'highest priority critically important (HPCIA)'. To be classed as HPCIAs, antibiotics must meet all of the following prioritisation factors (WHO, 2019):

1. They are used to treat high numbers of people for infections for which limited antibiotics are available.

2. They have high frequency use in human medicine or certain high-risk groups

3. They are used to treat infections in humans for which extensive evidence exists regarding transmission of resistant bacteria/genes from non-human sources

The classes of antibiotics which are currently considered HPCIAs are third and fourth generation cephalosporins, quinolones, glycopeptides, macrolides and ketolides, and polymixins (Scott *et al.*, 2019; WHO, 2019). To protect the use of these antibiotics, the WHO has recommended restriction of the use of antibiotics deemed crucial for human medicine in food producing animals, and that their use should be used as a last resort justified by the results of culture and susceptibility (Collignon *et al.*, 2016).

Antimicrobial resistance as a contemporary problem

Today, AMR has become an enormous health and welfare burden globally, although this burden is not equally distributed, with low income regions disproportionately affected (Bezabih *et al.*, 2021; Murray *et al.*, 2022). It has been estimated that in 2015, infections attributed to AMR bacteria accounted for 33,000 deaths in the European Union (EU) and European Economic Area (EEA), with the burden highest in those <1 year and >65 years old (Cassini *et al.*, 2019). The UK 2016 Review on Antimicrobial Resistance estimated that without intervention as many as 10 million human deaths worldwide could be attributable to AMR by 2050 (O'Neill, 2016). A more recent study estimated that in 2019, 4.95 million deaths globally were associated with bacterial AMR, and 1.27 million deaths were directly attributable (Murray *et al.*, 2022). The financial implications of AMR are also vast. Additional annual healthcare costs and productivity losses in the EU have been estimated to be €1.5 billion (ECDC/EMEA. Joint Technical Report, 2009). Economic costs to the USA alone as a result of AMR have been estimated to be as much as $55 billion per year, with $20 billion in medical/health costs and $35 billion in lost productivity, however the true costs could be much higher (Smith and Coast, 2013).

Some key factors remain present globally which continue to drive AMR, including ease of availability of antibiotics, clinical misuse (including lack of access to the correct antibiotics and to those of appropriate quality in some countries) and poor antibiotic stewardship, lack of surveillance and regulation of antibiotic use, lack of surveillance of resistance development, poor animal husbandry and continued excessive use of antibiotics in food producing animals (Chokshi *et al.*, 2019). Farming systems globally are associated with the routine use of subtherapeutic doses of antibiotics to maintain livestock health, growth promotion and productivity. One study predicted that in response to the increase in demand

for meat for human consumption, large-scale intensive farming systems will increase globally, leading to an overall increase in antibiotic consumption of 67% between 2010 and 2030, and in some countries specifically, the antibiotic use will nearly double (Van Boeckel *et al.*, 2015). However, more recently the projected global sales of veterinary antibiotics for livestock consumption are predicted to be lower up to 2030 (Tiseo *et al.*, 2020). This is due to recent decreases in sales, as demonstrated in the UK (Veterinary Medicines Directorate, 2021b) and introduction of stricter regulations and policies in important antibiotic-consuming food producing countries such as China (Tian *et al.*, 2021) and the USA (Sneeringer *et al.*, 2020). However, sales are still expected to increase across all continents (Tiseo *et al.*, 2020). While many countries in Europe, as well as Canada, Japan, Thailand and China now report and publish veterinary antibiotic sales, similar surveillance and reporting initiatives do not exist in many low and middle-income countries, notably countries such as Brazil, which produces and exports a large quantity of meat to other countries and also uses large quantities of antibiotics in its food animal production systems (Tiseo *et al.*, 2020; Dutra *et al.*, 2021).

Antimicrobial resistance in food-producing and companion animals

The importance of the link between antibiotic consumption by humans and animals and the presence of AMR within them must not be underestimated, and surveillance and judicious antibiotic stewardship across the human and veterinary sectors remains crucial. A recent study by Allel *et al.*, (2023) identified key drivers of AMR within humans and livestock (cattle, pigs and poultry) populations. The study found a significant relationship between carriage of AMR bacteria and consumption of antibiotics in both animals and humans, and a bidirectional relationship between consumption of antibiotics by animals and presence of AMR in important zoonotic pathogens, and between consumption of antibiotics by humans and presence of AMR within livestock animals. These findings demonstrate the importance of a One Health approach to AMR across the medical and veterinary sectors worldwide. Furthermore, consumption of antibiotics by livestock animals was significantly linked to AMR in critical and high importance pathogens as designated by the WHO, including oxacillin-resistant *Staphylococcus aureus*, carbapenem-resistant *Acinetobacter baumannii* and third-generation cephalosporin resistant (3GCR) *Escherichia coli (E. coli)* (Allel *et al.*, 2023). This has important implications for the potential transmission of AMR bacteria to both humans and animals which consume meat from livestock, particularly if it is consumed raw.

Because of the importance of food-producing animals to the human food chain and economy globally, studies focusing on antibiotic use and antimicrobial stewardship within livestock systems predominate, and comparatively less importance has been placed on companion animal species. However, due to the close relationship between humans and companion species, the use of common antibiotics and potential transmission routes, antimicrobial stewardship and AMR within companion animals is important (Guardabassi, Schwarz and Lloyd, 2004). It is noteworthy that many of the most frequently used antibiotics in human medicine, such as penicillins and cephalosporins are also among the most frequently prescribed in companion animal species (Mateus *et al.*, 2011; Buckland *et al.*, 2016; Singleton *et al.*, 2017). Additionally, many potentially pathogenic bacteria associated with companion animals are zoonotic, including *E. coli*, *Staphylococcus* spp., *Enterococcus* spp., *Klebsiella* spp., *Acinetobacter* spp., and *Pseudomonas* spp., and concerningly, are demonstrating increasing AMR (Umber and Bender, 2009). Furthermore, antibiotic consumption by companion animal species has been identified as a risk factor for their carriage and shedding of AMR bacteria (Damborg, Gaustad, *et al.*, 2011; Wedley *et al.*, 2017; Schmidt *et al.*, 2018). Antibiotics remain amongst the most commonly prescribed therapeutics for companion animals (Singleton *et al.*, 2018), and there are examples of routine use of HPCIAs in companion animal practice. There is increased fluoroquinolone prescription in certain settings such as emergency and critical care (Robbins *et al.*, 2020; Goggs *et al.*, 2021), in small mammal species where licensed antibiotic choices are limited such as rabbits and guinea pigs (Hedley, 2018) and frequent prescription of cefovecin (a third-generation cephalosporin) for cats (Mateus *etal.*, 2011; Buckland *et al.*, 2016; Singleton *et al.*, 2017). However, there is encouraging evidence to suggest that more prudent use of antibiotics is occurring in companion animal veterinary practice (Lehner *et al.*, 2020), and that interventions including providing educational materials and benchmarking are successful in reducing prescription of HPCIAs in dogs and cats (Singleton, Rayner, *et al.*, 2021).

The importance of *Escherichia coli*

E. coli is a Gram negative, facultative anaerobic rod-shaped bacterium of the genus *Escherichia*, which is part of the coliforms group within the family *Enterobacteriaceae,* one of the families within the order *Enterobacterales* (Adeolu *et al.*, 2016). *E. coli* has been described as a 'Jekyll and Hyde' organism (Day *et al.*, 2019), as it can be both a harmless commensal within the gut, or an important pathogen (Tenaillon *et al.*, 2010), depending on the strain and its location within the body. *E. coli* forms the majority of the non-anaerobic

gastrointestinal flora of mammals and birds; however, virulent intestinal strains such as Shiga-toxin producing *E. coli* (STEC) O157 are responsible for severe gastrointestinal disease and haemolytic uraemic syndrome, and extrapathogenic *E. coli* (ExPEC) strains are associated with disease outside of the gastrointestinal tract, most frequently urinary tract infections (Ballash *et al.*, 2023), but also other intra-abdominal infections, osteomyelitis, wound infections and sepsis (Johnson and Russo, 2002). *E. coli* is for the most part transmitted via the faeco-oral route (Russell and Jarvis, 2001), it is an important indicator organism for faecal contamination, and is used as a measure of environmental faecal pollution in river and bathing waters (Crowther *et al.*, 2011; Quilliam *et al.*, 2011), as well as a measure of contamination in food products (Doğan-Halkman *et al.*, 2003). Additionally, *E. coli* is an important sentinel organism for monitoring trends of AMR in populations, and alongside *S. aureus* is one of the most commonly monitored bacterial species in national and multinational AMR surveillance systems (Diallo *et al.*, 2020; Sijbom *et al.*, 2023).

Extended-spectrum beta-lactamases

Beta-lactam antibiotics (the penicillins and cephalosporins) are typically broad-spectrum in action, and are a staple of both human and veterinary medicine, with amoxycillin-clavulanic acid and cephalexin frequently used in companion animal practice (Mateus *et al.*, 2011). Beta-lactam antibiotics typically exert their action by inhibiting the formation of the peptidoglycan component of the bacterial cell wall, suppressing bacterial cell division or inducing bacterial rupture (Sawa, Kooguchi and Moriyama, 2020). Beta-lactamases confer resistance by hydrolysing the beta-lactam ring of penicillins and cephalosporins, altering the chemical structure of the drugs, thereby deactivating them (Fernandes, Amador and Prudêncio, 2013).

Extended-spectrum beta-lactamases (ESBLs), are a key AMR mechanism expressed by the *Enterobacterales*, and the main AMR mechanism studied in this thesis. ESBLs are enzymes which are encoded by specific plasmid-mediated genes and confer resistance to penicillins, cephalosporins, and a monobactam (aztreonam) (Bajpai *et al.*, 2017). ESBLs confer resistance to third and fourth generation cephalosporins, deemed HPCIAs by the WHO (Collignon *et al.*, 2016), and are frequently associated with co-resistance to other HPCIAs including fluoroquinolones and, more recently, carbapenems and colistin. Furthermore, ESBLs are frequently associated with multidrug resistance (MDR) (Livermore, 2009; Ibrahim *et al.*, 2023), defined as resistance to three or more antibiotic classes (Magiorakos *et al.*, 2012). Classically, they are susceptible to clavulanic acid; however, phenotypic resistance to this is

also possible if there is concurrent carriage of plasmid-mediated AmpC (pAmpC) beta-lactamase genes. The pAmpC genes are similar to ESBL genes in that they confer resistance to third generation cephalosporins and may be associated with MDR; however, they typically are resistant to beta-lactamase inhibitors such as clavulanic acid, and are classically susceptible to fourth generation cephalosporins such as cefepime (Meini *et al.*, 2019).

ESBL genes have been reported in many bacterial species, including *E. coli*, *Enterobacter* spp., *Klebsiella* spp., *Acinetobacter* spp., and *Pseudomonas* spp., and are readily acquired and transferred by horizontal transfer of plasmids (mobile genetic elements) (Sawa, Kooguchi and Moriyama, 2020). Importantly, many of these bacteria are not only ubiquitous in the environment, but also are responsible for infections of humans and animals, and zoonotic and nosocomial disease. Infections with ESBL-producing bacteria are associated with elevated morbidity and mortality rates as a result of reduced treatment options and delays in achieving appropriate treatment (Livermore and Hawkey, 2005), with the mortality rate being significantly higher for human patients infected with ESBL-producing *E. coli* compared to those with a non-ESBL-producing *E. coli* infection (Melzer and Petersen, 2007). Furthermore, the prevalence of human intestinal carriage of MDR ESBL-producing *E. coli* is increasing globally, both in the community and in hospital and care-based settings (Bezabih *et al.*, 2021, 2022).

ESBL discovery and evolution

The most clinically significant ESBL variants are the CTX-M enzymes, and ESBL-variants of TEM, SHV and OXA enzymes, encoded by *bla* genes, e.g. $bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV} and bla_{OXA}. It is important to note that whilst all $bla_{\text{CTX-M}}$ genes confer resistance to third and fourth generation cephalosporins, only certain variants of bla_{TEM}, bla_{SHV} and bla_{OXA} are ESBL-producing. First described in the early 1980s, TEM and SHV variants initially dominated until the 2000s and were largely associated with hospital outbreaks of *Klebsiella pneumoniae*, and to a lesser extent, *E. coli* (Cantón, González-Alba and Galán, 2012). However, in the early 2000s CTX-M variants, which originated from mobilisation of chromosomal *bla* genes from *Kluyvera* spp. and incorporation onto mobile genetic elements (Livermore, 2009; Cantón, González-Alba and Galán, 2012), underwent a sudden and exponential increase in prevalence and dispersal globally, predominantly associated with *E. coli* and importantly associated with both community and nosocomial spread (Cantón and Coque, 2006; Cantón, González-Alba and Galán, 2012). CTX-M variants have now displaced other ESBL variants to become the

most important ESBLs across much of Europe and Asia (Livermore, 2009), and continue to increase amongst the *Enterobacterales* globally (Bevan, Jones and Hawkey, 2017).

The importance of CTX-M

Bacteria carrying TEM and SHV ESBL variants frequently demonstrate phenotypic co-resistance to other classes of antibiotics, including tetracyclines, sulphonamides and aminoglycosides, due to co-carriage of these resistance genes on the same plasmid (Cantón and Coque, 2006). However, CTX-M variants are of key importance as not only have they rapidly become globally disseminated amongst humans, veterinary species and the environment, but frequently they demonstrate MDR and are commonly associated with concurrent quinolone resistance (Cantón and Coque, 2006). It has been suggested that >80% of $bla_{\text{CTX-M}}$ producing ESBL positive *E. coli* from human bacteraemia are also resistant to fluoroquinolones in the UK and Ireland (Livermore *et al.*, 2008; Livermore, 2009). Fluoroquinolone resistance may be a result of mutations within topoisomerase genes, and mediated by co-carriage of plasmid-mediated *qnr* genes, and/or the presence of the fluoroquinolone-modifying aminoglycoside-resistance gene *aac(6')-Ib-cr* (Cantón and Coque, 2006). Additionally, most *E. coli* which harbour the $bla_{\text{CTX-M-15}}$ gene demonstrate resistance to cefotaxime and ceftazidime (3GCR) (Livermore and Hawkey, 2005). Transfer and dissemination of resistance genes is frequently via horizontal transfer of plasmids. Whereas ESBL-producing bla_{TEM} and bla_{SHV} variants are associated with a few specific plasmids with varying transfer rates, $bla_{\text{CTX-M}}$ genes have been associated with both broad and narrow host range plasmids, and importantly have been linked to epidemic plasmids, with international dissemination of $bla_{\text{CTX-M-15}}$ specifically being associated with incompatibility (Inc) group FII (Cantón and Coque, 2006).

In the UK, the most prevalent $bla_{\text{CTX-M}}$ gene in human-derived *E. coli* is $bla_{\text{CTX-M-15}}$, which has been identified in *E. coli* isolated from blood, faeces and sewage (Day *et al.*, 2019; Ludden *et al.*, 2019), and is frequently associated with the globally disseminated pandemic *E. coli* sequence type (ST) 131 (Brodrick *et al.*, 2017), a major driver of ESBL spread worldwide (Rogers, Sidjabat and Paterson, 2011). In contrast, $bla_{\text{CTX-M-15}}$ is less frequently observed in livestock species, where $bla_{\text{CTX-M-1}}$ largely predominates as the most frequently identified bla_{ESBL} gene from meat, faeces and on-farm sampling (Day *et al.*, 2019; Ludden *et al.*, 2019; Veterinary Medicines Directorate, 2022). In horses, historically $bla_{\text{CTX-M-1}}$ has predominated; however, recent research has identified that $bla_{\text{CTX-M-15}}$ has emerged as the dominant variant in hospitalised horses in the UK (Isgren *et al.*, 2019). This trend is potentially being mirrored

in companion animal species. Although there are few data surrounding the exact CTX-M variants present in dogs in the UK, previous studies have historically identified *bla*$_{CTX\text{-}M\text{-}1}$ to be the predominant *bla*ESBL gene in dogs in Europe (Haenni *et al.*, 2014; Damborg *et al.*, 2015; Wedley *et al.*, 2017; Dupouy *et al.*, 2019); however, further studies have observed that *bla*$_{CTX\text{-}M\text{-}15}$ has increased in importance to be the most frequently isolated ESBL gene in UK canine and feline-derived ESBL-producing *E. coli* isolates obtained from clinical samples (Timofte *et al.*, 2016; Tuerena *et al.*, 2016; Singleton, Pongchaikul, *et al.*, 2021), displacing *bla*$_{CTX\text{-}M\text{-}1}$. This has also been identified in *E. coli* isolated from diseased canine and feline patients attending a veterinary hospital in Switzerland (Zogg *et al.*, 2018), and clinical isolates from dogs and cats in the USA (Shaheen *et al.*, 2013) echoing the increase in the prevalence of this gene in hospital settings in human patients. Additionally, a high prevalence of *bla*$_{CTX\text{-}M\text{-}15}$ and *bla*$_{CTX\text{-}M\text{-}14}$ has recently been identified in healthy dogs in the UK (Sealey *et al.*, 2022) and Romania (Cozma *et al.*, 2022). However, despite this, large scale data surrounding the prevalence of *E. coli* ESBL gene carriage by the healthy dog population in the UK remain limited.

Dogs as reservoir for antimicrobial-resistant bacteria

Dogs have previously been suggested to be important reservoirs of AMR-bacteria in the community (Guardabassi, Schwarz and Lloyd, 2004; Boehmer *et al.*, 2018; Zogg *et al.*, 2018; Rodríguez-González *et al.*, 2020; Marchetti *et al.*, 2021), particularly as shedding of these bacteria is largely asymptomatic. Additionally, carriage of AMR bacteria may be persistent, but highly dynamic, over time (Baede *et al.*, 2015). Domestic dogs have a unique relationship with humans, with access to several different environments, including within the home, within urban environments and rural environments such as farmland. Therefore, the potential for AMR-contamination and transmission across different environments and communities by dogs is high, particularly if faeces is not removed and disposed of appropriately, a particular concern with roaming stray dogs (Marchetti *et al.*, 2021). A study of canine faecal samples obtained from waste bins in public gardens in Denmark identified the presence of ESBL-producing *E. coli* within them (Damborg *et al.*, 2015), highlighting the risk of human and other animal exposure and the potential environmental AMR exposure risk posed by dog faeces. Furthermore, a study of healthy non-vet visiting Labradors in the UK demonstrated faecal carriage of ESBL-producing *E. coli* and that provision of a raw meat diet was a significant lifestyle risk factor for carriage (Schmidt *et al.*, 2015), highlighting the potential importance of human influences, such as diet choice, on AMR-bacterial carriage by pet dogs. Other factors such as recent antibiotic use and veterinary visits (Damborg, Gaustad,

et al., 2011; Wedley *et al.*, 2017; Schmidt *et al.*, 2018) have also been implicated as influences on AMR bacterial shedding by dogs.

The link between the veterinary hospital environment and AMR bacterial shedding by dogs has also been investigated. Veterinary hospital environments have been demonstrated to have AMR bacteria, including ESBL-producing *E. coli* present (Sidjabat *et al.*, 2006; Timofte *et al.*, 2016), and veterinary hospital staff have been shown to have a higher prevalence of faecal ESBL-producing *E. coli* compared to reports of faecal carriage in the community (Royden *et al.*, 2019). Such studies demonstrate a potentially risky environment for AMR bacterial transmission to dogs, but also an area where veterinary patients are likely to play an important role in environmental contamination. A study of rectal and buccal swabs obtained from dogs within 48 hours of hospital admission and then again at discharge identified that approximately 5% of dogs carried 3GCR *Enterobacterales* spp. initially, but this increased to nearly 25% at discharge. This study demonstrated that shedding of AMR bacteria by dogs may be a risk factor for hospital contamination and that AMR bacteria can be acquired by dogs during hospitalisation (Haenni *et al.*, 2022). A further longitudinal study observed similar strains of ESBL-producing *E. coli* and *K. pneumoniae* present in veterinary intensive care unit patients and their hospital environment, and that following discharge similar strains were then identified in the veterinary patients, their home environment and their owners, suggesting potential transmission of AMR bacteria from the hospital environment to the home via the pet (Schmitt *et al.*, 2021).

Dogs and their owners frequently share close contact, especially within the home where behaviours such as sharing of soft furnishings and beds, dogs sitting on the owners lap, and dogs licking owners hands and faces occur (Westgarth *et al.*, 2008), as well as owners kissing their pets (do Vale *et al.*, 2021). It is this close relationship, and the behaviours associated with it, which may pose a particularly high risk for transmission of AMR-bacteria between pets and their owners. In particular, risky behaviours around food such as sharing plates, utensils and allowing pets to eat from bare hands is reported, despite owners potentially being aware of the zoonotic disease potential (Dickson *et al.*, 2019). Dogs and humans in close contact, either within the home or within another close-contact environment such as a shelter or veterinary hospital environment, have been demonstrated to share AMR *E. coli* with similar resistance genes and resistance patterns (Sidjabat *et al.*, 2006; Toombs-Ruane *et al.*, 2020; Cozma *et al.*, 2022; Naziri, Poormaleknia and Ghaedi Oliyaei, 2022), and AMR *E. coli* of the same sequence type (Johnson *et al.*, 2016; Grönthal *et al.*, 2018). Such close contact is of particular concern with dogs fed a raw meat diet, where the potential for contact with

foodborne zoonotic pathogens is greater. Indeed, in a case series discussing an outbreak of *E. coli* O157:H7 in people in the UK which was epidemiologically linked to the provision of raw tripe fed to dogs, one of the patients had shared a toothbrush with the raw fed dog (Kaindama *et al.*, 2020).

Raw meat diets for dogs

The development of modern commercial diets has been suggested to be a major contributing factor to the longer and healthier lives of pet dogs (Laflamme *et al.*, 2008). However, there is an extensive and ever-expanding range of foods available now for dogs, providing a spectrum of choices for dog owners to select from. As demonstrated in table 1.1, these options range from pre-prepared conventional cooked diets, such as dry kibble and cooked wet food in tins, trays and sachets, alternative cooked diets such as insect-based and vegan/vegetarian options, to completely raw diets. Diets for dogs may also be pre-prepared, home-prepared or use a combination of both. Furthermore, newer options are also increasingly available such as subscription services which tailor-make the diet for the individual dog, and diets which utilise alternative processing methods such as cold-pressing, low temperature cooking or steaming. Increasingly, dog owners are looking to alternative diet choices for their pets, with raw meat diets (RMD) being an important option (Dodd *et al.*, 2020; Bulochova and Evans, 2021b). Although data surrounding the market share is limited, there was an increase in the number of plants registered as producing raw diets up to 2018, suggesting a response to increased demand (Withenshaw *et al*., 2020). Raw diets for pets are comprised of non-heat treated and unprocessed animal material, including muscle, bone, cartilage, skin and internal organs, and may be comprised of DEFRA category 3 animal by-products (https://www.gov.uk/guidance/using-animal-by-products-to-make-pet-food), or meat products intended for human consumption (so-called 'human grade' meat). There are different modes of raw diet feeding, including using pre-prepared commercial complete meals, provision of 'whole prey' carcasses, making home-prepared raw meals according to a recipe, adding raw meat as a protein source to a premixed complementary diet and adding raw meat to a conventional cooked kibble diet (Wales and Davies, 2021).

Table 1.1: *Types of pre-prepared and home-prepared (including 'do-it-yourself' (DIY) and tailor-made) foods available for dogs in the UK*

<table>
<tr><th colspan="4">Food Category</th></tr>
<tr><th colspan="2">Pre-prepared diets</th><th colspan="2">Home-prepared/DIY or tailor-made diets</th></tr>
<tr><td>Cooked wet or dry foods</td><td>1. Cooked conventional kibble^
 

2. Conventional tins/trays/sachets^
 

3. Grain-free food
 

4. Insect-based diets
 
</td><td>Cooked foods</td><td>1. Tailor made cooked subscription
 

2. Home-cooked diet
  </td></tr>
</table>

	5. Vegan/vegetarian diets  6. Semi-moist foods  		
Raw foods	1. Pre-prepared complete raw meals  	**Raw foods**	1. DIY using branded products***  2. 'Whole prey'  3. Completely DIY****
Others	1. Cold pressed food*  2. Low-temperature cooked food**		

	 3. Low-temperature steamed food (requires refrigeration) 		

^Includes prescription diets and breed-specific diets; *Advertised as being made from raw meat; **Marketed as alternative to using raw meat diet, ***Marketed as for use as component in at-home recipes; ****Can use combination of 'whole prey' and meat from butcher/supermarket, as well as fruit/vegetables.

There are multiple owner-reported physical and behavioural health benefits of a raw diet for pets, including improved skin and hair coat quality, improved muscle mass, cleaner teeth, calmer (or livelier/happier) demeanour and better overall general health and vitality (Morgan, Willis and Shepherd, 2017; Morelli *et al.*, 2019; Empert-Gallegos, Hill and Yam, 2020), as well as advantages such as increased palatability and enjoyment of food, and firmer, less smelly stools with reduced volume (Wales and Davies, 2021). Surveys from Finland of owner-reported prevalence of atopic dermatitis and gastrointestinal conditions, including inflammatory bowel disease and chronic enteropathies, in adult dogs suggested that provision of RMD components in puppyhood might reduce the prevalence of these conditions in later life (Hemida *et al.*, 2021a; Hemida *et al.*, 2021b; Vuori *et al.*, 2023). A frequent reason for feeding a raw diet to dogs as discussed by dog owners is that dogs are 'carnivores' evolved to eat raw meat and that the diet evokes the natural diet of wild canids, therefore is a more 'appropriate' food (Freeman and Michel, 2001; Morgan, Willis and Shepherd, 2017; Morelli *et al.*, 2019; Empert-Gallegos, Hill and Yam, 2020; Viegas *et al.*, 2020), and proponents of RMD report that the digestive system of cats and dogs is 'designed' to deal with any pathogens present in raw diets (Bulochova and Evans, 2021b). Additionally, heat treating or cooking food is argued to 'destroy' the nutrients within it (Michel *et al.*, 2008). While the decision surrounding what to feed a pet is multifaceted, emotive and complex, anthropomorphism of pets and treating them akin to a family member (including describing them in emotive, human terms such as 'children' or 'fur babies', or owners

referring to themselves as the pet's 'mummy', or 'pet parents'), does also play a role (Michel, 2006; Dickson *et al.*, 2019). Provision of the 'best nutrition possible' is of key importance for owners when choosing what food to buy for their pet (Schleicher, Cash and Freeman, 2019), and a raw diet is appealing due to its lack of processing, thus appearing more 'natural' and a 'healthier' option for pets compared to processed cooked commercial products (Michel *et al.*, 2008; Bulochova and Evans, 2021a). Additionally, raw diets are often comprised of multiple components selected and put together by the owner themselves, thus appealing to the caring aspect of food provision (Michel, 2006; Michel *et al.*, 2008), and the ability to have control over the component ingredients is important to many pet owners (Bulochova and Evans, 2021a). This desire for a healthier, less processed diet reflects many people's beliefs and choices surrounding their own diet, and that of their family (Michel *et al.*, 2008; Morgan, Willis and Shepherd, 2017). Furthermore, a mistrust of processed traditional commercial cooked diets, and the companies producing them, is apparent amongst owners who choose a raw diet (Bulochova and Evans, 2021b).

One of the first publicised, and still commonly-followed, raw diet plans was the Bones and Raw Food (BARF) diet, as proposed by Dr Ian Billinghurst, a veterinarian, in the early 1990s in which it is suggested to provide the majority of the diet as raw meaty bones, and the rest as a variety of foods akin to a wild dog's diet. This includes green vegetables to mimic the stomach contents of prey, offal, and eggs, dairy products (including milk and yoghurt), and a small amount of legumes and grains (Freeman and Michel, 2001). However, there are concerns with the nutritional balance and adequacy of raw diets (Vecchiato *et al.*, 2022), particularly home-prepared versions (Dillitzer, Becker and Kienzle, 2011; Hall *et al.*, 2020), as well as the microbiological risks of such a diet. Pre-prepared commercial raw brands available in the UK must undergo regular Animal and Plant Health Agency (APHA) sample testing for microbiological contamination with *Enterobacterales* and *Salmonella* spp. (https://www.gov.uk/guidance/laboratory-testing-requirements-for-animal-by-products-abps#how-much-bacteria-your-samples-can-contain). A sample from each product line must be tested, and a different product line must be present for each species of meat and offal and for each species of tripe included, although frequency of testing depends on factors specific to each individual pet food manufacturing facility (https://www.gov.uk/guidance/using-animal-by-products-to-make-pet-food). Therefore, a degree of safeguarding is in place in these diets, which is not possible to assess in home-prepared diets. In an international study of dog food choices made by owners, more dogs which were fed a raw diet were fed home-prepared food than commercial raw products

(89%, compared to 67%) (Dodd *et al.*, 2020). However, more recently in the UK it has been suggested that 7% of dogs (approximately 790,000 dogs) are fed a raw diet, divided into 5% fed pre-prepared raw diets, and 2% fed a home-prepared meal (PDSA, 2022), demonstrating a preference here for pre-prepared options. The internet, and social media in particular, are an important health information resource for pet owners (Thomas and Feng, 2020; Kogan, Little and Oxley, 2021), particularly for dietary information for raw-feeding owners (Connolly, Heinze and Freeman, 2014; Morelli *et al.*, 2019; Bulochova and Evans, 2021b; Wales and Davies, 2021). There are also many dedicated peer-to-peer groups and advice platforms for raw feeding communities on social media sites such as Facebook, Instagram and TikTok. However, the advice and information provided within these sources are largely unsubstantiated and based on informal opinions rather than scientific evidence. Importantly, these resources are often viewed as more important than advice from veterinary professionals, who are commonly viewed as having a lack of dietary knowledge or training surrounding nutrition (Morgan, Willis and Shepherd, 2017). Indeed, a recent study identified that owners who fed a raw diet frequently rated their own knowledge surrounding diet equal to or above that of a veterinary professional (Empert-Gallegos, Hill and Yam, 2020). Further, there remains an apparent mistrust within the raw feeding community towards veterinary professionals regarding diet (Connolly, Heinze and Freeman, 2014; Morgan, Willis and Shepherd, 2017).

Raw meat diets and zoonotic bacteria

Despite the numerous purported canine health benefits of RMDs, a number of studies globally have demonstrated bacteria with zoonotic and pathogenic potential within RMD for pets, therefore suggesting that this diet choice could pose an important animal and human health risk for infectious disease within the home. These diets are listed in table 1.2. Many of the studied diets were frozen, pre-prepared commercial diets intended for pet feeding only, and comprised of several different meat types. Several studies globally have identified *Enterobacterales* spp. (including virulent variants such as STEC O157:H7, *Salmonella* spp., and *Yersinia* spp.), as well as isolating *Listeria* spp., *Campylobacter* spp., *Clostridium* spp., *Brucella suis* and *Staphylococcus aureus* from RMD samples. Many of the studies in table 1.2 isolated several different bacteria from the RMD samples. However, few studies have investigated the presence of AMR bacteria within the samples (Nilsson, 2015; Baede *et al.*, 2017; van Bree *et al.*, 2018; Nüesch-Inderbinen *et al.*, 2019). Studies which have investigated the presence of AMR within RMD have demonstrated that there may be a high prevalence of ESBL-

producing and 3GCR-*E. coli* within them; in one study from The Netherlands, 80% of products tested had ESBL-producing *E. coli* present (van Bree *et al.*, 2018). The most frequently encountered ESBL gene type is $bla_{\text{CTX-M}}$, with $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-15}}$ most commonly isolated. Additionally, in a study where 23% of RMD samples had 3GCR-*E. coli* present, all isolates demonstrated the presence of the $bla_{\text{CMY-2}}$ gene (Nilsson, 2015).

Furthermore, as demonstrated in table 1.2, while many of the studies were undertaken in the USA or mainland Europe, little information exists regarding the microbiological and AMR risks associated with RMD available in the UK.

Table 1.2: *List of examples of previous studies which have isolated bacteria with zoonotic and pathogenic potential from raw meat diets (RMD) for pets, the country in which the study was undertaken, and the product type tested. Note: Many studies have tested for multiple bacteria so appear more than once in the table*

Bacteria isolated	**Authors**	**Country**	**Product/protein type**
Enterobacterales spp. (including *E. coli* STEC O157:H7)	Hellgren *et al.*, 2019	Sweden	Frozen RMD (multiple proteins)
	van Bree *et al.*, 2018	Netherlands	Frozen RMD (multiple proteins)
	Kaindama *et al.*, 2020	UK	Frozen RMD
	Nüesch-Inderbinen *et al.*, 2019	Switzerland	Frozen RMD (multiple proteins)
	Strohmeyer *et al.*, 2006	USA	Frozen RMD (multiple proteins)
	Vecchiato *et al.*, 2022	Germany	Frozen RMD (multiple proteins)
	Kananub *et al.*, 2020	Thailand	Frozen and freeze dried RMD
	Jones *et al.*, 2019	USA	RMD implicated in pet illness
	Treier *et al.*, 2021	Switzerland	Frozen RMD (multiple proteins)
	Gibson *et al.*, 2022	USA	Fresh and frozen RMD
	Nemser *et al*., 2014	USA	Frozen RMD (multiple proteins)
	Bottari *et al*., 2020	Italy	Frozen RMD (multiple proteins)
	Weese *et al.,* 2005	Canada	Frozen and freeze dried RMD
	Morelli *et al.,* 2020	Italy	Frozen RMD (multiple proteins)
AMR *E. coli*	van Bree *et al.*, 2018	Netherlands	Frozen RMD (multiple proteins)
	Nüesch-Inderbinen *et al*., 2019	Switzerland	Frozen RMD (multiple proteins)
	Nilsson, 2015	Sweden	Frozen RMD (multiple proteins)
	Baede *et al.*, 2017	Netherlands	Frozen commercial RMD for cats
Salmonella spp.	Hellgren *et al*., 2019	Sweden	Frozen RMD (multiple proteins)
	Fredriksson-Ahomaa *et al*., 2017	Finland	Frozen RMD (multiple proteins)
	Mehlenbacher *et al.*, 2012	USA	Frozen/dehydrated/freeze dried RMD
	van Bree *et al*., 2018	Netherlands	Frozen RMD (multiple proteins)
	Nüesch-Inderbinen *et al*., 2019	Switzerland	Frozen RMD (multiple proteins)
	Withenshaw, *et al.* 2020	UK	Surveillance data (isolates from RMD)
	Strohmeyer *et al*., 2006	USA	Frozen RMD (multiple proteins)

	Vecchiato *et al.*, 2022	Germany	Frozen RMD (multiple proteins)
	Kananub *et al.*, 2020	Thailand	Frozen and freeze dried RMD
	Jones *et al.*, 2019	USA	RMD implicated in animal illness
	Nemser *et al.*, 2014	USA	Frozen RMD (multiple proteins)
	Bottari *et al.,* 2020	Italy	Frozen RMD (multiple proteins)
	Weese *et al.,* 2005	Canada	Frozen and freeze dried RMD
	Morley *et al.*, 2006	USA	RMD implicated in animal illness
Campylobacter spp.	Hellgren *et al.*, 2019	Sweden	Frozen RMD (multiple proteins)
	Fredriksson-Ahomaa *et al.*, 2017	Finland	Frozen RMD (multiple proteins)
	Bojanić *et al.*, 2017	New Zealand	Frozen and fresh RMD
	Bottari *et al.*, 2020	Italy	Frozen RMD (multiple proteins)
Listeria spp.	van Bree *et al.*, 2018	Netherlands	Frozen RMD (multiple proteins)
	Kananub *et al.*, 2020	Thailand	Frozen and freeze dried RMD
	Jones *et al.*, 2019	USA	RMD implicated in pet illness
	Nemser *et al.*, 2014	USA	Frozen RMD (multiple proteins)
	Bottari *et al.*, 2020	Italy	Frozen RMD (multiple proteins)
	Morelli *et al.*, 2020	Italy	Frozen RMD (multiple proteins)
Brucella suis	van Dijk *et al.*, 2018	Netherlands	Imported raw hare meat
Clostridium spp.	Hellgren *et al.*, 2019	Sweden	Frozen RMD (multiple proteins)
	Weese *et al.*, 2005	Canada	Frozen and freeze dried RMD
	Morelli *et al.,* 2020	Italy	Frozen RMD (multiple proteins)
Yersinia spp.	Fredriksson-Ahomaa *et al.*, 2017	Finland	Frozen RMD (multiple proteins)
	Morelli *et al.*, 2020	Italy	Frozen RMD (multiple proteins)
Staphylococcus aureus	Kananub *et al.,* 2020	Thailand	Frozen and freeze dried RMD
	Weese *et al.,* 2005	USA	Frozen and freeze dried RMD

Studies have demonstrated that owners who feed RMD often believe freezing to be effective in eliminating most, if not all, bacteria present within the food (Bulochova and Evans, 2021b). However, as the studies in table 1.2 demonstrate, this is a misconception, as most studies tested frozen raw samples which were subsequently defrosted at standard refrigeration temperatures prior to testing. Indeed, studies have demonstrated very high bacterial counts in raw food samples following defrosting (Hellgren *et al.*, 2019; Kananub *et al.*, 2020; Vecchiato *et al.*, 2022). With regards to food preparation, storage and defrosting hygiene practices, there are readily available resources which detail safe practices, such as the UK Pet Food Responsible Raw Feeding for Cats and Dogs website, which includes a factsheet discussing safe handling of commercial raw food (https://www.ukpetfood.org/resource/raw-feeding-factsheet.html), and the APHA/Public Health England (PHE, now known as the United Kingdom Health Security Agency) guidance on handling raw pet foods and preventing

infection (https://www.gov.uk/guidance/raw-pet-foods-handling-and-preventing-infection). Despite this, there does seem to be some confusion as to what constitutes safe raw pet food handling practices among pet owners.

While many RMD-feeding pet owners have been documented to be aware of the potential presence of bacteria such as *E. coli*, *Salmonella* spp. and *Campylobacter* spp. associated with their pet food choice (Bulochova and Evans, 2021a), in a study of social media forums, confusion and disagreement surrounding methods of reducing or eliminating hazards has also been reported, and personal judgement regarding safety was frequently observed. In particular, owners reported avoiding raw poultry to reduce the risk of pathogens, or utilised supermarket-purchased meat as it was meant for human consumption, thus 'safer' (Bulochova and Evans, 2021b). In the same study, some owners who fed a raw meat diet described employing food hygiene practices which were insufficient, practices which were potentially risky such as rinsing meat, and a lack of concern regarding the need for safety precautions (Bulochova and Evans, 2021b). In a survey specifically investigating raw feeding pet owner's food preparation practices, many owners self-reported good hygiene practices, such as always washing their hands after preparing food and always using specific cleaning products to disinfect areas following preparation of raw food (Bulochova and Evans, 2021a). However, less than half of the owners surveyed utilised separate areas in the kitchen or separate utensils for preparing raw food, and only 46% of owners always defrosted raw meat in the fridge. This further demonstrates the spectrum of food hygiene and safety practices employed by pet owners who feed raw diets.

Further poor hygiene practices surrounding pet food bowls by owners have also previously been documented (Luisana *et al.*, 2022), and this may be of particular concern with regards to those who choose to feed a raw diet (Bulochova and Evans, 2021b). In a study where food bowls were experimentally contaminated with *Salmonella* spp. inoculated raw meat, *Salmonella* spp. contamination persisted despite cleaning measures such as soapy water and washing in a dishwasher, and this was hypothesised to be a result of remaining organic material and/or biofilm accumulation (Weese and Rousseau, 2006). Furthermore, in another study pet food bowls were found to be 17 times more likely to be contaminated with *Clostridium difficile* when the dog was fed a commercial raw diet compared to other diet types (Weese *et al.*, 2010).

Although, as previously mentioned, there are readily available internet-based resources describing safe food practices from independent and government agencies, a further

potential resource for dog owners regarding food storage, defrosting and hygiene could be the websites of the raw diet manufacturers themselves. However, there appears to be a wide range of quality and availability of information provided by these websites. A study by Bulochova and Evans (2021b) in the UK investigating the provision of food safety information on the websites of 33 RMD manufacturers and suppliers revealed that 61% of websites did not provide any consumer guidance, and only 15% provided guidance which was regarded as 'excellent'. Additionally, the standard of information provided by manufacturers which were approved by UK Pet Food (formerly the Pet Food Manufacturers Association) was rated as 'very good' or 'excellent', whereas 77% of the non-approved manufacturers did not provide guidance regarding safe practices surrounding raw food provision. Of the 13 websites which did provide food safety information, 85% provided guidance regarding freezing, thawing, handling and refrigeration, and 62% discussed cleaning and sanitisation strategies to prevent cross-contamination. Furthermore, manufacturers rarely included information regarding the presence of foodborne pathogens or the potentially increased risk to vulnerable members of the household.

According to Luisana *et al.* (2022), the majority of pet owners would expect to find guidelines regarding the handling and storage of their pet's food on the food label itself, demonstrating the importance of clear on-product labelling in the communication of this. However, unfortunately there also appears to be a wide discrepancy in the detail and availability of food defrosting, preparation, and storage hygiene guidance on raw pet food products. A previous study from the USA identified that few raw diet brands provided warnings on their product labelling that potentially harmful bacteria could be present, and that these bacteria could cause pet and human illness. Furthermore, approximately half of the brands examined provided information regarding cleaning food preparation surfaces and bowls/utensils with hot, soapy water but did not provide detail as to why this was needed (Mehlenbacher *et al.*, 2012). A further study which evaluated the bacterial contamination of RMD in the USA observed that none of the RMD products tested had any instructions for thawing or preparation present (Strohmeyer *et al.*, 2006). Therefore, it appears that an improvement in the provision of information regarding safe practices surrounding raw diets by US pet food manufacturers is needed; however, little data currently exist surrounding the availability of this information on labels on products available in the UK.

Zoonotic disease transmission, raw fed pets, and perception of risk

Despite the zoonotic potential of bacteria associated with RMD for pets, currently there are few reports of human disease associated with them. Indeed, a recent survey of RMD-feeding pet owners from a number of countries suggested that only 0.2% of respondents reported transmission of a pathogen from the RMD to a household member (Anturaniemi *et al.*, 2019). A UK survey identified that 90% of RMD-feeding pet owners reported no experience of foodborne illness as a result of the raw diet (Bulochova and Evans, 2021a). However, a limitation of this self-reporting method is that many of the bacterial contaminants discussed earlier may only produce mild clinical signs which may be self-limiting and not warrant medical attention and may not be attributed to the food by the owner. As such, the true prevalence may be underestimated, and there remains a particular concern regarding the risks of transmission to vulnerable members of society, such as the elderly, infants, and immunocompromised people. Whereas there are few data surrounding direct transmission of pathogens from the RMD to the owner, there are reports of transmission of pathogens leading to clinical disease within RMD-feeding households. As discussed previously, in the UK, an epidemiological link was identified between the provision of raw tripe to dogs and an outbreak of STEC O157:H7 (Kaindama *et al.*, 2020), and in the USA a link was made between the provision of contaminated raw turkey products and an outbreak of *Salmonella* Reading (Hassan *et al.*, 2019). In this outbreak, four of the affected people became ill after feeding their pets raw ground turkey pet food. More recently, a case study from Italy described the concomitant symptomatic infection with genetically associated *Campylobacter* spp. in a raw-fed puppy and its owner (Candellone *et al.*, 2023). There are also reports of pet illness associated with ingestion of contaminated raw meat diets. Disease as a result of *Salmonella* spp. infection is the most frequently reported, with the source of infection suspected to be RMD provided to two dogs suffering from septic peritonitis as a result of mesenteric lymphadenitis (Binagia and Levy, 2020), and two cases of suspected Salmonellosis in cats (Giacometti *et al.*, 2017). In addition, *Salmonella* Newport was detected in faecal, environmental, and raw meat samples in a greyhound breeding facility where an outbreak of diarrhoea and subsequent death of three puppies had occurred. Analysis of isolates led to the conclusion that *Salmonella* spp. was likely to have been introduced to the facility by contaminated raw meat (Morley *et al.*, 2006). Finally, whole genome sequencing linked cases of enterocolitis and death in puppies and kittens as a result of *Salmonella* spp. with

contaminated raw diets (Jones *et al.*, 2019). Links between other bacterial pathogens and disease in pets have also been described, including cases of canine acute polyradiculoneuritis linked to *Campylobacter* spp. associated with raw chicken consumption (Martinez-Anton *et al.*, 2018), and *Mycobacterium bovis* infection in cats suspected to be linked to contaminated RMD containing venison, where four owners and one veterinary surgeon were also found to have a high likelihood of latent tuberculosis infection (O'Halloran *et al.*, 2019; Mitchell *et al.*, 2021).

With regards to risk perception surrounding illness as a result of diet provision, a study of pet owners in general identified that they were more concerned with pets becoming ill than people as a result of pet food (Thomas and Feng, 2020). Although pet owners who feed RMD may be aware of the bacteriological risks associated with the provision of this diet, generally the perception of risk associated with contracting an infectious disease as a result of RMD provision is low (Empert-Gallegos, Hill and Yam, 2020; Viegas *et al.*, 2020). One study observed that only 36% of raw-feeding owners recognised that the result could be fatal, and that 89% of owners did not perceive the practice of feeding a raw diet to be a risk to themselves or their family members (Bulochova and Evans, 2021a). In contrast, a study of comments within a social media forum identified that some RMD-feeding owners have demonstrated concerns surrounding the potential bacterial risks and 'poisoning' for children and pets, or the risk of contracting bacterial disease as a result of being licked by a dog which has eaten 'pathogen-rich food' or as a result of poor food hygiene (Bulochova and Evans, 2021b). However, the vast majority of RMD-feeding owners have been reported to be fully confident in their food preparation and hygiene practices so that they do not pose a risk to themselves or their family members, and pet owners with high confidence in their own abilities to safely prepare RMD do not perceive themselves to be at risk from foodborne illness (Bulochova and Evans, 2021a). Therefore, pet owners may believe that 'good hygiene' measures surrounding RMD preparation (whatever they perceive them to be) may lessen or negate the potential bacteriological risks.

Although there are reports of clinical disease associated in pets fed RMD, many animals will shed potentially zoonotic bacteria asymptomatically, or with very mild transient clinical signs which are not attributed to the diet and may again lead to an underestimation of the true disease prevalence. This is a concern for the potential transmission of disease within the household and may lead pet owners to not perceive their pet, or the food they are fed, as an infectious disease risk. While there are studies which have examined the risk perception of acquiring an infectious disease from the raw diet itself, there remains a dearth of data

surrounding the risk perception of transmission of infectious disease via raw-fed pets. However, dogs fed RMD have been demonstrated to shed potentially zoonotic pathogens. One study from Canada identified that therapy dogs fed RMD were significantly more likely to test positive for *Salmonella* spp. than dogs fed non-raw diets (NRMD), with frequently identified serotypes including *S.* Typhimurium, *S.* Heidelberg and *S.* Kentucky (Lefebvre *et al.*, 2008), all of which have potential to cause human disease. Further studies from Brazil (Viegas *et al.*, 2020) and the UK (Groat *et al.*, 2022) demonstrated that dogs fed RMD had an increased likelihood of testing positive for *Salmonella* spp. and *Clostridium* spp. than those fed NRMD.

The lack of risk perception is of particular concern with regards to the risks of AMR bacteria transmission, which is arguably a less obvious concept for pet owners to perceive or appreciate, as it is unlikely to cause an immediate, direct problem, unlike bacterial diseases caused by STEC O157:H7, or salmonellosis. Studies which have investigated the AMR risks associated with raw diets themselves have been discussed above. However, provision of RMD has also been identified as a risk factor for canine and feline carriage of AMR bacteria (Schmidt *et al.*, 2015; Baede *et al.*, 2017; Wedley *et al.*, 2017; Sealey *et al.*, 2022), and dogs fed a raw diet have been demonstrated to shed AMR bacteria of concern, including ESBL-producing and MDR *E. coli*, and *E. coli* with phenotypic and genotypic resistance to HPCIAs (Lefebvre *et al.*, 2008; Mounsey *et al.*, 2022). Furthermore, dogs fed RMD may shed ESBL and AmpC-producing *Enterobacterales* over a protracted length of time, either continuously or intermittently (Baede *et al.*, 2015). Additionally, AMR, MDR and 3GCR-*E. coli* has been demonstrated to be significantly more likely to be shed in the faeces of dogs fed RMD than those fed NRMD (Groat *et al.*, 2022).

Potential routes of transmission of potentially pathogenic, zoonotic AMR bacteria to humans associated with feeding RMD are demonstrated in figure 1.2. There may be multiple routes of transmission present within the home, including direct routes such as contact with the contaminated raw food itself, or via close contact with the raw-fed pet, for example, allowing them to lick the hands and faces of household members. Indirect routes may also be important, for example, contamination of shared food areas such as the fridge or preparation spaces in the kitchen, or from handling contaminated fomites utilised by the raw-fed pet including pet toys and soft furnishings. Furthermore, there are risks of wider reaching contamination and AMR transmission from the raw food itself and the raw-fed pet. Environmental contamination, including farmland, beaches and public spaces may occur as a direct result of contamination by dog faeces, which in turn may lead to transmission of AMR

bacteria to grazing animals, other dogs which may ingest faeces, or humans who come into contact with the contaminated land. Additionally, poor disposal of waste food or contaminated packaging, or even recycling or composting of non-sterilised materials, may lead to additional environmental contamination. Finally, raw-fed pets may pose a risk of contamination within the veterinary clinical environment. Veterinary professionals have close and frequent contact with multiple patients each day, and as such, may be at higher risk of contracting bacteria from patients and contaminated environments such as kennels and bedding. Indeed, veterinary professionals have been identified previously as a high-risk population for faecal carriage of ESBL-producing *E. coli* (Royden *et al.*, 2019). Furthermore, bacterial shedding by raw-fed patients may pose a hazard for other at-risk veterinary patients, such as the elderly, the very young, patients undergoing surgery and those undergoing treatment with immunosuppressive medication such as those with autoimmune disease, or chemotherapy. This risk may occur because of direct contact, contamination of the hospital environment, or via patient-to-patient spread by veterinary professionals dealing with multiple patients.

Therefore, provision of RMD to pets has potential to be an infectious disease and AMR risk in the true 'One Health' sense, in that it may pose a risk to animals, humans and the environment.

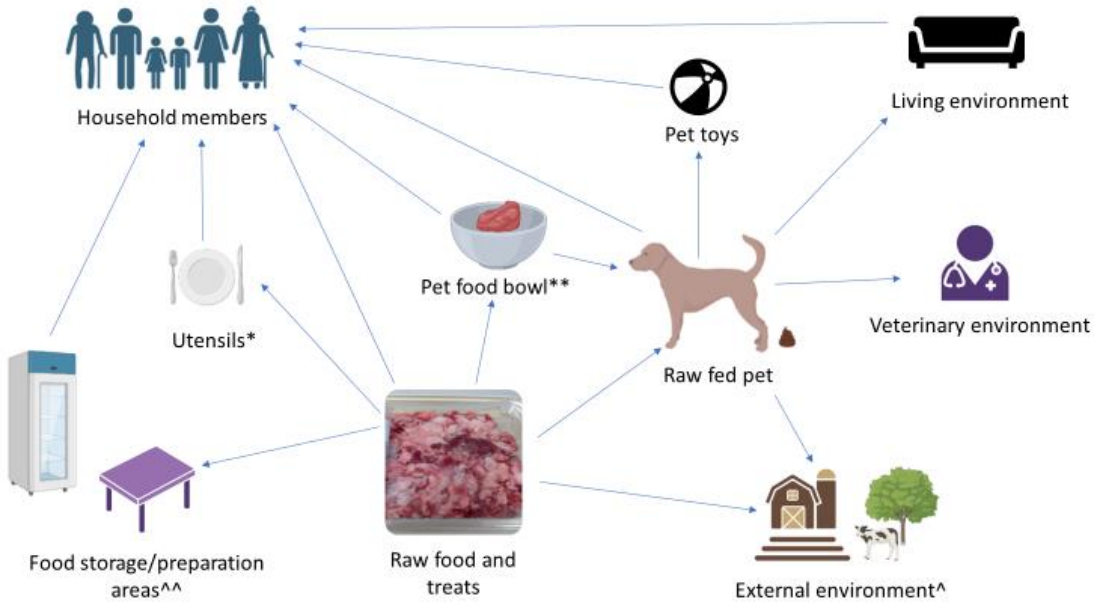


Figure 1.2: *Routes of transmission of zoonotic and AMR bacteria because of raw meat diets and the pets fed them. The routes of transmission are multiple and can be direct or indirect.*

*Transmission may occur because of direct contamination via the food itself, or secondarily because of the pet licking/contaminated saliva or faecal shedding by the raw-fed pet. Adapted from Wales and Davies (2021). *Using the same utensils for pet and human food preparation may be a direct source of transmission, or indirect if they are shared with the raw-fed pet (e.g. eating from same bowl or sharing a spoon); **Pet food bowls may be a source of transmission to the pet itself, or to other pets in the same household sharing a bowl. They may also be an indirect source of transmission to household members if uneaten food is left and children (for example) gain access; ^The external environment may be contaminated through faecal shedding of raw-fed pets, or via composting or disposal of biodegradable raw food packets; ^^Contamination of the food storage and preparation area may be direct as a result of food preparation, or indirect as a result of damaged or leaky packaging which may only become apparent during defrosting of frozen products.*

Aims of this thesis

The overall aims of this thesis were to determine the One Health risks associated with feeding a raw meat diet to dogs, with particular focus on AMR. It aimed to understand why UK dog owners might choose to feed an alternative raw diet rather than conventional cooked food, and their perception of risk associated with their diet choice. Additionally, it aimed to investigate the human and animal bacterial health hazards associated with raw meat diets available in the UK, and the potential for faecal shedding of AMR *E. coli* by dogs fed these diets. Finally, this thesis aimed to investigate the long-term carriage of AMR *E. coli* by dogs fed raw diets, and the potential for human co-carriage of these bacteria and environmental contamination within a household.

To achieve these aims, four studies were undertaken and are presented in chapters 2 to 5 in this thesis:

- Chapter 2 describes an online survey of UK dog owners, which aimed to investigate the factors associated with diet choice for dogs, as well as the sources of information sought by dog owners when deciding on their diet choice. It additionally aimed to investigate food hygiene practices undertaken by dog owners and their perception of the risks and benefits associated with their choice of diet for their pet.
- Chapter 3 describes a study investigating the preferred raw and cooked kibble diets available in the UK, including the favoured brands, meat proteins and sources as

discussed by dog owners. Laboratory analysis aimed to investigate the degree of contamination with *E. coli* and other *Enterobacteriaceae*, the presence of *Salmonella* spp. and AMR *E. coli*, and the phenotypic and genotypic resistance demonstrated by *E. coli* present within commonly purchased raw and cooked kibble diets.

- Chapter 4 describes a cross-sectional laboratory investigation of faecal carriage of AMR *E. coli* by dogs fed either a raw or a non-raw diet, including the phenotypic and genotypic resistance present. Alongside this, a questionnaire completed by dog owners aimed to understand the dog and owner lifestyle risk factors associated with ESBL-producing, 3GCR and MDR-*E. coli* carriage by dogs.
- Chapter 5 describes a longitudinal laboratory study of the faecal carriage of AMR *E. coli* by dogs fed either a raw- or non-raw diet within a household, alongside investigation of the concurrent carriage of AMR *E. coli* by dog owners and contamination of the household environment. Dog and owner lifestyle factors associated with the presence of ESBL-producing, 3GCR and MDR-*E. coli* were additionally assessed using a questionnaire.
- Chapter 6 presents an overall discussion of the findings of this thesis, ideas for further research stemming from the findings, and final conclusions.

Chapter 2: A Dogs' Dinner: Factors affecting food choice and feeding practices for UK dog owners feeding raw meat- based or conventional cooked diets

Published online August 2022 in Preventive Veterinary Medicine:

Morgan, G., Williams, N., Schmidt, V., Cookson, D., Symington, C., Pinchbeck, G. (2022), 'A Dog's Dinner: Factors affecting food choice and feeding practices for UK dog owners feeding raw meat-based or conventional cooked diets', *Preventive Veterinary Medicine*, 208, doi: 10.1016/j.prevetmed.2022.105741

2.1 Introduction

One of the most important decisions an owner makes during their dog ownership is what they choose to feed their pet. There is a vast range of food choices available, and while the majority of dog owners choose to feed a conventional cooked proprietary diet, an increasing number are looking to alternatives including raw meat-based diets (RMD) (Dodd *et al.*, 2020). What an owner chooses to feed is proposed to be a complex decision based on a combination of many factors (Michel, 2006), including beliefs regarding what constitutes an 'appropriate' food, the owners' perception of their dog's 'preference' for different foods, 'humanisation' of the dog and consideration of the dog as part of the family, social and cultural influences, and the owner's personal ideology for their own personal food choices, reflecting in their choices for their pet (Michel, 2006; Clemens, 2014; Dodd *et al.*, 2020; Viana, Mothé and Mothé, 2020). Additionally, the owner's pet owning history and prior experience will be likely to influence this choice. Selection of food is an area of the dog's care where the owner can actively control the wellbeing of their companion (Freeman *et al.*, 2013). As a result, decisions about food choice may be related to perceived health benefits or disease prevention (Rajagopaul *et al.*, 2016).

RMD utilise raw animal-derived ingredients such as muscle, bones and internal organs from mammals, poultry or fish, and may be either home-prepared e.g. using products from the supermarket or butchers, or ready-prepared commercial products (Freeman *et al.*, 2013). The feeding of RMD is an increasingly popular choice for pet dogs amongst dog owners globally (Schlesinger and Joffe, 2011; Hinney, 2018). A survey of pet owners in the USA and Australia identified that although commercial cooked diets comprised the majority of the diet

for ≥90% of dogs and cats, home prepared diets, raw food and table scraps comprised approximately 25% of the diet for 17% of dogs, with provision of bones and raw food at least weekly for 24% of dogs (Laflamme *et al.*, 2008). A more recent survey of dog owners from Australia, Canada, New Zealand, the UK and USA found that while conventional cooked commercial diets provided the majority of the diet for dogs, only 13% were fed this exclusively, with many being offered additional raw and/or homemade diets (Dodd *et al.*, 2020). Although data regarding the prevalence of raw feeding in the UK are limited, there was a steep increase in the number of pet food plants producing RMD in the UK up to 2018, which is likely to reflect an increase in popularity and demand of this diet choice (Withenshaw *et al*., 2020).

Dog owners who choose to feed RMD have been shown to hold particularly strong beliefs regarding the diet choice for their pet (Michel, 2006; Lenz *et al.*, 2009). While perception of their dog's 'preferred' food types and food enjoyment is an important factor in their diet choice, owners who choose RMD are more likely to be driven by the perceived health benefits when selecting their diet choice (Lenz *et al*., 2009; Morgan *et al.*, 2017). Nutritional quality and the perception of a healthier and more 'natural' diet (with respect to both the diet of ancestral wild canids and to non-processed or preserved ingredients) is also an important consideration (Morelli *et al.*, 2019; Empert-Gallegos, Hill and Yam, 2020). Data regarding pet feeding motivations and practices in the UK, including owners' hygiene practices surrounding food handling, preparation and storage, and views regarding the public health implications of such diets, are limited.

2.2 Aims

The aims of this study were to identify explanatory factors for diet choice and to explore the reasons, beliefs and sources of information behind owners' diet choices. Food hygiene and storage practices were investigated alongside analysis of risk perception for different food types, and specifically, RMD.

2.3 Materials and methods

A survey titled 'A Dog's Dinner: A survey investigating dog food selection by UK dog owners' and created using JISC online software was made available via the internet for approximately 6 weeks from the 19th of February to the 31st of March 2020 (Appendix 1). The survey was open to UK dog owners, regardless of dog food preference, and was advertised via social

media, at Crufts 2020 and via letters to a veterinary news publication and the Raw Feeding Veterinary Society (RFVS).

Questions were a combination of multiple choice, Likert scale and free text. For the food preparation and storage hygiene section, owners were directed to either a RMD or non-raw meat-based/cooked conventional diet (NRMD) specific set of questions, depending on their answer to the question 'Do you feed any raw animal material to your dog(s)'. Owners were requested to complete this section once on behalf of all dogs in the household if they were fed the same diet, or individually for each dog that was fed differently up to a possible total of 10 dogs per owner. A subset of questions regarding food preparation, storage and hygiene measures were asked only to owners who fed RMD. The remainder of the survey was completed once on behalf of the entire household, and the same set of questions was answered by all dog owners and included Likert questions on perceived health benefits and risks to the dog and any perceived public health risks associated with diet choice.

Ethics statement

All participant responses were anonymous and ethical approval was granted by the University of Liverpool Veterinary Research Ethics Committee (approval number VREC913).

Data analysis

Sample size calculations determined that a sample size of 1066 participants was required, using an estimated prevalence of raw feeding of 50%, a 3% precision and 95% confidence intervals.

Data were analysed at both 'dog' level and 'owner/household' level depending on the question.

RMD were classed as those fed raw animal material more than once weekly, and NRMD was classed as all diets comprising of cooked material (e.g., kibble, cans, trays and sachets of cooked commercial wet food, home cooked diets, vegetarian diets, etc). For this study, the very few owners who stated they fed raw animal material, such as a raw bone or raw meat scraps less than once weekly or as an occasional treat were reclassified as 'non-raw'.

Descriptive analyses included frequency and percentages (with 95% confidence intervals) of categorical and Likert scale responses and comparisons between RMD and NRMD responses were undertaken using the chi square test (or Fishers exact for any groups $N<5$). Significance was set at $p<0.05$. Univariable logistic regression was used to generate odds ratios with 95%

confidence intervals to identify dog and owner demographic explanatory variables associated with feeding either a RMD or NRMD. Two separate analyses were performed for owner/household responses and for individual 'dog' level data.

Explanatory variables with a liberal p value of <0.3 were selected for inclusion into multivariable logistic regression models to evaluate relationships between explanatory variables and the outcome. Correlations between variables were assessed and where highly correlated variables (correlation coefficient >0.7) were found, the most suitable variable was selected for inclusion into the multivariable regression model. Binary variables were assessed for correlation by examining proportions within each cell. A stepwise backwards elimination method was utilised to sequentially remove variables with a likelihood p value of >0.05. Eliminated variables were individually re-inserted back into the model and double checked at the end of modelling to ensure that significant or confounding variables had not been omitted. Finally, any biologically plausible interaction terms between variables were tested in the model before the final multivariable model was determined. The 'goodness of fit' of the final model was tested using the Hosmer-Lemeshow test.

The association between feeding either a RMD or NRMD and the food storage, preparation and hygiene practices reported were analysed by univariable logistic regression.

All statistical analyses were undertaken using SPSS 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.).

Qualitative analysis

Thematic analysis of the free text responses provided by dog owners to the dog health benefits and risks and public health questions was undertaken using an inductive approach in NVIVO 12 qualitative software (QSR International Pty Ltd. (2018) NVivo (Version 12)).

Responses were coded into 'raw' and 'non-raw' nodes. Following an iterative process of free text analysis, further nodes were generated based on common themes occurring within the free text and quotes from the free text answers were sorted into these nodes accordingly. Responses were compared qualitatively between RMD and NRMD.

2.4 Results

In total, 1831 dog owners completed the survey, detailing information for 3212 dogs; 915 (49.9%) indicating that they fed RMD and 916 (50.1%) that fed NRMD. This included 1754 (54.6%) dogs fed on RMD and 1458 (45.4%) fed NRMD.

1.Owner demographics and dog signalment

Owner demographics and univariable logistic regression results are shown in appendix table A1.1.

Multivariable analysis of owner factors associated with feeding RMD (Table 2.1) showed that dog owners who owned 2, 3 or 4 dogs were more likely to feed RMD compared to those who owned 1 dog, and those who fed RMD were less likely to have obtained their dog(s) from a friend or colleague. Dogs kept for breeding and working purposes, including farm, were more likely to be fed RMD (Table 2.1).

There were significant differences in reasons for diet choice ($p<0.05$) and sources of diet information $p<0.001$; dog owners who fed RMD were more likely to cite it being more natural, lack of trust of certain foods and behavioural and coat quality as reasons for their diet choice. Owners who fed NRMD were more likely to cite advice from a veterinary professional, safety concerns and cost. Dog owners who fed RMD were more likely to cite a pet food group on social media, dog breeder and a friend or family member as their main source of dietary information, compared to a veterinary surgeon or nurse.

Dog owners provided additional free text comments (RMD=2,612 comments, NRMD=2,058 comments) giving more in depth detail for their diet choice (Appendix tables A1.3-A1.4). Common themes from those who fed RMD were “believe it to be a more natural diet” (N=744), “stool consistency” (N=475), “coat quality” (N=378) and “lack of trust of certain foods” (N=209). Conversely, for NRMD, the most prominent answers were “advice from a veterinary professional” (N=410), “stool consistency” (N=325), “cost” (N=231) and “to address existing health concerns” (N=205). A number of additional themes (that were not listed as tick box options) regarding preventative health emerged, cited by owners feeding both RMD and NRMD, including dental health (N=10 RMD, N=4 NRMD), body condition (N=14 RMD, N=12 NRMD), nutritional content (N=20 RMD, N=56 NRMD) and general health (N=23 RMD, N=21 NRMD). ‘Convenience’ was also important for NRMD owners (N=25).

Table 2.1 *Multivariable regression model of owner-level (N=1831) explanatory variables significantly associated with RMD choice in a survey of diet choices made by UK dog owners*

Variable	Category	Odds ratio	CI	p value
Place obtained				
Friend/colleague	No	*Ref*		
	Yes	0.56	0.36, 0.88	0.01
Purpose of dog(s) in household				
Breeding	No	*Ref*		
	Yes	2.60	1.06, 6.37	0.04
Working/farm	No	*Ref*		
	Yes	1.79	1.05, 3.04	0.03
Other*	No	*Ref*		
	Yes	5.62	1.68, 18.73	0.01
Reason for diet choice				
More natural	No	*Ref*		
	Yes	19.06	14.18, 25.62	<0.001
Lack of trust	No	*Ref*		
	Yes	2.02	1.32, 3.10	<0.001
Behavioural reasons	No	*Ref*		
	Yes	1.91	1.21, 3.01	0.01
Coat quality	No	*Ref*		
	Yes	1.75	1.29, 2.38	<0.001
Advice from vet professional	No	*Ref*		
	Yes	0.43	0.28, 0.67	<0.001
Safety concerns	No	*Ref*		
	Yes	0.43	0.21, 0.90	0.02
Cost	No	*Ref*		
	Yes	0.31	0.21, 0.46	<0.001
Source of diet information	Veterinary surgeon/nurse	*Ref*		
	Advertisement	0.53	0.05, 6.38	
	Dog breeder	3.18	1.65, 6.12	
	Dog trainer	1.79	0.77, 4.19	
	Friend/family	2.84	1.45, 5.59	
	Other social media group	1.91	0.73, 5.02	<0.001
	Personal experience	0.90	0.54, 1.49	
	Pet food company website	0.68	0.27, 1.74	
	Pet food group on social media	17.07	6.52, 44.69	
	Rescue centre/charity	2.32	0.70, 7.75	
	Other	1.22	0.71, 2.08	
Number of dogs owned	1	*Ref*		
	2	1.42	1.03, 1.97	
	3	3.50	2.08,5.88	<0.001
	4	4.98	2.44, 10.15	
	5+	1.08	0.55, 2.13	

Ref= reference category, *denotes all breeds represented at less than 2%

Female entire (FE) and male entire (ME) dogs were significantly more likely to be fed RMD than male neutered (MN) or female neutered (FN) dogs (Table 2.2). Overall, young, and middle-aged dogs were more likely to be fed RMD than geriatric dogs, with the reference

category being dogs aged >12 years old as this was the category represented by the greatest number of dogs fed NRMD.

German Shepherd Dogs, Border Collies, Crossbreeds, and 'Other' breeds were more likely to be fed RMD compared to Labradors, which were the breed with the greatest representation of NRMD dogs. 'Other' included all breeds represented at less than 2% in this survey. Complete dog signalment data and univariable logistic regression results are shown in appendix table A1.2.

Table 2.2: *Multivariable regression model of dog-level (N=3212) explanatory variables significantly associated with RMD choice in a survey of diet choices made by UK dog owners.*

Variable	Category	Odds ratio	CI	p value
Dog sex	FN	*Ref*		
	FE	2.45	1.80, 3.33	
	ME	1.69	1.29, 2.22	<0.001
	MN	0.93	0.76, 1.14	
	Unknown	1.68	1.02, 2.77	
Dog breed	Labrador	*Ref*		
	Border Collie	1.67	1.08, 2.58	
	Cocker Spaniel	1.49	0.95, 2.34	
	Crossbreed	1.48	1.02, 2.16	<0.001
	GSD	5.21	2.61, 10.43	
	Others	2.01	1.44, 2.81	
	Unknown	1.62	0.97, 2.70	
Dog age	<6 months	1.98	1.03, 3.78	
	7-12 months	1.07	0.61, 1.89	
	1-4 years	1.41	1.02, 1.94	
	5-8 years	1.63	1.18, 2.26	0.022
	9-11 years	1.18	0.83,1.68	
	>12 years	*Ref*		
	Unknown	1.18	0.78, 1.81	

Ref= reference category

2. *Perceived health benefits and risks of RMD and NRMD*

Of the perceived health benefits and risks of each diet there were significant differences between the responses of owners who fed RMD and those who fed NRMD (Table 2.3).

A higher proportion of owners who fed RMD believed it to be beneficial with regards to a number of health factors, including for skin problems/allergies, coat health, dental disease and general digestive system health, compared to those who fed NRMD (Table 2.3). Virtually no RMD-feeding owners believed the diet to be a health risk for these factors.

By far the greatest risks of RMD as perceived by owners who fed NRMD were foreign bodies and bone splinters; however, far fewer owners who fed RMD indicated that they felt it constituted a health risk for these factors.

Feeding NRMD was seen as a health risk by owners who fed RMD for most of the health factors listed, with approximately 50% or more of RMD-feeding owners indicating that NRMD posed a risk for skin problems and allergies, coat health, dental disease and oral hygiene, general digestive system health, diarrhoea, anal sac clearance, and dog behaviour.

Table 2.3: *Dog health benefits and risks of feeding either RMD or NRMD as selected by dog owners in a survey of UK dog diet choices. The table details the percentage of RMD and NRMD feeding owners who perceived either health benefit, health risk, no effect or "don't know" for each health variable and the associated p value for the comparison (chi square). Owners who did not provide an answer for this section were omitted.*

	Owner Response Towards RMD						**Owner Response Towards NRMD**					
	Owner	**Health Benefit N (%)**	**Health Risk N (%)**	**No Effect N (%)**	**Don't Know N (%)**	**p value**	**Owner**	**Health Benefit N (%)**	**Health Risk N (%)**	**No Effect N (%)**	**Don't Know N (%)**	**p value**
Skin problems/ allergies	**RMD N=915**	92.1 (843)	0.2 (2)	3.6 (33)	4.0 (37)	<0.001	**RMD N=898**	7.5 (67)	67.8 (609)	10.9 (98)	13.8 (124)	<0.001
	NRMD N=903	25.0 (226)	9.2 (83)	25.9 (234)	39.9 (360)		**NRMD N=901**	55.7 (502)	6.3 (57)	19.9 (179)	18.1 (163)	
Coat health	**RMD N=915**	95.4 (873)	0.0 (0)	2.4 (22)	2.2 (20)	<0.001	**RMD N=898**	9.4 (84)	49.3 (443)	23.1 (207)	18.3 (164)	<0.001
	NRMD N=903	23.9 (216)	6.0 (54)	31.3 (283)	38.8 (350)		**NRMD N=902**	63.5 (573)	3.0 (27)	18.8 (170)	14.6 (132)	
Dental disease/ oral hygiene/ bad breath	**RMD N=915**	90.9 (832)	0.4 (4)	5.8 (53)	2.8 (26)	<0.001	**RMD N=898**	4.6 (41)	68.3 (613)	13.7 (123)	13.5 (121)	<0.001
	NRMD N=903	22.9 (207)	23.4 (211)	22.0 (199)	31.7 (286)		**NRMD N=902**	53.0 (478)	9.4 (85)	22.4 (202)	15.2 (137)	
Good general digestive system health	**RMD N=915**	96.5 (883)	0.0 (0)	1.4 (13)	2.1 (19)	<0.001	**RMD N=898**	7.2 (65)	62.5 (561)	14.5 (130)	15.8 (142)	<0.001
	NRMD N=903	18.3 (165)	34.0 (307)	15.2 (137)	32.6 (294)		**NRMD N=902**	70.0 (631)	3.7 (33)	13.4 (121)	13.0 (117)	
Vomiting	**RMD N=915**	44.3 (405)	1.4 (13)	37.8 (346)	16.5 (151)	<0.001	**RMD N=898**	2.6 (23)	36.5 (328)	30.2 (271)	30.7 (276)	<0.001

	NRMD N=903	4.5 (41)	42.3 (382)	17.8 (161)	35.3 (319)		**NRMD N=902**	30.8 (278)	3.3 (30)	43.8 (395)	22.1 (199)	
Diarrhoea	**RMD N=915**	60.3 (552)	1.3 (12)	27.0 (247)	11.4 (104)	<0.001	**RMD N=898**	3.5 (31)	51.1 (459)	21.6 (194)	23.8 (214)	<0.001
	NRMD N=903	6.0 (54)	45.3 (409)	15.1 (136)	33.7 (304)		**NRMD N=902**	36.3 (327)	5.1 (46)	39.1 (353)	19.5 (176)	
Anal sac clearance	**RMD N=915**	75.5 (691)	0.5 (5)	11.5 (105)	12.5 (114)	<0.001	**RMD N=898**	2.6 (23)	51.8 (465)	22.2 (199)	23.5 (211)	<0.001
	NRMD N=903	12.2 (110)	14.4 (130)	27.2 (246)	46.2 (417)		**NRMD N=901**	31.3 (282)	6.0 (54)	32.4 (292)	30.3 (273)	
Mobility	**RMD N=915**	72.3 (662)	0.0 (0)	15.5 (142)	12.1 (111)	<0.001	**RMD N=898**	6.6 (59)	39.1 (351)	28.0 (251)	26.4 (237)	<0.001
	NRMD N=903	8.2 (74)	8.7 (79)	40.3 (364)	42.7 (386)		**NRMD N=901**	47.2 (425)	1.8 (16)	28.6 (258)	22.4 (202)	
Performance	**RMD N=915**	76.7 (702)	0.1 (1)	11.4 (104)	11.8 (108)	<0.001	**RMD N=898**	5.3 (48)	41.3 (371)	25.8 (232)	27.5 (247)	<0.001
	NRMD N=903	9.1 (82)	6.8 (61)	41.0 (370)	43.2 (390)		**NRMD N=901**	41.6 (375)	2.0 (18)	34.0 (306)	22.4 (202)	
Behaviour	**RMD N=915**	73.8 (675)	0.0 (0)	15.6 (143)	10.6 (97)	<0.001	**RMD N=898**	4.0 (36)	53.9 (484)	19.7 (177)	22.4 (201)	<0.001
	NRMD N=903	9.1 (82)	8.9 (80)	40.5 (366)	41.5 (375)		**NRMD N=901**	33.5 (302)	4.4 (40)	38.2 (344)	23.9 (215)	
Foreign bodies	**RMD N=915**	15.2 (139)	16.2 (148)	50.2 (459)	18.5 (169)	<0.001	**RMD N=898**	6.3 (57)	18.4 (165)	47.4 (426)	27.8 (250)	<0.001
	NRMD N=903	2.1 (19)	62.8 (567)	8.5 (77)	26.6 (240)		**NRMD N=902**	34.1 (308)	2.5 (23)	46.8 (422)	16.5 (149)	
Bone splinters	**RMD N=915**	10.1 (92)	19.9 (182)	54.5 (499)	15.5 (142)	<0.001	**RMD N=898**	6.5 (58)	15.3 (137)	54.9 (493)	23.4 (210)	<0.001
	NRMD N=903	1.7 (15)	65.7 (593)	7.0 (63)	25.7 (232)		**NRMD N=892**	36.4 (325)	3.1 (28)	44.8 (400)	15.6 (139)	

Thematic analysis of owner responses from free text boxes discussing additional dog health risks for RMD and NRMD revealed several further themes, shown with supporting quotes in appendix tables A1.5- A1.6.

Owners who fed RMD, and those who fed NRMD, volunteered a number of additional risks of feeding RMD, including choking or unspecified risks associated with bones, constipation, cost, inconvenience/food freshness, general health concerns, poor quality/poor suppliers, lack of knowledge and safety (including generic risk due to lack of hygiene, nutritional risk; parasites/worms; pathogens/bacteria/contamination; and risk to human health/public health). Owners who fed NRMD cited obesity/problems regarding weight as an additional risk of RMD, conversely owners who fed RMD regarded feeding NRMD as a risk for weight problems.

Both groups of owners volunteered similar responses around additional health risks of NRMD, with concerns regarding ingredients being highlighted as important, including additives, fillers and ingredient quality. It was acknowledged by both groups that not all cooked, commercial kibble diets were the same and they were perceived to vary in quality.

A commonly cited 'other' health benefit of RMD was palatability, cited by both owners who fed RMD and NRMD. Owners who fed NRMD indicated that nutrition was an important health benefit of NRMD, with quotes centring around it being a nutritionally complete, balanced diet; whereas the main additional benefit of NRMD cited by owners who fed RMD was convenience (Appendix tables A1.7-A1.8).

3. Public Health perceptions and beliefs

There were significant differences in perceptions of risk between owners who fed RMD and those who fed NRMD (Table 2.4). NRMD feeding owners were more likely to perceive RMD diets as posing a risk to their dog, in contact dogs and people, whereas owners who fed RMD were more likely to perceive "no" or "maybe some" risk to these categories. Most owners who fed NRMD felt that there was no risk to their dog of feeding NRMD whereas owners who fed RMD believed feeding a NRMD did pose a risk to their dog. Most of both groups of owners felt that feeding NRMD posed no risk to themselves or in contact people.

Table 2.4: *Risk perception by UK dog owners regarding feeding RMD and NRMD selected in a survey of dog diet choices by owners who feed RMD (N=915) and NRMD (N=916)*

Level of risk	Diet type fed by Owner	Owner response to RMD % (N= 915 RMD, 916 NRMD)						Owner response to NRMD % (N= 915 RMD, 916 NRMD)					
		Yes, there is a risk	There may be some risk	There is no risk	Don't know	No answer	p value	Yes, there is a risk	There may be some risk	There is no risk	Don't know	No answer	p value
Risk to your dog	**RMD**	1.3 (12)	21.3 (195)	74.9 (685)	2.0 (18)	0.5 (5)	<0.001	43.8 (401)	35.4 (324)	14.2 (130)	5.0 (46)	1.5 (14)	<0.001
	NRMD	44.3 (406)	32.0 (293)	8.0 (73)	14.3 (131)	1.4 (13)		2.8 (26)	32.8 (300)	54.9 (503)	8.3 (76)	1.2 (11)	
Risk to you	**RMD**	4.3 (39)	32.3 (296)	62.1 (569)	0.8 (7)	0.4 (4)	<0.001	4.2 (38)	19.1 (175)	67.2 (615)	8.0 (73)	1.5 (14)	<0.001
	NRMD	46.9 (430)	24.8 (227)	14.5 (133)	12.3 (113)	1.4 (13)		0.2 (2)	6.4 (59)	84.3 (772)	7.9 (72)	1.2 (11)	
Risk to in-contact dogs	**RMD**	0.5 (5)	6.6 (60)	89.0 (814)	3.5 (32)	0.4 (4)	<0.001	3.0 (27)	12.0 (110)	72.6 (664)	10.9 (100)	1.5 (14)	<0.001
	NRMD	33.8 (310)	21.1 (194)	21.7 (199)	21.8 (200)	1.4 (13)		0.3 (3)	5.9 (54)	84.4 (773)	8.2 (75)	1.2 (11)	
Risk to in-contact people	**RMD**	1.9 (17)	16.8 (154)	78.4 (717)	2.5 (23)	0.4 (4)	<0.001	3.4 (31)	14.0 (128)	70.7 (647)	10.4 (95)	1.5 (14)	<0.001
	NRMD	39.5 (362)	18.7 (171)	20.9 (191)	19.5 (179)	1.4 (13)		0.1 (1)	5.0 (46)	85.4 (782)	8.3 (76)	1.2 (11)	

There were fewer free text comments regarding perceived specific risks of NRMD (N=757, RMD=499, NRMD=258) than RMD (N=1,336, RMD=539, NRMD=797) from both categories of owners (Appendix tables A1.9-A1.10). For owners who fed RMD, frequently mentioned specific risks of RMD pertained to good hygiene (or lack of) with regards to its use (N=177). Owners suggested there was a risk of pathogens and bacteria (N=57), however, a common theme was that these risks were reduced by appropriate hygiene measures (N=40).

However, owners who fed NRMD frequently cited pathogens and bacteria as perceived risks of RMD (N=177) as well increased risk of *Salmonella* spp. (N=141) and *Campylobacter* spp. (N=58) infection/transmission with RMD.

The more commonly cited risks of NRMD by both groups of owners involved ingredients (N=50 RMD, N=23 NRMD) and allergies (N=35 RMD, N=29 NRMD). The belief that the risk of *Salmonella* spp. was increased in NRMD was also often cited by owners who fed RMD (N=48).

4. Owner and Dog hygiene measures

There was no significant difference between the responses of dog owners who fed RMD and those who did not with regards to where in the household dogs slept or whether dogs licked human hands or faces. There was, however, a significant difference in where in the household dogs were fed; owners who fed RMD were less likely to feed their dog(s) indoors in a room other than the kitchen, but more likely to feed outside (Table 2.5).

Questions pertaining to dog bowl hygiene were asked at the dog level (N=3212). Dogs fed RMD were less likely to have food left and to have the bowl left down, and were more likely to have the bowl removed and cleaned after the meal, although they were also more likely to have any remaining food saved (Table 2.5).

Food bowls for dogs fed RMD were significantly more likely to be washed after every meal. Additionally, they were more likely to be washed by hand with bleach or washing up liquid, or in the dishwasher compared to rinsing out with water alone.

Table 2.5 *Univariable results for owner-level (N=1831) and dog-level (N=3212) hygiene measures comparing RMD and NRMD feeding responses in a survey of diet choices made by UK dog owners.*

Variable (Owner) **Totals**	**Category** (Owner)	**N** **1831**	**% of total**	**Diet choice % (N)** *Non-Raw* **50.0 (916)**	*Raw* **50.0 (915)**	**Odds ratio**	**CI**	**p value**
Where dog(s) in household eat	Indoors, in the kitchen	1317	71.9	70.6 (647)	73.2 (670)	*Ref*		
	Indoors, room other than kitchen	404	22.1	23.5 (215)	20.7 (189)	0.79	0.63, 0.99	
	Outside	86	4.7	4.1 (38)	5.2 (48)	3.31	1.98, 5.51	<0.001
	Other	21	1.1	1.4 (13)	0.9 (8)	1.34	0.56, 3.19	
	Unknown	3	0.2	0.3 (3)	0.0 (0)	0.5	0.05, 5.54	
Where dog(s) in household sleep	Indoors in room other than bedroom	903	49.3	51.9 (475)	46.8 (428)	*Ref*		
	Bedroom on human bed	454	24.8	23.9 (219)	25.7 (235)	1.19	0.95, 1.49	
	Bedroom on floor/in dog bed	419	22.9	21.5 (197)	24.3 (222)	1.25	0.99, 1.58	0.25
	Outside kennel	18	1	1.1 (10)	0.9 (8)	0.89	0.35, 2.27	
	Other	35	1.9	1.5 (14)	2.3 (21)	1.66	0.84, 3.31	
	Unknown	2	0.1	0.1 (1)	0.1 (1)	1.11	0.07, 17.80	
Whether dog(s) lick human face/hands	Never	164	9	9.8 (90)	8.1 (74)	*Ref*		
	Yes, but rarely	737	40.3	42.4 (388)	38.1 (349)	1.09	0.78, 1.54	
	Yes, quite often	559	30.5	28.3 (259)	32.8 (300)	1.41	0.99, 2.00	0.12

	Yes, frequently	364	19.9	19.2 (176)	20.5 (188)	1.3	0.90, 1.88	
	Unknown	7	0.4	0.3 (3)	0.4 (4)	1.62	0.35, 7.48	
Variable	**Category**	**N**	**% of total**	**Diet choice % (N)**		**Odds ratio**	**CI**	**p value**
(Dog)	(Dog)			*Non-Raw*	*Raw*			
Totals		**3212**		**45.4 (1458)**	**54.6 (1754)**			
What is done with bowl/feeding utensil after eating?	Food left, leave bowl down	222	6.9	12.9 (188)	1.9 (34)	*Ref*		<0.001
	Never food left, remove and clean bowl	1911	59.5	45.0 (656)	71.6 (1255)	10.58	7.25, 15.43	
	Never food left, leave bowl down	779	24.3	31.8 (464)	18.0 (315)	3.75	2.54, 5.56	
	Remove bowl, throw away food	144	4.5	5.2 (76)	3.9 (68)	4.95	3.03, 8.08	
	Remove bowl, save food	143	4.5	4.4 (64)	45.0 (790)	6.83	4.17, 11.16	
	Unknown	13	0.4	0.7 (10)	0.2 (3)	1.66	0.43, 6.34	
Bowl/feeding utensil washing method	Rinse out with water only	142	4.4	7.1 (103)	2.2 (39)	*Ref*		<0.001
	Hand wash with washing up liquid	2027	63.1	66.1 (964)	60.6 (1063)	2.91	1.99, 4.25	
	Dishwasher	892	27.8	24.1 (351)	30.8 (541)	4.07	2.75, 6.03	
	Hand wash with bleach	24	0.7	0.3 (4)	1.1 (20)	13.21	4.24, 41.08	
	Other	119	3.7	1.9 (28)	5.2 (91)	8.58	4.90, 15.05	
	Unknown	8	0.2	0.5 (8)	0.0 (0)	**	**	
Bowl/utensil washing frequency	Never	61	1.9	3.0 (44)	1.0 (17)	*Ref*		<0.001
	Less frequently	1638	51.0	67.8 (988)	37.1 (650)	1.70	0.97, 3.01	
	After every meal	1495	46.5	28.5 (416)	61.5 (1079)	6.71	3.79, 11.88	
	Unknown	18	0.6	0.7 (10)	0.5 (8)	2.07	0.70, 6.13	

5. Raw only data

An additional subset of questions regarding food preparation, cleaning measures and storage were asked to owners who fed RMD (Table 2.6). Nearly half of owners stored the RMD in a separate/dedicated pet-food fridge or freezer, although approximately 40% of respondents stored it in their own fridge or freezer. Most owners who fed RMD believed that freezing killed most bacteria and for RMD products that were supplied frozen, a wide range of defrosting places were utilised; most commonly the kitchen work surface at room temperature was used.

Approximately three-quarters of RMD-feeding owners prepared the dog food in the same place as their own food was prepared e.g., kitchen. When preparing their dog's food, most owners did not use a separate chopping board or utensils or wear gloves.

The majority of RMD-feedings owners indicated that they always cleaned the food preparation area and washed their hands immediately following preparation.

Table 2.6: *Survey responses (number (N) and percentage (%)) from UK dog owners who fed RMD (N=915) regarding food storage, preparation, and cleaning measures.*

Variable **Total**	**Response**	**N** **915**	**%**
Storage			
Raw food storage place	Separate/dedicated pet food fridge/freezer	420	45.9
	My own fridge/freezer	354	38.7
	Multiple places	129	14.1
	Non-temperature-controlled cupboard	4	0.4
	Other	5	0.5
	Unknown	3	0.3
Raw food defrosting place	Kitchen work surface, room temperature	242	26.4
	Fridge	186	20.3
	Work surface in dedicated pet food preparation area, room temperature	154	16.8
	Kitchen sink	108	11.8
	Dedicated pet food sink/microwave	39	4.3
	Dedicated/separate pet food fridge	34	3.7
	Kitchen microwave	10	1.1
	Other	110	12
	Not applicable to me	29	3.2
	Unknown	3	0.3
Opinion on freezing raw meat	Freezing meat kills most bacteria	410	44.8
	Freezing meat does not kill bacteria	202	22.1
	I don't have an opinion on freezing meat	190	20.8
	Freezing meat kills all bacteria	74	8.1
	I don't know	36	3.9
	Unknown	3	0.3
Preparation			
Raw food preparation place	Same area as my own food prepared e.g., kitchen	707	77.3

	Different area to where my food is prepared e.g., utility room, garage	191	20.9
	Multiple places	9	1.0
	Unknown	8	0.9
Separate chopping board/utensils	No	365	39.9
	Separate chopping board and utensils	259	28.3
	Separate utensils	156	17
	Separate chopping board	124	13.6
	Unknown	11	1.2
Wear gloves to prepare raw meat diet			
	Yes	128	14
	No	772	84.4
	Unknown	15	1.6
Cleaning measures			
Preparation area cleaned immediately			
	Always	739	80.8
	Usually	135	14.8
	Sometimes	27	3
	Never	7	0.8
	Unknown	7	0.8
Separate cleaning materials	Yes	485	53.0
	No	425	46.4
	Unknown	5	0.5
Hand wash after raw food preparation			
	1 (Always)	809	88.4
	2 (Less frequently)	81	8.9
	3 (Never)	20	2.2
	Unknown	5	0.5

2.5 Discussion

This study found dog breed, sex/neuter status and purpose were associated with diet choice. Dog age was also important, with younger dogs being more likely to be fed RMD. These results are in agreement with the findings of a previous survey-based study from Italy (Morelli *et al*., 2019), in which dogs fed a RMD commonly were medium to large breed and entire. Additionally, there was a peak in raw feeding in puppies <6 months of age. This may be explained by the finding that dog breeders were a significant source of information of RMD feeding (increased odds). Whilst obtaining a dog from a breeder was not significant for feeding RMD in the multivariable model in this study, it was significant in the univariable analysis. It would be expected that breeders could influence the diet choices of the puppies being sold, at least initially, as they may impart information to new dog owners including providing samples of food to go home with. Up to a third of respondents to a survey of the feeding practices of dog breeders in the USA and Canada fed RMD to breeding bitches and their puppies (Connolly *et al*., 2014).

In addition to dog breeders, the main sources of diet information for owners who fed RMD in this study were friends and/or family and, overwhelmingly, pet food groups on social media, as opposed to owners who fed NRMD who were more likely to seek information

regarding diet from a veterinary professional. In a previous study in the USA, 20% of owners who fed RMD identified online resources as their primary source of information regarding diet and nutrition, with only 9% consulting a veterinary professional or animal nutritionist (Morgan *et al*., 2017). Limited trust in veterinary professionals regarding pet diet was also reported by owners feeding RMD. The utilisation of information resources other than a veterinary professional for dietary advice and information for RMD has been seen in other survey-based study findings (Morelli *et al*., 2019), and others have also reported that owners who feed RMD lack trust in veterinary professionals with regards to pet diet and nutrition compared to owners who feed a conventional NRMD diet (Connolly *et al*., 2014; Rajagopaul *et al.*, 2016). In the UK, Empert-Gallegos *et al*. (2020) observed that owners who fed RMD rated their veterinary surgeon's knowledge regarding canine nutrition as lower, and their own knowledge higher, than owners who fed NRMD. Therefore, owners who choose RMD may not trust vets regarding diet advice as they believe they have limited knowledge.

The reasoning behind the choices made by owners regarding their diet, and the perceived health risks and benefits of the diets in this study, were clearly highly complex and based on a range of factors. There were distinct differences between owners who chose to feed RMD and those who chose NRMD. Owners who fed NRMD were most likely to choose the diet based on cost and advice from a veterinary professional. According to our model, safety concerns may also be one of the reasons NRMD feeders choose not to feed RMD. This result is similar to the findings of a previous UK study, where owners who fed NRMD cited terms such as 'expensive', 'time' and 'risk' as reasons for why they did not feed RMD (Empert-Gallegos *et al*., 2020).

The most likely reason for choosing RMD in this study was that it was perceived to be a more natural choice of diet, with other significant reasons being a lack of trust of certain foods, behavioural reasons, and improved coat quality. This is in agreement with other similar survey-based work, with 69% of owners who fed RMD in one recent study citing they chose the diet as they felt it respected the 'animal nature' of the dog (Viegas *et al.*, 2020), and 93% of owners in another study choosing RMD as they believed it was a more natural, 'species-appropriate' diet (Bulochova and Evans, 2021a). Additionally, the survey by Morelli *et al*., (2019) identified important reasons for choosing RMD were to respect the 'ancestral carnivorous nature' of the dog and to 'avoid commercial food', further supporting the findings in this study. It should be noted that in this study, the term 'natural' was not specifically defined, therefore as seen in the free text comments it could have been

interpreted in terms of ingredients (not processed or preserved) or in terms of the perceived diet of wild canids in nature. Both interpretations were discussed by owners in this study.

The association between the lack of trust of commercial NRMD and pet food manufacturers, and the choice to feed a non-commercial diet has been observed in previous work (Connolly, Heinze and Freeman, 2014) and has been linked to concerns regarding the origin of the constituent parts and ingredient contamination (Bulochova and Evans, 2021b; Dodd *et al*., 2020). One study found that owners who fed a proportion of more than 50% of their pet's diet as non-commercial food having increased concerns regarding commercial pet food and the pet food industry (Michel *et al.*, 2008). In this study, both a lack of trust of component ingredient quality and of large commercial pet food companies in general were discussed by RMD-feeding dog owners.

In this study, owners who fed RMD perceived a broad range of multiple health benefits to be associated with this diet choice, with strongly opposing views regarding NRMD This is unsurprising as previous studies have observed similar findings, reporting that owners who fed RMD were more likely to be driven by perceived health benefits and treatment effects compared to those who did not feed raw animal material (Lenz *et al*., 2009; Morgan *et al*., 2017). Furthermore, in previous survey- and netnography-based studies, the most common owner-reported health benefits of RMD were associated with muscle mass improvement, teeth, coat and general health (Bulochova and Evans, 2021b; Empert-Gallegos *et al*., 2020; Morelli *et al*., 2019). However, a critical review of the evidence surrounding the feeding of raw diets by Schlesinger and Joffe (2011) concluded that the evidence for nutritional benefit (or risk) was low level. There have been some studies since which have attempted to provide evidence in relation to the benefits of raw diets with respect to dental calculus (Marx *et al.*, 2016), urinary calcium and oxalate excretion (Dijcker *et al.*, 2012), digestibility and the faecal microbiome (Sandri *et al.*, 2017) and owner-reported reduction in development of atopy when fed in puppyhood (Hemida *et al.*, 2021). However, the body of published evidence to support the generalised claims of the benefits regarding RMD is lacking and further research is required to substantiate them.

There was some agreement in the most cited 'other' health benefits and risks of RMD and NRMD from the free text responses, with both sets of owners regarding palatability as an additional health benefit of RMD and discussing concerns regarding ingredients as an additional health risk of NRMD. Whilst both groups of owners commented on concerns regarding nutrition with regards to RMD, this was the most commonly cited additional health

risk of RMD for RMD-feeding owners, with many stressing the importance of 'research' into balancing the RMD properly. There is currently very little good quality evidence regarding the nutritional quality, 'optimum' composition, and benefits of RMD. This highlights the importance of further research into this area, as there is a clear desire from owners regarding nutritional information, and the importance of disseminating this information in a way that it reaches the desired audience. Interestingly, dog owners who feed RMD were less likely to consult a veterinary professional regarding their diet choices and more likely to seek advice from non-validated, anecdotal, or opinion-based resources.

This highlights an opportunity for veterinary professionals to better engage with owners who feed RMD, as they are more able to inform on diet composition, especially with regards to dogs at different life stages or disease states. Feeding nutritionally incomplete homemade RMD has been linked to nutritional deficiencies, secondary hyperparathyroidism, osteopenia and myelopathy in young dogs (Taylor *et al.*, 2009; Hall *et al.*, 2020).

Crucially, veterinary professionals are in a position to advise on food safety and public health risks, including transmission of zoonotic diseases including Shiga-toxin producing *E. coli* O157 (STEC), *Salmonella* spp., *Listeria* spp. and *Campylobacter* spp. These pathogens pose an infectious disease risk for both dogs (Morley *et al.*, 2006; Martinez-Anton *et al.*, 2018; Jones *et al.*, 2019; Binagia and Levy, 2020) and humans, particularly for vulnerable groups such as the immunocompromised or elderly, as discussed later.

However, as previously indicated, this communication should be open and seemingly non-judgemental to ensure constructive discussion regarding dietary concerns and choices (Wales and Davies, 2021).

A frequent health risk of RMD cited by owners who fed NRMD was regarding pathogens, with many owners being aware of zoonotic infectious agents such as *E. coli*, *Salmonella* spp. and *Campylobacter* spp. This, and the concerns regarding foreign bodies and bone fragments, supports the result of the regression model where concern regarding safety was one of the reasons for owners choosing to feed NRMD. Owners who fed RMD also reported awareness of pathogens, but it was not to the same degree. In this study, the vast majority of dog owners who fed RMD perceived RMD to present a low risk not only to their dog, but also to themselves and in-contact dogs and people; and the majority of both NRMD and NRMD-feeding owners overall felt there was no risk to owners or in-contacts from NRMD. Although there has not been a great deal of previous work regarding the owner perception of risk regarding diet choice, these results agree with similar findings of previous studies. Morelli *et*

al. (2019) observed that 94% of owners who fed RMD considered it safe for pets, Viegas *et al*. (2020) identified that 99% owners felt that the handling and feeding of RMD posed no risk to their own health, and Bulochova and Evans (2021a) reported that 89% of RMD-feeding owners did not perceive RMD to pose a risk of foodborne illness to either themselves or family members, and suggested a perception of low risk regarding foodborne illness and high confidence in the safety of RMD by RMD-feeding owners. Additionally, Lenz *et al*. (2009) observed that 70% of owners who did not feed a raw diet either disagreed with, or were indifferent to the statement 'diets containing raw meat are healthy for dogs', which is directly comparable to the result of this current survey where 76% of owners who fed NRMD answered either 'yes there is a risk' or 'there may be some risk' to their dog regarding the feeding of RMD.

Concerningly, some owners further commented that there was no risk presented to either themselves or their dog from RMD, with additional comments regarding 'scaremongering' in relation to feeding RMD. This suggests that owners who feed RMD are not necessarily aware of the risks that the food itself may pose nor the risks the dog fed the diet could pose with regards to bacterial carriage and shedding around the home and environment. This is of particular concern if the dogs are in contact with immunocompromised people, the elderly, or young children; and would also be of concern for veterinary practices which hospitalise these dogs alongside at-risk patients, or for attending veterinary staff who have close contact with dogs fed RMD during veterinary procedures.

NRMD feeders appear to be aware of the potential contact with bacteria and pathogens in RMD. RMD feeders cited 'good hygiene' was crucial when feeding this diet and that risks could be negated if practicing good food preparation hygiene measures. Although they did not state that bacteria and pathogens were risks, compared to dogs fed NRMD, dogs fed RMD were more likely to be fed outside, have the bowl cleaned immediately after feeding, have the bowl cleaned after every meal and cleaned with more stringent measures such as bleach or the dishwasher than rinsing with water alone. These results suggest that owners who feed RMD may be aware to some extent of the immediate potential foodborne pathogen transmission risks from the food itself, a finding consistent with those of Bulochova and Evans (2021b). On the contrary, in this study, a large proportion of owners who fed RMD also stored and defrosted the diet in the same area as their own food and 77% of owners prepared the diet in the same place as they prepared their own food, with approximately 40% of owners using the same utensils for both the RMD and their own food, suggesting inconsistent application of food safety practices. Additionally, the hazardous practice of defrosting food

at room temperature was common. These findings are consistent with previous work where owners who fed RMD reported awareness of good food safety practices, however did not consistently implement them (Bulochova and Evans, 2021a, 2021b). Guidelines for safe preparation, storage and handling of raw pet foods are readily available, such as the factsheet from the Pet Food Manufacturers Association (PFMA, available at https://www.pfma.org.uk/raw-feeding-factsheet) and UK Health Security Agency (UKHSA, available at https://www.gov.uk/guidance/raw-pet-foods-handling-and-preventing-infection).

From a public health point of view, the reduced awareness of the potential infectious disease risks posed by RMD as observed in this study is concerning. RMD has been shown by previous studies to harbour pathogenic and zoonotic bacteria including STEC, which can cause serious disease in the young, elderly and immunocompromised (Kaindama *et al.*, 2020; Treier *et al.*, 2021), *Salmonella* spp., *Listeria* spp., *Campylobacter* spp. and *Clostridium perfringens* (Weese *et al*., 2005; Strohmeyer *et al*., 2006; Nemser *et al*., 2014; Nilsson, 2015; van Bree *et al*., 2018; Hellgren *et al.*, 2019). Additionally, studies have shown that dogs fed RMD may shed *Salmonella* species asymptomatically in their faeces for up to 7 days following consumption of a contaminated RMD meal (Joffe and Schlesinger, 2002; Finley *et al.*, 2007). Finally, an emerging concern regarding the use of RMD is the potential risk for transmission of antimicrobial resistant bacteria (AMR). A growing number of studies have demonstrated the presence of AMR bacteria (including extended spectrum beta lactamase (ESBL) producing-*E. coli*) in RMD foods, and in the bacteria shed within faeces of dogs fed these diets (Leonard *et al.*, 2015; Nilsson, 2015; Schmidt *et al.*, 2015; Wedley *et al.*, 2017; van Bree *et al.*, 2018; Bacci *et al.*, 2019; Groat *et al.*, 2022). There is a lack of studies demonstrating direct transmission of these bacteria from RMD to humans, or dogs fed RMD to humans. However, a recent outbreak of four human cases of STEC in the UK were linked to the provision of raw tripe as dog food (Kaindama *et al.*, 2020). Co-carriage of ESBL-producing *Enterobacterales* has been identified in a small number of households between pet dogs and humans, with the main risk factor for canine carriage being a diet of RMD (van den Bunt *et al.*, 2020), suggesting that pet dogs may represent a further reservoir and potential route of transmission for ESBL-producing *Enterobacterales* to humans. Dogs and their owners have close and frequent physical contact, therefore transmission of bacteria between dog and human is plausible. Nevertheless, additional research is required to substantiate this further.

Of note is that the second-most frequently cited specific risk of NRMD by owners who fed RMD was *Salmonella* species. The perception that kibble is of particular risk for *Salmonella*

species transmission is commonly identified on RMD-specific social media and online resources. While it is true that *Salmonella* spp. contamination has been identified, albeit infrequently, in some commercial cooked/dehydrated foods following outbreak investigations in the USA (Behravesh *et al.*, 2010) and Germany (Schotte *et al.*, 2007), the evidence is clear that the risk of *Salmonella* spp. contamination remains far greater in RMD than NRMD (Strohmeyer *et al*., 2006; Nemser *et al*., 2014; Withenshaw *et al*., 2020). However, as the variety of pet foods available on the market continues to increase, further work to substantiate these findings is required.

There are some potential limitations to the methodology employed in this study such as inherent bias as a result of self-selection by the participants taking part. The subject of dog food choice is a particularly emotive topic, and this may encourage those who feel particularly strongly for or against one type of diet to participate. Such polarised views may not be representative of those of the wider population. The reliability of results is reliant on honesty from the participants when answering the questions, and they may be subject to bias or misinterpretation if participants answer vaguely or provide misleading responses. Furthermore, there are risks of bias in these results, particularly regarding food preparation and hygiene measures, in that owners may have answered what they think is the 'correct' answer rather than what they actually do, which could mask the true standard of food hygiene actually occurring within the study population. There is also likely to be some bias towards owners with fewer dogs, and from owners selectively completing the survey for certain dogs within their household to avoid having to complete the survey multiple times. This may lead to misclassification of an owner as RMD or NRMD-feeding if they have only answered the survey for a dog fed a certain diet type and missed another which was fed differently.

Finally, there is likely an over-representation of both owners who feed RMD and veterinary surgeons because of the participant recruitment process, which may not necessarily reflect the wider population, thus the frequency of RMD feeding here should not be viewed as representative of the UK population. The use of social media as a resource for diet information regarding RMD was potentially overrepresented in this study as a key component of the recruitment process was via social media. However, social media is undoubtedly an important and readily available means of communication, and the use and limitations of social media as a resource for pet nutrition information has been discussed previously (Hinney, 2018; Morelli *et al.*, 2019). Despite these limitations, clear differences

between the responses of owners who feed RMD and those who do not were demonstrated in this study population.

2.6 Conclusions

From this survey we have observed that owners who choose RMD seek dietary information from sources other than their veterinary surgeon, with resources such as social media being crucial. Reasons for diet choice appear to be multifactorial; however, a lack of trust of certain foods and the desire for a seemingly more natural diet choice were important, with an emphasis placed on 'good quality' and 'natural' ingredients.

Although owners who fed RMD were aware and concerned with the possible risks to their dog related to diet choice and appeared to practice some aspects of hygienic food preparation, they appeared less aware of the potential wider reaching infectious disease risks of RMD to in-contact dogs and people. This may represent a general lack of awareness, but there could also be an element of mis-trust of scientific information presented to them based on their own assessment and information from alternative sources, particularly if owners had not previously experienced illness themselves related to the food.

Although further research is required to clarify and quantify the level of risk associated with feeding RMD, from the findings of this study it is evident that any pertinent information regarding health and safety measures associated with RMD is not only accurate, but is also seen as credible to those who choose to feed this diet. Additionally, this information should be directed through relevant channels (such as social media) to ensure the wider target audience is reached.

Chapter 3: An investigation of the contamination and antimicrobial susceptibility of *Enterobacteriaceae* species present in raw and cooked kibble diets for dogs in the UK

3.1 Introduction

There are a diverse range of diet choices currently available for dog owners to select from to feed their pets, and while conventional cooked proprietary diets (including dry and semi-moist kibbles, tins and trays of wet food) make up the majority of pet dog diets, there is increasing availability of alternative options such as home-made diets, vegetarian/vegan foods, insect-based and raw meat-based diets (RMD). As such, many dogs are offered alternative options as a constituent part of their diet (Dodd *et al.*, 2020).

RMD are an increasingly popular diet choice. Broadly, RMD are composed of uncooked animal-derived material including muscle, internal organs, bones, skin and tendons (Freeman *et al.*, 2013). The 2022 PDSA Animal Welfare (PAW) report that surveyed a sample of dog owners which was demographically representative of the UK population (N=2569 dog owners), stated that 7% of dogs in the UK were fed RMD, with 5% fed pre-prepared RMD and 2% fed a home-made or home-prepared diet, although this may be an underestimation of the true prevalence within the UK dog-owning population (PDSA, 2022). Additional data, such as market share, is limited regarding the actual proportion of RMD fed to dogs in the UK. However, investigation of Animal and Plant Health Agency (APHA) surveillance data between the period of 2008-2018 indicated that the number of production plants registered to produce RMD increased greatly in that time period, suggesting a response to increased demand (Withenshaw *et al.*, 2020).

Raw materials, which are classed as category 3 animal by-products by the Department for Environment, Food and Rural Affairs (DEFRA) are allowed to be utilised in RMD for pets (https://www.gov.uk/guidance/animal-by-product-categories-site-approval-hygiene-and-disposal). This may include meat and carcasses passed fit for human consumption at the slaughterhouse, and animal material originally intended for human consumption but rejected for commercial reasons. It can also include material from animals which passed an antemortem inspection but were subsequently deemed unfit for human consumption (https://www.gov.uk/guidance/using-animal-by-products-to-make-pet-food.) Regular

product sampling must be undertaken for both *Enterobacteriaceae* and *Salmonella* spp. (https://www.gov.uk/guidance/laboratory-testing-requirements-for-animal-by-products-abps).

A variety of bacterial pathogens have been isolated from RMD worldwide including *Escherichia coli (E. coli), Salmonella* spp., *Campylobacter* spp, *Listeria* spp. and *Clostridium* spp. (Weese, Rousseau and Arroyo, 2005; Strohmeyer *et al.*, 2006; Mehlenbacher *et al.*, 2012; Nemser *et al.*, 2014; Bojanić *et al.*, 2017; van Bree *et al.*, 2018; Hellgren *et al.*, 2019; Bottari *et al.*, 2020; Kananub *et al.*, 2020; Treier *et al.*, 2021). In the UK, there have been limited studies specifically investigating the presence of bacterial pathogens in RMD; however, there have been a number of recalls involving RMD reported by the Food Standards Agency due to the presence of *Salmonella* spp. in particular (Food Standards Agency, 2021b, 2021a, 2022, all accessed March 2023). The number of *Salmonella* spp. isolations associated with raw pet food increased up to 2018, and this has been linked to the increase in number of plants registered to produce RMD in this time. Between 2014-2018, the number of *Salmonella* spp. isolations from RMD sampled by the APHA ranged from 26-244 isolations per year, compared to 4-27 isolations per year for cooked commercial kibble-based food, with the highest number of isolations occurring in both RMD and cooked kibble in 2018 (Withenshaw *et al*., 2020). In addition, in 2017 a cluster of human cases of Shiga-toxin producing (STEC) *E. coli* O157:H7 in the UK was epidemiologically linked back to the provision of contaminated RMD containing tripe (Kaindama *et al.*, 2020).

E. coli makes up part of the normal mammalian commensal intestinal flora (Johnson and Russo, 2002), and as such, is utilised as an indicator of faecal contamination of food products (Strohmeyer *et al.*, 2006). The EU absolute threshold for numbers of *E.coli* in raw pet food is 5×10^3 CFU/g (Commission Regulation (EU) No 142/2011) at the point of production; however, numbers in raw pet food samples in Europe commonly exceed this (Davies *et al*., 2019). RMD products are often described as comprising of 'human grade' meat which may lead to the perception of a better microbiological quality. However, an Italian study which sampled raw meat pet diet products (N=112) which were of 'human grade', but no longer intended for human consumption due to defects, manufacturing problems or commercial reasons, identified the presence of *E. coli* in 100% (N=52) of poultry samples, 100% (N=30) of pork samples and 93% (N=28) of beef samples tested (Bacci *et al*., 2019), as well as *Salmonella* spp. in 12% (N=6) of poultry and 13% (N=4) of pork samples.

Alongside the zoonotic disease concerns, there is increasing interest surrounding the potential for raw pet foods to be a source of antimicrobial-resistant (AMR) bacteria. Of particular interest is the presence of transmissible extended-spectrum beta lactamase (ESBL)-producing, and third generation cephalosporin resistant (3GCR), *Enterobacteriaceae.* Such resistances are of concern as they not only confer resistance to beta-lactam antibiotics, but also hydrolyse third generation cephalosporins including cefotaxime, ceftiofur, cefpodoxime and ceftazidime, which are highest priority critically important antibiotics (HPCIAs) (WHO, 2019), and are increasingly associated with multidrug resistance (Livermore and Hawkey, 2005; Livermore, 2008; Wedley *et al.*, 2017). A high prevalence of ESBL-producing and 3GCR-*Enterobacteriaceae* has been reported in pre-prepared RMD previously in European studies (Nilsson, 2015; van Bree *et al.*, 2018; Nüesch-Inderbinen *et al.*, 2019), as well as from meat products previously intended for the human food chain, but destined for pet food production (Bacci *et al.*, 2019). ESBL-producing *E. coli* has also been isolated from samples of fresh pre-packaged chicken, turkey and pork for human consumption purchased in UK supermarkets (Day *et al*., 2019; Ludden *et al*., 2019), however, this meat is intended to be cooked before consumption, which would kill both non-AMR and AMR-bacteria within it (James *et al.*, 2021)

3.2 Aims

Despite the interest in alternative diet choices and the growing canine and public health concerns regarding RMD, there are few studies investigating this aspect of dog ownership in the UK. The aims of this study were firstly to identify the most common RMD and non-raw diets (NRMD) selected by UK dog owners, their preferred treat types and from where owners obtained their dog's food. Secondly the study aimed to investigate the presence of *E. coli*, other *Enterobacteriaceae*, and *Salmonella* spp. within both commonly-purchased RMD and NRMD, as well as the presence of AMR and ESBL-producing *E. coli* within the most commonly fed diets.

3.3 Materials and methods

Methods for online survey

An online survey titled 'A Dog's Dinner: A survey investigating dog food selection by UK dog owners' was created using JISC online software. The survey was open to all UK dog owners, regardless of dog food preference and some findings including methods of dissemination

have been published (Morgan *et al.*, 2022) and are presented in chapter 2. A sub-section of the questionnaire involved questions specifically regarding the diet fed, including the types of food chosen, the sources from where foods were obtained, the preferred meat types (RMD only), preferred treat types and the preferred brands chosen. Dog owners were directed to either a raw-feeding or non-raw feeding specific set of questions, depending on their answer to the question "Do you feed any raw animal material to your dog(s)". Owners were requested to complete this section once on behalf of all dogs in the household if they were fed the same diet, or individually for each dog that was fed differently, up to a possible total of 10 dogs per owner. The survey was available online for approximately 6 weeks from the 19th of February to the 31st of March 2020.

Ethics statement

Ethical approval was granted by the University of Liverpool Veterinary Research Ethics Committee (approval number VREC913).

Statistical Methods and Study Design for online survey

The sample size of participants required to achieve statistical power for the online survey was calculated to be 1066, using an estimated prevalence of raw feeding of 50%, with 3% precision and 95% confidence intervals.

Statistical analysis was undertaken using SPSS 27 (IBM Corp. (released 2020). IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp.). The frequency and percentage (with 95% confidence intervals) of responses from participants feeding RMD and NRMD were computed. RMD were classed as those fed raw animal material more than once weekly, and NRMD being classed as all diets comprising of cooked material (e.g. kibble, cans, trays and sachets of cooked commercial wet food, home cooked diets, vegetarian diets, etc).

Descriptive analyses (frequency, percentage) was undertaken for both raw and non-raw food choices. Type of food preferred, source of food and types of treats were compared, and included options provided in the survey and those identified in the free text answers provided by owners. In addition, sources of non-pre-prepared raw meat provided were determined.

The frequency and percentage of types of meat fed raw either as a pre-prepared commercial meal or non-pre-prepared were identified. Finally, the top 20 brands of pre-prepared RMD and of NRMD were identified from the free text answers provided by dog owners.

Laboratory methods

The top 10 available brands of RMD and NRMD identified from the results of the survey were sampled. Samples from each (RMD 9-15 samples per brand, total 110 samples, NRMD 1-3 samples per brand, total 24 samples) were purchased between August 2020-October 2021 and tested to investigate the presence of *E. coli*, including AMR and ESBL-producing *E. coli*, *Salmonella* spp. and other *Enterobacteriaceae.*

Microbiological methods

Each food sample was assigned a unique number, and the brand, sample type (RMD, NRMD), batch number/lot code (where present), country of origin of ingredients and whether the product was produced in the UK was recorded. Sample packets were inspected for packaging material type and any evidence of damage or leakage. To ensure no cross-contamination of samples, RMD samples were stored frozen as per manufacturer instructions and defrosted fully in a refrigerated unit prior to testing within separate containers. NRMD samples were stored at room temperature and bags were opened only at the time of sampling. All samples tested were used within the 'use-by' date where this was provided, samples from three brands did not have a 'use-by' date provided; however, all samples were tested within one week of their delivery to the laboratory.

The amount of food (25g) to be tested was collected aseptically using sterile instruments from multiple sites within the food sample, and homogenised via stomaching in a sterile plastic stomaching bag for one minute with 225ml of buffered peptone water (BPW), at room temperature. Approximately 20ml of homogenate was poured into a sterile universal tube and incubated aerobically at 37^{o}C for 18-20h. The remainder of the RMD sample was placed into a sterile sealable bag and repeat frozen at -20^{o}C for further testing at a later date if required. NRMD bags were re-sealed and stored at room temperature.

Following incubation, a 5µl loopful of broth was used to inoculate one each of a chromogenic Harlequin *E. coli*/Coliform Agar (HECA) (Neogen, UK) and a HECA plate infused with 1µg/ml cefotaxime (HECA+Cx), and incubated overnight at 37^{o}C. Further broth (100µl) was added to 10ml of Rappaport Vassiliadis Broth (RVB) and incubated overnight at 42^{o}C for *Salmonella* species culture.

Following incubation, the HECA plates were analysed for the presence of typical *E. coli* (dark blue-violet colonies, 0.1mm-2mm diameter), and four colonies were picked and plated onto nutrient agar (NA) (Neogen, UK). The HECA+Cx plates were analysed for both typical blue *E.*

coli and other *Enterobacteriaceae* (rose pink colonies) and 2 colonies of each (if present) were picked and plated onto NA. One 5µl loopful of the incubated RVB was plated to Harlequin Chromogenic Agar for Salmonella Esterase (CASE) (Neogen, UK). The NA and CASE plates were incubated for 18-20h at 37°C.

Following incubation, the CASE plates were analysed for the presence of suspected *Salmonella* spp. (turquoise blue/green colonies) and if present, two colonies were picked and plated onto NA before overnight incubation at 37°C. Confirmation of *Salmonella* spp. was then undertaken via matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF).

Antimicrobial susceptibility testing (AST)

The *E. coli* isolates from plain HECA plates, and *Salmonella* spp. isolates, underwent antimicrobial susceptibility testing via the disc diffusion method using seven antibiotic discs chosen based on European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations (EUCAST, 2022). Isolates were inoculated into sterile saline to 0.5 McFarland using a 5µl loop, and a sterile cotton-tip swab was used to spread the inoculated saline onto Muller-Hinton agar (Neogen, UK) and antibiotic discs applied. Plates were incubated aerobically at 37°C for 18-20h. Antimicrobials tested were ampicillin 10µg, amoxycillin-clavulanic acid 20µg/10µg, ciprofloxacin 5µg, tigecycline 15µg, trimethoprim-sulphamethoxazole 1.25µg/23.75µg, amikacin 30µg and meropenem 10µg (MAST Group Ltd, Liverpool UK). A susceptible control strain of *E. coli* (ATCC 25922) was also tested to ensure disc efficacy.

Following incubation, zones of inhibition (ZOI) for each antibiotic disc were measured to the nearest millimetre. EUCAST clinical breakpoints (EUCAST, 2022) were used for interpretation for all antibiotics other than amoxycillin-clavulanic acid, where the breakpoint used for interpretation was as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2020). Isolates were defined as AMR if they demonstrated phenotypic resistance to less than three classes of antibiotics. Multidrug resistance (MDR) was defined as demonstrated phenotypic resistance to three or more classes of antibiotics tested on AST (Magiorakos *et al.*, 2012).

The *E. coli* isolates from HECA+Cx plates initially underwent the extended-spectrum beta-lactamase (ESBL) double-disc test to determine whether they were ESBL-producing or not, using cefotaxime 5µg, cefotaxime 5µg +clavulanic acid 10µg, ceftazidime 10µg and

ceftazidime 10µg +clavulanic acid 10µg discs (EUCAST ESBL detection set, MAST Group Ltd, Liverpool UK). Plates were incubated at 37°C for 18-20h. Isolates were deemed positive for ESBL-production if the ZOI surrounding the cephalosporin +clavulanic acid disc was a minimum of 5mm diameter larger than the ZOI for the corresponding cephalosporin disc alone for ≥1 antibiotic pairs; positive isolates were then continued to the full AST as described. Non-ESBL producing 3GCR isolates which did not demonstrate a typical positive result for ESBL production on the double disc test, but which demonstrated a pattern suggestive of AmpC production whereby there was no, or minimal, ZOI present surrounding the clavulanic acid disc(s), were also continued to full AST.

Bacterial enumeration of food samples

Bacterial enumeration was undertaken for food samples using the Miles and Misra method. An initial broth was made up to a 1/10 dilution (25g food in 225ml BPW) and 1ml was then added to 9ml BPW to make a 1/100 dilution. Three 20µl drops of the 1/100 dilution broth were placed onto a section of a HECA plate, followed by three 20µl drops of the 1/10 dilution broth onto a separate section. All plates were incubated overnight at 37°C.

Individual typical blue *E. coli* colonies and rose-pink colonies typical of other *Enterobacteriaceae* (such as *Enterobacter* spp.) were counted and an average of the counts of the three drops per dilution was calculated, followed by calculating the colony forming units (CFU)/g/L for each sample.

Bacterial counts were compared to the APHA acceptable levels for laboratory testing of *E. coli* and *Enterobacteriaceae* presence in animal by-products (ABP) as published at (https://www.gov.uk/guidance/laboratory-testing-requirements-for-animal-by-products-abps#how-much-bacteria-your-samples-can-contain), accessed July 2021. For the purpose of this study, the acceptable levels utilised were those presented for one sub-sample tested per sample, where samples would fail if one sub-sample tested had greater than 5000 CFU/g *E. coli* or *Enterobacteriaceae.*

Isolates which were phenotypically identified as *E. coli* underwent PCR for the *uspA* gene to confirm them as *E. coli* prior to undergoing whole genome sequencing. Methods were as per Anastasi *et al.*, 2010. Primers used were CCGATACGCTGCCAATCAGT (forward) and ACGCAGACCGTAGGCCAGAT (reverse), with an amplicon size of 884 base pairs.

Characterisation of E. coli resistance genes and whole genome sequencing (WGS)

DNA extraction was performed on ESBL-producing *E. coli* isolates using the QIAamp® DNA mini kit (Qiagen, Crawley, UK).

Genomic DNA samples were submitted to the Centre for Genomic Research, University of Liverpool for Illumina NEBNext Ultra II FS DNA Library Prep, which was completed following the manufacturer's protocol. Each library was quantified using Qubit and the size distribution assessed using the Fragment Analyzer. These final libraries were pooled in equimolar amounts using the Qubit and Fragment analyser data.

The quantity and quality of the pool was assessed by Bioanalyzer and subsequently by qPCR using the Illumina Library Quantification Kit from Kapa (KK4854) on a Roche Light Cycler LC480II according to manufacturer's instructions.

Following calculation of the molarity using qPCR data, template DNA was diluted to 300pM and denatured for 8 minutes at room temperature using freshly diluted 0.2 N sodium hydroxide (NaOH) and the reaction was subsequently terminated by the addition of 400mM TrisCl pH=8. To improve sequencing quality control 1% PhiX was spiked-in. The libraries were sequenced on the Illumina® NovaSeq 6000 platform (Illumina®, San Diego, USA) following the standard workflow over 1 lane of an S4 flow cell, generating 2 x 150 bp paired-end reads.

Bioinformatic analysis

Following sequencing, reads were assembled into contigs using SPAdes and contigs smaller than <200bps were removed. Quality control (QC) was undertaken on assemblies, and those which passed QC were subject to multi-locus sequence typing (MLST) by submitting locus allele sequences to pubmlst.org. e-BURST analysis was performed to group similar isolates based on the sharing of alleles, giving each isolate a e-BURST group assignment.

Gene prediction was carried out using Prokka. Detection of AMR genes was undertaken using Resistance Gene identifier (RGI) (https://card.mcmaster.ca/analyze/rgi), and plasmids were identified using PlasmidFinder and the *Enterobacteriaceae* plasmid marker database.

Salmonella spp. whole genome sequencing

DNA extraction and WGS was performed on *Salmonella* spp. isolates by the United Kingdom Health Security Agency, Gastrointestinal Bacteria Reference Unit.

Following DNA extraction, isolates were prepared for sequencing with Nextera XT DNA preparation kits, and sequenced on the Illumina HiSeq 2500 platform in rapid run mode to produce 100bp paired-end reads. Trimmomatic v0.40 (Bolger, Lohse and Usadel, 2014) was

used to quality trim fastq reads with bases removed from the trailing end that fell below a PHRED score of 30. The Metric Orientated Sequence Type (MOST) v1 (Tewolde *et al.*, 2016) was used for sequence type (ST) assignment and serotype was assigned using a combination of the Salmonella MLST database and SeqSero2 (Achtman *et al.*, 2012; Ashton *et al.*, 2016; Zhang *et al.*, 2019). FASTQ sequences were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive under the BioProject accession number PRJNA248792 (www.ncbi.nlm.nih.gov/bioproject/?term=248792). Raw sequence data files of isolates from this study were uploaded to EnteroBase (https://enterobase.warwick.ac.uk/) and short reads were assembled by EnteroBase using the then current backend pipelines (versions 3.61–4.1) including core genome Multi-Locus Sequence Types (cgMLST) analysis to produce a cgST as previously described (Chattaway, Chandra, *et al.*, 2019) using the cgMLST v2 HierCC v1 algorithm (Zhou *et al.*, 2018). The cgMLST quality parameters for analysis for Salmonella were met for all isolates (minimum size 4000 kbp, maximum size 5800 kbp, minimum N50 20 kbp, maximum number contigs 600, maximum low-quality sites 5 %, minimum taxonomic purity 70 % (Zhou *et al.*, 2020).

3.4 Results

Survey results

The most popular types of food for dogs fed RMD were pre-prepared raw meat and/or bone diets (78.1%), raw eggs (62.8%) and DIY/home-prepared raw meat and/or bone diets (58.8%), whereas the most popular type of food for dogs fed NRMD was overwhelmingly cooked commercial complete dry food (91.1%) (Table 3.1).

Table 3.1: *Frequency (N) and percentage (%) of types of food provided to dogs fed RMD (N=1754) and those fed NRMD (N=1458). Both food types included in the survey as multiple-selection answers and those detailed additionally as free text answers by dog owners within the 'other' category are listed.*

Type of Food	**% (N)**	
	Raw	*Non-Raw*
Total	**55.0 (1754)**	**45.0 (1458)**
Raw meat and/or bones (pre-prepared diet)	78.1 (1369)	-
Raw eggs	62.8 (1102)	-
Raw meat and/or bones (DIY/home-prepared diet)	58.8 (1032)	-

Dried food items (e.g. pig ears, rawhide chews, dried fish skin)	45.6 (800)	30.5 (444)
Cooked eggs	12.1 (212)	10.4 (152)
Cooked commercial complete dry food	9.6 (168)	91.1 (1326)
Cooked fresh meat and/or bones	8.5 (149)	18.3 (266)
Cooked commercial complete wet food	5.7 (100)	35.3 (513)
Vegetables	3.8 (67)	3.3 (48)
Fruit	2.4 (42)	0.9 (13)
Miscellaneous	2.0 (35)	1.3 (19)
Dairy	1.3 (23)	0.7 (10)
Oily fish	1.3 (23)	1.6 (23)
Vegetarian diet	1.0 (18)	2.8 (41)
Leftovers	0.9 (15)	0.9 (13)
Cold pressed food	0.5 (8)	0.3 (5)
Fresh fish	0.5 (8)	-
Bone broth	0.3 (6)	0.1 (2)
Dehydrated meat	0.3 (6)	-
Frozen Fish	0.3 (6)	-
Liver	0.3 (6)	0.1 (1)
Rabbit ears	0.3 (6)	-
Raw fish	0.3 (6)	-
Mussels	0.2 (3)	-
Air dried raw	0.1 (1)	-
Dehydrated offal	0.1 (2)	-
Fish	0.1 (2)	0.2 (3)
Freeze dried raw	0.1 (1)	0.1 (2)
Green tripe	0.1 (1)	-
Home cooked	0.1 (1)	0.3 (5)
Hooves	0.1 (1)	-
Whole prey	0.1 (1)	-
Starchy Carbohydrates	-	0.6 (9)

The main sources of food provided to dogs fed RMD were shop bought, pre-prepared frozen raw food (55.1%), raw food from an online supplier (48.2%) and fresh raw meat from the butcher or supermarket (41.2%). Again, the main source of food for dogs fed NRMD by far was shop bought or purchased online cooked dry kibble (84.6%) (Table 3.2). The predominant sources of non-pre-prepared raw meat for those who fed RMD were the supermarket (38.4%) and butcher (37.8%) (Appendix table A2.1). Sources of food that were mentioned at less than 1% were excluded.

Table 3.2: *Frequency (N) and percentage (%) of sources of the food provided to dogs fed RMD (N=1754) diet and those fed NRMD (N=1458) diet. The table is split into three sections which detail the sources most commonly offered to RMD-fed dogs, the sources most commonly offered to dogs fed NRMD and miscellaneous sources which were provided as additional or alternative 'other' sources by dog owners using the associated free text box provided in the survey.*

Source	**% (N)**	
	Raw	*Non-Raw*
Total	**55.0 (1754)**	**45.0 (1458)**
Shop bought, pre-prepared, frozen raw food	55.1 (966)	-
Raw food from an online supplier	48.2 (846)	-
Fresh raw meat from the butcher or supermarket	41.2 (723)	-
Fresh raw meat from another source e.g. specialist raw meat diet shop	29.1 (511)	-
Shop bought, pre-prepared, fresh raw food	9.9 (173)	-
Shop bought or purchased online cooked dry kibble	9.0 (157)	84.6 (1233)
Shop bought or purchased online, pre-prepared fresh cooked food e.g. tins, trays, sachets	5.8 (102)	32.6 (475)
Fresh meat from butcher or supermarket, but cook it before feeding	5.3 (93)	12.3 (179)
Shop bought or purchased online, pre-prepared frozen cooked food	4.0 (71)	3.6 (52)
Fresh meat from another source, but cook it before feeding	0.6 (10)	2.7 (40)
Abattoir	0.3 (5)	-
Farmers	0.1 (2)	-
Fishmonger	0.1 (2)	0.3 (4)
Game	0.5 (9)	-
Ourselves	0.1 (2)	0.5 (8)
Roadkill	0.1 (1)	-
Specialist supplier	0.3 (6)	-
Trainer	-	0.2 (3)
Vets	-	2.1 (30)

The most commonly fed types of raw meat provided to dogs as either a pre-prepared commercial raw diet or part of a non-pre-prepared DIY/home-prepared meal were offal (83.0%), beef (82.6%), lamb (79.0%), chicken (78.2%), turkey (75.0%) and duck (72.8%) (Table 3.3). Types of raw meat that were represented at less than 2% were excluded.

Table 3.3: *Frequency (N) and percentage (%) of types of meat provided to dogs fed RMD (N=1754), either as part of pre-prepared commercial raw diet or non-pre-prepared meat (meat types represented at <2% were excluded).*

Type of meat	**% (N)**
Total	**1754**
Offal (e.g. Tripe, heart, liver, kidney)	83.0 (1456)
Beef	82.6 (1448)
Lamb	79.0 (1386)
Chicken	78.2 (1372)
Turkey	75.0 (1315)
Duck	72.8 (1277)
Rabbit	65.2 (1143)
Venison	61.4 (1077)
Game (e.g. Pheasant, grouse, pigeon)	47.5 (834)
Pork	44.7 (784)
Fish	7.7 (135)
Goat	3.4 (59)
Kangaroo	2.9 (51)
Oily fish	2.8 (49)
Horse	2.6 (45)

The preferred types of treats given to dogs fed RMD and to those fed NRMD are detailed in appendix table A2.2. The most popular types of treat for dogs fed RMD were freeze-dried meat/fish treats (56.8%), raw bones (56.2%) and dried treats such as chicken feet, pig ears and rawhide (55.5%). By far, the most popular type of treat for dogs fed NRMD was shop bought cooked treats/biscuits (78.7%).

The proportions of the most popular brands of pre-prepared raw and non-raw cooked commercial complete dog food as identified in this survey are anonymously presented in appendix tables A2.3 and A2.4.

Packaging materials and traceability information available on sample packs

Of the ten brands studied, six had batch numbers present on the sample packs , although it was not always clearly stated as some were present on sticky labels which came unglued, or had printed numbers on the packets which were presumed to be batch numbers, although were not explicitly labelled as such (Appendix table A2.5). Five brands clearly stated that the

meat ingredients were sourced from the UK, and five had an unknown meat source but terminology such as 'organic' and 'ethically sourced' were used instead. Whether the products were made in the UK was not clear for all brands, and only two brands stated specifically that they were made in the UK; however, others stated they used British ingredients or used terminology such as 'packed in the UK. The sample packs themselves were not swabbed for evidence of contamination; however, samples from four brands were damaged on arrival and as such did not have sealed contents, and samples from eight brands did not have leakproof packaging. Samples from two brands were presented in cardboard packaging which subsequently became compromised on defrosting. Figures 3.1-3.3 demonstrate some of the damaged and contaminated packaging observed in this study. Figure 3.4 demonstrates fluid leakage in the bottom of a defrosting box following defrosting of a sample from one brand tested.

Figure 3.1: *Shattered rigid plastic packaging and open film seal from samples from two different brands of RMD*

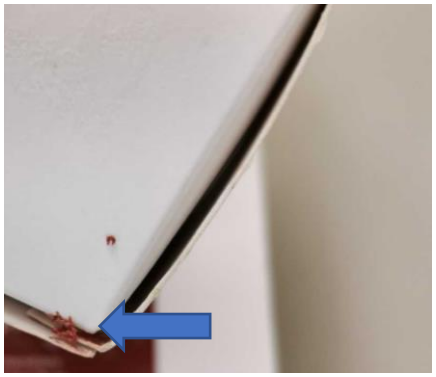


Figure 3.2: *Frozen raw material evident on outside of cardboard packaging of RMD sample prior to defrosting*

Figure 3.3: *Disintegrated cardboard packaging following defrosting of a RMD sample*

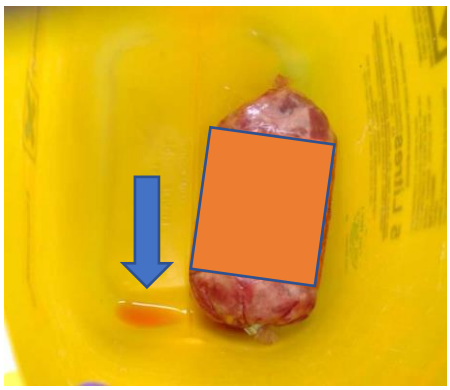


Figure 3.4: *Leaked bloody fluid following defrosting of a RMD sample*

Enumeration of colonies typical of E. coli and other Enterobacteriaceae.

Bacterial enumeration for colonies typical of *E. coli* and other *Enterobacteriaceae* was undertaken on 110 RMD samples and 24 NRMD samples from 10 brands each (Table 3.4, full enumeration results presented in appendix table A2.6). No target bacteria were isolated from any of the NRMD samples.

Of the food samples tested, 24.5% (27/110) had bacterial counts for *E. coli* greater than 5000 CFU/g, and therefore would fail DEFRA testing, and 30.9% (34/110) for other *Enterobacteriaceae.* Additionally, 20.0% (22/110) of samples had counts of both *E. coli* and other *Enterobacteriaceae* present within the same sample which exceeded 5000 CFU/g. Of the brands tested, 80% (8/10) had at least one sample tested which had counts of both *E. coli* and other *Enterobacteriaceae* greater than 5000 CFU/g, and for one brand, 60% of samples tested had *E. coli* counts greater than 5000 CFU/g, and 70% had other *Enterobacteriaceae* counts greater than 5000 CFU/g. The highest CFU/g for *E. coli* was

associated with minced feathered pigeon, and the highest CFU/g for other *Enterobacteriaceae* was associated with a pork and chicken mix.

Table 3.4: *Bacterial enumeration from RMD samples, illustrating the number of samples per brand tested, the number of samples with >5000 CFU/g of E. coli and other Enterobacteriaceae, the maximum CFU/g of E. coli and other Enterobacteriaceae isolated within a sample from each brand, and the RMD ingredients within the sample associated with this count.*

Anonymised brand	**N samples**	**N samples with >5000 CFU/g *E. coli***	**Maximum CFU/g identified**	**Protein type**	**N samples with >5000 CFU/g other *Enterobacteriaceae***	**Maximum CFU/g identified**	**Protein type**
B1	13	3	3.0E+04	Beef	5	8.7E+03	Chicken, tripe
B2	15	3	3.8E+04	Offal	4	1.5E+04	Beef
B3	14	3	2.57E+05	Lamb	3	1.05E+05	Lamb
B4	9	0	1.0E+03	Beef	2	6.7E+04	Goat
B5	10	2	6.2E+04	Pork, chicken	4	2.8E+05	Pork, chicken
B6	9	4	2.9E+05	Beef, offal	3	6.0E+04	Tripe, heart
B7	10	6	4.7E+05	Pigeon with feather	7	2.0E+05	Pork, chicken
B8	10	1	5.5E+03	Turkey	0	2.7E+03	Turkey
B9	10	1	1.1E+05	Chicken	3	1.7E+05	Chicken
B10	10	4	3.4E+05	Lamb tripe	3	9.8E+04	Beef

Antimicrobial susceptibility testing results

The majority of tested RMD samples (99.1%, 109/110) grew *E. coli,* and AMR-isolates with phenotypic resistance to at least one class of antibiotic, were isolated from 39.1% (43/110) of samples (Table 3.5). Of concern, fluoroquinolone (ciprofloxacin)-resistant *E. coli* was present in 8.2% of samples, and multidrug-resistant (MDR) *E. coli* was isolated from 7.3% (8/110) of samples. No resistance to tigecycline or meropenem was observed.

AMR *E. coli* was isolated from a number of different meat proteins, whilst MDR *E. coli* was isolated from samples containing goat, turkey and chicken only. *E. coli* was not isolated from any of the NRMD samples tested.

Table 3.5: *Percentage (%) and number (N) of RMD samples with resistance to antibiotics tested in this study, an AMR phenotype and a multidrug-resistant (MDR) phenotype detected, and their associated component protein ingredients*

Antibiotic	**% (N) RMD samples with at least one resistant *E. coli* detected**	**Component protein(s)**
Total samples	*110*	
Ampicillin	30.0 (33)	Lamb, chicken, fish, turkey, offal, tripe, goat, duck
Amoxycillin-clavulanic acid	1.8 (2)	Beef
Ciprofloxacin	8.2 (9)	Chicken, fish, goat, turkey, goose, duck
Tigecycline	0.0 (0)	N/A
TMS	14.5 (16)	Chicken, fish, offal, tripe, turkey, goat, beef
Amikacin	5.5 (6)	Chicken, offal, tripe, fish, game, lamb
Meropenem	0.0 (0)	N/A
AMR	39.1 (43)	Chicken, lamb, fish, turkey, offal, tripe, goat, duck, beef, goose
MDR	7.3 (8)	Goat, turkey, chicken

%=percentage; N/A=non-applicable

3GCR-*E. coli* (including ESBL-producing and non-ESBL producing *E. coli*) was identified from 16.4% (18/110) of samples of RMD tested, and phenotypic ESBL-producing *E. coli* (as determined by a positive double-disc test result) was isolated from 13.6% (15/110) of samples. MDR-ESBL-producing *E. coli* was isolated from 10.0% (11/110) and fluoroquinolone resistant ESBL-producing *E. coli* was isolated from 5.5% (6/110) (Table 3.6). As expected, resistance to ampicillin was observed in isolates from all 18 samples with 3GCR-*E. coli*

present. Resistance to ciprofloxacin and TMS were both observed in 40% (6/15) of samples with ESBL-producing *E. coli* present.

Table 3.6: *Presence of 3GCR- E. coli (including ESBL-producing and non-ESBL producing E. coli), ESBL-producing E. coli and MDR-E. coli in RMD samples (data presented at sample level), their associated antibiotic resistance profile and component protein ingredients*

Brand	Sample ID	Component protein(s)	ESBL-*E. coli*	3GCR-*E. coli*	MDR-*E. coli*	Antibiotic Resistance Profile (S/R)*								
						Amp	AmxC	Cip	Tig	TMS	Ami	Mer	Ctx	Ctz
B1	4	Chicken, tripe	Y	Y	Y	R	S	S	S	R	S	S	R	R
	8	Chicken, tripe	Y	Y	Y	R	S	S	S	S	R	S	S	R
	12	Chicken, tripe	Y	Y	N	R	S	S	S	S	S	S	S	R
	13	Offal, salmon	Y	Y	N	R	S	S	S	S	S	S	R	R
B2	3	Duck	N	Y	Y	R	R	S	S	R	S	S	R	R
	11	Duck	Y	Y	N	R	S	S	S	S	S	S	R	S
	13	Beef	Y	Y	Y	R	S	R	S	S	S	S	R	R
B3	5	Duck	Y	Y	N	R	S	S	S	S	S	S	R	S
	8	Lamb	Y	Y	N	R	S	S	S	S	S	S	R	R
	12	Game, tripe	Y	Y	Y	R	S	R	S	R	S	S	R	R
	13	Beef, tripe	Y	Y	N	R	S	S	S	S	S	S	R	R
B4	1	Goat	Y	Y	Y	R	S	R	S	R	S	S	R	S
	5	Goat	Y	Y	Y	R	S	R	S	R	S	S	R	S
B5	1	Duck	N	Y	Y	R	R	S	S	S	S	S	R	R
	7	Duck	N	Y	Y	R	R	S	S	S	S	S	R	R
B6	1	Lamb	Y	Y	Y	R	S	R	S	R	S	S	R	R
	2	Chicken, beef, lamb, tripe, offal	Y	Y	Y	R	S	R	S	R	S	S	R	S
B7	1	Pork, chicken	Y	Y	N	R	S	S	S	S	S	S	R	S
% (N) samples demonstrating *E. coli* with resistance to an antibiotic						100 (18)	17 (3)	33 (6)	0 (0)	39 (7)	6 (1)	0 (0)	89 (16)	67 (12)

*AmxC: Amoxycillin-clavulanic acid; Amp: Ampicillin; Tig: Tigecycline; TMS: Trimethoprim sulphamethoxazole; Ami: Amikacin; Cip: Ciprofloxacin; Mer: Meropenem; Ctx: Cefotaxime; Ctz: Ceftazidime. S: sensitive; R: resistant.

Of the samples where ESBL-producing *E. coli* was present, 46.7% (7/15) comprised of tripe and/or offal as a component ingredient, and 33.3% (5/15) comprised, at least in part, of chicken. 3GCR-*E. coli* was most frequently isolated from samples containing offal/tripe (38.9%, 7/18) and duck (27.8%, 5/18) (Appendix table A2.7).

No 3GCR-*E. coli* was isolated from any NRMD samples.

Whole genome sequencing results (isolate level)

3GCR *E. coli* isolates which demonstrated ESBL-production on the double disc test, or were non-ESBL producing and suspected of having AmpC production, and which demonstrated a unique resistance phenotype within a sample underwent WGS (N=17). Of these, 13 were phenotypic ESBL-producing *E. coli*, as determined by the double-disc test, and four were suspected to have their ESBL phenotype 'masked' due to the presence of pAmpC genes. Representative isolates were sent from all food samples other than brand B4 (samples BE1 and BE5), as isolates were not available.

Eleven distinct sequence types (STs) were identified. The most frequently observed ST was ST10 (N=4). Food samples with ST10 contained duck, lamb, beef, tripe, pork and chicken. Other STs represented by more than one isolate were ST58, ST69 and ST1629 (N=2 for each). There was no distinct relationship between the food protein types and the STs observed, other than for ST1629 where both *E. coli* isolates were from a combined chicken and tripe product (Table 3.7).

Multiple AMR genes were identified in the isolates in this study (Table 3.7). In terms of ESBL-encoding genes, $bla_{\text{CTX-M}}$ genes were present in 10 isolates (59%). The most frequently identified $bla_{\text{CTX-M}}$ gene was $bla_{\text{CTX-M-15}}$, present in seven isolates (41%), which were associated with a range of STs. The $bla_{\text{CTX-M-1}}$ gene was identified in one isolate, which was ST10. The $bla_{\text{CTX-M-27}}$ and $bla_{\text{CTX-M-55}}$ genes were present in one isolate each (ST69 and ST58, respectively). One isolate, which was ST58, carried both $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-107}}$ genes. bla_{TEM} genes were identified in 47% (8/17) of isolates; however, the only ESBL-encoding variant isolated was $bla_{\text{TEM-52}}$, which was identified in two isolates (both ST 1629). The ESBL-encoding $bla_{\text{SHV-7}}$ gene was identified in one isolate (ST10) only. The bla_{OXA} gene was not observed in any of the isolates. Five isolates did not have $bla_{\text{CTX-M}}$, bla_{TEM} or bla_{SHV} ESBL genes present; however, four

of these did have the AmpC gene *bla*$_{CMY-2}$ present (Table 3.7). These isolates were 3GCR-*E. coli*, and demonstrated phenotypic amoxycillin-clavulanic acid resistance on AST.

Of the ten MDR isolates, four were associated with the presence of *bla*$_{CTX-M-15}$, and were ST48, ST58, ST542 and ST4681. The isolates were associated with raw food samples containing chicken (N=1), tripe (N=2), lamb (N=1), game (N=1) and beef (N=1). The *qnrS1* gene, associated with quinolone resistance, was present in 35% (6/17) of isolates. Of these, five isolates were associated with concurrent presence of *bla*$_{CTX-M-15}$ and one isolate was associated with concurrent *bla*$_{CTX-M-1}$. STs associated with the presence of *qnrS1* were ST48, ST58, ST542, ST4096 and ST4681. However, only three of the isolates which carried the *qnrS1* gene demonstrated phenotypic fluoroquinolone resistance. Additionally, one ST69 isolate which demonstrated phenotypic fluoroquinolone resistance carried both *gyrA* and *parC* gene variants, alongside concurrent *bla*$_{CTX-M-27}$. In terms of trimethoprim-sulphamethoxazole (TMS) resistance, the *dfr* gene (trimethoprim resistance) was found in 24% (4/17) of isolates, and the *sul* gene (sulphamethoxazole resistance) in 35% (6/17) of isolates. All isolates which carried the *dfr* gene also carried the *sul* gene, and coincided with phenotypic TMS resistance. Interestingly, two of the isolates (ST48 and ST542) which carried both *dfr* and *sul* genes, and demonstrated phenotypic TMS resistance, also carried *qnrS1* and *bla*$_{CTX-M-15}$.

Multiple genes encoding aminoglycoside modifying enzymes were present in isolates in this study; however, only one isolate (ST1629) demonstrated phenotypic resistance to amikacin, the test aminoglycoside in this study, where resistance genes *aph(3'')-Ib* and *aph(6)-Id* were present. Additional genes of interest present which were not specifically tested for phenotypic resistance included those encoding chloramphenicol and fosfomycin resistance.

Table 3.7: *Food protein, sequence type, phenotypic antimicrobial resistance as determined by disc diffusion and resistance genes present for ESBL-producing/3GCR- E. coli isolates from raw food samples in this study which were sent for whole genome sequencing (data presented at isolate level). Note: No resistance to meropenem or tigecycline was observed, so these have been omitted from this table.*

Isolate ID	Meat protein (s)	ST	CTX-M	TEM	SHV	CMY	*qnr*	*gyr*	*parC*	*tet*	*sul*	*dfrA*	aminoglycoside resistance genes	chloramphenicol resistance genes	Amp	Amx C	Cip	TMS	Ami	Ctx	Ctz
F92	Duck	10	1		7										R	S	S	S	S	R	R
F104	Lamb	10	15	216											R	S	S	S	S	S	R
F118	Beef, tripe	10	15	216											R	S	S	S	S	S	R
F199	Pork, chicken	10								B, R			*aph(3'')-Ib, aph(6)-Id*		R	S	S	S	S	R	R
F9	Chicken, tripe	48	15	1			S1			A, M	2, 3	12, 14	*aadA2, ant(3'')-Iia, aph(3'')-Ib, aph(6)-Id*	*cmlA6*	R	S	S	R	S	R	R
F68	Duck	58	55				S1						*aac(3)-Iid, aph(3'')-Ib*		R	S	S	S	S	R	R
F184	Lamb	58	15, 107				S1								R	S	R	R	S	R	S
F157	Duck	69		1		2				A					R	R	S	S	S	R	R
F185	Chicken, beef, tripe, lamb, offal	69	27					X	X	A	2	17	*aadA5,aph(3'')-Ib,aph(6)-Id*		R	S	R	R	S	R	S
F154	Duck	155				2				A					R	R	S	S	S	R	R
F113	Game, tripe	542	15	1			S1			A, B, R	2	14	*aph(3'')-Ib, aph(3')-Ia, aph(6)-Id*		R	S	R	R	S	R	R
F57	Duck	602				2									R	R	S	S	S	R	R
F11	Chicken, tripe	1629		52						A	2		*aph(3'')-Ib, aph(6)-Id*		R	S	S	S	R	R	R

F33	Chicken, tripe	1629		52						A	2		*aph(3'')-Ib, aph(6)-Id*		R	S	S	S	S	R	R
F36	Offal, salmon	4096	15				S1								R	S	S	S	S	R	R
F80	Beef	4681	15				S1								R	S	R	S	S	R	S
F56	Duck	6958		1		2					2	14	*aph(3'')-Ib, aph(6)-Id*	*catB9*	R	R	S	R	S	R	S

ST: Sequence type. Amp: ampicillin; AmxC: amoxycillin-clavulanic acid; Ami: amikacin; Cip: ciprofloxacin; TMS: Trimethoprim-sulphamethoxazole; Ctx: cefotaxime; Ctz: ceftazidime

Plasmid analysis

Incompatibility (Inc) group plasmids associated with ESBL genes of interest in the ESBL-producing *E. coli* isolates in this study are presented in appendix table A2.8. IncF was the most frequently identified plasmid group in food isolates (N=7 IncF types). Within this, plasmid type IncFIB was identified most commonly. In this study $bla_{CTX\text{-}M\text{-}15}$ was the most frequently identified ESBL gene, and this was associated with multiple IncF plasmids, as well as IncH and IncI group plasmids. One food isolate carried $bla_{CTX\text{-}M\text{-}55}$ (ST58), and as well as being associated with IncF group plasmids, it was the only food isolate associated with plasmid IncX1. Four food isolates carried $bla_{CMY\text{-}2}$, and all but one of these were associated with IncFIB and IncFIC, with the fourth isolate (ST602) being linked to IncI2(Delta) only. All but two of the MDR food isolates were associated with the presence of IncF plasmids. For the two that were not associated with IncF plasmids, one (ST602) was associated with IncI2(Delta), the other did not have an identified Inc group plasmid present.

Salmonella spp. results

Of the RMD samples, 17.3% (19/110) had turquoise colonies present on CASE agar, indicating presumptive *Salmonella* spp. No presumptive *Salmonella* spp. colonies were isolated from NRMD samples.

Following this, five (4.5%, 5/110) RMD samples from two different brands were confirmed by whole genome sequencing to have *Salmonella enterica* present (Table 3.8). A diverse range of *S. enterica* serotypes were identified, with four separate serotypes being isolated; *S.* Kottbus*, S.* Typhimurium*, S.* Indiana*, S.* Enteriditis, and one separate subspecies; *S. diarizonae.* Within each brand, each specific *S. enterica* serotype was associated with a specific food protein type. Two samples which contained duck from brand B2 were separately contaminated with two different serotypes (*S.* Kottbus and *S.* Indiana). *S.* Kottbus and *S.* Typhimurium isolates demonstrated resistance to ampicillin on AST; however, no further antimicrobial resistance was observed.

Table 3.8: *Sample number, raw meat protein type, brand, sequence type and S. enterica serotype, alongside antimicrobial susceptibility results of isolates confirmed as Salmonella isolated from raw food samples in this study*

Brand	Sample number	Sample type	Sequence type	*Salmonella* Serotype	Antibiotic type*						
					Amp	AmxC	Tig	TMS	Ami	Cip	Mer
2	3A	Duck	582	Kottbus	R	S	S	S	S	S	S
2	3B	Duck	582	Kottbus	R	S	S	S	S	S	S
2	5A	Tripe	34	Typhimurium (monophasic)	R	S	S	S	S	S	S
2	11A	Duck	17	Indiana	S	S	S	S	S	S	S
2	11B	Duck	17	Indiana	S	S	S	S	S	S	S
10	2A	Goose	11	Enteritidis	S	S	S	S	S	S	S
10	1A	Lamb tripe	432	*diarizonae* (subsp.)	S	S	S	S	S	S	S

*Amp: Ampicillin; AmxC: Amoxycillin-clavulanic acid, Tig: Tigecycline; TMS: Trimethoprim sulphamethoxazole; Ami: Amikacin; Cip: Ciprofloxacin; Mer: Meropenem. S: sensitive; R: resistant

3.5 Discussion

This study demonstrated distinct differences in the food choices made by dog owners who feed RMD and those who do not. It also demonstrated the distinct microbiological contamination risks associated with RMD provided to dogs in the UK, alongside highlighting the potential AMR risks. RMD samples in this study were frequently contaminated with high numbers of *E. coli* and other *Enterobacteriaceae*, as well as having *Salmonella* spp. isolated, and were associated with the presence of *E. coli* which demonstrated resistance to HPCIAs.

While conventional commercial cooked diets remain the staple diet for the majority of dogs worldwide, other choices are increasing in popularity. A survey of pet owners in the USA and Australia identified that home prepared diets, raw food and table scraps comprised approximately a quarter of the diet for 17.3% of dogs, with provision of bones and raw food at least weekly for nearly a quarter of dogs (Laflamme *et al.*, 2008). A more recent survey of dog owners within English-speaking countries including the USA, Canada, Australia, New Zealand and the UK also observed that while conventional commercial feeds were provided to the majority of pet dogs, only 13% of dogs were fed this exclusively, with many being provided additions of homemade food and/or RMD (Dodd *et al.*, 2020). Additionally, 40.3% of the respondents of a recent internet-based survey of pet food preferences of dog owners in Brazil indicated that they fed RMD, with the majority adopting the diet within the previous

year, further suggesting increasing popularity of this diet choice (Viegas *et al.*, 2020). In the present study, approximately 50% of respondents indicated that they fed RMD at least once per week which is a higher proportion than reported previously. However, this is unlikely to be entirely representative of the diet choices of the dog owning population in the UK, with RMD likely to have been over-represented in this study due in part to the participant recruitment methods and an element of self-selection bias due to an unexpectedly high uptake of the survey within the RMD feeding community. However, as mentioned previously, as in other countries the popularity of RMD within the UK is likely to be increasing. Additional research is required to validate this further.

Whilst there was a broader range of food types provided to dogs fed RMD than NRMD observed, the most frequently provided type of RMD provided to dogs in this study was pre-prepared raw meat and/or bones. The greater use of pre-prepared diets may reflect the concerns of owners regarding correct diet formulation and the desire to ensure adequate nutrition, but also may also reflect convenience, brand familiarity and the increasing use of internet resources and social media for dietary information with the ready use of targeted advertising via these communication streams. Cooked commercial complete dry food was by far the most commonly provided food type for dogs fed NRMD, with >90% of NRMD-fed dogs being provided this as at least a component part of the diet. This is a similar finding to previous research (Laflamme *et al.*, 2008; Dodd *et al.*, 2020).

Although over half of the RMD in this study was reportedly purchased frozen from a shop, nearly 50% was also purchased from an online supplier, again indicating the importance of internet-based resources. There is a greater availability of products and choice online, and an added convenience of delivery straight to the consumer, and this result potentially echoes the increasing desire for online shopping amongst people in general (Brand, Schwanen and Anable, 2020). However, purchasing food through this method could potentially pose further risks as delivery relies on the cold chain remaining uninterrupted, and if disruption or delay occurs at any point the RMD could be exposed to warmer temperatures, thus allowing proliferation of bacteria. Additionally, packaging may be damaged in transit, resulting in content leakage. This survey was conducted prior to the coronavirus pandemic; however, it is anticipated that the proportion of products purchased online may have increased further because of this. There are limited data currently surrounding the proportions of pet food purchased online in the UK; however, studies from China and Romania suggested that online purchasing increased during the pandemic and remain an important choice for pet owners (Xiao, Wang and Li, 2021; Cozma, Cosma and Văleanu, 2022).

The most frequently fed RMD proteins within this study were offal such as tripe, heart, liver, kidney (83.0%), as well as beef, lamb, chicken, turkey and duck. These results are broadly similar to the study by Morelli *et al.*, (2019) who observed that beef, chicken and turkey were preferred, with type of offal analysed separately, and Groat *et al.*, 2022, who observed that most dogs fed RMD were fed a mix of meats, with chicken, red meat and tripe being the most frequently chosen. In the present study, approximately half of dogs fed RMD were fed freeze-dried meat/fish, dried foodstuffs such as pig ears, chicken feet and hide and raw bones as treats, which may again echo the desire indicated previously by owners feeding RMD to provide non-processed products which are perceived as 'more natural' in general (Bulochova and Evans, 2021b), a finding also observed in chapter 2, whereas >70% of dogs fed NRMD were provided shop bought cooked treats/biscuits.

E. coli was isolated from all but one of the RMD samples tested in this study; however, no *E. coli* was cultured from any of the NRMD kibble samples. This is in agreement with recent research from the USA where similarly, no *E. coli* was isolated from samples tested of commercially available conventional diets with no uncooked components (Gibson *et al.*, 2022). Freezing raw meat is often believed to reduce or negate the risks associated with any microbiological contaminants present, as demonstrated in chapter 2. However, this was not the case for *E. coli*, other *Enterobacteriaceae* and *Salmonella* spp in this study. All RMD samples tested in this study were originally purchased as frozen products prior to defrosting within their original unopened packaging for testing, indicating that contamination was most likely present prior to defrosting, and importantly, that the freezing process did not kill these bacteria. Additionally, not only was contamination present, but very high bacterial counts were evident, suggesting a high degree of contamination of the original product prior to the freezing process. This was also the case for frozen commercially available RMD in Thailand (Kananub *et al.*, 2020) and in Italy, where samples were found to be contaminated with *Salmonella* spp., *E. coli* O157:H7, *Listeria monocytogenes* and *Campylobacter* spp., despite the freezing process (Bottari *et al.*, 2020).

In this study, *E. coli* and other *Enterobacteriaceae* could be enumerated at >5000 CFU/g, and therefore would fail APHA sub-sample testing, in a quarter and a third of RMD samples tested respectively. Nine out of ten brands tested had at least one sample tested which had counts of *E. coli* or other *Enterobacteriaceae* which were greater than those deemed acceptable by DEFRA/APHA. Additionally, some of the bacterial counts present were very high. This highlights that RMD samples in this study were frequently contaminated with bacteria which can be pathogenic and cause zoonotic disease, often to a concerningly high degree. This

finding is in agreement with those of previous studies (Weese, Rousseau and Arroyo, 2005). In one Swedish study, bacteria belonging to the family *Enterobacteriaceae*, including *E. coli*, was present at a level which exceeded EU regulations for raw meat intended for pet food production in 52% of RMD samples (Hellgren *et al.*, 2019), and in a study from Switzerland, 73% of samples tested exceeded these limits (Nüesch-Inderbinen *et al.*, 2019). In another study from Italy, RMD products purchased online and received frozen were found to be highly contaminated with *E. coli* immediately following defrosting, as well as having *Listeria* spp., *Clostridia* spp. and *Yersinia* spp. present, and were suggested to be of poor microbiological quality initially, but demonstrated distinct worsening of quality if products were improperly refrigerated, or not utilised immediately following defrosting (Morelli *et al.*, 2020). All brands of food tested in this study were received frozen, stored in the freezer at -20^{o}C until they were due to be tested, then defrosted overnight in the fridge prior to testing. While some bacterial multiplication could have occurred during the defrosting process within the laboratory, this is unlikely due to the refrigeration throughout, and rapid processing of samples once defrosted. If there were any breaks in the cold chain during the packing and delivery process, this may have allowed bacterial multiplication; however, this mirrors the process by which owners would receive and utilise the foods, therefore is representative of the microbiological quality of the products received by consumers. All packs were received with insulating packing of different types, and some were more successful at keeping foodstuffs frozen than others, with some leakage of package contents identified in some cases. Nevertheless, it is most likely that a high degree of bacterial contamination was already present in the samples, and highlights the importance of safe storage (refrigeration at 0-4^{o}C) and defrosting processes for these diets. Additionally, it highlights that RMD products may have poor microbiological quality prior to freezing, thus more needs to be done in manufacturing to minimise contamination, both at source by reviewing the raw materials utilised or during the production process. Previous research has demonstrated that dog owners utilise a number of different methods for defrosting and preparing RMD (Bulochova and Evans, 2021a; Morgan *et al.*, 2022), with poor practices regularly employed, potentially indicating some confusion as to appropriate measures for defrosting RMD. Defrosting processes have been demonstrated previously to be important for food safety, and time-temperature abuse has been shown to be an important factor in the increase in bacteria in contaminated raw meat products, thus increasing the risk of foodborne disease (Roccato *et al.*,2015).

Not all brands tested in this study included detailed instructions for safe defrosting of the product on their product packaging, and therefore highlights an area where improvements

are needed regarding safe handling of RMD. RMD in this study were frequently contaminated with AMR *E. coli*, with approximately 16% of samples tested having 3GCR-*E. coli* present, and 14% having ESBL-producing *E. coli* present. Additionally, 10% of samples tested had MDR ESBL-producing *E. coli* present, with phenotypic resistance to TMS and/or ciprofloxacin observed alongside ESBL-production within many of these isolates. AMR *E. coli* was not isolated from any NRMD samples, a finding similar to that of Baede *et al.*, 2017. It is concerning that *E. coli* which demonstrated concurrent phenotypic resistance to both fluoroquinolones and third generation cephalosporins (cefotaxime, ceftazidime) was isolated from approximately 6% of RMD samples. These represent classes of HPCIAs as determined by the World Health Organisation (Veterinary Medicines Directorate, 2015; Collignon *et al.*, 2016). The presence of ESBL-producing and 3GCR-*E. coli* was associated most frequently with samples containing offal/tripe and poultry meat (chicken and duck respectively); however, there was no distinct link between meat type and the presence of phenotypic fluoroquinolone resistance, with resistance demonstrated in *E. coli* isolated from RMD samples containing a range of proteins, including beef, game, goat, lamb, chicken and tripe. These meat types were often mixed in combinations in the food samples; however, there were single-protein samples of goat, lamb and beef. The prevalence of 3GCR and ESBL-producing *E. coli* in pre-prepared RMD samples in the present study is lower than that previously reported by smaller studies in Europe. A study of 51 samples of RMD available in Switzerland observed that approximately 61% of samples tested had ESBL-producing *E. coli* present, with the majority of affected samples involving products of cattle or poultry origin (Nüesch-Inderbinen *et al.*, 2019). An additional smaller study of 35 samples from eight brands available in The Netherlands reported that 80% of RMD samples present had ESBL-producing *E. coli* isolated (van Bree *et al.*, 2018). Finally, a study from Sweden identified that 23% of 39 samples tested had 3GCR- *E. coli* present (Nilsson, 2015), and all of the 3GCR *E. coli* harboured the $bla_{\text{CMY-2}}$ gene, which was also the most frequently observed bla_{CMY} gene in the present study.

There remains limited evidence currently regarding the AMR risks specifically from pre-prepared RMD available in the UK and elsewhere for comparison. However, there are studies of AMR- *E. coli* contamination in meats destined for the human food chain and for pet food. One national study of meat samples purchased from retailers for human consumption in the UK identified that 65% of chicken samples had ESBL-producing *E. coli* present (Day *et al.*, 2019), another more localised study identified ESBL-producing *E. coli* in 18% of meat products from UK supermarkets, with the majority of products being chicken (Ludden *et al.*, 2019), and

while the majority of products were of UK origin, products were also imported from a range of other countries, highlighting the multinational origin of meat products entering both the human and pet food chains. Finally, a study from Italy identified ESBL-producing and MDR *E. coli* in meat products that were previously packaged at a mass retailer for human consumption but were no longer suitable and became pet foods (Bacci *et al.*, 2019). It is important to note that meat sold for human consumption would be intended to be cooked, which would mitigate the risk of AMR-bacteria (James *et al.*, 2021). A concern regarding DIY/home-prepared raw diets is that the meats used are still likely to harbour zoonotic and AMR bacteria, whereas pre-prepared RMDs must undergo testing to ensure bacteria do not exceed acceptable levels; it is not possible to measure the risk posed by meats from unknown sources prepared within the home. However, it could also be argued that there is potential for more opportunity for cross-contamination within pre-prepared diets in the manufacturing process, particularly where more than one protein type is included in the product, with ingredients potentially from more than one country.

In the present study, while a UK origin was stated on the sample packets for 50% of the RMD brands tested, the remainder did not specifically state the country of origin of the meats used. Additionally, 60% of the brands tested had a batch number clearly present on the sample packets, but whether the food was produced in the UK was not clear for a number of brands. This is a concern because it would seem that there is a lack of traceability and provenance of product present, which would prove an issue if there was an outbreak of disease potentially associated with the product. The importance of traceability of RMD ingredients was highlighted by the case of raw hare meat which was imported into the UK from The Netherlands and originated in Argentina and intended for use in RMD, but found to be contaminated with *Brucella suis* (Frost, 2017), therefore this is an area of RMD production which requires attention.

A variety of AMR genes were identified in the ESBL-producing *E. coli* isolates from RMD samples. The predominant bla_{ESBL} gene identified was $bla_{\text{CTX-M-15}}$, present in 41% of isolates. Presence of the $bla_{\text{CTX-M-15}}$ gene was frequently observed alongside co-carriage of additional plasmid-mediated resistance genes such as *qnrS1*, which mediates fluoroquinolone resistance, and genes encoding resistance to other antibiotic classes such as tetracyclines, trimethoprim-sulphamethoxazole and aminoglycosides. The predominance of $bla_{\text{CTX-M-15}}$ is of concern as it is carried on mobile transferrable genetic elements which frequently harbour resistance genes to other antimicrobials, including fluoroquinolones, thus increasing the risk of conferring MDR (Baba Ahmed-Kazi Tani *et al*., 2013). Only one isolate demonstrated the

presence of *bla*$_{CTX\text{-}M\text{-}1}$. Again, there is little data available from pre-prepared RMDs for comparison; however, this finding contrasts with the findings of Nüesch-Inderbinen *et al.*, 2019, who observed that *bla*$_{CTX\text{-}M\text{-}1}$ was the most frequently detected *bla*$_{ESBL}$ gene in ESBL-producing *E. coli* isolated from RMD samples commercially available in Switzerland, although *bla*$_{CTX\text{-}M\text{-}15}$ was the second-most frequently detected *bla*$_{ESBL}$ gene. Isolates harbouring the *bla*$_{CTX\text{-}M\text{-}15}$ gene in the present study were not associated with any specific meat protein type. The *bla*$_{CTX\text{-}M\text{-}15}$ gene is widely disseminated globally, and is the most prevalent *bla*$_{CTX\text{-}M}$ gene within human clinical (Cantón, González-Alba and Galán, 2012; Bevan, Jones and Hawkey, 2017; Day *et al.*, 2019) and companion animal (Shaheen *et al.*, 2011; Liu, Thungrat and Boothe, 2016) ESBL-producing *E. coli* isolates. In food-producing animals in Europe, the *bla*$_{CTX\text{-}M\text{-}1}$ gene predominates (Bevan, Jones and Hawkey, 2017), and is frequently associated with chicken meat in the UK (Day *et al.*, 2019; Veterinary Medicines Directorate, 2021b), as well as being the most frequently identified *bla*$_{CTX\text{-}M}$ gene in *E. coli* isolated from healthy pigs at slaughter in the UK (Veterinary Medicines Directorate, 2022). However, the *bla*$_{CTX\text{-}M\text{-}15}$ gene has also been isolated from ESBL-producing *E. coli* from livestock in the UK, including from pig meat (Veterinary Medicines Directorate, 2022), and from faeces of chicken, cattle and pigs (Ludden *et al.*, 2019). Although *bla*$_{CTX\text{-}M}$ genes were the predominant *bla*$_{ESBL}$ genes in this study, *bla*$_{TEM\text{-}52}$ was also isolated from two RMD samples containing a combination of chicken and tripe. This ESBL-gene has previously been observed in *E. coli* isolated from UK produced broiler chickens and turkeys (Randall *et al.*, 2011). Finally, the plasmid-mediated AmpC (pAmpC) resistance gene *bla*$_{CMY\text{-}2}$ was identified in four samples, all of which were raw duck, were single-protein, and obtained from two different brands. In all cases, isolates were resistant to ampicillin, amoxycillin-clavulanic acid and a third-generation cephalosporin. The *bla*$_{CMY\text{-}2}$ gene has been identified in broilers and chicken meat within Europe previously (Voets *et al.*, 2013; Solà-Ginés *et al.*, 2015), and from a ducks in China (Ma *et al.*, 2012; Zheng *et al.*, 2022); however, to the author's knowledge this is the first report of *bla*$_{CMY\text{-}2}$ being present in products containing duck meat in the UK, although the country of origin of the meat was unknown, again highlighting the importance and need for improved traceability of RMD ingredients.

A number of STs were identified in ESBL-producing *E. coli* isolates in the present study, with the most frequently encountered being ST10. *E. coli* ST10 belongs to a global extraintestinal pathogenic *E. coli* lineage of increasing importance in human infections (Bojesen *et al*., 2022). ST10 *E. coli* is frequently associated with ESBL-genes, in particular, *bla*$_{CTX\text{-}M}$ genes (Oteo *et al.*, 2009; Cormier *et al.*, 2019). Of the MDR isolates, ST48 *E. coli* is a single-locus variant of ST10,

and alongside ST10 is part of clonal complex (CC) 10. CC10 was the most frequently identified CC in *E. coli* isolated from RMD in this study. This is unsurprising as CC10 is widely disseminated globally and often associated with the presence of ESBL and fluoroquinolone resistance genes. Three of the five CC10 isolates in this study harboured $bla_{\text{CTX-M-15}}$ and one harboured both $bla_{\text{CTX-M-1}}$ and $bla_{\text{SHV-7}}$. Previous studies have demonstrated the concurrent presence of other genes, including those encoding resistance to aminoglycosides, tetracyclines, sulphonamides and phenicols (Liu *et al.*, 2021), which were also observed in the ST48 isolate in this study. This suggests that this CC could pose a risk for the harbouring and transmission of extensive resistance genes. Other STs of interest which were identified in ESBL-producing *E. coli* isolated from RMD samples in this study were ST58, ST69, ST602 and ST155. *E. coli* ST58 and ST69 are globally disseminated uropathogens and have previously been associated with bla_{ESBL} and AMR gene carriage (Novais *et al*., 2013; de Souza da Silva *et al*., 2020; Reid *et al*., 2022). *E. coli* ST58 has been isolated from livestock and food sources previously (Reid *et al*., 2022), including raw pet food, where it was associated with the carriage of ESBL genes $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-3}}$ and $bla_{\text{CTX-M-15}}$ in samples comprising of chicken, lamb and beef respectively (Nüesch-Inderbinen *et al.*, 2019). In the present study, $bla_{\text{CTX-M-1}}$ was not identified in the ST58 isolates, however, concurrent carriage of $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-107}}$ was observed in one isolate from a sample comprising of raw lamb, and $bla_{\text{CTX-M-55}}$ from a sample of raw duck. *E. coli* ST155 is an important extraintestinal pathogenic *E. coli* (ExPEC strain with zoonotic potential, previously identified in beef cattle faeces, chicken meat and human blood in the UK, as well as in RMD samples (Ludden *et al.*, 2019; Nüesch-Inderbinen *et al.*, 2019).

While the presence of certain AMR genes was not always observed with demonstrated phenotypic resistance, it is concerning that such a range of AMR genes was present, frequently in combination. These genes are potentially transmissible through mechanisms such as mobile plasmids and as such these isolates could act as a reservoir for MDR. There are a multitude of RMD brands in the UK which utilise meat products sourced from both within the UK and abroad, therefore larger scale studies are required to investigate the problem with regards to AMR *E. coli* presence in UK-fed RMDs. Further, the findings of the current study indicate that contamination with AMR *E. coli* is also a problem with RMD fed to dogs in the UK. This is concerning from an animal health and welfare point of view, but also from a One Health aspect. Dogs fed RMD have been shown to shed AMR *E. coli* in their faeces, and the provision of a raw diet has been demonstrated to be a risk factor for the

carriage of ESBL-producing *E. coli* by healthy dogs (Schmidt *et al*., 2015; Wedley *et al*., 2017; Runesvärd *et al*., 2020; van den Bunt *et al*., 2020; Groat *et al*., 2022).

With regards to the plasmids identified via whole genome sequencing, the most commonly identified plasmids were of the IncF group. The IncF group plasmids are frequently encountered in among the *Enterobacterales*; however, they are important as they are able to integrate genes conferring a wide range of resistance to multiple antibiotic classes, as well as co-harbouring ESBL and plasmid-mediated quinolone genes, and plasmid-mediated virulence traits (Yang *et al.*, 2015). Of additional interest are the plasmids associated with $bla_{\text{TEM-52}}$ ESBL gene carriage in two *E. coli* ST1629 isolates this study. This gene was associated with plasmids IncFII, IncI1-I, IncX1 and IncY. Interestingly, the latter two plasmids were only associated with this ST and $bla_{\text{TEM-52}}$ gene presence, suggesting that ST1629 could be a potential source of plasmid-mediated bla_{TEM} ESBL gene dissemination, or potentially clonal spread of antimicrobial resistant ST1629. The $bla_{\text{TEM-52}}$ gene has, however, been associated with IncX1 plasmids in broiler meat, beef and human samples (Bielak *et al.*, 2011). Additionally, plasmids harbouring $bla_{\text{TEM-52}}$ carriage have been identified in ESBL-producing *Salmonella enterica* isolated from chicken meat (Matsumoto *et al.*, 2014). It must be stressed, however, that although the plasmids listed were those associated with the presence of particular resistance genes in this study, it is not possible to determine which specific plasmids the genes were present on from these data alone. Additional investigative work is needed, such as conjugation experiments, to determine this.

Five (4.5%) RMD samples from two brands in this study were contaminated with *Salmonella enterica*, with five different serotypes/subspecies identified, each associated with a unique meat protein type. The reported prevalence of *Salmonella* spp. contamination in RMD in studies in Europe, Canada and the USA is wide ranging, from 4%-25% (Weese, Rousseau and Arroyo, 2005; Strohmeyer *et al.*, 2006; Mehlenbacher *et al.*, 2012; Nemser *et al.*, 2014; Hellgren *et al.*, 2019; Nüesch-Inderbinen *et al.*, 2019), therefore the prevalence identified in the present study is at the lower end of that range. However, no meat containing *Salmonella* spp. should be present within pet food at retail, and samples which test positive for *Salmonella* spp. at production should be removed from entering sale, therefore the presence of any *Salmonella* spp. contamination is concerning. *S*. Enteriditis and *S.* Typhimurium are among the top five serotypes resulting in human infection reported to the UK Health Security Agency (UKHSA) (Chattaway, Dallman, *et al.*, 2019); however, all serotypes present in the RMD samples in this study have the potential to cause disease in humans, again highlighting the One Health concerns associated with RMD. Concerningly, a study of experimentally-

inoculated raw meat identified that *Salmonella* spp. persisted in pet food bowls despite standard cleaning methods, including bleach, scrubbing with soap and washing in the dishwasher (Weese and Rousseau, 2006). Furthermore, while much of the *Salmonella* spp. contamination occurred in samples containing poultry, S. *enterica* subspecies *diarizonae* was isolated from a sample containing lamb tripe. This is unsurprising as *S. enterica* subsp. *diarizonae* is commonly associated with reptiles and sheep; however, human infections have been linked to the consumption of sheep meat (Giner-Lamia *et al.*, 2019), demonstrating the zoonotic potential of this subspecies.

The presence of *Salmonella* spp. in RMD samples is not only a zoonotic disease concern, but also an animal health and welfare issue. Furthermore, *Salmonella* spp. has been isolated from dried raw pet treats, highlighting a potentially overlooked source of contamination (Morgan *et al.*, 2023, presented in Appendix 5). The provision of *Salmonella*-contaminated RMD to pets has been implicated as a cause of mesenteric lymphadenitis in two dogs (Binagia and Levy, 2020), diarrhoea and death in Greyhound puppies (Morley *et al.*, 2006), enterocolitis and death in a series of puppies and kittens (Jones *et al.*, 2019) as well as being highly suspected as the cause of two cases of salmonellosis in cats (Giacometti *et al.*, 2017).

Limitations

There were some limitations to this study. The food samples chosen to test were selected based on the preferences of the dog owners who responded to the survey, not on market share, therefore can only offer a snapshot of the possible levels of contamination present in dog foods (RMD and NRMD) available in the UK. There are a multitude of brands available, and different brands which were not sampled may have different levels of contamination. Additionally, only a limited number of samples were tested per brand, particularly of NRMD. This may have underestimated the contamination present within a brand, or indeed may have overestimated if a particularly contaminated batch was tested, or (for RMD) if bacterial proliferation had occurred due to a break in the cold chain in transit during the order packing and delivery process, or during defrosting, although this was deemed unlikely as discussed earlier. Furthermore, this study only tested pre-packaged samples of RMD, and did not include home-prepared/DIY diets, which may have differing levels of contamination as discussed earlier. A larger scale, wider reaching study would consolidate the findings of this study further. There may be an underestimation of the presence of *Salmonella* spp. in the food samples as the method of isolating *Salmonella* spp. using the CASE agar is likely to have selected only for *S. enterica*, which may mean that a small number of other *Salmonella*

subspecies could have been missed. Finally, WGS was only undertaken on 3GCR and ESBL-producing *E. coli* isolates, and further WGS on non-ESBL *E. coli* was beyond the scope of this study due to funding limitations. There may be further resistance genes of interest in the AMR- *E. coli* isolates which were not 3GCR/ESBL-producing, and this warrants further investigation. Additionally, analysis of *E. coli* and *Salmonella* spp. virulence factors present would provide further depth surrounding the potential health risks associated with RMD. Furthermore, we were only able to identify the plasmids present which were associated with ESBL gene presence; however, further in-depth investigation is required to determine which plasmids genes were carried on specifically, and how transmissible these may be.

3.6 Conclusions

In conclusion, this study has demonstrated that RMDs provided to dogs in the UK may be frequently contaminated with high levels of potentially pathogenic and zoonotic bacteria. In addition, there were concerning levels of AMR-*E. coli* present, with resistance to critically important antibiotic classes demonstrated. Therefore, RMD for pets are potentially an important One Health concern. Pre-prepared RMDs are often sold as 'human-grade', which may suggest a perceived greater level of quality and safety; however, all meats which are utilised within RMDs are graded as at least DEFRA category 3 ABPs, and as demonstrated, this does not negate the microbiological risks. It is crucial that veterinary professionals, medical staff, pet food retailers and dog owners are aware of these risks, and if dog owners do choose to feed RMD, it is vital that strict hygiene measures are practiced throughout the food storage, defrosting and preparation processes, including using separate food storage and preparation facilities, practising thorough hand washing, and disinfection of food bowls and food preparation areas after feeding.

Chapter 4: Raw meat diets are an important risk factor for antimicrobial-resistant *E. coli* carriage by dogs in the UK

4.1 Introduction

Raw meat diets (RMD) remain an increasingly popular diet choice for dogs, and while conventional cooked kibble-based diets continue to be a staple for the majority of dogs, RMD is increasingly fed as at least a constituent part of the diet for many (Dodd *et al.*, 2020; Morgan *et al.*, 2022; PDSA, 2022). RMDs are comprised of muscle, bone, skin, cartilage, tendon and organs from livestock and wild animals, which have not undergone heat treatment or cooking during the food production process (Freeman *et al.*, 2013; Davies, Lawes and Wales, 2019), and may be provided in a commercial pre-prepared format, or home-prepared. RMDs for dogs and cats have been demonstrated to harbour pathogenic and zoonotic organisms, including *E. coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter* spp., amongst others (Davies, Lawes and Wales, 2019; Kaindama *et al.*, 2020).

There is an increasing body of evidence to suggest that pets fed RMD pose a risk for zoonotic bacteria shedding. Studies have demonstrated carriage of *E. coli*, *Salmonella* spp., *Campylobacter* spp. and *Clostridium* spp. by dogs and cats fed RMD globally (Morley *et al.*, 2006; Finley *et al.*, 2007; Lefebvre *et al.*, 2008; Leonard *et al.*, 2015; Baede *et al.*, 2017; Runesvärd *et al.*, 2020; Viegas *et al.*, 2020; Groat *et al.*, 2022; Usmael *et al.*, 2022). Additionally, shedding of antimicrobial-resistant (AMR) bacteria by companion animals fed RMD is of increasing concern. Provision of RMD has been identified as a risk factor for faecal carriage of AMR *E. coli* by dogs (van den Bunt *et al.*, 2020), with greater proportions of dogs fed RMD shedding extended-spectrum beta-lactamase producing (ESBL)-*E. coli* than those fed non-raw diets (NRMD) (Runesvärd *et al.*, 2020).

In the UK, provision of RMD has also been identified as a risk factor for faecal carriage of AMR *E. coli* in both healthy, non-veterinary visiting dogs (Schmidt *et al.*, 2015) and those visiting veterinary practices (Wedley *et al.*, 2017). Additionally, feeding RMD was identified as a risk factor for carriage of third-generation cephalosporin resistant (3GCR) *E. coli* in rural-living dogs (Sealey *et al.*, 2022). A recent study from the UK indicated that AMR, 3GCR and multidrug resistant (MDR) *E. coli* were significantly more likely to be shed by dogs fed RMD,

compared to those fed NRMD, with 54% of dogs fed RMD shedding AMR *E. coli*, compared to 17% of dogs fed NRMD (Groat *et al.*, 2022).

Plasmid mediated AMR genes mediating resistance, or reducing susceptibility, to critically important antibiotics such as third generation cephalosporins (bla_{ESBL} genes including those of $bla_{CTX\text{-}M}$ group 1 and bla_{CMY}) and quinolones (such as *qnr* genes) have been identified in *E. coli* isolated from companion animals, and have been associated with those fed RMD (Baede *et al.*, 2017; Groat *et al.*, 2022; Mounsey *et al.*, 2022; Sealey *et al.*, 2022). These genes often occur concurrently, and are often co-harboured on mobile genetic elements, increasing the potential for transmission of MDR.

Dogs and their owners share close and frequent contact, therefore the risk posed by RMD with regards to zoonotic disease and AMR is a potential public health concern. Despite the popularity and interest surrounding RMD, there remains little data surrounding the potential AMR risks associated with their provision as a diet for dogs, particularly in the UK.

4.2 Aims

The aims of this study were to investigate the presence of AMR *E. coli* in the faeces of dogs fed either RMD or NRMD in the UK, with focus on 3GCR- *E. coli,* ESBL-producing *E. coli* and MDR-*E. coli*, alongside investigation of the AMR genes harboured by *E. coli* isolates via whole genome sequencing (WGS). Additionally, this study aimed to determine the dog and owner lifestyle risk factors for the carriage of AMR *E. coli* in canine faeces.

4.3 Materials and methods

Participant recruitment and survey design

This study was cross-sectional in design and data were collected between October 2020-August 2021. Participant recruitment was via email contact of dog owners who had previously participated in related studies (chapters 2 and 3) and had agreed to be contacted further, and additionally through social media and word of mouth. Following recruitment, participating households were sent a questionnaire and a faecal sample collection kit via Royal Mail. The kit comprised of an information sheet, consent form, sampling instructions (available in appendix 3), gloves and a faecal sample pot, as well as UN3373-compliant biohazard packaging to return the sample in. Completed questionnaires and collected canine faecal samples were received by the laboratory by prepaid first class return post. Participant

details were anonymised and each sample and corresponding questionnaire was assigned a unique identification number.

Dog owners were requested to collect one sample from a freshly evacuated stool at one time point from their dog. For multidog households, owners were requested to select one dog at random to participate in the study.

The questionnaire discussed dog lifestyle and clinical factors including diet, recent antibiotic treatment and veterinary visits, recent diarrhoea and treatment, contact with other animals and access to communal areas such as dog kennels, dog shows and public parks. It also collected data on owner factors including age, location in the country, receipt of antibiotics and place of work (full questionnaire in appendix 3). Questions were multiple choice, with additional free text boxes included for owners to expand on their answers where appropriate.

Based on prior research from the UK (Groat *et al.*, 2022), the percentage of RMD-fed dogs hypothesised to carry ESBL-producing *E. coli* was estimated to be 30%, compared to 5% in dogs fed NRMD. To achieve 80% power to detect differences with 95% confidence in ESBL-producing *E. coli* carriage between dogs fed RMD and those fed NRMD, it was calculated that a minimum of 36 dogs in each group would be required.

Microbiology: Bacterial Culture and Sensitivity

A 1g sample of faeces was homogenised in 4ml buffered peptone water (BPW) and incubated aerobically overnight at 37°C. Following incubation, a 5µl loopful of the BPW broth was inoculated onto plain chromogenic Harlequin *E. coli*/Coliform Agar (HECA) (Neogen, UK) and HECA with 1µg/ml cefoxatime (HECA+Cx); all plates were incubated at 37°C for 18-20h. If present, four colonies typical of *E. coli* (dark blue-violet colonies, 0.1mm-2mm diameter) were picked from the HECA plate, and two colonies were picked from the HECA+Cx plate. All picked colonies were individually plated onto nutrient agar (NA) (Neogen, UK) plates and incubated at 37°C for 18-20h.

E. coli isolates from plain HECA plates underwent antimicrobial susceptibility testing (AST) via the disc diffusion method. Antibiotic discs were chosen representing antimicrobials used in dogs and humans, and in compliance with European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations (EUCAST, 2022). Isolates were inoculated into sterile saline to 0.5 McFarland using a 5µl loop, then the inoculated saline was spread onto Muller-Hinton agar (Neogen, UK) using a sterile cotton-tip swab and antibiotic discs

applied. Plates were then incubated aerobically at 37°C for 18-20 h. Antimicrobials tested were ampicillin 10 µg, amoxycillin-clavulanic acid 20 µg/10 µg, ciprofloxacin 5 µg, tigecycline 15 µg, trimethoprim-sulphamethoxazole 1.25 µg/23.75 µg, amikacin 30 µg and meropenem 10µg (MAST Group Ltd, Liverpool UK). A susceptible control strain of *E. coli* (ATCC 25922) was also tested.

Following incubation, zones of inhibition (ZOI) for each antibiotic disc were measured to the nearest millimetre. Human clinical breakpoints used for interpretation were as recommended by EUCAST (EUCAST, 2022) for all antibiotics other than amoxycillin-clavulanic acid, where the breakpoint used for interpretation was as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2020). Isolates were defined as AMR if they demonstrated phenotypic resistance to less than three classes of antibiotics. Multidrug resistance (MDR) was defined as demonstrated phenotypic resistance to three or more classes of antibiotics (Magiorakos *et al.*, 2012).

The *E. coli* isolates from HECA+Cx plates initially underwent the extended-spectrum beta-lactamase (ESBL) double-disc test to determine whether they were ESBL-producing or not, using cefotaxime 5µg, cefotaxime 5µg +clavulanic acid 10µg, ceftazidime 10µg and ceftazidime 10µg +clavulanic acid 10µg discs (EUCAST ESBL detection set, MAST Group Ltd, Liverpool UK). Plates were incubated at 37°C for 18-20h. Isolates were deemed positive for ESBL-production if the ZOI surrounding the cephalosporin +clavulanic acid disc was a minimum of 5mm diameter larger than the ZOI for the corresponding cephalosporin disc alone for ≥1 antibiotic pairs; positive isolates were then continued to the full AST as described. Non-ESBL producing 3GCR isolates which did not demonstrate a typical positive result for ESBL production on the double disc test, but which demonstrated a pattern suggestive of AmpC production whereby there was no, or minimal, ZOI present surrounding the clavulanic acid disc(s), were also continued to full AST

Confirmation of E. coli identification

All isolates were confirmed as *E. coli* by PCR of the *usp*A gene. Methods were as per Anastasi *et al.*, (2010). Primers used were CCGATACGCTGCCAATCAGT (forward) and ACGCAGACCGTAGGCCAGAT (reverse), with an amplicon size of 884 base pairs.

Characterisation of E. coli resistance genes and whole genome sequencing (WGS)

DNA extraction was performed on ESBL-producing *E. coli* isolates using the QIAamp® DNA mini kit (Qiagen, Crawley, UK).

Genomic DNA samples were submitted to the Centre for Genomic Research, University of Liverpool for Illumina NEBNext Ultra II FS DNA Library Prep, completed following the manufacturer's protocol. Each library was quantified using Qubit and the size distribution assessed using the fragment analyser. These final libraries were pooled in equimolar amounts using the Qubit and fragment analyser data. The quantity and quality of the pool was assessed by Bioanalyzer and subsequently by qPCR using the Illumina Library Quantification Kit from Kapa (KK4854) on a Roche Light Cycler LC480II according to manufacturer's instructions.

Following calculation of the molarity using qPCR data, template DNA was diluted to 300pM and denatured for 8 minutes at room temperature using freshly diluted 0.2 N sodium hydroxide (NaOH) and the reaction was subsequently terminated by the addition of 400mM TrisCl pH=8. To improve sequencing quality control 1% PhiX was spiked-in. The libraries were sequenced on the Illumina® NovaSeq 6000 platform (Illumina®, San Diego, USA) following the standard workflow over 1 lane of an S4 flow cell, generating 2 x 150 bp paired-end reads.

Bioinformatic analysis

Following sequencing, reads were assembled into contigs using SPAdes and contigs smaller than <200bps were removed. Quality control (QC) was undertaken on assemblies, and those which passed QC were subject to multi-locus sequence typing (MLST) by submitting locus allele sequences to pubmlst.org. eBURST analysis was performed to group similar isolates based on the sharing of alleles, giving each isolate a e-BURST group assignment.

Gene prediction was carried out using Prokka. Detection of AMR genes was undertaken using Resistance Gene identifier (RGI) (https://card.mcmaster.ca/analyze/rgi). Plasmids were identified using PlasmidFinder and the *Enterobacteriaceae* plasmid marker database.

Data analysis

Data analysis was undertaken in Microsoft Excel (Microsoft Corp. (2019) and SPSS 27 (IBM Corp. (released 2020). IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp.). Descriptive analysis was undertaken to determine the frequency and percentage of antimicrobial-resistant *E. coli* present at sample and isolate level for dogs fed RMD or NRMD, alongside calculation of 95% confidence intervals. Comparisons between dogs fed RMD and NRMD were undertaken using the chi square test (Fisher's exact test for groups of N<5), and significance was set at $p < 0.05$.

Descriptive analysis of categorical questionnaire response data (frequency, percentage) was undertaken. Based on the accompanying laboratory results, three outcomes were analysed; 'presence of ESBL- producing *E. coli*', 'presence of 3GCR-*E. coli*' and 'presence of MDR-*E. coli*'. Comparisons were undertaken using the chi square test (Fisher's exact for groups of N<5), and statistical significance was set at $p < 0.05$. Odds ratios and 95% confidence intervals were generated by univariable logistic regression to identify explanatory variables associated with the three outcomes. Multivariable logistic models were created to investigate the explanatory variables associated with the three outcomes. Univariable explanatory variables with a liberal p value of <0.3 were selected for inclusion into each multivariable model. Correlations between each variable were assessed, and where a high correlation coefficient (>0.7) was identified, only the variable deemed most suitable was selected for inclusion into the model. A backwards elimination method was utilised to sequentially remove variables with a p value of >0.05 until all remaining variables were significant at $p < 0.05$. Variables which had been eliminated were individually reinserted back into the model and checked to ensure that any confounding or significant variables had not been omitted. Plausible interactions between variables were also tested in the model to ensure no significant interactions had been missed and then 'goodness of fit' of the final model was tested using the Hosmer-Lemeshow test.

Ethics statement

All participation was anonymous and ethical approval was granted by the University of Liverpool Veterinary Ethics Committee (approval number VREC935).

4.4 Results

Microbiology: Sample Level Bacterial Culture and Sensitivity

A total of 432 (193 RMD-fed, 239 NRMD-fed) canine faecal samples were received. *Escherichia coli* was isolated from 92.6% (400/432; 191 RMD, 209 NRMD) of samples. AMR *E. coli* was isolated from 39.4% (76/193) of RMD-fed and 13.8% (N=33/239) of NRMD-fed dogs ($p<0.001$) (Table 1). Dogs which were fed RMD carried significantly more 3GCR-*E. coli* ($p<0.001$), ESBL-producing *E. coli* ($p<0.001$), fluoroquinolone-resistant (FQR) ESBL-producing *E. coli* and multidrug-resistant (MDR) ESBL-producing *E. coli* ($p<0.001$) than dogs fed NRMD (Table 4.1).

Of the dogs fed RMD, approximately one third shed 3GCR-*E. coli* in their faeces, and a quarter shed ESBL-producing *E. coli*. Additionally, 17% of RMD-fed dogs shed MDR ESBL-producing *E. coli*, compared to 1% of those fed NRMD.

Table 4.1: *Sample level data (Number (N) and percentage (%)) describing the overall phenotypic antimicrobial resistance demonstrated by E. coli isolated from dogs fed either a raw (RMD, N=193) or non-raw (NRMD, N=239) diet*

Phenotypic resistance	Diet choice % (N)				p value
	RMD (44.7%, N=193)		NRMD (55.3%, N=239)		
	N	% (95% CI)	N	% (95% CI)	
AMR *E. coli*	76	39.4 (32.8-46.4)	33	13.8 (10.0-18.8)	<0.001
Third-generation cephalosporin resistant *E. coli**	63	32.6 (26.4-39.5)	12	5.0 (2.9-8.6)	<0.001
ESBL- producing *E. coli*	47	24.4 (18.8-30.9)	4	1.7 (0.7-4.2)	<0.001
MDR ESBL- producing *E. coli*	32	16.6 (12.0-22.5)	3	1.3 (0.4-3.6)	<0.001
Fluoroquinolone-resistant ESBL-producing *E. coli*	21	10.9 (7.2-16.1)	2	0.8 (0.2-3.0)	<0.001

**Includes both ESBL-producing and non-ESBL producing third-generation cephalosporin-resistant E. coli*

Phenotypic antimicrobial resistance results for AMR *E. coli* are shown in table 4.2. Dogs fed RMD carried significantly greater proportions of AMR-*E. coli* with resistance to ampicillin (p<0.001), amoxycillin-clavulanic acid (p 0.02) and TMS (p<0.001) in their faeces compared to dogs fed NRMD. Tigecycline resistance was observed in AMR-*E. coli* from two dogs fed RMD; however, no resistance to tigecycline was observed in dogs fed NRMD. Resistance to meropenem was not observed in any isolates from dogs fed either RMD or NRMD.

Table 4.2: *Number (N) and percentage (%) of RMD-fed (N=193) and NRMD-fed (N=239) faecal samples with AMR-E. coli identified which demonstrated resistance to antibiotics tested in the present study*

Resistance phenotype*	**Diet choice % (N)^**				**p value**
	RMD (44.7%, N=193)		**NRMD (55.3%, N=239)**		
	N	**% (95% CI)**	**N**	**% (95% CI)**	
Total samples: 432					
Amp	64	33.2 (26.9-40.1)	31	13.0 (9.3-17.8)	<0.001
AmxC	25	13 (8.9-18.4)	15	6.3 (3.8-10.1)	0.02
Cip	8	4.1 (2.1-8.0)	3	1.3 (0.4-3.6)	0.07
TMS	37	19.2 (14.2-25.3)	18	7.5 (4.8-11.6)	<0.001
Tig	2	1 (0.3-3.7)	0	0	0.19
Ami	5	2.6 (1.1-5.9)	2	0.8 (0.2-3.0)	0.25
Mer	0	0	0	0	NA
MDR	12	6.2 (3.6-10.6)	7	2.9 (1.4-5.9)	0.11

**Amp: ampicillin; AmxC: amoxycillin-clavulanic acid; Cip: ciprofloxacin; TMS: trimethoprim-sulphamethoxazole; Tig: tigecycline; Ami: amikacin; Mer: meropenem; MDR: multidrug resistance. ^RMD: raw meat diet; NRMD: non-raw diet*

At sample level, dogs which were fed RMD shed significantly more ESBL-producing *E. coli* in their faeces which demonstrated resistance to ampicillin (p<0.001), amoxycillin-clavulanic acid (p<0.001), ciprofloxacin (p<0.001) and TMS (<0.001) than dogs fed NRMD (Table 4.3). Approximately 11% of dogs fed RMD shed ESBL-producing *E. coli* with co-resistance to ciprofloxacin, compared to approximately 1% of NRMD-fed dogs. No resistance to tigecycline, amikacin or meropenem was observed in the ESBL-producing *E. coli* from dogs fed RMD or NRMD.

Table 4.3: *Number (N) and percentage (%) of RMD-fed (N=193) and NRMD-fed (N=239) samples with ESBL-producing E. coli identified which demonstrated resistance to antibiotics tested in the present study*

Resistance phenotype*	Diet choice % (N)^				p value
	RMD (44.7%, N=193)		NRMD (55.3%, N=239)		
	N	% (95% CI)	N	% (95% CI)	
Total samples: 432					
Amp	47	24.4 (18.8-30.9)	4	1.7 (0.7-4.2)	<0.001
AmxC	13	6.7 (4.0-11.2)	1	0.4 (0.1-2.3)	<0.001
Cip	21	10.9 (7.2-16.1)	2	0.8 (0.2-3.0)	<0.001
TMS	23	11.9 (8.1-17.2)	2	0.8 (0.2-3.0)	<0.001
Tig	0	0	0	0	NA
Ami	0	0	0	0	NA
Mer	0	0	0	0	NA

**Amp: ampicillin; AmxC: amoxycillin-clavulanic acid; Cip: ciprofloxacin; TMS: trimethoprim-sulphamethoxazole; Tig: tigecycline; Ami: amikacin; Mer: meropenem; MDR: multidrug resistance. ^RMD: raw meat diet; NRMD: non-raw diet*

Microbiology: Isolate level

Eighty-seven 3GCR *E. coli* isolates which demonstrated ESBL-production on the double disc test, or were non-ESBL producing and suspected of having AmpC production, and which had unique resistance profiles on AST within a sample, underwent whole genome sequencing (WGS). Of the isolates which underwent WGS, 75 were from RMD-fed dog faeces and 12 were from NRMD-dog faeces (Table 4.4). There was a greater number and more varied resistance profiles demonstrated in the isolates from RMD fed dogs, with 20 different profiles identified, compared to 7 profiles in dogs fed NRMD. The most frequently encountered profile in both RMD and NRMD dogs was resistance to ampicillin, amoxycillin-clavulanic acid, cefotaxime and ceftazidime (N=11 RMD, N=3 NRMD). The second most frequently identified profile in RMD-fed dogs (N=8) demonstrated resistance to a wide range of antibiotics, including concurrent 3GCR and FQR.

Table 4.4: *Resistance profiles of E. coli isolates sent for whole genome sequencing which were 3GCR (including those which were ESBL-producing) and had unique profiles on AST, and the number of isolates with each profile*

Resistance profile*	N isolates (RMD)	N isolates (NRMD)
Total	**75**	**12**
Amp, AmxC, Ctx, Ctz	11	3
Amp, Cip, TMS, Ctx, Ctz	8	1
Amp, Ctx, Ctz	7	1
Amp, Cip, Ctx, Ctz	5	2
Amp, TMS, Ctx	5	1
Amp, Ctx	5	0
Amp, AmxC, TMS, Ctz	4	2
Amp, AmxC, Ctz	4	2
Amp, Cip, TMS, Ctx	4	0
Amp, TMS, Ctx, Ctz	4	0
Amp, Cip, Ctx	3	0
Amp, TMS, Ctz	3	0
Amp, Ctz	3	0
Amp, AmxC, TMS, Ctx, Ctz	2	0
Amp, Cip, Ctz	2	0
Amp, AmxC, Cip, TMS, Ctz	1	0
Amp, AmxC, Cip, Ctx, Ctz	1	0
Amp, AmxC	1	0
Amp, TMS	1	0
Amp	1	0

*Amp: ampicillin; AmxC: amoxycillin-clavulanic acid; Cip: ciprofloxacin; TMS: Trimethoprim-sulphamethoxazole; Ctx: cefotaxime; Ctz: ceftazidime

Multiple, varied AMR genes were identified on WGS and full results are presented in appendix table A3.1. A summary of the ESBL and pAmpC genes present in *E. coli* isolates from dogs fed either RMD or NRMD, as well as the presence of other AMR genes of interest are presented in table 4.5.

The *E. coli* isolates detected from dogs fed RMD had a wider variety of AMR genes than dogs fed NRMD (Table 4.5). The predominant ESBL-genes in both RMD and NRMD-originating isolates were $bla_{\text{CTX-M}}$, with $bla_{\text{CTX-M-15}}$ being the most frequently isolated (19%, 14/75 RMD; 25% 3/12 NRMD). A wide range of $bla_{\text{CTX-M}}$ genes was present in RMD isolates, with 12 different genes being identified, compared to two different $bla_{\text{CTX-M}}$ genes identified in NRMD isolates ($bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-1}}$). Within the RMD isolates, $bla_{\text{CTX-M-55}}$ was the second-most

frequently isolated ESBL-gene (12%, 9/75); however, this gene was not present in NRMD-originating isolates. Multiple *bla*$_{TEM}$ genes were identified in the isolates, with *bla*$_{TEM-1}$ being the most frequently isolated (appendix table A3.1); however, in terms of ESBL-producing *bla*$_{TEM}$ genes, *bla*$_{TEM-52}$ and *bla*$_{TEM-60}$ were isolated in RMD *E. coli* isolates only. Additionally, two inhibitor-resistant *bla*$_{TEM}$ genes were identified, *bla*$_{TEM-78}$ (N=3 RMD, N=1 NRMD) and *bla*$_{TEM-185}$ (N=3 RMD only). The ESBL-producing *bla*$_{SHV-66}$ gene was only identified in RMD isolates. The ESBL *bla*$_{OXA-45}$ gene was infrequently observed, and was identified in one isolate each from RMD and NRMD-fed dogs.

Additionally, pAmpC genes were mainly observed in RMD isolates. By far the most frequently observed pAmpC gene was *bla*$_{CMY-2}$, present in 21% (16/75) of RMD *E. coli* isolates, whereas this gene was only identified in one NRMD isolate. With regards to genes associated with quinolone resistance, five separate *qnr* genes were observed in RMD isolates, and only two in NRMD isolates. The *qnrS1* gene was most frequently isolated, present in 20% (15/75) of RMD isolates. Both RMD and NRMD isolates demonstrated the presence of variants of the *parC* and *gyrA* genes which mediate quinolone resistance. Concerningly, one RMD isolate carried the *aac(6')-Ib-cr* gene, which can simultaneously result in fluoroquinolone and aminoglycoside resistance. One RMD *E. coli* isolate was found to harbour the *arr-2* gene which encodes rifampin resistance, and the colistin-resistance encoding *mcr-4* gene was identified in one NRMD-originating isolate.

Table 4.5: *Summary table of ESBL and pAmpC genes identified in E. coli isolates from RMD-fed (N=75 isolates) and NRMD-fed (N=12 isolates) dogs via whole genome sequencing, demonstrating percentage (%) and number (N) of genes present within the isolates submitted for sequencing. *bla$_{TEM-78}$ and bla$_{TEM-185}$ are inhibitor-resistant genes*

Genotype		Diet choice			
		RMD (75)		NRMD (12)	
		N	% (95% CI)	N	% (95% CI)
ESBL genes					
bla$_{CTX-M}$	CTX-M-1	5	6.7 (2.9-14.7)	1	8.3 (1.5-35.4)
	CTX-M-2	1	1.3 (0.2-7.2)	0	0
	CTX-M-9	1	1.3 (0.2-7.2)	0	0
	CTX-M-14	2	1.3 (0.2-7.2)	0	0
	CTX-M-15	14	18.7 (11.5-28.9)	3	25.0 (8.9-54.2)
	CTX-M-24	1	1.3 (0.2-7.2)	0	0
	CTX-M-27	1	1.3 (0.2-7.2)	0	0
	CTX-M-32	2	2.7 (0.7-9.2)	0	0
	CTX-M-55	9	12.0 (6.4-21.3)	0	0
	CTX-M-60	1	1.3 (0.2-7.2)	0	0

	CTX-M-65	1	1.3 (0.2-7.2)	0	0
	CTX-M-123	1	1.3 (0.2-7.2)	0	0
bla$_{TEM}$	TEM-52	2	2.7 (0.7-9.2)	0	0
	TEM-60	1	1.3 (0.2-7.2)	0	0
	TEM-78*	3	4.0 (1.4-11.1)	1	8.3 (1.5-35.4)
	TEM-185*	3	4.0 (1.4-11.1)	0	0
bla$_{SHV}$	SHV-66	10	7.4-22.8)	0	0
bla$_{OXA}$	OXA-45	1	1.3 (0.2-7.2)	1	8.3 (1.5-35.4)
pAmpC genes					
bla$_{CMY}$	CMY-2	16	21.3 (13.6-31.9)	1	8.3 (1.5-35.4)
	CMY-4	1	1.3 (0.2-7.2)	0	0
	CMY-6	1	1.3 (0.2-7.2)	0	0
	CMY-44	0	0	1	8.3 (1.5-35.4)
	CMY-58	1	1.3 (0.2-7.2)	0	0
	CMY-59	1	1.3 (0.2-7.2)	0	0
	CMY-100	1	1.3 (0.2-7.2)	0	0
	CMY-132	2	2.7 (0.7-9.2)	0	0
bla$_{DHA}$	DHA-1	1	1.3 (0.2-7.2)	1	8.3 (1.5-35.4)
Quinolone resistance associated genes					
qnr	*B4*	1	1.3 (0.2-7.2)	1	8.3 (1.5-35.4)
	S1	15	20.0 (12.5-30.4)	2	16.7 (4.7-44.8)
	S2	1	1.3 (0.2-7.2)	0	0
	S7	1	1.3 (0.2-7.2)	0	0
	S15	1	1.3 (0.2-7.2)	0	0
parC		9	12.0 (6.4-21.3)	1	8.3 (1.5-35.4)
gyrA		18	24.0 (15.8-34.8)	3	25.0 (8.9-54.2)
aac(6')-Ib-cr		1	1.3 (0.2-7.2)	0	0
Colistin resistance associated gene					
mcr-4		0	0	1	8.3 (1.5-35.4)
Rifampin resistance associated gene					
arr-2		1	1.3 (0.2-7.2)	0	0
Other antibiotic classes					
Tetracyclines		40	53.3 (42.2-64.2)	4	33.3 (13.8-60.9)
Aminoglycosides		49	65.3 (54.1-75.1)	7	58.3 (32.0-80.7)
TMS		38	50.1 (39.6-61.7)	6	50.0 (25.4-70.6)
Chloramphenicol		15	20.0 (12.5-30.4)	0	0

Figure 4.1 shows the ESBL, pAmpC and plasmid-mediated quinolone-resistance associated *qnr* genes present for each isolate, alongside the phenotypic AST results and the associated sequence type (ST) and clonal complex (CC) identified. All isolates demonstrated resistance to ampicillin, and phenotypic 3GCR was indicated in all but three isolates. Ciprofloxacin resistance was demonstrated by 31% (27/87) of isolates (N=24 RMD, N=3 NRMD) and resistance to TMS was observed in 41% (36/87) of isolates (N=32 RMD, N=4 NRMD). No phenotypic resistance to tigecycline, amikacin or meropenem was identified. MDR

phenotypes were present in 79% (69/87) of isolates (N=59 RMD, N=10 NRMD). Fifty-one distinct *E. coli* sequence types (STs) were identified (N=45 RMD, N =10 NRMD), with two novel STs identified across three RMD isolates. The most frequently observed STs from RMD dogs were ST38 (N=5), ST117 (N=4), ST602 (N=4) and ST752 (N=5), whereas the most common STs in isolates from NRMD dogs were ST75 (N=2) and ST88 (N=2).

Multiple plasmid-mediated AMR genes were often observed concurrently, particularly within RMD-isolates. The presence of the $bla_{\text{CTX-M-15}}$ gene was frequently associated with the presence of *qnrS1* across a range of STs. This was the case for 9 isolates (N=7 RMD, N=2 NRMD), and of these, 8 isolates demonstrated MDR on AST. One isolate (ST533), from a RMD-fed dog harboured both *qnrS1* and *qnrS15*, was MDR, and demonstrated FQR and 3GCR. It was, however, not associated with the presence of $bla_{\text{CTX-M}}$ genes, but *bla*$\text{SHV}_{\text{-66}}$ and $bla_{\text{CMY-2}}$ were present. A further isolate of interest from an RMD-fed dog (ST351) carried $bla_{\text{CTX-M-27}}$, $bla_{\text{CTX-M-123}}$, $bla_{\text{TEM-185}}$ and *qnrS1*. and was phenotypically MDR, with FQR and 3GCR. Both of the isolates which carried the $bla_{\text{DHA-1}}$ gene (N=1 RMD, N=1 NRMD) also concurrently carried *qnrB4*, and were the only isolates associated with the carriage of this particular *qnr* gene. Both of these isolates demonstrated phenotypic resistance to amoxycillin-clavulanic acid, but only one demonstrated phenotypic FQR (RMD-fed).

All ST101 and ST752 isolates harboured the $bla_{\text{CTX-M-55}}$ gene. All but one of the isolates which carried $bla_{\text{CTX-M-55}}$ demonstrated phenotypic MDR to combinations of ampicillin, ciprofloxacin, TMS, cefotaxime and ceftazidime. All isolates which harboured the $bla_{\text{TEM-78}}$ gene demonstrated phenotypic amoxycillin-clavulanic acid resistance, alongside being MDR.

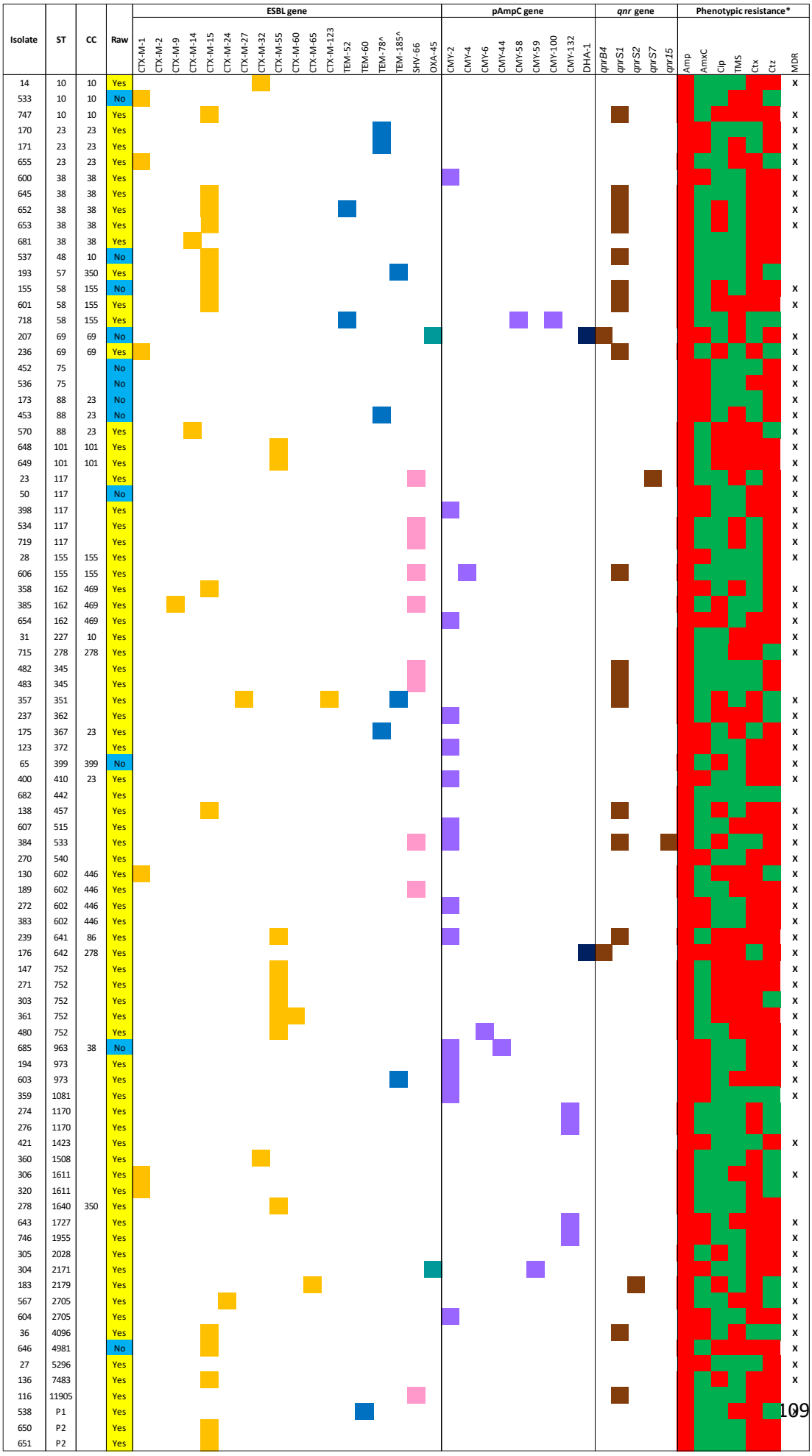

ESBL gene
pAmpC gene
qnr gene
Phenotypic resistance*
Isolate
ST
CC
Raw
CTX-M-1
CTX-M-2
CTX-M-9
CTX-M-14
CTX-M-15
CTX-M-24
CTX-M-27
CTX-M-32
CTX-M-55
CTX-M-60
CTX-M-65
CTX-M-123
TEM-52
TEM-60
TEM-78^
TEM-185^
SHV-66
OXA-45
CMY-2
CMY-4
CMY-6
CMY-44
CMY-58
CMY-59
CMY-100
CMY-132
DHA-1
qnrB4
qnrS1
qnrS2
qnrS7
qnr15
Amp
AmxC
Cip
TMS
Ctx
Ctz
MDR
14 10 10 Yes x
533 10 10 No
747 10 10 Yes x
170 23 23 Yes x
171 23 23 Yes x
655 23 23 Yes x
600 38 38 Yes x
645 38 38 Yes x
652 38 38 Yes x
653 38 38 Yes x
681 38 38 Yes
537 48 10 No
193 57 350 Yes
155 58 155 No x
601 58 155 Yes x
718 58 155 Yes
207 69 69 No x
236 69 69 Yes x
452 75 No x
536 75 No x
173 88 23 No x
453 88 23 No x
570 88 23 Yes x
648 101 101 Yes x
649 101 101 Yes x
23 117 Yes x
50 117 No x
398 117 Yes x
534 117 Yes x
719 117 Yes x
28 155 155 Yes x
606 155 155 Yes
358 162 469 Yes x
385 162 469 Yes x
654 162 469 Yes x
31 227 10 Yes x
715 278 278 Yes x
482 345 Yes
483 345 Yes
357 351 Yes x
237 362 Yes x
175 367 23 Yes x
123 372 Yes x
65 399 399 No x
400 410 23 Yes x
682 442 Yes
138 457 Yes x
607 515 Yes x
384 533 Yes x
270 540 Yes x
130 602 446 Yes x
189 602 446 Yes x
272 602 446 Yes x
383 602 446 Yes x
239 641 86 Yes x
176 642 278 Yes x
147 752 Yes x
271 752 Yes x
303 752 Yes x
361 752 Yes x
480 752 Yes x
685 963 38 No x
194 973 Yes x
603 973 Yes x
359 1081 Yes x
274 1170 Yes
276 1170 Yes
421 1423 Yes x
360 1508 Yes
306 1611 Yes x
320 1611 Yes
278 1640 350 Yes
643 1727 Yes x
746 1955 Yes x
305 2028 Yes x
304 2171 Yes x
183 2179 Yes x
567 2705 Yes x
604 2705 Yes x
36 4096 Yes x
646 4981 No x
27 5296 Yes x
136 7483 Yes x
116 11905 Yes
538 P1 Yes
650 P2 Yes
651 P2 Yes

***Figure 4.1**: ESBL, pAmpC and quinolone resistance associated qnr genes associated with each isolate which underwent whole genome sequencing, alongside the sequence type (ST) and clonal complex (CC) identified and phenotypic resistance demonstrated via disc diffusion. For the 'raw' column, a yellow box denotes a raw-fed dog isolate, whereas a blue box denotes a non-raw fed dog isolate. For the genes, a coloured box indicates presence of a gene. For the phenotypic resistance, a red box denotes a resistance and a green box denotes susceptible. Although amikacin, tigecycline and meropenem were all tested via disc diffusion, no resistance was observed and they have been omitted from this figure. *Amp: ampicillin; AmxC: amoxycillin-clavulanic acid; Cip: ciprofloxacin; TMS: trimethoprim-sulphamethoxazole; Ctx: cefotaxime; Ctz: ceftazidime; MDR: multidrug resistance. ^inhibitor-resistant genes*

Multiple combinations of plasmid replicon types were associated with *bla*$_{ESBL}$ gene carriage in the present study (Table 4.6). In particular, *bla*$_{CTX\text{-}M\text{-}15}$ carriage was associated with many plasmid groups including multiple IncF group plasmids, IncB/O/K/Z, IncI1-I(gamma) and IncX1. Isolates which carried *bla*$_{CTX\text{-}M\text{-}55}$ were associated with a number of different plasmid groups, including IncF, IncH. IncI and IncX (IncX4); however, there were some associations of particular plasmids with specific STs. All isolates except one were associated with IncFIB. Plasmid IncFII was identified in all ST752 isolates, and ST641 was the only *bla*$_{CTX\text{-}M\text{-}55}$-carrying ST which had IncH group plasmids IncHI2A and IncHI2 present. The ST1640 isolate which did not have IncFIB present was instead associated with IncFII and IncX4. One isolate which had *bla*$_{TEM\text{-}52}$ present (ESBL gene, ST58) was associated with IncFI group plasmids and IncI1-I(gamma). The other *bla*$_{TEM\text{-}52}$-carrying isolate (ST38) concurrently carried *bla*$_{CTX\text{-}M\text{-}15}$; however, no associated plasmids were identified with this isolate. The four isolates which carried inhibitor-resistant *bla*$_{TEM\text{-}78}$ were associated with the presence of plasmid IncFIB. One isolate (ST57) which carried both *bla*$_{CTX\text{-}M\text{-}15}$ and inhibitor resistant *bla*$_{TEM\text{-}185}$ was associated with the presence of the IncFII plasmid only.

With regards to *bla*$_{SHV\text{-}66}$, eight isolates which carried this gene were associated with the presence of IncFIB, and one isolate had plasmids IncFIA(HI1) and IncFIB(K) present. One isolate (ST155, isolated from a raw-fed dog) which carried *bla*$_{SHV\text{-}66}$ was associated with IncHI1B(pNDM-CIT). Both isolates which carried *bla*$_{OXA\text{-}45}$ were associated with IncFII.

Finally, plasmids associated with *bla*$_{CMY\text{-}2}$ carriage in the present study were IncFIB IncI1-I(gamma) and IncI2(Delta). One isolate did not have any Inc group plasmid identified.

Table 4.6: *Inc group plasmids associated with sequence types (STs) and ESBL genes of interest from ESBL-producing E. coli isolated from RMD-fed (N=75 isolates) and NRMD-fed (N=12 isolates) dog faeces in the present study. *Inhibitor-resistant* bla_{TEM}

Beta-lactam resistance gene	Gene type	STs associated	Plasmids associated
bla_{CTX-M}	1	10, 23, 69, 602, 1611	IncFIA|AP001918, IncFIB(AP001918)|AP001918, IncFIC(FII)|AP001918, IncFII(pCoo)|CR942285, IncFII|AY458016, IncI1-I(gamma)|AP005147, IncY|K02380
	2	362	IncB/O/K/Z|CU928147, IncB/O/K/Z|FN868832, IncFIB(AP001918)|AP001918, IncFII(pCoo)|CR942285, IncI1-I(gamma)|AP005147
	9	278	IncI1-I(gamma)|AP005147
	14	38, 88	IncB/O/K/Z|FN868832, IncFIB(AP001918)|AP001918, IncFIC(FII)|AP001918, IncFII(pHN7A8)|JN232517, IncFII|AY458016
	15	10, 38, 48, 57, 58, 162, 457, 1170, 4981, 7843, P2	IncB/O/K/Z|CU928147, IncB/O/K/Z|FN868832, IncFIA|AP001918, IncFIB(AP001918)|AP001918, IncFIB(H89-PhagePlasmid)|HG530657, IncFIB(K)|JN233704, IncFIC(FII)|AP001918, IncFII(pCoo)|CR942285, IncFII|AY458016, IncI1-I(gamma)|AP005147, IncI2|KP347127, IncR|DQ449578, IncX1|EU370913, IncX4|FN543504
	24	2705	IncI1-I(gamma)|AP005147
	27	351	IncFIB(AP001918)|AP001918, IncFII|AY458016, IncHI2A|BX664015, IncHI2|BX664015

	32	10, 1508	IncFIB(AP001918)|AP001918, IncFII(29)|CP003035, IncHI2A|BX664015, IncHI2|BX664015, IncI2(Delta)|AP002527, IncR|DQ449578
	55	101, 641, 752, 1640	IncFIB(AP001918)|AP001918, IncFIC(FII)|AP001918, IncFII(29)|CP003035, IncFII(pHN7A8)|JN232517, IncFII(pSE11)|AP009242, IncFII|AY458016, IncHI2A|BX664015, IncHI2|BX664015, IncI1-I(gamma)|AP005147, IncX4|FN543504
	60	752	IncFIB(AP001918)|AP001918, IncFII(pSE11)|AP009242, IncFII|AY458016
	65	2179	IncFIB(AP001918)|AP001918, IncFIC(FII)|AP001918, IncI1-I(gamma)|AP005147
	123	351	IncFIB(AP001918)|AP001918, IncFII|AY458016, IncHI2A|BX664015, IncHI2|BX664015
bla$_{TEM}$	52	38, 58	IncFIA|AP001918, IncFIB(AP001918)|AP001918, IncFIC(FII)|AP001918, IncI1-I(gamma)|AP005147
	78*	23, 88, 367	IncFIA|AP001918, IncFIB(AP001918)|AP001918, IncFII(pCoo)|CR942285, IncX4|FN543504, IncY|K02380
	185*	57	IncFII|AY458016
bla$_{SHV}$	66	117, 155, 162, 345, 533, 602, 11905	IncB/O/K/Z|CU928147, IncB/O/K/Z|FN868832, IncFIA(HI1)|AF250878, IncFIA|AP001918, IncFIB(AP001918)|AP001918, IncFIB(H89-PhagePlasmid)|HG530657, IncFIB(K)|JN233704, IncFIC(FII)|AP001918, IncFII(pHN7A8)|JN232517, IncFII(pRSB107)|AJ851089, IncFII(pSE11)|AP009242, IncFII|AY458016, IncHI1B(pNDM-CIT)|JX182975, IncI1-I(gamma)|AP005147, IncX1|EU370913, IncX3|JN247852, IncY|K02380
bla$_{OXA}$	45	69, 2171	IncFIA|AP001918, IncFIB(AP001918)|AP001918, IncFIB(pLF82-PhagePlasmid)|CU638872, IncFIC(FII)|AP001918, IncFII|AY458016, IncI2(Delta)|AP002527

	2	38, 117, 162, 362, 372, 410, 515, 533, 602, 641, 973, 1081, 1727, 1955, 2705	IncB/O/K/Z|CU928147, IncFIB(AP001918)|AP001918, IncFIB(H89-PhagePlasmid)|HG530657, IncFIC(FII)|AP001918, IncFII(pCoo)|CR942285, IncFII(pHN7A8)|JN232517, IncFII(pRSB107)|AJ851089, IncFII(pSE11)|AP009242, IncFII|AY458016, IncHI2A|BX664015, IncHI2|BX664015, IncI1-I(gamma)|AP005147, IncI2(Delta)|AP002527, IncI2|KP347127, IncX1|EU370913, IncX3|JN247852, IncY|K02380
	4	155	IncFIB(AP001918)|AP001918, IncFII(pHN7A8)|JN232517, IncHI1B(pNDM-CIT)|JX182975, IncI1-I(gamma)|AP005147, IncX3|JN247852
	6	752	IncFIB(AP001918)|AP001918, IncFII(pSE11)|AP009242, IncFII|AY458016,
	44	963	IncFIB(AP001918)|AP001918, IncFII(29)|CP003035
bla_{CMY}	58	58	IncFIA|AP001918, IncFIB(AP001918)|AP001918, IncFIC(FII)|AP001918, IncI1-I(gamma)|AP005147
	59	2171	IncFIB(AP001918)|AP001918, IncFIB(pLF82-PhagePlasmid)|CU638872, IncFIC(FII)|AP001918, IncFII|AY458016, IncI2(Delta)|AP002527
	100	58	IncFIA|AP001918, IncFIB(AP001918)|AP001918, IncFIC(FII)|AP001918, IncI1-I(gamma)|AP005147
	132	1170	IncFIB(AP001918)|AP001918, IncFIC(FII)|AP001918, IncFII|AY458016, IncR|DQ449578
bla_{DHA}	1	69, 642	IncFIA|AP001918, IncFIB(AP001918)|AP001918, IncFIC(FII)|AP001918, IncFII|AY458016

Survey data analysis

A total of 432 surveys were received. Participant demographics and univariable logistic regression results are shown in appendix tables A3.3-A3.5. Multivariable analysis demonstrated a number of dog and owner lifestyle risk factors for carriage of overall 3GCR-*E. coli*, ESBL-producing *E. coli* and MDR *E. coli* by dogs.

Risk factors for carriage 3GCR-*E. coli*, ESBL-producing *E. coli* and MDR *E. coli* are demonstrated in tables 4.7, 4.8 and 4.9, respectively. There were also some common risk factors across all three outcomes, dogs fed a raw diet and dogs which had received antibiotics in the last 3 months were significantly more likely to shed 3GCR, ESBL-producing and MDR *E. coli*. Dog owners were asked to report the type of antibiotic prescribed (if known), these descriptive results are shown in appendix table A3.2. The most frequently prescribed antibiotic was amoxycillin-clavulanic acid (N=19 dogs), followed by metronidazole (N=7 dogs). Veterinary visits in the last 3 months were also common to all outcomes, dogs which had visited for an emergency appointment were more likely to shed 3GCR and ESBL-producing *E. coli*, whereas dogs which had attended a veterinary clinic in general were more likely to shed MDR *E. coli*. Dogs which were fed shop bought cooked treats/biscuits were less likely to shed 3GCR, ESBL-producing or MDR *E. coli.*

There were some risk factors which were unique for 3GCR and ESBL-producing *E. coli* carriage. Dogs which attended dog shows or whose owner worked in a nursery were more likely to carry 3GCR-*E. coli*; however, dogs were less likely to carry 3GCR-*E. coli* with increasing age (Table 4.7). Dogs that visited care homes, for example “Pets As Therapy” (PAT) dogs were more likely to carry ESBL-producing *E. coli* in their faeces (Table 4.8).

Table 4.7: *Final multivariable regression model describing explanatory variables significantly associated with dog (N=432) faecal carriage of 3GCR- E. coli in the present study*

Variable	Category	Odds Ratio*	CI	p value
Fed a raw diet	Yes	10.8	4.93, 23.75	<0.001
	No	*Ref*		
Type of treat fed				
Shop bought cooked treats/biscuits	Yes	0.50	0.27, 0.93	0.03
	No	*Ref*		
Dog received antibiotics in last 3 months	Yes	5.03	1.84, 13.81	<0.01
	No	*Ref*		
Regular access to communal places				
Dog shows	Yes	2.60	1.15, 5.87	0.02
	No	*Ref*		
Dog age (years)	Linear	0.91	0.84, 0.99	0.02
Reason for most recent vet visit	No visit	*Ref*		0.02
	Routine	2.28	0.94, 5.51	
	Non-emergency problem	0.75	0.34, 1.62	
	Emergency	5.12	1.31, 20.02	
Residents in house place of work				
Nursery	Yes	29.92	2.06, 435.50	0.01
	No	*Ref*		

**Ref: reference category*

Hosmer-Lemeshow goodness of fit result: 0.471

Table 4.8: *Final multivariable regression model describing explanatory variables significantly associated with dog (N=432) faecal carriage of ESBL-producing E. coli in the present study*

Variable	Category	Odds Ratio*	CI	p value
Fed a raw diet	Yes	24.34	7.09, 83.55	<0.001
	No	*Ref*		
Diet changed in last 3 months	Yes	0.24	0.07, 0.86	0.03
	No	*Ref*		
Type of treat fed				
Shop bought cooked treats/biscuits	Yes	0.34	0.16, 0.72	0.01
	No	*Ref*		
Dog received antibiotics in last 3 months	Yes	5.98	1.71, 29.92	0.01
	No	*Ref*		
Reason for most recent vet visit	No visit	*Ref*		0.04
	Routine	2.73	0.92, 8.07	
	Non-emergency problem	1.20	0.51, 2.82	

	Emergency	6.38	1.45, 28.01	
Dog visits care homes (e.g. PAT dog)	Yes	7.11	1.14, 44.38	0.04
	No	*Ref*		

**Ref: reference category*

Hosmer-Lemeshow goodness of fit result: 0.103

Table 4.9: *Final multivariable regression model describing explanatory variables significantly associated with dog (N=432) faecal carriage of MDR- E. coli in the present study*

Variable	Category	Odds Ratio*	CI	p value
Fed a raw diet	Yes	22.9	5.87, 89.58	<0.001
	No	*Ref*		
Diet changed in last 3 months	Yes	0.15	0.03, 0.77	0.02
	No	*Ref*		
Type of treat fed				
Shop bought cooked treats/biscuits	Yes	0.41	0.19, 0.90	0.03
	No	*Ref*		
Dog received antibiotics in last 3 months	Yes	6.32	1.84, 21.67	0.003
	No	*Ref*		
Vet visit within last 3 months	Yes	2.2	1.01, 4.80	0.048
	No	*Ref*		

**Ref: reference category*

Hosmer-Lemeshow goodness of fit result: 0.876

4.5 Discussion

This study has provided further evidence on a larger scale that provision of RMD to dogs in the UK is a significant risk factor for faecal carriage of AMR *E. coli*, and that dogs fed RMD are significantly more likely to shed AMR *E. coli* in their faeces than those fed a cooked diet. The provision of raw meat to dogs has been identified as a risk factor for AMR *E. coli* carriage globally (Lefebvre *et al.*, 2008; Baede *et al.*, 2015; Leonard *et al.*, 2015; Runesvärd *et al.*, 2020; van den Bunt *et al.*, 2020) as well as in previous studies in the UK (Wedley *et al.*, 2017; Sealey *et al.*, 2022). One study demonstrated RMD to be a significant risk factor for resistance to amoxycillin-clavulanic acid and 3GCR in particular in healthy, non-veterinary visiting, non-antimicrobial treated dogs (Schmidt *et al.*, 2015).

Although provision of RMD was by far the most significant risk factor for AMR *E. coli* shedding by dogs in this study, there were additional risk factors identified for carriage of all three categories of resistance tested (ESBL-producing *E. coli*, 3GCR-*E. coli* and MDR-*E. coli*). The provision of antibiotics in the last 3 months was a significant risk factor in all three models in this study, and has been identified as a risk factor for carriage of AMR *E. coli* by dogs over this timeframe previously (Gandolfi-Decristophoris *et al.*, 2013; Wedley *et al.*, 2017). Treatment with specific antibiotics has been linked with AMR *E. coli* carriage in dogs; the provision of oral cephalexin has been associated with selection of bla_{CMY-2} producing *E. coli* (Damborg, Gaustad, *et al.*, 2011), and carriage of MDR *E. coli* has been attributed to the use of fluoroquinolones (Gibson *et al.*, 2011; Leite-Martins *et al.*, 2014). Schmidt *et al.*, 2018 observed that administration of amoxycillin-clavulanic acid and cefovecin both increased the risk of MDR *E. coli* carriage, and usage of cephalosporins and fluoroquinolones both increased the risk of FQR in dogs. Fluoroquinolone use was not widely reported in the present study, with amoxycillin-clavulanic acid being the most frequently prescribed antibiotic reported.

It is interesting that visiting a veterinary practice in the last 3 months was a risk factor for AMR *E. coli* carriage, with an emergency visit specifically being significant for ESBL-producing and 3GCR *E. coli*. Previous studies have identified veterinary hospitals as sources of ESBL-producing *E. coli* (Timofte *et al.*, 2016; Schmitt *et al.*, 2021), with carriage by staff (Royden *et al.*, 2019) and patients being reported, and a further study identified frequent carriage of AMR *E. coli* by vet-visiting dogs, with resistance to ampicillin, tetracycline and trimethoprim most commonly detected (Wedley *et al.*, 2017). As opposed to previous studies where hospitalisation and length of stay was a significant risk factor for MDR *E. coli* (Gibson *et al.*, 2011; Tuerena *et al.*, 2016; Haenni *et al.*, 2022), hospitalisation was not significant for any of the AMR outcomes in the present study. However, as discussed, antibiotic treatment was, and it is possible that dogs receiving emergency care may be more likely to receive antibiotics. Dogs which visited care homes (for example as "Pets As Therapy" dogs) were more likely to carry ESBL-producing *E. coli*. A study from Switzerland also identified that pets which lived in or visited nursing homes carried ESBL-producing *E. coli*; however, the prevalence was not at a higher rate than dogs living in the community (Gandolfi-Decristophoris *et al.*, 2013). Further research is required into this area, as a high prevalence of AMR *E. coli* has been identified in people in residential care homes and nursing homes (Ludden *et al.*, 2015; Overdevest *et al.*, 2016; Van Dulm *et al.*, 2019), therefore it could suggest a potential high-risk area for transmission of ESBL-producing *E. coli* from humans to canines.

Dogs that were fed shop bought cooked treats were less likely to carry ESBL-producing, 3GCR and MDR- *E. coli*. Dogs fed a dry kibble diet have been shown previously to excrete significantly less ESBL-producing *E. coli* in their faeces than those fed RMD (Runesvärd *et al.*, 2020). There are limited data with regards to AMR *E. coli* carriage and types of treat fed specifically; however, it stands to reason that dogs fed cooked treats and biscuits would also be less likely to shed AMR bacteria.

In the present study, RMD-fed dogs carried significantly more AMR *E. coli* than those fed NRMD, with a quarter of dogs fed RMD carrying ESBL-producing *E. coli*, and approximately one third of dogs fed RMD carrying 3GCR-*E. coli*. Previous studies have also observed significantly greater ESBL-producing *E. coli* carriage in dogs fed RMD than those fed NRMD. In a small study from Sweden of 25 dogs, 52% of those fed RMD carried ESBL-producing *E. coli*, compared to 4% of dogs fed dry food (Runesvärd *et al.*, 2020). The findings of the present study are further supported by those of a recent smaller UK study which identified that dogs fed RMD were significantly more likely to carry AMR, MDR and 3GCR-*E. coli* than dogs fed NRMD (Groat *et al.*, 2022). However, whereas the prevalence of 3GCR-*E. coli* was similar between the findings of Groat *et al.* (2022) and the present study (31% and 32.6%, respectively), there was a reduction in overall AMR and MDR *E. coli* in the present study, with 39.4% of RMD-fed dogs carrying AMR *E. coli* and 17% carrying MDR *E. coli* (compared to 54% and 25%, respectively).

There was a significantly greater prevalence of phenotypic resistance to ampicillin, amoxycillin-clavulanic acid, TMS and ciprofloxacin in the ESBL-producing *E. coli* isolated from dogs fed RMD than NRMD in this study. Similarly, significantly greater proportions of non-ESBL producing *E. coli* resistant to ampicillin, amoxycillin-clavulanic acid and TMS were also observed from RMD-fed dogs; however, there was no significant difference identified between dogs fed either diet for phenotypic ciprofloxacin resistance. High levels of phenotypic resistance to ampicillin, amoxycillin-clavulanic acid and/or TMS have been reported in dogs fed RMD previously (Schmidt *et al.*, 2015; Runesvärd *et al.*, 2020; Groat *et al.*, 2022). The findings of the present study are interesting as two previous studies demonstrated no (Groat *et al.*, 2022) or uncommon (Schmidt *et al.*, 2015) phenotypic fluoroquinolone resistance in *E. coli* isolated from healthy adult dogs in the UK. However, in contrast to this, a further study of 16-week-old puppies identified that provision of a raw diet was the most substantial risk factor for FQR *E. coli* carriage (Mounsey *et al.*, 2022). Interestingly, in the study by Schmidt *et al*. (2015), when ciprofloxacin resistance did occur, it was associated with MDR. In the present study, greater proportions of ciprofloxacin-

resistance were observed in the ESBL-producing *E. coli*, and frequently were associated with MDR. Concurrent MDR and FQR-*E. coli* has been reported previously in dogs (Platell *et al*., 2011) and ESBL-producing *E. coli* often demonstrate co-resistance to other antibiotic classes, with concurrent phenotypic fluoroquinolone resistance and ESBL production frequently observed in humans (Lautenbach *et al.*, 2001; Bartoloni *et al.*, 2013; Palma *et al.*, 2017). No carbapenem resistance was demonstrated in dogs fed either diet in the present study, a finding which echoes that of Runesvärd *et al*., 2020.

A greater number of ESBL-producing *E. coli* isolates with unique resistance phenotypes were obtained from RMD-fed dogs than NRMD, though this may reflect the greater absolute number of ESBL-producing *E. coli* isolated from RMD-fed dogs. However, on WGS, isolates from RMD-fed dogs additionally demonstrated more varied STs and a greater diversity of ESBL genes. The most frequently observed genes were $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-55}}$ and $bla_{\text{SHV-66}}$. While $bla_{\text{CTX-M-15}}$ was identified, albeit far less frequently, in dogs fed NRMD, no $bla_{\text{CTX-M-55}}$ or $bla_{\text{SHV-66}}$ was present in isolates from NRMD-fed dogs. The presence of $bla_{\text{CTX-M-15}}$ was frequently associated with concurrent *qnrS1* carriage, which reduces susceptibility to quinolones, as well as MDR, and was present across a range of STs, including one novel ST. Of particular interest is ST38, identified in five of the RMD isolates. ST38 has been described as belonging to a global extraintestinal pathogenic lineage (Manges *et al.*, 2019), isolated from a range of sources, and has been previously documented in dogs in Korea (Tamang, Nam, *et al.*, 2012). In one study in Switzerland, it was isolated from dogs and dog owners, although was associated with the carriage of $bla_{\text{CTX-M-14}}$ and not associated with provision of raw dog food (Schmitt *et al.*, 2021). In the current study, one ST38 isolate carried $bla_{\text{CTX-M-14}}$, however the remaining isolates were associated with carriage of $bla_{\text{CTX-M-15}}$ and *qnrS1*. There are few studies which have specifically investigated the resistance genes present in *E. coli* isolated from dogs fed raw diets. However, previous research by the author (see chapter 3) identified $bla_{\text{CTX-M-15}}$ to be the most prevalent bla_{ESBL} gene in samples of UK raw pet food. While previous studies have demonstrated a predominance of $bla_{\text{CTX-M-1}}$ in the UK healthy dog population (Wedley *et al.*, 2017; Mounsey *et al.*, 2022), this gene was only observed in 5 isolates from RMD-fed and one isolate from NRMD-fed dogs in the present study. The dominance of $bla_{\text{CTX-M-15}}$ across a range of STs in this study is interesting and, along with the WGS findings from other studies (Timofte *et al.*, 2016; Singleton, Pongchaikul, *et al.*, 2021; Sealey *et al.*, 2022), may demonstrate an increase in this particular gene within the canine population in the UK in general, alongside a decrease in $bla_{\text{CTX-M-1}}$ carriage, as well as potentially an increased risk of $bla_{\text{CTX-M-15}}$ carriage in RMD-fed dogs. A recent study of canine

faecal *E. coli* from dogs in the South West of England demonstrated a predominance of the $bla_{\text{CTX-M-15}}$ gene in urban dogs, but not rural dogs; however, excretion of *E. coli* with $bla_{\text{CTX-M}}$ genes was significantly associated with RMD-feeding in both urban and rural dogs (Sealey *et al.*, 2022). The $bla_{\text{CTX-M-15}}$ gene has been identified as the most frequently isolated $bla_{\text{CTX-M}}$ gene in *E. coli* from dogs in other countries including the USA (Lv *et al.*, 2013), Canada (Cormier *et al.*, 2019) and Portugal (Carvalho *et al.*, 2021). It is also the most commonly identified *bla*ESBL gene associated with human *E. coli* infections in the UK (Woodford, 2008; Woodford, Turton and Livermore, 2011). Furthermore, this trend of increasing dominance of $bla_{\text{CTX-M-15}}$ has also been identified in hospitalised horses in the UK (Isgren *et al.*, 2019).

The second-most frequently encountered bla_{ESBL} gene was $bla_{\text{CTX-M-55}}$, associated with 4 STs; ST101, ST641, ST752 and ST1640 identified in RMD-fed dogs only. $bla_{\text{CTX-M-55}}$ is derived from $bla_{\text{CTX-M-15}}$ (He *et al.*, 2015) and is frequently identified in humans in China (Zhang *et al.*, 2014), as well as being reported in food producing animals and pets in China (Sun *et al.*, 2010; Lv *et al.*, 2013). However, $bla_{\text{CTX-M-55}}$ is infrequently identified in dogs elsewhere, reported previously in Korea (Tamang, Nam, *et al.*, 2012), Canada (Cormier *et al.*, 2019), Portugal (Carvalho *et al.*, 2021), France (Lupo *et al.*, 2018), Switzerland (Zogg *et al.*, 2018) and the Netherlands (Baede *et al.*, 2015). The high prevalence of $bla_{\text{CTX-M-55}}$ in the present study is a particularly interesting finding, as to the author's knowledge, it has only been reported once before in dogs in the UK, in *E. coli* isolates from clinical samples (Bortolami *et al.*, 2019) ; however, previous research by the author (see chapter 3) identified $bla_{\text{CTX-M-55}}$ in a sample of duck flavoured RMD. All isolates which carried $bla_{\text{CTX-M-55}}$ in this study, except one, demonstrated MDR. $bla_{\text{CTX-M-55}}$ has been identified in healthy pigs at slaughter in the UK (Veterinary Medicines Directorate, 2022), and was the most frequently identified bla_{ESBL} gene in healthy broilers (Veterinary Medicines Directorate, 2021a), where it was associated with ST101 and ST752. Therefore $bla_{\text{CTX-M-55}}$ could be an emerging bla_{ESBL} gene of interest within Europe, as well as within the UK dog population and may be associated with provision of raw meat, particularly poultry.

The $bla_{\text{CTX-M-32}}$ gene was identified in isolates from two RMD-fed dogs in the present study (ST10 and ST1508). Again this is infrequently identified in dogs; however, has previously been associated with cattle (Findlay *et al.*, 2020) and pigs, where it was also associated with ST10 (Veterinary Medicines Directorate, 2022). One previous study observed it in a rural-living dog (Sealey *et al.*, 2022) in the UK, and others identified it in low numbers in dogs in the Netherlands (Baede *et al.*, 2015), Portugal (Carvalho *et al.*, 2021) and France (Haenni *et al.*, 2014). Additionally, a study in the Netherlands of RMD-fed cats identified faecal $bla_{\text{CTX-M-32}}$

carriage, as well as identifying *bla*$_{\text{CTX-M-32}}$ presence in samples of beef and chicken RMD (Baede *et al.*, 2017).

A further interesting finding within this study was the identification of *bla*$_{\text{SHV-66}}$ in *E. coli* isolated from dogs fed RMD, which was not present in *E. coli* isolated from NRMD-fed dogs. *bla*$_{\text{SHV-66}}$ is usually more frequently associated with *Klebsiella* spp (Shibu *et al.*, 2021; Imkamp *et al.*, 2022); however, a study from the UK identified its presence in *E. coli* isolated from horses (Isgren, 2020). Other ESBL-producing *bla*$_{\text{SHV}}$ genes, in particular *bla*$_{\text{SHV-12}}$, have been associated with *E. coli* isolated from dogs (Liu, Thungrat and Boothe, 2016; Alonso *et al.*, 2017; Boehmer *et al.*, 2018; Zogg *et al.*, 2018; Dupouy *et al.*, 2019). Two studies in the UK have identified *bla*$_{\text{SHV-12}}$ carriage in canine *E. coli* from single dogs (Singleton, Pongchaikul, *et al.*, 2021; Sealey *et al.*, 2022); however, other UK studies did not isolate any *bla*$_{\text{SHV}}$ genes from canine faecal *E. coli* (Wedley *et al.*, 2017; Schmidt *et al.*, 2018; Groat *et al.*, 2022; Mounsey *et al.*, 2022). To the author's knowledge, this is the first report of *bla*$_{\text{SHV-66}}$ presence in ESBL-producing *E. coli* isolated from dogs which may suggest that *bla*$_{\text{SHV-66}}$ is an emerging *bla*$_{\text{ESBL}}$ gene of concern. Three isolates from RMD-fed dogs which carried *bla*$_{\text{SHV-66}}$ in the present study were identified as ST117. ST117 is an avian pathogenic *E. coli* strain (Ronco *et al.*, 2017; Cormier *et al.*, 2019), which has also been isolated from dairy calves (Kim *et al.*, 2017) where it was found to be MDR. It is also of clinical importance as an extraintestinal pathogenic *E. coli* (ExPEC) strain which has been identified in chicken meat used as a food source and implicated in human urinary tract infections (Vincent *et al.*, 2010). ST117 has been isolated previously in cattle and dogs in the UK, where 3GCR isolates were found to carry *bla*$_{\text{CTX-M-14}}$ (Sealey *et al.*, 2022); however, there was no carriage of *bla*$_{\text{SHV-66}}$ identified.

It is unsurprising that the most prevalent pAmpC gene in this study was *bla*$_{\text{CMY-2}}$, present across a range of STs, as this is the most frequently isolated pAmpC gene from *E. coli* of animal and human origin (Denisuik *et al.*, 2013; Hansen *et al.*, 2016). The *bla*$_{\text{CMY-2}}$ gene was been identified in *E. coli* from livestock (Findlay *et al.*, 2020; Veterinary Medicines Directorate, 2021a, 2022; Zheng *et al.*, 2022), as well as in samples of poultry meat, pork and beef (Voets *et al.*, 2013; Hansen *et al.*, 2016; Clemente *et al.*, 2021). Additionally, *bla*$_{\text{CMY-2}}$ has been demonstrated in *E. coli* isolated from raw pet food (Nilsson, 2015; Baede *et al.*, 2017), and previous research investigating AMR-*E. coli* presence in raw dog food samples in the UK also identified *bla*$_{\text{CMY-2}}$ in samples comprising of duck meat (see chapter 3). Dogs have been frequently shown to carry *E. coli* which harbours *bla*$_{\text{CMY-2}}$ in previous studies (Tamang, Nam, *et al.*, 2012; Baede *et al.*, 2015; Hansen *et al.*, 2016; Rodríguez-González *et al.*, 2020; Haenni *et al.*, 2022; Sealey *et al.*, 2022), and a link between oral administration of cefalexin and

selection of *bla*$_{CMY-2}$ production by *E. coli* isolated from dogs has been observed (Damborg, Gaustad, *et al.*, 2011). However, of interest in the present study, although it was isolated from *E. coli* from one NRMD-fed dog, far more *E. coli* isolates from RMD-fed dogs were demonstrated to carry this gene, therefore suggesting that provision of RMD is also a risk for *bla*$_{CMY-2}$ carriage. This finding is also supported by the phenotypic AMR findings that dogs fed RMD carried significantly greater 3GCR-*E. coli* and multivariable model results demonstrating provision of RMD to be a risk factor for 3GCR-*E. coli* carriage by dogs.

Of concern was the identification of the *arr-2* (ST641, isolated from a single RMD-fed dog) and *mcr-4* (ST4981, isolated from a NRMD-fed dog) genes in this study. The *arr-2* gene confers plasmid-mediated resistance to rifampicin, and in this study occurred alongside carriage of *bla*$_{CTX-M-55}$ and plasmids IncHI2A and IncHI2. The *mcr-4* gene confers plasmid-mediated resistance to colistin, and was associated with co-carriage of *bla*$_{CTX-M-15}$ and plasmid IncI2. Both of these isolates were phenotypically MDR. The *mcr-4* gene has previously been reported in *K. pneumoniae* isolated from canine faeces in China (Hamame *et al.*, 2022), and *arr-2* has been reported in *E. coli* isolated from humans and chickens (Hopkins *et al.*, 2014; Tang *et al.*, 2022); however, to the author's knowledge, this is the first report of isolation of either of these genes from canine *E. coli*.

Additional work is required to investigate these genes further, including identifying whether they are phenotypically expressed, if they are transferrable and whether any other lifestyle factors (other than diet) could contribute to their carriage.

Multiple plasmids were identified associated with *bla*$_{ESBL}$ gene carriage in the present study, including multiple IncF group plasmids. Plasmid IncFII has been linked to *bla*$_{CTX-M-15}$ gene carriage in dogs previously, where it was suggested to have exchanged from a human reservoir (Dahmen *et al.*, 2013). Plasmid-associated *bla*$_{CTX-M-55}$ carriage has previously been observed with plasmids IncFIB and IncFIC(FII) in ESBL-producing *E. coli* isolated from chickens (Yoon and Lee, 2022). Additionally, plasmids IncFIB, IncFII, IncHI2 and IncI1-I (gamma) were associated with the carriage of the *bla*$_{CTX-M-55}$ gene in ESBL-producing *E. coli* isolated from a sick pig in China (Zhang *et al.*, 2021). One isolate carrying *bla*$_{SHV-66}$ was found to harbour the IncHI1B(pNDM-CIT) plasmid. This plasmid has been reported with the *ndm-1* metallobetalactamase gene carriage which encodes for carbapenem resistance (Pillai, McGeer and Low, 2011), however, no carbapenemase genes were evident in this study. This could indicate an example of divergent evolution between two close contact populations (human and canine); however, as carbapenem antibiotics are antibiotics of last resort in

human medicine, and not routinely utilised in companion animals there is no selection pressure in canine populations to maintain this gene. Nevertheless, there have been reports previously of carbapenemase gene expression in *E. coli* isolated from companion animals around the world (da Silva *et al.*, 2022).

The carriage of bla_{CMY-2} gene has been linked with IncI1 plasmids in *E. coli* isolated from dogs in Italy (García-Fernández *et al.*, 2008), France (Haenni *et al.*, 2014, 2022), as well as in *E. coli* isolated from cephalexin-treated dogs in Denmark (Damborg, Gaustad, *et al.*, 2011). Additionally a study of stray and hospitalised dog faeces in the Republic of Korea identified bla_{CMY-2} association with plasmids IncFIB and IncI1-I(gamma), amongst others (Tamang, Nam, *et al.*, 2012).

Further research is required to investigate the potential for transmission and co-carriage of AMR *E. coli* between dogs, their owners and the environment. Studies have demonstrated the potential for dissemination of ESBL-producing *E. coli* within a veterinary hospital environment (Timofte *et al.*, 2016), with the same ST being shared between the environmental and canine clinical samples, as well as between a veterinary hospital intensive care unit (ICU), hospitalised ICU companion animal patients, their owners and their home following patient discharge from hospital (Schmitt *et al.*, 2021). Additionally, antibiotic resistance profiles have been demonstrated to be the same in *E. coli* isolates from dogs and their owners (Naziri, Poormaleknia and Ghaedi Oliyaei, 2022) and ESBL and AmpC-producing *E. coli* of the same strain has been identified between human patients with urinary tract infections and pet dogs in the same household, suggesting within-household transmission does occur (Johnson *et al.*, 2016; Toombs-Ruane *et al.*, 2020).

Few studies have investigated the risks of transmission and co-carriage of AMR *E. coli* within a pet-owning household in relation to provision of a raw diet specifically. A study from the UK identified a common *E. coli* lineage (ST744) carried by a raw fed puppy and isolated from a human urinary tract infection within a local area (Mounsey *et al.*, 2022). A previous study from The Netherlands identified co-carriage of ESBL-producing *E. coli* between dogs and their owners in a small number of households, and observed that provision of RMD was a risk factor for ESBL-producing *E. coli* carriage in dogs (van den Bunt *et al.*, 2020). Carriage of AMR *E. coli* of STs which are known to be of clinical importance in human medicine has been identified in the present study to a greater degree in dogs fed RMD, associated with mobile transmissible genetic elements. Therefore, it stands to reason that dogs fed RMD could pose

an increased public health risk for transmission of AMR *E. coli*, however further research is required to investigate this risk.

Limitations

There were some limitations to this study. Firstly, there may have been an element of bias from the recruitment methods used and participant self-selection. Recruitment was largely via direct contact using email of dog owners who had previously taken part in related studies, and via social media. Therefore, this excluded dog owners without internet or social media access. Additionally, survey responses relied on owner responses which rely on honesty and could be subject to recall bias. With regards to limitations in the microbiological work, the media used (HECA) is utilised as the chromogenic nature allows easy recognition of *E. coli* colonies. However, some colonies could be missed if there was a slight deviation from the expected colour for any reason. Typical colonies were navy blue, however, there was some variation observed in this. This could lead to underestimation of *E. coli* presence at sample level. A set number of *E. coli* picks were taken from each agar plate. This method aims to obtain a representative sample by sampling multiple colonies at random; however, does mean that there could be an over- or underrepresentation of the level of AMR present by chance, depending on the colonies picked. Faecal sampling was undertaken at one time point only; bla_{ESBL} gene carriage has previously been demonstrated to be transient (van den Bunt *et al.*, 2020) and therefore may not have been present at the time of sampling in the present study, which may underestimate the prevalence of bla_{ESBL} gene carriage. Finally, the presence of the AMR-genes identified by WGS in this study was not always associated with phenotypic resistance; interpretation of the AMR genes must be undertaken with caution as their presence does not necessarily indicate that resistance will be demonstrated. Further research is also needed to determine the transmissibility of genes, and their phenotypic presentation, particularly those genes which were identified for the first time in canine samples.

4.6 Conclusions

This study has contributed to the growing body of evidence to suggest that provision of RMD to dogs is a potential public health concern. In the present study, dogs fed RMD were found to shed significantly greater proportions of AMR *E. coli* than dogs fed NRMD, as well as shedding bacteria which demonstrated resistance to critically important antibiotics. STs and ESBL genes were identified which are linked to those identified in livestock, as well as being

present in humans and associated with clinical disease in humans and animals. This constitutes a potential One Health concern, as well as a concern for animal welfare. Further research is required to investigate the risks of co-carriage and transmission of AMR *E. coli* with respect to dogs, their owners and their environment, nevertheless, provision of RMD as a pet food choice should be considered with caution and efforts should be made to continue to educate and engage with pet owners, pet food retailers, veterinary and medical professionals with regards to the potential AMR bacteria risks associated with RMD feeding.

Chapter 5: A study to explore the household carriage of extended-spectrum beta-lactamase-producing and third-generation cephalosporin-resistant *E. coli* in raw and non-raw fed dogs, their owners and their home environment- a longitudinal study

5.1 Introduction

Extended-spectrum beta-lactamases (ESBLs) and third generation cephalosporin resistance (3GCR) due to plasmid mediated AmpC beta-lactamases are important antimicrobial resistance (AMR) mechanisms in the *Enterobacterales*, including *E. coli*. ESBL-producing *E. coli* is increasingly prevalent within human and companion animal veterinary hospital settings and the community, and concerningly is often multidrug-resistant (MDR), with co-resistance to fluoroquinolones frequently observed (Livermore and Hawkey, 2005; Livermore, 2009; Cozma *et al.*, 2018; Bortolami *et al.*, 2019; Royden *et al.*, 2019; Bezabih *et al.*, 2021; Singleton *et al.*, 2021). Additionally, clinical infections with ESBL-producing *E. coli* are frequently associated with increased mortality rates (Livermore, 2009; Marchetti *et al.*, 2020).

Healthy and clinically unwell dogs have been identified as potential reservoirs for ESBL- and AmpC-producing *E. coli* globally (Tamang, Nam, *et al.*, 2012; Damborg *et al.*, 2015; Carvalho *et al.*, 2016; Boehmer *et al.*, 2018; Cormier *et al.*, 2019; Dupouy *et al.*, 2019; Karkaba *et al.*, 2019; Abreu-salinas *et al.*, 2020; Rodríguez-González *et al.*, 2020; Marchetti *et al.*, 2021), and feeding a raw meat based diet (RMD) has been identified as a risk factor for canine carriage of these bacteria, as well as for carriage of *E. coli* which is resistant to other classes of antibiotics such as the fluoroquinolones (Baede *et al.*, 2015; Schmidt *et al.*, 2015; Wedley *et al.*, 2017; Runesvärd *et al.*, 2020; Groat *et al.*, 2022; Mounsey *et al.*, 2022; Sealey *et al.*, 2022).

Dog owners share close and frequent contact with their pets within the household (Westgarth *et al.*, 2008) thus there is potential for transmission of AMR-*E. coli* isolates and genes between them (Dickson *et al.*, 2019). Transmission is likely to be facilitated by behaviours practiced within the home by household members, including allowing the pet to lick the owners' hands and face, and sharing of dinner plates and utensils (Dickson *et al.*,

2019), practices also discussed in chapter 2 of this thesis. Previous studies have identified that dogs and their owners frequently carry *E. coli* which demonstrate similar antimicrobial resistance patterns and expression of bla_{ESBL} genes (Carvalho *et al.*, 2016; Naziri, Poormaleknia and Ghaedi Oliyaei, 2022). Furthermore, in households where a member has a urinary tract infection caused by an ESBL-producing *E. coli*, whole genome sequencing (WGS) has identified carriage of *E. coli* of the same strain by other co-habiting household members, including pet dogs, demonstrating that within-home transmission is possible (Toombs-Ruane *et al.*, 2020). Additionally, contamination and persistence of ESBL-producing *E. coli* is possible within the environment where ESBL-producing *E. coli* shedding dogs are present. ESBL-producing *E. coli* has been isolated from surfaces in veterinary hospitals (Timofte *et al.*, 2016; Schmitt *et al.*, 2021), as well as from household surfaces in a home with a persistently colonised dog. Contaminated surfaces included not only dog food and water bowls, but also carpets and the kitchen sponge (Schmitt *et al.*, 2021), highlighting household items as a potential route of transmission between dogs and their owners.

There are limited studies which have investigated the longitudinal carriage of ESBL-producing and 3GCR-*E. coli* in dogs, particularly with respect to their diet, and currently there are no data from the UK pertaining to this. The findings of such studies are important, as dogs fed RMD have been demonstrated to shed AMR-*E. coli* to a greater degree than dogs fed conventional non-raw kibble-based diet (Non-raw meat diets, NRMD) (Runesvärd *et al.*, 2020; Groat *et al.*, 2022; thesis chapter 4), thus could potentially pose a higher risk for transmission of these bacteria within the home.

5.2 Aims

This exploratory study aimed to investigate the longitudinal co-carriage of AMR-*E. coli* by dogs, their owners and their household environment, with a particular focus on ESBL-producing and 3GCR-*E. coli*. Additionally, it aimed to investigate any difference in carriage amongst those dogs fed either RMD or NRMD.

5.3 Materials and methods

Recruitment and sampling

RMD and NRMD-feeding households were recruited via direct contact of dog owners who had participated in previous studies and who agreed to be contacted again regarding further research. RMD- feeding households were defined as those where all dogs in the household were fed raw meat components in their diet at least once per week. Households were recruited from within a two-hour radius of the University of Liverpool.

Canine faecal samples, human faecal swabs and three swabs from the environment, which were the dog food bowl, water bowl and floor immediately surrounding the food bowl, were collected approximately once a month between June-December 2022. Sample packs included a faecal sample collection pot for all dogs and a sterile dry cotton-tip swab for all participating humans within the household, a sterile dry cotton-tip swab for each dog bowl and a single sterile dry cotton-tip swab for the water bowl and floor per household and were posted to owners via Royal Mail. Questionnaires asking about dog and human lifestyle factors were included within the sample collection packs. Copies of the questionnaires are included in appendix 4.

Owners were requested to collect a single freshly evacuated stool sample from each dog within the household, and a swab of their own stool at one time point per sampling point. They were additionally instructed to swab the food bowl of each dog, as well as the water bowl and a 10cm^2 area of the floor surrounding the food bowl at a single time point after the dog had finished eating but before any cleaning was undertaken. Full sample collection instructions are included in appendix 4.

Completed sample packs and questionnaires were received by return first-class post, and arrived at the laboratory within 1-2 days of the owners posting them. Households were assigned a unique number, and participants were anonymised as 'person N' or 'dog N' within these. Results were anonymised; however, the unique household number allowed tracking of the participants across the study stages. Owners were requested to keep the person and dog numbers the same throughout the study. Following the initial study (T0), a follow up email was sent to owners to confirm that they would like to continue to participate in the longitudinal aspect of the study (T1 onwards). Households which had no AMR *E. coli* identified at T0 were not invited to participate further. Across the duration of the study, owners were contacted once weekly for a maximum of two times by email if sample packs had not been returned.

As this was an exploratory study, no sample size power calculation was undertaken.

Microbiological methods

Returned sample packs were stored in a refrigerated unit between 0-4^{o}C, and samples were processed within 24-72 hours of their receipt by the laboratory. A 1g sample of dog faeces, the human faecal swabs and the environmental swabs were incubated individually at 37^{o}C aerobically overnight in 4ml buffered peptone water (BPW). Following incubation, a 5µl loopful of the BPW broth was inoculated onto one chromogenic Harlequin *E. coli*/Coliform

Agar (HECA) (Neogen, UK) plate and one HECA plate with 1µg/ml cefoxatime (HECA+Cx), and incubated at 37°C for 18-20h. If present, four typical *E. coli* colonies (dark blue-violet colonies, 0.1mm-2mm diameter) were picked from the HECA plate, and two from the HECA+Cx plate, and subsequently plated onto nutrient agar (NA) (Neogen, UK). NA plates were incubated at 37°C for 18-20h.

E. coli isolates from plain HECA plates underwent antimicrobial susceptibility testing (AST) via the disc diffusion method. Antibiotic discs were chosen based on European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations (EUCAST, 2022). Isolates were inoculated into sterile saline to 0.5 McFarland using a 5µl loop, and the inoculated saline was spread onto Mueller-Hinton agar (Neogen, UK) using a sterile cotton-tip swab, then antibiotic discs were applied. Plates were then incubated aerobically at 37°C for 18-20 h. Antimicrobials tested were ampicillin 10 µg, amoxycillin-clavulanic acid 20 µg/10 µg, ciprofloxacin 5 µg, tigecycline 15 µg, trimethoprim-sulphamethoxazole 1.25 µg/23.75 µg, amikacin 30 µg and meropenem 10µg (MAST Group Ltd, Liverpool UK). A susceptible control strain of *E. coli* (ATCC 25922) was also tested.

Following incubation, zones of inhibition (ZOI) for each antibiotic disc were measured to the nearest millimetre. Breakpoints used for interpretation were as recommended by EUCAST (EUCAST, 2022) for all antibiotics other than amoxycillin-clavulanic acid, where the breakpoint used for interpretation was as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2020). Isolates were defined as AMR if they demonstrated phenotypic resistance to less than three classes of antibiotics. Multidrug resistance (MDR) was defined as demonstrated phenotypic resistance to three or more classes of antibiotics on AST (Magiorakos *et al.*, 2012).

The *E. coli* isolates from HECA+Cx plates initially underwent the extended-spectrum beta-lactamase (ESBL) double-disc test to determine whether they were ESBL-producing or not, using cefotaxime 5µg, cefotaxime 5µg +clavulanic acid 10µg, ceftazidime 10µg and ceftazidime 10µg +clavulanic acid 10µg discs (EUCAST ESBL detection set, MAST Group Ltd, Liverpool UK). Plates were incubated at 37°C for 18-20h. Isolates were deemed positive for ESBL-production if the ZOI surrounding the cephalosporin +clavulanic acid disc was a minimum of 5mm diameter larger than the ZOI for the corresponding cephalosporin disc alone for ≥1 antibiotic pairs; positive isolates were then continued to the full AST as described. Non-ESBL producing 3GCR isolates which did not demonstrate a typical positive result for ESBL production on the double disc test, but which demonstrated a pattern

suggestive of AmpC production whereby there was no, or minimal, ZOI present surrounding the clavulanic acid disc(s), were also continued to full AST

PCR methods

Isolates which were phenotypically identified as *E. coli* underwent PCR for the *uspA* gene to confirm their identity as *E. coli* (Anastasi *et al.*, 2010). Isolates confirmed as *E. coli* and which were ESBL-producing, as well as those which did not demonstrate the classic ESBL pattern on the double disc test but which were phenotypically 3GCR, underwent further PCR assay testing to determine the presence of ESBL and AmpC genes (bla_{CTX-M}, bla_{TEM}, bla_{SHV}, bla_{OXA}, bla_{SHV} and bla_{CITM}) which could be responsible for this resistance phenotype (Table 5.1).

Table 5.1: *Forward and reverse primer sequences, amplicon sizes and references used for E. coli and blaESBL gene identification in this study*.

Target gene	Forward primer	Reverse primer	Amplicon size (base pairs)	Reference
uspA	CCGATACGCTGCCAATCAGT	ACGCAGACCGTAGGCCAGAT	884	Anastasi *et al.*, 2010
bla_{CTX-M}	ATGTGCAGYACCAGTAARGTKATGGC	TGGGTRAARTARGTSACCAGAAYCAGCGG	593	Boyd *et al.*, 2004
bla_{TEM}	CATTTCCGTGTCGCCCTTATTC	CGTTCATCCATAGTTGCCTGAC	800	Dallenne *et al.*, 2010
bla_{SHV}	AGCCGCTTGAGCAAATTAAAC	ATCCCGCAGATAAATCACCAC	713	Dallenne *et al.*, 2010
bla_{OXA}	GGCACCAGATTCAACTTTCAAG	GACCCCAAGTTTCCTGTAAGTG	564	Dallenne *et al.*, 2010
bla_{CITM}	TGGCCAGAACTGACAGGCAAA	TTTCTCCTGAACGTGGCTGGC	462	Pérez-Pérez and Hanson, 2002

Questionnaire

Dog lifestyle factors from the questionnaires included diet, recent antibiotic treatment and recent veterinary hospitalisation. Owner lifestyle factors included recent hospital visits, antibiotic treatment and travel. For the initial study, questions regarding hospital visits and antibiotic therapy for both dog and owner were asked regarding the previous 3 months; however, for follow up questionnaires, owners were asked to detail any changes in these factors for themselves or their dog since the previous sampling time point. Descriptive analysis of categorical questionnaire response data (frequency, percentage) was undertaken. Based on the accompanying laboratory results, three outcomes were analysed, which were 'presence of ESBL- producing *E. coli*', 'presence of phenotypic 3GCR-*E. coli*' and 'presence of

phenotypic MDR-*E. coli*'. Comparisons were undertaken using the chi square test (Fisher's exact for groups of N<5), and statistical significance was set at p <0.05. Statistical analysis was performed using Microsoft Excel (Microsoft Corp. (2019)) and SPSS 27 (IBM Corp. (released 2020). IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp.).

Ethics statement

Ethical approval for this study was granted by the University of Liverpool Veterinary Ethics committee (approval number VREC1160).

5.4 Results

In total, 19 households participated in the initial (T0) study (N=8 RMD, N=9 NRMD, N=2 which fed both RMD and NRMD, where individual dogs within a household were fed different diets), providing samples for 36 dogs (N=20 RMD, N=16 NRMD), 27 people (N=12 RMD households, N=12 NRMD households, N=3 from households which fed both), 36 food bowls (N=20 RMD, N=16 NRMD), 19 water bowls (N=8 RMD, N=9 NRMD, N=2 which fed both RMD and NRMD) and 19 floor swabs (N as per water bowls). Of these households, six (N=5 NRMD, N=1 both RMD and NRMD) were not invited to continue after the initial study as no AMR-*E. coli* was identified in any of their samples. Three (N=3 RMD) households dropped out and did not respond to follow up invitations, so were lost to follow up. Thus, ten households continued to the longitudinal study (T1 onwards). Nine households (9/19, 47.4%) continued to the end of the longitudinal study (N=5 RMD, N=4 NRMD) as one further household (N=1 both RMD and NRMD) was lost to follow up after T1 (Table 5.2).

Table 5.2: *Study participant households, the diet fed to their dog, number of samples provided and their participation across the study from T0 (initial study) to T4 (final sampling period).*

Household	Dog diet[a]	Sample type (N)					Participation				
		Dog	Human	Food bowl	Water bowl	Floor	Initial (T0)	T1	T2	T3	T4
11	RMD	1	1	1	1	1	Y	Y	Y	Y	Y
15	RMD	2	2	2	1	1	Y	Y	Y	Y	Y
19	RMD	2	1	2	1	1	Y	Y	Y	Y	Y
21	RMD	2	2	2	1	1	Y	Y	Y	Y	Y
13	RMD	3	1	3	1	1	Y	Y	Y	Y	Y
9	NRMD	3	2	3	1	1	Y	Y	N	Y	Y
6	NRMD	1	1	1	1	1	Y	Y	Y	Y	Y
20	NRMD	1	2	1	1	1	Y	Y	Y	Y	Y

7	NRMD	1	1	1	1	1	Y	Y	Y	Y	Y
4	BOTH*	2	2	2	1	1	Y	Y^	N	N	N
3	RMD	2	2	2	1	1	Y^	N	N	N	N
18	RMD	4	1	4	1	1	Y^	N	N	N	N
22	RMD	2	2	2	1	1	Y^	N	N	N	N
1	NRMD	1	1	1	1	1	Y^^				
10	NRMD	2	2	2	1	1	Y^^				
12	BOTH**	3	1	3	1	1	Y^^				
16	NRMD	1	1	1	1	1	Y^^				
24	NRMD	1	1	1	1	1	Y^^				
17	NRMD	2	1	2	1	1	Y^^				
Totals		**36**	**27**	**36**	**19**	**19**					

*1 RMD-fed dog, 1 NRMD-fed dog, **1 RMD-fed dog, 2 NRMD-fed dogs; ^Lost to follow up; ^^No antimicrobial resistance identified so not followed; [a]RMD: raw meat diet, NRMD: non-raw diet

E. coli growth

E. coli was identified in 54.3% (75/138) of samples at T0, and was present in at least one sample from all households which participated. Within T0, *E. coli* was isolated from 94.4% (34/36) of dogs (N=20 RMD, N=14 NRMD), 85.2% (23/27) of people (N=10 RMD, N=10 NRMD, N=3 both), 22.2% (8/36) of food bowls (N=6 RMD, N=2 NRMD), 31.6% (6/19) of water bowls (N=4 RMD, N=1 NRMD, N=1 both) and 10.5% (2/19) of floor swabs (N=1 RMD, N=1 both). For consecutive follow ups (T1-T4), *E. coli* was isolated frequently from dog and human faecal samples; however, was infrequently isolated from food bowls, water bowls and floor swabs (Appendix table A4.1).

AMR E. coli

The presence of AMR-*E. coli* within households feeding dogs on either RMD or NRMD across the duration of the study is demonstrated in figure 5.1, alongside the associated resistance patterns identified at the sample level. AMR-*E. coli* was identified in samples from 68.4% (13/19) of households at T0 of which 8/19 fed RMD, 4/19 fed NRMD and 1/19 fed both. A greater diversity in resistance phenotypes was demonstrated in *E. coli* isolates from samples obtained from RMD-fed households. Within the RMD-households at T0, AMR-*E. coli* was isolated from 10 dogs and 7 people, and in the NRMD-household they were isolated from 4 dogs and 3 people. AMR-*E. coli* was isolated intermittently across the study from food and water bowls (more frequently) in both RMD and NRMD-fed households. MDR-*E. coli* was isolated from 26.3% (5/19) households at T0, of which 4 fed RMD and 1 fed NRMD.

Five (26.3%) households (4 RMD, 1 NRMD) demonstrated the presence of AMR-*E. coli* at every time point across the duration of the study, although it was not always isolated from the same participant within the household at each time point, and was identified in dog, human and environmental samples in different households. Three dogs from three separate households (all RMD) demonstrated the presence of AMR-*E. coli* across consecutive study time points. *E. coli* isolated from one dog (dog 1, household 11, RMD) demonstrated the same resistance patterns at consecutive time points, whereas *E. coli* isolated from other dogs had different resistance patterns at each time point (e.g. dog 1, household 21, RMD). Interestingly, AMR-*E. coli* was isolated from all three dogs in household 13 at follow up T3, and all demonstrated the same resistance pattern. Across the study timepoints, MDR-*E. coli* was isolated from a total of 17 samples (N=14 RMD, N=3 NRMD) and the most frequently observed resistance pattern was to ampicillin, amoxycillin-clavulanic acid and TMS, all of which were isolated from samples from dogs or people from households which fed RMD.

For the household where both RMD and NRMD were fed, at T0 only the RMD-fed dog was found to carry AMR-*E. coli*, alongside both owners, and the resistance pattern was the same for the dog and one of the owners. However, at T1, both dogs carried AMR-*E. coli*, but demonstrated different resistance phenotypes. Unfortunately, this household was lost to subsequent follow up.

AMR E. coli proportions

The proportion of samples from which *E. coli* was isolated which demonstrated resistance to each class of antimicrobial tested at each stage of the study are shown in table 5.3. Across the study, resistance to ampicillin was the most frequently observed resistance phenotype within samples from households which fed RMD, and resistance to amoxycillin-clavulanic acid was intermittently observed. However, at least one sample from an NRMD household demonstrated the presence of *E. coli* which was resistant to amoxycillin-clavulanic acid at each time point, and at T0 it was the most frequently demonstrated resistance phenotype in *E. coli* within samples from NRMD-households. Resistance to ciprofloxacin was observed in at least one sample from RMD-fed households at each study time point; however, was only identified in one human sample from a NRMD-fed household at T0, and not identified again in any samples in later study stages.

Resistance to amikacin was only identified in *E. coli* from three samples (one dog, two people) from one household (RMD) at T0 and not identified in any samples thereafter. No resistance to meropenem was observed at any time point.

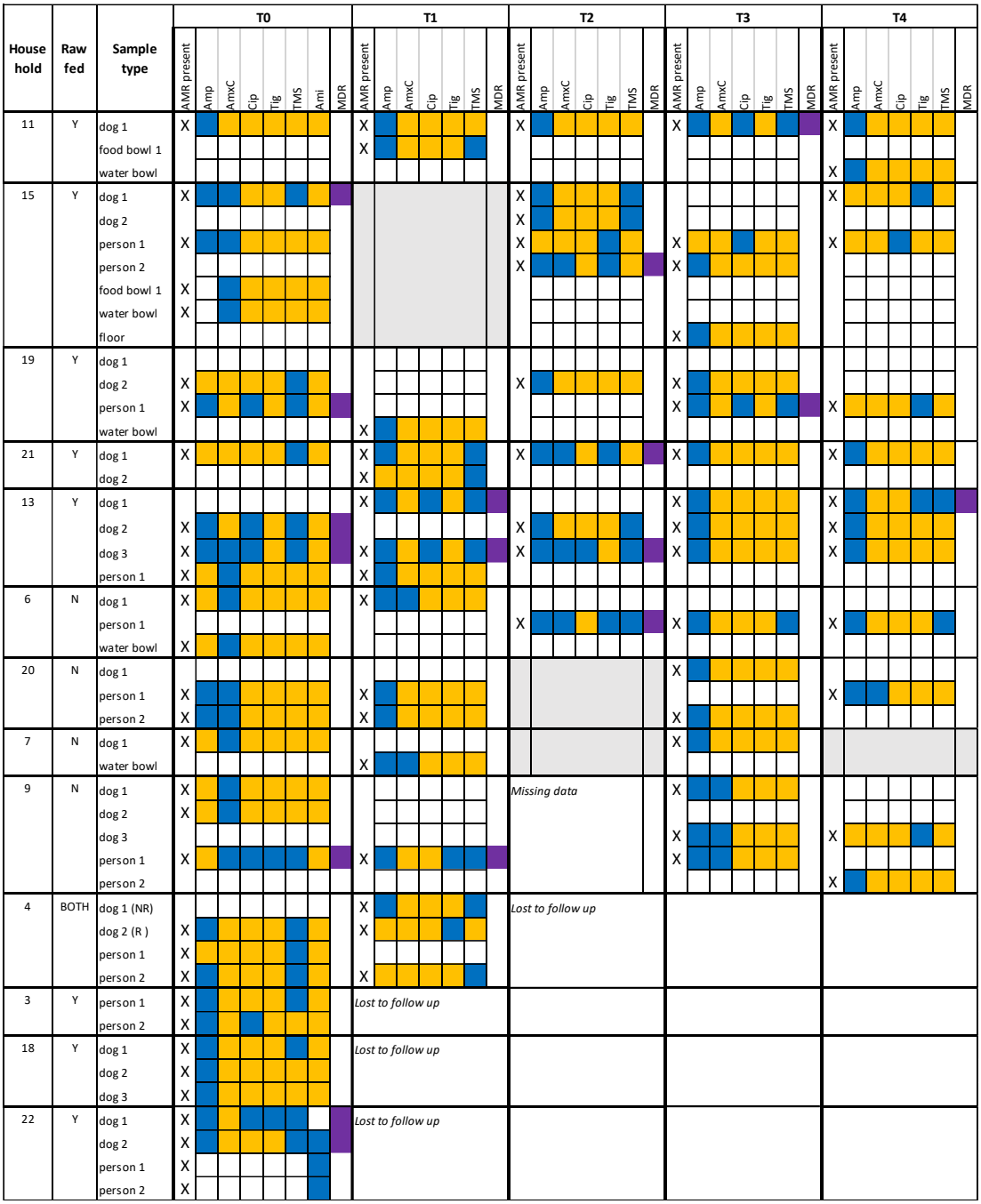


Figure 5.1: *Antimicrobial resistance phenotypes of AMRE. coli isolates which demonstrated AMR over the duration of the study, at sample level. A cross denotes AMR-E. coli was present, then the coloured boxes following indicate the phenotypic AMR pattern observed. A blue denotes resistance, whereas a yellow box denotes susceptibility. A purple box denotes the presence of MDR. A grey box denotes no AMR identified at that study stage. Amp: ampicillin;*

AmxC: amoxycillin-clavulanic acid; Cip: ciprofloxacin; Tig: tigecycline; TMS: trimethoprim-sulphamethoxazole; Ami: amikacin; MDR: multidrug resistance

Table 5.3: *Percentage (%) and number (N) of samples with AMR-E. coli present which demonstrated resistance to each antimicrobial at each stage of the study from RMD households, NRMD households and households which fed both. Dogs from households which fed 'both' were placed into either the RMD or NRMD category depending on the diet stated by their owner; however, owners and environmental swabs from those households were classed as within the 'both' category.*

Antimicrobial	**T0 (N=138 samples)**			**T1 (N=72 samples)**			**T2 (N=53 samples)**		**T3 (N=63 samples)**		**T3 (N=63 samples)**	
	RMD (N=65) % (N)	*NRMD* (N=60) % (N)	*Both* (N=13) % (N)	*RMD* (N=38) % (N)	*NRMD* (N=27) % (N)	*Both* (N=7) % (N)	*RMD* (N=37) % (N)	*NRMD* (N=16) % (N)	*RMD* (N=37) % (N)	*NRMD* (N=26) % (N)	*RMD* (N=37) % (N)	*NRMD* (N=26) % (N)
Amp	21.5 (14)	3.3 (2)	7.7 (1)	18.4 (7)	22.2 (6)	0.0 (0)	21.6 (8)	6.3 (1)	24.3 (9)	26.9 (7)	16.2 (6)	11.5 (3)
Aug	9.2 (6)	13.3 (8)	0.0 (0)	0.0 (0)	7.4 (2)	0.0 (0)	8.1 (3)	6.3 (1)	0.0 (0)	11.5 (3)	0.0 (0)	3.8 (1)
Cip	7.7 (5)	1.7 (1)	0.0 (0)	5.3 (2)	0.0 (0)	0.0 (0)	2.7 (1)	0.0 (0)	8.1 (3)	0.0 (0)	2.7 (1)	0.0 (0)
Tig	1.5 (1)	1.7 (1)	0.0 (0)	2.6 (1)	3.7 (1)	0.0 (0)	8.1 (3)	6.3 (1)	0.0 (0)	0.0 (0)	8.1 (3)	3.8 (1)
TMS	16.9 (11)	1.7 (1)	15.4 (2)	13.2 (5)	7.4 (2)	14.3 (1)	10.8 (4)	6.3 (1)	5.4 (2)	3.8 (1)	2.7 (1)	3.8 (1)
Ami	4.6 (3)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)

3GCR E. coli within households

The presence of 3GCR *E. coli* (including ESBL-producing and non-ESBL producing *E. coli*) and ESBL-producing *E. coli* within households feeding either RMD or NRMD across the duration of the study is demonstrated in figure 5.2, alongside the associated resistance patterns identified at sample level. At T0, 36.8% (7/19, all RMD-feeding) households had 3GCR-*E. coli* present and 26.3% (5/19, all RMD-feeding) households demonstrated the presence of ESBL-producing *E. coli* within at least one sample type. Of these households, two were lost to follow up after T0, one household did not have any 3GCR or ESBL-producing *E. coli* isolated after T1, and two households did not have any 3GCR or ESBL-producing *E. coli* isolated after T2. ESBL-producing *E. coli* was isolated from two households (households 13 and 19) at every time point, although not always within the same participant. Person 1 in household 19 was found to carry ESBL-producing *E. coli* at each study time point and with consistently the same resistance pattern. On investigation of their questionnaire responses, this person was found to have visited either their GP or attended hospital as an outpatient at every sampling timepoint other than T0.

One household which fed both RMD and NRMD had ESBL-producing *E. coli* present at T2, isolated from the RMD-fed dog only. However, this household was subsequently lost to follow up. Interestingly, only one NRMD-household demonstrated the presence of 3GCR-*E. coli*, although not ESBL-producing, at T3 only, where *E. coli* isolated from one person and two dogs all demonstrated the same resistance pattern at sample level. Investigation of the questionnaire responses from this household revealed that one dog at this study stage had received cephalexin for management of pyoderma.

Resistance patterns varied across the study stages, and often appeared intermittently. Only one resistance phenotype was consistently observed across the study stages, which was resistance to ampicillin, ciprofloxacin, TMS, cefotaxime and ceftazidime.

No 3GCR *E. coli* was isolated from any environmental swabs (food bowl, water bowl or floor swab) from any household at any study stage.

Proportions of samples and dogs with 3GCR E. coli present

Across the study, 34 samples (N=28 dogs (N=26 RMD, N=2 NRMD) and N=6 people (N=5 RMD, N=1 NRMD)) demonstrated the presence of 3GCR-*E. coli*. Of these 85.3% (29/34) were MDR. Table 5.4 shows the proportion of samples at each study stage which demonstrated the

presence 3GCR, ESBL-producing, MDR-3GCR and fluoroquinolone-resistant 3GCR *E. coli*. Many of these samples were from dogs, with only two from owners (1 from a RMD household consistently every study stage, 1 from a NRMD household at T3 only).

The proportion of dogs from which phenotypic 3GCR-*E. coli* was isolated again ranged from 12% to 39%, and at all study stages except T3, these dogs were RMD-fed only; however, 12.5% of the dogs which carried 3GCR-*E. coli* at T3 were fed NRMD. The proportion of dogs within the study from which ESBL-producing *E. coli* was isolated ranged across the study stages from approximately 12% to 39%, all of which were RMD-fed. The proportion of MDR 3GCR *E. coli* isolated from dogs ranged from 12% to 31%. *E. coli* which was concurrently 3GCR and fluoroquinolone resistant was isolated from 6% to 22% of dogs across the study, all of which were RMD-fed.

ESBL genes and associated resistance phenotypes

The most frequently identified bla_{ESBL} gene was $bla_{CTX\text{-}M}$ (85.7%; 24/28 of samples harbouring ESBL-producing *E. coli*) (Figure 5.2). The presence of this gene was frequently associated with concurrent fluoroquinolone resistance, and isolates were commonly MDR. bla_{TEM} was identified in 42.9% (12/28) of samples, and bla_{SHV} from 17.9% (5/28) of samples; however, further sequencing is required to determine whether the bla_{TEM} and bla_{SHV} genes were ESBL variants, particularly in samples where $bla_{CTX\text{-}M}$ was also present. Whereas the $bla_{CTX\text{-}M}$ gene was identified in one dog owner (household 19) consistently at every study stage as well as being identified frequently in samples from RMD-fed dogs, the bla_{TEM} gene was present in ESBL-producing *E. coli* isolated from RMD-fed dogs only. bla_{SHV} was present in one dog owner (household 19) and T0 and T1 only and was isolated sporadically from RMD-fed dog samples (one dog at T0, T1 and T3 respectively). No bla_{OXA} was identified in any *E. coli* from samples at any study stage.

The bla_{CITM} gene was identified in 23.5% (8/34) of samples harbouring 3GCR *E. coli*. Two of these samples had concurrent *bla* genes present; one had $bla_{CTX\text{-}M}$ and the other had bla_{TEM}, and both were samples from RMD-fed dogs. 3GCR-*E. coli* isolated from the six remaining samples was associated with the presence of the bla_{CITM} gene only, therefore the resistance phenotype was likely to be a result of the AmpC mechanism. In all samples where the bla_{CITM} gene was present (3 from separate RMD households, 3 from the same NRMD household), concurrent 3GCR and phenotypic ampicillin and amoxycillin-clavulanic acid resistance was demonstrated.

Although shedding of ESBL-producing *E. coli* was consistent across a number of study stages for some household members (household 13 dog 1, household 13 dog 2 and household 19 person 1), for many study participants the shedding was intermittent and the patterns of ESBL genes was dynamic across study stages. Additionally, the ESBL gene patterns and associated AMR phenotypes were similar but not the same across household members or study stage in most cases. Exceptions to this were in household 19 where *E. coli* harboured by the owner and dog both had $bla_{\text{CTX-M}}$, and both demonstrated a very similar resistance phenotype at T3 and T4. Additionally, in household 9, *E. coli* isolated from both dogs and the owner was found to carry bla_{CITM} and all demonstrated the same resistance phenotype at T3. Isolates which were 3GCR and demonstrated a unique resistance phenotype have been submitted for whole genome sequencing. Thus, further detail surrounding *E. coli* co-carriage and similarities within the household and across study stages, such as *E. coli* sequence types and more detail with regards to the resistance genes and plasmids present, should be clearer once these data are available.

Table 5.4: *Percentage (%) and number (N) of samples containing ESBL-producing, 3GCR (including ESBL-producing and non-ESBL-producing E. coli), MDR-3GCR or FQR-3GCR E. coli from dogs and humans across this study*

Sample	Diet type	T0 (%, N)				T1 (%, N)				T2 (%, N)				T3 (%, N)				T4 (%, N)			
		N dogs=36, N humans=27				*N dogs=18, N humans=15*				*N dogs=13, N humans=11*				*N dogs=16, N humans=13*				*N dogs=16, N humans=13*			
		ESBL*	3GCR*	MDR*	FQ*	ESBL	3GCR	MDR	FQ	ESBL	3GCR	MDR	FQ	ESBL	3GCR	MDR	FQ	ESBL	3GCR	MDR	FQ
Dog	**Total**	22.2 (8)	27.8 (10)	27.8 (10)	16.7 (6)	38.9 (7)	38.9 (7)	27.8 (5)	22.2 (4)	15.4 (2)	23.1 (3)	15.4 (2)	0.0 (0)	25.0 (4)	37.5 (6)	31.3 (5)	12.5 (2)	12.5 (2)	12.5 (2)	12.5 (2)	6.3 (1)
	RMD	22.2 (8)	27.8 (10)	27.8 (10)	16.7 (6)	38.9 (7)	38.9 (7)	27.8 (5)	22.2 (4)	15.4 (2)	23.1 (3)	15.4 (2)	0.0 (0)	25.0 (4)	25.0 (4)	18.8 (3)	12.5 (2)	12.5 (2)	12.5 (2)	12.5 (2)	6.3 (1)
	NRMD	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	12.5 (2)	12.5 (2)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Human	**Total**	3.7 (1)	3.7 (1)	3.7 (1)	3.7 (1)	6.7 (1)	6.7 (1)	6.7 (1)	6.7 (1)	9.1 (1)	9.1 (1)	9.1 (1)	9.1 (1)	7.7 (1)	15.4 (2)	15.4 (2)	7.7 (1)	7.7 (1)	7.7 (1)	7.7 (1)	7.7 (1)
	RMD	3.7 (1)	3.7 (1)	3.7 (1)	3.7 (1)	6.7 (1)	6.7 (1)	6.7 (1)	6.7 (1)	9.1 (1)	9.1 (1)	9.1 (1)	9.1 (1)	7.7 (1)	7.7 (1)	7.7 (1)	7.7 (1)	7.7 (1)	7.7 (1)	7.7 (1)	7.7 (1)
	NRMD	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	7.7 (1)	7.7 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)

*ESBL: Extended-spectrum beta-lactamase producing; 3GCR: Third-generation cephalosporin-producing; MDR: Multidrug-resistant; FQR: Fluoroquinolone-resistant

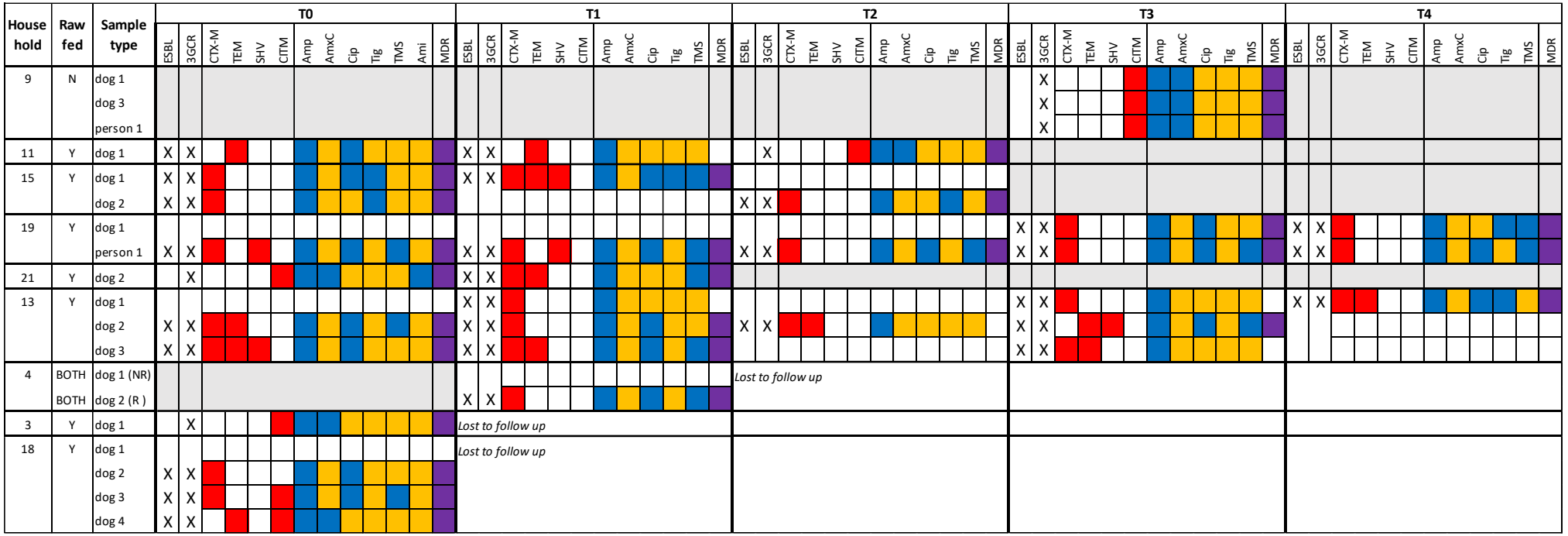


Figure 5.2: *ESBL-producing and 3GCR-E. coli across the study at sample level, alongside phenotypic AMR pattern and associated bla genes identified by PCR from dogs and humans in this study which demonstrated ESBL-producing and 3GCR E. coli phenotypes. A cross denotes presence of ESBL-producing or 3GCR phenotype. A red box denotes presence or absence of bla genes. A blue box denotes antimicrobial resistance, whereas a yellow box denotes susceptibility. A purple box denotes MDR. A grey box indicates no ESBL or 3GCR-E. coli identified within study stage. Amp: ampicillin; AmxC: amoxycillin-clavulanic acid; Cip: ciprofloxacin; Tig: tigecycline; TMS: trimethoprim-sulphamethoxazole; Ami: amikacin; MDR: multidrug resistance*

Questionnaire data

Questionnaire data were analysed to determine the dog and owner risk factors across the study which had an association with the overall prevalence of faecal ESBL-producing, 3GCR and MDR-*E. coli* in dogs. Due to the low numbers of human participants with positive results, this analysis was not conducted for the owners. Overall prevalence of ESBL-producing *E. coli* in dogs was 24.2% (23/99), 3GCR-*E. coli* was 28.3% (28/99) and MDR-*E. coli* was 25.3% (25/99). Factors associated with ESBL-producing *E. coli*, 3GCR-*E. coli* and MDR-*E. coli* by dogs are demonstrated in tables 5.5, 5.6 and 5.7, respectively. Risk factors associated with the investigated resistance categories were provision of RMD to the dog (p <0.001, all categories) and if the owner visited a (human) hospital, either as a patient or staff (p <0.001 for ESBL-producing *E. coli*, p <0.01 for 3GCR- and MDR-*E. coli*).

Table 5.5: *Dog and owner factors associated with faecal carriage of ESBL-producing E. coli by dogs (N=99 samples) in this study.*

Variable	**Category**	**N (total samples) 99**	**% of total**	**ESBL-*E. coli* present %, N**		**p value (chi sq)**
				Yes **24.2 (23)**	***No*** **75.8 (76)**	
Dog Factors						
Fed raw	Yes	61	61.6	37.7 (23)	62.3 (38)	<0.001
	No	38	38.4	0.0 (0)	100.0 (38)	
Antibiotics received	Yes	15	15.2	13.3 (2)	86.7 (13)	0.51*
	No	84	84.8	25.0 (21)	75.0 (63)	
Hospitalised	Yes	6	6.1	16.7 (1)	83.5 (5)	1.00*
	No	93	93.9	23.7 (22)	76.3 (71)	
Owner Factors						
Antibiotics received	Yes	5	5.1	60.0 (3)	40.0 (2)	0.08*
	No	94	94.9	21.3 (20)	78.7 (74)	
Visited hospital	Yes	34	35.4	44.1 (15)	55.9 (19)	<0.001
	No	62	64.6	12.9 (8)	87.1 (54)	
	Unknown	3	3.0			
Travelled abroad	Yes	7	7.1	28.6 (2)	71.4 (5)	0.52*
	No	92	92.9	22.8 (21)	77.2 (71)	

*denotes Fishers Exact

Table 5.6: *Dog and owner factors associated with faecal carriage of 3GCR-E. coli by dogs (N=99 samples) in this study.*

Variable	Category	N (total samples) 99	% of total	3GCR-*E. coli* present %, N		p value (chi sq)
				Yes 28.3 (28)	*No* 71.8 (71)	
Dog Factors						
Fed raw	Yes	61	61.6	42.6 (26)	57.4 (35)	<0.001*
	No	38	38.4	5.3 (2)	94.7 (36)	
Antibiotics received	Yes	15	15.2	20.0 (3)	80.0 (12)	0.55*
	No	84	84.8	29.8 (25)	70.3 (59)	
Hospitalised	Yes	6	6.1	16.7 (1)	83.3 (6)	0.62*
	No	93	93.9	29.0 (27)	71.0 (66)	
Owner Factors						
Antibiotics received	Yes	5	5.1	60.0 (3)	40.0 (2)	0.14*
	No	94	94.9	26.6 (25)	73.4 (69)	
Visited hospital	Yes	34	35.4	47.1 (16)	52.9 (18)	<0.01
	No	62	64.6	19.4 (12)	80.6 (50)	
	Unknown	3	3.0			
Travelled abroad	Yes	7	7.1	28.6 (2)	71.4 (5)	1.00*
	No	92	92.9	28.3 (26)	71.7 (66)	

*denotes Fishers Exact

Table 5.7*: Dog and owner factors associated with faecal carriage of MDR-E. coli by dogs (N=99 samples) in this study.*

Variable	Category	N (total samples) 99	% of total	MDR-*E. coli* present %, N		p value (chi sq)
				Yes 25.3 (25)	*No* 74.7 (74)	
Dog Factors						
Fed raw	Yes	61	61.6	37.7 (23)	62.3 (38)	<0.001
	No	38	38.4	5.3 (2)	94.7 (36)	
Antibiotics received	Yes	15	15.2	20.0 (3)	80.0 (12)	0.75*
	No	84	84.8	26.2 (22)	73.8 (62)	
Hospitalised	Yes	6	6.1	16.7 (1)	83.3 (5)	1.00*
	No	93	93.9	25.8 (24)	74.2 (69)	
Owner Factors						
Antibiotics received	Yes	5	5.1	40.0 (2)	60.0 (3)	0.60*
	No	94	94.9	24.5 (23)	75.5 (71)	
Visited hospital	Yes	34	35.4	41.2 (14)	58.8 (22)	0.01

	No	62	64.6	17.7 (11)	82.3 (51)	
	Unknown	3	3.0			
Travelled abroad	Yes	7	7.1	28.6 (2)	71.4 (5)	1.00*
	No	92	92.9	25.0 (23)	75.0 (69)	

*denotes Fishers Exact

5.5 Discussion

This study has provided further evidence to suggest that provision of RMD is associated with faecal carriage of AMR-*E. coli* by dogs in the UK. Furthermore, dogs may shed AMR- *E. coli* over a prolonged period, and dogs fed RMD shed this more frequently than those fed NRMD. Additionally, this study has indicated that dogs fed RMD shed *E. coli* with important resistance mechanisms, whether consistently or intermittently, and the *E. coli* is often co-resistant to other important classes of antibiotics such as fluoroquinolones, and MDR.

A similar pattern in ESBL-producing *E. coli* shedding has been observed previously. A study of the longitudinal shedding of ESBL-producing and AmpC-producing *E. coli* in dogs in the Netherlands also identified continuous shedding of these bacteria in some dogs, but mainly it was intermittent and highly dynamic, with frequent changes in the ESBL profile. Provision of RMD was also identified as a risk factor for ESBL- and AmpC-producing *E. coli* shedding (Baede *et al.*, 2015). These findings potentially suggest that while dogs fed RMD are at higher risk for shedding ESBL-producing *E. coli* than those fed NRMD, these bacteria may not persist for an extended length of time in the gut. Additionally, the varying ESBL gene patterns and AMR phenotypes demonstrated for most dogs could indicate repeated exposure.

Human participants also shed AMR-*E. coli* over time in the present study, and although numbers were small, this was observed to a greater degree in people from RMD-fed households than NRMD. Interestingly, the study did not identify a high degree of ESBL-producing or 3GCR-*E. coli* isolated from people. One person from an RMD household consistently shed ESBL-producing *E. coli* with the same resistance phenotype over time, and was found to have visited either their GP or attended hospital as an outpatient at every sampling timepoint other than T0. Another person from a NRMD household was found to shed 3GCR-*E. coli* at one time point only (as did two of their dogs). At this time point, one of the dogs in the household was reported to have received cephalexin for pyoderma. Previous

studies have demonstrated that treatment with cephalexin may select for 3GCR-*E. coli* harbouring the bla_{CMY-2} gene (Damborg, Gaustad, *et al.*, 2011), and that this effect can occur rapidly following treatment (Schmidt *et al.*, 2018). In the present study, the *E. coli* isolates which demonstrated 3GCR, but did not have a definitive ESBL phenotype, harboured the bla_{CITM} gene, which has been shown previously to correspond with bla_{CMY-2} on gene amplicon sequencing (Tuerena *et al.*, 2016).

The low prevalence of ESBL-producing *E. coli* in humans was surprising; however, this may have been a result of the sampling methodology. Whereas owners were asked to provide a faecal sample for their dogs, they provided a swab for themselves. The swabs had varying amounts of sample on them and so a low prevalence of ESBL-producing *E. coli* may be related to low sample volume and thus reduced detection. The use of rectal swabs for detection of AMR *Enterobacteriaceae* has been demonstrated previously to be inferior to collection of a stool sample due to variation in faecal sample quantity (D'agata *et al.*, 2002) or if the concentration of AMR organisms in the stool is very low (Lautenbach *et al.*, 2005). Additionally, in the present study owners were requested to take a swab of the stool itself, rather than sampling per rectum, therefore faecal density may be lower again. It was, however, deemed more appropriate to use swabs for sample collection in this study to encourage participant compliance. The global pooled prevalence of human intestinal ESBL-producing *E. coli* carriage between 2003-2018 has been estimated to be 16.5%, with a lower prevalence of 6.0% in Europe, and an 8-fold increase in global prevalence over that time period (Bezabih *et al.*, 2021). In the UK, the prevalence of 3GCR-*E. coli* carriage in healthy humans has been estimated to be 3.1% (Leonard *et al.*, 2018). High rates of human ESBL-producing *E. coli* carriage have been identified in clinical and long-term care settings in the UK (Livermore and Hawkey, 2005; Brodrick *et al.*, 2017), and a cross-sectional study of human faecal samples routinely submitted from the community and hospital inpatients identified a prevalence of ESBL-producing *E. coli* of 11% (Day *et al.*, 2019). However, there are limited longitudinal studies of healthy human carriage of ESBL-producing *E. coli* in the UK for comparison.

Whilst no ESBL-producing *E. coli* was isolated from the environmental swabs, both fully susceptible and non-ESBL producing AMR- *E. coli* was isolated from food bowls, water bowls, and in one sampling time point, from the floor, in both RMD and NRMD-households (albeit

less frequently and to a lesser degree in NRMD households). This indicates that contamination of the environment was present, albeit to a lesser extent than identified in the canine faecal carriage within households. Although dog owners were instructed to obtain environmental samples prior to cleaning up after the dog, cleaning of the food bowls, water bowls and floor prior to sampling cannot be excluded and may explain the low detection rate in the present study. Additionally, taking part in the present study may have led to dog owners altering their cleaning behaviours across the duration of their participation. Interestingly however, a previous study of veterinary hospital environmental contamination isolated AMR-*E. coli* from rectal and buccal swabs of veterinary patients and did not find it in the environment. AMR-*Enterobacter cloacae* was, however, found to be the most prevalent environmental contaminant, as well as isolating it from the rectal swab of three dogs and buccal swab from one dog (Haenni *et al.*, 2022), indicating that other *Enterobacterales* may be of importance in the environment.

Furthermore, while this study focussed on AMR, it did not investigate the presence of important virulent non-AMR *E. coli* variants within samples, such as Shiga-toxin producing *E. coli* (STEC). An outbreak of STEC O157:H7 in the UK was linked to raw tripe provision to dogs (Kaindama *et al.*, 2020), and further studies have isolated STEC from RMD for pets in Switzerland and the USA (Nemser *et al.*, 2014; Treier *et al.*, 2021). This further highlights the importance of good food and water bowl hygiene and regular cleaning to reduce the risk of transmission from these items.

The most prevalent bla_{ESBL} gene group in the present study was $bla_{\text{CTX-M}}$. $bla_{\text{CTX-M}}$ genes are globally disseminated, most frequently identified bla_{ESBL} genes in *E. coli,* isolated from humans and animals (Bevan, Jones and Hawkey, 2017), with $bla_{\text{CTX-M-15}}$ predominating in humans in the UK (Livermore and Hawkey, 2005; Day *et al.*, 2019; Ludden *et al.*, 2019). However, recent studies have identified $bla_{\text{CTX-M-15}}$ as increasingly isolated from canine *E. coli* in the UK (Timofte *et al.*, 2016; Singleton, Pongchaikul, *et al.*, 2021; Sealey *et al.*, 2022) alongside frequent concurrent fluoroquinolone resistance. This finding was also observed in chapter 4 of this thesis. In-depth analysis using next generation sequencing will enable further identification of the $bla_{\text{CTX-M}}$ genes present in the present study to determine the presence of $bla_{\text{CTX-M-15}}$, as well as any co-carriage of genes conferring resistance to other antibiotic classes. It will also allow sequence typing of the *E. coli* isolates to determine any

relationships or co-carriage within households, and whether persistence is occurring in households where ESBL-producing *E. coli* is detected in consecutive study time points. Additionally, determination of the mobile genetic elements present (plasmids) would provide information regarding transmissibility and dissemination of plasmid-mediated resistance genes.

Analysis of accompanying questionnaire data indicated two important household factors that were significantly associated with carriage of ESBL-producing, 3GCR- and MDR-*E. coli* by dogs in the households over the study duration, namely provision of RMD to dogs, and owners visiting a hospital. Provision of RMD has been indicated as a risk factor for AMR-*E. coli* carriage by dogs in previous mainly cross-sectional studies (Schmidt *et al.*, 2015; Wedley *et al.*, 2017; Runesvärd *et al.*, 2020; Groat *et al.*, 2022; Mounsey *et al.*, 2022; Sealey *et al.*, 2022), however, there are minimal studies which have investigated the longitudinal effects of feeding this diet. Whilst cross-sectional studies are useful to estimate the prevalence, they may be limited due to their only sampling at single time points. As indicated by the findings of the present study, and that of previous studies (Baede *et al.*, 2015; van den Bunt *et al.*, 2020), ESBL-producing *E. coli* shedding by dogs is likely to be dynamic and transient, thus single time-point sampling may under or overestimate the true prevalence in the population. A more recent longitudinal study from The Netherlands indicated the prevalence of ESBL-producing *E. coli* in dogs to be 10.6%, which interestingly is much less than that identified in the present study of 24.2%, and also identified provision of RMD to be a risk factor for ESBL-producing *E. coli* carriage (van den Bunt *et al.*, 2020). Moreover, this study also identified that the participant dogs, and the ESBL-genes identified, were not the same at each study stage, which further supports the findings of the present study.

It is interesting that hospital visits for owners (either as a patient or employee) were associated with carriage of 3GCR, ESBL-producing and MDR-*E. coli* carriage in dogs. The prevalence of ESBL-producing *E. coli* has been identified as higher in healthcare settings than the community (Bezabih *et al.*, 2022), and thought to be driven, at least in the UK, by $bla_{\text{CTX-M-15}}$ (Livermore and Hawkey, 2005). Studies have demonstrated co-carriage and potential bidirectional transmission of AMR-*E. coli* between companion animals and humans in the same household (Carvalho *et al.*, 2016; Grönthal *et al.*, 2018; Toombs-Ruane *et al.*, 2020). Additionally, dogs and young children in the same household have been found to both carry

either ESBL- or AmpC-producing *E. coli*, and were reported to have shared food and utensils (Ljungquist *et al.*, 2016), further highlighting the importance of good food hygiene within the household, especially for at-risk groups such as infants. No children under the age of 16 were included in the present study; however, this would be an important consideration for future research, particularly as young children may have close contact with pets but are unlikely to practice good food and hand hygiene around them. In the present study, there was limited evidence of co-carriage of ESBL-producing *E. coli* between dogs and owners in the same household, a finding which is similar to that of van den Bunt *et al.* (2020), where co-carriage was infrequent. However, as stated previously, carriage may be transient and may have been missed at the time of sampling, and human samples were limited by small volumes in many cases. Additionally, further information would be obtained from WGS and sequence typing of the *E. coli* isolates. Further research is required to investigate the potential effects of owner hospital attendance on household dog AMR-*E. coli* carriage, and the potential for transmission and co-carriage between dogs and their owners in this situation.

The present study has identified potential associations between RMD-feeding and longitudinal shedding of ESBL-producing *E. coli* and 3GCR-*E. coli* by dogs, but this must be interpreted with caution. Further research is required on a larger scale focusing on concurrent sampling of dog diets at the same time as faecal sampling, alongside next generation sequencing, which would enable in-depth investigation of ESBL-producing *E. coli* transmission from food to dog, as well as within the home. Additionally, analysis of questionnaire data in future studies including risk factor analysis would allow deeper investigation of the associations identified in the present study.

The findings of the present study have potential to be of importance not only within the home, but also within a clinical setting. As ESBL-producing *E. coli* may not persist for a prolonged length of time in the gut without repeated re-colonisation, to reduce the risk of shedding of AMR-bacteria by RMD-fed patients and contamination of the clinical setting, one consideration could be to change the patient diet to a non-raw food prior to admission for routine procedures. However, further research is required to determine whether a diet change would indeed be successful in reducing shedding, and how long prior to admission a diet change would need to be instigated.

Limitations

This study had some limitations. It was an exploratory study and so only a limited number of households were included to start with; however, there was significant loss to follow up. The original study design involved household visits by researchers, and so households were recruited within a two-hour driving distance from the University of Liverpool. However, due to Covid-19 precautions and household preference, the study design was changed to remote and all packs were sent and received via Royal Mail. This remote nature may have led to a reduction in sample return rate as it meant dog owners had to visit a Post Office to return the completed sample pack. Additionally, there may have been some bias introduced by the study design as it was limited to a small area of the country, so the findings may not necessarily represent the true population. Finally, the study participants were recruited by directly contacting those who had agreed to be contacted further following participation in previous studies, which is likely to have introduced a selection bias, and questionnaire responses were reliant on honest and accurate completion by the owners thus could be subject to recall bias.

Due to the small numbers of samples received and AMR-positive results in the final study stages, further modelling on questionnaire data such as multivariable modelling was not undertaken. This meant that associations only between household factors and AMR outcomes could be suggested. Further research with larger numbers would allow more in-depth analysis of the risk factors identified in this study for ESBL-producing, 3GCR- and MDR-*E. coli*.

The study duration was limited by time and financial constraints, so only five sampling points over approximately six months were achieved. Due to the nature of sampling, there may be an underestimation of the prevalence of ESBL-producing, 3GCR- and MDR-*E. coli* for humans and the environment in this study. Swabs were provided for human stool sampling as this was felt to be less unpleasant for dog owners and encourage participation; however, there was a large variation in sample volume received. A low prevalence of ESBL-producing *E. coli* was expected and so small sample volume may have limited the ability to pick up any ESBL-producing *E. coli* present in the stool. Additionally, swabs were dry which may have limited the ability to collect a sample from the food bowl and floor. Furthermore, the possibility that

owners may have cleaned the bowls and floor prior to sampling cannot be excluded. A larger stool sample from the owners and saline-soaked swabs for environmental sampling would potentially improve this methodology for future research. Additionally, due to the potentially transient nature of intestinal ESBL-producing *E. coli* carriage, the length of time between sampling periods may have led to an underestimation of the prevalence as colonisation may have come and gone in the sampling timeframe. Therefore, more frequent sampling for future studies may better inform this.

Finally, limited genotyping was undertaken in this study. Further in-depth analysis using WGS will allow analysis of the particular genes that are present, as well as *E. coli* sequence typing, to investigate trends and potential co-carriage observed in this study in greater detail. DNA from ESBL-producing and 3GCR-*E. coli* and which demonstrated a unique AMR has been extracted and sent to the Centre for Genomic Research at the University of Liverpool and is currently undergoing WGS. Furthermore, in cases where an unusual phenotype was demonstrated on AST, for example in the non-ESBL-producing *E. coli* isolates which were resistant to amoxycillin-clavulanic acid but not ampicillin, WGS would allow further confirmation of species and underlying resistance mechanisms. These isolates have not been sent for WGS as part of the present study as efforts were focussed on ESBL-producing and 3GCR-*E. coli*; however, this would be a useful to investigate in future research.

5.4 Conclusions

To the author's knowledge, this is the first study to investigate longitudinal carriage of AMR-*E. coli* by dogs and household members co-carriage and how this varies by diet provision in the UK. Despite the limitations, this study has provided further evidence that provision of RMD to dogs is associated with shedding of AMR-*E. coli* which demonstrates important resistance mechanisms to critically important antibiotics, and that this shedding occurs longitudinally and may be associated with repeated re-colonisation. Additional research is required to investigate this association further; however, it highlights the potential one-health risk posed by provision of RMD to dogs.

Chapter 6: General Discussion

The overall aim of this study was to investigate and understand the potential public health risks associated with feeding raw meat diets (RMD) to dogs, with particular focus on antimicrobial resistance (AMR). In addressing this aim, a number of important findings have emerged surrounding the risks associated with the foods themselves, as well as those associated with the carriage of AMR bacteria by dogs fed these diets, and the potential wider-reaching implications this may have within the home. Additionally, some key concerns surrounding the products, their labelling, and traceability and how they are handled by consumers, and the perception and misinformation surrounding the diet have been recognised.

A growing body of evidence has demonstrated the presence of zoonotic pathogens in RMD (van Bree *et al.*, 2018; Hellgren *et al.*, 2019; Nüesch-Inderbinen *et al.*, 2019; Treier *et al.*, 2021; Gibson *et al.*, 2022), and within the present study, RMD samples were contaminated with high levels of *E. coli*, and frequently AMR-*E. coli,* suggesting that these diets not only pose a risk for zoonosis, but also could be a route of transmission of AMR within the home. Furthermore, the feeding of RMD to dogs as a potential route for transmission of AMR-*E. coli,* has been demonstrated by previous studies, with provision of RMD being identified as a risk factor for AMR-*E. coli* carriage by dogs (Schmidt *et al.*, 2015; Wedley *et al.*, 2017; van den Bunt *et al.*, 2020; Sealey *et al.*, 2022). Additionally, studies from the UK (Groat *et al.*, 2022) and Sweden (Runesvärd *et al.*, 2020) observed that dogs fed RMD shed third-generation cephalosporin-resistant (3GCR), extended-spectrum beta-lactamase (EBSL)-producing and multidrug-resistant (MDR)-*E. coli* to a greater degree than those fed non-raw diets (NRMD). However, these studies were both limited by a small study population. The present study has contributed to this evidence base on a larger scale within the UK, and demonstrated that not only was RMD itself contaminated with 3GCR-, ESBL-producing and MDR-*E. coli*, but also that dogs fed RMD were significantly more likely to shed these bacteria, compared to those fed NRMD. Dogs fed RMD shed these bacteria both intermittently and over consecutive time periods, suggesting that either persistence of carriage was present in some cases, or repeated exposure occurred in some dogs throughout the study. On whole genome sequencing, *E. coli* isolates from RMD-fed dogs were found to harbour multiple plasmid-

mediated AMR-genes with the potential to confer resistance to various classes of antibiotics. These findings are of importance as these genes are present on mobile genetic elements and could be transferred horizontally to pathogenic bacteria or importantly, to host commensal microbial flora.

The present study identified some *E. coli* sequence types (STs) and AMR genes of key importance with regards to public health. While the majority of unique STs were identified in RMD-fed dog isolates, there were some isolates which crossed over between RMD foods (chapter 3) and RMD-fed dogs (chapter 4), which were ST155, ST602, ST4096, ST10, ST58 and ST69. Previous studies have identified these STs in *E. coli* isolates from human infections and from livestock faeces and meat. A study of human and food chain derived *E. coli* isolates from England identified ST155 in beef cattle faeces, chicken meat and human blood (Day *et al.*, 2019; Ludden *et al.*, 2019). ST602 has been identified as one of the most frequently isolated STs from livestock in the UK, notably chicken (both in faeces on the farm and in the meat sold within the supermarket) (Ludden *et al.*, 2019), and a further study observed that ST602 was dominant in both chickens and chicken meat in food chain derived samples (Day *et al.*, 2019). ST10 *E.coli* has been linked with $bla_{\text{CTX-M-15}}$ gene presence in pigs in Portugal (Fournier *et al.*, 2020) and sheep in Tunisia (Sghaier *et al.*, 2019), $bla_{\text{CTX-M-1}}$ in dogs in France (Dahmen *et al.*, 2013), and $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-15}}$ in humans in Germany (Gerhold *et al.*, 2016). ST58 *E. coli* has been isolated from livestock globally, including cattle, pigs and poultry, as well as from wildlife and humans (Reid *et al.*, 2022). *E. coli* ST69 has been isolated from milk samples from dairy cows with mastitis in Brazil (dos Santos Alves *et al.*, 2023), cattle faeces in Italy (Giufrè *et al.*, 2021) and broiler meat and chickens in Denmark (Agersø *et al.*, 2014). ST69 is infrequently isolated from companion animals, however, a study in Finland identified ST69 *E. coli* being co-carried by humans and dogs in the same household, where it was associated with carriage of CTX-M group 9 (Grönthal *et al.*, 2018). The identification of STs in RMD food samples which have previously been associated with livestock, and the presence of these STs within the *E. coli* isolated from RMD-fed dogs demonstrates the potential importance of raw livestock meats as potential transmission sources of STs in dogs that are associated with pathogenicity and/or antimicrobial resistance genes.

Three specific bla_{ESBL} genes isolated in this present study are of particular interest: $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-55}}$ and $bla_{\text{SHV-66}}$. The $bla_{\text{CTX-M-15}}$ gene is the most frequently isolated bla_{ESBL} gene in

human *E. coli* worldwide (Livermore, 2009; Day *et al.*, 2019), and has been isolated from canine *E. coli* and from veterinary clinical environments in the UK (Timofte *et al.*, 2016; Tuerena *et al.*, 2016; Singleton, Pongchaikul, *et al.*, 2021). Due to the close and frequent contact dogs have with their owners, it could be hypothesised that the appearance of this gene in canine isolates reflects transmission from human to dog, particularly as this gene is not highly prevalent in livestock, in which $bla_{\text{CTX-M-1}}$ predominates (Meunier *et al.*, 2006; Ludden *et al.*, 2019; Veterinary Medicines Directorate, 2022). Furthermore, the findings of the present cross-sectional study indicated that $bla_{\text{CTX-M-15}}$ was the most frequently isolated bla_{ESBL} gene in both RMD and NRMD-fed dogs, suggesting that this gene is of increasing importance in the general UK canine population, which is of concern due to its frequent co-carriage alongside additional plasmid mediated resistance genes conferring MDR.

The identification of $bla_{\text{CTX-M-55}}$ and $bla_{\text{SHV-66}}$ as the second and third-most prevalent bla_{ESBL} genes in *E. coli* in RMD-fed dogs in the present study was an unexpected finding, and of interest as $bla_{\text{CTX-M-55}}$ has only been reported once previously in dogs in the UK, in clinical *E. coli* isolates (Bortolami *et al*., 2019), and $bla_{\text{SHV-66}}$ would appear to be novel in UK canine *E. coli*. The $bla_{\text{CTX-M-55}}$ gene is highly prevalent in *E. coli* isolates from humans and animals in China (Sun *et al.*, 2010; Lv *et al.*, 2013; Zhang *et al.*, 2014), and has been identified in *E. coli* isolates from broiler farms in Brazil, one of the world's leading exporters of poultry meat (Cunha *et al.*, 2017; Menck-Costa *et al.*, 2022). Additionally, it has been isolated infrequently in a few cases in dogs in European countries (Lupo *et al.*, 2018; Carvalho *et al.*, 2021), including from *E. coli* cultured from urine and wound samples from sick dogs admitted to a veterinary hospital in Switzerland (Zogg *et al.*, 2018), and from the faeces of a raw-fed dog in the Netherlands (Baede *et al.*, 2015). In the UK the $bla_{\text{CTX-M-55}}$ gene has not been identified in *E. coli* isolated from healthy dogs or humans, however it was isolated in broilers in 2020 and pigs at slaughter in 2021 (Veterinary Medicines Directorate, 2021b, 2022). The implications of this are twofold; one implication is that RMD-fed dogs may have shed this gene as a result of consumption of contaminated raw meat in their diet, particularly as this gene was not identified in isolates from NRMD-fed dogs. The other is that the appearance of this gene may be important for surveillance of AMR. It is possible that this gene is emerging within dogs and livestock in Europe. Additionally, it is possible that this gene may have reached the UK in imported pig and poultry food, which could potentially explain its appearance in broilers and

pigs at slaughter in this country. Finally, importation of raw meat to be used in RMD products from countries globally where the *bla*$_{CTX\text{-}M\text{-}55}$ gene is prevalent in livestock may have led to the appearance of this gene in RMD-fed dogs. While further research and surveillance are needed, this highlights the need for critical evaluation of using imported raw products in the introduction of novel AMR genes to the population, and manufacturers of RMD should be aware of this risk when utilising imported products. The present study highlighted the variation between RMD suppliers and sample packets in the level of information provided regarding the country of origin of ingredients and presence of batch numbers. Lack of traceability information has been highlighted previously in a study of *Salmonella* spp. presence in raw pet treats (Morgan *et al.*, 2023) (Appendix 5), and this should be an area for concern and improvement for manufacturers. The importance of bacterial zoonotic disease surveillance with regards to imported meat products intended for use in RMD has been highlighted previously, where hare meat imported into the UK via The Netherlands, originating in Argentina had been found to be contaminated with *Brucella suis* (Frost, 2017). Furthermore, imported frozen chicken intended for human consumption has been demonstrated to carry significantly more *Salmonella* spp. than home produced chicken (Janecko *et al.*, 2023). Surveillance of AMR in relation to imported raw meat products is currently not undertaken, and must be considered for the future as this is potentially an overlooked source of novel AMR genes. In addition to the bacterial concerns, imported RMD may also be a source of other emerging zoonotic diseases, which are not native to the UK, including parasites. A recent case of tongueworm (*Linguatula serrata*) in an untravelled UK dog was suggested to be linked to the provision of a raw meat diet (Campbell and Jones, 2023). *L. serrata* infection is associated with the consumption of raw ruminant offal, particularly liver, and although it is frequently observed in countries in the Middle East, Asia and Africa it is rarely observed in Northern Europe (Tappe and Warrell, 2020). Previous reported cases in the UK have been in dogs imported to the UK from Romania (Villedieu *et al.*, 2017; Macrelli and Mackintosh, 2022).

The reason for the appearance of *bla*$_{SHV\text{-}66}$ at high prevalence in RMD-fed dogs is less clear. The *bla*$_{SHV\text{-}66}$ gene is more likely to be associated with other *Enterobacterales* such as *Klebsiella* spp. and *Enterobacter* spp. (Du *et al.*, 2020; Imkamp *et al.*, 2022); however, it has been isolated from equine faecal *E. coli* isolates in the UK obtained from hospitalised horses

(Isgren, 2020). While it is possible that RMD-fed dogs may have eaten horse meat contaminated with *E. coli* which carried bla_{SHV-66}, it is unlikely to be the sole cause given that horse meat was not an especially common component in RMD as discussed by dog owners in the present study, with <3% of dogs being fed it. A further potential transmission route could be from dogs consuming horse faeces, which may be more likely than from consuming horse meat in the UK, particularly due to the close contact some dogs have with horses in environments such as livery yards, stables and farms, as well as if they are walked on pasture land or bridleways. In the present study, two of the nine dogs which shed *E. coli* that harboured the bla_{SHV-66} gene had contact with horses listed on their lifestyle risk factors questionnaire, and one dog attended agility classes at an indoor arena at a stable. Therefore, the link between equine and canine carriage of *E. coli* harbouring the bla_{SHV-66} gene is an area which warrants further research.

The findings of the present study are not only a concern with regards to the risk of AMR-transmission within the home, particularly to the vulnerable individuals such as the immunocompromised, elderly and infants, but a specific potential concern is the use of RMD-fed dogs as therapy dogs. Therapy dogs come into contact with potentially vulnerable and high-risk people in care homes, schools, hospices and nursing facilities, therefore posing a potentially significant risk to their health. The Pets As Therapy (PAT) Dogs charity states that, due to the Royal College of Nursing and an increasing number of education authorities and NHS Trust Infection Prevention and Control Policies, PAT Dogs should not be fed raw diets (https://petsastherapy.org/information/volunteer-policies-and-procedures/volunteer-policies-and-procedures). Despite this, in the present study 31 RMD-fed dogs from the online survey were reported to act as therapy dogs. In a previous study in Canada, therapy dogs which were fed RMD were been demonstrated to shed more *Salmonella* spp. and 3GCR-*E. coli* than those fed NRMD (Lefebvre *et al.*, 2008), therefore highlighting the potential risks posed. Dog owners who feed RMD have previously been demonstrated to be aware of some risks associated with RMD; however, they may underestimate or downplay those risks (Bulochova and Evans, 2021b), or believe that perceived health benefits of the diet choice outweigh them. This was again an important finding within the present study; owners who fed RMD did not believe that the diet or their RMD-fed dog posed a risk to in-contact dogs or people, further indicating the need for additional education and strategies to increase

awareness of the potential human health risks posed by RMD-fed dogs, particularly to vulnerable people. On the other hand, within the present cross-sectional study dogs that visited care homes were more likely to carry ESBL-producing *E. coli* in their faeces, which could suggest that there is also a risk to dogs of AMR-transmission from the care home residents and environment. Although a study from Switzerland did not find that dogs which visited or lived in long-term residential care facilities had a higher prevalence of ESBL-producing *E. coli* than the general canine population (Gandolfi-Decristophoris *et al.*, 2013), residents of nursing and care homes have been identified as having a high prevalence of AMR-*E. coli* in the UK (Rosello *et al.*, 2017), Ireland (Ludden *et al.*, 2015) and the Netherlands (Overdevest *et al.*, 2016; Van Dulm *et al.*, 2019). Data surrounding the risks of transmission of AMR-bacteria between dogs and humans in the care home environment are limited, therefore further research is required.

Although there was no evidence of longitudinal environmental contamination specifically with ESBL-producing *E. coli* identified in the present study, there was evidence of AMR-*E. coli* being present in food bowls, water bowls and the floor, and where this occurred, the resistance pattern demonstrated often was the same, or similar between the *E. coli* isolated from the environmental swabs and either a dog, or less frequently, a person in the household. This finding occurred more frequently in RMD-feeding households and demonstrates potential transmission of AMR-*E. coli* within the household and that RMD provision is not just a direct risk for the dogs and potentially people, but also a risk for environmental contamination. This finding is of particular concern for households where animals share food and water bowls, thus potentiating transmission between pets, and for households where at-risk individuals, e.g., immune-compromised people, may be involved with the cleaning of pet food bowls, or where young children are present who may be at risk from playing with or around the food bowls (Lambertini *et al.*, 2016). The issue of environmental contamination from dogs shedding AMR-*E. coli* has also been highlighted in the hospital environment. Studies have demonstrated commonalities in the STs and AMR-genes identified between ESBL-producing *E. coli* isolated from veterinary clinical patients and swabs of hospital surfaces (Timofte *et al.*, 2016; Schmitt *et al.*, 2021). While it is indeed plausible that transmission may occur from hospital environments to patients, it is likely that shedding of AMR-bacteria by patients would contaminate the hospital environment, and that

if RMD-fed dogs shed ESBL-producing and 3GCR-*E. coli* to a greater degree than those fed NRMD, then as patients they are likely to pose a greater risk for hospital contamination. As a result, RMD-fed patients may pose a significant risk to hospitalised immunocompromised, elderly or young veterinary patients, and separate housing for RMD-fed patients could be recommended. Additionally, veterinary staff have close and frequent contact with multiple patients daily, and as such not only are they at potentially greater risk of zoonotic disease from RMD-fed patients, but also may inadvertently be a risk for transmission of AMR-*E. coli* between patients. Therefore, strict barrier nursing and hand washing protocols are crucial for reducing this risk of spread when handling RMD-fed patients. While there is evidence for AMR-*E. coli* persisting for up to three months in patients which have received antibiotics (Schmidt *et al.*, 2018), there is little evidence for the length of time RMD-fed patients may shed these bacteria. The findings of the present study and others (Baede *et al.*, 2015; van den Bunt *et al.*, 2020) suggest that the situation is complicated, and that RMD-fed patients may shed ESBL-producing *E. coli* both intermittently and consistently and therefore it is difficult to recommend a length of time for which RMD-fed patients should not be fed this diet prior to hospitalisation. However, this is an area for potential future research as this information could be used to inform on veterinary practice protocols for patients undergoing elective procedures, to reduce the risk of AMR-*E. coli* contamination of the hospital environment.

Potentially risky pet food preparation practices may increase the potential for AMR-bacteria transmission within the home (Thomas and Feng, 2020). Practices such as feeding the dog in the kitchen, utilising the same utensils and food preparation surfaces for preparation of dog and human food, and sharing utensils with dogs are reported (Dickson *et al.*, 2019; Thomas and Feng, 2020; Bulochova and Evans, 2021a; Luisana *et al.*, 2022), and were also identified in the present study, demonstrating a possible lack of knowledge, understanding or concern regarding the potential infectious disease risks surrounding pet food provision. Furthermore, there appears to be some confusion and misinformation surrounding the 'correct' and safe food preparation practices with regards to RMD (Bulochova and Evans, 2021b), which may reflect the finding of this study that owners who feed RMD are significantly more likely to seek dietary advice from unsubstantiated resources such as social media, rather than a veterinary professional, a key finding from chapter 2. There appears to be a distinct problem

with the communication and perception of risks surrounding RMD to stakeholders, which include pet owners, food retailers and manufacturers. An emphasis was put on the importance of the owners doing their own research regarding diets; however, the level of research being done, and what 'research' meant to dog owners was not clear. Some owners mentioned 'scientific studies', others discussed forms of evidence such as books and websites or social media. This suggests a breakdown in communication regarding diets between dog owners who feed RMD and veterinary professionals. The limitation in trust in the veterinary professional's ability to provide independent advice regarding diets has similarly been observed in previous studies (Connolly, Heinze and Freeman, 2014; Morgan, Willis and Shepherd, 2017; Empert-Gallegos, Hill and Yam, 2020), and the importance of social media for dissemination of dietary advice has been identified previously (Kogan, Little and Oxley, 2021). Therefore, the importance of social media to dog owners as a readily-available and easy to access resource must not be underestimated. However, resources such as social media and websites are not peer reviewed and often based on unsubstantiated and anecdotal evidence, which may be important from the point of view of perpetuation of misinformation. Thus, a vital area for future research would be to gain understanding of the barriers to both veterinary professionals and RMD-feeding owners with regards to communication surrounding RMD, and to understand why RMD-feeding owners would rather 'do their own research' from unsubstantiated resources, with a focus on understanding what 'research' and 'scientific evidence' actually means to them. This is turn would aim to change how important messaging surrounding infectious disease risks and raw feeding is perceived and understood, how resources based on solid scientific evidence get to the intended audience and become trusted, and improve the veterinary professional-raw feeding owner relationship. Furthermore, it is important that veterinary professionals have access to up-to-date scientific knowledge regarding the pros and cons of feeding RMD to pass on to their clients, and the creation of an easily accessible evidence-based article or handout which could be used to aid communication with clients in consultation or given to clients to read in their own time, could be beneficial.

A theme that was present within the current research was that dog owners were more concerned about the numerous purported health benefits of RMD and about their pet becoming unwell as a result of their diet than they were about becoming ill themselves, a

theme which has also been identified in previous research (Thomas and Feng, 2020). Frequently, RMD was chosen due to the perceived values of it being 'healthier' and 'more natural', and the owners could have more control over the component ingredients. Conventional cooked proprietary diets such as kibble were viewed with mistrust due to component ingredients such as 'additives' and 'fillers', and a lack of trust towards the pet food companies was identified. Furthermore, owners who chose RMD commented on the risks of contamination of NRMD with (for example) heavy metals, and in some instances, they believed NRMD to be contaminated with *Salmonella* spp. to a greater degree than RMD. While it is true that in the USA, Canada and Germany there have been isolated reports of *Salmonella* spp. contamination of NRMD (Schotte *et al.*, 2007) including incidences of human salmonellosis which were traced back to contaminated kibble (Centers for Disease Control and Prevention (CDC), 2008; Behravesh *et al.*, 2010; Imanishi *et al*., 2014), there have been no reports of this in the UK. However, there are numerous studies which have isolated *Salmonella* spp. from commercially available RMD in countries worldwide (Weese, Rousseau and Arroyo, 2005; Finley *et al.*, 2008b; van Bree *et al.*, 2018; Hellgren *et al.*, 2019; Bottari *et al.*, 2020; Kananub *et al.*, 2020; Vecchiato *et al.*, 2022), and where RMD and NRMD have been investigated within the same study, RMD samples have been demonstrated to be contaminated with *Salmonella* spp. more frequently than NRMD (Strohmeyer *et al.*, 2006; Nemser *et al.*, 2014). The findings of the present study support this further; *Salmonella* spp. was isolated from 4.5% of RMD samples tested; however, no *Salmonella* spp. was isolated from any of the NRMD samples.

An obvious route to reducing the risk of zoonotic disease and AMR-gene transmission from RMD would be to cook it, as cooking for 2 minutes at 70^{o}C has been demonstrated to kill non-AMR bacteria, and is suggested to be sufficient to kill AMR-bacteria in food products (James *et al.*, 2021). However, the products utilised by RMD-feeding owners are not heat-treated or processed in any way, therefore other measures of reducing the risk posed by this diet are required. In light of the findings of the present study, one suggestion could be that RMD manufacturers have an important role in improving the messaging surrounding the importance of safe food handling and storage, and regarding the potential zoonotic disease risks and AMR, and that this messaging could be stronger. Although only ten brands were tested in this study, there was distinct variation in the instruction provided to owners

regarding defrosting, food preparation and safe handling/hand washing. Some brands did provide further information on their websites which may not have been printed onto packets; however, the relevance of this is questionable if it is not directly available to the dog owner at the times of highest risk, such as food defrosting and preparation. Furthermore, a previous study identified that food safety information and warnings provided to dog owners on RMD manufacturers' websites were lacking in sufficient detail to enable owners to undertake appropriate food hygiene practices (Bulochova and Evans, 2021b). While there are useful resources available for pet owners to aid safe practices when utilising RMD, such as the APHA/UKHSA website (https://www.gov.uk/guidance/raw-pet-foods-handling-and-preventing-infection), and the UK Pet Food safe handling poster (https://www.ukpetfood.org/resource/handling-commercial-raw-pet-food-safely-poster.html), the advice is vague or suggests reading the RMD packet for defrosting instructions, which are not always present, further iterating the need for improvement in the clarity and detail in the information provided on the RMD packet regarding storage, defrosting and food preparation. Many different defrosting, storage and handling practices were discussed by dog owners in chapter 2 of the present study. This variability in reported practices suggests that currently messaging is not clear or consistent, and not getting to enough of the intended audience. This highlights the need for further research into how messaging surrounding good pet food hygiene practices could better reach owners and how messaging can be made consistent between different manufacturers and products, as well as highlighting the important role RMD manufacturers themselves must play within this.

However, the problem surrounding ensuring owner and pet safety with regards to infectious disease is not solely one of refining messaging, education and signposting to resources, and care must be taken not to appear to alienate or put complete responsibility on the end consumer. Dog owners who purchase pre-prepared RMD do so in the belief that the product is of a certain microbiological quality, and that component ingredients are also of a high standard. Therefore, pet food producers also have a responsibility to ensure that their product is as 'safe' as it can be. The findings of the present research would suggest that a high level of bacterial contamination was already present in the foods at the time of defrosting, and that improvement is needed to reduce this, whether that it is in the selection of the quality of type of initial ingredients which make up the diets, or within the production

process itself. Additionally, it does suggest that the current protocol for sample testing of pre-prepared raw diets may not be sufficiently stringent. Finally, improvements in traceability of ingredients utilised in raw diets are required. The findings of the present study indicated that at product level, batch numbers were not always present on samples and country of origin of meats was often vague. Therefore, for both disease outbreak and future AMR monitoring, this needs to be improved.

There are some interesting potential future methods of reducing bacterial load in RMD without using heat treatment or processing which appear to be effective, such as the use of bacteriophage. Bacteriophage preparations have been approved for improving food safety in areas of the human food chain in the USA, such as poultry, meat and egg production (Moye, Woolston and Sulakvelidze, 2018), and a study of the use of bacteriophage to reduce *Salmonella* spp. contamination in experimentally inoculated kibble for dogs was successful (Heyse *et al.*, 2015). Additionally, it was found to be effective for reducing *Salmonella* spp. in raw pet food ingredients (Soffer *et al.*, 2016). However, bacteriophage preparations for use in pet food are still in the research stages and are not currently available for commercial use. Therefore, for the present time a focus on reducing bacterial contamination in the first place, and improving product quality and traceability, remains crucial.

Limitations

Although some important findings have been generated within the present study, there are some limitations to the research, further to those described in individual studies. A limitation of the online survey is that utilising a questionnaire format which discusses an emotive topic such as pet diet choice is likely to encourage those individuals with a particularly strong opinion on the subject to take part, leading to polarisation of results, and as such may not be truly representative of the feelings of the general population. Without further qualitative analysis of free text responses, the nuances of opinion and decision making are likely to be missed.

There were some additional limitations in the sampling strategy for the microbiological aspects of this study. For the cross-sectional study, owners were requested to select one dog at random (if they were within a multidog household) to obtain a sample from on behalf of their household. This put the onus on the owner to select the dog, and although they were

requested to select a dog at random, there may have been some bias in their selection. Furthermore, this created an artificial binary situation of 'raw-fed' or 'non-raw-fed' households, which was accounted for by utilising a broad definition of a raw-feeding household being where dogs were fed raw items at least once per week. This may have oversimplified the true situation as many dogs are likely to have a mixed diet, and this may vary week to week. Furthermore, in households where dog diets are mixed, different dogs within the household may have different percentages of the diet being made up of raw or cooked materials. Although very few households in this study reported that dogs within the household were fed different diets, these variations could have an impact on the gut bacteria present. Additionally, by selecting only one dog, the AMR within the household may have been under or overestimated. As seen in the longitudinal study, at any time point multiple dogs within a household may or may not shed ESBL-producing *E. coli*, and this can vary over time, with different dogs within the household shedding at each time point, therefore sampling one dog at one point in time may miss the AMR present within the household.

With regards to the sampling of dog foods, a limitation of this exploratory aspect of the present study was that the sampling strategy was based upon the information provided by dog owners in relation to their preferred brands, rather than market share data (which is not currently available). Additional research on a larger scale, ideally with repeated sampling over time of a greater variety of brands and food sources, is required to support these findings further. In addition, only pre-prepared RMDs were sampled, although 2% of RMDs may be home-prepared (PDSA, 2022), which has the potential for greater contamination concerns, as while manufacturers of pre-prepared diets must submit samples for microbiological testing, there is no way of knowing the level of contamination which may occur within and around the home as a result of home-prepared diets. Furthermore, although the foods themselves comprise the majority proportion of the dog's diet, an important consideration is that often the diet will be supplemented with treat items, which themselves are likely to be DEFRA category 3 animal by-products. Moreover, these items may not have undergone any heat-treatment or processing, may also be imported materials, and have previously been demonstrated to be contaminated with pathogens such as *Salmonella* spp. (Morgan *et al.*, 2023) (Appendix 5). These items were not tested as part of the present study, but may be an important additional source of AMR-*E. coli* (and other zoonoses) within

the home, particularly as these items tend towards being dried items which rehydrate when chewed, take increased time to consume and may be present in the house for a prolonged period of time. Furthermore, the perception of risk with regards to these treats may be lower due to their dry nature. Therefore, future research should include these food items when investigating AMR-*E. coli* in the context of pet diets and One Health.

Further work

In addition to the suggestions made in individual studies within this thesis, further research should focus on some key areas. The first is to focus on awareness, education and communication strategies to identify and investigate the reasons for owners' breakdown in trust with veterinary professionals and conventional cooked food manufacturing companies. The present study focused predominantly on infectious disease and AMR; however, this is one component of a bigger picture. Despite widely publicised government campaigns to raise awareness of AMR, and multiple studies now demonstrating the microbiological risks surrounding RMD, the present study has indicated that this is not necessarily the biggest concern to owners. Therefore, to achieve better engagement surrounding these issues with owners, then further research may be required into other aspects of RMD which owners may put more 'value' on, such as investigation into the anecdotal health benefits, nutritional studies and research surrounding the prevalence of 'other' physical health risks to the dog brought up by owners in the present study such as bone impactions, foreign bodies, fractured teeth and pathologies as a result of nutritional imbalances/deficiencies. Additionally, further study into veterinary professionals' beliefs surrounding the risks and benefits of RMD and the challenges they face with regards to communication surrounding diets with dog owners would be beneficial. This research would be best done using qualitative techniques such as focus groups and individual interviews and would aim to bridge the communication gap that currently appears to exist between owners and veterinary professionals, and identify factors which could lead to a behaviour change around food choice and food preparation and hygiene measures.

Additional microbiological research would be beneficial to investigate the transmissibility of the plasmid-mediated AMR genes identified in this study, using methods such as bacterial conjugation experiments to further determine the specific plasmids associated with

particular AMR genes. Further phenotypic testing would also be useful to confirm any phenotypic expression of the *mcr-4* and rifampicin genes identified on whole genome sequencing. Finally, the present study focussed on the AMR-*E. coli* aspects within the samples; however, this is again only one component. An important additional area for investigation would be to determine the prevalence of pathogenic strains of *E. coli* within the isolates, including STEC O157 and investigation of virulence factors present within the isolates.

From a clinical veterinary medicine point of view, further research into the duration of shedding of AMR-*E. coli* by dogs fed RMD would be useful, and particularly if this shedding could be reduced by a diet change to NRMD, although compliance with a diet change by some owners may be challenging due to the multifactorial reasons for why they feed RMD. This has potential relevance for admission and hospitalisation of veterinary patients undergoing elective procedures, as any measure to reduce shedding of AMR and MDR-bacteria in the clinical setting would be beneficial to reduce the risk of transmission within the hospital, particularly to vulnerable patients such as those which are immunosuppressed, those in intensive care, the elderly or very young. Furthermore, despite many veterinary hospitals adopting a policy of prohibiting RMD feeding within the hospital, this does not address the shedding risk of the patient which may be fed RMD up to the point of admission. There are no current standardised protocols for managing RMD patients within the hospital environment, and while measures such as barrier nursing and placing these patients in isolation have been adopted by many veterinary practices, the findings of the present study, and further research into the transmission risks of RMD-fed patients, could be useful in the creation of a standard policy for management of these patients within the veterinary hospital.

Finally, an important consideration is that this study identified contamination of RMD samples with AMR-*E. coli*, and an association between RMD provision and carriage of AMR-*E. coli* by dogs was identified in both the cross-sectional and longitudinal studies. However, care must be taken not to overstate this association as samples of the dogs' specific diets were not taken at the same time as the faecal samples, consequently a direct link cannot be proven. Furthermore, provision of antibiotics and veterinary visits within the last three months were also identified as important risk factors for AMR-*E. coli* carriage. Therefore, a

suggestion for further research to investigate and solidify this association would be to simultaneously test the dog diets, dog faeces and home environment for the presence of AMR-*E. coli* and undertake whole genome sequencing to identify any co-carriage of *E. coli* STs and resistance genes. Nevertheless, RMD diets, and their provision have been identified as an important, and arguably avoidable, risk factor for AMR-*E. coli* carriage by dogs, and potentially their owners, in the present study.

Final conclusions

The present study has demonstrated that RMDs for dogs are a potentially significant One Health concern, posing a risk for both animal and human welfare. These diets, and the dogs fed them, have been demonstrated to carry high levels of AMR-*E. coli*, including a high proportion of isolates which demonstrated important resistance mechanisms such as ESBL-production and AmpC, and which were phenotypically resistant to critically-important antibiotics such as fluoroquinolones and third-generation cephalosporins. Additionally, multiple plasmid-mediated AMR genes were present concurrently within these *E. coli* isolates, highlighting the potential for transmission of MDR. Furthermore, AMR-*E. coli* were demonstrated to be shed by dogs fed RMD over a protracted period of time, and were present in the home environment. Further research is required to clarify and quantify the associated risks, particularly with regards to the risks of in-home transmission and the clinical risk of AMR-bacteria in RMD to human and veterinary patients. The non-AMR risks of RMD also need to be investigated, including the non-AMR pathogenic bacterial risks (for example, STEC *E. coli* O157, *Listeria* spp.), and the physical pathologies caused to dogs as a result of RMD. Furthermore, the introduction of routine surveillance of AMR bacteria within RMD products would be pertinent given the findings of the current research. A large-scale national study of RMD products available for dogs and cats in the UK is currently being undertaken by the Food Standards Agency and this will provide valuable insights into the scale of the AMR and zoonotic disease currently in the UK RMD market. The feeding of raw meat diets is no longer a niche diet choice for pets, and interest in the diet choice remains high amongst pet owners. Therefore, a multifaceted and integrated approach to their use, involving government bodies, RMD manufacturers and retailers, veterinary and medical professionals and pet owners, is vital for the future management of the AMR and zoonotic disease risks associated with them.

Appendices

Appendix 1: Appendices for Chapter 2

A1.1 JISC online survey cover letter, consent declaration and questionnaire

A Dog's Dinner: Survey Investigating Dog Food Selection by UK Dog Owners (JISC Online Survey Transcript)

Page 1: Introduction

You are being invited to participate in a research study conducted by the University of Liverpool.

As part of research into dog food selection in the UK, we are asking dog owners to fill in the following questionnaire regarding their choice of diet for their dog. Your participation in this study will help us better understand people's reasoning for their diet choice, their main sources of information when choosing what to feed, and their beliefs behind their selection of diet.
This questionnaire is open to **all UK dog owners**, regardless of dog food preference.

Before you decide whether to participate, it is important that you understand why the research is being conducted and what it will involve if you do choose to take part. Please consider the following information carefully. Researcher contact details are listed below should you have any further questions.

Reading this information and completing the survey will be considered as consent to participate in this study. **You must be at least 18 years old to participate.**
At the end of the survey you will be asked whether you would like to be contacted further regarding future related research studies, and given the opportunity to enter our prize draw to **win a £50 Marks and Spencer voucher.**

This project has been fully approved by the ethics committee at the University of Liverpool and funded by the Veterinary Medicines Directorate (VMD). The Veterinary Medicines Directorate (VMD) is an executive agency, sponsored by the Department for Environment, Food & Rural Affairs, which protects animal health, public health and the environment.

Thank you in advance for your participation.

Why am I being invited to take part and what will happen if I decide to participate?

You are being invited to take part because you are a UK dog owner.

If you decide to take part you will need to complete the following questionnaire, which will take **30-60** minutes, depending on how many dogs you complete the questionnaire for (around **5-10** minutes per dog).

Participation is **entirely** voluntary and you do not have to take part in the study. You do not have to give a reason if you do not wish to take part.

You are free to withdraw at any time until you have selected the 'Finish' button on the final page of the questionnaire, after which it will not be possible to withdraw responses.

How do I answer the questions?

Please answer the questions by selecting the appropriate answer box. Some questions will allow you to select more than one answer.

For answers where more detail is required we have provided a box for you to type your answer.

Some questions are more general, others are specific to your individual dog(s), and you will be given the option to fill these out for each dog in your household.

How will my data be used?

The data you provide will be stored securely for 7 years, in line with the data protection requirements at the University of Liverpool and GDPR. At the end of the questionnaire you will be given the option to enter our prize draw and provide an email address to be contacted further regarding future related studies. Any email address you provide will be used only to contact you further at a later date and will not be used to personally identify your survey responses.

If you do not agree to be contacted further, your data will remain anonymous.

All data is strictly confidential, will be used for this specific project only, and will be available only to the investigators. Data will be aggregated and no individuals will be identifiable from any published data. The Veterinary Medicines Directorate will receive aggregated & anonymised data.

What if I am unhappy or there is a problem?

If you are unhappy, or there is a problem, please contact the researchers listed below and we will try to help. If you remain unhappy or have a complaint which you feel you cannot communicate directly to our researchers then you should contact the Research Ethics and Integrity Office on 0151 794 8290 (ethics@liv.ac.uk).

When contacting the Research Governance Officer, please provide details of the name or description of the study so that it can be identified, the researchers involved, and the details of the complaint you wish to make.

Who can I contact for further details?

- **Miss Genever Morgan**

  Institute of Infection and Global Health, University of Liverpool Leahurst Campus, Chester High Road, CH64 7TE.

  Email: ddsurv20@liverpool.ac.uk

- **Professor Nicola Williams**

  Institute of Infection and Global Health, University of Liverpool Leahurst Campus, Chester High Road, CH64 7TE.

  Email: ddsurv20@liv.ac.uk

Consent to participate

Please confirm that you have read and understood the above information, are over 18 years old and consent to participating in this study:

I have read the above information and I consent to participating in this study.

Yes

Page 2: Your dog(s)

This first section is about your dog(s) and a little about your interaction with them.

How many dogs are in your household?

Please provide some information about your dog(s): *(picture of table included)*

	Dog name	Dog breed	Sex				How old is your dog?	How long have you owned this dog?
			Male, neutered	Male, not neutered	Female, neutered	Female, not neutered		
Dog 1							Please select	Please select
Dog 2							Please select	Please select
Dog 3							Please select	Please select
Dog 4							Please select	Please select
Dog 5							Please select	Please select

Where did you obtain your dog(s)? Please select all that apply:

- [ ] Breeder in the UK
- [ ] Website e.g. Gumtree
- [ ] Rescue Centre/charity in the UK
- [ ] Imported from a breeder abroad
- [ ] Imported from a rescue centre/charity abroad
- [ ] Given to me as a gift
- [ ] Obtained from a friend/colleague
- [ ] Other

(Text box pops up when 'Other' selected)

What is the purpose of your dog(s)? Please select all that apply:

- [ ] Pet
- [ ] Working/Farm dog
- [ ] Assistance/Guide dog
- [ ] Pets As Therapy (PAT) dog
- [ ] Breeding dog
- [ ] Show dog
- [ ] Other

(Text box pops up when 'Other' selected)

Where does your dog(s) **mainly** sleep?

- ( ) Outside kennel
- (•) Indoors in a room other than a bedroom
- ( ) Bedroom on floor/in a dog bed

- Bedroom on human bed
- Other

(Text box pops up when 'Other' selected)

Where does your dog(s) **mainly** eat their meals?

- Outside
- Indoors, in the kitchen
- Indoors, in a room other than the kitchen
- Other

(Text box pops up when 'Other' selected)

Does your dog(s) lick your hands/face?

	Never	Yes, but rarely	Yes, quite often	Yes, frequently
Frequency	☐	☐	☐	☐

Are all the dogs in your household fed the same type(s) of food (e.g. dry biscuit/kibble, raw diet, cooked diet, mix of foods)?

- Yes
- No

Page 3: Your dog food choice

We are now going to ask you some questions regarding your dog(s), your food choice and how you prepare their food.

Please complete the following sections for each dog in your household individually unless they are all fed the same, in which case please fill out the following questions on behalf of all of your dogs collectively.

Do you feed **any** raw animal material to your dog(s), either within their meals or as a treat (including raw meat, bones, eggs, dried meat treats)

- Yes – directs to 'Page 4: Your dog food choices (a)

- ○ No- directs to ‘Page 5: Your dog food choices (b)

Page 4: Your food choices (a) *respondents who selected ‘yes’ to Page 3 directed here*

Dog name

About your dog food selection

What type of food do you feed your dog? Please tick all that apply:

Please select at least 1 answer(s).

- ☐ Cooked commercial complete wet food
- ☐ Raw meat and/or bones (pre-prepared diet)
- ☐ Raw meat and/or bones (DIY/home-prepared diet)
- ☐ Cooked fresh meat and/or bones
- ☐ Cooked commercial complete dry food
- ☐ Raw eggs
- ☐ Cooked eggs
- ☐ Vegetarian diet
- ☐ Dried food items (e.g. pig ears, rawhide chews, dried fish skin)
- ☐ Other

(Text box pops up when ‘Other’ selected)

How often do you feed raw meat/bones to your dog?

- ○ Every meal
- ○ 5+ days per week
- ○ 3-4 days per week
- ○ 1-2 days per week
- ○ Less than once a week/as an occasional treat

What category do you feed? Please select all that apply:

Please select at least 1 answer(s).

- ☐ Shop bought, pre-prepared, frozen raw food
- ☐ Shop bought, pre-prepared, fresh raw food
- ☐ Fresh raw meat from the butcher or supermarket
- ☐ Fresh raw meat from another source e.g. specialist raw meat diet shop
- ☐ Raw food from an online supplier
- ☐ Shop bought or purchased online, pre-prepared frozen cooked food
- ☐ Shop bought or purchased online, pre-prepared fresh cooked food e.g tins, trays, sachets
- ☐ Shop bought or purchased online cooked dry kibble
- ☐ Fresh meat from butcher or supermarket, but cook it before feeding
- ☐ Fresh meat from another source, but cook it before feeding
- ☐ Other

(Text box pops up when ‘Other’ selected)

Which brands of pre-prepared food do you prefer to buy? Please list up to 3. If you do not buy pre-prepared food please write 'N/A'.

If you feed fresh raw meat that is not pre-prepared, where do you purchase this from? Please select all that apply.

- ☐ Supermarket
- ☐ Butcher
- ☐ Farm shop
- ☐ Market stall
- ☐ Abattoir

- [ ] Not applicable
- [ ] Other

(Text box pops up when ‘Other’ selected)

What type(s) of raw meat, either as part of a pre-prepared meal or bought from the supplier fresh, do you prefer to feed your dog? Please tick all that apply:

- [ ] Beef
- [ ] Pork
- [ ] Chicken
- [ ] Lamb
- [ ] Venison
- [ ] Turkey
- [ ] Rabbit
- [ ] Duck
- [ ] Game (e.g. Pheasant, grouse, pigeon)
- [ ] Offal (e.g. Tripe, heart, liver, kidney)
- [ ] Other

(Text box pops up when ‘Other’ selected)

How long have you been feeding raw meat/bones to your dog?

- ○ The entire time I have owned my dog
- ○ Less than 6 months
- ○ 6-12 months
- ○ Longer than 12 months

Do you feed any additional treats?

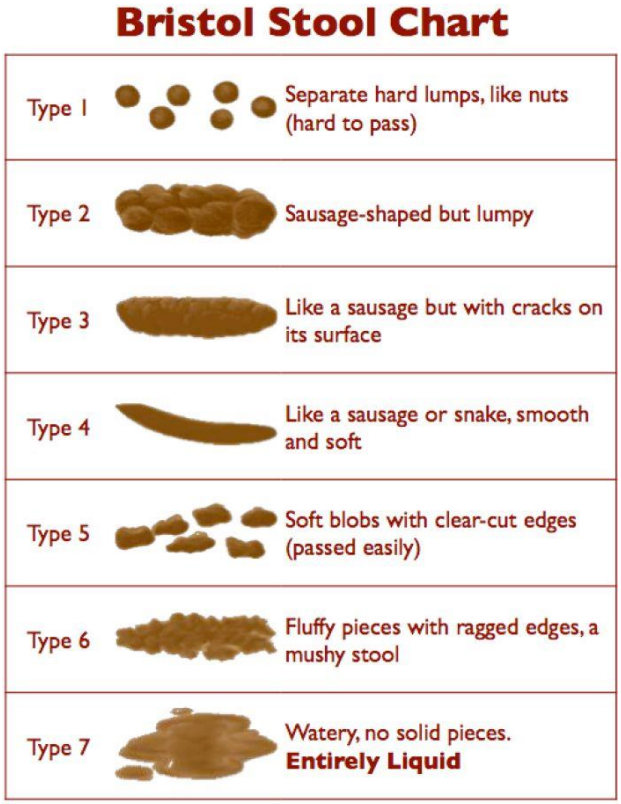
- ○ Yes
- ○ No

What types of treat do you feed? Please select all that apply:

- ☐ Shop bought cooked treats/biscuits
- ☐ Freeze dried meat/fish treats
- ☐ Dried treats (e.g. pig ears, rawhide, chicken feet)
- ☐ Raw meat (including body parts such as feet, hooves)
- ☐ Raw bones
- ☐ Cooked meat
- ☐ Cooked bones
- ☐ I don't feed any treats
- ☐ Other

(Text box pops up when 'Other' selected)

Using the Bristol Stool Chart below please indicate using the drop-down list which number most closely resembles your dog's normal stool consistency on an average day:



:

About the storage and preparation of your dog's raw meat food

Do you wear gloves to prepare the raw meat/bones?

- ○ Yes
- ○ No

Where do you store the raw meat components of your dog's food?

- ☐ In my own fridge/freezer
- ☐ In a separate fridge/freezer
- ☐ Non-temperature-controlled storage cupboard
- ☐ Other

(Text box pops up when 'Other' selected)

What is your opinion on freezing raw meat?

	Freezing meat kills all bacteria	Freezing meat kills most bacteria	Freezing meat does not kill bacteria	I don't have an opinion on freezing meat	I don't know
Opinion	☐	☐	☐	☐	☐

Where do you **prepare** the raw meat components of your dog's food?

- ☐ In the same area as my own food is prepared e.g. kitchen
- ☐ In a different area to where my own food is prepared e.g. utility room, shed

Please state where:

If you purchase frozen raw meat diets, where do you defrost this prior to feeding?

- Kitchen sink
- Kitchen microwave
- Dedicated pet food sink or microwave
- On kitchen work surface at room temperature
- On work surface in dedicated pet food preparation area at room temperature
- Not applicable to me
- Other

(Text box pops up when 'Other' selected)

Do you have separate chopping boards and/or utensils for preparation of the raw meat?

- Separate chopping board
- Separate utensils
- Separate chopping board and utensils
- No

Do you clean the food preparation area immediately after preparing the raw meat?

	Always	Usually	Sometimes	Never
Frequency	☐	☐	☐	☐

Do you have separate cleaning materials (e.g. cloths, sponges) for your dog's raw food preparation areas?

- Yes
- No

On a scale of 1-5, how often do you wash your hands after preparing your dog's raw meat (1= Always, 5= Never)?

	1	2	3	4	5

Number	☐	☐	☐	☐	☐

What do you do after your dog has finished eating?

- ○ I remove the bowl/feeding utensil and save the food if there is still food present
- ○ I remove the bowl/feeding utensil and throw away any remaining food
- ○ I leave the bowl/feeding utensil in case my dog would like to come back to it later
- ○ There is never any leftover food and I leave the bowl/feeding utensil down
- ○ There is never any leftover food and I remove and clean the bowl/feeding utensil

On a scale of 1-5, how often do you wash your dog's bowl after they have finished eating (1= After every meal, 5= Never)

	1	2	3	4	5
Number	☐	☐	☐	☐	☐

How do you clean your dog's bowls?

- ○ Rinse out with water only
- ○ Hand wash with kitchen washing up liquid
- ○ Hand wash with bleach
- ○ Dishwasher
- ○ Other

(Text box pops up when 'Other' selected)

Do you have another dog you would like to tell us about?

- ○ Yes- directs back to 'Page 3: Your dog food choice', can add up to 5 dogs
- ○ No- directs to 'Page 6: Reasons and beliefs behind your diet choice'

Page 5: Your food choices (b) *respondents who selected 'no' to Page 3 directed here*

Dog name

About your dog food selection

What type of food do you feed your dog? Please tick all that apply:

Please select at least 1 answer(s).

- ☐ Cooked commercial complete wet food
- ☐ Cooked commercial complete dry food
- ☐ Cooked fresh meat and/or bones
- ☐ Cooked eggs
- ☐ Vegetarian diet
- ☐ Dried food items (e.g. pig ears, rawhide chews, dried fish skin)
- ☐ Other

(Text box pops up when 'Other' selected)

What category do you feed? Please select all that apply:

Please select at least 1 answer(s).

- ☐ Shop bought or purchased online, pre-prepared frozen cooked food
- ☐ Shop bought or purchased online, pre-prepared fresh cooked food e.g tins, trays, sachets
- ☐ Shop bought or purchased online- cooked dry kibble
- ☐ Fresh meat from butcher or supermarket, but cook it before feeding
- ☐ Fresh meat from another source, but cook it before feeding
- ☐ Other

(Text box pops up when 'Other' selected)

Which brands of pre-prepared food do you prefer to buy? Please list up to 3. If you do not buy pre-prepared food please write 'N/A'.

Do you feed any additional treats?

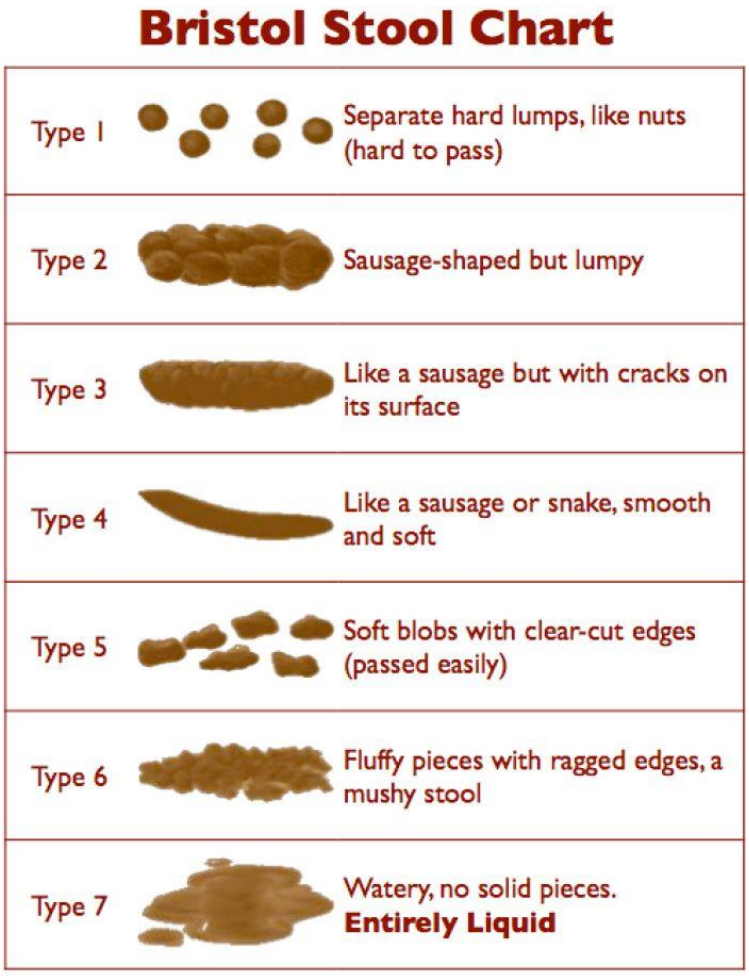
- ○ Yes
- ○ No

What types of treat do you feed? Please select all that apply:

- ☐ Shop bought cooked treats/biscuits
- ☐ Freeze dried meat/fish treats
- ☐ Dried treats (e.g. pig ears, rawhide, chicken feet)
- ☐ Cooked meat
- ☐ Cooked bones
- ☐ I don't feed any treats
- ☐ Other

(Text box pops up when ‘Other’ selected)

Using the Bristol Stool Chart below please indicate using the drop-down list which number most closely resembles your dog's normal stool consistency on an average day:



About the storage and preparation of your dog's food

What do you do after your dog has finished eating?

- I remove the bowl/feeding utensil and save the food if there is still food present
- I remove the bowl/feeding utensil and throw away any remaining food
- I leave the bowl/feeding utensil in case my dog would like to come back to it later
- There is never any leftover food and I leave the bowl/feeding utensil down

- ○ There is never any leftover food and I remove and clean the bowl/feeding utensil

On a scale of 1-5, how often do you wash your dog's bowl after they have finished eating (1= After every meal, 5= Never)

	1	2	3	4	5
Number	☐	☐	☐	☐	☐

How do you clean your dog's bowls?

- ○ Rinse out with water only
- ○ Hand wash with kitchen washing up liquid
- ○ Hand wash with bleach
- ○ Dishwasher
- ○ Other

(Text box pops up when 'Other' selected)

Do you have another dog you would like to tell us about?

- ○ Yes- directs back to 'Page 3: Your dog food choice', can add up to 5 dogs
- ○ No- directs to 'Page 6: Reasons and beliefs behind your diet choice'

Page 6: Reasons and beliefs behind your diet choice

This section is about the reasons for your choice of dog food, and your beliefs behind your choices.

Your reasons for choosing your dog's current diet

What was your **main** source of information when deciding on the diet for your dog? Please choose one option:

- ○ Veterinary surgeon/nurse
- ○ Advice from dog breeder
- ○ Rescue centre/charity

- Friend/family
- Personal experience from previous pet ownership
- Dog trainer
- Advertisement
- Pet food group on social media (please state which below)
- Other social media group e.g. breed specific group (please state which below)
- Pet food company website (please state which below)
- Other

(Text box pops up when 'Other' selected)

Please give us some more information about your main source of information, if applicable:

What was the reason for choosing the current diet for your dog? Please select **up to** three options:

- [ ] Breeder advice/came with a starter pack as a puppy
- [ ] Advice from rescue centre/charity
- [ ] Advice from a veterinary professional (vet or nurse)
- [ ] Believe it to be a more natural choice of diet
- [ ] Coat quality
- [ ] Stool consistency
- [ ] Behavioural reasons
- [ ] Cost
- [ ] Safety concerns
- [ ] Lack of trust of certain foods (please detail below)
- [ ] My dog will not eat/does not like certain foods (please detail below)

☐ To address existing health concerns (please detail below)

☐ Other

(Text box pops up when 'Other' selected)

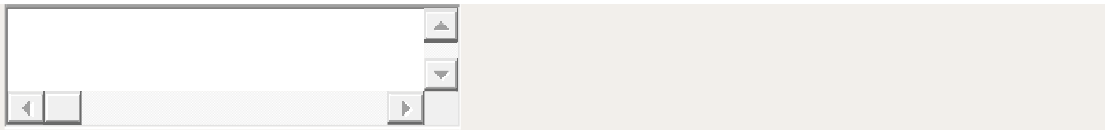
Your beliefs with regards to food choice

The following questions are regarding your beliefs and opinions with regard to diet selection and asks about your beliefs for both commercial cooked foods and raw meat diets. Please answer all questions regardless of what type of food you feed your dog. In each case please pick the answer that **most closely** resembles your opinion.

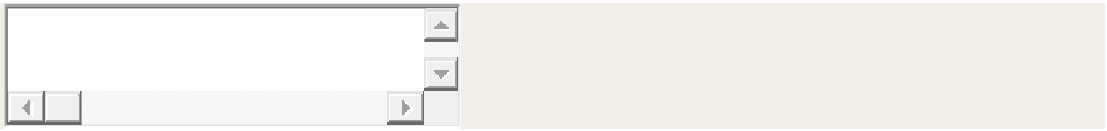
Do you think feeding a **raw meat diet** has a positive or negative effect on aspects of canine health? Please select the option which closest matches your opinion on the following categories of canine health and the effect (health benefit or risk) of feeding raw diet:

	Health benefit	No effect	Health risk	Don't know
Skin problems/allergies	☐	☐	☐	☐
Coat health	☐	☐	☐	☐
Dental disease/oral hygiene/bad breath	☐	☐	☐	☐
Good general digestive system health	☐	☐	☐	☐
Vomiting	☐	☐	☐	☐
Diarrhoea	☐	☐	☐	☐
Anal gland clearance	☐	☐	☐	☐
Mobility	☐	☐	☐	☐
Performance	☐	☐	☐	☐
Behaviour	☐	☐	☐	☐
Foreign bodies (getting something stuck in the stomach/intestines)	☐	☐	☐	☐
Bone splinters	☐	☐	☐	☐

Do you think there are any other **benefits** of feeding a raw meat diet not listed above? Please tell us:



Do you think there are any other **risks** of feeding a raw meat diet not listed above? Please tell us:



Do you think feeding a **commercial, cooked** diet (e.g. cooked fresh meat, wet complete diet or kibble) has a positive or negative effect on aspects of canine health? Please select the option which closest matches your opinion on the following categories of canine health and the effect (health benefit or risk) of feeding a commercial, cooked diet:

	Health benefit	No effect	Health risk	Don't know
Skin problems/allergies	☐	☐	☐	☐
Coat health	☐	☐	☐	☐
Dental disease/oral hygiene/bad breath	☐	☐	☐	☐
Good general digestive system health	☐	☐	☐	☐
Vomiting	☐	☐	☐	☐
Diarrhoea	☐	☐	☐	☐
Anal gland clearance	☐	☐	☐	☐
Mobility	☐	☐	☐	☐
Performance	☐	☐	☐	☐
Behaviour	☐	☐	☐	☐
Foreign bodies (getting something stuck in the stomach/intestines)	☐	☐	☐	☐
Bone splinters	☐	☐	☐	☐

Do you think there are any other **benefits** of feeding a cooked, commercial diet not listed above? Please tell us:

Do you think there are any other **risks** of feeding a cooked, commercial diet not listed above? Please tell us:

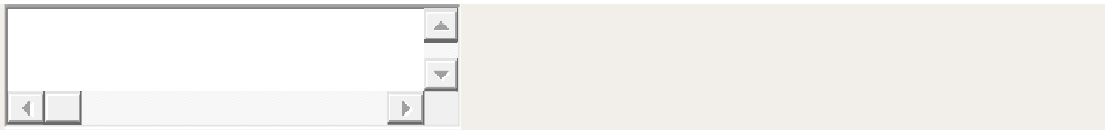
Have you personally noticed any of these benefits or risks since feeding your dog on his/her current diet?

- ○ Not applicable to me
- ○ No
- ○ Yes

Do you believe there are any **health risks** to the following categories associated with feeding a **raw** diet? Please select the option which **closest matches** your opinion:

	Yes, there is a risk	There may be some risk	No, there is no risk	Don't know
Your dog's health	☐	☐	☐	☐
Your own health	☐	☐	☐	☐
The health of other dogs that your dog comes into contact with	☐	☐	☐	☐
The health of other people that your dog comes into contact with	☐	☐	☐	☐

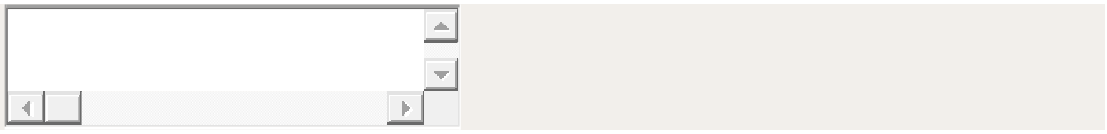
Please detail any specific risks related to the above categories that you believe could be present:



Do you believe there are any **health risks** to the following categories associated with feeding a **commercial, cooked diet** (e.g. cooked fresh meat, wet complete diet or kibble)? Please select the option which **closest matches** your opinion:

	Yes, there is a risk	There may be some risk	No, there is no risk	Don't know
Your dog's health	☐	☐	☐	☐
Your own health	☐	☐	☐	☐
The health of other dogs that your dog comes into contact with	☐	☐	☐	☐
The health of other people that your dog comes into contact with	☐	☐	☐	☐

Please detail any specific risks related to the above categories that you believe could be present:



Page 7: About you and your household

This final section asks a few short questions about you and your household.

Your age (years):

- 18-25
- 26-40
- 41-59
- 60-74
- 75+

Do you consider yourself:

- Male
- Female
- Other
- Prefer not to say

What region of the country do you live in? (drop down menu)

- North East
- North West
- Yorkshire and the Humber
- East Midlands
- West Midlands
- East of England
- Greater London
- South East
- South West
- Wales
- Scotland
- Northern Ireland

Page 8: End of questions

Thank you for completing this survey. Please now **continue to the next page to submit your answers,** learn more about further related research and enter our prize draw for the chance to win a £50 Marks and Spencer voucher!

Page 9: Win a £50 Marks and Spencer voucher!

We plan to continue and expand our investigation into dog food choices and the risks associated with these with future research studies. We would like to contact you

further regarding participation in these further projects. Please be aware that your participation is **completely voluntary** and there is **no obligation** to take part if you are contacted.

As a thank you for your participation in our study, you have the opportunity to enter our prize draw to win a £50 Marks and Spencer voucher.
Please indicate below whether you would like to enter the competition and if you would agree to be contacted regarding further related studies. Please also provide us with a contact email address. If you do not wish to participate further, please indicate by selecting the appropriate box.

Would you agree to be contacted further regarding future studies as explained above?

- ◉ Yes, I agree to be contacted further. I would like to be entered into the prize draw.
- ○ I would like to enter the prize draw but do not wish to be contacted about further participation.
- ○ No, I would not like to enter the prize draw and do not wish to participate further.

My contact email address is: (text box only visible if respondent clicks 'Yes, I agree to be contacted further. I would like to be entered into the prize draw'

FINISH BUTTON

Page 10: Final page- Thank you for participating

Thank you very much for taking the time to complete this study.

If you have chosen to participate further or enter the prize draw, we will be in contact soon.

Table A1.1: *Owner demographics (n=1831) and results of univariable analysis of factors associated with diet choice (either raw or non- raw diet)*

Variable	**Category** (O)	**N**	**% of total**	**Diet choice % (N)**		**Odds ratio**	**95% CI**	**p value** (chi sq.)
				Non Raw	*Raw*			
Totals		**1831**		**50.0 (916)**	**50.0 (915)**			
Age	18-25	230	12.6	57.4 (133)	42.2 (97)	*Ref*		
	26-40	642	35.1	56.7 (364)	43.3 (278)	1.05	0.77, 1.42	
	41-59	677	37.0	43.6 (295)	56.4 (382)	1.78	1.31, 2.40	<0.01
	60-74	261	14.3	44.1 (115)	55.9 (146)	1.74	1.22, 2.49	
	75+	18	1.0	50.0 (9)	50.0 (9)	1.37	0.52, 3.58	
	Unknown	3	0.2	0.0 (0)	100 (3)	**	**	
Gender	Female	1699	92.8	49.9 (848)	50.1 (851)	*Ref*		
	Male	109	6.0	53.2 (58)	46.8 (51)	0.88	0.59, 1.29	
	Other	3	0.2	0.0 (0)	100 (3)	**	**	0.40
	Prefer not to say	14	0.8	57.1 (8)	42.9 (6)	0.75	0.26, 2.16	
	Unknown	6	0.3	33.3 (2)	66.7 (4)	1.99	0.36, 10.91	
Location	North West	322	17.6	61.8 (199)	38.2 (123)	*Ref*		
	East of England	138	7.5	52.9 (73)	47.1 (65)	1.44	0.96, 2.16	
	Greater London	57	3.1	42.1 (24)	57.9 (33)	2.22	1.26, 3.94	
	North East	79	4.3	32.9 (26)	67.1 (53)	3.30	1.96, 5.55	
	East Midlands	108	5.9	42.6 (46)	57.4 (62)	2.18	1.40, 3.40	
	Northern Ireland	24	1.3	45.8 (11)	54.2 (13)	1.91	0.83, 4.40	
	Scotland	156	8.5	55.8 (87)	44.2 (69)	1.28	0.87, 1.89	<0.01
	South East	290	15.8	42.4 (123)	57.6 (167)	2.20	1.59, 3.04	
	South West	254	13.9	46.9 (119)	53.1 (135)	1.84	1.31, 2.56	
	Wales	102	5.6	57.8 (59)	42.2 (43)	1.18	0.75, 1.85	
	West Midlands	112	6.1	49.1 (55)	50.9 (57)	1.68	1.09, 2.59	

	Yorkshire	178	9.7	51.1 (91)	48.9 (87)	1.55	1.07, 2.24	
	Unknown	11	0.6	27.3 (3)	72.2 (8)	4.31	1.12, 16.57	
Number of dogs owned	1	965	52.7	57.3 (553)	42.7 (412)	*Ref*		
	2	524	28.6	49.4 (259)	50.6 (265)	1.373	1.11, 1.70	
	3	180	9.8	28.9 (52)	71.1 (128)	3.304	2.34, 4.67	<0.01
	4	76	4.2	28.9 (22)	71.1 (54)	3.295	1.98, 5.50	
	5 or more	86	4.7	34.9 (30)	65.1 (56)	2.506	1.58, 3.98	
General purpose of dog(s) in household	Pet only	1469	80.2	53.8 (791)	46.2 (678)	*Ref*		
	Pet and other purpose	322	17.6	35.4 (114)	64.6 (208)	2.13	1.66, 2.73	<0.01
	Purpose other than pet	40	2.2	27.5 (11)	72.5 (29)	3.08	1.53, 6.20	
Main source of dog diet information	Veterinary surgeon/nurse	428	23.4	81.5 (349)	18.5 (79)	*Ref*		
	Advice from dog breeder	112	6.1	35.7 (40)	64.3 (72)	7.95	5.03, 12.56	
	Dog trainer	61	3.3	32.8 (20)	67.2 (41)	9.06	5.03, 16.30	
	Friend/family	143	7.8	26.6 (38)	73.4 (105)	12.21	7.83, 19.03	
	Personal experience	521	28.5	51.6 (269)	48.4 (252)	4.14	3.07, 5.58	
	Pet food company website	47	2.6	57.4 (27)	42.6 (20)	3.27	1.75, 6.13	<0.01
	Pet food group on social media	116	6.3	6.0 (7)	94.0 (109)	68.79	30.84, 153.45	
	Other social media group	43	2.3	25.6 (11)	74.4 (32)	12.85	6.21, 26.60	
	Rescue centre/charity	20	1.1	60.0 (12)	40.0 (8)	2.95	1.17, 7.45	
	Advertisement	7	0.4	71.4 (5)	28.6 (2)	1.77	0.34, 9.27	
	Other	332	18.1	41.6 (138)	58.4 (194)	6.21	4.48, 8.62	
	Unknown	1	0.1	0.00 (0)	100.0 (1)	**	**	**
Reasons for diet choice	Advice from veterinary professional	503	27.5	81.1 (408)	18.9 (95)	0.14	0.11, 0.19	<0.01
	Advice from rescue/charity	42	2.3	78.6 (33)	21.4 (9)	0.27	0.13, 0.60	<0.01
	Behavioural reasons	195	10.6	28.7 (56)	71.3 (139)	2.75	1.99, 3.81	<0.01
	More natural choice of diet	863	47.1	14.1 (122)	85.9 (741)	27.72	21.54, 35.67	<0.01
	Breeder advice/came with starter pack	164	9.0	44.5 (73)	55.5 (91)	0.78	0.58, 1.08	0.14

	Coat quality	560	30.6	33.8 (189)	66.2 (371)	2.62	2.13, 3.23	<0.01
	Cost	298	16.3	75.5 (225)	24.5 (73)	0.27	0.20, 0.35	<0.01
	Lack of trust of certain foods	264	14.4	24.6 (65)	75.4 (199)	3.64	2.70, 4.90	<0.01
	Dog does not like/will not eat certain foods	183	10.0	55.2 (101)	44.8 (82)	0.79	0.58, 1.08	0.14
	Safety concerns	72	3.9	68.1 (49)	31.9 (23)	0.46	0.28, 0.76	<0.01
	Stool consistency	790	43.1	41.1 (325)	58.9 (465)	1.88	1.56, 2.27	<0.01
	Address underlying health concerns	381	20.8	50.7 (193)	49.3 (188)	0.97	0.77, 1.21	0.78
	Other	227	12.4	58.1 (132)	41.9 (95)	0.69	0.52, 0.91	0.01
	Unknown	4	0.2	75.0 (3)	25.0 (1)	0.33	0.04, 3.21	0.62
Specific purpose of dog(s) in household	Pet	1786	97.5	50.5 (902)	49.5 (884)	0.40	0.21, 0.76	<0.01
	Assistance/guide dog	14	0.8	50.0 (7)	50.0 (7)	1.00	0.35, 2.86	1.00
	Breeding	54	2.9	22.2 (12)	77.8 (42)	3.62	1.89, 6.92	<0.01
	Pets As Therapy (PAT)	48	2.6	35.4 (17)	64.6 (31)	1.85	1.02, 3.37	0.04
	Agility/dog sport	119	6.5	41.2 (49)	58.8 (70)	1.46	1.00, 2.13	0.05
	Show	84	4.6	22.6 (19)	77.4 (65)	3.60	2.14, 6.06	<0.01
	Working/farm	125	6.8	37.6 (47)	62.4 (78)	1.72	1.18, 2.50	<0.01
	Other	30	1.6	16.7 (5)	83.3 (25)	5.11	1.95, 13.40	<0.01
Where dog(s) in household obtained	Breeder in the UK	1132	61.8	45.8 (519)	54.2 (613)	1.54	1.28, 1.87	<0.01
	Breeder abroad	42	2.3	23.8 (10)	76.2 (32)	3.28	1.60, 6.70	<0.01
	Friend/colleague	208	11.4	62.0 (129)	38.0 (79)	0.58	0.43, 0.77	<0.01
	Gift	35	1.9	51.4 (18)	48.6 (17)	0.94	0.48, 1.84	0.86
	Rescue/charity abroad	76	4.2	46.1 (35)	53.9 (41)	1.18	0.74, 1.87	0.49
	Rescue/charity in the UK	444	24.2	49.8 (221)	50.2 (223)	1.01	0.82, 1.25	0.92
	Website e.g. Gumtree	196	10.7	49.5 (97)	50.5 (99)	1.02	0.76, 1.38	0.89
	Other	201	11.0	51.2 (103)	48.8 (98)	0.94	0.71, 1.27	0.70

** denotes unable to calculate (n too small/ no comparison)

Table A1.2 *Dog (n= 3212) signalment and demographics results of univariable analysis of factors associated with diet choice (either raw or non- raw diet)*

Variable (Dog) **Totals**	**Category** (Dog)	**N** **3212**	**% of total**	**Diet choice % (N)** *Non Raw* **45.4 (1458)**	*Raw* **54.6 (1754)**	**Odds ratio**	**95% CI**	**p value** (chi sq.)
Breed	Labrador	170	5.3	58.2 (99)	41.8 (71)	*Ref*		
	Border Collie	177	5.5	48.0 (85)	52.0 (92)	1.51	0.99, 2.31	
	Crossbreed	416	13.0	54.1 (225)	45.9 (191)	1.18	0.83, 1.70	
	Cocker Spaniel	152	4.7	46.7 (71)	53.3 (81)	1.59	1.02, 2.47	<0.01
	GSD	65	2.0	20.0 (13)	80.0 (52)	5.58	2.82, 11.01	
	Others	1323	41.2	42.6 (563)	57.4 (760)	1.88	1.36, 2.60	
	Unknown	909	28.3	44.2 (402)	55.8 (507)	1.76	1.26, 2.45	
KC group	Crossbreed	416	13.0	54.1 (225)	45.9 (191)	*Ref*		
	Hound	145	4.5	40.0 (58)	60.0 (87)	1.77	1.20, 2.59	
	Pastoral	341	10.6	37.5 (128)	62.5 (213)	1.96	1.46, 2.62	
	Terrier	171	5.3	52.6 (90)	47.4 (81)	1.06	0.74, 1.51	
	Toy	148	4.6	48.0 (71)	52.0 (77)	1.09	0.75, 1.58	<0.01
	Utility	136	4.2	38.2 (52)	61.8 (84)	1.90	1.28, 2.83	
	Working	97	3.0	23.7 (23)	76.3 (74)	3.79	2.28, 6.29	
	Gundog	609	19.0	47.5 (289)	52.5 (320)	1.30	1.02, 1.67	
	Unknown	909	28.3	44.2 (402)	55.8 (507)	1.49	1.18, 1.88	
	Unrecognised	240	7.5	47.5 (114)	52.5 (126)	1.30	0.95, 1.79	
Sex	FN	805	25.1	51.7 (416)	48.3 (389)	*Ref*		
	FE	341	10.6	28.2 (96)	71.8 (245)	2.73	2.08, 3.59	
	MN	720	22.4	53.5 (385)	46.5 (335)	0.93	0.76, 1.14	<0.01
	ME	408	12.7	36.8 (150)	63.2 (258)	1.84	1.44, 2.35	
	Unknown	938	29.2	43.8 (411)	56.2 (527)	1.37	1.14, 1.66	

Age (> 12 years as ref)	<6 months	68	2.1	25.0 (17)	75.0 (51)	4.04	2.19, 7.47	
	7-12 months	79	2.5	40.5 (32)	59.5 (47)	1.98	1.17, 3.35	
	1-4 years	816	25.4	42.2 (344)	57.8 (472)	1.85	1.36, 2.52	
	5-8 years	618	19.2	44.5 (275)	55.5 (343)	1.68	1.23, 2.31	<0.01
	9=11 years	337	10.5	54.0 (182)	46.0 (155)	1.15	0.81, 1.63	
	>12 years	209	6.5	57.4 (120)	42.6 (89)	*Ref*		
	Unknown	1085	33.8	45.0 (488)	55.0 (597)	1.65	1.22, 2.23	
Time owned	<3 months	113	3.5	41.6 (47)	58.4 (66)	0.98	0.60, 1.58	
	4-6 months	95	3.0	27.4 (26)	72.6 (69)	1.85	1.07, 3.17	
	7-9 months	77	2.4	33.8 (26)	66.2 (51)	1.36	0.78, 2.38	
	1 year	178	5.5	41.0 (73)	59.0 (105)	*Ref*		
	2 years	263	8.2	45.6 (120)	54.4 (143)	0.83	0.56, 1.22	
	3 years	221	6.9	47.5 (105)	52.5 (116)	0.77	0.52, 1.14	
	4 years	181	5.6	44.8 (81)	55.2 (100)	0.86	0.56, 1.30	
	5 years	166	5.2	49.4 (82)	50.6 (84)	0.71	0.46, 1.09	<0.01
	6 years	150	4.7	45.3 (68)	54.7 (82)	0.84	0.54, 1.30	
	7 years	115	3.6	52.2 (60)	47.8 (55)	0.64	0.40, 1.02	
	8 years	111	3.5	48.6 (54)	51.4 (57)	0.73	0.46, 1.18	
	9 years	84	2.6	54.8 (46)	45.2 (38)	0.57	0.34, 0.97	
	10 years	103	3.2	55.3 (57)	44.7 (46)	0.56	0.34, 0.92	
	11 years	75	2.3	41.3 (31)	58.7 (44)	0.99	0.57, 1.71	
	>12 years	129	4.0	55.8 (72)	44.2 (57)	0.55	0.35, 0.87	
	Unknown	1151	35.8	44.3 (510)	55.7 (641)	0.87	0.63, 1.20	

'Others' in breed represents all breeds represented at less than 2% in this survey.

Full quote tables for reasons for diet choice and health benefits/risks of diet choice

Table A1.3. *Reasons for raw diet choice volunteered by dog owners who fed RMD (N= 915) in response to the question 'What was the reason for choosing the current diet for your dog'. Reasons suggested for diet choice were selected from multiple choice answer options. Owners could select up to three options, and could elaborate further on their choices in a free text box. Additional reasons listed at the bottom of the table were further themes identified in the free text.*

Reasons Suggested for Diet Choice (raw feeders)	**N**	**Example Quotation**
Breeder Advice/ Came with a Starter Pack as a Puppy	91	"Following an IBD diagnosis our breeder recommended we researched the benefits of raw."
Advice from a Veterinary Professional	94	"Holistic Vet advised." "Decision was made after many months of illness from puppyhood to 11 months old...After no improvement in 4 months of treatment and continued weight loss, blood allergy panel was done. Results showed food allergies: Rice, Wheat, Corn, Soya, Potato. Vet advised unable to find a suitable commercial dog food and suggested homemade diet."
Believe it to be a More Natural Choice of Diet	744	"Dogs are carnivores so they eat meat, that's what they would eat in the wild had we not domesticated them. It's natural." "I don't 'believe' it to be more natural. It is! What was fed before kibble was invented?"
Coat Quality	378	"He was overweight and being an older dog I wanted him to have the best for his joints and coat." "I couldn't find a kibble she liked and would eat consistently and her stools were very loose on kibble and weight gain was poor. She was also very itchy and coat was thin, so wondered whether other ingredients in kibble weren't being tolerated by her very well."
Stool Consistency	475	"Firm stools to avoid anal gland issues, good coat, keep weight on even keel, overall health, close to what dog would fed in wild." "*Dog Name* failed to gain weight whilst on dry food despite trying lots of varieties/styles. His poo was frequent and soft. Vet tests found no underlying health issues so we decided to try raw, he quickly gained weight which is now stable."
Behavioural Reasons	143	"To improve and maintain optimum health of ex puppy farm and behaviourally difficult rescue dogs." "There are more than three reasons! Dental health, so odour, smelled well-formed stools, relaxed behaviour and more."

Cost	76	"Feeding raw costs me less than home cooked and my dog is allergic to chicken and intolerant to beef lamb and pork." "Cheaper to feed her with frozen meat than pre-prepared pet food."
Lack of Trust of Certain Foods	209	"I don't trust processed foods. especially when they're made by Mars or Nestle." "I don't trust big corporations to have my pets' best interests at heart. It's just profit for them."
My Dog Will Not Eat/ Doesn't Like Certain Foods	86	"*Dog Name* kept going off his food. Tried him on raw and he's never turned up his nose again." "My first dog wouldn't eat "dog food", so I tried raw."
To Address Existing Health Concerns	201	"*Dog Name* has allergies which are unmanageable on dry food." "Dog doesn't tolerate dry food and gets pancreatitis No problems at all since being fed raw diet." "I had a dog with lymphoma and was seeking ways to support her body condition. I came across a book which made a convincing case for feeding a raw/home cooked diet to dogs with cancer."
Additional reasons	**N**	**Example Quotation**
Body Condition	14	"They have well defined musculature and a neat tummy tuck." "Could not get weight down in kibble so raw diet recommended. Now at ideal weight." "She was not putting on weight, once on raw she blossomed."
Convenience	1	"Less waste and always a good consistency."
Dental Health	10	"All dogs healthy and great teeth. No discolouration or dental problems." "Better general health including oral. Teeth have no plaque."
Enrichment	2	"She also has a positive, enriching experience when eating her meals." "I think frozen raw bones as a treat give my dog an outlet for chewing and provide her with some enrichment."
General Advice	4	"Behaviourist recommendation." "Follow advice given in Ian Billinghurst Grow Your Pups With Bones and Tom Lonsdale BARF."
General Health	23	"To improve and maintain optimum health." "Continued to feed a diet I knew worked and came with many health benefits." "I believe raw food has improved the health of my pets."
Nutritional Content	20	"Raw food has no additives and more natural ingredients." "Nutritional content and quality of the food." "Dalmations need a low purine diet and by feeding raw I know exactly what she's eating."
Owner Control of Diet	5	"Going full DIY raw allows us to fully control his diet." "With raw feeding I can tailor their individual requirements much more easily than with kibble."
Owner Enjoyment	1	"I enjoy sourcing, preparing and providing my pup's food."
Owner Health	2	"My allergies flare up if Dog Name eats starches." "Having had mast cell tumours I want to avoid any histamine triggers."
Palatability	11	"Dog Name kept going off his food. Tried him on raw and he's never turned up his nose again." "And they absolutely love their food, no adding stuff to try to tempt my dogs to eat or left overs standing in bowls all day!"

Preventative Health	2	"Prevents any stomach upset." "Concern with using only kibble because of risks of GDV."
Previous Experience	9	"Knew the benefits of raw from previous experience." "Personal experience (have fed a raw diet life long with great results)."
Research	8	"In-depth look at various scientific studies on how adding fresh/raw affects canine health." "Own research and nutrition reviews."
Suitability	3	"I think every dog is different, my previous dog would never touch raw in any form but my current girl loves it." "I think it works well for each of my dogs."

Table A1.4: *Reasons for non-raw diet choice volunteered by dog owners who fed NRMD (N= 916) in response to the question 'What was the reason for choosing the current diet for your dog'. Reasons suggested for diet choice were selected from multiple choice answer options. Owners could select up to three options, and could elaborate further on their choices in a free text box. Additional reasons listed at the bottom of the table were further themes identified in the free text.*

Reasons Suggested for Diet Choice (non-raw feeders)	**N**	**Example Quotation**
Breeder Advice/ Came with a Starter Pack as a Puppy	73	"I feed *Brand Name* kibble as this is what my pup was weaned on to and she came with a starter pack."
Advice from Rescue Centre/ Charity	34	"The 2 rescue foster dogs are kibble as the rescue wants that fed."
Advice from a Veterinary Professional	410	"Advice from vet to have him on a commercial dry food for sensitive digestion, add medications/supplements to meal, add yoghurt/milk to make food softer." "*Brand Name* is recommended by vets."
Believe it to be a More Natural Choice of Diet	122	"Breed appropriate food i.e., salmon for skin & coat etc."
Coat Quality	190	"Wanted a high quality, high protein, preservative-free dog food that was as convenient as feeding kibble but more nutritious and results in better coat condition and stools."
Stool Consistency	325	"We struggled to get firm stools with some earlier foods, especially when he was under 1yr."
Behavioural Reasons	57	"Not too much protein as I understood it can exacerbate aggressive traits when she was younger." "Alertness."
Cost	231	"I work for *Company Name* and therefore, this was the natural choice as I receive a discount." "I want to provide the best nutrition that I can afford to buy, and want to protect my dogs from health issues that can be avoided or managed with diet."

Safety Concerns	54	"I don't want to give him raw meat and bones due to safety concerns re potential infection (or injury from jagged bones)." "I previously fed raw. When my dog was 3.5 he nearly died due to an autoimmune condition. We suppressed his immune system with medication and I was advised that continuing to feed raw at that time would be unsafe. I researched high quality kibble and had recommendations from friends."
Lack of Trust of Certain Foods	73	"*Brand Name* was ranked top on most websites by far. Started in raw and stools were perfect but vet kept putting me off. Now worried about *Brand Name* as it has been links to a heart scare in the US so am introducing *Brand Name* wet."
My Dog will not Eat/ Doesn't Like Certain Foods	106	"Both dogs are quite fussy and will not eat any other source of wet food." "Dog stopped eating previous kibble, tried free trial and worked well for him."
To Address Existing Health Concerns	205	"*Dog Name* on a derm diet to address atopy." "Hypoallergenic and gets bladder stones if not on the right food." "The food we give *Dog Name* is supposed to be good for dogs with epilepsy." "She suffers with pancreatitis so did some research on the food with the lowest fat content."
Additional reasons	**N**	**Example Quotation**
Body Condition	12	"Ensure a good weight is maintained." "Very slim dog, needs quite fatty tasty food to maintain good body score."
Compromise	3	"Compromise between what partner wants to feed and what I want to feed." "Not all members of the family agreed with raw feeding."
Convenience	25	"Easy to obtain dried kibble as part of weekly family food shopping." "I feed her whilst out walking/ training and it's a lot easier to carry kibble than raw minced meat!" "Carrying around semi defrosted raw meat is not feasible for us." "Do not have the facilities to safely store large quantities of raw food."
Dental Health	4	"Dry food keeps teeth healthy." "Dry food for better dental hygiene."
Enrichment	1	"We believe adding small amounts of carbs/ veg to the kibble provides the dog with a certain enrichment allowing them to smell and taste different foods."
Ethical Reasons	7	"Ethically sourced. They carry out no animal testing themselves." "Don't want meat in house." "Animal welfare and quality of produce govern my decision making when choosing dog food."

General Advice	4	"I chose grain free due to allergies, when they were dieting they were hungry a lot, so my current food was recommended by my trainer." "The addition of carbs to the diet came as advice from Toller showing community as a way to help satiate growing 'teenage' juniors without upsetting their stomachs by giving too much high protein kibble."
General Health	21	"Food didn't upset his stomach." "My dog's overall health and well-being." "Dogs work well and are super fit and healthy."
Nutritional Content	56	"I understand that manufactured dry dog food is specially designed to give my dog the perfect balance of protein, CHO, fat, vitamins and minerals and is safe for them to eat." "We think it's a good nutritious food for him." "It provides all the nutrition they need."
Owner Control	1	"I make her food so I know exactly what's in it."
Palatability	20	"He seems to go off foods very quickly and he has been consistent on this food for 4 years." "Trial and error to what they enjoyed the most."
Preventative Health	3	"Wanted a kibble that didn't swell greatly when wet, to help prevent GDV." "I want to protect my dogs from health issues that can be avoided or managed with diet."
Previous Experience	19	"I have fed *Brand Nam*e raw complete food in the past but the housekeeping routine made me aware of the risks to all family members in my family when thawing it out during hot weather." "It's what I've always known. I trust dog food to be formulated correctly."
Suitability	6	"I feel it is the best food available that suits my dog." "Quality of food and what suits my dogs."

Table A1.5. *Additional health risks of feeding the dog a RMD as volunteered by owners who chose to feed either RMD (N= 915) (i) or NRMD (N=916) (ii). Risks discussed are themes identified from the free text responses to the question 'Do you think there are any other risks of feeding a raw diet not listed above?' which was a follow-on question from the multiple-choice question 'Do you think feeding a raw diet has a positive or negative effect on aspects of canine health?'*

Other Risks of RMD (RMD feeders) (i)	**Example Quotation**	**Other Risks of RMD (NRMD feeders) (ii)**	**Example Quotation**
Choking, or other mention of risk of bones	"Only if not fed properly, e.g. not enough bone content, wrong type of bones." "Choking hazards with some animal materials."	Choking, or other mention of risk of bones	"Choking on bones." "Bones could lodge in the throat as he is only a small dog."
Constipation	"Some dogs swallow things whole which can cause an issue and too much bone leads to constipation."	Constipation	"Too much bone being fed causing constipation." "Massive constipation in dogs I walk for others that eat a raw diet."
Cost	"More expensive to buy."	Cost	"My main deterrents are freezer/storage space and cost."
Dehydration	"She doesn't drink a huge amount of water as she gets most of her fluids from her food, so this can sometimes be a concern that she's not hydrated enough."	General Health Concerns	"Many related adverse conditions." "Pancreatitis. Heart disease. Infections." "Pancreatitis as high fat Makes renal failure worse as high phosphate."

General Health Concerns	"Yes, if people feed too many weight bearing bones. I've known of a dog with immune medicated conditions not be able to tolerate raw. Also know of one dog who had a large amount of bone pieces that the dog couldn't pass. That dog was incredibly unhealthy, with many health issues though, and fed too many large bones."	Inconvenience/ freshness	"Keeping it fresh on holiday and long distance hiking trips with no freezer." "Difficult to keep fresh."	
Inconvenience/ freshness	"If want to buy fresh can only buy in fairly small quantities as very bulky to store."	Lack of Knowledge	"Poorly educated owners feeing inappropriate raw foods causing health problems to their dogs." "Minimal research been done by the raw food companies."	
Lack of Enrichment	"It can be more challenging with regards to enrichment options and using raw food as treats."	Obesity/ problems with weight	"Over feeding - obesity." "Obesity. As a vet nurse I have seen many obese dogs which are fed raw."	
Lack of Knowledge	"The only Risk with raw feeding is when people feed without understanding." "Those not doing there research properly before starting a raw diet can be a risk." "Inexperienced owners beginning a raw diet without substantial research and knowledge."	Poor Quality/ Poor Suppliers	"Quality of raw food is not guaranteed and may be of poor quality or contaminated." "Disease risk if not purchased from proper manufacturers."	
Poor Quality/ Poor Suppliers	"I think the only risk is if you don't use a reputable supplier." "There are risks if people feed inappropriate bones or if people buy from a non registered supplier (unregulated by Defra, or not a member of the PFMA)."	Safety	AMR	"Risk of acquisition of antibiotic resistant bacteria into flora."

Safety	Generic Risk acknowledged but not specified	"Just maintain proper hygiene. That's all." "Same risks as preparing meat for human consumption." "Obvious care should be used when handling raw food."		Generic Risk acknowledged but not specified	"Appropriate and safe and hygienic storage of raw food." "I was always very careful about separating and handling the raw food - always kept in sealed containers in the fridge away from other foods. Always washed hands thoroughly after feeding which I still do." "I just don't feel it's hygienic."
	Nutritional Risk	"Full research on suitable foods and ensuring the right percentages to give them a balanced diet are very important to minimise problems occurring from a raw diet." "Nutritional deficiency if someone attempts a DIY diet without conducting proper research."		Nutritional Risk	"Risk of malnutrition ie not giving the correct balance of ingredients all the time." "Dietary deficiencies especially in home made diets." "Calcium:phos unknown so risk for growing animals. TB. Secondary hyper parathyroidism."
	Parasites/ worms	"Parasites if raw meat isn't frozen for at least a week."		Parasites/ worms	"Yes- worm eggs can be present in some meats and can only be killed off by cooking." "Giardia." "Parasites infection."

	Pathogens, bacteria, disease	"Like any raw food there is an increased microbial hazard that needs to be managed for all the food consumed here."		Pathogens, bacteria, disease	"Salmonella, campylobacter, e.coli." "I would be concerned about risks to both pet and owners of things like salmonella, campylobactor bacteria from raw feed." "Bacteria shedding."
	Risk to humans/ public health	"Campylobacter in both pets and owners." "There could be risks to human health if hygiene standards are not maintained."		Risk to humans/ public health	"Risk to human health (touching raw components as well as bacteria from the food being spread to the feeding environment such as floors, walls etc." "Zoonotic risk to humans."
	Specific mention of risk towards Immuno-compromised, young or elderly	"Risk to immune suppressed adults & children." "Of course there are risks with everything, more so because I am immune compromised & i prepare & feed the dogs. I assessed the risks for both myself, my family and the dogs, the benefits far outweigh the risks & having good hygiene standards minimizes the risks to the humans in our family."		Specific mention of risk towards Immuno-compromised, young or elderly	"Human public health: immunocompromised, young and elderly." "Bacteria passing to humans especially if immunocompromised." "Risks to owners -especially immunocompromised adults, the elderly, pregnant women and children."

Table A1.6 *Additional health risks of feeding the dog a NRMD as provided by owners who chose to feed either RMD (N= 915) (i) or NRMD (N=916) (ii). Risks discussed are themes identified from the free text responses to the question 'Do you think there are any other risks of feeding a cooked, commercial diet not listed above?' which was a follow-on question from the multiple-choice question 'Do you think feeding a cooked, commercial diet (e.g. cooked fresh meat, wet complete diet or kibble) has a positive or negative effect on aspects of canine health?'*

Other Risks of NRMD (RMD feeders) (i)		**Example Quotation**	**Other Risks of NRMD (NRMD feeders) (ii)**		**Example Quotation**
Concerns with Faeces (aside from diarrhoea)		"Much smellier, larger and looser stools."	Concerns with faeces (aside from diarrhoea)		"Cheaper foods may cause intolerance- itchy skin, flatulent, poor stool consistency poor dental health."
Cost		"Expensive."	Cost		"No risks, but too expensive most of the time. You're paying for water and packaging."
Dehydration		"Dehydration issues/ kidney stones."	Dehydration		"Possible dehydration."
Health Concerns	General	"Long term illnesses." "Risk of giving your dog a life where he never felt his best." "Poor overall health, increased risk of disease."	Health Concerns	General	"Commercial biscuits and wet food carry health risks due to the poor quality ingredients as well as the additives to make it tasty. More diabetes and health issues around these days esp with cats."
	Auto-immune Disease	"Obesity and pathology of modern life like cancers and autoimmune diseases are more		DCM/ heart disease	"Heart disease with grain-free diets."

		likely to be a risk due to altering of the foods chemical state."			
	GDV	"Increased risk of GDV."		GDV	"Gorging food can lead to aerophagia and bloat." "Dry food with no water after exercise can cause GDV."
	Cancer	"Dog are more at risk of developing illnesses such as cancer, big pet brands have led to a lot of dog and cat deaths world wide." "Yes I believe there is a correlation between this diet and cancer. Obviously I have no scientific data on this but my work within the pet industry for 25 years has led me to feel very strongly that this is the case."	Ingredients		"Labelling is misleading." "Additives Colourings Preservatives Beet pulp Grain Potato Unnecessary things Meal Derivatives."
	DCM/ heart disease	"Grain free diets causing DCM."	Lack of enjoyment/ boredom		"Not much variety for dogs."
	Diabetes	"More likely to be obese and suffer from diabetes and pancreatitis."	Problems regarding weight		"Over feeding - obesity." "Over/underfeeding is common because it can be difficult to find the correct amount for individual dogs. Commonly, the amounts suggested by manufacturers can lead to over feeding."

	Kidney disease/ kidney stones	"Dry food increases risk of kidney failure."	Poor quality/ concerns with commercial		"Poor quality ingredients from some suppliers, difficult to assess quality." "Risks if poor quality detriment to gut health, behaviour, weight."
	Liver disease	"Yes, cancer, kidney failure, liver failure, autoimmune disease which will lead to an early death."	Safety	Nutritional Risks	"Nutritional imbalances also exist in these diets, particularly in cheap diets where little to no research has been done into appropriate composition." "Potentially lower in minerals and vitamins than raw diet."
	Pancreatitis	"Pancreatic damage from a carb heavy diet."		Pathogens, bacteria, disease	"The possibility of contamination." "Possible use of diseased or contamination meat products."
	Shortened life-span	"Shortened life span due to low quality food."		Improper storage	"Storage issues causing mould."
	Thyroid disease	"Higher risk of disease like cancers, liver/kidney problems, thyroid conditions etc."			
Harder to tailor		"Not being able to tailor ingredients to a specific dogs needs."			

Ingredients	"Too many fillers not enough protein. Not clearly labelled or misleading quality content of protein." "There is a clause about dog food which makes it legal for there to be anything in the dog food - it's full of additives and unnatural ingredients and products that aren't biologically available for a canine."
Lack of enjoyment/ boredom	"Boredom! Same bland stuff every day for life....that's just mean."
Problems regarding weight	"Easy to over feed, obesity problems." "Weight loss/gain."

Poor quality/ concerns with commercial		"The whole process of extrusion ... I have been to a dog food factory and witnessed the process from start to finish .... the fact that you have to coat the end kibble with all its 'marvellous' ingredients with fat and digest just to get the dog to eat it says a lot about kibble." "Most commercial food is poor quality with high carbohydrate content and GM fillers like soya hulls which are not appropriate for canines."
	Nutritional Risks	"Unbalanced, not enough natural vitamin nutrition content, cause deficiencies, causes kidney stones and diabetes on kibbles." "Required nutritional values destroyed by cooking."

Safety	Parasites	"Low stomach acids due to lacking demand for strong acids from lifeless, carb-laden diets leave the dog more vulnerable when bacteria are ingested. Higher likelihood of pancreatitis and other inflammatory diseases and parasite overload due to unhealthy gut environment."	
	Pathogens, bacteria, disease	"Many brands of kibble have been recalled on mass due to salmonella contamination." "Recalls, salmonella, fungus and bacteria, rancid fats."	
	Improper storage	"There is a small risk of salmonella and listeria if people dont store food correctly." "Improper storage could attract pests, particularly flies."	

Table A1.7. *Additional health benefits of feeding the dog a RMD as provided by owners who chose to feed either RMD (N= 915) (i) or NRMD (N=916) (ii). Benefits discussed are themes identified from the free text responses to the question 'Do you think there are any other benefits of feeding a raw diet not listed above?' which was a follow-on question from the multiple-choice question 'Do you think feeding a raw diet has a positive or negative effect on aspects of canine health?'*

Other Benefits of RMD (RMD feeders) (i)	**Example Quotation**	**Other Benefits of Raw (NRMD feeders) (ii)**	**Example Quotation**
Body Condition	"Better weight management, fewer obesity problems when fed raw." "Much easier to keep your dog at a healthy weight, with good lean muscles." "lower risk of obesity when feeding raw correctly."	Body Condition	"Weight, I had one dog who I was seeing and she was obese, tried every other diet under the sun to get her to lose weight, switched her to raw food with help from one of the nurses in the practice and made a huge difference."
Convenience	"Often cheaper for the owner, easier to obtain for us personally. Can always give off cuts to dog to prevent any food wastage on our part."	Enrichment	"May better satisfy a chewing behaviour." "Mental stimulation..."
Cost	"Less expensive."	General Health and Wellbeing	"Better for the dog if the dog can manage on a raw."
Enrichment	"Dog interested in food, bones provide stimulation that kibble doesn't." "Dog getting pleasure from variety and freshness of their meals. Raw meaty bones and whole prey are eaten slowly	Knowledge and Control of Ingredients	"It is easier to find out which ingredient might be affecting your dog's health." "Knowing and being able to control exactly what's in the dog's food."

	and offer enrichment and often help with stress reduction and relaxation."		
Ethical Advantages	"Easy. Sustainable. Plastic free."	More Natural	"Maybe it's more natural, and mimics what they would be eating." "It feels more natural for a dog to eat raw food."
General Health and Wellbeing	"In my 20+ years of experience it has meant far less health problems and has led to longevity and greater activity in older age." "Two of my dogs are coming up for 14yrs. They are both Rhodesian ridgebacks. So far they are showing no signs of cancer., skin growths, bad teeth or any of the other symptoms older dogs on commercial dog foods seem to be prone to."	Nutritional Content	"Often more wholesome, transparent food ingredients." "No additives - eg preservatives, sugar, cereal."
Knowledge and Control of Ingredients	"Complete understanding of what's going into your dog. No chemicals, bad additives, colours etc. Natural is best." "Absolute control of intake."	Owner Enjoyment	"Gives owners the belief they care for their dogs more by spending more time preparing food!" "Owner entertainment."

Longevity	"They live long and healthy lives, 13-16 years for my previous dogs." "Longevity of life, better joint outcomes in old age, enrichment for dogs eating real bones and food, variety for dogs."	Palatability	"Palatability and variation." "The dogs probably enjoy it more that kibble."
More Natural	"It's a more natural diet and contains no harmful byproducts." "Raw is the most natural food for dogs."	Stool Consistency	"Been told stools are fewer and former, and less smelly." "I could not say whether a raw diet had health benefits. I always worried about him getting the correct balance of nutrients. The only visible benefit I saw was an improvement in stool firmness."
Nutritional Content	"A purer diet, easier to spot and manage allergies, higher meat content and you know exactly what is in their food." "A balanced diet with various proteins and inclusive of correct ratios of bone, muscle meat and offal."		
Owner Enjoyment/ Strengthens human-animal bond	"The dogs love their food. I don't feed bones unless ground. Good food strengthens the bond between human and dog and thus improves behaviour."		

Palatability	"Dog seems happier to eat his food. Was very picky on kibble and choked on kibble more." "General happiness given to the dog vs dry food. For them to actually be excited for their meals and enjoy them."	
Preventative Health	"Lower risk of cancer, pancreatitis etc." "Less risk of GDV. Does not swell in the stomach the same way as extruded kibble unless you also feed cold pressed which breaks down."	
Stool Consistency	"Chronic diarrhoea often disappears and stool volume and odour can be significantly reduced. " " Consistent stool consistency & small size."	

Table A1.8. *Additional health benefits of feeding the dog a NRMD as provided by owners who chose to feed either RMD (N= 915) (i) or NRMD (N=916) (ii). Benefits discussed are themes identified from the free text responses to the question 'Do you think there are any other benefits of feeding a cooked, commercial diet?' which was a follow-on question from the multiple-choice question 'Do you think feeding a cooked, commercial diet (e.g. cooked fresh meat, wet complete diet or kibble) has a positive or negative effect on aspects of canine health?'*

Other Benefits of NRMD (RMD feeders) (i)	**Example Quotation**	**Other Benefits of NRMD (NRMD feeders) (ii)**	**Example Quotation**	
Consistency	"Consistency."	Better weight management/ regulation of feeding	"Easier to weigh and thus have body condition score control." "Fixed amount feeding easier to regulate weight."	
Convenience	"Easier to manage than raw. I feed tins and kibble on holiday." "Ease or feeding and convenience Easier to store Easier to use food as treats to 'remove the bowl'."	Improved stool consistency	"Weight loss achieved by excluding canned food and better stool consistency."	
Cost	"Can be cheaper than raw."	Consistency	"Years of research and study. Consistent feeding regime." "I would assume due to standards that the food is more consistent than a raw diet, allowing more control over what the dog eats."	
General Health	"Any good quality kibble will have benefits over a poor quality one so improvements would likely be seen in health/coat etc."	Convenience	General	"Easy and convenient to store and serve." "Less faffy to prepare. Treats are not as messy.... Aka, do not have to carry around raw meat when out."

Nutrition	"Nutritionally well balanced." "Easier to make sure dog has other nutrients in diet that are not in raw meat." "Contains all required nutrients, vitamins and minerals - no risk of imbalance."			Regarding training	"Useful to use as 'treats' as a training tool, especially if on a weight control diet. Convenient."
Safety	General hygiene	"You know they are safe."	Cost	"Cost. Consistency. Convenience." "Can be cheaper and easily accessible."	
	Lower risk of contamination, bacteria etc.	"Less likely to risk salmonella etc." "Less risk of infection/ contamination."	Nutrition	"I am happier knowing that he gets a nutritionally complete diet." "Peace of mind that it is prepared by a nutritionist. Safer for growing dogs in terms of mineral balance." "Avoiding dietary insufficiency."	
Suitability	"I know that some dogs are only suited by commercial diets." "Only if the dog cannot eat raw food."		Safety	General hygiene	"No concerns about kids in the household." "Convenience. Long shelf life. No/minimal risk to humans or other animals coming into contact with food."
Ability to tailor to specific needs	"If a dog has a very specific need then a veterinary prescription food could be the answer such as bladder issues."			Lower risk of contamination, bacteria etc.	"Easy and complete diet available with no raw contaminants to household." "Lower risk of contamination with bacteria, virus, parasites…."
Variety/ palatability	"Lots of variety."		Suitability	"Designed with dog in mind." "More balanced and better suited for dogs."	

	Ability to tailor to specific needs	"Convenient, balanced and researched to make sure my dog gets everything that they need. I can also tailor this nutrition to life stage and medical conditions they may develop in the future." "There are tailored diets designed to help with a variety of health issues which may not be achievable for an individual owner to achieve."
	Variety/ palatability	"Some of them can still provide enrichment in similar way to raw meats, with a bit of imagination ie puzzle feeders/kongs/working for food etc." "That the dog enjoys the food."

Complete quote tables for perceived wider risks of diet choice

Table A1.9: *Specific risks of NRMD as discussed by owners who feed NRMD and RMD in response to the question 'Please detail any specific risks related to the above categories that you believe could be present', which was a follow on free text question to the multiple-choice question 'Do you believe there are any health risks to the following categories associated with feeding a commercial, cooked diet (e.g. cooked fresh meat, wet complete diet or kibble)'*

Risks of NRMD as discussed by NRMD feeders				**Risks NRMD as discussed by RMD feeders**			
	Node	**N**	**Example Quotation**		**Node**	**N**	**Example Quotation**
	Allergies	29	"Cheap food can cause health & allergy problems." "Whether there is risk greatly depends on if your dog is allergic"		Allergies	35	"Not knowing the ingredients causing … allergic reaction." "Higher risk of allergies, digestive problems etc for the dog"
	Anal Gland Expression	1	"anal gland issues"		Anal Gland Expression	1	"Hence big stools and possible anal gland issues."

	Cancer	1	"Have heard raw food has lower cancer rates"		Cancer	8	"Cancer causing additives, allergies." " some chemicals in cheap dog food leading to cancer in canines" "known carcinogens in some commercial pet food"
					Behaviour	10	"Dog can be hyperactive on processed kibble" "I believe commercial foods cause many health and behavioural problems."
Cardiac Health	Cardiac Health	1	"Potential risk of heart disease with grain free diets"	Cardiac Health	Cardiac Health	2	"Obesity, heart failure" "Insufficient taurine caused by adding legumes to dog food causes heart problems."
	DCM	3	"There appears to be some correlation with particular types of diet and DCM." "Link between grain free food and DCM" "grain free diets and the link towards DCM"		DCM*	1	"Recent cases of DCM linked to kibble."
	Coat Health	1	"negative effects on the dogs health including but not limited to kidney, liver, stomach, coat"		Coat Health	2	"they always had oily smelly coats." "If that dogs coat wasn't in good condition and in turn had some skin condition"
	Cost	8	"Cheap food can cause health & allergy problems." "Cheap ingredients may be harmful" "Risk that lower cost products are nutritionally poor."		Dehydration	5	"Dog needs to drink more water to process food" "I believe too much dehydrated food causes dogs to drink excessively"
	Dehydration	1	"Need to ensure enough water when feeding dried food to stop dehydration"		Dental Health	19	"Bad dental hygiene." "Kibble…enables plaque build-up on teeth"
	Dental Health	7	"Poor quality diets may lead to poor dental or GI health." "Potential dental issues around consuming a commercial cooked diet" "risk of poor dental hygiene on wet food."		Diabetes	1	"Salmonella, imbalanced diet, highly processed ingredients, lack of transparency, cancer, diabetes"

Digestive Health	Digestive Health	11	“perhaps upset stomach if the food doesn't agree with the dog.” “Risk to dogs gut health if food is poor quality”	Digestive Health	Digestive Health	27	“upsets my dogs’ stomach.” “Allergies for dog and poor digestion.” “Digestive and immune health from high carbs”
	Diarrhoea	1	Risk to dogs health if the diet the owner chooses causes diarrhoea		Diarrhoea	5	“catching bugs from frequent D and V.” “Return of Diarrhoea”
	GDV	2	“dry kibble could play a part in GDVs” “Risk of bloat.”		Faeces	11	“The faeces are usually runny /slimy difficult to remove” “Dogs generally have soft stools fed on a commercial diet. so, a risk to small children”
General Risk	Less Risk Feeding Non-Raw	3	“Not really ‘no risk’ but I think its generally less likely to cause harm.”		GDV**	1	“Gastric torsion (bloat)”
	No Risk	17	“Don’t think there is any” “Nothing”		Vomiting	1	“frequent D and V.”
	Equivalent Risk	3	“I'm unconvinced that raw is significantly better than a good quality kibble”	General Risk	General Health Concerns	13	“Dog's general health and behaviour” “It's not healthy for her to eat kibble.”
	Unspecified Risk	3	“If the dog reacts to the food there can be a risk” “Potential to cause health problems from a dry diet?”		No Risk	6	“I assume there is no particular risk generally.”
	Hepatic Health	1	“negative effects on the dogs health including but not limited to kidney, liver”		Equivalent Risk	6	“There are risks no matter what you feed” “ALL diets carry some risk: choking, salmonella, etc

							but these are the same whether raw or kibble fed”
	Hygiene	6	“Still need to exercise good hygiene handling the food” “Hygiene rules apply also to cooked foods”		Unspecified Risk	12	“I think risk here is lower but still present.”
Ingredients	Ingredients	23	“Some risk if dog does not react well to ingredients in commercial food” “Ingredients may change and not be good” “Food may not be of highest quality ingredients.”		Hygiene	25	“Risk of illness if not following good hygiene” “Hygiene still important with kibble as it also contains bacteria potentially harmful to humans”
	Additives	6	“Harmful additives.” “additives may affect health over prolonged periods” “Unknown additives”		Immune System	5	“Digestive and immune health from high carbs, fillers and artificially added vitamins and minerals” “low immune system”
Nutrition	Nutrition	39	“Risk of manufacturer putting imbalanced ingredients ie vitamin toxicity (rare)” “Unbalanced foods may cause nutritional risks”	Ingredients	Ingredients	50	“Processed, ingredients added that are not conductive to canine health such as cereals for bulk” “Contents of some dog food is unreliable so may have ingredients dog is allergic to”
	Risk negated by Nutrition	7	“No risk provided diet is balanced and complete and appropriate for the dog's needs.”		Additives	22	“Too many unknown additives” “Not always very high nutritional content, full of artificial additives”
	Obstructions	2	“dog eating way to quickly and inhaling a kibble”		Nutrition	33	“Rubbish, poorly nutritious, high fat foods.” “Not getting basic nutrients needed for a healthy body”
	Pathogens & Bacteria	14	“Still potential for bacterial contamination” “All food can have bacterial contamination.”		Obstructions	2	“Cooked bones could splinter.” “Foreign bodies within food”
Pathogens & Bacteria	Parasites	2	“Allergies to storage mites” “Storage mite allergy, red meat intolerance”	Pathogens & Bacteria	Pathogens & Bacteria	28	“Bacteria contamination within food” “If food not stored in the correct way bacteria will grow and offer the same risks as raw food.” “Bacteria present in the food sometimes”

	Salmonella	4	“Salmonella is still present in wet food and kibble.” “Few studies have found Salmonella from kibble” “Salmonella from contaminated food”		Campylobacter	1	“Campylobacter”
	Quality of Food	26	“Risk if food quality is poor or contaminated” “Some commercial foods are known to be of very bad quality and ingredients.”		E. coli	4	“E. coli”
	Recalls	1	“Occasionally there are food recall alerts when something has been added/omitted to prepared food”		Listeria	1	“Listeria”
	Renal Health	4	“Feeding ‘dry food’ water must always be available or kidney problems could occur” “potentially dangerous in the long term condition of the kidneys.”		Salmonella	48	“I have seen some kibble brand recalls due to Salmonella” “Salmonella contamination has been found more regularly in Kibble! That's a health risk”
	Risk to In-Contact Dogs	1	“Dogs eating food and licking other dogs that may be allergic to products in the food”		Parasites	6	“I believe fewer raw fed dogs have parasites” “storage mites”
	Risk to In-Contact People	1	“All food can have bacterial contamination. So care with immunocompromised people of children.”		Quality of Food	26	“If poor quality food poorer all-round health” “Bad quality commercial diets can cause various issues”
	Skin Health	2	“Dietary intolerance may lead to dermatological disease.”		Recalls	24	“Poor health of the dog and many instances of Salmonella driven kibble recalls” “Number of recalls on commercial made dog food.”
	Storage and Manufacture	18	“There could always be the risk of contamination somewhere in the production chain” “If a bag is damp etc causing mould.”		Renal Health	2	“now deceased dog was on *Brand Name* and had to be tested for kidney damage” “Too much dehydrated food causes dogs to drink excessively and must risk damage to their kidneys.”
	Weight Management	9	“Some dog food brands like Pedigree have high sugar content so the dogs can put more weight on” “Possible overfeeding and weight gain”		Risk to In-Contact Dogs	5	“If that dogs coat wasn’t in good condition and in turn had some skin condition that could be

							contagious.” “Threats to overall health of pets and those that come into contact.”
					Risk to In-Contact People	5	“Don't know what's in the food so could cause an allergic reaction to someone handling the food” “Bacteria build up in the mouth can spread to people”
					Skin Health	10	“My dogs skin deteriorates on commercial kibble.” “It can affect their skin and make them sluggish I find”
					Storage and Manufacture	21	“Contamination of kibble at source, incorrect and dangerous mistakes in the making of the food.” “Still possible contamination or fungal deterioration due to poor packaging/storage”
					Weight Management	15	“Long term risk from eating highly processed, non-species appropriate food including obesity” “Overfeeding from bad feeding guides and hidden sugars”

Table A1.10: *Specific risks of RMD as discussed by owners who feed NRMD and RMD in response to the question 'Please detail any specific risks related to the above categories that you believe could be present', which was a follow-on free text question to the multiple-choice question 'Do you believe there are any health risks to the following categories associated with feeding a raw diet'*

Risks of RMD as discussed by NRMD Feeders				**Risks of RMD as discussed by RMD Feeders**			
	Node	**N**	**Example Quotation**		**Node**	**N**	**Example Quotation**
	Anal Gland Expression	1	"anal glands not effectively emptied."		Allergies	1	"Some risk with allergies if I feed my dog raw chicken."
	Behaviour	3	"behaviour issues (high protein)." "Behaviour may become more aggressive"	Digestive Issues	Digestive Issues	5	"fresh raw food could cause digestive issues" "Certain raw food can also be too rich for some dogs also eg offal"
	Dental Health	6	"Tooth fracture" "Slab fractures of teeth"		Constipation	1	"The balance of bone and meat and veg can result in constipation if not judged correctly"
Digestive Issues	Digestive Issues	10	"Stomach bugs/issues" "I feel the raw diet may be too rich for my dogs, but cannot base this on fact."	General Risk	Equivalent Risk	22	"Everything has a risk" "No difference to feeding dry"
	Constipation	1	"constipation (bone)"		Less Risk Feeding Raw	5	"we have fed mainly raw for over 60 years and have had fewer problems while feeding raw" "I believe there to be less risk by feeding raw. The stools are a much better consistency to clean up"

	Diarrhoea	3	“v+ & d+” “Bacterial spread, choking, diarrhoea”		No Risk	22	“Why could there possibly be a risk, bar stupid scaremongering?” “No risks from me and my dog.”
	Vomiting	2	“v+ & d+” “The chance of causing vomiting and diarrhoea probably from salmonella etc”		Unspecified Risk	4	“The usual risk that come with feeding raw” “Dogs are animals and they pose a health risk by their very nature.”
General Risk	Equivalent Risk	3	“I don't think there is much of an increased risk with dogs licking after raw food than at any other time” “There is risk with any food product!”	Hygiene	Hygiene	177	“If you don’t follow good hygiene practices there’s a risk of contamination as with any raw meat”
	No Risk	1	“If there was significant risk to people or animals surely it would be sold in the UK”		Risk negated by Hygiene	40	“The human element could be minimised by practicing good hygiene” “Food preparation is the risk for us, but we practise good hygiene”
	Unspecified Risk	9	“Fact of raw food, especially chicken”		Lack of Research	8	“Lack of knowledge/education is an issue” “A risk if people do not research how to feed a balanced raw diet properly”
Hygiene	Hygiene	49	“Requires more diligence dealing with raw meat products hygienically” “Preparing raw meat and lack of hygiene.”		Nutrition	27	“insufficient nutrition from an unbalanced diet.” “You need to ensure dog gets all required nutrients via a balanced diet”

	Risk negated to Hygiene	2	“As long as you are hygienic I see no risk.” “All down to source of food and good food hygiene so shouldn't be a problem”	Obstructions	Bone Splinters	18	“bone obstruction in dog.” “in raw food diet there may be some bones that have not been processed that are too big for a dog”
	Lack of Research	1	“Also, the risk of the diet not providing the correct nutrients for your dog if you prepared it with little knowledge about necessary ingredients for a healthy dog”		Choking & Other Obstructions	8	“Eating too quickly with bone can lead to choking if dog is a ‘gulper’ supervision needed” “Risk to dog of choking on bone - mitigated by supervision”
	Nutrition	25	“Incomplete nutrition” “Not balanced or specific to life stage.”	Pathogens & Bacteria	Pathogens & Bacteria	57	“Problems with parasites or bacteria being spread” “Risk of bacterial infection if not stored correctly”
Obstructions	Bone Splinters	28	“Relating to bone pieces piercing the bowel etc.”		Campylobacter	4	“Campylobacter”
	Choking & Other Obstructions	7	“Choking on bones.” “choking on cartilage/gristle to dog”		E. Coli	2	“bacteria such as E. coli”
	Foreign Bodies	15	“gastric FB” “Foreign bodies insides stomach/intestines Illness related to ingesting raw meat”		Parasites	10	“Possible parasite issues?”
Pathogens & Bacteria	Pathogens & Bacteria	177	“Passing on bacteria” “Potential risk of harmful bacteria and infectious diseases”		Salmonella	25	“There could be a small risk to me around food prep & bacteria eg salmonella”
	AMR	1	“Transmission of antibiotic resistant bacteria.”		Tuberculosis	1	“TB”

	Campylobacter	58	“I've had campylobacter from a dog that was raw fed, it jumped up and licked me before I could stop it” “Zoonotic diseases like Campylobacter”		Zoonoses	2	“Zoonotic disease but normal hygiene will prevent unless immune compromised individual”
	E.Coli	44	“Infections such as E.coli” “The spread of lethal bugs like e-coli”		Quality of Food	15	“Dog’s health - bad batch of food” “Poor food hygiene and poor food quality could present risks to me.”
	Giardia	2	“Giardia”		Risk to In-Contact Dogs	2	“Infected meat can make the dog and own ill, risk of passing infection or worms onto other dogs” “If my dog were to have salmonella poisoning and lick a person or dog who wasn’t healthy this would be a risk.”
	Listeria	5	“Shedding of harmful bacteria has been seen (i.e. Listeria)”		Risk to In-Contact People	8	“Raw food carries bacteria, others could come in contact if it’s not done properly” “Like any raw meat if you don’t clean up you could make people sick”
	Parasites	50	“Possible worm issues” “Parasite transmission.”		Immunocompromised, Elderly & Children	40	“you need to be mindful of him licking other people especially if they may have a health condition” “Risk to children & immune suppressed adults” “Worry of

							dog name licking children on the face after eating raw"
	Salmonella	141	"If people don't clean correctly after their dog has finished eating then the risk of salmonella is high." "Salmonella and other bacterial contaminants associated with raw meats" "Salmonella is the only thing I know can be a risk"		Licking	34	"Spreading bacteria from dog to human by licking." "Things like salmonella could be passed especially from a lick"
	Tuberulosis	2	"Tuberculosis"		Weight Management	1	"rare they overeat which could be a problem. Usually fit and healthy"
	Zoonoses	30	"Transmissible diseases/zoonosis" "Risk of spread of zoonotic diseases"				
	Quality of Food	25	"I would be concerned that the raw food may not be at it's freshest" "Poorly manufactured raw food could harbour harmful bacteria"				
	Risk to In-Contact Dogs	17	"If food is left down too long can be a risk to your dog or other animals." "Bacterial infection and illness to humans and other dogs."				
	Risk to In-Contact People	30	"Would want to risk my dog transferring bacteria from raw meat to humans" "Transfer of bacteria between dogs and humans as not everyone takes hygiene very seriously." "Transmission of bacteria from raw meat to myself and my surroundings"				

	Immunocompromised, Elderly & Children	33	“I worry about bacteria as I’m immune-comprised” “dog comes into contact with immunocompromised people or children then likely to be more at risk”				
	Licking	15	“Cross contamination from bowl, worktops, and dog licking people and toys/bed etc” “If the dog eats raw food and then licks other people it could spread bacteria”				
	Weight Management	1	“Ensuring that a nutritionally and calorie-sufficient diet is being provided every day - easier to overfeed/underfeed without guidelines”				

Appendix 2: Appendices for Chapter 3

Table A2.1: *Frequency (N) and percentage (%) of sources of non-pre-prepared raw food provided to dogs fed RMD (n=1754) diet. Both food sources included in the survey as multiple-selection answers and those detailed additionally as free text answers by dog owners within the 'other' category are listed. Sources represented at <1% were excluded.*

Source	**% (N)**
Total	**1754**
Supermarket	38.4 (673)
Butcher	37.8 (663)
Farm Shop	13.3 (234)
Dedicated raw supplier	7.5 (131)
Abattoir	4.9 (86)
Market Stall	3.8 (67)
Online	1.7 (29)
Pet shop	1.7 (29)
Game dealer	1.5 (27)
Hunter	1.3 (22)

Table A2.2: *Frequency (N) and percentage (%) of types of treats provided to dogs fed RMD (N=1754) diet and those fed NRMD (N=1458) diet. Both treat types included in the survey as multiple-selection answers and those detailed additionally as free text answers by dog owners within the 'other' category are listed (indicated by *).*

Treats	**% (N)**	
	Raw	*Non-Raw*
Total	**55.0 (1754)**	**45.0 (1458)**
Freeze dried meat/fish treats	56.8 (997)	27.5 (401)
Raw bones	56.2 (986)	-
Dried treats (e.g. pig ears, rawhide, chicken feet)	55.5 (973)	35.0 (510)
Raw meat (including body parts such as feet, hooves)	43.0 (754)	-
Shop bought cooked treats/biscuits	36.5 (640)	78.7 (1148)
Cooked meat	18.1 (318)	27.3 (398)
Homemade treats*	3.9 (68)	2.0 (29)
Dehydrated meat *	3.4 (59)	0.7 (10)
Vegetables *	3.1 (55)	6.5 (95)
Fruit *	2.1 (36)	2.0 (29)
Dairy *	1.9 (33)	2.8 (41)
Dehydrated offal *	1.9 (34)	0.1 (2)

Miscellaneous *	1.9 (33)	1.9 (27)
Liver *	1.5 (27)	1.0 (14)
Oily fish*	1.4 (24)	0.5 (7)
Fish *	1.1 (19)	0.7 (10)
Cooked bones	1.0 (17)	5.6 (81)
Dried/frozen rabbit ears *	0.7 (12)	-
Leftovers *	0.7 (12)	0.8 (11)
Filled bones*	0.3 (5)	-
I don't feed any treats	2.4 (42)	1.7 (83)

Table A2.3: *Frequency (N) and percentage (%) of the top 20 brands of pre-prepared raw diets provided to dogs fed RMD in this survey*.*

Brand	**% (N)**
Total	**1754**
DA	17.0 (298)
NU	13.5 (236)
NM	13.0 (228)
DB	12.4 (217)
PR	10.6 (185)
BD	6.7 (118)
BU	5.9 (103)
BE	5.0 (88)
MV	3.8 (66)
NR	3.5 (62)
HR	3.3 (57)
FF	3.2 (56)
CR	3.1 (55)
JN	3.0 (52)
RF	2.9 (50)
HE	2.4 (42)
LW	2.2 (38)
TD	2.1 (36)
AL	1.6 (28)
KB	1.5 (26)

*Samples from first 10 brands selected for microbiological testing

Table A2.4: *Frequency (N) and percentage (%) of the top 20 brands of pre-prepared non-raw diets provided to dogs fed a non-raw diet in this survey*.*

Brand	**% (N)**
Total	**1458**
RC	12.6 (184)
JW	8.4 (122)
HI	7.7 (112)
FG	6.2 (91)
LK	6.2 (91)
WW	5.4 (79)
AG	4.7 (68)
HA	4.6 (67)
BU	4.5 (65)
TA	4.5 (65)
MW	4.3 (62)
CH	3.8 (56)
SK	3.8 (55)
PU	3.6 (53)
BU	3.2 (47)
NM	2.6 (38)
PE	2.5 (37)
AV	2.5 (36)
EU	2.3 (33)
BA	2.2 (32)

*Samples from first 10 brands selected for microbiological testing

Table A2.5: *Presence of batch numbers on packets and source of meats used as ingredients in RMD brands tested in this study, alongside whether the products were made in the UK.*

Anonymised Brand	Batch Number Present	Meat Source	Product Made in UK	Packet material	Pack damaged on arrival	Leakproof packet	Other Information
B1	No	Unknown	Unknown	Film/thin plastic	No	No	
B2	Yes	UK	Yes	Plastic tub	Yes	No	
B3	No	Unknown	States manufactured in Britain	Plastic with film lid	Yes	No	
B4	Yes	UK	States British ingredients	Plastic tub	Yes	No	Batch number on sticky label for some products, not present on all. Numbers printed on back of some packs but unclear if batch number
B5	Yes	Unknown	Yes	Cardboard	No	No	Batch number on sticky label. Meat source unknown, states organic and ethically sourced
B6	No	UK	States packed in UK	Cardboard	Yes	No	
B7	No	Unknown	Unknown	Plastic with film lid	No	Yes	
B8	Yes	UK	States British meat	Plastic with film lid	No	Yes	

B9	Yes	UK	States British meat	Plastic film wrapped with metal clamps on ends	No	No	
B10	Yes	Unknown	Unknown	Thin flexible plastic with film lid	No	No	

Table A2.6. *Bacterial enumeration results for RMD (N=110 samples). Samples would fail DEFRA testing if they are found to contain bacterial counts of E. coli or other Enterobacteriaceae greater than 5000 CFU/g.*

Brand	Sample number	Flavour	Average *E. coli* CFU/g	Average other Enterobacteriaceae CFU/g	Pass *E. coli*? (1 sample tested, fail if >5000 CFU/g)	Pass *Enterobacter*? (1 sample tested, fail if <5000 CFU/g)
B1	NM1	Lamb with chicken	0	500	Pass	Pass
	NM2	Chicken and salmon	0	1000	Pass	Pass
	NM3	Beef	26000	7167	**Fail**	**Fail**
	NM4	Chicken with tripe	1333	8667	Pass	**Fail**
	NM5	Lamb with chicken	0	333	Pass	Pass
	NM6	Chicken and salmon	0	333	Pass	Pass
	NM7	Beef	26667	6333	**Fail**	**Fail**
	NM8	Chicken with tripe	833	3500	Pass	Pass
	NM9	Lamb with chicken	0	167	Pass	Pass

	NM10	Chicken and salmon	0	833	Pass	Pass
	NM11	Beef	29833	7333	**Fail**	**Fail**
	NM12	Chicken with tripe	2833	7167	Pass	**Fail**
	NM13	Mixed offal and salmon	0	1167	Pass	Pass
	NU1	Tripe	333	167	Pass	Pass
B2	NU2	Lamb	0	0	Pass	Pass
	NU3	Duck	0	0	Pass	Pass
	NU4	Offal	37667	13167	**Fail**	**Fail**
	NU5	Tripe	4500	3500	Pass	Pass
	NU6	Turkey	500	667	Pass	Pass
	NU7	Turkey	0	0	Pass	Pass
	NU8	Chicken	0	0	Pass	Pass
	NU9	Beef	3667	8500	Pass	**Fail**
	NU10	Rabbit	0	0	Pass	Pass
	NU11	Duck	0	167	Pass	Pass
	NU12	Offal	21500	12000	**Fail**	**Fail**
	NU13	Beef	10833	14667	**Fail**	**Fail**
	NU14	Lamb	0	0	Pass	Pass
	NU15	Chicken	0	167	Pass	Pass
	DU1	Beef	5667	10000	**Fail**	**Fail**
B3	DU2	Chicken	0	0	Pass	Pass
	DU3	Offal	0	0	Pass	Pass
	DU4	Rabbit	0	0	Pass	Pass
	DU5	Duck	167	500	Pass	Pass

	DU6	Game and tripe	167	1000	Pass	Pass
	DU7	Duck	167	167	Pass	Pass
	DU8	Lamb	256667	105000	**Fail**	**Fail**
	DU9	Chicken	0	0	Pass	Pass
	DU10	Turkey	0	0	Pass	Pass
	DU11	Chicken and salmon	0	167	Pass	Pass
	DU12	Game and tripe	167	0	Pass	Pass
	DU13	Beef and tripe	0	0	Pass	Pass
	DU14	Lamb	126667	68333	**Fail**	**Fail**
	BE1	Goat	833	40833	Pass	**Fail**
B4	BE2	Beef	1000	12167	Pass	**Fail**
	BE3	Lamb	0	2333	Pass	Pass
	BE4	Beef	0	667	Pass	Pass
	BE5	Goat	833	67333	Pass	**Fail**
	BE6	Lamb	0	1333	Pass	Pass
	BE7	Chicken	0	833	Pass	Pass
	BE8	Turkey	0	167	Pass	Pass
	BE9	Turkey	0	0	Pass	Pass
	PR1	Duck	833	102667	Pass	**Fail**
B5	PR2	Beef tripe mince	0	0	Pass	Pass
	PR3	Chicken (carcass mince)	0	0	Pass	Pass
	PR4	Pork, Chicken	62167	280000	**Fail**	**Fail**
	PR5	Turkey	18000	10667	**Fail**	**Fail**
	PR6	Chicken	1000	2167	Pass	Pass

	PR7	Duck	1333	17333	Pass	**Fail**
	PR8	Beef mince	0	667	Pass	Pass
	PR9	Chicken	833	1167	Pass	Pass
	PR10	Beef tripe mince	167	0	Pass	Pass
B6	NA1	All lamb	20000	50000	**Fail**	**Fail**
	NA2	Chicken, beef, beef tripe, lamb and beef offal	3667	2667	Pass	Pass
	NA3	Duck	0	0	Pass	Pass
	NA4	Chicken	0	0	Pass	Pass
	NA5	Venison	11500	1667	**Fail**	Pass
	NA6	Chicken	167	0	Pass	Pass
	NA7	Duck	0	0	Pass	Pass
	NA8	Tripe and heart	141667	60000	**Fail**	**Fail**
	NA9	Beef and offal	288333	58333	**Fail**	**Fail**
B7	DB1	Pork mince with chicken	11000	200000	**Fail**	**Fail**
	DB2	Duck mince	0	1000	Pass	Pass
	DB3	Minced pigeon	473333	0	**Fail**	Pass
	DB4	Lamb, fish with turkey	131667	130000	**Fail**	**Fail**
	DB5	Ox tripe mince	500	38333	Pass	**Fail**
	DB6	Tripe and oily fish	1000	13833	Pass	**Fail**
	DB7	Pig pluck mince	18000	5667	**Fail**	**Fail**
	DB8	Ox mince	56667	73333	**Fail**	**Fail**
	DB9	Venison	28333	56667	**Fail**	**Fail**
	DB10	Chicken mince	333	1667	Pass	Pass
	CR1	80/20 chicken mince	0	1500	Pass	Pass

B8	CR2	80/20 turkey mince	5500	1833	**Fail**	Pass
	CR3	Wild boar and duck	333	333	Pass	Pass
	CR4	80/20 chicken mince	0	1500	Pass	Pass
	CR5	80/20 turkey mince	4333	2667	Pass	Pass
	CR6	80/20 beef and tripe mince	0	167	Pass	Pass
	CR7	Rabbit and venison	0	0	Pass	Pass
	CR8	70/30 lamb	833	167	Pass	Pass
	CR9	Duck and venison	0	0	Pass	Pass
	CR10	70/30 beef	0	1667	Pass	Pass
B9	HR1	Chicken	110000	170000	**Fail**	**Fail**
	HR2	Beef	167	0	Pass	Pass
	HR3	Lean lamb	500	4833	Pass	Pass
	HR4	Lean turkey	0	1500	Pass	Pass
	HR5	Venison	2500	10333	Pass	**Fail**
	HR6	Turkey	0	0	Pass	Pass
	HR7	Lamb	333	1000	Pass	Pass
	HR8	Duck	0	833	Pass	Pass
	HR9	Duck	833	17667	Pass	**Fail**
	HR10	Pork	0	0	Pass	Pass
B10	FF1	Lamb tripe	341667	50000	**Fail**	**Fail**
	FF2	Goose	0	667	Pass	Pass
	FF3	Lamb and lamb tripe	18167	6500	**Fail**	**Fail**
	FF4	Beef	500	98333	Pass	**Fail**
	FF5	Pork	167	1667	Pass	Pass

	FF6	Lamb 80/10/10	7000	3667	**Fail**	Pass
	FF7	Duck	0	3000	Pass	Pass
	FF8	Duck and lamb tripe 80/10/10	33167	1500	Fail	Pass
	FF9	Turkey and lamb	1167	500	Pass	Pass
	FF10	Chicken and salmon	2500	3667	Pass	Pass

Table A2.7: *Percentage (%) and number (N) of RMD samples with ESBL-producing and 3GCR E. coli present, and the associated food protein types*

Protein type	**% (N) samples ESBL- *E. coli***	**% (N) samples 3GCR- *E. coli***
Total	**15**	**18**
Offal/Tripe	46.7 (7)	33.3 (6)
Chicken	31.3 (5)	22.2 (4)
Beef	18.8 (3)	16.7 (3)
Lamb	18.8 (3)	16.7 (3)
Duck	12.5 (2)	27.8 (5)
Goat	12.5 (2)	11.1 (2)
Fish	6.3 (1)	5.6 (1)
Game	6.3 (1)	5.6 (1)
Pork	6.3 (1)	5.6 (1)
Pigeon	0.0 (0)	0.0 (0)
Rabbit	0.0 (0)	0.0 (0)
Turkey	0.0 (0)	0.0 (0)
Venison	0.0 (0)	0.0 (0)
Other	0.0 (0)	0.0 (0)

Table A2.8: *Inc group plasmids associated with STs and ESBL genes of interest from ESBL-producing E. coli isolated from raw dog food*

ESBL gene	**Gene number**	**STs associated**	**Plasmids associated**
bla$_{CTX-M}$	1	10	IncI1-I(gamma)
	15	10, 48, 542, 4096, 4681	IncFIA(HI1), IncFIA, IncFIB, IncFIB(K), IncFIC(FII), IncFII(pCoo), IncFII|AY458016, IncHI1A, IncHI1B(R27), IncI1-I(gamma)
	27	69	IncFIA, IncFIB, IncFIC(FII)
	55	58	IncFIA, IncFIB, IncFII, IncX1
bla$_{TEM}$	52	1629	IncFII, IncI1-I(gamma), IncX1, IncY
bla$_{SHV}$	7	10	IncI1-I(gamma)
bla$_{CMY}$	2	69, 155, 602, 6958	IncB/O/K/Z, IncFIA, IncFIB, IncFIC(FII), IncI1-I(gamma), IncI2(Delta)

Appendix 3: Appendices for Chapter 4

Study information letter

A Dog's Dinner Phase 2: Study of Canine Faecal Bacteria

You are being invited to participate in a research study. Before you decide whether to participate, it is important for you to understand why the research is being conducted and what it will involve if you do choose to take part. Please consider the following information. Researcher contact details are listed below should you have any further questions.

Once you have read this information sheet, please indicate your consent to participate in this study by completing the accompanying consent form.

This study has full ethical approval from the University of Liverpool.

What is the purpose of the study?

Choice of food is an important consideration for dog owners, and is an area of ownership where owners have direct impact on the care of their dog. Therefore, the decision on what to feed is something many owners think about very carefully. This study aims to investigate and compare the types of bacteria present within the faeces of dogs that are fed on a range of diets including those fed cooked and raw meat, along with the presence of any bacteria which are resistant to antibiotics.

Why am I being invited to take part and what will happen if I take part?

You are being invited to take part because you are a UK dog owner who has already completed the online 'Dog's Dinner' survey, and agreed to be contacted further, or have responded to an advertisement for this study and indicated your willingness to participate.

If you decide to take part you in this phase of the study, you will need to complete the accompanying short questionnaire, which will take around 10 minutes, and supply a small sample of your dog's faeces (poo) using the collection pot provided. Please return the completed questionnaire, consent form and faecal sample together using the pre-paid envelope provided.

Participation is entirely voluntary and you do not have to take part in this study. You do not have to give a reason if you do not wish to take part.

Are there any benefits or risks in taking part?

There are no direct benefits or risks to you associated with taking part in this study.

What will happen if I want to stop taking part?

If you want to stop taking part in this study you can contact the named personnel at the end of this letter, using the details provided. If you wish to withdraw from the study, you may do so up to 14 days following our receipt of your completed questionnaire, and may request removal of your questionnaire data and destruction of the faecal sample you have provided, as well as any associated

microbiological data. After 14 days it will not be possible to remove this as it will have been anonymised and incorporated into our analysis.

What will happen to the results of the study?

The results of this study will be used to determine the types of bacteria present in the faeces of dogs fed raw diets and those fed cooked diets, whether there is an increased risk of bacteria which have resistance to antibiotics or could possibly cause illness in dogs and humans. We hope the results help us better understand if there are any bacterial risks to both dogs and humans of feeding different diets.

Ultimately, we would like to provide information which could lead to feeding practices which are safer for both dog and owner.

Due to anonymisation of samples and data, it will not be possible to inform you of individual faecal sample test results.

How will my data be used?

The data you provide will be stored securely for 10 years in line with data protection requirements at the University of Liverpool and GDPR. All data is strictly confidential and will be used for this specific project only, and a limited number of people will have access to it.

The University processes personal data as part of its research and teaching activities in accordance with the lawful basis of 'public task', and in accordance with the University's purpose of "advancing education, learning and research for the public benefit".

Under UK data protection legislation, the University acts as the Data Controller for personal data collected as part of the University's research. The Principal Investigator acts as the Data Processor for this study, and any queries relating to the handling of your personal data can be sent to Professor Nicola Williams (Principal Investigator) using the contact details below.

What if I am unhappy or if there is a problem?

If you are unhappy, or if there is a problem, please feel free to contact the researcher listed below and we will try to help. If you remain unhappy or have a complaint which you feel you cannot communicate directly to the researcher then you should contact the Research Ethics and Integrity Office on 0151 794 8290 (ethics@liv.ac.uk). When contacting the Research Governance Officer, please provide details of the name or description of the study (so that it can be identified), the researcher involved, and the details of the complaint you wish to make.

Who can I contact for further details?

Miss Genever Morgan
Institute of Infection and Global Health
University of Liverpool, Leahurst Campus
Chester High Road

CH64 7TE
Email: ddsurv20@liverpool.ac.uk

Professor Nicola Williams
Institute of Infection and Global Health
University of Liverpool, Leahurst Campus
Chester High Road
CH64 7TE
Email: ddsurv20@liv.ac.uk

Participant consent form

Title of the research project: **A Dog's Dinner Phase 2: A cross sectional study of canine faecal bacteria**

Name of researcher(s): Genever Morgan, Professor Nicola Williams, Dr Gina Pinchbeck, Dr Vanessa Schmidt

Please initial box

1. I confirm that I have read and have understood the information sheet dated 30/3/20 for the above study, or it has been read to me. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that taking part in the study involves completion of the attached questionnaire and submission of a faecal sample from my dog.

3. I understand that my participation is voluntary, that I am free to stop taking part and can withdraw from the study at any time up to 14 days from our receipt of your completed questionnaire without giving any reason and without my rights being affected. In addition, I understand that I am free to decline to answer any particular question(s). ☐

4. I understand that I can ask for access to the information I provide and I can request the destruction of that information, plus destruction of the sample I have provided and any associated microbiological data, if I wish at any time prior to 14 days from our receipt of your completed questionnaire. I understand that following 14 days I will no longer be able to request access to or withdrawal of the information or sample I provide. ☐

5. I understand that the information I provide will be held securely and in line with data protection requirements at the University of Liverpool. Data will be stored for up to 10 years by the University of Liverpool. ☐

6. I understand that signed consent forms and original questionnaires will be held securely and in line with data protection requirements at the University of Liverpool. ☐

7. I agree that my data may be shared within the research team named above and used in future research if reviewed and approved by the ethics committee. I ☐

understand that my data will be **fully anonymised** and will not be identifiable in any published reports.

8. I agree to being contacted at a later date and invited to take part in future studies **(OPTIONAL)**. I understand that I am only agreeing to receive information and I am under no obligation to take part in any future studies. If you decide not to consent to being contacted in the future it will not have any influence on your involvement in this particular research study ☐
9. I agree to take part in the above study. ☐

Please sign this page to indicate your consent to participate in this study.

_____	_____	_____
Participant name	Date	Signature
_____	_____	_____
Name of person taking consent *(if applicable)*	Date	Signature

The faecal sample you provide will be specifically tested for *E.coli* and *Salmonella* in this study. We may wish to test for additional zoonotic pathogens or new antibiotic resistance mechanisms that may emerge in future, therefore intend to keep the sample you provide after the study has finished. This will be stored anonymously.

If you DO NOT wish for us to keep the sample for further testing and would prefer it was destroyed following the completion of this study, please indicate by ticking this box ☐

Principal Investigator

Professor Nicola Williams

Institute of Infection and Global Health
Health

University of Liverpool, Leahurst Campus
Campus

Chester High Road

CH64 7TE.

ddsurv20@liverpool.ac.uk

PhD Student Investigator

Miss Genever Morgan

Institute of Infection and Global

University of Liverpool, Leahurst

Chester High Road

CH64 7TE.

ddsurv20@liverpool.ac.uk

Sample collection instructions

Instructions for collection of your dog's sample

Please read these instructions carefully before collecting your sample

In your collection pack there should be **1 x 15ml collection pot**, **1 x pair of gloves**, **1 x wooden spatula**, **1 x Specisafe collection pot holder**, **1 x pre-paid postal bag**.

Picture 1: Collection pack components

Put on your gloves and open your sample pot.

Using the spatula, pick up a small sample of your dog's faeces (preferably a freshly evacuated stool). As an example, a sample the size of your fingernail would be suitable.

Place both the sample and spatula into the sample pot as shown in picture 2. Screw the lid tightly closed.

Picture 2: Place wooden spatula and sample inside the collection pot and screw tightly closed.

Remove your gloves and dispose of them. Please wash your hands.

Place your sample pot into the Specisafe collection pot holder and push closed as shown in picture 3. Please ensure it is fully closed.

Picture 3: Place sample pot into Specisafe collection pot holder and push tightly closed.

Place the Specisafe containing your sample pot into the enclosed pre-paid postal bag, along with your signed consent form and completed questionnaire.

Place into a Royal Mail post box.

If you have any problems or concerns regarding the faecal sample collection process, please contact the research team via email at ddsurv20@liverpool.ac.uk

Thank you very much for your participation

Questionnaire

A Dog's Dinner Phase 2: Cross Sectional Study of Canine Faecal Bacteria

Please complete this questionnaire and return it with the faecal sample and your signed consent form using the enclosed prepaid packing bag.

Answering the questions

This questionnaire consists of **seven** short sections about your dog and its healthcare, their food, and your household.

Section One: Your dog
Section Two: Your dog's food
Section Three: Antibiotic treatment
Section Four: Diarrhoea
Section Five: Vet visits and hospitalisation
Section Six: Preventative healthcare and exposure
Section Seven: Your household

Please indicate your answers by marking an 'x' in the box provided e.g. [X] or writing in BLOCK CAPITAL letters.

Please use a blue or black ballpoint pen. If you want to change your answer, or you make a mistake, please indicate clearly by filling in the box completely and placing a cross in the correct box.

Checklist

Please ensure the following are included in your prepaid packing bag before returning to us:

- ☐ Signed consent form
- ☐ Faecal sample collected in enclosed pot
- ☐ Completed questionnaire

Many thanks for your participation

Section One: About your dog

Q1. Dog name:

Q2. Is your dog (please put a cross in the appropriate box):

Male, neutered	Male, not neutered	Female, neutered	Female, not neutered

Q3. Dog Age: …..years ……months

Q4 Length of time owned: …..years ……months

Section Two: Your dog's food

Q5. What **categories** of food do you feed your dog? **Please tick all that apply**:

- ☐ Cooked commercial complete wet food
- ☐ Raw meat and/or bones (pre-prepared diet)
- ☐ Raw meat and/or bones (DIY/home-prepared diet)
- ☐ Cooked fresh meat and/or bones
- ☐ Cooked commercial complete dry food/kibble
- ☐ Vegetarian diet
- ☐ Other (Please detail below)

Q6. Do you **currently** feed any **raw meat/bones** to your dog?

- ☐ Yes (Please go to Q7)
- ☐ No (Please go to Q8)

Q7. What **type(s) of raw meat**, either as part of a pre-prepared meal or bought from the supplier fresh, do you prefer to feed your dog? **Please tick all that you currently feed**:

- ☐ Beef
- ☐ Pork
- ☐ Chicken
- ☐ Lamb
- ☐ Venison
- ☐ Turkey
- ☐ Rabbit

- ☐ Duck
- ☐ Game (e.g. Pheasant, grouse, pigeon)
- ☐ Offal (e.g. Tripe, heart, liver, kidney)
- ☐ Fish
- ☐ Other (please detail below)

Q8. What are you **currently** feeding your dog? *Please list all food items and include the name of the brand and/or the type of meat(s)/foodstuff that you use, e.g. 'James Wellbeloved Turkey and Rice dry biscuits, Natures Menu Original Beef Nuggets, raw egg and raw chicken breast'.*

Q9. Has this **changed in the last 3 months**?

- ☐ Yes
- ☐ No

Q10. If there has been a change in the last 3 months, what were you feeding **previously**?

Q11. What types of **treats** are you **currently** feeding your dog? please tick all that apply

- ☐ Shop bought cooked treats/biscuits
- ☐ Freeze dried meat/fish treats
- ☐ Dried treats (e.g. pig ears, chicken feet)
- ☐ Raw meat (including body parts such as feet, hooves)
- ☐ Raw bones
- ☐ Cooked meat
- ☐ Cooked bones
- ☐ I don't feed any treats
- ☐ Other (please detail below):

Q12. Is your dog ever fed **human food scraps/titbits**?

- ☐ No
- ☐ Rarely
- ☐ Occasionally as a treat
- ☐ Frequently
- ☐ Don't know

Q13. Is your dog a **scavenger (e.g. eats things on walks, steals from bins, eats faeces or carcasses)**?

- ☐ No
- ☐ Yes, Sometimes
- ☐ Yes, frequently
- ☐ Don't know

Section Three: Antibiotic treatment

Q14. In the **last 3 months**, has your dog received antibiotics for any condition?

- ☐ No (please go to Q20)
- ☐ Yes (please go to Q15)

Q15. Is your dog **currently** receiving antibiotics?

- ☐ No (please go to Q16)

☐ Yes (please go to Q17)

Q16. If no, how **long ago** was the **most recent** course of antibiotics prescribed?

☐ 1 week ago or less
☐ 2-4 weeks ago
☐ 4-8 weeks ago
☐ More than 8 weeks ago

Q17. How **long was the most recent course** of antibiotics prescribed for your dog?

☐ One-off short acting injection (<24 hours)
☐ One-off long acting injection (lasts up to 2 weeks)
☐ Oral antibiotics up to 5 days
☐ Oral antibiotics up to 10 days
☐ Oral antibiotics up to 2 weeks
☐ Oral antibiotics up to 3 weeks
☐ Oral antibiotics for longer than 3 weeks

Q18. What was the **name** of the most recent antibiotic prescribed? Please detail the brand name on the label or type of antibiotic (if known)

Q19. What were the antibiotics **prescribed for**? Please detail the **problem or condition** your vet prescribed the course of antibiotics for (if known)

Section Four: Diarrhoea

Q20. In the **last 3 months**, has your dog had diarrhoea/loose stools?

- ☐ No (Please go to Q28)
- ☐ Yes (Please go to Q21)

Q21. How **long ago** did your dog have diarrhoea?

- ☐ Currently has diarrhoea/diarrhoea in the last week/always has diarrhoea
- ☐ 1- 2 weeks ago
- ☐ 2-4 weeks ago
- ☐ 4-8 weeks ago
- ☐ More than 8 weeks ago

Q22. Has your dog had **repeated episodes** of diarrhoea in the last 3 months?

- ☐ Yes
- ☐ No

Q23. If yes, **how many** episodes of diarrhoea has your dog had in the last 3 months?

- ☐ 1-2 episodes
- ☐ 3-4 episodes
- ☐ 5+ episodes
- ☐ Constant diarrhoea

Q24. Was **any treatment** given?

- ☐ No, it resolved by itself
- ☐ No, but I did feed a bland diet (please detail below what you fed)
- ☐ Yes, a home remedy
- ☐ Yes, over the counter medication from a shop
- ☐ Yes, veterinary prescribed treatment

Q25. If your dog received treatment, **what** was it (if known)?

Q26. Were any **faecal (poo) samples** taken by your vets to investigate the diarrhoea?

- ☐ Yes
- ☐ No

Q27. If yes, what were the **results** (if known)?

Section Five: Vet visits and hospitalisation

Q28. In the **last 3 months**, has your dog been in a veterinary practice for any condition?

- ☐ No (Please go to Q32)
- ☐ Yes (Please go to Q29)

Q29. **How many** visits to the vets has your dog had in the **last 3 months**?

- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5 or more

Q30. What was the **reason** for the most recent vet visit?

- ☐ Routine (e.g. vaccination, long term medication check-up, wormer/flea treatment check)
- ☐ Non-emergency problem/concern (*please give further detail below*)

☐ Emergency visit (*please give further detail below*)

Q31. Was your dog **hospitalised**?

☐ No
☐ Yes, for the day only
☐ Yes, for longer than 24 hours

Section Six: Preventative healthcare and exposure

Q32. Do you give your dog any **anti-parasite treatment** (e.g. for fleas, worms, etc)

☐ No
☐ Yes, vet prescribed treatment
☐ Yes, over the counter/shop bought treatment
☐ Yes, natural remedy

If yes, please detail what you use:

Q33. Does your dog **regularly come into contact** with any of the following (Please tick all that apply)?

☐ Other dogs
☐ Cats
☐ Small mammals/rodents (e.g. rats, mice, hamsters, degus)
☐ Horses
☐ Farm animals
☐ Wildlife
☐ Reptiles/snakes
☐ No other regular animal contact

☐ Other (please state)

Q34. Does your dog **attend or have regular access to** any of the following? (Please tick all that apply)

☐ Dog training classes
☐ Doggy day care
☐ Group dog walking
☐ Dog shows
☐ Dog parks
☐ Farm land
☐ Public parks/towpaths/footpaths
☐ Other (please state)

Q35. Does your dog regularly visit **human** care homes, nurseries, etc (e.g. Pets As Therapy dogs)?

☐ Yes
☐ No

Q36. If yes, please detail where your dog regularly visits and in what capacity:

Section Seven: Your household

Q37. How many people **permanently** reside in your household? ☐

Q38. How many people are aged **65 years or over**? ☐

Q39. How many people are aged **5 years or younger**? ☐

Q40. Do any of the permanent residents in your household **work** in the following?

- ☐ Hospital/GP surgery
- ☐ Care home
- ☐ Nursery
- ☐ Primary school
- ☐ Livestock farm
- ☐ Dog boarding kennels
- ☐ Petting zoo

Q41. Have any of the permanent residents in your household **received antibiotics** in the last **3 months**?

- ☐ Yes
- ☐ No

Q42. Have any of the permanent residents in your household **been hospitalised** in the last **3 months**?

- ☐ Yes
- ☐ No

End of Questions

Thank you very much for completing this questionnaire and for your continued participation in this study.

Table A3.1: *Sequence type, phenotypic antimicrobial resistance as determined by disc diffusion and resistance genes present as determined by whole genome sequencing for extended-spectrum beta-lactamase (ESBL)-producing/third-generation cephalosporin resistant (3GCR)- E. coli isolates (N=75 RMD-fed, N=12 NRMD-fed) from dog faecal samples in the present study NB: No resistance to amikacin, meropenem or tigecycline was observed.*

																		Phenotypic resistance on AST								
Sample ID	**ST**	**Raw YN**	**CTX-M**	**TEM**	**SHV**	**OXA**	***Escherichia coli* ampC1 β-lactamase**	**CMY**	***Escherichia coli* ampC**	***DHA-1***	***qnr***	***parC***	***gyrA***	***tet***	***sul***	***dfrA***	**aminoglycoside resistance genes**	**chloramphenicol resistance genes**	**Amp**	**Aug**	**Cip**	**Tig**	**TMS**	**Ami**	**Mer**	**Ctx**	**Ctz**
14	10	Yes	32				x		x					B, R			*aph3'')-Ib, aph(6)-Id*		R	S	S	S	S	S	S	R	R
747	10	Yes	15	1			x		x		S1			A	2	14	*aph(3'')-Ib, aph(6)-Id*		R	S	R	S	R	S	S	R	R
533	10	No	1				x		x						2	17	*aadA5*		R	S	S	S	R	S	S	R	S
170	23	Yes		78			x		x					B, R	2				R	R	S	S	S	S	S	S	R
171	23	Yes		78			x		x					B, R	2				R	R	S	S	R	S	S	S	R
655	23	Yes	1				x		x				x		2	17	*aadA5*		R	S	S	S	R	S	S	R	S
600	38	Yes					x	2	x										R	R	S	S	S	S	S	R	R
645	38	Yes	15				x		x		S1			B, R					R	S	S	S	S	S	S	R	R
652	38	Yes	15	52			x		x		S1								R	S	R	S	S	S	S	R	R
653	38	Yes	15				x		x		S1								R	S	R	S	S	S	S	R	R
681	38	Yes	14	1			x		x							1	*aadA2, ant(3'')-Ila*	*catI*	R	S	S	S	S	S	S	R	R
537	48	No	15				x		x		S1				2				R	S	S	S	S	S	S	R	R

193	57	Yes	15	185			x		x					B, R	1				R	S	S	S	S	S	S	R	S
601	58	Yes	15	1			x		x		S1			A	2	14	*aph(3'')-Ib, aph(3')-Ia, aph(6)-Id*		R	S	R	S	R	S	S	R	R
718	58	Yes		52			x	58, 100	x						3	12	*aadA2, aadA25, ant(3'')-IIa*		R	S	S	S	R	S	S	S	S
155	58	No	15	1			x		x		S1			A			*aac(3)-IId, ant(3'')-IIa, aph(3')-Ia*		R	S	R	S	S	S	S	R	R
236	69	Yes	1	1			x		x		S1			B, R			*aac(3)-IId, ant(3'')-IIa , aph(3')-Ia, aph(6)-Id*		R	S	R	S	S	S	S	R	S
207	69	No		1		1, 45	x		x	2	B4			A	1, 2	17	*aadA5, ant(3'')-IIa*		R	R	S	S	R	S	S	S	R
452	75	No					x		x										R	R	S	S	S	S	S	S	R
536	75	No					x		x								*aadA22*		R	R	S	S	S	S	S	R	R
570	88	Yes	14				x		x			x		B, R	1, 2		*ant(3'')-IIa*		R	S	R	S	R	S	S	R	S
173	88	No					x		x						2		*aph(3'')-Ib, aph(6)-Id*		R	R	S	S	S	S	S	S	R
453	88	No		78			x		x				x	B, R	1, 2		*ant(3'')-IIa, aph(3'')-Ib, aph(3')-Ia, aph(6)-Id*		R	R	S	S	R	S	S	S	R
648	101	Yes	55	1			x		x			x	x	A	2	1	*ant(3'')-IIa, aph(3'')-Ib, aph(6)-Id*		R	S	R	S	R	S	S	R	R
649	101	Yes	55	1			x		x			x	x	A	2	1	*ant(3'')-IIa, aph(3'')-Ib, aph(6)-Id*		R	S	R	S	R	S	S	R	R

23	117	Yes		1	66		x		x		S7		x		2, 3	12	*aadA2, aadA25, ant(3'')-Ila*	*cmlA6*	R	S	S	S	R	S	S	S	R
398	117	Yes					x	2	x					A					R	R	S	S	S	S	S	R	R
534	117	Yes		1	66		x		x						2, 3	12	*aadA2, aadA25, ant(3'')-Ila*	*cmlA6*	R	S	S	S	R	S	S	S	R
719	117	Yes		1	66		x		x				x		2, 3	12	*aadA2, aadA5, aadA25, ant(3'')-Ila*		R	S	S	S	R	S	S	S	R
50	117	No					x		x				x						R	R	S	S	S	S	S	R	R
28	155	Yes					x		x										R	R	S	S	S	S	S	S	R
606	155	Yes			66		x	4	x		S1			A			*ant(3'')-Ila*		R	S	S	S	S	S	S	S	R
358	162	Yes	15				x		x			x	x				*ant(3'')-Ila*		R	R	S	S	R	S	S	S	R
385	162	Yes		1	66		x		x			x	x				*ant(3'')-Ila*		R	S	R	S	S	S	S	S	R
654	162	Yes		1			x	2	x			x	x	B, R					R	R	R	S	S	S	S	R	R
31	227	Yes		1			x		x					A	2	14	*aph(3'')-Ib, aph(6)-Id*		R	S	S	S	R	S	S	R	R
715	278	Yes	9				x		x					A	1	16	*aadA2*		R	S	S	S	R	S	S	R	S
482	345	Yes			66		x		x		S1				2				R	S	S	S	S	S	S	S	R
483	345	Yes			66		x		x		S1			A			*aadA17, aph(3'')-Ib*		R	S	S	S	S	S	S	S	R
357	351	Yes	27, 123	135, 185			x		x		S1			A, B, R		1, 14	*aadA8, aadA25, ant(3'')-Ila*		R	S	R	S	S	S	S	R	S
237	362	Yes	2				x	2	x				x	A	1, 2		*ant(3'')-Ila, aph(3')-Ia*	*catI*	R	S	R	S	R	S	S	R	S

175	367	Yes		78			x		x					B, R	2		*aph(3'')-Ib, aph(3')-Ia, aph(6)-Id*		R	R	S	S	R	S	S	S	R
123	372	Yes						2	x										R	R	S	S	S	S	S	R	R
65	399	No					x		x										R	S	R	S	S	S	S	R	R
400	410	Yes					x	2	x										R	R	S	S	S	S	S	R	R
682	442	Yes					x		x								*aadA22, aph(3')-Ia*		R	S	S	S	S	S	S	S	S
138	457	Yes	15				x		x		S1			A			*aac(3)-IId*		R	S	R	S	S	S	S	R	R
607	515	Yes					x	2	x					A	1, 2	12	*aadA2, aph(3'')-Ib, aph(6)-Id*		R	S	S	S	R	S	S	R	R
384	533	Yes			66		x	2	x		S1, S15						*ant(3'')-IIa*		R	S	R	S	S	S	S	S	R
270	540	Yes		1		1	x		x					B, R	3		*aadA2, aadA12, aadA22, aadA23, aadA24, ant(3'')-IIa, aph(3')-Ia*	*catI*	R	R	S	S	S	S	S	R	R
130	602	Yes	1				x		x			x	x	B, R	2	17	*aadA5, aph(3'')-Ib, aph(6)-Id*		R	S	R	S	R	S	S	R	S
189	602	Yes			66		x		x				x	A	2, 3	12	*aadA2, aadA8b, ant(3'')-IIa*	*cmlA6*	R	S	S	S	R	S	S	R	R
272	602	Yes					x	2	x										R	R	S	S	S	S	S	R	R
383	602	Yes					x		x										R	R	S	S	S	S	S	R	R
239	641	Yes	55	1			x	2	x		S1			A	3	14	*aac(3)-IId, ant(3'')-IIa,*	*cmlA6*	R	S	R	S	R	S	S	R	R

																	aph(3')-Ia, aph(6)-Id										
176	642	Yes					x		x	2	B4				1	17	*aadA5*		R	R	R	S	R	S	S	S	R
147	752	Yes	55	209			x		x				x	A	2, 3	14	*aadA2, ant(3'')-IIa, aph(3'')-Ib, aph(6)-Id*	*cmlA6*	R	S	R	S	R	S	S	R	R
271	752	Yes	55	150			x		x				x	A	2, 3	1, 14	*aadA3, aadA15, ant(3'')-IIa, aph(3'')-Ib, aph(6)-Id*	*cmlA6*	R	S	R	S	R	S	S	R	R
303	752	Yes	55	209			x		x				x	A	2, 3	14	*aadA2, ant(3'')-IIa, aph(3'')-Ib, aph(6)-Id*	*cmlA6*	R	S	R	S	R	S	S	R	S
361	752	Yes	55, 60	209			x		x				x	A	2, 3	14	*aadA2, ant(3'')-IIa, aph(3'')-Ib, aph(6)-Id*	*cmlA6*	R	S	R	S	R	S	S	R	R
480	752	Yes	55	209			x	6	x				x	A, B(P)	2, 3	14	*aadA2, ant(3'')-IIa, aph(3'')-Ib, aph(6)-Id*	*cmlA6*	R	S	S	S	R	S	S	R	R
685	963	No					x	2, 44	x										R	R	S	S	S	S	S	R	R
194	973	Yes						2	x										R	R	S	S	S	S	S	R	R
603	973	Yes		104, 185				2	x					B, R	2		*aph(3'')-Ib, aph(6)-Id*		R	R	S	S	R	S	S	R	R
359	1081	Yes					x	2	x					B, R	2		*aph(3'')-Ib, aph(6)-Id*		R	R	S	S	S	S	S	S	S
274	1170	Yes	15	1				132						A, M		12	*aadA2, sgm, ant(3'')-IIa*	*cmlA6*	R	S	S	S	S	S	S	R	S

276	1170	Yes	15					132						A, M		12	*aadA2, sgm, ant(3'')-Ila*	*cmlA6*	R	S	S	S	S	S	S	R	S
421	1423	Yes					x		x										R	R	S	S	S	S	S	S	R
360	1508	Yes	32				x		x					B, R			*aac(6')-Ib7, aph(3'')-Ib, aph(6)-Id*		R	S	S	S	S	S	S	R	S
306	1611	Yes	1				x		x										R	S	S	S	R	S	S	R	S
320	1611	Yes	1				x		x										R	S	S	S	S	S	S	R	S
278	1640	Yes	55	1			x		x										R	S	S	S	S	S	S	R	R
643	1727	Yes					x	2	x						2	14	*aph(3'')-Ib, aph(6)-Id*		R	R	S	S	R	S	S	R	R
746	1955	Yes					x	2	x									*cmx*	R	R	S	S	S	S	S	R	R
305	2028	Yes					x		x										R	S	R	S	S	S	S	R	R
304	2171	Yes				45	x	59	x					A			*aph(3')-Ia*		R	R	S	S	S	S	S	R	R
183	2179	Yes	65	1		1	x		x		S2	x	x				*aac(6')-Ib-cr*		R	S	R	S	S	S	S	R	S
567	2705	Yes	24	1			x		x						2	1	*ant(3'')-Ila*		R	S	S	S	R	S	S	R	S
604	2705	Yes					x	2	x										R	R	S	S	S	S	S	R	R
36	4096	Yes	15				x		x		S1			Y			*aph(3')-Ia*		R	R	S	S	R	S	S	S	R
646	4981	No	15	1			x		x			x	x	B, R	2	17	*aadA5, aph(3'')-Ib, aph(3')-Ia, APH(6)-Id*		R	S	R	S	R	S	S	R	R
27	5296	Yes					x		x										R	R	S	S	S	S	S	S	R
136	7483	Yes	15				x		x			x	x	A	1		*aph(3')-Ia*		R	S	R	S	S	S	S	R	R
116	11905	Yes		1	66, 123		x		x		S1			A			*aac(3)-IIe, ant(3'')-Ila, aph(3')-Ia*		R	S	S	S	S	S	S	R	R

538	P1	Yes		60			x										*aac(6')-Iy*		R	S	S	S	R	S	S	R	S
650	P2	Yes	15	1			x		x										R	S	S	S	S	S	S	R	R
651	P2	Yes	15	1			x		x										R	S	S	S	S	S	S	R	R

Table A3.2*: Types of antibiotics prescribed to dogs in the present study as recalled by dog owners, and the number (N) and percentage (%) of faecal samples (N=432) with 3GCR-, ESBL-producing and MDR-E. coli present*

Variable	**Category**	**N (total samples)**	**% of total**	**3GCR-*E. coli* present % (N)**		**ESBL-*E. coli* present % (N)**		**MDR-*E. coli* present % (N)**	
				Yes	***No***	***Yes***	***No***	***Yes***	***No***
		432		**17.4 (75)**	**82.6 (357)**	**11.8 (51)**	**88.2 (381)**	**8.1 (35)**	**91.9 (397)**
Antibiotic type	Amoxycillin-clavulanic acid	19	4.4	9.3 (7)	3.4 (12)	7.8 (4)	3.9 (15)	8.6 (3)	4.0 (16)
	Cefalexin	5	1.2	2.7 (2)	0.8 (3)	2.0 (1)	1.1 (4)	0.0 (0)	1.3 (5)
	Marbofloxacin	1	0.2	0.0 (0)	0.3 (1)	0.0 (0)	0.3 (1)	0.0 (0)	0.3 (1)
	Metronidazole	7	1.6	0.0 (0)	2.0 (7)	0.0 (0)	1.8 (7)	0.0 (0)	1.8 (7)
	Type not known	1	0.2	0.0 (0)	0.3 (1)	0.0 (0)	0.3 (1)	0.0 (0)	0.3 (1)
	Not applicable/ None prescribed	385	89.1	81.3 (61)	90.8 (324)	80.4 (41)	90.3 (344)	80.0 (28)	89.9 (357)
	Unknown	14	3.2	6.7 (5)	2.5 (9)	9.8 (5)	2.4 (9)	14.3 (5)	2.3 (9)

Table A3.3: *Univariable analysis of explanatory factors associated with dog faecal carriage of 3GCR-E. coli, analysed at sample level (N=432 dogs). Ref=reference category*

Variable	Category	N (total samples)	% of total	3GCR-*E. coli* present % (N)		Odds ratio	95% CI	p value
				Yes	*No*			
		432		**17.4 (75)**	**82.6 (357)**			
1. Food								
Fed raw diet	Yes	193	55.3	32. 6 (63)	67.4 (130)	9.17	4.77, 17.63	<0.001
	No	239	44.7	5.0 (12)	95.0 (227)	*Ref*		
Type of diet fed	Pre-prepared raw yes	157	36.3	34.3 (54)	65.6 (103)	6.34	3.65, 11.03	<0.001
	Pre-prepared raw no	275	63.7	7.6 (21)	92.4 (254)	*Ref*		
	DIY/home-prepared raw yes	101	23.4	29.7 (30)	70.3 (71)	2.69	1.58, 4.56	<0.001
	DIY/home-prepared raw no	331	76.6	13.6 (45)	86.4 (286)	*Ref*		
	Cooked meat/bones yes	43	10.0	9.3 (4)	90.7 (39)	0.46	0.16, 1.33	0.15
	Cooked meat/bones no	389	90.0	18.3 (71)	81.7 (318)	*Ref*		
	Cooked commercial complete dry/kibble yes	254	58.8	7.5 (19)	92.5 (235)	0.18	0.10, 0.31	<0.001
	Cooked commercial complete dry/kibble no	178	41.2	31.5 (56)	68.5 (122)	*Ref*		
	Cooked commercial complete wet yes	108	25.0	4.6 (5)	95.4 (103)	0.18	0.07, 0.45	<0.001
	Cooked commercial complete wet no	324	75.0	21.6 (70)	78.4 (254)	*Ref*		
	Vegetarian/vegan yes	4	0.9	25.0 (1)	75.0 (4)	1.60	0.16, 15.54	0.69
	Vegetarian/vegan no	428	99.1	17.3 (74)	82.7 (354)	*Ref*		
	Other yes	39	9.0	15.4 (6)	84.6 (33)	0.85	0.34, 2.12	0.73

	Other no	393	91.0	17.6 (69)	82.4 (324)	*Ref*		
Diet changed in last 3 months	Yes	83	19.2	15.7 (13)	84.3 (70)	0.87	0.45, 1.67	0.68
	No	347	80.3	17.6 (61)	82.4 (286)	*Ref*		
	Unknown	2	0.5	50.0 (1)	50.0 (1)	NA	NA	
Types of treat fed	Shop bought cooked treats/biscuits yes	273	63.2	11.4 (31)	88.6 (242)	0.33	0.20, 0.56	<0.001
	Shop bought cooked treats/biscuits no	159	36.8	27.2 (44)	72.3 (115)	*Ref*		
	Freeze dried treats yes	136	31.5	27.9 (38)	72.1 (98)	2.71	1.63, 4.52	<0.001
	Freeze dried treats no	296	68.5	12.5 (37)	87.5 (259)	*Ref*		
	Dried treats yes	159	36.8	24.5 (39)	75.5 (120)	2.14	1.29, 3.54	<0.01
	Dried treats no	273	63.2	13.2 (36)	86.8 (237)	*Ref*		
	Raw meat yes	75	17.4	32.0 (24)	68.0 (51)	2.82	1.60, 4.99	<0.001
	Raw meat no	357	82.6	14.3 (51)	85.7 (306)	*Ref*		
	Raw bones yes	97	22.5	30.9 (30)	69.1 (67)	2.89	1.69, 4.92	<0.001
	Raw bones no	335	77.5	13.4 (45)	86.6 (290)	*Ref*		
	Cooked meat yes	104	24.1	12.5 (13)	87.5 (91)	0.61	0.32, 1.17	0.14
	Cooked meat no	328	75.9	18.9 (62)	81.1 (266)	*Ref*		
	Cooked bones yes	10	2.3	10.0 (1)	90.0 (9)	0.52	0.07, 4.19	0.54
	Cooked bones no	422	97.7	17.5 (74)	82.5 (348)	*Ref*		
	I don't feed any treats yes	16	3.7	12.5 (2)	87.5 (14)	0.67	0.15, 3.01	0.60
	I don't feed any treats no	416	96.3	17.5 (73)	82.5 (343)	*Ref*		
	Other treats yes	110	25.5	16.4 (18)	83.6 (92)	0.91	0.51, 1.63	0.75
	Other treats no	322	74.5	17.7 (57)	82.3 (265)	*Ref*		
Human food/titbits given	Frequently	70	16.2	11.4 (8)	88.6 (62)	0.57	0.23, 1.43	0.13
	Occasionally as a treat	169	39.1	14.8 (25)	85.2 (144)	0.76	0.38, 1.54	

	Rarely	109	25.2	23.9 (26)	76.1 (83)	1.38	0.68, 2.81	
	No	81	18.8	18.5 (15)	81.5 (66)	*Ref*		
	Unknown	3	0.7	33.3 (1)	66.7 (2)	NA		
Dog scavenges	Yes frequently	74	17.1	13.5 (10)	86.5 (64)	0.71	0.33, 1.53	0.64
	Yes sometimes	165	38.2	18.2 (30)	81.8 (135)	1.01	0.59, 1.74	
	No	189	43.8	18.0 (34)	82.0 (155)	*Ref*		
	Unknown	4	0.9	25.0 (1)	75.0 (3)	NA		
2. Antibiotic use								
Antibiotics in last 3 months	Yes	47	10.9	27.7 (13)	72.3 (34)	1.99	1.00, 3.99	0.05
	No	385	89.1	16.1 (62)	83.9 (323)	*Ref*		
Currently receiving antibiotics	Yes	5	1.2	20.0 (1)	80.0 (4)	1.21	0.13, 10.97	0.87
	No	426	98.6	17.1 (73)	82.9 (353	*Ref*		
	Unknown	1	0.2	100.0 (1)	0.0 (0)	NA		
Most recent antibiotic course	1 week ago or less	4	0.9	50.0 (2)	50.0 (2)	5.23	0.73, 38.03	0.18
	2-8 weeks ago	17	3.9	23.5 (4)	76.5 (13)	1.62	0.51, 5.13	
	More than 8 weeks ago	22	5.1	27.3 (6)	72.7 (16)	1.97	0.74, 5.23	
	Not applicable	388	89.8	16.0 (62)	84.0 (326)	*Ref*		
	Unknown	1	0.2	100.0 (1)	0.0 (0)	NA		
Duration of most recent course	One off injection	3	0.7	33.3 (1)	66.7 (2)	2.65	0.24, 29.66	0.09
	Oral antibiotics up to 5 days	16	3.7	18.8 (3)	81.3 (13)	1.22	0.34, 4.42	
	Oral antibiotics up to 10 days	20	4.6	40.0 (8)	60.0 (12)	3.53	1.39, 8.90	
	Oral antibiotics for 2 weeks or longer	7	1.6	28.6 (2)	71.4 (5)	2.12	0.40, 11.17	

	Not applicable	384	88.9	15.9 (61)	84.1 (323)	*Ref*		
	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
3. Diarrhoea								
Diarrhoea/loose stools last 3 months	Yes	138	31.9	14.5 (20)	85.5 (118)	0.73	0.42, 1.28	0.28
	No	293	67.8	18.8 (55)	81.2 (238)	*Ref*		
	Unknown	1	0.2	0.0 (0)	100.0 (1)			
Most recent episode	Currently has/always has/in the last week	18	4.2	5.6 (1)	94.4 (17)	0.26	0.03, 1.99	0.73
	1-2 weeks ago	35	8.1	20.0 (7)	80.0 (28)	1.10	0.46, 2.66	
	2-4 weeks ago	36	8.3	16.7 (6)	83.3 (30)	0.88	0.35, 2.22	
	4-8 weeks ago	28	6.5	10.7 (3)	89.3 (25)	0.53	0.15, 1.82	
	More than 8 weeks ago	21	4.9	19.0 (4)	81.0 (17)	1.04	0.34, 3.21	
	Not applicable	292	67.6	18.5 (54)	81.5 (238)	*Ref*		
	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
Repeated episodes in last 3 months	Yes	46	10.6	10.9 (5)	89.1 (41)	0.53	0.20, 1.40	0.42
	No	91	21.1	16.5 (15)	82.5 (76)	0.85	0.46, 1.60	
	Not applicable	293	67.8	18.8 (55)	81.2 (238)	*Ref*		
	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
Number of episodes in last 3 months	Constant diarrhoea/up to 2 episodes	26	6.0	7.7 (2)	92.3 (24)	0.38	0.09, 1.64	0.41
	3-4 episodes	15	3.5	26.7 (4)	73.3 (11)	1.66	0.51, 5.36	
	5 or more episodes	17	3.9	11.8 (2)	88.2 (15)	0.61	0.14, 2.72	
	Not applicable	372	86.1	18.0 (67)	82.0 (305)	*Ref*		
	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
Treatment given	None, resolved by itself (yes)	71	16.4	14.1 (10)	85.9 (61)	0.75	0.36, 1.53	0.43
	None, resolved by itself (no)	361	83.6	18.0 (65)	82.0 (296)	*Ref*		

	Bland diet (yes)	25	5.8	16.0 (4)	84.0 (21)	0.90	0.30, 2.71	0.85
	Bland diet (no)	407	94.2	17.4 (71)	82.6 (336)	*Ref*		
	Home remedy (yes)	11	2.5	27.5 (3)	72.7 (8)	1.82	0.47, 7.02	0.39
	Home remedy (no)	421	97.5	17.1 (72)	82.9 (349)	*Ref*		
	Over the counter medication from a shop (yes)	25	5.8	4.0 (1)	96.0 (24)	0.19	0.03, 1.41	0.10
	Over the counter medication from a shop (no)	407	94.2	18.2 (74)	81.8 (333)	*Ref*		
	Veterinary prescribed treatment (yes)	19	4.4	10.5 (2)	89.5 (17)	0.55	0.12, 2.42	0.43
	Veterinary prescribed treatment (no)	413	95.6	17.7 (73)	82.3 (340)	*Ref*		
4.Vet visits								
Visit to vet in the last 3 months	Yes	189	43.8	17.5 (33)	82.5 (156)	1.01	0.61, 1.67	0.98
	No	242	46.0	17.4 (42)	82.6 (200)	*Ref*		
	Unknown	1	0.2	0.0 (0)	100.0 (0)	NA		
Number of vet visits	1	102	23.6	16.7 (17)	83.3 (85)	0.98	0.53, 1.81	0.83
	2	43	10.0	14.0 (6)	86.0 (37)	0.79	0.31, 2.00	
	3	22	5.1	22.7 (5)	77.3 (17)	1.44	0.50, 4.11	
	4	8	1.9	12.5 (1)	87.5 (7)	0.70	0.08, 5.82	
	5 or more visits	14	3.2	28.6 (4)	71.4 (10)	1.95	0.58, 6.53	
	Not applicable	241	55.8	17.0 (41)	83.0 (200)	*Ref*		
	Unknown	2	0.5	50.0 (1)	50.0 (1)	NA		
Reason for visit	Emergency	16	3.7	43.8 (7)	56.3 (9)	3.79	1.34, 10.77	0.06
	Non-emergency problem/concern	111	25.7	14.4 (16)	85.6 (95)	0.82	0.44, 1.54	
	Routine visit	62	14.4	17.7 (11)	82.3 (51)	1.05	0.51, 2.19	
	Not applicable	241	55.8	17.0 (41)	83.0 (17)	*Ref*		

	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
Patient hospitalised	For the day only	32	7.4	12.5 (4)	87.5 (28)	0.70	0.23, 2.10	0.73
	For longer than 24 hours	3	0.7	33.3 (1)	66.7 (2)	2.45	0.22, 27.67	
	No	153	35.4	19.0 (29)	81.0 (124)	1.15	0.69, 1.94	
	Not applicable	242	56.0	16.9 (41)	83.1 (124)	*Ref*		
	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
5. Preventative healthcare and exposure to other animals and carehomes								
Antiparasite treatment given	No treatment (yes)	69	16.0	31.9 (22)	68.1 (47)	2.74	1.52, 4.91	<0.001
	No treatment (no)	363	84.0	14.6 (53)	85.4 (310)	*Ref*		
	Vet prescribed treatment (yes)	264	61.1	11.7 (31)	88.3 (233)	0.38	0.23, 0.62	<0.001
	Vet prescribed treatment (no)	168	38.9	26.2 (44)	73.8 (124)	*Ref*		
	Over the counter/shop bought treatment (yes)	41	9.5	12.2 (5)	87.8 (36)	0.64	0.24, 1.68	0.36
	Over the counter/shop bought treatment (no)	391	90.5	17.9 (70)	82.1 (321)	*Ref*		
	Natural remedy (yes)	72	16.7	25.0 (18)	75.0 (54)	1.77	0.97, 3.24	0.06
	Natural remedy (no)	360	83.3	15.8 (57)	84.2 (303)	*Ref*		
Regular contact with other animals	Dogs (yes)	380	88.0	17.1 (65)	82.9 (315)	0.88	0.41, 1.82	0.70
	Dogs (no)	52	12.0	19.2 (10)	80.8 (42)	*Ref*		
	Cats (yes)	149	34.5	12.1 (18)	87.9 (131)	0.55	0.31, 0.97	0.04
	Cats (no)	283	65.5	20.1 (57)	79.9 (226)	*Ref*		
	Small mammals/rodents (yes)	56	13.0	12.5 (7)	87.5 (49)	0.65	0.28, 1.49	0.31
	Small mammals/rodents (no)	376	87.0	18.1 (68)	81.9 (308)	*Ref*		
	Horses (yes)	81	18.8	13.6 (11)	86.4 (70)	0.71	0.35, 1.41	0.32
	Horses (no)	351	81.3	18.2 (64)	81.8 (287)	*Ref*		

	Farm animals (yes)	75	17.4	13.3 (10)	86.7 (65)		0.69	0.34, 1.42	0.31
	Farm animals (no)	357	82.6	18.2 (65)	81.8 (292)	*Ref*			
	Wildlife (yes)	126	29.2	16.7 (21)	83.3 (105)		0.93	0.54, 1.62	0.81
	Wildlife (no)	306	70.8	17.6 (54)	82.4 (252)	*Ref*			
	Reptiles/snakes (yes)	13	3.0	15.4 (2)	84.6 (11)		0.86	0.19, 3.97	0.85
	Reptiles/snakes (no)	419	97.0	17.4 (73)	82.6 (346)	*Ref*			
	Chickens/poultry (yes)	29	6.7	13.8 (4)	86.2 (25)		0.75	0.25, 2.22	0.60
	Chickens/poultry (no)	403	93.3	17.6 (71)	82.4 (332)	*Ref*			
	No other regular contact (yes)	26	6.0	23.1 (6)	76.9 (20)		1.47	0.57, 3.78	0.43
	No other regular contact (no)	406	94.0	17.0 (69)	83.0 (337)	*Ref*			
	Other (yes)	41	9.5	14.6 (6)	85.4 (35)		0.80	0.32, 1.98	0.63
	Other (no)	391	90.5	17.6 (69)	82.4 (332)	*Ref*			
Regular access to communal areas	Dog training classes (yes)	77	17.8	23.4 (18)	76.6 (59)		1.60	0.88, 2.90	0.13
	Dog training classes (no)	355	82.2	16.1 (57)	83.9 (298)	*Ref*			
	Doggy daycare (yes)	26	6.0	30.8 (8)	69.2 (18)		2.25	0.94, 5.38	0.07
	Doggy daycare (no)	406	94.0	16.5 (67)	83.5 (339)	*Ref*			
	Group dog walking (yes)	67	15.5	22.4 (15)	77.6 (52)		1.47	0.78, 2.77	0.24
	Group dog walking (no)	365	84.5	16.4 (60)	83.6 (305)	*Ref*			
	Dog shows (yes)	40	9.3	32.5 (13)	67.5 (27)		2.56	1.25, 5.22	0.01
	Dog shows (no)	391	90.7	15.9 (62)	84.1 (329)	*Ref*			
	Dog parks (yes)	77	17.8	14.3 (11)	85.7 (66)		0.76	0.38, 1.52	0.43
	Dog parks (no)	355	82.2	18.0 (64)	82.0 (291)	*Ref*			
	Farm land (yes)	222	51.4	15.8 (35)	84.2 (187)		0.80	0.48, 1.31	0.37
	Farm land (no)	210	48.6	19.0 (40)	81.0 (170)	*Ref*			

	Public parks/towpaths/footpaths (yes)	354	81.9	17.8 (63)	82.2 (291)	1.19	0.61, 2.33	0.61
	Public parks/towpaths/footpaths (no)	78	18.1	15.4 (12)	84.6 (66)	*Ref*		
	Other (yes)	70	16.2	17.1 (12)	82.9 (58)	0.98	0.50, 1.94	0.96
	Other (no)	362	83.8	17.4 (63)	82.6 (299)	*Ref*		
	No regular access to places listed (yes)	18	4.2	5.6 (1)	94.4 (17)	0.27	0.04, 2.06	0.21
	No regular access to places listed (no)	414	95.8	17.9 (74)	82.1 (340)	*Ref*		
Visit human carehomes (e.g. PAT dog)	Yes	8	1.9	37.5 (3)	62.5 (5)	2.91	0.68, 12.4	0.15
	No	421	97.5	17.1 (72)	82.9 (349)	*Ref*		
	Unknown	3	0.7	0.0 (0)	100.0 (3)	NA		
6. Household data								
Number of people in household	1	66	15.3	19.7 (13)	80.3 (53)	*Ref*		0.92
	2	220	50.9	18.2 (40)	81.8 (180)	0.91	0.45, 1.82	
	3	69	16.0	15.9 (11)	84.1 (58)	0.77	0.32, 1.87	
	4	58	13.4	13.8 (8)	86.2 (50)	0.65	0.25, 1.71	
	5 or more	17	3.9	17.6 (3)	82.4 (14)	0.87	0.22, 3.50	
	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
Residents present aged 65 or over?	Yes	87	20.1	18.4 (16)	81.6 (71)	1.08	0.59, 1.99	0.80
	No	382	79.2	17.3 (59)	82.7 (283)	*Ref*		
	Unknown	3	0.7	0.0 (0)	100.0 (3)	NA		
Residents present aged 5 or younger?	Yes	27	6.3	3.7 (1)	96.3 (26)	0.17	0.02, 1.28	0.09
	No	402	93.1	18.4 (74)	81.6 (328)	*Ref*		
	Unknown	3	0.7	0.0 (0)	100.0 (3)	NA		
Resident works in riskier areas	Hospital/GP surgery (yes)	27	6.3	25.9 (7)	74.1 (20)	1.74	0.71, 4.26	0.23
	Hospital/GP surgery (no)	405	93.8	16.8 (68)	83.2 (337)	*Ref*		

	Carehome (yes)	8	1.9	25.0 (2)	75.0 (6)	1.6	0.32, 8.10	0.57
	Carehome (no)	424	98.1	17.2 (73)	82.3 (351)	*Ref*		
	Nursery (yes)	3	0.7	66.7 (2)	33.3 (1)	9.75	0.87, 108.99	0.06
	Nursery (no)	429	99.3	17.0 (73)	83.0 (356)	*Ref*		
	Primary school (yes)	11	2.5	27.3 (3)	72.7 (8)	1.82	0.47, 7.02	0.37
	Primary school (no)	421	97.5	17.1 (72)	82.9 (349)	*Ref*		
-	Livestock farm (yes)	13	3.0	15.4 (2)	84.6 (11)	0.86	0.19, 3.97	0.85
	Livestock farm (no)	419	97.0	17.4 (73)	82.6 (346)	*Ref*		
	Dog boarding kennels (yes)	9	2.1	33.3 (3)	66.7 (6)	2.44	0.60, 9.97	0.22
	Dog boarding kennels (no)	423	97.9	17.0 (72)	83.0 (351)	*Ref*		
	Petting zoo (yes)	2	0.5	50.0 (1)	50.0 (1)	4.81	0.30, 77.79	0.27
	Petting zoo (no)	430	99.5	17.2 (74)	82.8 (356)	*Ref*		
	Veterinary practice (yes)	113	26.2	8.0 (9)	92.0 (104)	0.33	0.16, 0.69	0.00
	Veterinary practice (no)	319	73.8	20.7 (66)	79.3 (253)	*Ref*		
	No other risky workplace (yes)	259	60.0	19.3 (50)	80.7 (209)	1.42	0.84, 2.39	0.19
	No other risky workplace (no)	173	40.0	14.5 (25)	85.5 (148)	*Ref*		
Resident received antibiotics last 3 months	Yes	51	11.8	15.7 (8)	84.3 (43)	0.86	0.39, 1.92	0.71
	No	377	87.3	17.8 (67)	82.2 (310)	*Ref*		
	Unknown	4	0.9	0.0 (0)	100.0 (4)	NA		
Resident hospitalised in last 3 months	Yes	15	3.5	13.3 (2)	86.7 (13)	0.71	0.16, 3.23	0.66
	No	412	95.4	17.7 (73)	82.3 (339)	*Ref*		
	Unknown	5	1.2	0.0 (0)	100.0 (5)	NA		

Region of country	East Midlands	28	6.5	14.4 (4)	85.7 (24)	2.06	0.42, 10.01	0.37
	East of England	27	6.3	14.8 (4)	85.2 (23)	2.15	0.44, 10.46	
	Greater London	12	2.8	33.3 (4)	66.7 (8)	6.17	1.15, 33.11	
	North East and Yorkshire	40	9.3	5.0 (2)	95.0 (38)	*Ref*		
	North West	104	24.1	14.4 (15)	85.6 (89)	2.08	0.59, 7.61	
	Northern Ireland	4	0.9	50.0 (2)	50.0 (2)	12.33	1.26, 121.30	
	Scotland	20	4.6	15.0 (3)	85.0 (17)	2.18	0.40, 11.92	
	South East	73	16.9	20.5 (15)	79.5 (58)	3.19	0.86, 11.78	
	South West	47	10.9	25.5 (12)	74.5 (35)	4.23	1.10, 16.26	
	Wales	30	6.9	20.0 (6)	80.0 (24)	3.08	0.70, 13.52	
	West Midlands	36	8.3	13.9 (5)	86.1 (31)	1.99	0.44, 8.99	
	Unknown	11	2.5	18.2 (2)	81.8 (9)	NA		
7. Dog data								
Dog sex	Female entire	48	11.1	29.2 (14)	70.8 (34)	2.78	1.29, 6.03	0.08
	Female neutered	163	37.7	12.9 (21)	87.1 (142)	*Ref*		
	Male entire	68	15.7	19.1 (13)	80.9 (55)	1.60	0.75, 3.41	
	Male neutered	150	34.7	18.0 (27)	82.0 (123)	1.48	0.80, 2.76	
	Unknown	3	0.7	0.0 (0)	100.0 (3)	NA		
Dog age	<12 months	29	6.7	24.1 (7)	75.9 (22)	0.92	0.86, 0.99	0.02
	1 year	16	3.7	6.3 (1)	93.8 (15)			

	2 years	42	9.7	28.6 (12)	71.4 (30)			
	3 years	38	8.8	31.6 (12)	68.4 (26)			
	4 years	36	8.3	8.3 (3)	91.7 (33)			
	5 years	37	8.6	18.9 (7)	81.1 (30)			
	6 years	36	8.3	25.0 (9)	75.0 (27)			
	7 years	34	7.9	14.7 (5)	85.3 (29)			
	8 years or older	157	36.9	12.1 (19)	87.9 (138)			
	Unknown	7	1.6	0.0 (0)	100.0 (7)	NA		
Dog age[2]	<12 months	29	6.7	24.1 (7)	75.9 (22)	0.99	0.99, 1.00	0.24
	1 year	16	3.7	6.3 (1)	93.8 (15)			
	2 years	42	9.7	28.6 (12)	71.4 (30)			
	3 years	38	8.8	31.6 (12)	68.4 (26)			
	4 years	36	8.3	8.3 (3)	91.7 (33)			
	5 years	37	8.6	18.9 (7)	81.1 (30)			
	6 years	36	8.3	25.0 (9)	75.0 (27)			
	7 years	34	7.9	14.7 (5)	85.3 (29)			
	8 years or older	157	36.9	12.1 (19)	87.9 (138)			
	Unknown	7	1.6	0.0 (0)	100.0 (7)	NA		
Length of time owned (8+ combined)	<12 months	44	10.4	22.7 (10)	77.3 (34)	0.91	0.84, 0.98	0.02
	1 year	23	5.4	13.0 (3)	87.0 (20)			
	2 years	49	11.5	24.5 (12)	75.5 (37)			
	3 years	43	10.1	27.9 (12)	72.1 (31)			
	4 years	57	13.4	19.3 (11)	80.7 (46)			
	5 years	36	8.5	22.2 (8)	77.8 (28)			

	6 years	31	7.3	12.9 (4)	87.1 (27)			
	7 years	28	6.6	10.7 (3)	89.3 (25)			
	8 years or longer	114	26.8	10.5 (12)	89.5 (102)			
	Unknown	7	1.6	0.0 (0)	100.0 (7)	NA		

Table A3.4: *Univariable analysis of explanatory factors associated with dog faecal carriage of ESBL-producing E. coli, analysed at sample level (N=432 dogs). Ref=reference category*

Variable	**Category**	**N (total samples)**	**% of total**	**ESBL-*E. coli* present % (N)**		**Odds ratio**	**95% CI**	**p value**
				Yes	***No***			
		432		**11.8 (51)**	**88.2 (381)**			
1. Food								
Fed raw diet	Yes	193	55.3	24.4 (47)	75.6 (146)	18.91	6.68, 53.59	<0.001
	No	239	44.7	1.7 (4)	98.3 (235)			
Type of diet fed	Pre-prepared raw yes	157	36.3	24.8 (39)	75.2 (118)	7.24	3.66, 14.33	<0.001
	Pre-prepared raw no	275	63.7	4.4 (12)	95.6 (263)	*Ref*		
	DIY/home-prepared raw yes	101	23.4	21.8 (22)	78.2 (79)	2.90	1.58, 5.32	<0.001
	DIY/home-prepared raw no	331	76.6	8.8 (29)	91.2 (302)	*Ref*		
	Cooked meat/bones yes	43	10.0	4.7 (2)	95.3 (41)	0.34	0.08, 1.44	0.14
	Cooked meat/bones no	389	90.0	12.6 (49)	87.4 (340)	*Ref*		
	Cooked commercial complete dry/kibble yes	254	58.8	3.1 (8)	96.9 (246)	0.10	0.05, 0.22	<0.001
	Cooked commercial complete dry/kibble no	178	41.2	24.2 (43)	75.8 (135)	*Ref*		

	Cooked commercial complete wet yes	108	25.0	2.8 (3)	97.2 (105)	0.16	0.05, 0.54	0.00
	Cooked commercial complete wet no	324	75.0	14.8 (48)	85.2 (276)	*Ref*		
	Vegetarian/vegan yes	4	0.9	25.0 (1)	75.0 (3)	2.52	0.26, 24.69	0.43
	Vegetarian/vegan no	428	99.1	11.7 (50)	88.3 (378)	*Ref*		
	Other yes	39	9.0	5.1 (2)	94.9 (37)	0.38	0.09, 1.62	0.19
	Other no	393	91.0	12.5 (49)	87.5 (344)	*Ref*		
Diet changed in last 3 months	Yes	83	19.2	4.8 (4)	95.2 (79)	0.33	0.12, 0.95	0.04
	No	347	80.3	13.3 (46)	86.7 (301)	*Ref*		
	Unknown	2	0.5	50.0 (1)	50.0 (1)	NA		
Types of treat fed	Shop bought cooked treats/biscuits yes	273	63.2	6.6 (18)	83.4 (255)	0.27	0.15, 0.50	<0.001
	Shop bought cooked treats/biscuits no	159	36.8	20.8 (33)	79.2 (126)	*Ref*		
	Freeze dried treats yes	136	31.5	19.9 (27)	80.1 (109)	2.81	1.55, 5.08	<0.001
	Freeze dried treats no	296	68.5	8.1 (24)	91.9 (272)	*Ref*		
	Dried treats yes	159	36.8	15.7 (25)	84.3 (134)	1.77	0.99, 3.19	0.06
	Dried treats no	273	63.2	9.5 (26)	90.5 (247)	*Ref*		
	Raw meat yes	75	17.4	18.7 (14)	81.3 (61)	1.99	1.01, 3.89	0.05
	Raw meat no	357	82.6	10.4 (37)	89.6 (320)	*Ref*		
	Raw bones yes	97	22.5	19.6 (19)	80.4 (78)	2.31	1.24, 4.29	0.01
	Raw bones no	335	77.5	9.6 (32)	90.4 (303)	*Ref*		
	Cooked meat yes	104	24.1	7.7 (8)	92.3 (96)	0.55	0.25, 1.22	0.14
	Cooked meat no	328	75.9	13.1 (43)	86.9 (285)	*Ref*		
	Cooked bones yes	10	2.3	0.0 (0)	100.0 (10)			0.62
	Cooked bones no	422	97.7	12.1 (51)	87.9 (371)	*Ref*		

	I don't feed any treats yes	16	3.7	12.5 (2)	87.5 (14)	1.07	0.24, 4.85	0.93
	I don't feed any treats no	416	96.3	11.8 (49)	88.2 (367)	*Ref*		
	Other treats yes	110	25.5	13.6 (15)	86.4 (95)	1.25	0.66, 2.39	0.49
	Other treats no	322	74.5	11.2 (36)	88.8 (286)	*Ref*		
Human food/titbits given	Frequently	70	16.2	5.7 (4)	94.3 (66)	0.55	0.16, 1.92	0.01
	Occasionally as a treat	169	39.1	8.9 (15)	91.1 (154)	0.89	0.36, 2.19	
	Rarely	109	25.2	21.1 (23)	78.9 (86)	2.44	1.03, 5.78	
	No	81	18.8	9.9 (8)	90.1 (73)	*Ref*		
	Unknown	3	0.7	33.3 (1)	66.7 (2)	NA		
Dog scavenges	Yes frequently	74	17.1	8.1 (6)	91.9 (68)	0.67	0.26, 1.72	0.51
	Yes sometimes	165	38.2	13.3 (22)	86.7 (143)	1.17	0.62, 2.20	
	No	189	43.8	11.6 (22)	88.4 (167)	*Ref*		
	Unknown	4	0.9	25.0 (1)	75.0 (3)	NA		
2. Antibiotic use								
Antibiotics in last 3 months	Yes	47	10.9	21.3 (10)	78.7 (37)	2.27	1.05, 4.90	0.04
	No	385	89.1	10.6 (41)	89.4 (344)	*Ref*		
Currently receiving antibiotics	Yes	5	1.2	20.0 (1)	80.0 (4)	1.92	0.21, 17.56	0.56
	No	426	98.6	11.5 (49)	88.5 (377)	*Ref*		
	Unknown	1	0.2	100.0 (1)	0.0 (0)	NA		
Most recent antibiotic course	Less than 8 weeks ago	21	4.9	19.0 (4)	81.0 (17)	1.94	0.62, 6.03	0.32
	More than 8 weeks ago	22	5.1	18.2 (4)	81.8 (18)	1.83	0.59, 5.67	
	Not applicable	388	89.8	10.8 (42)	89.2 (346)	*Ref*		
	Unknown	1	0.2	100.0 (1)	0.0 (0)	NA		

Duration of most recent course	One off injection	3	0.7	33.3 (1)	66.7 (2)	4.18	0.37, 47.14	0.17
	Oral antibiotics up to 5 days	16	3.7	12.5 (2)	87.5 (14)	1.20	0.26, 5.45	
	Oral antibiotics up to 10 days	20	4.6	25.0 (5)	75.0 (15)	2.79	0.96, 8.07	
	Oral antibiotics for 2 weeks or longer	7	1.6	28.6 (2)	71.4 (5)	3.35	0.63, 17.80	
	Not applicable	384	88.9	10.7 (41)	89.3 (343)	*Ref*		
	Unknown	2	0.5			NA		
3. Diarrhoea								
Diarrhoea/loose stools last 3 months	Yes	138	31.9	9.4 (13)	90.6 (125)	0.70	0.36, 1.36	0.24
	No	293	67.8	13.0 (38)	87.0 (255)	*Ref*		
	Unknown	1	0.2	0.0 (0)	100.0 (1)	NA		
Most recent episode	Currently has/always has/in the last week	18	4.2	5.6 (1)	94.4 (17)	0.41	0.05, 3.14	0.91
	1-2 weeks ago	35	8.1	11.4 (4)	88.6 (31)	0.89	0.30, 2.66	
	2-4 weeks ago	36	8.3	11.1 (4)	88.9 (32)	0.86	0.29, 2.58	
	4-8 weeks ago	28	6.5	7.1 (2)	92.9 (26)	0.53	0.12, 2.33	
	More than 8 weeks ago	21	4.9	14.3 (3)	85.7 (18)	1.15	0.32, 4.09	
	Not applicable	292	67.6	12.7 (37)	87.3 (255)	*Ref*		
	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
Repeated episodes in last 3 months	Yes	46	10.6	4.3 (2)	95.7 (44)	0.31	0.07, 1.31	0.28
	No	91	21.1	12.1 (11)	87.9 (80)	0.92	0.45, 1.89	
	Not applicable	293	67.8	13.0 (38)	87.0 (255)	*Ref*		
	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
Number of episodes in last 3 months	Constant diarrhoea/up to 2 episodes	26	6.0	3.8 (1)	96.2 (25)	0.28	0.04, 2.09	0.54
	3-4 episodes	15	3.5	13.3 (2)	86.7 (13)	1.06	0.23, 4.86	

	5 or more episodes	17	3.9	5.9 (1)	94.1 (16)	0.43	0.06, 3.34	
	Not applicable	372	86.1	12.6 (47)	87.4 (325)	*Ref*		
	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
Treatment given	None, resolved by itself (yes)	71	16.4	9.9 (7)	90.1 (64)	0.79	0.34, 1.83	0.58
	None, resolved by itself (no)	361	83.6	12.2 (44)	87.8 (317)	*Ref*		
	Bland diet (yes)	25	5.8	8.0 (2)	92.0 (23)	0.64	0.15, 2.78	0.55
	Bland diet (no)	407	94.2	12.0 (49)	88.0 (358)	*Ref*		
	Home remedy (yes)	11	2.5	18.2 (2)	81.8 (9)	1.69	0.35, 8.04	0.51
	Home remedy (no)	421	97.5	11.6 (49)	88.4 (372)	*Ref*		
	Over the counter medication from a shop (yes)	25	5.8	0.0 (0)	100.0 (25)			0.06
	Over the counter medication from a shop (no)	407	94.2	12.5 (51)	87.5 (356)	*Ref*		
	Veterinary prescribed treatment (yes)	19	4.4	10.5 (2)	89.5 (17)	0.87	0.20, 3.90	0.86
	Veterinary prescribed treatment (no)	413	95.6	11.9 (49)	88.1 (364)	*Ref*		
4.Vet visits								
Visit to vet in the last 3 months	Yes	189	43.8	13.2 (25)	86.8 (164)	1.27	0.71, 2.27	0.43
	No	242	46.0	10.7 (26)	89.3 (216)	*Ref*		
	Unknown	1	0.2	0.0 (0)	100.0 (1)	NA		
Number of vet visits	1	102	23.6	14.7 (15)	85.3 (87)	1.49	0.75, 2.96	0.91
	2	43	10.0	11.6 (5)	88.4 (38)	1.14	0.41, 3.15	
	3	22	5.1	9.1 (2)	90.9 (20)	0.86	0.91, 3.92	
	4	8	1.9	12.5 (1)	87.5 (7)	1.23	0.15, 10.45	
	5 or more visits	14	3.2	14.3 (2)	85.7 (12)	1.44	0.31, 6.81	
	Not applicable	241	55.8	10.4 (25)	89.6 (216)	*Ref*		

	Unknown	2	0.5	50.0 (1)	50.0 (1)	NA		
Reason for visit	Emergency	16	3.7	37.5 (6)	62.5 (10)	5.18	1.74, 15.47	0.03
	Non-emergency problem/concern	111	25.7	11.7 (13)	88.3 (98)	1.15	0.56, 2.34	
	Routine visit	62	14.4	11.3 (7)	88.7 (55)	1.10	0.45, 2.68	
	Not applicable	241	55.8	10.4 (25)	89.6 (216)	*Ref*		
	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
Patient hospitalised	For the day only	32	7.4	6.3 (2)	93.8 (30)	0.60	0.13, 2.57	0.26
	For longer than 24 hours	3	0.7	33.3 (1)	66.7 (2)	4.34	0.38, 49.59	
	No	153	35.4	15.0 (23)	85.0 (130)	1.54	0.84, 2.82	
	Not applicable	242	56.0	10.3 (25)	89.7 (217)	*Ref*		
	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
5. Preventative healthcare and exposure to other animals and carehomes								
Antiparasite treatment given	No treatment (yes)	69	16.0	23.2 (16)	76.8 (53)	2.83	1.46, 5.47	0.00
	No treatment (no)	363	84.0	9.4 (35)	90.4 (328)	*Ref*		
	Vet prescribed treatment (yes)	264	61.1	6.9 (18)	93.1 (246)	0.30	0.16, 0.55	<0.001
	Vet prescribed treatment (no)	168	38.9	19.6 (33)	80.4 (135)	*Ref*		
	Over the counter/shop bought treatment (yes)	41	9.5	12.2 (5)	87.8 (36)	1.04	0.39, 2.79	0.94
	Over the counter/shop bought treatment (no)	391	90.5	11.8 (46)	88.2 (345)	*Ref*		
	Natural remedy (yes)	72	16.7	18.1 (13)	81.9 (59)	1.87	0.94, 3.72	0.08
	Natural remedy (no)	360	83.3	10.6 (38)	89.4 (322)	*Ref*		
Regular contact with other animals	Dogs (yes)	380	88.0	12.1 (46)	87.9 (334)	1.30	0.49, 3.42	0.60
	Dogs (no)	52	12.0	9.6 (5)	90.4 (47)	*Ref*		

	Cats (yes)	149	34.5	9.4 (14)	90.6 (135)	0.69	0.36, 1.32	0.26
	Cats (no)	283	65.5	13.1 (37)	86.9 (246)	*Ref*		
	Small mammals/rodents (yes)	56	13.0	8.9 (5)	91.1 (51)	0.70	0.27, 1.85	0.48
	Small mammals/rodents (no)	376	87.0	12.2 (46)	87.8 (330)	*Ref*		
	Horses (yes)	81	18.8	9.9 (8)	90.1 (73)	0.79	0.35, 1.74	0.55
	Horses (no)	351	81.3	12.3 (43)	87.7 (308)	*Ref*		
	Farm animals (yes)	75	17.4	9.3 (7)	90.7 (68)	0.73	0.32, 1.70	0.47
	Farm animals (no)	357	82.6	12.3 (44)	87.7 (313)	*Ref*		
	Wildlife (yes)	126	29.2	11.1 (14)	88.9 (112)	0.91	0.47, 1.75	0.77
	Wildlife (no)	306	70.8	12.1 (37)	87.9 (269)	*Ref*		
	Reptiles/snakes (yes)	13	3.0	15.4 (2)	84.6 (11)	1.37	0.30, 6.38	0.69
	Reptiles/snakes (no)	419	97.0	11.7 (49)	88.3 (370)	*Ref*		
	Chickens/poultry (yes)	29	6.7	10.3 (3)	89.7 (26)	0.85	0.25, 2.93	0.80
	Chickens/poultry (no)	403	93.3	11.9 (48)	88.1 (355)	*Ref*		
	No other regular contact (yes)	26	6.0	7.7 (2)	92.3 (24)	0.97	0.28, 3.36	0.97
	No other regular contact (no)	406	94.0	11.8 (48)	88.2 (358)	*Ref*		
	Other (yes)	41	9.5	12.2 (5)	87.8 (36)	1.04	0.39, 2.79	0.94
	Other (no)	391	90.5	11.8 (46)	88.2 (345)	*Ref*		
Regular access to communal areas	Dog training classes (yes)	77	17.8	16.9 (13)	83.1 (64)	1.69	0.85, 3.36	0.13
	Dog training classes (no)	355	82.2	10.7 (38)	89.3 (317)	*Ref*		
	Doggy daycare (yes)	26	6.0	23.1 (6)	76.9 (20)	2.41	0.92, 6.31	0.07
	Doggy daycare (no)	406	94.0	11.1 (45)	88.9 (361)	*Ref*		
	Group dog walking (yes)	67	15.5	17.9 (12)	82.1 (55)	1.82	0.90, 3.70	0.10
	Group dog walking (no)	365	84.5	10.7 (39)	89.3 (326)	*Ref*		

	Dog shows (yes)	40	9.3	22.5 (9)	77.5 (31)	2.41	1.08, 5.41	0.03
	Dog shows (no)	391	90.7	10.7 (42)	89.3 (349)	*Ref*		
	Dog parks (yes)	77	17.8	10.4 (8)	89.6 (69)	0.84	0.38, 1.87	0.67
	Dog parks (no)	355	82.2	12.1 (43)	87.9 (312)	*Ref*		
	Farm land (yes)	222	51.4	10.8 (24)	89.2 (198)	0.82	0.46, 1.48	0.51
	Farm land (no)	210	48.6	12.9 (27)	87.1 (183)	*Ref*		
	Public parks/towpaths/footpaths (yes)	354	81.9	11.6 (41)	88.4 (313)	0.89	0.43, 1.87	0.76
	Public parks/towpaths/footpaths (no)	78	18.1	12.8 (10)	87.2 (68)	*Ref*		
	Other (yes)	70	16.2	7.1 (5)	92.9 (65)	0.53	0.20, 1.38	0.19
	Other (no)	362	83.8	12.7 (46)	87.3 (316)	*Ref*		
	No regular access to places listed (yes)	18	4.2	5.6 (1)	94.4 (17)	0.43	0.06, 3.29	0.42
	No regular access to places listed (no)	414	95.8	12.1 (50)	87.9 (364)	*Ref*		
Visit human carehomes (e.g. PAT dog)	Yes	8	1.9	37.5 (3)	62.5 (5)	4.66	1.80, 20.13	0.04
	No	421	97.5	11.4 (48)	88.6 (373)	*Ref*		
	Unknown	3	0.7	0.0 (0)	100.0 (3)	NA		
6. Household data								
Number of people in household	1	66	15.3	13.6 (9)	86.4 (57)	*Ref*		0.91
	2	220	50.9	11.4 (25)	88.6 (195)	0.81	0.36, 1.84	
	3	69	16.0	10.1 (7)	89.9 (62)	0.72	0.25, 1.05	
	4	58	13.4	12.1 (7)	87.9 (51)	0.87	0.30, 2.50	
	5 or more	17	3.9	17.6 (3)	82.4 (14)	1.36	0.32, 5.68	
	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
Residents present aged 65 or over	Yes	87	20.1	16.1 (14)	83.9 (73)	1.58	0.81, 3.08	0.18

	No	382	79.2	9.7 (37)	79.8 (305)	*Ref*		
	Unknown	3	0.7	0.0 (0)	100.0 (3)	NA		
Residents present aged 5 or younger	Yes	27	6.3	7.4 (2)	92.6 (25)	0.58	0.13, 2.51	0.46
	No	402	93.1	12.2 (49)	87.8 (353)	*Ref*		
	Unknown	3	0.7	0.0 (0)	100.0 (3)	NA		
Resident works in riskier areas	Hospital/GP surgery (yes)	27	6.3	18.5 (5)	81.5 (22)	1.77	0.64, 4.91	0.27
	Hospital/GP surgery (no)	405	93.8	11.4 (46)	88.6 (359)	*Ref*		
	Carehome (yes)	8	1.9	12.5 (1)	87.5 (7)	1.07	0.13, 8.87	0.95
	Carehome (no)	424	98.1	11.8 (50)	88.2 (374)	*Ref*		
	Nursery (yes)	3	0.7	66.7 (2)	33.3 (1)	15.51	1.38, 174.2	0.03
	Nursery (no)	429	99.3	11.4 (49)	88.6 (380)	*Ref*		
	Primary school (yes)	11	2.5	9.1 (1)	90.9 (10)	0.74	0.09, 5.92	0.78
	Primary school (no)	421	97.5	11.9 (50)	88.1 (371)	*Ref*		
	Livestock farm (yes)	13	3.0	15.4 (2)	84.6 (11)	1.37	0.30, 6.38	0.69
	Livestock farm (no)	419	97.0	11.7 (49)	88.3 (370)	*Ref*		
	Dog boarding kennels (yes)	9	2.1	33.3 (3)	66.7 (6)	3.91	0.95, 16.13	0.06
	Dog boarding kennels (no)	423	97.9	11.3 (48)	88.7 (375)	*Ref*		
	Petting zoo (yes)	2	0.5	0.0 (0)	100.0 (2)			1.00
	Petting zoo (no)	430	99.5	11.9 (51)	88.1 (379)	*Ref*		
	Veterinary practice (yes)	113	26.2	5.3 (6)	94.7 (107)	0.34	0.14, 0.82	0.02
	Veterinary practice (no)	319	73.8	14.1 (45)	85.9 (274)	*Ref*		
	No other risky workplace (yes)	259	60.0	13.1 (34)	86.9 (225)	1.39	0.75, 2.57	0.30
	No other risky workplace (no)	173	40.0	9.8 (17)	90.2 (156)	*Ref*		

Resident received antibiotics last 3 months	Yes	51	11.8	11.8 (6)	88.2 (45)	0.98	0.40, 2.44	0.97
	No	377	87.3	11.9 (45)	88.1 (332)	*Ref*		
	Unknown	4	0.9	0.0 (0)	100.0 (4)	NA		
Resident hospitalised in last 3 months	Yes	15	3.5	13.3 (2)	86.7 (13)	1.14	0.25, 5.20	0.87
	No	412	95.4	11.9 (49)	88.1 (363)	*Ref*		
	Unknown	5	1.2	0.0 (0)	100.0 (5)	NA		
Region of country	East Midlands	28	6.5	3.6 (1)	96.4 (27)	0.7	0.06, 8.16	0.26
	East of England	27	6.3	14.8 (4)	85.2 (23)	3.3	0.56, 19.49	
	Greater London	12	2.8	16.7 (2)	83.3 (10)	3.8	0.48, 30.42	
	North East and Yorkshire	40	9.3	5.0 (2)	95.0 (38)	*Ref*		
	North West	104	24.1	8.7 (9)	91.3 (95)	1.8	0.37, 8.72	
	Wales, Scotland and Northern Ireland	54	12.8	9.3 (5)	90.7 (49)	1.94	0.36, 10.55	
	South East	73	16.9	16.4 (12)	83.6 (61)	3.74	0.79, 17.62	
	South West	47	10.9	21.3 (10)	78.7 (37)	5.14	1.05, 25.04	
	West Midlands	36	8.3	11.1 (4)	88.9 (32)	2.38	0.41, 13.82	
	Unknown	11	2.5	18.2 (2)	81.8 (9)	NA		
7. Dog data								
Dog sex	Female entire	48	11.1	20.8 (10)	79.2 (38)	2.8	1.15, 6.80	0.15
	Female neutered	163	37.7	8.6 (14)	91.4 (149)	*Ref*		
	Male entire	68	15.7	13.2 (9)	86.8 (59)	1.62	0.67, 3.95	

	Male neutered	150	34.7	12.0 (18)	88.0 (132)	1.45	0.70, 3.03	
	Unknown	3	0.7	0.0 (0)	100.0 (3)	NA		
Dog age	<12 months	29	6.7	13.8 (4)	86.2 (25)	0.96	0.88, 1.03	0.26
	1 year	16	3.7	6.3 (1)	93.8 (15)			
	2 years	42	9.7	16.7 (7)	83.3 (35)			
	3 years	38	8.8	15.8 (6)	84.2 (32)			
	4 years	36	8.3	8.3 (3)	91.7 (33)			
	5 years	37	8.6	10.8 (4)	89.2 (33)			
	6 years	36	8.3	22.2 (8)	77.8 (28)			
	7 years	34	7.9	11.8 (4)	88.2 (30)			
	8 years or older	157	36.9	8.9 (14)	91.1 (143)			
	Unknown	7	1.6	0.0 (0)	100.0 (7)	NA		
Length of time owned	<12 months	44	10.4	13.6 (6)	86.4 (38)	0.96	0.88, 1.05	0.35
	1 year	23	5.4	8.7 (2)	91.3 (21)			
	2 years	49	11.5	12.2 (6)	87.8 (43)			
	3 years	43	10.1	16.3 (7)	83.7 (43)			
	4 years	57	13.4	14.0 (8)	86.0 (49)			
	5 years	36	8.5	16.7 (6)	83.3 (30)			
	6 years	31	7.3	12.9 (4)	87.1 (27)			
	7 years	28	6.6	7.1 (2)	92.9 (26)			
	8 years or longer	114	26.8	8.8 (10)	91.2 (104)			
	Unknown	7	1.6	0.0 (0)	100.0 (7)	NA		

Table A3.5: *Univariable analysis of explanatory factors associated with dog faecal carriage of multidrug resistant (MDR)-E. coli, analysed at sample level (N=432 dogs). Ref=reference category*

Variable	Category	N (total samples)	% of total	MDR-*E. coli* present % (N)		Odds ratio	95% CI	p value
				Yes	*No*			
		432		8.1 (35)	91.9 (397)			
1. Food								
Fed raw diet	Yes	193	55.3	18.1 (35)	81.9 (158)	17.43	5.27, 57.64	<0.001
	No	239	44.7	1.3 (3)	98.7 (236)	*Ref*		
Type of diet fed	Pre-prepared raw yes	157	36.3	17.8 (28)	82.2 (129)	5.75	2.71, 12.20	<0.001
	Pre-prepared raw no	275	63.7	3.6 (10)	96.4 (265)	*Ref*		
	DIY/home-prepared raw yes	101	23.4	15.8 (16)	84.2 (85)	2.64	1.33, 5.26	0.01
	DIY/home-prepared raw no	331	76.6	6.6 (22)	93.4 (309)	*Ref*		
	Cooked meat/bones yes	43	10.0	4.7 (2)	95.3 (41)	0.48	0.11, 2.06	0.32
	Cooked meat/bones no	389	90.0	9.3 (36)	90.7 (353)	*Ref*		
	Cooked commercial complete dry/kibble yes	254	58.8	2.4 (6)	97.6 (248)	0.11	0.05, 0.27	<0.001
	Cooked commercial complete dry/kibble no	178	41.2	18.0 (32)	82.0 (146)	*Ref*		

	Cooked commercial complete wet yes	108	25.0	1.9 (2)	98.1 (106)	0.15	0.04, 0.64	0.02
	Cooked commercial complete wet no	324	75.0	11.1 (36)	88.9 (288)	*Ref*		
	Vegetarian/vegan yes	4	0.9	25.0 (1)	75.0 (3)	3.52	0.36, 34.72	0.28
	Vegetarian/vegan no	428	99.1	8.6 (37)	91.4 (391)	*Ref*		
	Other yes	39	9.0	5.1 (2)	94.9 (37)	0.54	0.12, 2.32	0.4
	Other no	393	91.0	9.2 (36)	90.8 (357)	*Ref*		
Diet changed in last 3 months	Yes	83	19.2	1.2 (1)	98.8 (82)	0.21	0.05, 0.91	0.04
	No	347	80.3	9.8 (34)	90.2 (313)	*Ref*		
	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
Types of treat fed	Shop bought cooked treats/biscuits yes	273	63.2	4.4 (12)	95.6 (261)	0.24	0.12, 0.48	<0.001
	Shop bought cooked treats/biscuits no	159	36.8	16.4 (26)	83.6 (133)	*Ref*		
	Freeze dried treats yes	136	31.5	12.5 (17)	87.5 (119)	1.87	0.95, 3.67	0.07
	Freeze dried treats no	296	68.5	7.1 (21)	92.9 (275)	*Ref*		
	Dried treats yes	159	36.8	12.6 (20)	87.4 (139)	2.04	1.04, 3.98	0.04
	Dried treats no	273	63.2	6.6 (18)	93.4 (255)	*Ref*		

	Raw meat yes	75	17.4	16.0 (12)	84.0 (63)	2.43	1.16, 5.06	0.02
	Raw meat no	357	82.6	7.3 (26)	92.7 (331)	*Ref*		
	Raw bones yes	97	22.5	15.5 (15)	84.5 (82)	2.48	1.24, 4.97	0.01
	Raw bones no	335	77.5	6.9 (23)	93.1 (312)	*Ref*		
	Cooked meat yes	104	24.1	4.8 (5)	95.2 (99)	0.45	0.17, 1.19	0.11
	Cooked meat no	328	75.9	10.1 (33)	89.9 (295)	*Ref*		
	Cooked bones yes	10	2.3	0.0 (0)	100.0 (10)			1.00
	Cooked bones no	422	97.7	8.3 (35)	91.7 (387)			
	I don't feed any treats yes	16	3.7	12.5 (2)	87.5 (14)	1.51	0.33, 6.90	0.6
	I don't feed any treats no	416	96.3	7.9 (33)	92.1 (383)	*Ref*		
	Other treats yes	110	25.5	11.8 (13)	88.2 (97)	1.59	0.78, 3.32	0.2
	Other treats no	322	74.5	7.8 (25)	92.2 (297)	*Ref*		
Human food/titbits given	Frequently	70	16.2	4.3 (3)	95.7 (67)	0.47	0.12, 1.91	0.04
	Occasionally as a treat	169	39.1	6.5 (11)	93.5 (158)	0.74	0.27, 1.98	
	Rarely	109	25.2	15.6 (17)	84.4 (92)	1.95	0.77, 4.96	
	No	81	18.8	8.6 (7)	91.4 (74)	*Ref*		

	Unknown	3	0.7	33.3 (1)	66.7 (2)	NA		
Dog scavenges	Yes frequently	74	17.1	4.1 (3)	95.9 (71)	0.46	0.13, 1.62	0.19
	Yes sometimes	165	38.2	11.5 (19)	88.5 (146)	1.41	0.70, 2.84	
	No	189	43.8	8.5 (16)	91.5 (173)	*Ref*		
	Unknown	4	0.9	0.0 (0)	100.0 (4)	NA		
2. Antibiotic use								
Antibiotics in last 3 months	Yes	47	10.9	17.0 (8)	83.0 (39)	2.43	1.04, 5.66	0.04
	No	385	89.1	7.8 (30)	92.2 (355)	*Ref*		
Currently receiving antibiotics	Yes	5	1.2	20.0 (1)	80.0 (4)	2.71	0.30, 24.88	0.38
	No	426	98.6	8.5 (36)	91.5 (390)	*Ref*		
	Unknown	1	0.2	0.0 (0)	100.0 (1)	NA		
Most recent antibiotic course	1 week ago or less	4	0.9	0.0 (0)	100.0 (4)			0.39
	2-8 weeks ago	17	3.9	17.6 (3)	82.4 (14)			
	More than 8 weeks ago	22	5.1	13.6 (3)	86.4 (19)			
	Not applicable	388	89.8	8.0 (31)	92.0 (357)	*Ref*		
	Unknown	1	0.2	0.0 (0)	100.0 (1)	NA		

Duration of most recent course	One off injection	3	0.7	33.3 (1)	66.7 (2)	5.9	0.52, 66.97	0.08
	Oral antibiotics up to 5 days	16	3.7	6.3 (1)	93.8 (15)	0.79	0.10, 6.16	
	Oral antibiotics up to 10 days	20	4.6	25.0 (5)	75.0 (15)	3.93	1.34, 11.57	
	Oral antibiotics for 2 weeks or longer	7	1.6	14.3 (1)	85.7 (6)	1.97	0.23, 16.88	
	Not applicable	384	88.9	7.8 (30)	92.2 (354)	*Ref*		
	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
3. Diarrhoea								
Diarrhoea/loose stools last 3 months	Yes	138	31.9	6.5 (9)	93.5 (129)	0.64	0.29, 1.38	0.25
	No	293	67.8	9.9 (29)	90.1 (264)	*Ref*		
	Unknown	1	0.2	0.0 (0)	100.0 (1)	NA		
Most recent episode	Currently has/always has/in the last week	18	4.2	5.6 (1)	94.4 (17)			0.57
	1-2 weeks ago	35	8.1	11.4 (4)	88.6 (31)			
	2-4 weeks ago	36	8.3	8.3 (3)	91.7 (33)			
	4-8 weeks ago	28	6.5	0.0 (0)	100.0 (28)			
	More than 8 weeks ago	21	4.9	4.8 (1)	95.2 (20)			
	Not applicable	292	67.6	9.9 (29)	90.1 (263)			

	Unknown	2	0.5	0.0 (0)	100.0 (2)			
Most recent episode (categories combined so no zeros)	Currently has/always has/in the last week	18	4.2	5.6 (1)	94.4 (17)	0.53	0.07, 4.15	0.53
	1-2 weeks ago	35	8.1	11.4 (4)	88.6 (31)	1.17	0.39, 3.55	
	2-4 weeks ago	36	8.3	8.3 (3)	91.7 (33)	0.82	0.24, 2.86	
	More than 4 weeks ago	49	11.3	2.0 (1)	98.0 (48)	0.19	0.03, 1.42	
	Not applicable	292	67.6	9.9 (29)	90.1 (263)	*Ref*		
	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
Repeated episodes in last 3 months	Yes	46	10.6	4.2 (2)	95.7 (44)	0.41	0.10, 1.80	0.44
	No	91	21.1	7.7 (7)	92.3 (84)	0.76	0.32, 1.80	
	Not applicable	293	67.8	9.9 (29)	90.1 (264)	*Ref*		
	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
Number of episodes in last 3 months (combined)	Constant diarrhoea/up to 2 episodes	26	6.0	3.8 (1)	96.2 (25)	0.39	0.05, 2.93	0.76
	3-4 episodes	15	3.5	6.7 (1)	93.3 (14)	0.69	0.09, 5.39	
	5 or more episodes	17	3.9	5.9 (1)	94.1 (16)	0.6	0.08, 4.68	
	Not applicable	372	86.1	9.4 (35)	90.6 (337)	*Ref*		

	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
Treatment given	None, resolved by itself (yes)	71	16.4	5.6 (4)	94.4 (67)	0.57	0.20, 1.67	0.31
	None, resolved by itself (no)	361	83.6	9.4 (34)	90.6 (327)	*Ref*		
	Bland diet (yes)	25	5.8	4.0 (1)	96.0 (24)	0.42	0.06, 3.17	0.4
	Bland diet (no)	407	94.2	9.1 (37)	90.9 (370)	*Ref*		
	Home remedy (yes)	11	2.5	18.2 (2)	81.8 (9)	2.38	0.50, 11.42	0.28
	Home remedy (no)	421	97.5	8.6 (36)	91.4 (385)	*Ref*		
	Over the counter medication from a shop (yes)	25	5.8	0.0 (0)	100.0 (25)			0.15
	Over the counter medication from a shop (no)	407	94.2	9.3 (38)	90.7 (369)			
	Veterinary prescribed treatment (yes)	19	4.4	10.5 (2)	89.5 (17)	1.23	0.27, 5.55	0.790
	Veterinary prescribed treatment (no)	413	95.6	8.7 (36)	91.3 (377)	*Ref*		
4.Vet visits								
Visit to vet in the last 3 months	Yes	189	43.8	11.1 (21)	88.9 (168)	1.65	0.85, 3.23	0.14
	No	242	46.0	7.0 (17)	93.0 (225)			
	Unknown	1	0.2	0.0 (0)	100.0 (1)			
Number of vet visits	1	102	23.6	12.7 (13)	87.3 (89)	2.05	0.95, 4.45	0.57

	2	43	10.0	11.6 (5)	88.4 (38)	1.85	0.64, 5.35	
	3	22	5.1	9.1 (2)	90.9 (20)	1.41	0.30, 6.56	
	4	8	1.9	12.5 (1)	87.5 (7)	2.01	0.23, 17.35	
	5 or more visits	14	3.2	7.1 (1)	92.9 (13)	1.08	0.13, 8.80	
	Not applicable	241	55.8	6.6 (16)	93.4 (225)	*Ref*		
	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
Reason for visit	Emergency	16	3.7	37.5 (6)	62.5 (10)	8.44	2.72, 26.17	0.003
	Non-emergency problem/concern	111	25.7	9.9 (11)	90.1 (100)	1.55	0.69, 3.45	
	Routine visit	62	14.4	8.1 (5)	91.9 (57)	1.23	0.43, 3.51	
	Not applicable	241	55.8	6.6 (16)	93.4 (225)	*Ref*		
	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
Reason for visit Categories collapsed	All (emergency/non-emergency/routine)	189	43.7	11.6 (22)	88.4 (167)			
	No visit/not applicable	241	55.8	6.6 (16)	93.4 (225)	*Ref*		
	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
Patient hospitalised	For the day only	32	7.4	3.1 (1)	96.9 (31)	0.46	0.06, 3.56	0.06

	For longer than 24 hours	3	0.7	33.3 (1)	66.7 (2)	7.06	0.61, 82.12	
	No	153	35.4	13.1 (20)	86.9 (133)	2.12	1.06, 4.24	
	Not applicable	242	56.0	6.6 (16)	93.4 (226)	*Ref*		
	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
5. Preventative healthcare and exposure to other animals and carehomes								
Antiparasite treatment given	No treatment (yes)	69	16.0	15.9 (11)	84.1 (58)	2.36	1.11, 5.02	0.03
	No treatment (no)	363	84.0	7.4 (27)	92.6 (336)	*Ref*		
	Vet prescribed treatment (yes)	264	61.1	5.3 (14)	94.7 (250)	0.34	0.17, 0.67	0.002
	Vet prescribed treatment (no)	168	38.9	14.3 (24)	85.7 (144)	*Ref*		
	Over the counter/shop bought treatment (yes)	41	9.5	7.3 (3)	92.7 (38)	0.8	0.24, 2.74	0.73
	Over the counter/shop bought treatment (no)	391	90.5	9.0 (35)	91.0 (356)	*Ref*		
	Natural remedy (yes)	72	16.7	13.9 (10)	86.1 (62)	1.91	0.88, 4.14	0.1
	Natural remedy (no)	360	83.3	7.8 (28)	92.2 (332)	*Ref*		
Regular contact with other animals	Dogs (yes)	380	88.0	7.7 (34)	92.3 (346)	1.18	0.40, 3.47	0.77
	Dogs (no)	52	12.0	7.7 (4)	92.3 (48)	*Ref*		
	Cats (yes)	149	34.5	7.4 (11)	92.6 (138)	0.76	0.36, 1.57	0.45

Cats (no)	283	65.5	9.5 (27)	90.5 (256)	*Ref*		
Small mammals/rodents (yes)	56	13.0	3.6 (2)	96.4 (54)	0.35	0.08, 1.40	0.16
Small mammals/rodents (no)	376	87.0	9.6 (36)	90.4 (340)	*Ref*		
Horses (yes)	81	18.8	2.5 (2)	97.5 (79)	0.22	0.52, 0.94	0.04
Horses (no)	351	81.3	10.3 (36)	89.7 (315)	*Ref*		
Farm animals (yes)	75	17.4	4.0 (3)	96.0 (72)	0.38	0.12, 1.28	0.12
Farm animals (no)	357	82.6	9.8 (35)	90.2 (322)	*Ref*		
Wildlife (yes)	126	29.2	7.1 (9)	92.9 (117)	0.74	0.34, 1.60	0.44
Wildlife (no)	306	70.8	9.5 (29)	90.5 (277)	*Ref*		
Reptiles/snakes (yes)	13	3.0	15.4 (2)	84.6 (11)	1.93	0.41, 9.07	0.40
Reptiles/snakes (no)	419	97.0	8.6 (36)	91.4 (383)	*Ref*		
Chickens/poultry (yes)	29	6.7	3.4 (1)	96.6 (28)	0.35	0.05, 2.67	0.31
Chickens/poultry (no)	403	93.3	9.2 (37)	90.8 (366)	*Ref*		
No other regular contact (yes)	26	6.0	7.7 (2)	92.3 (24)	0.86	0.19, 3.77	0.84
No other regular contact (no)	406	94.0	8.9 (36)	91.1 (370)	*Ref*		
Other (yes)	41	9.5	4.9 (2)	95.1 (39)	0.51	0.12, 2.18	0.36

	Other (no)	391	90.5	9.2 (36)	90.8 (355)	*Ref*		
Regular access to communal areas	Dog training classes (yes)	77	17.8	14.3 (11)	85.7 (66)	2.03	0.96, 4.28	0.07
	Dog training classes (no)	355	82.2	7.6 (27)	92.4 (328)	*Ref*		
	Doggy daycare (yes)	26	6.0	19.2 (5)	80.8 (21)	2.69	0.95, 7.60	0.06
	Doggy daycare (no)	406	94.0	8.1 (33)	91.9 (373)	*Ref*		
	Group dog walking (yes)	67	15.5	13.4 (9)	86.6 (58)	1.8	0.81, 3.99	0.15
	Group dog walking (no)	365	84.5	7.9 (29)	92.1 (336)	*Ref*		
	Dog shows (yes)	40	9.3	17.5 (7)	82.5 (33)	2.46	1.01, 6.02	0.05
	Dog shows (no)	391	90.7	7.9 (31)	92.1 (360)	*Ref*		
	Dog parks (yes)	77	17.8	6.5 (5)	93.5 (72)	0.68	0.26, 1.80	0.43
	Dog parks (no)	355	82.2	9.3 (33)	90.7 (322)	*Ref*		
	Farm land (yes)	222	51.4	6.3 (14)	93.7 (208)	0.52	0.26, 1.04	0.06
	Farm land (no)	210	48.6	11.4 (24)	88.6 (186)	*Ref*		
	Public parks/towpaths/footpaths (yes)	354	81.9	9.0 (32)	91.0 (322)	1.19	0.48, 2.96	0.7
	Public parks/towpaths/footpaths (no)	78	18.1	7.7 (6)	92.3 (72)	*Ref*		
	Other (yes)	70	16.2	5.7 (4)	94.3 (66)	0.59	0.20, 1.70	0.33

	Other (no)	362	83.8	9.4 (34)	90.6 (328)	*Ref*		
	No regular access to places listed (yes)	18	4.2	5.6 (1)	94.4 (17)	0.6	0.08, 4.63	0.62
	No regular access to places listed (no)	414	95.8	8.9 (37)	91.1 (377)	*Ref*		
Visit human carehomes (e.g. PAT dog)	Yes	8	1.9	25.0 (2)	75.0 (6)	3.57	0.69, 18.31	0.13
	No	421	97.5	8.6 (36)	91.4 (385)	*Ref*		
	Unknown	3	0.7	0.0 (0)	100.0 (3)	NA		
6. Household data								
Number of people in household	1	66	15.3	10.6 (7)	89.4 (59)	*Ref*		0.83
	2	220	50.9	8.2 (18)	91.8 (202)	0.75	0.30, 1.89	
	3	69	16.0	7.2 (5)	92.8 (64)	0.66	0.20, 2.19	
	4	58	13.4	12.1 (7)	87.9 (51)	1.16	0.38, 3.52	
	5 or more	17	3.9	5.9 (1)	95.1 (16)	0.53	0.06, 4.60	
	Unknown	2	0.5	0.0 (0)	100.0 (2)	NA		
Residents present aged 65 or over?	Yes	87	20.1	11.5 (10)	88.5 (77)	1.46	0.68, 3.13	0.34
	No	382	79.2	8.2 (28)	91.8 (314)	*Ref*		
	Unknown	3	0.7	0.0 (0)	100.0 (3)	NA		

Residents present aged 5 or younger?	Yes	27	6.3	7.4 (2)	92.6 (25)	0.81	0.19, 3.57	0.78
	No	402	93.1	9.0 (36)	91.0 (366)	*Ref*		
	Unknown	3	0.7	0.0 (0)	100.0 (3)	NA		
Resident works in riskier areas	Hospital/GP surgery (yes)	27	6.3	11.1 (3)	88.9 (24)	1.32	0.38, 4.61	0.66
	Hospital/GP surgery (no)	405	93.8	8.6 (35)	91.4 (370)	*Ref*		
	Carehome (yes)	8	1.9	12.5 (1)	87.5 (7)	1.5	0.18, 12.48	0.71
	Carehome (no)	424	98.1	8.7 (37)	91.3 (387)	*Ref*		
	Nursery (yes)	3	0.7	66.7 (2)	33.3 (1)	21.8	1.93, 246.67	0.01
	Nursery (no)	429	99.3	8.4 (36)	91.6 (393)	*Ref*		
	Primary school (yes)	11	2.5	9.1 (1)	90.9 (10)	1.04	0.13, 8.33	0.97
	Primary school (no)	421	97.5	8.8 (37)	91.2 (384)	*Ref*		
	Livestock farm (yes)	13	3.0	7.7 (1)	92.3 (12)	0.86	0.11, 6.80	0.88
	Livestock farm (no)	419	97.0	8.8 (37)	91.2 (382)	*Ref*		
	Dog boarding kennels (yes)	9	2.1	33.3 (3)	66.7 (6)	5.54	1.32, 23.13	0.02
	Dog boarding kennels (no)	423	97.9	8.3 (35)	91.7 (388)	*Ref*		
	Petting zoo (yes)	2	0.5	0.0 (0)	100.0 (2)			1.0

	Petting zoo (no)	430	99.5	8.8 (38)	91.2 (392)	*Ref*		
	Veterinary practice (yes)	113	26.2	3.5 (4)	96.5 (109)	0.31	0.11, 0.89	0.03
	Veterinary practice (no)	319	73.8	10.7 (34)	89.3 (285)	*Ref*		
	No other risky workplace (yes)	259	60.0	10.0 (26)	90.0 (233)	1.5	0.73, 3.05	0.27
	No other risky workplace (no)	173	40.0	6.9 (12)	93.1 (161)	*Ref*		
Resident received antibiotics last 3 months	Yes	51	11.8	9.8 (5)	90.2 (46)	1.13	0.42, 3.05	0.81
	No	377	87.3	8.8 (33)	91.2 (344)	*Ref*		
	Unknown	4	0.9	0.0 (0)	100.0 (4)	NA		
Resident hospitalised in last 3 months	Yes	15	3.5	13.3 (2)	86.7 (13)	1.61	0.35, 7.40	0.54
	No	412	95.4	8.7 (36)	91.3 (376)	*Ref*		
	Unknown	5	1.2	0.0 (0)	100.0 (5)	NA		
Region of country (combined)	East Midlands	28	6.5	3.6 (1)	96.4 (27)	0.70	0.06, 8.16	0.78
NE as ref	East of England	27	6.3	7.4 (2)	92.6 (25)	1.52	0.20, 11.50	
	Greater London	12	2.8	16.7 (2)	83.3 (10)	3.80	0.48, 30.42	
	North East and Yorkshire	40	9.3	5.0 (2)	95.0 (38)	*Ref*		

	North West	104	24.1	7.7 (8)	92.3 (96)	1.58	0.32, 7.80	
	Wales, Scotland and Northern Ireland	54	12.8	9.3 (5)	90.7 (49)	1.94	0.36, 10.55	
	South East	73	16.9	9.6 (7)	90.4 (66)	2.02	0.40, 10.20	
	South West	47	10.9	14.9 (7)	85.1 (40)	3.33	0.65, 17.02	
	West Midlands	36	8.3	8.3 (3)	91.7 (33)	1.73	0.27, 10.97	
	Unknown	11	2.5	9.1 (1)	90.9 (10)	NA		
Region of country (combined)	East Midlands	28	6.5	3.6 (1)	96.4 (27)	0.44	0.05, 3.71	0.78
NW as ref	East of England	27	6.3	7.4 (2)	92.6 (25)	0.96	0.19, 4.81	
	Greater London	12	2.8	16.7 (2)	83.3 (10)	2.4	0.45, 12.89	
	North East and Yorkshire	40	9.3	5.0 (2)	95.0 (38)	0.63	0.13, 3.11	
	North West	104	24.1	7.7 (8)	92.3 (96)	*Ref*		
	Wales, Scotland and Northern Ireland	54	12.8	9.3 (5)	90.7 (49)	1.22	0.38, 3.94	
	South East	73	16.9	9.6 (7)	90.4 (66)	1.27	0.44, 3.68	
	South West	47	10.9	14.9 (7)	85.1 (40)	2.1	0.71, 6.18	
	West Midlands	36	8.3	8.3 (3)	91.7 (33)	1.09	0.27, 4.36	

	Unknown	11	2.5	9.1 (1)	90.9 (10)	NA		
7. Dog data								
Dog sex	Female entire	48	11.1	10.7 (6)	87.5 (42)	2.19	0.75, 6.36	0.29
	Female neutered	163	37.7	6.1 (10)	93.9 (153)	*Ref*		
	Male entire	68	15.7	13.2 (9)	86.8 (59)	2.33	0.90, 6.03	
	Male neutered	150	34.7	8.7 (13)	91.3 (137)	1.45	0.62, 3.42	
	Unknown	3	0.7	0.0 (0)	100.0 (3)	NA		
Dog age	<12 months	29	6.7	10.3 (3)	89.7 (26)	*Ref*		0.08
	1 year	16	3.7	6.3 (1)	93.8 (15)	0.58	0.06, 6.06	
	2 years	42	9.7	16.7 (7)	83.3 (35)	1.73	0.41, 7.35	
	3 years	38	8.8	15.8 (6)	84.2 (32)	1.63	0.37, 7.13	
	4 years	36	8.3	8.3 (3)	91.7 (33)	0.79	0.15, 4.23	
	5 years	37	8.6	10.8 (4)	89.2 (33)	1.05	0.22, 5.11	
	6 years	36	8.3	2.8 (1)	97.2 (35)	0.25	0.02, 2.52	
	7 years	34	7.9	8.8 (3)	91.2 (31)	0.84	0.16, 4.51	
	8 years or older	157	36.9	6.4 (10)	93.6 (147)	0.59	0.15, 2.29	

	Unknown	7	1.6	0.0 (0)	100.0 (7)	NA		
Length of time owned	<12 months	44	10.4	11.4 (5)	88.6 (39)	*Ref*		0.12
	1 year	23	5.4	8.7 (2)	91.3 (21)	0.74	0.13, 4.16	
	2 years	49	11.5	12.2 (6)	87.8 (43)	1.09	0.31, 3.85	
	3 years	43	10.1	16.3 (7)	83.7 (36)	1.52	0.44, 5.21	
	4 years	57	13.4	8.8 (5)	91.2 (52)	0.75	0.20, 2.77	
	5 years	36	8.5	8.3 (3)	91.7 (33)	0.71	0.16, 3.19	
	6 years	31	7.3	6.5 (2)	93.5 (29)	0.54	0.10, 2.97	
	7 years or longer	142	32.9	5.6 (8)	94.4 (134)	0.51	0.15, 1.70	
	Unknown	7	1.6	0.0 (0)	100.0 (7)	NA		

Appendix 4: Appendices for Chapter 5

Study information letter

A Dog's Dinner: Longitudinal Study of Persistence and Transmission of Antimicrobial Resistant Bacteria Between Dogs, Owners and their Home

You are being invited to participate in a research study. Before you decide whether to participate, it is important for you to understand why the research is being conducted and what it will involve if you do choose to take part. Please consider the following information. Researcher contact details are listed below should you have any further questions.

Once you have read this information sheet, please indicate your consent to participate in this study by completing the accompanying consent form.

This project has been fully approved by the ethics committee at the University of Liverpool and is funded by the Veterinary Medicines Directorate (VMD)*.

What is the purpose of the study?

Antibiotic resistance is one of the greatest global threats to human and animal health today, with a growing number of bacterial infections becoming harder to treat as the antibiotics used to treat them become less effective (World Health Organisation). Antibiotic resistance occurs when bacteria develop mechanisms to make them less susceptible to the drugs used to treat them.

Dogs and their owners often have close and frequent contact, which may lead to an increased risk of transmission of bacteria between them such as *E. coli* and *Salmonella* spp., including variants which are resistant to antibiotics. *E. coli* are present as normal gut bacteria in all species; however, some variants can be harmful, can cause disease if they infect areas other than the gut (such as the bladder/kidneys), and importantly can carry genes which confer antibiotic resistance, which can also be transferred to other species of bacteria.

This study aims to investigate the presence of antimicrobial-resistant *E. coli* and *Salmonella* spp. in the faeces of dogs fed a range of diets (including those fed cooked, commercial kibble-based diets and raw meat-based diets), in and around their food bowls (the environment) and in the faeces of the adult owners/household members (>16 years of age) in the household, alongside investigating the potential risk factors for dog and owner carriage of these bacteria. To further investigate the persistence of these bacteria in the household, the study will aim to follow some households and their dogs for 12 months.

The study will comprise of an initial questionnaire and collection of samples (part a), followed by a shorter follow-up questionnaire and sample collection once per month for 12 months (part b) if you agree to take part in the further sampling.

Why am I being invited to take part and what will happen if I take part?

You are being invited to take part because you are a UK dog owner who has already completed a previous 'Dogs Dinner' cross sectional study, and agreed to be contacted further, or have responded to an advertisement for this study and indicated your willingness to participate.

This study will compromise of two parts:

(a) If you decide to take part in this initial study, you will need to complete a short questionnaire (which will take around 15 minutes) and supply a small sample of your dog's faeces (poo), a swab sample of your own stool, stool samples from other members of your household who are aged 16 years and older, and take swab samples of your dog's food bowl, water bowl and the area of the floor where your dog eats (the environment) using the collection pots and swabs provided. Please return the completed questionnaire, consent form and samples together using the pre-paid envelope provided.
(b) We will then contact a selection of households to follow for up to 12 months. If you decide to take part in this part of the study you will need to complete a very short (5 minute) follow up questionnaire and provide repeat samples of your dog's faeces, your own stool and environmental swabs once per month for 12 months.

Participation is entirely voluntary and you do not have to take part in either part of this study. You can also participate in part (a) of the study but choose not to continue to part (b). You do not have to give a reason if you do not wish to take part and are also free to withdraw at any point.

All members of the household who are aged 16 years or above may participate in this study. All members of the household who agree to take part in this study are requested to complete an individual consent form, however, only the person who will be collecting the dog faecal sample and environmental samples needs to complete this section of the consent form.

Are there any benefits or risks in taking part?

There is a risk of contracting an infection from handling faeces as they may contain bacteria that can cause illness in people, such as diarrhoea, however there should not be any further risk than when you would normally pick up your dog's faeces for disposal. We have provided you with a pair of disposable gloves and instructions for how to collect a sample safely and to minimise any risks. We also advise you to wash hands after collection of faeces. If you are immunocompromised or unable to put on the disposable gloves, we would ask that another adult within your household collects the sample as those with underlying health issues may be more susceptible to infection.
All instructions are provided in the sample collection instructions leaflet, however should you feel ill after collecting the sample then we would recommend you contact your GP

What will happen if I want to stop taking part?

If you want to stop taking part in this study you can contact the named personnel at the end of this letter, using the details provided. If you wish to withdraw from the study, you may do so up to 14 days following our receipt of your completed questionnaire, and may request removal of your questionnaire data and destruction of the faecal sample you have provided, as well as any associated microbiological data. After 14 days it will not be possible to remove this as the results will have been anonymised and incorporated into our analysis.

What will happen to the results of the study?

The results of this study will be used to determine the presence of antimicrobial-resistant *E. coli* and *Salmonella* spp. (bacteria) in the faeces of dogs fed a range of diets, and whether these bacteria are also present in and around the dog food and water bowls and in the faeces of their owners, which would potentially indicate transmission within the home. They will also help us better understand the factors which may increase the risk of these bacteria being present.

Antimicrobial resistance is of increasing concern in all species, therefore we would like to understand whether there is a possible route of transmission of bacteria within the home between dogs and their

owners. Ultimately, we would like to provide information which could increase awareness of antimicrobial resistance amongst dog owners, and lead to pet care practices which are safer for both dog and owner.

All results will be fully anonymised, and published results of this study will be used in scientific journal articles and presentations. Due to anonymisation of samples and data, it will not be possible to inform you of individual faecal sample or environmental swab test results, however we can provide a short report of the overall findings once they are available on request.

How will my data be used?

The data you provide will be stored securely for 10 years in line with data protection requirements at the University of Liverpool and GDPR. All data is strictly confidential and will be used for this specific project only, and a limited number of people will have access to it.

The University processes personal data as part of its research and teaching activities in accordance with the lawful basis of 'public task', and in accordance with the University's purpose of "advancing education, learning and research for the public benefit".

Under UK data protection legislation, the University acts as the Data Controller for personal data collected as part of the University's research. The Principal Investigator acts as the Data Processor for this study, and any queries relating to the handling of your personal data can be sent to Professor Nicola Williams (Principal Investigator) using the contact details below.

What if I am unhappy or if there is a problem?

If you are unhappy, or if there is a problem, please feel free to contact the researcher listed below and we will try to help. If you remain unhappy or have a complaint which you feel you cannot communicate directly to the researcher then you should contact the Research Ethics and Integrity Office on 0151 794 8290 (ethics@liv.ac.uk). When contacting the Research Governance Officer, please provide details of the name or description of the study (so that it can be identified), the researchers involved, and the details of the complaint you wish to make.

Who can I contact for further details?

Miss Genever Morgan
Department of Livestock and One Health
University of Liverpool, Leahurst Campus
Chester High Road
CH64 7TE
Email: ddsurv20@liv.ac.uk

Professor Nicola Williams
Department of Livestock and One Health
University of Liverpool, Leahurst Campus
Chester High Road
CH64 7TE
Email: ddsurv20@liverpool.ac.uk

***** The Veterinary Medicines Directorate (VMD) is an executive agency, sponsored by the Department for Environment, Food & Rural Affairs, which protects animal health, public health and the environment.

Participant consent form

Title of the research project: **A Dog's Dinner: Longitudinal Study of Persistence and Transmission of Antimicrobial Resistant Enteric Bacteria Between Dogs, Owners and their Home**

Name of researcher(s): Genever Morgan, Professor Nicola Williams, Dr Vanessa Schmidt, Dr Gina Pinchbeck

Please complete BOTH sides of this consent form

Please initial box

1. I confirm that I have read and have understood the information sheet dated 25/10/21 for the above study, or it has been read to me. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that taking part in the study involves completion of the attached questionnaire, submission of a faecal sample from myself, my dog and swabs of my dog's food bowl, water bowl and floor area around the bowls. ☐

3. I am happy to provide a faecal sample from myself and consent to its use in this study. ☐

4. I am happy to provide a faecal sample from my dog, and swabs of my dog's food bowl, water bowl and floor area around the bowls, and consent to their use in this study **(IF APPLICABLE).** ☐

5. I understand that my participation is voluntary, that I am free to stop taking part and can withdraw from the study at any time up to 14 days from our receipt of your completed questionnaire without giving any reason and without my rights being affected. In addition, I understand that I am free to decline to answer any particular question(s). ☐

6. I understand that I can ask for access to the information I provide and I can request the destruction of that information, plus destruction of the sample I have provided and any associated microbiological data, if I wish at any time prior to 14 days from our receipt of your completed questionnaire. I understand that following 14 days I will no longer be able to request access to or withdrawal of the information or sample I provide. ☐

7. I understand that the information I provide will be held securely and in line with data protection requirements at the University of Liverpool. Data will be stored for up to 10 years by the University of Liverpool. ☐

8. I understand that signed consent forms and original questionnaires will be held securely and in line with data protection requirements at the University of Liverpool. ☐

9. I agree that my data may be shared within the research team named above and used in futu research if reviewed and approved by the ethics committee. I understand that my data will be **anonymised** and will not be identifiable in any published reports ☐

10. I consent to taking part in the longitudinal aspect of this study, which will involve monthly questionnaires and provision of samples **(OPTIONAL)**. I understand that I am under no obligation to take part in any further study (NB: If you decide not to consent to be involved in any further study it will not have any influence on your involvement in this particular research study). ☐

11. The faecal and environmental samples you provide will be specifically tested for *E. coli* and *Salmonella* in this study. We may wish to test for additional zoonotic pathogens or new antibiotic resistance mechanisms that may emerge in future, therefore intend to keep the sample you provide after the study has finished. **This will be stored anonymously.** Please indicate whether you consent to our storing the following samples for use at a later date **(OPTIONAL)**:

- Your stool sample ☐
- Your dog's faecal sample ☐

12. I agree to take part in the above study. ☐

Please now sign this page to indicate your consent to participate in this study.

_____	_____	_____
Participant name	Date	Signature
_____	_____	_____
Name of person taking consent *(if applicable)*	Date	Signature

Principal Investigator

Professor Nicola Williams

Institute of Infection and Global Health

University of Liverpool, Leahurst Campus

Chester High Road

CH64 7TE.

ddsurv20@liverpool.ac.uk

PhD Student Investigator

Miss Genever Morgan

Institute of Infection and Global Health

University of Liverpool, Leahurst Campus

Chester High Road

CH64 7TE.

ddsurv20@liverpool.ac.uk

Sample collection instructions

Instructions for collection of your dog's sample

Please read these instructions carefully before collecting your sample

In your collection pack there should be **1 x 15ml collection pot**, **1 x pair of gloves**, **1 x wooden spatula**, **1 x clear plastic zip lock collection bag**, **1 x absorbent tissue**

Put on your gloves and open your sample pot.

Using the spatula, pick up a small sample of your dog's faeces (preferably a freshly evacuated stool). As an example, a sample the size of your fingernail would be suitable.

Place both the sample and spatula into the sample pot as shown in picture 2. Screw the lid tightly closed.

Picture 1: Place wooden spatula and sample inside the collection pot and screw tightly closed.

Wrap the sample pot in the absorbent tissue and place into the clear zip lock bag.

Remove your gloves and dispose of them. Please wash your hands.

Place the zip lock bag in the large 95KPa bag along with your environmental sample swabs and your own stool sample and place this bag into the cardboard postal box, along with your completed questionnaire and consent form.

Take your sample collection kit to a Royal Mail post office if returning by post, or email the research team to inform us that your samples are ready for collection

If you have any problems or concerns regarding the faecal sample collection process, please contact the research team via email at ddsurv20@liverpool.ac.uk

Thank you very much for your participation

Instructions for stool sampling

Please read these instructions carefully before collecting the samples

In your collection pack there should be **1 x sterile dry swabs, 1 x pair of gloves, 1 x clear plastic zip lock collection bag, 1 x absorbent tissue**

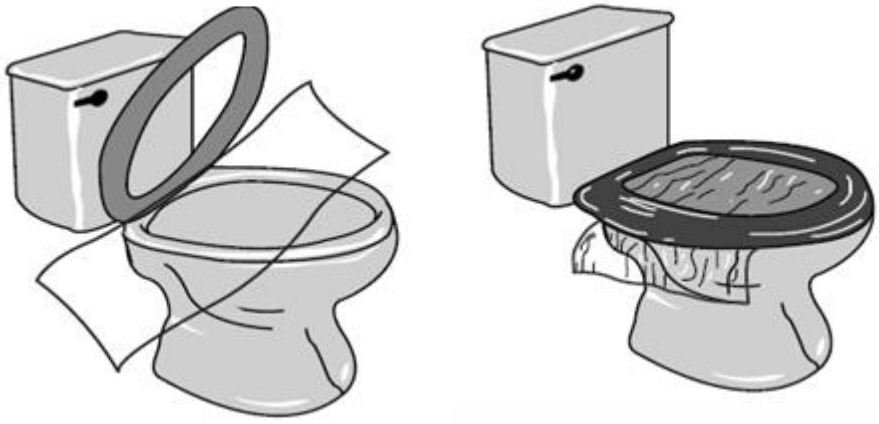
Please label your dry swab tube to indicate that the swab is a human stool sample

Wear gloves when collecting the sample

Please add your **name and the date of sampling** to the label of the sterile swab labelled 'human stool sample swab'.

Place something inside the toilet bowl to catch the stool to ensure it does not contact the inside of the toilet bowl (e.g. line the toilet bowl with toilet roll, place an empty plastic container in the toilet bowl, or use plastic wrap e.g. clingfilm as shown in pictures 1 and 2 below).

Pictures 1 and 2:



1. *Place plastic wrap over rim of toilet. 2. Lower seat prior to collection of sample to hold plastic wrap in place.*

Open the sterile swab, taking care not to touch the tip, and use it to take a sample of the stool.

Place the swab back into the tube, wrap it in the absorbent tissue provided and place into the clear plastic zip lock bag provided.

Dispose of your gloves and any items used to collect the stool sample in the bin, then wash your hands.

Place the zip lock bag in the large 95KPa bag along with your environmental sample swabs and your dog's faecal sample and place this bag into the cardboard postal box, along with your completed questionnaire and consent form.

Take your sample collection kit to a Royal Mail post office if returning by post, or email the research team to inform us that your samples are ready for collection

If you have any problems or concerns regarding the sample collection process, please contact the research team via email at ddsurv20@liverpool.ac.uk

Thank you very much for your participation

Instructions for environmental swab sampling

Please read these instructions carefully before collecting the samples

In your collection pack there should be **3 x sterile dry swabs in tubes (labelled A, B and C), 1 x pair of gloves, 1 x clear plastic zip lock collection bag, 1 x absorbent tissue, 1 x 10x10cm paper frame**

Please add your name and date of sampling to the label of your dry swab tubes. The labels have been pre-filled to indicate the swab from your dog's food bowl (Swab A), the water bowl (Swab B) and the floor area (Swab C).

Food bowl (Swab A)

Put on your gloves and open one of the sterile dry swabs, taking care not to touch the tip of the swab.

After your dog has finished eating, rub the swab along the entire inside surface of your dog's food bowl (the inside edge and bottom of the bowl). Rotate the swab so that the entire surface has touched the food bowl (Fig 1 and 2).

Place the swab into the tube and close tightly.

Fig 1 and 2: Swab entire surface of dog food bowl (edge and bottom of bowl)

Water bowl (Swab B)

Open the second sterile dry swab, taking care not to touch the tip of the swab.

Rub the swab along the entire inside edge of your dog's empty water bowl at the level of the water line and around the inside where the walls of the bowl meet the bottom of the bowl. Rotate the swab so that the entire surface has touched the water bowl.

Place the swab into the tube and close tightly.

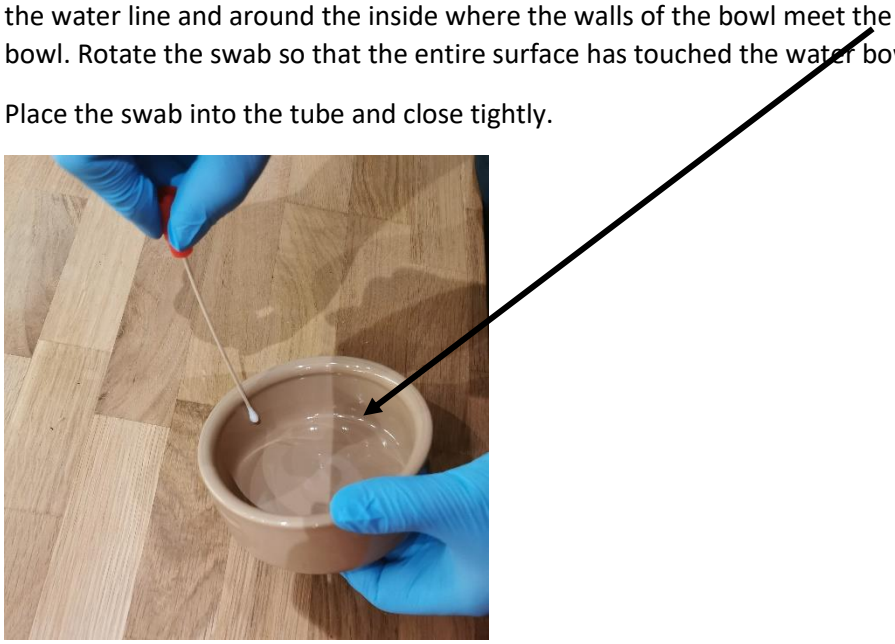


Fig 3: Swab along entire inside edge of empty water bowl at level of water line

Floor around food bowl (Swab C)

Place the 10x10cm paper frame onto the floor area surrounding where your dog's food bowl is placed. Open the final sterile dry swab, taking care not to touch the tip of the swab.

Rub the swab back and forth within the 10x10cm frame area, rotate the swab so that the entire surface has touched the floor area.

Place the swab into the tube and close tightly.

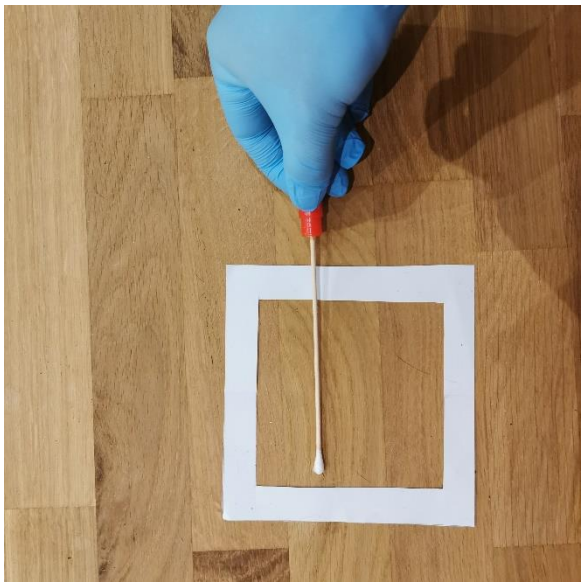


Fig 4: Swab entire area within 10x10cm frame

Finally, wrap all 3 swab tubes in the absorbent tissue and place inside the plastic ziplock bag.

Place the ziplock bag in the large 95KPa bag along with your dog faecal sample and your own stool sample and place this bag into the cardboard postal box, along with your completed questionnaire and consent form.

Take your sample collection kit to a Royal Mail post office if returning by post, or email the research team to inform us that your samples are ready for collection

If you have any problems or concerns regarding the sample collection process, please contact the research team via email at ddsurv20@liverpool.ac.uk

Thank you very much for your participation

I

Initial questionnaire: Human participant

A Dog's Dinner: Longitudinal Study of Persistence and Transmission of Antimicrobial Resistant Enteric Bacteria Between Dogs, Owners and their Home: Human Participant Questionnaire

This questionnaire is to be completed for every member of the household participating in the study over 16 years old

Please indicate your answers by marking an 'x' in the box provided or writing in BLOCK CAPITAL letters.

Please use a blue or black ballpoint pen. If you want to change your answer, or you make a mistake, please indicate clearly by filling in the box completely and placing a cross in the correct box.

Many thanks for your participation

Q1: What age group do you fit in to?

- ☐ 16-25
- ☐ 26-35
- ☐ 36-45
- ☐ 46-55
- ☐ 56-64
- ☐ 65 or over

Q2: Are you the main caregiver for the dog(s) in your household?

- ☐ Yes
- ☐ Sometimes
- ☐ No

Q3: Do you currently work in any of the following?

- ☐ Hospital
- ☐ GP surgery
- ☐ Care home/nursing home
- ☐ Nursery/children's day care
- ☐ Primary school

☐ Secondary school
☐ Livestock farm
☐ Horse riding stables/livery yard
☐ Dog boarding kennels
☐ Zoo/petting zoo
☐ None of the above

Q4: Have you received any antibiotics (e.g. injections, tablets, liquid, powder) in the last 3 months?

☐ No

☐ Yes *(please detail the name of the antibiotics, if known, below)*

Q5: If yes, are you currently receiving antibiotics?

☐ No

☐ Yes *(please detail the name of the antibiotics, if known, below)*

Q6: Have you attended a hospital in the last month?

☐ No

☐ Yes, as a patient

☐ Yes, as a visitor

Q7: Which of the following do you currently eat as part of your diet?

☐ White meat (e.g. poultry)
☐ Red meat (e.g. beef, lamb, pork)
☐ Fish
☐ Vegetarian diet
☐ Vegan diet
☐ Other (please detail below)

Q8: Do you regularly eat any rare or raw meat as part of your diet (e.g. steak, burgers)?

☐ No

☐ Yes *(please detail below)*

Q9: Have you travelled abroad in the last 3 months?

☐ No

☐ Yes *(please detail travel destination(s) below)*

Date of stool sample: ……/……/…….

END OF QUESTIONS

Thank you once again for your participation

Follow up questionnaire: Human participant

A Dog's Dinner: Longitudinal Study of Persistence and Transmission of Antimicrobial Resistant Enteric Bacteria Between Dogs, Owners and their Home

Monthly follow up sheet (Human Participant)

Please complete one copy of this sheet per participant

Please let us know of any changes that have occurred since the last sampling pack.

Q1. Person Name: Participant Number:

Q2. Have you received any antibiotics in the last month?

☐ Yes *(please provide name of antibiotic if known)*

☐ No

☐ Q3. Have you visited a GP surgery in the last month?

☐ Yes

☐ No

Q4. Have you visited a hospital in the last month?

☐ Yes, as an outpatient

☐ Yes, I was hospitalised

☐ No

Q5. Have you travelled abroad in the last month?

☐ No

☐ Yes *(please detail travel destination(s) below)*

Date of stool sample: ...../...../..... **Date of environmental samples:** ...../...../.....

Initial questionnaire: Dog

A Dog's Dinner: Longitudinal Study of Persistence and Transmission of Antimicrobial Resistant Enteric Bacteria Between Dogs, Owners and their Home: Dog and Household Questionnaire

Please complete this questionnaire **once on behalf of each dog in your household** and return it with your signed consent form, faecal samples and environmental swabs using the enclosed prepaid packing bag.

Answering the questions

This questionnaire consists of **three** short sections about your dog and its healthcare, their food, and your household.

Section One: Your dog, its general health and lifestyle
Section Two: Your dog's food
Section Three: You and your family

Please indicate your answers by marking an 'x' in the box provided e.g. [X] or writing in BLOCK CAPITAL letters.

Please use a blue or black ballpoint pen. If you want to change your answer, or you make a mistake, please indicate clearly by filling in the box completely and placing a cross in the correct box.

Checklist

Please ensure the following are included in your prepaid packing bag before returning to us:

- ☐ Signed consent form
- ☐ Dog faecal sample, human stool samples and environmental swabs
- ☐ Completed questionnaires (this one, plus separate completed human participant questionnaires)

Many thanks for your participation

Your dog, its general health and lifestyle

Q1: Dog Name:

Q2: How old is your dog? ……..years …… months

Q3: Is your dog:

☐ Male, Not neutered
☐ Male, Neutered
☐ Female, Not neutered
☐ Female, Neutered

Q4: How many dogs live permanently in your household? …….

Q5: Have any new dogs been brought into your household in the last 3 months?

☐ No

☐ Yes

Q6: Has your dog suffered from diarrhoea/loose stools in the last 3 months?

☐ No (*please go to Q7*)

☐ Yes *(please complete Q6a below)*

Q6a: How many episodes of diarrhoea/loose stools has your dog had in the last 3 months?

☐ 1-2 episodes
☐ 3-4 episodes
☐ 5 or more episodes
☐ Constant diarrhoea

Please detail if any tests were done, if a diagnosis was made or cause for diarrhoea was found, and what treatment was given (if any):

Q7: Has your dog received any antibiotics (e.g. injections, tablets, liquid, powder) in the last 3 months?

☐ No

☐ Yes *(please detail the name of the antibiotics, if known, below)*

Q8: Has your dog attended a veterinary practice in the last 3 months?

☐ No *(Please go to Q10)*

☐ Yes *(Please go to Q9)*

Q9: If yes, was your dog hospitalised?

☐ No
☐ Yes, for the day only
☐ Yes for 24 hours or longer

Q10: Does your dog regularly (at least once per week) attend any of the following *(please tick all that apply)*?

☐ Doggy day care
☐ Dog training classes
☐ Group dog walking
☐ Dog shows
☐ Dog parks
☐ Farm land
☐ Public parks
☐ Towpaths/footpaths/bridleways
☐ Beaches
☐ Other *(please detail)*

Q11: Does your dog have regular (at least once per week) close contact with any of the following *(please tick all that apply)*?

- ☐ Other dogs
- ☐ Cats
- ☐ Small mammals/rodents (e.g. rats, mice, hamsters, rabbits)
- ☐ Horses
- ☐ Farm animals (e.g. cattle, sheep, pigs)
- ☐ Poultry (e.g. chickens, ducks, geese)
- ☐ Wildlife (e.g. squirrels, rats, foxes, badgers, wild birds)
- ☐ Reptiles/snakes
- ☐ No other regular animal contact
- ☐ Other *(please detail)*

Q12: Has your dog visited any human hospitals, care homes, nursing homes, nurseries, etc (e.g., Pets as Therapy dogs) in the last month?

- ☐ No
- ☐ Yes *(please detail)*

Q13. Where in the house does your dog usually sleep *(please tick all that apply)*?

- ☐ In a dog bed or crate on the floor in my bedroom
- ☐ In a dog bed or crate on the floor in another person's bedroom
- ☐ In a dog bed or crate on the floor in a room other than a bedroom (e.g. kitchen, living room)
- ☐ In/on my bed
- ☐ On the sofa
- ☐ Other *(please detail)*

Q14: Does your dog lick your hands and/or face?

- ☐ No
- ☐ Yes, sometimes
- ☐ Yes, frequently
- ☐ Don't know

Your dog's food

Q15a. What category of food(s) are you **currently** feeding your dog *(please tick all that apply)*:

- ☐ Cooked commercial complete wet food
- ☐ Raw meat and/or bones (pre-prepared diet)
- ☐ Raw meat and/or bones (DIY/home-prepared diet)
- ☐ Cooked fresh meat and/or bones
- ☐ Cooked commercial complete dry food/kibble
- ☐ Cooked DIY/home-prepared diet
- ☐ Vegetarian/Vegan diet
- ☐ Other e.g. insect-based diet *(please detail)*

Q15b: Please list what your dog is ***currently*** fed (e.g. Nature's Menu nuggets, James Wellbeloved kibble, home cooked diet ingredients):

Q16: Do you feed any raw meat and/or bones to your dog **at least once per week**?

- ☐ Yes *(please go to Q17)*
- ☐ No *(please go to Q18)*

Q17: What type(s) of raw meat, either as part of a pre-prepared meal or component parts of the diet bought from a shop/supplier are you currently feeding your dog *(tick all that apply)*?

- ☐ Beef
- ☐ Pork
- ☐ Chicken
- ☐ Lamb
- ☐ Venison
- ☐ Turkey
- ☐ Rabbit
- ☐ Duck
- ☐ Fish
- ☐ Game (e.g. Pheasant, grouse, pigeon)
- ☐ Tripe
- ☐ Offal (e.g. Heart, liver, kidney)
- ☐ Other

Q18: Has your dog's diet changed in the last 3 months?

- ☐ No
- ☐ Yes *(please detail the changes in the box below)*

Q19: What types of treats are you currently feeding your dog? *(please tick all that apply)*:

- ☐ Shop bought cooked treats/biscuits
- ☐ Freeze dried meat/fish treats
- ☐ Dried treats (e.g. pig ears, chicken feet, pizzle sticks, hooves)
- ☐ Dehydrated treats
- ☐ Raw meat (including items such as raw ears, duck necks, beef trachea, etc)
- ☐ Raw bones
- ☐ Cooked meat
- ☐ Cooked bones
- ☐ Vegetable-based treats
- ☐ I don't feed any treats
- ☐ Other *(please detail below)*

Q20: How frequently does your dog scavenge items (e.g. eat things they shouldn't do on walks, steal from a bin, eat faeces or carcasses)?

- ☐ Never *(please go to Q22)*
- ☐ Yes, sometimes
- ☐ Yes, frequently
- ☐ Yes, every walk
- ☐ Don't know

Q21: What items does your dog scavenge *(please tick all that apply)*?

- ☐ Food items
- ☐ Dead animals/carcasses
- ☐ Items from the bin
- ☐ Other dog's faeces
- ☐ Other animals' (other than dog) faeces
- ☐ Don't know
- ☐ Not applicable

Q22: Where does your dog usually eat their meals?

- ☐ In the kitchen
- ☐ In the utility room
- ☐ Outside/in an outbuilding or shed
- ☐ In another room in the house *(please detail)*

About you and your family *(Please complete the section **once only** on behalf of your household)*

Q23: How many people currently reside in your household? ☐

Q24: How many people are aged 65 years or over? ☐

Q25: How many people are aged 5 years or less? ☐

Please now complete the accompanying human participant questionnaire (one questionnaire per person over 16 years old who is participating in this study)

Date of dog faecal sample: ……/……/…..

Date of environmental swabs (food bowl, water bowl, area of floor around food bowl): ……/……/…..

END OF QUESTIONS

Thank you once again for your participation

Follow up questionnaire dog

A Dog's Dinner: Longitudinal Study of Persistence and Transmission of Antimicrobial Resistant Enteric Bacteria Between Dogs, Owners and their Home

Monthly follow up sheet (Dog)

Please complete one copy of this sheet per dog in your household

Please let us know of any changes that have occurred since the last sampling pack.

Q1. Dog Name: Dog Number:

Q2. Has this dog had any change in their diet in the last month?

☐ Yes *(please detail on the other side of the page)*

☐ No

Q3. Has this dog received any antibiotics in the last month?

☐ Yes *(please detail on the other side of the page)*

☐ No

Q4. Has this dog been hospitalised in a veterinary practice in the last month?

☐ Yes *(please detail on the other side of the page)*

☐ No

Date of faecal sample collection: ...../...../.....

Thank you very much for your continued participation

Please detail any changes since the last sampling pack on this page

Changes in diet:

Antibiotics given (name of antibiotics if known, reason for treatment):

Reason for hospitalisation:

Table A1: *Presence of E. coli growth on HECA agar for each sample at each stage of the study*

Household	Raw	Sample	*E. coli* growth on HECA				
			Initial (T0)	T1	T2	T3	T4
20	N	dog 1	Y	Y	Y	Y	Y
20	N	person 1	Y	Y	N	Y	Y
20	N	person 2	Y	Y	Y	Y	N
20	N	food bowl	N	N	N	N	N
20	N	water bowl	N	N	N	N	N
20	N	floor	N	N	N	N	Y
21	Y	dog 1	Y	Y	Y	Y	Y
21	Y	dog 2	Y	Y	Y	Y	Y
21	Y	person 1	Y	N	N	N	N
21	Y	person 2	Y	Y	Y	Y	Y
21	Y	food bowl 1	N	N	N	N	N
21	Y	food bowl 2	N	N	Y	N	N
21	Y	water bowl	N	N	N	N	N
21	Y	floor	N	N	Y	N	N
15	Y	dog 1	Y	Y	Y	Y	Y
15	Y	dog 2	Y	Y	Y	Y	Y
15	Y	person 1	Y	Y	Y	Y	Y
15	Y	person 2	Y	Y	Y	Y	Y
15	Y	food bowl 1	Y	N	N	N	N
15	Y	food bowl 2	N	N	N	N	N
15	Y	water bowl	Y	N	N	N	N
15	Y	floor	N	Y	N	Y	N
7	N	dog 1	Y	Y	Y	Y	Y
7	N	person 1	Y	Y	N	N	Y
7	N	food bowl	N	N	N	N	N
7	N	water bowl	N	N	N	N	N
7	N	floor	N	N	N	N	N
6	N	dog 1	Y	Y	N	N	Y
6	N	person 1	N	Y	Y	Y	Y
6	N	food bowl	N	N	N	N	N
6	N	water bowl	Y	N	N	N	N
6	N	floor	N	N	N	N	N
9	N	dog 1	Y	N	Missing data	Y	Y
9	N	dog 2	Y	Y		Y	Y

9	N	dog 3	Y	Y		Y	Y
9	N	person 1	Y	Y		Y	Y
9	N	person 2	Y	Y		N	Y
9	N	food bowl 1	N	N		N	N
9	N	food bowl 2	N	N		N	N
9	N	food bowl 3	N	N		N	N
9	N	water bowl	N	Y		N	N
9	N	floor	N	N		N	N
13	Y	dog 1	Y	Y	Y	Y	Y
13	Y	dog 2	Y	Y	Y	Y	Y
13	Y	dog 3	Y	Y	Y	Y	Y
13	Y	person 1	Y	Y	Y	Y	Y
13	Y	food bowl 1	N	N	N	N	N
13	Y	food bowl 2	N	N	N	N	N
13	Y	food bowl 3	N	N	N	N	N
13	Y	water bowl	N	N	N	N	N
13	Y	floor	N	N	N	N	N
11	Y	dog 1	Y	Y	Y	Y	Y
11	Y	person 1	N	Y	Y	Y	Y
11	Y	food bowl 1	N	Y	N	N	N
11	Y	water bowl	N	N	N	N	Y
11	Y	floor	N	N	N	N	N
19	Y	dog 1	Y	Y	Y	Y	Y
19	Y	dog 2	Y	Y	Y	Y	Y
19	Y	person 1	Y	Y	Y	Y	Y
19	Y	food bowl 1	Y	N	N	N	N
19	Y	food bowl 2	N	N	N	N	N
19	Y	water bowl	Y	Y	N	N	N
19	Y	floor	N	N	N	N	N
4	N	dog 1	Y	Y	*		
4	Y	dog 2	Y	Y			
4	B	person 1	Y	Y			
4	B	person 2	Y	Y			
4	B	food bowl 1	N	N			
4	B	food bowl 2	N	N			
4	B	water bowl 1	N	N			
4	B	water bowl 2	N	N			
4	B	floor	N	N			
18	Y	dog 1	Y	*			
18	Y	dog 2	Y				
18	Y	dog 3	Y				
18	Y	dog 4	Y				
18	Y	person 1	Y				

18	Y	food bowl 1	N				
18	Y	food bowl 2	N				
18	Y	food bowl 3	N				
18	Y	food bowl 4	Y				
18	Y	water bowl	Y				
18	Y	floor	N				
3	Y	dog 1	Y	*			
3	Y	dog 2	Y				
3	Y	person 1	Y				
3	Y	person 2	N				
3	Y	food bowl 1	Y				
3	Y	food bowl 2	Y				
3	Y	water bowl	N				
3	Y	floor	N				
22	Y	dog 1	Y	*			
22	Y	dog 2	Y				
22	Y	person 1	Y				
22	Y	person 2	Y				
22	Y	food bowl 1	N				
22	Y	food bowl 2	N				
22	Y	water bowl	Y				
22	Y	floor	Y				
24	N	dog 1	Y	**			
24	N	person 1	Y				
24	N	food bowl	N				
24	N	water bowl	N				
24	N	floor	N				
17	N	dog 1	N	**			
17	N	dog 2	N				
17	N	person 1	Y				
17	N	food bowl 1	N				
17	N	food bowl 2	N				
17	N	water bowl	N				
17	N	floor	N				
12	N	dog 1	Y	**			
12	Y	dog 2	Y				
12	N	dog 3	Y				
12	B	person 1	Y				
12	B	food bowl 1	Y				
12	B	food bowl 2	Y				
12	B	food bowl 3	Y				
12	B	water bowl	Y				
12	B	floor	Y				

10	N	dog 1	Y	**			
10	N	dog 2	Y				
10	N	person 1	Y				
10	N	person 2	Y				
10	N	food bowl 1	N				
10	N	food bowl 2	N				
10	N	water bowl	N				
10	N	floor	N				
1	N	dog 1	Y	**			
1	N	person 1	Y				
1	N	food bowl 1	N				
1	N	water bowl	N				
1	N	floor	N				
16	N	dog 1	Y	**			
16	N	person 1	N				
16	N	food bowl 1	N				
16	N	water bowl	N				
16	N	floor	N				

*Lost to follow up; **No antimicrobial resistance identified on initial study, so no further participation

Appendix 5: Published paper: A study on the isolation of *Salmonella* species of public health concern from commonly-fed dried meat dog treats

Genever Morgan[a,c], Mikhela Saal[a], Aoife Corr[a], Claire Jenkins[b], Marie Anne Chattaway[b], Gina Pinchbeck[a], Nicola Williams[a]

[a] Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Leahurst Campus, Chester High Road, Neston, CH64 7TE

[b] United Kingdom Health Security Agency, Gastrointestinal Bacteria Reference Unit, Colindale, London, NW9 5HT

[c] Corresponding author email address: Genever.Morgan@liverpool.ac.uk

Published in the Veterinary Record, January 2023, doi: https://doi.org/10.1002/vetr.2642

Key Words: Salmonella, dog, treats, raw, dried

Abstract

Background
Dried non-heat-treated meat treats, such as ears, skin and tails are popular supplementary dog foods. Previous studies have demonstrated *Salmonella* spp. contamination on treats, particularly in pig ears and chicken products. This small exploratory cross-sectional study investigated *Salmonella* spp. presence in dried treats available in the UK.
Methods
A selection of dried treats from local pet shops and online retailers underwent bacterial culture for *Salmonella* spp. and antimicrobial susceptibility testing, with *Salmonella* serotype determined by whole genome sequencing.
Results
Eighty-four samples were tested, with 16% *Salmonella* spp. positive. Five *Salmonella* serotypes were identified, each associated with specific treat types. An antimicrobial resistant phenotype was identified in 39% of isolates. All serotypes identified are known to cause human infection.
Limitations
This study was limited by a small sample size and limited number of retail sources.
Conclusion
Salmonella spp. of public health concern were present in some dried dog treats in this study. Dog owners, pet food retailers and veterinary professionals should be aware of the potential zoonotic disease risk associated with these treats, and appropriate hygiene measures, including thorough hand washing, should be utilised if they are fed.

Introduction

Non-heat processed meat items, which include raw meat diets (RMD) and air dried, freeze dried or dehydrated treats, are an increasingly popular diet choice for dogs (Dodd *et al.*, 2020). These foodstuffs have not undergone any cooking or heat treatment as part of the production process, however the process used for treat production must have proven in sampling tests to destroy *Salmonella* (Department for Environment Food and Rural Affairs, no date). Items used as treats or chews may include body parts from a range of animals such as ears, snouts, tendons, skin, trachea, tails, bull penis, hooves and feet (Wong *et al.*, 2007; Freeman *et al.*, 2013). Previous studies have demonstrated that dog owners who choose to feed non-processed meat items do so as they believe them to be a more natural and healthier choice for their pet (Lenz *et al.*, 2009; Morgan, Willis and Shepherd, 2017; Morelli *et al.*, 2019; Viegas *et al.*, 2020), and that these items provide perceived benefits such as mental stimulation, increased satisfaction in food, and allow the dog to exhibit more natural chewing behaviour (Marx *et al.*, 2016; Morelli *et al.*, 2019; Wales and Davies, 2021).

Dried, non-processed dog chews are composed of category 3 animal by-products (ABP) as per DEFRA regulation, and may include raw abattoir material passed fit for human consumption but that is unwanted due to commercial reasons, and material from animals which passed an ante-mortem test but was deemed unfit for human consumption (Department for Environment Food and Rural Affairs, no date).

While there is an increasing body of research examining RMDs for dogs, there remains relatively limited evidence regarding the microbiological risks of ABPs used as dog treats. *Salmonella* spp. contamination has previously been reported in dried and dehydrated treats in the UK and elsewhere (Clark *et al.*, 2001; Willis, 2001; White *et al.*, 2003; Centers for Disease Control and Prevention (CDC), 2006; Finley *et al.*, 2007, 2008a; Wong *et al.*, 2007; Adley *et al.*, 2011; Yukawa *et al.*, 2019), with pig ear treats, raw hide and chicken products frequently represented even among treats where they were expected to have been heat-treated (Willis, 2001)

The present small exploratory cross-sectional study aimed to investigate the presence of *Salmonella* spp. in a selection of dried natural dog treats readily available in the UK.

Materials and methods

A selection of dried natural dog treats was purchased from a convenience sample of suppliers. Treats were purchased in-person from an independent pet shop (supplier A) and a large nationwide chain pet shop in Merseyside (Supplier B), and from two nationwide-supplying online retailers (Suppliers C and D) during September-October 2021. Treat type selection was opportunistic and at random, depending on availability at the time of shop or website visit. Purchases were made on two visits two weeks apart from supplier A, whereas one-time purchases were made from supplier B, C and D. Information regarding packaging type and labelling was recorded.

Whole treats were placed into individual sterile sealable bags and homogenised with 25ml of buffered peptone water (BPW). The broth was then poured into a sterile universal tube and incubated overnight at 37°C, after which 100µl was added to 5ml of Rappaport-Vassiliadis broth (RVB) and incubated overnight at 42°C.

Harlequin Chromogenic Agar for Salmonella Esterase (CASE) (Neogen, UK) was inoculated with the RVB and incubated for 18-20h at 37°C. CASE plates were examined for turquoise colonies characteristic of suspected *Salmonella* spp., and if present, two individual colonies were then plated onto nutrient agar and incubated overnight at 37°C. Confirmation of *Salmonella* spp. was undertaken using matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF).

Isolates underwent whole genome sequencing (WGS) at the Gastrointestinal Bacteria Reference Unit (GBRU) within the United Kingdom Health Security Agency (UKHSA). For isolates confirmed as *Salmonella* spp., DNA was extracted using the QIAamp® DNA mini kit (Qiagen, Crawley, UK). Following DNA extraction, isolates were prepared for WGS with Nextera XT DNA preparation kits, and sequenced on the Illumina HiSeq 2500 platform in rapid run mode to produce 100bp paired-end reads. Trimmomatic v0.40 (Bolger, Lohse and Usadel, 2014) was used to quality trim fastq reads with bases removed from the trailing end that fell below a PHRED score of 30. The Metric Orientated Sequence Type (most) v1 (Tewolde *et al.*, 2016) was used for sequence type (ST) assignment and serotype was assigned using a combination of the Salmonella MLST database and SeqSero2 (Achtman *et al.*, 2012; Ashton *et al.*, 2016; Zhang *et al.*, 2019).

FASTQ sequences were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive under the BioProject accession number PRJNA248792 (www.ncbi.nlm.nih.gov/bioproject/?term=248792). Raw sequence data files of isolates from this study were uploaded to EnteroBase (https://enterobase.warwick.ac.uk/) and short reads were assembled by EnteroBase using the then current backend pipelines (versions 3.61–4.1) including core genome Multi-Locus Sequence Types (cgMLST) analysis to produce a cgST as previously described (Chattaway, Chandra, *et al.*, 2019) using the cgMLST v2 HierCC v1 algorithm (Zhou *et al.*, 2018). All 13 isolates met the cgMLST quality parameters for Salmonella (minimum size 4000 kbp, maximum size 5800 kbp, minimum N50 20 kbp, maximum number contigs 600, maximum low-quality sites 5 %, minimum taxonomic purity 70 % (Zhou *et al.*, 2020)) for analysis. Hierarchical Clustering (HierCC of cgMLST) is a multi-level clustering scheme for population assignments based on cgMLSTs (Zhou *et al.*, 2020) and previous studies have shown that analyzing strains at the 5 allelic threshold is appropriate to detect clusters or closely related clones (Pearce *et al.*, 2018; Chattaway, Chandra, *et al.*, 2019; Larkin *et al.*, 2022). Therefore, HierCC was analysed at the five allelic level (HC5 – strains linked within five cgMLST alleles) for microbiologically linked human cases. The minimum spanning tree was created in EnteroBase for each pathogen using the MSTree v2 algorithm and visualizing on GrapeTree (Zhou *et al.*, 2018).

Salmonella spp. isolates underwent antimicrobial susceptibility testing via disc diffusion. Antimicrobials tested were ampicillin 10 µg, amoxycillin-clavulanic acid 20 µg/10 µg, ciprofloxacin 5 µg, tigecycline 15 µg, trimethoprim-sulphamethoxazole (TMS) 1.25 µg/23.75 µg, amikacin 30 µg and meropenem 10µg (MAST Group Ltd, Liverpool UK). Isolates were inoculated into sterile saline to 0.5 McFarland Units (MFU) and a 5µl loopful was spread on to Muller-Hinton agar (Neogen, UK). Discs were placed and plates incubated aerobically for 18-20h at 37°C. Following incubation, the zones of inhibition were measured and susceptibility interpreted. Breakpoints and screening concentration criteria used for interpretation were as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (EUCAST, 2022). Data processing and descriptive statistics were carried out using Microsoft Excel 2016.

No animal subject, human participant or personal data collection was required for this study, hence ethical approval was not required.

Results

Eighty-four samples were tested from a selection of treat types. Animal proteins represented were buffalo/bison (N=25), chicken (N=19), beef (N=13), lamb (N=4), pork (N=4), duck (N=3), rabbit (N=3), camel (N=3) and other unspecified sources sold as ‘bronchos’, tendons and ‘pizzle sticks’ (N=10). Full data regarding treat type, supplier and *Salmonella* spp. presence is provided in supplementary table 1.

Sample packaging varied greatly. Supplier A treats (N=43) were provided unpackaged with no labelling or traceability information present. Treats were in separate baskets based on treat type and purchased by placing into paper bags. Supplier B treats (N=4) were individually wrapped in branded plastic sealed packets; Supplier C treats (N=21) were delivered in a box

comprising some loose unpackaged ear treats and other items provided in branded sealed bags. Treats purchased from Supplier D (n=16) presented as multiple items in clear plastic bags with no labelling. Country of origin was unknown for the majority of treats (70%, 59/84), 5% (4/84) stated they were produced in the UK, and 25% (21/84) stated materials were sourced from the UK and Europe on their website (supplementary table 1).

Salmonella enterica was isolated from 16% (95% CI 7.8-23.2; n=13) of the treats tested. The types of treats that tested positive for *S. enterica* were dried bull's penis 'pizzle sticks' (67%, 95% CI 20.8-93.9; N=2/3), bison ears (24%, 95% CI 11.5-43.4; N=6/25), furry rabbit ears (67%, 95% CI 20.8-93.9; N=2/3) and dried chicken treats (60%, 95% CI 23.1-88.2; N=3/5). All treats which had *Salmonella* spp. isolated were purchased from the same independent pet shop purchased on two separate visits.

Five different *S. enterica* serotypes were identified via WGS (Table 1), identified as *S.* Anatum, *S.* Derby, *S.* Dublin, *S.* Infantis and *S.* Typhimurium (monophasic). Each specific serotype was isolated from a single treat type only. Data were compared to all sequences in the UKHSA database. All serotypes detected were known to cause human infection (Chattaway, Dallman, *et al.*, 2019). The most frequently isolated serotype was *S.* Derby (46%, 6/13) isolated from bison ears, *S.* Dublin was identified in two pizzle stick samples. As well as identifying a diverse range of serotypes via WGS, HierCC analysis (cluster analysis for population assignments based on the core genome) indicated that even within serotypes, the populations were genetically diverse (Table 1). Figure 1 shows the population structure of Salmonella species isolated from the different dog treats and which have also been identified in human cases, at the HierCC 5 allelic level. Dog treat types were associated with single specific *S. enterica* serotypes with the exception of chicken, which was associated with two serotypes. Isolates associated with human clinical cases were found in two of the five serotypes.

Table 1: Treat number and type, *S. enterica* serotype identification, sequence type (ST), HierCC HC5, and associated antimicrobial susceptibility testing results for isolates confirmed as *Salmonella* in this study. All isolates were isolated from treats from the same supplier obtained over two separate visits.

Treat no.	SRA Accession Number	Treat type	Visit no.	ST	HierCC HC5	*Salmonella* Serotype	Antibiotic type*						
							Aug	Amp	Tig	TMS	Ami	Cip	Mer
13	SRR18529427	Pizzle stick	1	10	301902	Dublin	S	S	S	S	S	S	S
14	SRR18529420	Pizzle stick	1	10	301891	Dublin	S	S	S	S	S	S	S
15	SRR18488403	Bison ear	1	40	298030^	Derby	S	S	R	S	S	S	S
16	SRR18488404	Bison ear	1	682	298030^	Derby	S	S	R	S	S	S	S
21	SRR18488367	Furry rabbit ear	1	32	301731	Infantis	S	S	S	S	S	S	S
22	SRR18488418	Furry rabbit ear	1	32	301762	Infantis	S	S	R	S	S	S	S
34	SRR18488400	Bison ear	2	682	67536^	Derby	S	S	S	S	S	S	S
35	SRR18488407	Bison ear	2	682	165407^	Derby	S	S	S	S	S	S	S
36	SRR18488414	Bison ear	2	682	301761	Derby	S	S	S	S	S	S	S
37	SRR18488402	Bison ear	2	682	67536	Derby	S	S	S	S	S	S	S
43	SRR18529396	Chicken treat	2	64	301899	Anatum	S	S	S	S	S	S	S
44	SRR18488364	Chicken treat	2	34	1597^	Typhimurium (monophasic)	S	R	S	S	S	S	S
47	SRR18545478	Chicken treat	2	34	1597^	Typhimurium (monophasic)	S	R	S	S	S	S	S

*Aug: Amoxycillin-clavulanic acid; Amp: Ampicillin; Tig: Tigecycline; TMS: Trimethoprim sulphamethoxazole; Ami: Amikacin; Cip: Ciprofloxacin; Mer: Meropenem. S: sensitive; R: resistant. ST: Sequence type; HierCC HC5: Hierarchical Clustering of cgMLST number at the five allelic level. ^Contains genetically linked human cases

Figure 1

Of the confirmed isolates of *S. enterica*, 39% (5/13) demonstrated resistance to at least one antibiotic class. Resistance to tigecycline was observed in 23% (3/13) of isolates, which were serotypes Derby and Infantis. Ampicillin resistance was detected in 15% (2/13) of isolates, which were serotype Typhimurium (monophasic). No resistance was observed to other antibiotics.

Discussion

This small exploratory study provided further evidence that dried natural dog treats available in the UK can be contaminated with *S. enterica*. Previous studies globally have demonstrated a wide range (2-51%) of *Salmonella* spp. prevalence in such treats, frequently from raw hide and pig ears (Clark *et al.*, 2001; Willis, 2001; White *et al.*, 2003; Wong *et al.*, 2007; Finley *et al.*, 2008a; Adley *et al.*, 2011; Yukawa *et al.*, 2019). Non-processed dog treats derived from raw animal material contaminated with *S. enterica* are known to be a source of gastrointestinal infectious disease in humans; there have been at least three outbreaks of human salmonellosis linked to dog treats in the USA and Canada, attributed to *S. enterica* serotype Infantis (Clark *et al.*, 2001), *S.* Thompson (Centers for Disease Control and Prevention (CDC), 2006) and *S.* Newport (Pitout *et al.*, 2003). Dried 'natural' dog treats are an increasingly popular supplementary food choice, and the types of dried treats available are diverse; the present study has demonstrated the presence of *Salmonella* spp. in a range of commonly selected treats other than pig ears and raw hide.

A variety of *Salmonella* spp. serotypes were identified in this study. *S.* Typhimurium and *S.* Infantis are among the top five serotypes resulting in human infection reported to the UKHSA (Chattaway, Dallman, *et al.*, 2019). However, the most commonly isolated in this study was *S.* Derby, all isolated from bison ear treats, and several strains were found to be genetically highly similar to human cases, alongside *S*. Typhimurium strains (Table 1, Figure 1). Additionally, *S.* Typhimurium is regularly reported in the top five reported serotypes in human cases in Europe (Ferrari *et al.*, 2019) and is most commonly associated with pigs and poultry. Indeed, *S.* Typhimurium and *S.* Derby have been previously isolated from pork and poultry foodstuffs intended for pet food production in Italy (Bacci *et al.*, 2019). *S.* Derby is a common cause of human salmonellosis in France (Sévellec *et al.*, 2020) and was implicated in a foodborne disease outbreak in Germany, linked to the consumption of raw pork products (Simon *et al.*, 2018).

While *Salmonella* spp. infection typically causes self-limiting gastroenteritis in otherwise healthy humans, it poses a much higher risk in the immunocompromised, young and elderly, and can result in severe infections. *S.* Dublin is a cattle-adapted serotype, isolated in this study from pizzle stick treats. Although no microbiologically linked human cases were detected, this serovar is capable of causing severe invasive illness in humans which can result

in septicaemia, hospitalisation and death (Harvey *et al.*, 2018). Antimicrobial resistance within *Salmonella* spp. is also of concern, and whilst resistance to ampicillin and tigecycline was identified in some isolates in the present study, no multidrug resistance was observed.

The risk of transmission to humans has been linked to lack of appropriate hand hygiene following handling of the dog treats and/or contact with animals that may shed *S. enterica* in their faeces after consuming the treats (Clark *et al.*, 2001; Pitout *et al.*, 2003; Centers for Disease Control and Prevention (CDC), 2006). It is well documented that dogs can be asymptomatic carriers of *Salmonella* spp. and infected dogs often appear clinically well (Sanchez *et al.*, 2002; Lowden *et al.*, 2015; Reimschuessel *et al.*, 2017). Previously identified risk factors for *Salmonella* spp. carriage include the feeding of offal and raw animal products (Lefebvre *et al.*, 2008; Leonard *et al.*, 2015; Bataller *et al.*, 2020; Viegas *et al.*, 2020; Groat *et al.*, 2022; Usmael *et al.*, 2022) and dogs have been shown to asymptomatically shed *Salmonella* spp. in their faeces for up to a week following ingestion of infected food (Finley *et al.*, 2007). There is also a clinical disease risk to dogs, including diarrhoea (Morley *et al.*, 2006; Reimschuessel *et al.*, 2017; Usmael *et al.*, 2022), and reports of non-enteric infections in dogs with additional comorbidities (Andruzzi *et al.*, 2020; Hertzer *et al.*, 2021; Williams and Towle, 2021).

This study has highlighted a potential One Health concern regarding natural treat products, with some isolates from these products being genetically highly similar to human case isolates, although epidemiological investigations would be needed to establish exposures. These items are often provided to pet dogs both as treats and as a popular natural alternative to traditional anthelminthics. Rehydration (via saliva during chewing) of treats may reactivate foodborne pathogens inactive in the dehydrated state. The treats may also take increased time to chew and consume than conventional cooked treats, so may be in the household environment for a prolonged period, posing an elevated risk of contamination. Studies have shown that few pet owners perceive dry food items, including dried natural treats, to pose a microbiological risk (Thomas and Feng, 2020) and that owners who feed raw animal products generally perceive their diet choice as low risk for foodborne illness (Empert-Gallegos, Hill and Yam, 2020; Viegas *et al.*, 2020; Bulochova and Evans, 2021b, 2021a).

The government guidelines for the packaging of ABPs as treats states that dog chews must be packed in unused packaging. However, the treats contaminated with *Salmonella* spp. in this study were sold as loose items able to be picked up by hand and purchased in paper bags. Again, this is a public health concern and demonstrates the need for further education regarding safe storage and handling surrounding ABPs. Furthermore, for many treats no country of origin was indicated, this potentially poses a risk of importation of *Salmonella* spp. serotypes not commonly reported in the UK via these products and highlights the importance of clear package labelling for traceability. DEFRA guidance states that the production process for dog chews must be proven via testing to destroy *Salmonella*, and an ABP will fail DEFRA testing if any *Salmonella* spp. colonies are identified within tested samples (https://www.gov.uk/guidance/laboratory-testing-requirements-for-animal-by-products-abps#how-much-bacteria-your-samples-can-contain). Therefore, the treats identified as

contaminated with *Salmonella* spp. in this study would be expected to fail testing at a DEFRA-accredited laboratory.

There are some limitations to this study. It was a small, exploratory investigation, and while UK-wide online suppliers were sought, in-person visits to independent pet shops were only carried out in a small area. Therefore, the findings may not accurately represent the prevalence of *Salmonella* spp. contamination in treats available across the UK. All contaminated treats were from the same independent pet shop, which could represent a localised problem, but could also potentially be a result of contamination at the suppliers or within the supply chain, and without further environmental sampling it would not be possible to identify where within the production chain contamination occurred. However, cross-contamination within the shop itself was deemed unlikely for a number of reasons; treats were separated within the shop into separate baskets based on treat type and were purchased on separate occasions, and importantly the serotypes identified were treat-specific and there was genetic diversity within the population (Figure 1). Additional limitations were that treats were picked opportunistically and only a small number of some types were selected depending on availability at the time of visits. Finally, the method of isolating *Salmonella* spp. using chromogenic agar is likely to have only selected for *Salmonella* subsp. *enterica*, therefore a small number of other *Salmonella* subspecies may have been missed.

Nevertheless, this study has demonstrated the presence of *Salmonella* spp. contamination in dried natural dog treats readily available and commonly purchased by dog owners. Larger studies are required to quantify the risk further. Veterinary staff, retailers and dog owners should be made aware of these risks. Efforts should be made to educate dog owners further regarding the potential risks posed by these treats if they choose to feed them, especially in households with higher-risk individuals present, such as immunocompromised individuals or young children. The importance of hygienic practice surrounding their use should be stressed (https://www.gov.uk/guidance/raw-pet-foods-handling-and-preventing-infection) particularly regarding hand washing after use and consideration against feeding them within the home environment.

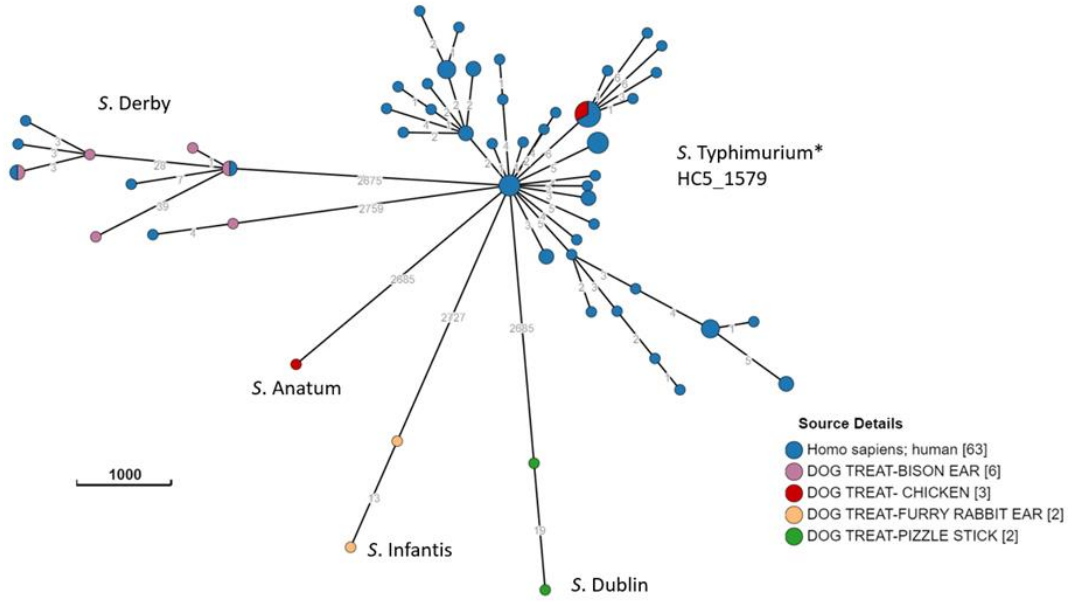


Figure 1: *Grape tree illustrating the population structure of Salmonella isolated from dog treats in this study and which have been identified in human cases, at the HierCC 5 allelic level. S. Typhimurium HC5_1579 is a genetically diverse cluster and dog treats from this study were highly similar genetically to the subcluster HC2_299262. Dog treat types were associated with single serotypes with the exception of chicken, which was associated with two serotypes. Isolates from two serotypes (S. Derby and S. Typhimurium) were also associated with human clinical cases.*

Author contribution

Laboratory work at the University of Liverpool was undertaken by M.S, A.C, G.M and N.W.

Whole genome sequencing was performed and analysed by C.J and M.A.C at UKHSA.

N.W and G.M supervised the project. All authors discussed the results. G.M wrote the manuscript with support from N.W, G.P, M.A.C and C.J

Acknowledgements

With thanks to the Veterinary Microbiology Diagnostic Laboratory at the University of Liverpool for undertaking MALDI-TOF analysis, and to the Veterinary Medicines Directorate (VMD) for their review and feedback on this paper.

Funding and competing interests

Funding for this project was provided by the University of Liverpool and the Veterinary Medicines Directorate (VMD).

CJ and MAC are affiliated to the National Institute for Health Research Health Protection Research Unit (NIHR HPRU) in Gastrointestinal Infections and Genomics and Enabling Data at University of Liverpool and University of Warwick respectively in partnership with the UK Health Security Agency (UKHSA). CJ and MAC are based at UKHSA. The views expressed are those of the author(s) and not necessarily those of the NIHR, the Department of Health and Social Care or the UK Health Security Agency.

Data Availability Statement

Data that supports the findings of this study are available in the supplementary material of this article. Additionally, FASTQ sequences from this study are available in the National Center for Biotechnology Information (NCBI) Sequence Read Archive under the BioProject accession number PRJNA248792 (www.ncbi.nlm.nih.gov/bioproject/?term=248792). Raw sequence data files of isolates from this study have been uploaded to EnteroBase (https://enterobase.warwick.ac.uk/)

Ethical Statement

No animal subject, human participant or personal data collection was required for this study, hence ethical approval was not required.

Supplementary material

Supplementary Table 1: *Complete sample data for dog treats tested in this study, including treat type, place purchased, packaging type, presence of labelling and branding, country of origin (if known) and whether S. enterica was identified*

Treat number	Treat type	Place purchased	Packaging type	Labelling present	Branded	Country of origin	*S. enterica* present
1	Broncho	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	N
2	Broncho	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	N
3	Broncho	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	N
4	Broncho	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	N
5	Tendons	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	N
6	Tendons	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	N
7	Tendons	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	N
8	Cowtails	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	N
9	Cowtails	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	N
10	Hairy cow ears	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	N
11	Hairy cow ears	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	N
12	Pizzle Sticks	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	N
13	Pizzle Sticks	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	Y

14	Pizzle Sticks	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	Y
15	Bison Ear	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	Y
16	Bison Ear	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	Y
17	Pig snouts	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	N
18	Pig snouts	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	N
19	Pig snouts	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	N
20	Furry Rabbit Ears	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	N
21	Furry Rabbit Ears	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	Y
22	Furry Rabbit Ears	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	Y
23	Cow scalp	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	N
24	Cow scalp	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	N
25	Cow scalp	A (visit 1)	Unpackaged, loose in baskets	N	N	Unknown	N
26	Trachea with beef filling	B	Individually wrapped in plastic	Y	Y	States produced in UK	N
27	Beef Hide	B	Individually wrapped in plastic	Y	Y	States produced in UK	N
28	Beef Hide	B	Individually wrapped in plastic	Y	Y	States produced in UK	N
29	Pork hide	B	Individually wrapped in plastic	Y	Y	States produced in UK	N
30	Chicken feet	A (visit 2)	Unpackaged, loose in baskets	N	N	Unknown	N
31	Chicken feet	A (visit 2)	Unpackaged, loose in baskets	N	N	Unknown	N
32	Chicken feet	A (visit 2)	Unpackaged, loose in baskets	N	N	Unknown	N
33	Chicken feet	A (visit 2)	Unpackaged, loose in baskets	N	N	Unknown	N
34	Bison Ear	A (visit 2)	Unpackaged, loose in baskets	N	N	Unknown	Y
35	Bison Ear	A (visit 2)	Unpackaged, loose in baskets	N	N	Unknown	Y
36	Bison Ear	A (visit 2)	Unpackaged, loose in baskets	N	N	Unknown	Y
37	Bison Ear	A (visit 2)	Unpackaged, loose in baskets	N	N	Unknown	Y
38	Lamb Ear	A (visit 2)	Unpackaged, loose in baskets	N	N	Unknown	N
39	Lamb Ear	A (visit 2)	Unpackaged, loose in baskets	N	N	Unknown	N
40	Lamb Ear	A (visit 2)	Unpackaged, loose in baskets	N	N	Unknown	N
41	Lamb Ear	A (visit 2)	Unpackaged, loose in baskets	N	N	Unknown	N
42	Lamb Ear	A (visit 2)	Unpackaged, loose in baskets	N	N	Unknown	N
43	Chicken treat	A (visit 2)	Unpackaged, loose in baskets	N	N	Unknown	Y
44	Chicken treat	A (visit 2)	Unpackaged, loose in baskets	N	N	Unknown	Y
45	Chicken treat	A (visit 2)	Unpackaged, loose in baskets	N	N	Unknown	N
46	Chicken treat	A (visit 2)	Unpackaged, loose in baskets	N	N	Unknown	N
47	Chicken treat	A (visit 2)	Unpackaged, loose in baskets	N	N	Unknown	Y
48	Chicken necks	C	Individually wrapped in plastic	Y	Y	States UK and Europe on website	N
49	Chicken necks	C	Individually wrapped in plastic	Y	Y	States UK and Europe on website	N
50	Chicken necks	C	Individually wrapped in plastic	Y	Y	States UK and Europe on website	N
51	Chicken wings	C	Individually wrapped in plastic	Y	Y	States UK and Europe on website	N
52	Chicken wings	C	Individually wrapped in plastic	Y	Y	States UK and Europe on website	N
53	Chicken wings	C	Individually wrapped in plastic	Y	Y	States UK and Europe on website	N
54	Duck wings	C	Individually wrapped in plastic	Y	Y	States UK and Europe on website	N

55	Duck wings	C	Individually wrapped in plastic	Y	Y	States UK and Europe on website	N
56	Duck wings	C	Individually wrapped in plastic	Y	Y	States UK and Europe on website	N
57	Camel skin	C	Individually wrapped in plastic	Y	Y	States UK and Europe on website	N
58	Camel skin	C	Individually wrapped in plastic	Y	Y	States UK and Europe on website	N
59	Camel skin	C	Individually wrapped in plastic	Y	Y	States UK and Europe on website	N
60	Chicken feet	C	Individually wrapped in plastic	Y	Y	States UK and Europe on website	N
61	Chicken feet	C	Individually wrapped in plastic	Y	Y	States UK and Europe on website	N
62	Chicken feet	C	Individually wrapped in plastic	Y	Y	States UK and Europe on website	N
63	Beef testicles	C	Individually wrapped in plastic	Y	Y	States UK and Europe on website	N
64	Beef testicles	C	Individually wrapped in plastic	Y	Y	States UK and Europe on website	N
65	Beef testicles	C	Individually wrapped in plastic	Y	Y	States UK and Europe on website	N
66	Buffalo ears	C	Unpackaged, loose in box	Y	Y	States UK and Europe on website	N
67	Buffalo ears	C	Unpackaged, loose in box	Y	Y	States UK and Europe on website	N
68	Buffalo ears	C	Unpackaged, loose in box	Y	Y	States UK and Europe on website	N
69	Buffalo ears	D	Clear plastic bag	N	N	Unknown	N
70	Buffalo ears	D	Clear plastic bag	N	N	Unknown	N
71	Buffalo ears	D	Clear plastic bag	N	N	Unknown	N
72	Buffalo ears	D	Clear plastic bag	N	N	Unknown	N
73	Buffalo ears	D	Clear plastic bag	N	N	Unknown	N
74	Buffalo ears	D	Clear plastic bag	N	N	Unknown	N
75	Buffalo ears	D	Clear plastic bag	N	N	Unknown	N
76	Buffalo ears	D	Clear plastic bag	N	N	Unknown	N
77	Buffalo ears	D	Clear plastic bag	N	N	Unknown	N
78	Buffalo ears	D	Clear plastic bag	N	N	Unknown	N
79	Buffalo ears	D	Clear plastic bag	N	N	Unknown	N
80	Buffalo ears	D	Clear plastic bag	N	N	Unknown	N
81	Buffalo ears	D	Clear plastic bag	N	N	Unknown	N
82	Buffalo ears	D	Clear plastic bag	N	N	Unknown	N
83	Buffalo ears	D	Clear plastic bag	N	N	Unknown	N
84	Buffalo ears	D	Clear plastic bag	N	N	Unknown	N

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