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Antimicrobial resistance risk factors and characterisation of faecal *E. coli* isolated from healthy Labrador retrievers in the United Kingdom

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ABSTRACT

Antimicrobial resistant bacteria are increasingly detected from canine samples but few studies have examined commensal isolates in healthy community dogs. We aimed to characterise faecal *Escherichia coli* from 73 healthy non-veterinarian-visiting and non-antimicrobial treated Labrador retrievers, recruited from dog shows in the North West United Kingdom between November 2010 and June 2011. Each enrolled dog provided one faecal sample for our study. *E. coli* were isolated from 72/73 (99%) faecal samples. Disc diffusion susceptibility tests were determined for a range of antimicrobials, including phenotypic extended-spectrum beta-lactamase (ESBL) and AmpC-production. PCR assay detected phylogenetic groups and resistance genes (bla_{CTX-M}, bla_{SHV}, bla_{TEM}, bla_{OXA}, bla_{CIT}, *qnr*), and conjugation experiments were performed to investigate potential transfer of mobile genetic elements. Multivariable logistic regression examined potential risk factors from owner-questionnaires for the presence of antimicrobial resistant faecal *E. coli*. Antimicrobial resistant, multi-drug resistant (≥3 antimicrobial classes; MDR) and AmpC-producing *E. coli* were detected in 63%, 30% and 16% of samples, respectively. ESBL-producing *E. coli* was detected from only one sample and conjugation experiments found that bla_{CTX-M} and bla_{CIT} were transferred from commensal *E. coli* to a recipient strain. Most isolates were phylogenetic groups B1 and A. Group B2 isolates were associated with lower prevalence of resistance to at least one antimicrobial ($P < 0.001$) and MDR ($P < 0.001$). Significant at $P < 0.003$, was the consumption of raw meat for clavulanate–amoxicillin (OR: 9.57; 95% CI: 2.0–45.7) and third generation cephalosporin resistance (3GCR) (OR: 10.9; 95% CI: 2.2–54.0). AMR *E. coli* were surprisingly prevalent in this group of non-antimicrobial treated and non-veterinarian-visiting dogs and consumption of raw meat was a significant risk factor for antimicrobial resistance. These findings are of concern due to the increasing popularity of raw-meat canine diets, and the potential for opportunistic infection, zoonotic transmission and transmission of antimicrobial resistant determinants from commensal isolates to potential pathogenic bacteria.

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1. Introduction

Escherichia coli is the main aerobe of the gastrointestinal flora in humans and other animals (Tenaillon et al., 2010), and has been widely studied as an indicator of antimicrobial selection pressure (Gronvold et al., 2010). A stable gastrointestinal flora is important for health and acts as a colonisation barrier against pathogens (Vollaard and Clasener, 1994; Dethlefsen et al., 2007); this may be disturbed by a number of factors including disease, diet and antimicrobial therapy (Stecher and Hardt, 2008; Jernberg et al., 2010; Vincent et al., 2010).

Of particular concern is the emergence and dissemination of extended spectrum beta-lactamase (ESBL) and AmpC-producing *E. coli* that are resistant to a variety of beta-lactam antimicrobials including third generation cephalosporins (3GCR) (Livermore and Hawkey, 2005; Thomson, 2010). Genes encoding these enzymes are carried on plasmids, often in conjunction with other antimicrobial resistance determinants, enabling horizontal transmission of multidrug resistance (MDR) (Zhao et al., 2001; Li et al., 2007; Karczmarczyk et al., 2011; Dahmen et al., 2012). ESBL-, AmpC-producing and MDR *E. coli* have been detected in healthy (De Graef et al., 2004; Carattoli et al., 2005; Costa et al., 2008; Wedley et al., 2011) and sick dogs (Carattoli et al., 2005; Pomba et al., 2009; Gibson et al., 2011a), and increased detection has been associated with exposure to antimicrobials and veterinary healthcare (Moreno et al., 2008; Damborg et al., 2011; Gibson et al., 2011a, b).

E. coli populations include commensal and pathogenic strains. Compared to commensal strains, pathogenic strains are more likely to carry a range of virulence genes that can facilitate disease (Johnson and Russo, 2002; Nowrouzian et al., 2006). Gut colonisation by ExPEC (extra-intestinal pathogenic *E. coli*) strains is a prerequisite for extra-intestinal infections. The gut of healthy humans and other animals can be a reservoir of ExPEC strains (Johnson et al., 2003; Russo and Johnson, 2003), which are potentially zoonotic (Johnson et al., 2009), and may be shared between humans and pets within households (Johnson et al., 2008). Food, particularly chicken meat, is also a potential source of ExPEC strains for humans and dogs (Johnson et al., 2007, 2009; Vincent et al., 2010).

Phylogenetic grouping is a simple and inexpensive method to investigate the genetic background, potential pathogenicity, and antimicrobial resistance traits of *E. coli* isolates (Sato et al., 2014). A PCR assay to assign *E. coli* isolates to four major phylogenetic groups: A, B1, B2 and D (Clermont et al., 2000) has been widely used and recently updated (Doumith et al., 2012). Additionally, the original method (Clermont et al., 2000) has been revised (Clermont et al., 2013) to assign isolates to eight different phylogenetic groups: A, B1, B2, C, D, E, F and *Escherichia* Clade I. Phylo-groups B2, D, E and F are more likely to be involved in extra-intestinal infections compared to A, B1 or C (Picard et al., 1999; Moissenet et al., 2010; Tenaillon et al., 2010) and Clade isolates are thought to reside outside of the gut (Walk et al., 2009).

The distribution of these phylo-groups amongst different hosts may depend on characteristics such as body mass, diet and environment (Gordon and Cowling, 2003; Escobar-Paramo et al., 2004; Tenaillon et al., 2010). In healthy humans, phylo-group A generally predominates followed by B2, B1 and D, whereas in animals group B1 predominates followed by A, B2 and D (Tenaillon et al., 2010). Diversity due to host diet has also been reported with group A predominating in carnivores and omnivores, and group B1 in herbivores (Escobar-Paramo et al., 2006; Baldy-Chudzik et al., 2008; Carlos et al., 2010).

Antimicrobial resistance has been linked to the non-B2 phylo-groups in people, cattle, pigs and dogs (Johnson et al., 2003, 2009; Moreno et al., 2008). In dogs, phylo-group D isolates are more likely to be antimicrobial resistant, including fluoroquinolone, 3GCR and MDR (Platell et al., 2011; Tamang et al., 2012; Sato et al., 2014) and group B2 are more likely to be antimicrobial susceptible (Johnson et al., 2009; Platell et al., 2011; Sato et al., 2014). However ESBL-producing fluoroquinolone resistant and MDR ExPEC strains, that further challenge therapeutic regimes are emerging amongst human clinical isolates, and have been reported in dogs (Russo and Johnson, 2003; Johnson et al., 2009; Platell et al., 2010).

Previous studies have concentrated on clinical isolates and the effects of potential risk factors, in particular antimicrobial pressure. However, few studies have examined canine gastrointestinal *E. coli* populations under natural conditions. The aim of this study was to determine the prevalence of antimicrobial resistance and determine phylogenetic groups amongst faecal *E. coli* from a group of healthy non-vet visiting and non-antimicrobial treated dogs. In addition, we aimed to examine the association of these findings with potential risk factors.

2. Methods

2.1. Study population

The prevalence of antimicrobial resistant faecal *E. coli* in healthy non-antimicrobial treated and non-veterinarian-visiting dogs was hypothesised to be low. Simple sample size estimates to determine prevalence showed that with an expected prevalence of 5%, precision of 5% and 95% confidence, 73 dogs would be required. Labrador retriever dogs were recruited from two dog shows in the North West UK between November 2010 and June 2011. One healthy dog of any age was enrolled from each household following a clinical examination. Dogs that had received topical or systemic antimicrobial therapy, had been admitted to a veterinary clinic within the last 12 months, or were determined not to be healthy were excluded. All dog owners gave written informed consent before enrolment in this study, and completed a two-page questionnaire regarding potential risk factors for the carriage of antimicrobial resistant bacteria that was administered at recruitment by a veterinarian. Time to complete the questionnaire was 1–2 min and it was either submitted at recruitment or returned with the sample by first-class post. The questionnaire had been previously used (Wedley et al., 2014) and consisted of simple closed questions with tick box responses and space for additional information. A “Don’t Know” response was