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Patterns of antimicrobial resistance in pathogenic *Escherichia coli* isolates from cases of calf enteritis during the spring-calving season

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ABSTRACT

Neonatal enteritis is a common condition of young calves and can be caused by pathogenic strains of *Escherichia coli*. We hypothesised that on-farm antimicrobial use would result in an increased frequency of resistance in these strains during the calving season. We also sought to determine if the frequency of resistance reflected on-farm antimicrobial use. Faecal samples were collected from cases of calf enteritis on 14 spring-calving dairy farms during two 3 week periods: Period 1 – February 11th through March 2nd 2008 and Period 2 – April 14th through May 5th 2008. *E. coli* were cultured from these samples, pathogenic strains were identified and antimicrobial susceptibility testing was carried out on these pathogenic isolates. Antimicrobial prescribing data were collected from each farm for the previous 12 months as an indicator of antimicrobial use. The correlation between antimicrobial use and resistance was assessed using Spearman's correlation coefficient. Logistic regression analysis was used to investigate the relationship between resistance, sampling period and pathotype. Penicillins and aminopenicillins, streptomycin, and tetracyclines were the most frequently prescribed antimicrobials and the greatest frequencies of resistance were detected to these 3 antimicrobial classes. A strong correlation ($\rho = 0.879$) was observed between overall antimicrobial use and frequencies of antimicrobial resistance on farms. Sampling period was significant in the regression model for ampicillin resistance while pathotype was significant in the models for streptomycin, tetracycline and trimethoprim/sulphamethoxazole resistance. The frequencies of resistance observed have implications for veterinary therapeutics and prudent antimicrobial use. Resistance did not increase during the calving season and factors other than antimicrobial use, such as calf age and bacterial pathotype, may influence the occurrence of resistance in pathogenic *E. coli*.

1. Introduction

The Irish dairy industry is predominantly based around grass production resulting in a highly seasonal (spring-calving) and relatively compact calving season (Mee, 2004). Consequently, the number of young calves on-farm

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increases rapidly which may have implications for disease. Calf health was recently identified as a major animal health priority by farmers and animal health experts in Ireland (More et al., 2010). Neonatal calf enteritis is the most frequently diagnosed fatal condition in calves less than one month of age in Ireland (Anonymous, 2009) and it is accepted that the incidence of this condition increases over the calving season (Bazeley, 2003). Rotavirus and *Cryptosporidium* spp. were the most frequently detected pathogens in samples of neonatal enteritis submitted to Regional Veterinary Laboratories in Ireland in 2009. *Escherichia coli* K99 was detected in less than 5% of these samples; samples were not tested for other pathogenic strains of *E. coli* (Anonymous, 2009).

Antimicrobials are frequently prescribed for the treatment of neonatal calf enteritis to combat bacterial overgrowth in the small intestine and to prevent or treat associated bacteraemia (Constable, 2004). However, there is some debate about the necessity of such therapy and the potential for imprudent antimicrobial use to select for antimicrobial resistance in zoonotic and commensal bacteria (Aarestrup, 1999; Grove-White, 2007).

We hypothesised that antimicrobial use for the treatment of enteritis may act as a selective pressure on pathogenic *E. coli* resulting in the accumulation of more resistant strains during the calving season with implications for antimicrobial prescribing and clinical outcomes.

The objectives of this study were to determine:

1. If the frequency of antimicrobial resistance among pathogenic strains of *E. coli* remains constant throughout the calving season.
2. If the frequency of antimicrobial resistance among pathogenic *E. coli* isolates reflects on-farm antimicrobial use.

2. Materials and methods

2.1. Farm selection

Private veterinary practitioners in the Cork region were requested to identify farms suitable for recruitment to this study. The selection criteria for inclusion were farm type (dairy herds), cow herd size (>75 cows), calving pattern (spring-calving) and a willingness to participate in the study. Fourteen spring-calving dairy herds were recruited to this study. Cow herd size on these farms ranged from 75 to 400 cows with an average herd size of 148 cows. The average date for the start of the calving season was January 16th with a range from January 1st to February 1st. The average duration of the calving season on these 14 farms was 14 weeks, with a range from 8 to 20 weeks. Six farms also had a concurrent beef enterprise where all male animals born on the farm were raised to slaughter.

2.2. Prescribing data

Private veterinary practitioners were requested to supply all antimicrobial prescribing data for each farm for the 12-month period May 2007–May 2008. The antimicrobial prescribing data collected included the prescribing date, drug name, amount of drug prescribed and active ingredient. The prescribing data did not distinguish between antimicrobial usage in the various animal groups on the farms. Volumes of all antimicrobials prescribed were converted to grams of active substance using the information provided on the product datasheet as published on the website of the Irish Medicines Board (www.imb.ie). In order to standardise amounts of antimicrobials used on each farm the number of defined animal daily doses for a 50 kg calf (ADD50) was calculated for each orally or parenterally administered antimicrobial prescribed on each farm using the formula:

$$\text{ADD50} = \frac{\text{weight of active substance prescribed (mg)}}{\text{recommended daily dose (mg/kg)}} \times 50\,\text{kg}$$

2.3. Sampling and faecal scoring

Farms were visited daily during two three-week periods, denoted as Period 1 – February 11th through March 2nd 2008 and Period 2 – April 14th through May 5th 2008. Faecal scoring of calves less than 30 days of age was carried out using a previously reported system to identify calves with diarrhoea (Perez et al., 1998). Calves with a cumulative faecal score of 4 or greater were sampled by rectal swab using rayon tipped swabs (Copan, USA) and placed in Amies agar gel transport medium for transport to the laboratory. Calves with repeated episodes of diarrhoea which met the sampling criteria were sampled during the first diarrhoeic episode only and only untreated calves were sampled in order to avoid a selection bias. Swabs were processed within 8 h of sampling. The presence of other enteropathogens in these samples was not established.

2.4. Bacteriological culture and identification

Swabs were streaked directly onto MacConkey agar plates (Oxoid, UK) and incubated for 24 h at 37 °C. Up to five colonies displaying the characteristics of *E. coli* (large lactose-fermenting colonies, coliform odour) were sub-cultured onto Harlequin Tryptone Bile Glucuronide agar (TBGA) (Lab M Ltd., UK) and incubated for 24 h at 37 °C. Colonies displaying the characteristics of *E. coli* (blue-green colour) on TBGA were assumed to be *E. coli* (Frampton and Restaino, 1993; Perez et al., 1986), sub-cultured onto 5% sheep blood agar then stored on cryo-beads at −70 °C for subsequent analysis. A multiplex PCR assay was carried out to detect the gene encoding heat stable enterotoxin (STa) of enterotoxigenic *E. coli* (ETEC) and the *eae* gene of attaching and effacing *E. coli* (AEEC) as described by Franck et al. (1998); both ETEC and AEEC have been identified as causative agents of calf enteritis (DebRoy and Maddox, 2001). While isolates of *E. coli* expressing the afimbrial adhesin CS31A are frequently isolated from cases of calf septicaemia and may be considered extra-intestinal pathogenic *E. coli* (EXPEC), they are also frequently isolated from cases of calf diarrhoea (Girardeau et al., 1988; Mercado et al., 2003).