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Epidemiology of extended spectrum beta-lactamase *E. coli* (CTX-M-15) on a commercial dairy farm

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ABSTRACT

The epidemiology of an extended spectrum beta-lactamase *Escherichia coli* (CTX-M-15) was observed and described on a commercial dairy farm located in the United Kingdom. During 2008 longitudinal sampling of faecal pat samples from different cattle groups comprising milking and non-milking cows, calving cows, calves, and the environment was carried out. The proportion of CTX-M-15 *E. coli* positive samples was significantly ($p < 0.0.01$) higher in milking cows (30.3%, $CI_{95\%}$ 26.8; 33.8) than in the herd as a whole (17.0%, $CI_{95\%}$ 14.9; 19.0). In 2008 95.6% of sampled calves tested positive for CTX-M-15 *E. coli* at two days of age. A more detailed investigation in 2009 revealed that cows and heifers were approximately eight times more likely to test positive in the 10 days after calving than the 9 days before (OR 7.6, $CI_{95\%}$ 2.32; 24.9). The CTX-M15 *E. coli* was also readily isolated from the immediate calving pen environment, including the water troughs. A cyclic pattern was apparent where cows immediately after calving and as high yielders were highly positive, but where the prevalence decreased during the dry period. The increased prevalence of the CTX-M-15 *E. coli* in certain cattle groups and farm environments including calving pens suggested that husbandry, antimicrobial usage and hygiene may play a significant role on a farm with regards to the epidemiology of CTX-M-15. This may offer a practical opportunity to reduce further dissemination through good practice and hygiene around calving.

1. Introduction

Extended spectrum β-lactamases (ESBLs) are enzymes produced by bacteria that can degrade and confer resistance to some of the most commonly used antibiotics in hospitals including penicillins, cephalosporins and monobactams (Bonnet, 2004). The first UK isolation of a CTX-M ESBL in *Escherichia coli* from food producing animals was from diarrhoeic calves at a Welsh dairy farm in the autumn of 2004 (Teale et al., 2005). Further molecular studies indicated that this CTX-M-14 ESBL was located on an IncK plasmid (pCT) and was present in a range of different *E. coli* PFGE types (Liebana et al., 2006).

Farm animals are now recognised as important carriers and a significant potential reservoir of ESBL *E. coli* and Salmonella in some countries (Carattoli, 2008). CTX-M ESBL *E. coli* have been isolated from cattle in Europe including Germany (CTX-M-1; Guerra et al., 2007), Spain (CTX-M-1; Brinas et al., 2005), France (CTX-M-1 and 15; Meunier et al., 2006) and worldwide in other countries including Hong-Kong (CTX-M-3, 13, 14 and 24; Duan et al., 2006) and Japan (CTX-M-2; Shiraki et al., 2004). To date,

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there has only been published a single longitudinal study of CTX-M *E. coli* prevalence at the farm level in different cattle groups by our research group at the Veterinary Laboratories Agency (Liebana et al., 2006). This study left unanswered questions about the association of CTX-M *E. coli* with cattle age, group (milking, dry or heifer) and husbandry, as well as incidence rates, risk factors and transmission dynamics for the carriage of the CTX-M gene by cattle. Subsequently an alternative dairy farm was selected for more detailed investigation and to consider whether any measures could be implemented on farms to prevent further dissemination of CTX-M *E. coli*.

2. Materials and methods

2.1. Farm details

A single CTX-M-15 positive farm located in the United Kingdom was enrolled for observation over 2 years. The farm was a large commercial mixed arable and dairy unit. The dairy herd was made up of largely pedigree Holstein cattle. There were approximately 280 lactating cows and 140 heifer replacements. Cattle management and husbandry was typical of many similar dairy enterprises in the UK, although notably the high yielding lactating cows were housed all year round in standard cubicle accommodation (straw/ash bedding, with automated scrapers). Low yielding lactating cows had access to grazing during the summer. The herd had an all year round calving pattern and calves were reared in conventional individual pens, moving to group pens within a few days of birth. Weaning was at six weeks of age. The farm comprised four different sites (A–D) which specialised in different husbandry activities in the cycle for milk production. The milking and calving cows were located at site A. After calving the bull calves remained at site A for up to 14 days before being sent for meat. The heifer (young female cattle which have not previously borne a calf) calves were moved after birth from site A to site B within 48 h until 20–24 weeks of age when they were transferred to site D. They returned to site B for mating at approximately 15 months. Pregnant calf heifers and dry cows were maintained at site C prior to transfer to site A for calving. After calving heifers became cows. The term "dry" is used to describe cows that have reached the end of one lactation and are due to calve, so as to start a new lactation period. Typically, the dry period is around 6–8 weeks duration. During this time dry cows are typically managed as a separate group from the milking herd. High yielders refers to cows in the early part of lactation, typically the first 3 months, when daily milk yield is at peak. Low yielders refers to cows in the later part of lactation, typically after 3 months, when daily milk yield declines.

A list of veterinary medicines used on the farm was compiled from the farm medicines log book maintained by the farmer. Veterinary medicines containing antimicrobial products were widely used for the treatment and prophylaxis of mastitis in the milking groups and other conditions. The products included cephquinome and cefalonium for mastitis prophylaxis at drying off, cephquinome and novobiocin/neomycin/procaine/penicillin/dihydrostreptomycin for mastitis therapy in milking cows, and ceftiofur, marbofloxacin, tylosin, amoxicillin clavulanate and penicillin and streptomycin for a wide range of infections in any of the cattle groups.

2.2. Farm selection

In 2006, a CTX-M-15 ESBL *E. coli* was identified in a septic neonatal dairy heifer calf from this farm submitted to the VLA for diagnostic testing under the Scanning Surveillance Programme. Further serological typing showed that this was not the human CTX-M-15 ST131 O25b:H4 UK pandemic strain (Lau et al., 2008; Nicolas-Chanoine et al., 2008). Other calves in the group had also recently presented as sick and there had been several mortalities. The start of the study was slightly delayed to 2008 due an outbreak of Foot and Mouth disease in the UK.

2.3. Sampling of cattle groups

Fresh floor faecal samples were collected from individual adult, calving cows and calves and tested for the presence of CTX-M *E. coli*.

2.3.1. Adult cattle

Six prospective visits (bimonthly) were systematically scheduled during 2008 and at each visit approximately 55 individual pat samples were randomly collected from four groups of cattle comprising bulling heifers, dry cows, high and low yield lactating cows. The number of fresh floor faecal pat samples collected for the longitudinal study was based on a sample size calculation using the results from a previous sampling visit in January 2007 which estimated the prevalence of CTX-M *E. coli* to be 15%. Collection of 55 pat samples (100 g) from each group enabled estimation of prevalence within each group to $\pm$5% based on the assumption that the true prevalence was 15% and the group size was 70. This sample size would also provide 95% confidence that the true prevalence was less than 4% if all samples tested negative for CTX-M *E. coli*. The data was combined for the entire year to estimate prevalence in different groups or at each collection for longitudinal seasonal analysis.

2.3.2. Longitudinal study of calves in 2008

During 2008, 976 pat samples were collected from 142 calves from birth to 161 days. The data was analysed from birth to 21 and 161 days for those pat samples testing positive for CTX-M *E. coli*.

2.3.3. Calving cattle in 2008

A total of 62 cows were pat sampled in the periparturient period (days -4 to +11).

2.3.4. Calving cattle in 2009

A total of 24 animals (16 cows and 8 heifers) were sampled pre-calving and followed through into the post-calving period, together with a further nine cows that were followed in the post-calving period as they had just calved at the start of the study. Consequently not all individuals were sampled both pre- and post-calving and results are drawn from the test status of individual cattle. Individual