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Risk factors for quinolone-resistant *Escherichia coli* in feces from preweaned dairy calves and postpartum dairy cows

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ABSTRACT

Quinolone resistance may emerge in gut bacteria (e.g., in *Escherichia coli*) of animals. Such bacteria could cause infections in the animal itself or be transmitted to humans via the food chain. Quinolone resistance is also observed in fecal *E. coli* of healthy dairy cattle, but the prevalence varies between farms, not solely as a result of varying degree of fluoroquinolone exposure. The objective of this study was to identify risk factors for the fecal shedding of quinolone-resistant *E. coli* (QREC) from dairy calves and postpartum cows. Rectal swabs from 15 preweaned calves and 5 postpartum cows per farm were collected on 23 Swedish dairy farms to determine the prevalence of QREC. Risk factors for the shedding of QREC were investigated using multivariable statistical models. Quinolone-resistant *E. coli* were found on all but one farm. Factors associated with QREC shedding by calves were being younger than 18 d, being fed milk from cows treated with antimicrobials, recent use of fluoroquinolones in the herd, carriage of QREC by postpartum cows, and using the calving area never or rarely as a sick pen compared with often. Factors associated with QREC shedding by cows were calving in group pens or freestalls compared with single pens or tiestalls, purchasing cattle, sharing animal transports with other farmers, and poor farm hygiene. Proper biosecurity and improved hygiene, as well as minimizing fluoroquinolone exposure and waste milk feeding, may be important factors to reduce the burden of QREC on dairy farms.

Key words: calf, postpartum cow, quinolone-resistant *Escherichia coli*, risk factor

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INTRODUCTION

Microorganisms with decreased susceptibility to antimicrobials are one of the greatest threats for human health according to the European Center for Disease Prevention and Control (ECDC, 2007). Special concerns have been raised about the development of resistance to quinolones, which are identified by the World Health Organization (2012) as critically important antimicrobials for human medicine. Results from several surveillance programs in Europe show an increase of some quinolone-resistant pathogens affecting both humans and food-producing animals (EFSA, 2014). In young dairy calves, fluoroquinolone-resistant *Escherichia coli* have been increasingly involved in bacterial infections (Marchese et al., 2012; Cummings et al., 2014). Like most resistance phenotypes, quinolone-resistant *E. coli* (**QREC**) are more common in calves than in older cattle (Kaesbohrer et al., 2012; Swedres-Svarm 2013). The fecal microbiota of calves may therefore serve as a reservoir for QREC, causing intractable infections in cattle. Although QREC is less common in feces from older cattle, studies on adult animals indicate that the shedding of some antimicrobial-resistant *E. coli* increases around parturition (Watson et al., 2012; Callens et al., 2015). The dam's microbiota might therefore be a reservoir of resistant strains for her offspring (Callens et al., 2015). Hence, it is important to elucidate whether QREC is found in the feces of postpartum cows and what factors affect the shedding of QREC from them.

Knowledge about the emergence and epidemiology of quinolone resistance in fecal *E. coli* from cattle is limited. In our previous study, we identified a few risk factors for the shedding of QREC by calves; one such factor was usage of fluoroquinolones for cows (Duse et al., 2015). Although historic and recent usage of fluoroquinolones has been linked to the presence of QREC (Pereira et al., 2014a; Duse et al., 2015), QREC have also been isolated from animals with no known exposure to quinolones (Taylor et al., 2009; Duse et al., 2015). These findings indicate that factors other than exposure to fluoroquinolones may be involved in the epidemiology of QREC. We were therefore interested in seeing how farms with high and low prevalence of QREC differed from each other. The objectives of this study were to identify risk factors for the shedding of QREC from preweaned dairy calves and postpartum dairy cows. In particular, we looked at feeding and management factors for preweaned dairy calves. Such knowledge could form the basis for recommendations to reduce the prevalence of QREC on dairy farms.

MATERIALS AND METHODS

Study Design and Selection of Farms

We aimed to study differences in management factors on farms where QREC was common and farms where QREC was rare or absent. For this purpose, the study was designed as a case-control study. This study design is useful when the case condition is rare or when time limits extensive sampling (Dohoo et al., 2010). Because historic use of fluoroquinolones and being located in south or east Sweden were found to be significant risk factors for QREC in Duse et al. (2015), we selected only farms matching these criteria to avoid confounding (Dohoo et al., 2010). Moreover, because the farm environment was sampled for a parallel study, we wanted to include farms that were as similar in housing as possible; thus, only farms with insulated freestall barns were eligible for inclusion. Cases and control farms were selected from 84 farms. Based on a previous estimate on the mean within-sample prevalence of fecal QREC for 3 calves on each farm (Duse et al., 2015), we defined cases as farms where the mean within-sample prevalence of QREC exceeded 10% (n = 14 farms) and controls as those farms where none of the calves had QREC (n = 20 farms). In descending order of QREC prevalence, case farms were asked to take part in the study and 10 farms were recruited; the remaining 4 did not want to participate for various reasons. A total of 11 control farms were asked in random order to participate until 10 farms could be enrolled. Finally, 3 of the 84 farms with moderate mean prevalence of QREC (0.02–0.8%) were enrolled. These 3 farms did not match the criteria for cases or controls but were selected due to the presence of *E. coli* carrying genes encoding extended-spectrum β-lactamases (**ESBL**), which was of interest for a parallel study in which shedding of *E. coli* resistant to third-generation cephalosporins was studied. Table 1 contains descriptive statistics of the enrolled farms.

Farm Visits

Each farm was visited once between November 2013 and March 2014. The corresponding author interviewed the farmer or the herdsman regarding herd management, assessed the general hygiene on the farm, and collected fecal samples from calves and postpartum cows. The interview questions were categorized into the following subsets: farm characteristics (number of cattle on the farm, average milk yield, and predominant milking system), veterinary services in the previous 4 mo, management of preweaned dairy calves, management of the calving area and routines around calving, feeding of milk from cows treated with antimicrobials, and biosecurity factors (purchasing cattle, sharing equipment with other livestock farmers, visitor restrictions, and requirements for visitors to use protective clothing). For more details, see Supplemental Tables S1 and S2 (http://dx.doi.org/10.3168/jds.2015-9453). Most questions were semi-closed with space available for the investigator to make notes if alternatives to the options were given. Answers to all questions were given by the herdsman or the farmer, except those about veterinary services, which were compiled from veterinary invoices/records and on-farm records, in cooperation with the respondents. The questionnaire (in Swedish) is available upon request from the corresponding author. Approximate estimates on historic usage of fluoroquinolones in cows and calves from up to 2 yr before the sampling date were obtained from our previous study (Duse et al., 2015). The interview did not include questions that could interfere with the personal integrity of the farmers and hence, an ethical approval for research on humans was not deemed necessary, according to the act on ethical review of research involving humans (SFS 2003:460) in the Swedish Code of Statutes. The majority of the questions in the interview were tested on 6 other farmers before the study and no corrections had to be made to the questionnaire.

To assess hygiene on the farm, specific locations (group calf pens, single calf pens, feed and water troughs in calf pens, young stock area, calving area, dry cow area, lactating cow area, and milk feeding equipment) were scored on predefined scales of 0 to 3, where 0 was cleanest and 3 dirtiest (Supplemental Table S3; http://dx.doi.org/10.3168/jds.2015-9453). A mean hygienic score for each farm was calculated as the sum of all scores divided by the number of locations evaluated.

Fecal samples were collected rectally with Amie's charcoal culture swabs (Copan Diagnostics Inc., Murrieta, CA) from up to 15 calves and up to 5 cows in each herd. The samples were immediately mailed at