Risk factors for antimicrobial resistance in fecal *Escherichia coli* from preweaned dairy calves

Anna Duse,*†¹ Karin Persson Waller,*† Ulf Emanuelson,† Helle Ericsson Unnerstad,* Ylva Persson,*‡ and Björn Bengtsson*

*Department of Animal Health and Antimicrobial Strategies, National Veterinary Institute, SE-751 89 Uppsala, Sweden

†Department of Clinical Sciences, Swedish University of Agricultural Sciences, SE-750 07 Uppsala, Sweden

‡Växa Sverige, SE-751 05 Uppsala, Sweden

ABSTRACT

The primary objective of this study was to investigate calf and farm factors associated with antimicrobial-resistant *Escherichia coli* in the feces of preweaned dairy calves in Sweden. In particular, we investigated the effects of feeding calves colostrum and milk from cows treated with antimicrobials. The secondary objective was to describe the prevalence of resistant *E. coli* in feces of preweaned dairy calves in Sweden. Fecal samples from 3 calves, aged 7 to 28 d, from 243 farms were analyzed for the within-sample prevalence of *E. coli* resistant to nalidixic acid, streptomycin, and cefotaxime using selective agars supplemented with antimicrobials. In addition, resistance to 12 antimicrobials was tested in one randomly selected *E. coli* isolate per calf. Information was collected from the farmers via questionnaires regarding the use of colostrum and milk from cows treated with antimicrobials as calf feed and other uses of antimicrobials in the herd. Multivariable zero-inflated negative binomial and logistic regression models were used to assess the effect of various risk factors for shedding of resistant *E. coli*. *Escherichia coli* resistant to streptomycin, nalidixic acid, or cefotaxime were isolated from 90, 49, and 11% of the calves, respectively. Resistance to at least one antimicrobial was found in a random isolate of *E. coli* from 48% of the calves. Feeding colostrum from cows treated with antimicrobials at drying off did not affect the prevalence of resistant *E. coli*. In contrast, feeding milk from cows treated with antimicrobials during lactation resulted in significantly more nalidixic acid- and streptomycin-resistant *E. coli* than when such milk was discarded; no significant effect was seen for other resistance traits. Furthermore, an interaction was found between feeding milk from cows treated with antimicrobials and use of fluoroquinolones in cows. In general, the prevalence of resistance was lower for older calves and calves on

small farms. Other factors that were associated with the shedding of resistant *E. coli* were administration of oral dihydrostreptomycin to calves, administration of systemic tetracycline and ceftiofur to cows and calves, housing of the calves, predominant breed of the herd, and geographic location of the farm. The presence of resistant *E. coli* in calves was clearly due to multiple factors, but minimizing the feeding of milk from cows treated with antimicrobials during lactation should lower the prevalence of resistant *E. coli* in the gastrointestinal tract of the calves.

Key words: waste milk, antibiotic resistance, dairy calf, risk factor

INTRODUCTION

Infections caused by antimicrobial-resistant bacteria represent an increasing threat to human and animal health (Wernli et al., 2011). Resistance is seen not only in pathogenic bacteria but also in commensal bacteria (Summers, 2002), such as *Escherichia coli*, which are ubiquitous in the feces of animals and humans (Sørum and Sunde, 2001). Although commensal *E. coli* strains seldom cause infections, they can harbor resistance genes that may be transferred to pathogenic or opportunistic bacteria, leading to intractable infections (Sørum and Sunde, 2001; Summers, 2002). Commensal *E. coli* from cattle feces are often less resistant to antimicrobials than *E. coli* from feces of other food animals (European Food Safety Authority, 2011). Young calves are an exception; they often harbor *E. coli* resistant to multiple antimicrobials (DeFrancesco et al., 2004; Sato et al., 2005; SVARM, 2006). Resistant isolates of *E. coli* from calves may therefore be a significant source of resistance genes for other bacteria in the vicinity (Donaldson et al., 2006; Di Labio et al., 2007).

Antimicrobial resistance is often a consequence of antimicrobial use (van den Bogaard and Stobberingh, 2000). Frequent use of antimicrobials for individual calves has been associated with increased shedding of resistant *E. coli* (Berge et al., 2005, 2006; Di Labio et al., 2007); however, the effect of antimicrobial therapy

Received June 2, 2014.

Accepted September 23, 2014.

¹Corresponding author: anna.duse@sva.se

on resistance in *E. coli* in the gastrointestinal tract of calves seems to be transient (Berge et al., 2006). Overall, the prevalence of resistant *E. coli* in the feces of young calves is high even when the calves have not previously been treated with antimicrobials (Khachatryan et al., 2004; de Verdier et al., 2012). It is therefore likely that there are factors of importance other than antimicrobial treatment.

In several studies, the occurrence of resistant *E. coli* in calf feces has been linked to the age of the calf and it seems to be most abundant during milk feeding (Khachatryan et al., 2004; Watson et al., 2012). Resistance peaks between 2 and 4 wk of age, followed by a gradual decline (Khachatryan et al., 2004; Berge et al., 2005; Watson et al., 2012). During the period when the prevalence of resistance peaks, it is likely that calves are exposed to antimicrobials via ingestion of milk or colostrum from cows treated with antimicrobials. Feeding milk from treated cows is a common practice on many dairy farms (Brunton et al., 2012; Duse et al., 2013). Such milk may contain residues of antimicrobials (Pereira et al., 2014; Randall et al., 2014), which may impose selection pressure on the calves' gastrointestinal bacteria and favor resistant strains. This practice may explain the high prevalence of resistant *E. coli* in pre-weaned calves (Brunton et al., 2012; Aust et al., 2013; Duse et al., 2013). A few studies have investigated the effect of this feeding practice. Wray et al. (1990) found a significantly higher MIC of streptomycin, but not of ampicillin, in *E. coli* from calves fed antimicrobial-containing milk than those fed milk replacer. In another study, a higher prevalence of resistant *E. coli*, but not of resistant enterococci, was found in calves fed nonpasteurized waste milk compared with bulk tank milk (Aust et al., 2013). Xu et al. (2013) found significantly more *E. coli* producing extended spectrum β -lactamases (**ESBL**) among calves from farms that fed milk containing antimicrobial residues than from farms that discarded such milk. Brunton et al. (2014) observed a tendency toward more calves that shed ESBL-producing *E. coli* and in higher absolute number among calves fed waste milk with antimicrobial residues than calves fed milk replacer. The results of these studies indicate that feeding milk from cows treated with antimicrobials may have some effect on the shedding of resistant *E. coli* from calves. On the other hand, to our knowledge, the shedding of resistant *E. coli* from calves fed colostrum or transition milk from cows treated with antimicrobials at drying off has not been studied.

Some of the previous studies in this field failed to take other potential risk factors into account (Wray et al., 1990; Aust et al., 2012; Brunton et al., 2014), although the experiments were conducted under commercial practice, where many factors might influence

the shedding of resistant *E. coli* by calves (Call et al., 2008). In addition, conclusions are often drawn after monitoring the susceptibility of only a single or a few colonies from each calf (Wray et al., 1990; Aust et al., 2013; Xu et al., 2013). The accuracy of this approach is greatly affected by the within-sample prevalence of resistant bacteria (Scott et al., 2011) and by variations in antimicrobial susceptibility among isolates of the same sample (Villarroel et al., 2006).

The primary objective of our study was to investigate the effect of feeding colostrum or milk from cows treated with antimicrobials on the shedding of resistant *E. coli* in feces from preweaned calves between 7 and 28 d. This relationship was assessed taking the use of antimicrobials and other potential risk factors into account. The secondary objective was to describe the prevalence of antimicrobial resistance among *E. coli* from preweaned dairy calves in Sweden.

MATERIALS AND METHODS

Selection of Herds

The number of farms included in the study was based on the assumption that 25% of Swedish dairy farms feed colostrum from cows treated with antimicrobials at drying off to calves and that this would increase the prevalence of streptomycin resistance in fecal *E. coli* from calves from 30 to 50%. To demonstrate this difference with 95% confidence and 80% power, 270 farms had to be included in the study.

The farms were recruited via a questionnaire survey conducted among 457 Swedish dairy farmers in May 2011. The survey inquired about farming practices related to feeding of colostrum and milk from cows treated with antimicrobials (Duse et al., 2013). As the final question in the survey, respondents were given the opportunity to participate in the current study by submitting calf fecal samples for susceptibility testing. Farmers wanting more information before deciding to participate were contacted via telephone in random order before possible inclusion. Of those who agreed to participate, only farms with at least 30 cows were enrolled to simulate a future scenario with larger farms. In total, 287 Swedish dairy farmers were recruited. The median herd size in the study was 75 cows, compared with 66 for all Swedish dairy farms in 2013 with more than 30 cows. (A. Grönvall, Swedish Board of Agriculture, Jönköping, Sweden, personal communication). The farms in the study were located in East (20%), South (54%), and North (26%) Sweden; these divisions are in accordance with the highest subdivision (lands/NUTS1) using the Nomenclature of Territorial Units for Statistics (European Union, 2003). The figures for

all Swedish dairy farms (≥ 30 cows) in 2013 were 18, 64, and 23% for East, South, and North Sweden, respectively (A. Grönvall, Swedish Board of Agriculture, Jönköping, Sweden, personal communication). Thus, in terms of herd size and geographic location, the farms in the study were considered representative of Swedish dairy farms with at least 30 cows.

Sampling

Each farm was randomly assigned a starting date for sampling between October 2011 and September 2012, but samples could be submitted until the end of the study in October 2012. Sampling materials, instructions, and submission forms were provided by the National Veterinary Institute (SVA) in Uppsala, Sweden. On each farm, 3 calves were sampled once by the farmer or farm employees. Sampled calves were between 7 and 28 d, visually healthy, and never treated with antimicrobials. Fecal samples were collected from the rectum with Amie's charcoal culture swabs (Copan Diagnostics Inc., Murrieta, CA) and mailed to SVA. Farmers were encouraged to send samples on Sunday through Thursday to ensure that the samples would reach the laboratory before the weekend. Farmers were also requested to fill out a purpose-specific submission form containing the sampling date and age, sex, and type of housing for each sampled calf. The experimental design and all handling of animals were approved by the Uppsala Ethical Committee, Sweden (reference number C88/11).

Sample Analyses

Fecal samples were analyzed within 48 h after collection. In short, the percentage of *E. coli* resistant to streptomycin (Sm^r), nalidixic acid (Nal^r), and cefotaxime (Ctx^r) with respect to total *E. coli* was determined for each sample. Cefotaxime-resistant isolates of *E. coli* were also investigated for genes encoding ESBL or plasmid-mediated AmpC β -lactamases (*pAmpC*). In addition, susceptibility to 12 antimicrobials was determined for one randomly selected isolate of *E. coli* from each sample (details below).

Sample Preparation. Upon arrival, each rectal swab was mixed by vortex in 1 mL of 0.9% saline to release fecal content. This fecal suspension constituted the nondiluted suspension (10^0) from which 10-fold dilutions down to 10^{-5} were made in 0.9% saline to enumerate (in cfu) *E. coli* in the suspensions. The total count of *E. coli* per sample suspension was estimated by plating 50 μL of dilutions 10^{-3} and 10^{-5} on one-half each of a MacConkey agar plate (Difco, Basingstoke, UK). The count of Sm^r *E. coli* was estimated by plating

50 μL of dilutions 10^{-1} and 10^{-3} on one-half each of a MacConkey agar plate supplemented with 32 $\mu\text{g}/\text{mL}$ streptomycin sulfate salt (Sigma-Aldrich Co., St. Louis, MO) each. The count of Nal^r *E. coli* was estimated by plating 50 μL of dilutions 10^0 and 10^{-2} on one-half each of a MacConkey agar plate supplemented with 32 $\mu\text{g}/\text{mL}$ nalidixic acid sodium salt (Sigma-Aldrich Co.). The count of Ctx^r *E. coli* was estimated by plating 100 μL of the nondiluted suspension (10^0) on a MacConkey agar plate supplemented with 1 $\mu\text{g}/\text{mL}$ cefotaxime sodium salt (Sigma-Aldrich Co.). Dilutions were selected based on a pilot study (A. Duse, unpublished data). The inoculated agar plates were incubated overnight at 37°C .

Within-Sample Prevalence of Antimicrobial Resistant *E. coli*. Red or pink colonies with a colony morphology resembling *E. coli* were enumerated on plates with and without supplemented antimicrobials. In a pilot study (A. Duse, unpublished data) with these methods, at least 97% of the colonies meeting the above criteria were confirmed as *E. coli* by testing for production of tryptophanase (indole) and β -D-glucopyranosiduronic acid (PGUA). Isolates of *E. coli* growing on an agar plate supplemented with antimicrobials were regarded as resistant. To determine the proportion of resistant *E. coli* (%) in each sample suspension and for each antimicrobial, the number of resistant *E. coli* isolates was divided by the total number of isolates and multiplied by 100. Quality control was conducted for each new batch of agar, using *E. coli* ATCC 25922 as a susceptible reference strain, and *E. coli* 4.7 and *E. coli* 1.6, obtained from the European Union Reference Laboratory–Antimicrobial Resistance (EURL-AR, National Food Institute, Lyngby, Denmark), as Nal^r/Sm^r and Ctx^r reference strains, respectively. The quality control results were all within acceptable ranges. The methods described above to measure the within-sample prevalence of resistant *E. coli* were modified from the methods used by Vieira et al. (2008).

Antimicrobial Susceptibility Testing of a Random Isolate of *E. coli*. One lactose-fermenting colony with typical *E. coli* morphology was randomly selected from the MacConkey agar plate without antimicrobial. This colony was subcultured on horse blood agar (Oxoid, Basingstoke, UK) incubated overnight at 37°C , and tested for production of tryptophanase (indole), using the spot indole test (Miller and Wright, 1982). Lactose- and indole-positive isolates with typical colony morphology were considered *E. coli*. These isolates were tested for the susceptibility to 12 antimicrobials by determination of MIC with broth microdilution. Testing was completed according to recommendations by the Clinical and Laboratory Institute (CLSI, 2013) using VetMIC panels (National Veterinary Institute, Uppsala, Sweden) and cation-adjusted Mueller Hinton broth

Table 1. The proportion of resistance and the MIC₅₀ and MIC₉₀ among isolates of *Escherichia coli* from feces of preweaned dairy calves in Sweden for 12 antimicrobials (n = 729)¹

Antimicrobial	Range tested (mg/L)	Cut-off value (mg/L)	Resistance (%)	95% CI	MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)
Ampicillin	1–128	>8	19.3	16.4; 22.2	2	>128
Cefotaxime	0.016–2	>0.25	1.0	0.3; 1.7	0.06	0.12
Chloramphenicol	2–64	>16	3.3	2.0; 4.6	4	8
Ciprofloxacin	0.008–1	>0.06	10.4	8.2; 12.6	0.03	0.12
Florfenicol	4–32	>16	0.1	0.0; 0.4	8	8
Gentamicin	0.12–16	>2	0.3	0.0; 0.7	1	1
Kanamycin	8–16	>8	5.5	3.8; 7.1	8	8
Nalidixic acid	1–128	>16	9.6	7.5; 11.7	4	8
Streptomycin	2–256	>16	39.5	36.0; 43.1	8	256
Sulfametoxazole	8–1,024	>64	31.3	27.9; 34.7	32	>1,024
Tetracycline	1–128	>8	23.3	20.3; 26.4	2	64
Trimethoprim	0.12–16	>2	8.0	6.0; 9.9	0.5	1

¹Cut-off value according to the epidemiological cut-off values established by EUCAST; MIC₅₀ and MIC₉₀ = MIC that inhibit 50 and 90% of the isolates, respectively.

(Becton Dickinson, Cockeysville, MD). Antimicrobials and ranges are given in Table 1. Quality control, using the reference strain *E. coli* ATCC 25922, was conducted in parallel with each batch of isolates; all results were within acceptable ranges. Epidemiological cut-off values (Table 1) issued by the European Committee on Antimicrobial Susceptibility Testing (EUCAST; www.eucast.org) were used to classify isolates as susceptible or resistant.

Detection of Genes Encoding for ESBL or *pAmpC* β -Lactamases. One lactose-fermenting colony with *E. coli* morphology was randomly selected from the cefotaxime-supplemented MacConkey agar plate. This colony was subcultured on horse blood agar (Oxoid) and incubated overnight at 37°C. Isolates were considered as *E. coli* if positive in the spot indole test (Miller and Wright, 1982). Confirmed isolates were screened by multiplex-PCR for detection of the following gene groups: plasmid-mediated AmpC (*pAmpC*; Pérez-Pérez and Hanson, 2002) and *bla*_{CTX-M} (Woodford et al., 2006). Specific gene variants were determined by sequencing using BigDye v1.1 (Life Technologies, Grand Island, NY) and the following primers: *bla*_{CTX-M-1}, 5'-CAGAATAAGGAATCCCATGGTT-3' and 5'-GGCGATAAACAAAAACGGAAT-3'; and *bla*_{CMY-2}, 5'-AAATCGTTATGCT(G/C)CGCTCT-3' and 5'-CATGGGATTTTCCTTGCTGT-3' (Sundsfjord et al., 2004).

Collection and Management of Risk Factor Data

Risk factor data for the 243 farms were collected via 3 channels: national database records, a questionnaire survey conducted before the study (questionnaire 1), and a second one conducted in relation to the sampling (questionnaire 2).

Data Obtained via National Databases. Information regarding geographic location and the predominant herd breed (Table 2) was obtained from the Swedish Official Milk Recording Scheme (SOMRS). The farms geographic location was defined by the highest subdivision (lands/NUTS1) using the Nomenclature of Territorial Units for Statistics (East Sweden, South Sweden including the islands, and North Sweden; European Union, 2003). The predominant breed of the cows on the farm was defined as Swedish Holstein or Swedish Red when more than 75% of the cows were of these breeds. When none of these breeds exceeded 75% of the cows in the herd, the breed was classified as mixed. Additionally, recorded data on veterinary use of antimicrobials during the 2 preceding years were collected from the dairy disease database (DDD) administered by Växa Sverige (Mörk et al., 2010).

Questionnaire 1. A web-based questionnaire survey was conducted in 2011 to obtain information describing farming practices related to feeding milk and colostrum from cows treated with antimicrobials to calves (Duse et al., 2013). The questionnaire is described in detail in Duse et al. (2013). Colostrum and transition milk (i.e., milk from the second milking to the fourth day after calving) from a cow that was treated with antimicrobials at dry off were designated waste colostrum (WC) and waste transition milk (WT), respectively. Milk produced by a cow that was treated with antimicrobials during lactation was denoted as waste milk (WM). The farms were categorized as “always to occasionally” or “never” feeding WC to calves (Table 2). The farms were likewise categorized with respect to WT (Table 2). Finally, farms were categorized with respect to the feeding of WM to calves: “always to occasionally” (during the treatment period, the statutory withdrawal period, or both) or “never” (Table 2). Besides categorization of

Table 2. Farm- and calf-level variables in the univariable analysis for antimicrobial-resistant *Escherichia coli* in feces from preweaned dairy calves (n = 243 farms, 729 calves)

Variable	Category (no. of farms or calves)
Farm-level	
Type of production	Nonorganic (195), organic (47)
Herd size	28 to 62 cows (81), 63 to 98 cows (80), ≥ 100 cows (82)
Housing of lactating cows	Insulated freestall (137), cold freestall (32), tiestall (63), other (10)
Milking system	Automatic milking system (115), milking parlor (59), tiestall milking (62), other (6)
Geographic location	East Sweden (50), South Sweden and islands (135), North Sweden (58)
Predominant breed	Swedish Holstein (64), Swedish Red (38), mixed breeds (141)
Feeding colostrum from cows treated with dry cow antimicrobials (WC)	Always to occasionally (204), never (37), unknown (2)
Feeding transition milk from cows treated with dry cow antimicrobials (WT)	Always to occasionally (196), never (45), unknown (2)
Feeding milk from cows treated with antimicrobials during lactation (WM)	Always to occasionally during the treatment and withdrawal period (138), always to occasionally during the withdrawal period only (61), never (43), unknown (1)
Use of nursing cows treated with antimicrobials	At least occasionally (17), never (226)
Using antimicrobial treatment at drying off	Never (24), at least occasionally (219)
Cows treated with long-acting ¹ dry cow antimicrobials (% of all cows)	0 to 10% (127), 11 to 50% (97), more than 50% (17), unknown (2)
Dry cow treatments with short-acting ² antimicrobials	Never (202), at least occasionally (38), unknown (3)
Calf-level	
Sampling season	January to March (213), April to June (171), July to September (181), October to December (164)
Sex	Heifer (406), bull (323)
Age	7 to 11 d (222), 12 to 19 d (255), 20 to 28 d (252)
Housing of preweaned calves	Single pens (433), group pens with calves less than 30 d (123), group pens with at least one calf above 30 d (143), other (30)

¹Long-acting dry cow antimicrobials provide sustained concentrations when administered intramammary and must be administered at least 4 wk before calving to deliver milk for human consumption 4 d after calving. In this study, long-acting dry cow antimicrobials were benzylpenicillin with either framycetin or dihydrostreptomycin.

²Short-acting antimicrobials for dry cow therapy do not provide sustained concentrations and therefore, repeated administration is necessary. These formulas can be used in lieu of long-acting formulas to avoid subtherapeutic concentrations over several days and to minimize the risk of residues in milk. In this study, short-acting dry cow antimicrobials were either benzylpenicillin alone or in combination with dihydrostreptomycin.

farms based on the feeding of WC, WT, and WM, the questionnaire surveyed the production type (organic vs. nonorganic) of the farm (Table 2).

Questionnaire 2. The purpose of the second survey was to obtain information on the types of antimicrobials used on the farms, and treatment incidence (**TI**) of cows and preweaned calves. As in the first questionnaire, the second one was conducted using the online survey platform Easyresearch (Questback; <http://www.questback.com>) via an e-mail link. The questionnaire used conditional branching to follow-up questions based on the answers to the previous question; only closed-ended questions were used. Questions about antimicrobials included the names of the active substance, names of the available preparations, and pictures thereof. The respondents were asked to provide a crude measure of the frequency of treatments for the most commonly used antimicrobials in Sweden (Växa Sverige, 2012). The scale, in descending order of frequency, was “several times a week,” “several times a month,” “several times a year,” “few times a year,” and “never.”

Antimicrobial consumption was described both as a binary variable (using/not using) and mean annual TI (Table 3). To estimate the TI, the response options from questionnaire 2 were transformed to an estimated number of treatments per year as follows. “Several times a week” was set to approximately correspond to 104 cases (2×52 wk), “several times a month” to 48 cases (4×12 mo), “several times a year” to 12 cases, “few times a year” as 4 cases, and “never.” This number was then related to the herd size and multiplied by 100 to obtain an estimated treatment incidence (**TI_e**), expressed as cases per 100 cow-years.

Likewise, the mean number of recorded cases of antimicrobial treatments from the DDD during a year was related to the herd size of the farm and multiplied by 100 to obtain the recorded treatment incidence (**TI_r**), expressed as cases per 100 cow-years. The coverage of the DDD in Sweden is less than 100% due to underreporting (Mörk et al., 2010). To avoid underestimation, the highest value of either the **TI_e** or the **TI_r** for each farm was therefore used as the TI for further analyses.

Table 3. Mean, 95% CI, minimum, median, and maximum of the annual treatment incidence (TI) for the most commonly used antimicrobials for cows and preweaned dairy calves in 243 Swedish dairy farms

Antimicrobial (% of farms where data were available)	Route	No. of farms (%) ¹	Annual TI (cases per 100 cow-years)				
			Mean	95% CI	Minimum	Median	Maximum
Cows							
Benzylpenicillin (99.6%)	Systemic	239 (98.8)	27.7	26.2; 29.2	0	22.5	111.6
Benzylpenicillin or benzylpenicillin/DHS ² combination (99.2%)	IMM ³	137 (56.4)	6.3	5.6; 7.0	0	2.8	78.7
Ceftiofur (98.4%)	Systemic	47 (19.7)	1.4	1.2; 1.9	0	0	20.0
Fluoroquinolones (99.2%)	Systemic	205 (85.1)	6.6	6.2; 7.0	0	5.8	30.0
Trimethoprim-sulfa combination (97.9%)	Systemic	68 (28.6)	1.7	1.5; 1.9	0	0	18.0
Tetracycline (98.8%)	Intrauterine	77 (32)	2.2	1.1; 3.3	0	0	37.5
Tetracycline (97.9%)	Systemic	144 (60.5)	4.5	4.2; 4.8	0	3.3	30.7
Preweaned calves							
Benzylpenicillin (99.2%)	Systemic	194 (80.5)	6.5	6.0; 7.0	0	5.3	56.5
Benzylpenicillin/DHS combination (98.7%)	Systemic	44 (18.3)	1.1	0.9; 1.3	0	0	17.1
Ceftiofur (99.2%)	Systemic	2 (0.8)	0.1	0.05; 0.2	0	0	9.6
DHS ² (99.1%)	Oral	46 (19.1)	1.6	1.3; 1.9	0	0	21.8
Fluoroquinolones (97.9%)	Systemic	29 (11.9)	0.6	0.5; 0.7	0	0	13.5
Trimethoprim-sulfa combination (97.9%)	Systemic	55 (23.1)	2.0	1.5; 2.5	0	0	68.6
Tetracycline (98.8%)	Systemic	71 (29.2)	1.8	1.5; 2.1	0	0	34.3

¹Number and proportion of farms where the antimicrobial is used.²Dihydrostreptomycin.³Intramammary.

As underreporting is even more common for antimicrobial treatments of calves (Mörk et al., 2009), only the TI_e was used as the TI of antimicrobial treatments of preweaned dairy calves (Table 3).

Besides information regarding antimicrobial consumption in cows and preweaned calves, data were also obtained from questionnaire 2 regarding current herd size, predominant housing type for lactating cows and predominant milking system [i.e., automatic milking system (AMS), milking parlor, tiestall milking, or other; Table 2].

Neither of the questionnaires contained questions that could interfere with the personal integrity of the respondents. Hence, in accordance with the act on ethical review of research involving humans (SFS 2003:460) in the Swedish Code of Statutes, an ethical approval for research on humans was not deemed necessary. In accordance with the Helsinki Declaration, several precautions to maintain the personal integrity of the farmers were implemented. These included decoding the farm identification number in the data, keeping personal information confidential, and removing survey data and e-mails from the online platform. The questionnaires in Swedish can be obtained upon request from the corresponding author.

Statistical Analysis

Data were entered in a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA) and exported into Stata 11 (release 11.2; Stata Statistical Software, College Station, TX) for analysis. The association of poten-

tial predictor variables to the within-sample prevalence of Sm^r or NaI^r *E. coli* was assessed using zero-inflated negative binomial regression with the ZINB function in Stata. A robust variance estimator was used to account for the clustering of observations at the herd level. Zero-inflated negative binomial regression models are used to handle count data for which the variance is greater than the mean (over-dispersion) and where there is an excess of zero counts (observations with zero resistant *E. coli* isolates; Dohoo et al., 2010). These types of models include the negative binomial part (which models the within-sample prevalence of resistant *E. coli* isolates in the sample) and the logistic regression part (which models the probability of isolating zero resistant *E. coli* isolates in the sample; Dohoo et al., 2010).

Potential predictor variables (Tables 2 and 3) were tested both in the logistic and negative binomial parts of the model. Only a small proportion of the calves (77 out of 729) carried Ctx^r *E. coli*; therefore, it was not possible to use the within-sample prevalence of Ctx^r *E. coli* as an outcome variable in the analyses. Instead, this variable was modeled as a binary outcome variable; that is, the calves had either at least one Ctx^r *E. coli* isolate or none. The association of potential predictor variables (Tables 2 and 3) with the presence or absence of Ctx^r *E. coli* or with antimicrobial resistance of a random isolate of *E. coli* was assessed by univariable logistic regression using the LOGIT function in Stata. A robust variance estimator was used to account for the clustering of observations at the herd level.

Collinearity was assessed pair-wise by calculation of Spearman rank correlations. Variables with a *P*-value

<0.2 were considered for multivariable analysis, provided that there was no collinearity ($r < |0.7|$) between them. The assumption of linearity between continuous predictor variables and outcome variables was checked visually with the LINTREND function in Stata. Continuous variables that were not linearly related to the outcomes were categorized using either biologically important cut-off values or cut-off values that resulted in even-sized groups. Antimicrobial use (Table 3) was included as either continuous or categorical variables (using/not using), depending on whether the assumption of linearity was fulfilled. Categories with too few observations (<5) were amalgamated with another category to constitute a biologically plausible one.

For each outcome, a single multivariable model was developed using a manual, backward-stepwise procedure. Potential confounders were considered in every model as variables that if present, changed the coefficient for one or more variable by more than 30% (Houe et al., 2004). All variables with a P -value ≤ 0.05 were retained in the final model. All biologically plausible interactions between remaining main effects were tested; a P -value ≤ 0.05 was considered evidence of interaction. Model fit was investigated by generating and comparing expected probabilities to observed probabilities. To assess the fit of binary dependent variables, we used the Hosmer-Lemeshow goodness-of-fit test with the data partitioned in up to 10 deciles. Models were developed for resistance in a random isolate of *E. coli* for ampicillin (**Am^r**), nalidixic acid (**Nal^r**), ciprofloxacin (**Ci^r**), streptomycin (**Sm^r**), tetracycline (**Tc^r**), sulfamethoxazole (**Su^r**), chloramphenicol (**Cm^r**), and kanamycin (**Km^r**). A multivariable model could not be developed for trimethoprim due to the lack of significant predictor variables, nor could it be developed for florfenicol or gentamicin due to low prevalence/absence of resistant isolates.

Significant predictors of the final zero-inflated negative binomial regression models were described with coefficients and robust standard errors, separated in the logistic and the negative binomial part of the model (Tables 4 and 5). Significant predictors of the final logistic regression models were described with coefficients, robust standard errors, and odds ratios (Tables 6 to 8).

RESULTS

Samples and Farms

Sampling material and questionnaires were distributed to 286 of the 287 farms. One farm withdrew before the designated sampling period. In total, 756 fecal samples were received from 248 farms (87% response). Of these samples, 14 were from calves outside the correct age

span and 4 were from farms that had already submitted samples from 3 calves. These samples were therefore excluded. Four farms submitted only 2 samples each and 1 farm submitted only 1 sample; these data were also excluded. In total, samples from 729 calves on 243 farms were used. The median age of the calves was 15 d, ranging from 7 to 28 d. The distribution of the calf age, sex, and housing type, as well as the distribution of samples obtained in different seasons are described in Table 2. The median herd size for the 243 farms was 72 cows, ranging from 28 to 1,175 cows. Table 2 shows the distribution of farms within groups by herd size, production type (nonorganic or organic), predominant milking system (AMS, milking parlor, tiestall milking, or other), housing of lactating cows, geographic location, and predominant herd breed.

Antimicrobial Susceptibility

Within-Sample Prevalence of Antimicrobial Resistant *E. coli*. Streptomycin-resistant *E. coli* were isolated from 90% of the calves, representing 96% of the 243 farms. Streptomycin-resistant *E. coli* were isolated from all 3 calves (82% of the farms), 2 calves (8%), only 1 calf (6%), and no calf (4%). The within-sample prevalence of Sm^r *E. coli* for individual calves ranged from 0 to 100%, where the median, 25th, and 75th percentiles were 4, 0.07, and 32%, respectively.

Nalidixic acid-resistant *E. coli* were isolated from 49% of the calves, representing 60% of the 243 farms. Nalidixic acid-resistant *E. coli* were isolated from no calf (40% of the farms), all 3 calves (37%), 2 calves (14%), and only 1 calf (9%). The within-sample prevalence of Nal^r *E. coli* for individual calves ranged from 0 to 100% and the median, 25th, and 75th percentiles were 0, 0, and 0.5%, respectively.

Cefotaxime-resistant *E. coli* were isolated from 11% of the calves, representing 18% of the 243 farms. Cefotaxime-resistant *E. coli* were isolated from no calf (82% of the farms), only 1 calf (9%), 2 calves (5%), and all calves (4%). The within-sample prevalence of Ctx^r *E. coli* for individual calves ranged from 0 to 78%, and the median, 25th, and 75th percentiles were all 0%.

Antimicrobial Susceptibility Testing of a Random Isolate of *E. coli*. Of the 729 randomly selected *E. coli* isolates subjected to antimicrobial susceptibility testing, 352 (48%) were resistant to one or more antimicrobials. Distribution of MIC and the proportion of resistance to antimicrobials tested are presented in Table 1. Of the isolates, 197 (27%) were resistant to 3 or more antimicrobials (multiresistant) and 61 (8%) to 5 or more. Of the 197 multiresistant isolates, the most common phenotype was resistance to streptomycin, sulfamethoxazole, and tetracycline (115 isolates).

Table 4. Risk factors for an increased prevalence of streptomycin-resistant *Escherichia coli* in fecal samples from preweaned dairy calves (n = 729) according to a multivariable zero-inflated negative binomial regression model

Variable	Level or unit	Coefficient	Robust SE ¹ (β)	Level P-value	Overall P-value
Negative binomial (within-sample prevalence of streptomycin-resistant <i>E. coli</i>)					
Intercept		−1.78	0.28	—	
Age of calf at sampling	Days	−0.03	0.01	0.002	0.002
Feeding WM ² to calves	0: Never	Referent	—	—	0.03
	1: Always to occasionally	0.43	0.21	0.03	
	2: Unknown	0.73	0.21	0.01	
Herd size	0: 28 to 62 cows	Referent	—	—	0.01
	1: 63 to 98 cows	0.49	0.17	0.004	
	2: 100 to 1,175 cows	0.41	0.19	0.03	
Oral DHS ³ for calves	0: No	Referent	—	—	<0.001
	1: Yes	0.52	0.16	0.001	
	2: Unknown	1.55	0.49	0.002	
Systemic penicillin and DHS for calves	0: No	Referent	—	—	0.05
	1: Yes	0.34	0.16	0.04	
	2: Unknown	−0.12	0.15	0.42	
Sampling season	0: October to December	Referent	—	—	0.02
	1: January to March	0.61	0.21	0.004	
	2: April to June	0.26	0.23	0.27	
	3: July to September	0.52	0.22	0.02	
Logistic (probability of zero streptomycin-resistant <i>E. coli</i>)					
Intercept		−2.52	0.73	—	
Age of calf at sampling	Days	0.06	0.03	0.05	0.05
Cows treated with long-acting dry cow antimicrobials	0: 0 to 10% of the cows	Referent	—	—	<0.001
	1: 11 to 50% of the cows	−0.89	0.44	0.05	
	2: More than 50% of the cows	−21.93	0.68	<0.001	
	3: Unknown	0.32	1.48	0.83	
Dry cow treatment with short-acting antimicrobials	0: No	Referent	—	—	<0.001
	1: Yes	−21.73	0.76	<0.001	
	3: Unknown	−19.60	1.75	<0.001	
Oral DHS for calves	0: No	Referent	—	—	<0.001
	1: Yes	−22.15	0.40	<0.001	
	2: Unknown	−18.06	1.74	<0.001	
Sampling season	0: October to December	Referent	—	—	<0.001
	1: January to March	−1.28	0.49	0.01	
	2: April to June	−2.55	0.70	<0.001	
	3: July to September	−3.85	1.44	0.008	

¹Robust standard errors (adjusted by clustering within herds).²Milk produced by a cow that was treated with antimicrobials during lactation.³Dihydrostreptomycin.

Detection of Genes Encoding ESBL or pAmpC β -Lactamases. Cefotaxime-resistant *E. coli* were isolated from 81 calves. Nine of these calves (1% of all calves) from 5 farms carried *E. coli* with genes encoding either ESBL or transferrable pAmpC. Four of these isolates carried *bla*_{CTX-M-15}, 1 carried *bla*_{CTX-M-1}, and 4 carried *bla*_{CMY-2}. The remaining 72 isolates did not carry any of these transferable genes; decreased susceptibility to cefotaxime among these could be the result of mutations causing hyperproduction of AmpC β -lactamases.

Risk Factors Associated With the Shedding of Antimicrobial-Resistant *E. coli*

Feeding Colostrum or Milk From Cows Treated With Antimicrobials to Calves. Feeding of WC

(Figure 1) or WT was not significantly associated with a high within-sample prevalence of Sm^r or Nal^r *E. coli* (Tables 4 and 5). Likewise, the odds of isolating at least one Ctx^r *E. coli* (Table 6) or a random *E. coli* resistant to any of the other tested antimicrobials (Tables 7 and 8) were not significantly higher among calves on farms that fed either WC or WT than on farms that discarded WC or WT.

Calves from farms that fed WM to calves had a higher within-sample prevalence of Sm^r and Nal^r *E. coli* than calves on farms where WM was not fed to calves (Figure 2). We detected no significant difference between feeding WM produced during the withdrawal period and feeding WM during both the treatment and the withdrawal period (Figure 2). Therefore, these 2 categories were amalgamated into one in the analyses.

Table 5. Risk factors for increased prevalence of nalidixic acid-resistant *Escherichia coli* in fecal samples from preweaned dairy calves (n = 720), according to a multivariable zero-inflated negative binomial regression model

Variable	Level or unit	Coefficient	Robust SE ¹ (β)	Level <i>P</i> -value	Overall <i>P</i> -value
Negative binomial (within-sample prevalence of nalidixic acid-resistant <i>E. coli</i>)					
Intercept		−3.07	0.45	—	
Age of calf at sampling	Days	−0.10	0.02	<0.001	<0.001
Geographic location	0: North Sweden	Referent	—	—	0.01
	1: South Sweden and islands	0.97	0.33	0.003	
	2: East Sweden	0.97	0.37	0.009	
Herd size	0: 28 to 62 cows	Referent	—	—	0.03
	1: 63 to 98 cows	1.00	0.38	0.009	
	2: 100 to 1,175 cows	0.59	0.36	0.10	
Oral DHS ² for calves	0: No	Referent	—	—	<0.001
	1: Yes	0.68	0.29	0.02	
	2: Unknown	−1.55	0.45	0.001	
Systemic fluoroquinolone for cows × feeding WM ³ to calves	0: Yes, Yes	Referent	—	—	0.003
	1: No, No	−2.85	0.82	0.001	
	2: Yes, No	−0.58	0.27	0.03	
	3: No, Yes	−0.30	0.43	0.48	
Systemic trimethoprim-sulfa for calves	0: No	Referent	—	—	<0.001
	1: Yes	0.60	0.27	0.03	
	2: Unknown	1.55	0.39	<0.001	
Logistic (probability of zero nalidixic acid-resistant <i>E. coli</i>)					
Intercept		1.65	0.54	—	
Herd size	0: 28 to 62 cows	Referent	—	—	<0.001
	1: 63 to 98 cows	−1.33	0.41	0.001	
	2: 100 to 1,175 cows	−2.83	0.80	<0.001	
South Sweden × predominantly Swedish Holstein breed	0: No, No	Referent	—	—	<0.001
	1: Yes, No	−1.65	0.47	<0.001	
	2: No, Yes	−2.81	1.18	0.02	
	3: Yes, Yes	−1.70	0.61	0.005	
Systemic fluoroquinolone for cows × feeding WM to calves	0: Yes, Yes	Referent	—	—	0.02
	1: No, No	1.89	0.75	0.01	
	2: Yes, No	−0.08	0.49	0.86	
	3: No, Yes	1.07	0.56	0.05	

¹Robust standard errors (adjusted for clustering within herds).²Dihydrostreptomycin.³Milk produced by a cow that was treated with antimicrobials during lactation was here denoted as waste milk.

We detected a significant interaction between using fluoroquinolones in cows and feeding WM to calves. Farms that did both had a significantly higher within-sample prevalence of Nal^r *E. coli* among their calves than farms that neither fed WM to calves nor treated cows with fluoroquinolones (Table 5).

Feeding WM to calves also resulted in increased likelihood of Nal^r (Table 8) or Sm^r (Table 7) in a random isolate of *E. coli* compared with not feeding WM to calves. However, the prevalence of resistance to the remaining tested antimicrobials was not significantly higher for calves on farms feeding WM to calves, al-

Table 6. Risk factors for the presence of at least 1 cefotaxime-resistant isolate of *Escherichia coli* in feces from preweaned dairy calves (n = 723), according to a multivariable logistic regression model

Variable	Level or unit	Coefficient	Robust SE ¹ (β)	Odds ratio	95% CI	Level <i>P</i> -value	Overall <i>P</i> -value
Intercept		−3.54	0.50				
Calf housing	0: Single pens	Referent	—	—	—	—	0.002
	1: Group pens	0.97	0.30	2.65	1.48; 4.75	0.001	
	2: Other	−0.93	1.07	0.40	0.05; 3.23	0.39	
Herd size	0: 28 to 62 cows	Referent	—	—	—	—	0.04
	1: 63 to 98 cows	1.21	0.53	3.36	1.19; 9.51	0.02	
	2: 100 to 1,175 cows	1.31	0.53	3.69	1.31; 10.4	0.01	
Systemic ceftiofur treatments of calves	0: No	Referent	—	—	—	—	<0.001
	1: Yes	0.90	0.21	2.46	1.62; 3.73	<0.001	

¹Robust standard errors (adjusted for clustering within herds).

Table 7. Risk factors for resistance to ampicillin, streptomycin, tetracycline or kanamycin in a random isolate of *Escherichia coli* in feces from preweaned dairy calves (n = 726–729), according to multivariable logistic regression models

Resistance phenotype	Variable	Level or unit	Coefficient	Robust SE (β) ¹	Odds ratio	95% CI	Level <i>P</i> -value	Overall <i>P</i> -value
Ampicillin	Intercept		−0.77	0.39				
	Age of calf at sampling	Days	−0.09	0.02	0.91	0.88; 0.95	<0.001	
	Predominant milking system	0: Tie stall milking	Referent	—	—	—	—	<0.001
		1: Milking parlor	1.10	0.37	3.01	1.46; 6.21	0.003	
		2: Automatic milking system (AMS)	0.38	0.33	1.46	0.77; 2.79	0.25	
		3: Other	1.70	0.49	5.46	2.07; 14.39	0.001	
	Systemic ceftiofur treatments of cows	0: No	Referent	—	—	—	—	0.01
		1: Yes	0.65	0.24	1.92	1.19; 3.10	0.01	
		2: Unknown	1.26	0.90	3.54	0.61; 20.5	0.158	
			−0.36	0.33	—	—	—	
Streptomycin	Intercept		−0.06	0.01	0.95	0.92; 0.97	<0.001	
	Age of calf at sampling	Days	−0.06	0.01	0.95	0.92; 0.97	<0.001	
	Feeding WM ² to calves	0: Never	Referent	—	—	—	—	0.05
		1: Always to occasionally	0.49	0.25	1.62	1.00; 2.65	0.05	
	Herd size	0: 28 to 62 cows	Referent	—	—	—	0.025	0.04
		1: 63 to 98 cows	0.51	0.22	1.67	1.09; 2.56	0.02	
		2: 100 to 1,175 cows	0.42	0.21	1.57	1.02; 2.27	0.04	
	Oral DHS ³ for calves	0: No	Referent	—	—	—	—	0.001
		1: Yes	0.55	0.22	1.73	1.14; 2.64	0.01	
		2: Unknown	1.16	0.30	3.19	1.76; 5.78	<0.001	
Tetracycline	Intercept		−2.01	0.48				
	Age of calf at sampling	Days	−0.06	0.02	0.94	0.91; 0.97	<0.001	
	Calf housing	0: Group pens with calves above 30 d	Referent	—	—	—	—	0.03
		1: Single pens	0.70	0.31	2.01	1.10; 3.65	0.02	
		2: Group pens with calves below 30 d	0.77	0.35	2.17	1.09; 4.31	0.03	
		3: Other	1.40	0.54	4.06	1.42; 11.64	0.01	
	Herd size	0: 28 to 62 cows	Referent	—	—	—	0.025	0.03
		1: 63 to 98 cows	0.77	0.29	2.15	1.23; 3.77	0.01	
		2: 100 to 1,175 cows	0.57	0.30	1.76	0.99; 3.15	0.05	
	Predominant milking system	0: Tiestall milking	Referent	—	—	—	—	<0.001
		1: Milking parlor	0.68	0.33	1.97	1.03; 3.74	0.04	
		2: AMS	0.20	0.31	1.22	0.67; 2.24	0.51	
		3: Other	1.64	0.42	5.14	2.27; 11.63	<0.001	
	Systemic tetracycline treatments of cows	Cases per 100 cow years	0.07	0.02	1.07	1.03; 1.11	0.001	
	Systemic tetracycline treatments of calves	0: No	Referent	—	—	—	—	0.04
		1: Yes	0.58	0.24	1.79	1.12; 2.84	0.001	
		2: Unknown	−0.35	0.94	0.71	0.11; 4.31	0.71	
Kanamycin	Intercept		−2.78	0.31				
	Age of calf at sampling	0: 7 to 11 d	Referent	—	—	—	—	0.04
		1: 12 to 19 d	0.09	0.38	0.91	0.44; 1.91	0.81	
		2: 20 to 28 d	−1.18	0.48	0.31	0.12; 0.79	0.02	
	Herd size	Number of cows	0.002	0.0005	1.00	1.00; 1.00	<0.001	

¹Robust standard errors (adjusted for clustering within herds).

²Milk produced by a cow that was treated with antimicrobials during lactation was here denoted as waste milk.

³Dihydrostreptomycin.

Figure 1. The mean within-sample prevalence and 95% CI (—) of streptomycin- and nalidixic acid-resistant *Escherichia coli* from feces of calves raised on farms that never fed colostrum from cows given antimicrobial treatments at drying off (dark gray bars) or farms that fed such colostrum at least occasionally (light gray bars). Different letters indicate a significant difference ($P < 0.05$) in the mean within-sample prevalence for streptomycin-resistant or nalidixic acid-resistant *E. coli* isolates, respectively (results from univariable analysis).

though the association to Ci^r *E. coli* was borderline ($P = 0.06$; results not shown).

Use of Antimicrobials in Cows and Preweaned Calves. All farmers that submitted samples completed questionnaire 2. The annual TI for each antimicrobial is given in Table 3. A higher within-sample prevalence of Sm^r and Nal^r *E. coli* was observed among calves from farms that treated calves with oral preparations containing dihydrostreptomycin (DHS) than calves from farms that did not (Tables 4 and 5). Oral treatment with DHS also increased the likelihood of Nal^r , Ci^r , or Sm^r in randomly selected isolates of *E. coli* (Tables 7 and 8).

Calves from farms using short-acting intramammary antimicrobials at drying off were more likely to shed Sm^r *E. coli* than farms that did not use them, but the within-sample prevalence was unaffected (Table 4). The probability of isolating Sm^r *E. coli* also increased with an increasing proportion of cows per farm that were treated with long-acting dry cow antimicrobials (Table 4).

Farms that treated calves with systemic trimethoprim-sulfonamide had a higher within-sample prevalence of Nal^r *E. coli* compared with farms that did not (Table 5). Systemic treatment of cows with ceftiofur almost doubled the odds of isolating Am^r *E. coli* (Table 7), and treatment of calves with the same drug increased the odds of finding Ctx^r *E. coli* by a factor of 2.5 (Table 6). Additionally, the likelihood of finding Tc^r , Nal^r , and Ci^r in a random *E. coli* increased with increasing TI of systemic tetracycline for cows (Tables 7 and 8). Systemic

tetracycline for treatment of calves increased the odds of isolating Tc^r or Cm^r *E. coli* (Tables 7 and 8).

Farm Characteristics. The within-sample prevalence of Nal^r and Sm^r *E. coli* was significantly higher on farms with more than 62 cows than on farms with fewer than 62 cows. Calves on large farms were also more likely to have Ctx^r (Table 6), Sm^r , and Tc^r (Table 7) in a random *E. coli*. The odds of Km^r , Nal^r , and Ci^r in a random *E. coli* also increased with herd size (Tables 7 and 8). A significant interaction was found between predominant herd breed and geographic location, meaning that calves on farms located in North or East Sweden and with predominantly Swedish Red or mixed-breed cows had a lower probability of Nal^r *E. coli* compared with calves on farms in South Sweden with predominantly Swedish Holstein cows (Table 5). However, a significantly higher prevalence of Cm^r *E. coli* was found in calves on farms in South Sweden. It was also more likely to find Nal^r or Ci^r in a random *E. coli* from Swedish Holstein herds than Swedish Red or mixed-breed herds (Table 8). However, calves from Swedish Holstein herds were less likely to shed Cm^r *E. coli* (Table 8). Use of a milking parlor or other milking facilities, was associated with increased odds of Am^r and Tc^r compared with AMS or tiestall milking (Table 7).

Calf Factors. Increasing age of the calf (days) was associated with a decline in the within-sample prevalence of Sm^r (Table 4) and Nal^r (Table 5) *E. coli*, and a decreasing probability of isolating *E. coli* resistant to all tested antimicrobials except cefotaxime (Tables 7 and 8). Cefotaxime-resistant *E. coli* were more common in calves housed in groups rather than in single pens (Table 6). Tetracycline-resistant *E. coli* were found more often in calves housed in groups together with young calves or in single pens than in group pen where at least one calf was >30 d (Table 7). Calves were less likely to shed a high proportion of Sm^r *E. coli* between October and December than during the rest of the year (Tables 4).

DISCUSSION

The main objective of this study was to investigate the effect of feeding colostrum or milk from cows treated with antimicrobials on the shedding of resistant *E. coli* in feces from preweaned calves. Such milk or colostrum often contains antimicrobial residues (Pereira et al., 2014; Randall et al., 2014) and feeding it to calves would therefore favor resistant strains in the gastrointestinal tracts of calves. However, feeding WC or WT was not associated with an increase in the shedding of resistant *E. coli*. The lack of a significant association may be due to insufficient power but could also indicate

Table 8. Risk factors for resistance to nalidixic acid, ciprofloxacin, sulfametoxazole or chloramphenicol among a random isolate of *Escherichia coli* in feces from preweaned dairy calves (n = 720 – 729), according to multivariable logistic regression models

Resistance phenotype	Variable	Level or unit	Coefficient	Robust SE ¹ (β)	Odds ratios	95% CI	Level <i>P</i> -value	Overall <i>P</i> -value
Nalidixic acid	Intercept		−2.53	0.75				
	Age of calf at sampling	Days	−0.08	0.02	0.92	0.88; 0.96	—	0.001
	Herd size	Number of cows	0.001	0.0007	1.00	1.00; 1.00	—	
	Feeding WM ² to calves lactation to calves	0: Never	Referent	—	—	—	—	0.04
		1: Always to occasionally	1.47	0.72	4.34	0.06; 0.94	0.04	
	Oral DHS ³ for calves	0: No	Referent	—	—	—	—	0.04
		1: Yes	0.81	0.35	2.26	1.13; 4.48	0.02	
	Predominant breed	0: Swedish Holstein	Referent	—	—	—	—	0.02
		1: Swedish Red	−0.93	0.54	0.39	0.13; 1.12	0.08	
		2: Mixed breeds	−0.82	0.31	0.44	0.24; 0.82	0.009	
Ciprofloxacin	Systemic tetracycline treatments of cows	Cases per 100 cow years	0.07	0.03	1.07	1.02; 1.13	—	0.01
	Intercept		−1.20	0.43				
	Age of calf at sampling	Days	−0.07	0.02	0.93	0.89; 0.98	—	0.002
	Herd size	Number of cows	0.002	0.0007	1.00	1.00; 1.00	—	0.01
	Oral DHS for calves	0: No	Referent	—	—	—	—	0.03
		1: Yes	0.73	0.32	2.07	1.10; 3.91	—	
	Predominant breed	0: Swedish Holstein	Referent	—	—	—	—	0.007
		1: Swedish Red	−1.08	0.50	0.34	0.13; 0.91	0.03	
		2: Mixed breeds	−0.89	0.31	0.41	0.23; 0.75	0.004	
	Systemic tetracycline treatments of cows	Cases per 100 cow-years	0.07	0.03	1.07	1.02; 1.13	—	0.007
Sulfametoxazole	Intercept		0.02	0.25				
	Age of calf at sampling	Days	−0.64	0.01	0.94	0.91; 0.96	<0.001	
Chloramphenicol	Intercept		−1.46	0.72				
	Age of calf at sampling	Days	−0.06	0.03	0.94	0.89; 0.99	0.05	0.05
	Located in South Sweden	0: No	Referent	—	—	—	—	0.03
		1: Yes	−1.32	0.61	0.27	0.08; 0.88	0.03	
	Predominant breed	0: Swedish Red	Referent	—	—	—	—	0.03
		1: Swedish Holstein	−2.69	1.09	0.07	0.01; 0.58	0.01	
		2: Mixed breeds	−1.18	0.59	0.31	0.10; 0.98	0.05	
	Systemic tetracycline treatments of calves	0: No	Referent	—	—	—	—	0.01
		1: Yes	1.39	0.56	4.02	1.34; 12.11	0.01	

¹Robust standard errors (adjusted for clustering within herds).²Milk produced by a cow that was treated with antimicrobials during lactation was here denoted as waste milk.³Dihydrostreptomycin.

Figure 2. The mean within-sample prevalence and 95% CI (—) of streptomycin- and nalidixic acid-resistant *Escherichia coli* from feces of calves raised on farms that never fed calves milk from cows given lactation antimicrobial treatments (light gray bars), fed such milk during the withdrawal period only (medium gray bars), or during both the treatment and the withdrawal period (dark gray bars). Different letters indicate a significant difference ($P < 0.05$) in the mean within-sample prevalence for streptomycin-resistant or nalidixic acid-resistant *E. coli* isolates, respectively (results from univariable analysis).

that, by the time of parturition, the antimicrobials in colostrum either were eliminated or were present in such low concentrations that the susceptibility of *E. coli* in the gastrointestinal tract of preweaned calves was unaffected. Therefore, ingestion of WC or WT was probably not important for the shedding of resistant *E. coli*. To our knowledge, this is the first study to investigate the effect of feeding WC and WT on the shedding of resistant *E. coli* in feces of preweaned calves.

Calves from farms that fed WM to calves were more likely to shed Sm^r or Nal^r *E. coli* than calves on farms that discarded such milk, which is in agreement with previous studies (Wray et al., 1990; Aust et al., 2013; Xu et al., 2013). In Sweden, the bulk of WM is from cows treated with benzylpenicillin (Växa Sverige, 2012). Bearing in mind that *E. coli* is intrinsically resistant to benzylpenicillin and that this drug is, to some extent, hydrolyzed in the abomasum of the calves (Giguère et al., 2013), we hypothesized that milk containing benzylpenicillin residues would have a negligible effect on the susceptibility of fecal *E. coli*. It is therefore unlikely that the increased shedding of Nal^r or Sm^r *E. coli* observed in this study was directly caused by a selective pressure exerted by benzylpenicillin residues in milk. It could be the result of compositional changes in the gastrointestinal flora caused by this drug (Bridges et al., 1953; Dubos et al., 1963), but is more likely due to selective pressure from WM from cows treated with broad-spectrum antimicrobials (e.g., fluoroquinolones). It can be assumed that farms that fed WM from cows treated with benzylpenicillin also fed WM from cows treated with broad-spectrum antimicrobials, but in-

formation on specific strategies for the latter was not sought in this study. Nonetheless, the significant interaction between fluoroquinolone use in cows and feeding WM to calves indicates that the shedding of *E. coli* with certain resistance traits would be more abundant after feeding WM from cows treated with the same or a related antimicrobial.

On the other hand, the probability of finding *E. coli* resistant to other tested antimicrobials was not significantly affected by feeding WM to calves. Wray et al. (1990) and Aust et al. (2013) also observed that such feeding practices affected only some resistance traits and not others. This phenomenon may be because different mechanisms account for the selection of resistant traits or that different methods exist for susceptibility testing. By using selective media for Sm^r , Nal^r , and Ctx^r , the susceptibility of multiple isolates was tested simultaneously (Scott et al., 2011). This method was therefore more sensitive to subtle differences in the prevalence of resistance than methods based on susceptibility testing of only a single or few isolates. If selective media had been used for all tested antimicrobials in this study, feeding WM might have been significantly associated with other resistance traits.

We can assume that WM produced during ongoing treatment contains higher concentrations of antimicrobial residues than WM from the withdrawal period. Ingestion of VM from the treatment period would therefore be more likely to cause an increase in the prevalence of resistant *E. coli*. However, we found no significant difference in the shedding of Nal^r or Sm^r *E. coli* in calves on farms that fed WM during treatment compared with farms where VM was fed only during the withdrawal period. This indicates that low concentrations of antimicrobials are sufficient to exert a selective pressure on the gastrointestinal flora, as reported elsewhere (Gullberg et al., 2011).

The effect of feeding WM seems to be relatively subtle and was clearly not the main cause of shedding resistant *E. coli*. However, shedding of resistant *E. coli* persists longer among WM-fed calves (Brunton et al., 2014). Hence, this feeding practice is probably important in the maintenance of resistant bacteria in the intestinal flora. It is therefore recommended to minimize the feeding of VM to reduce the prevalence of resistant *E. coli* in the gut of calves.

The purpose was also to investigate whether antimicrobial use in preweaned dairy calves and cows was associated per se with increased shedding of resistant *E. coli*. Our results showed that therapy with some antimicrobials for both cows and calves was positively associated with the presence of some resistance phenotypes. Oral administration of DHS to calves increased the shedding of Sm^r , Nal^r , and Ct^r *E. coli*. Previous

studies have shown that oral DHS may select for *E. coli* resistant to multiple types of antimicrobials, probably because of limited absorption from the gastrointestinal tract (Gaines et al., 1978).

The use of both long- and short-acting intramammary dry cow antimicrobials increased the shedding of Sm^r *E. coli* in the calves. This result is likely because most of the products licensed for dry cow therapy in Sweden contain an aminoglycoside, often DHS (Växa Sverige, 2012). Likewise, Mollenkopf et al. (2010) observed that intramammary-administered dry cow antimicrobials influence the shedding of resistant *E. coli* from the treated cow. The environments of dairy farms probably favor the sharing of bacteria among animals; meaning that resistant bacteria shed by cows may propagate to other age categories on the farm, including calves. Aminoglycosides such as DHS are assumed to have a synergistic effect with benzylpenicillin, and a combination of the 2 would therefore be beneficial in the treatment of infections caused by bacteria with moderate susceptibility to both antimicrobials (Giguère et al., 2013). The synergistic effect may be of limited value, however, because many gram-negative bacteria causing intramammary infections have only moderate susceptibility to benzylpenicillin. Furthermore, β -lactamase-producing staphylococci are fully resistant to benzylpenicillin (Giguère et al., 2013). The value of adding aminoglycosides to intramammary tubes can therefore be questioned, and such combination preparations may not be justified due to their potential selection for resistant *E. coli*, as observed in this study. Unfortunately, long-acting antimicrobials without an aminoglycoside component are not yet available in Sweden (Medical Products Agency, 2014).

Systemically administered tetracycline was associated with higher odds for Tc^r, Cm^r, and Nal^r in a random *E. coli*. Most of the Cm^r and Nal^r *E. coli* isolates were also resistant to tetracycline (results not shown), and the link between use and resistance is therefore probably due to co-selection of resistance determinants. Likewise, almost two-thirds of the randomly selected *E. coli* isolates from calves having at least one Nal^r *E. coli* isolate were resistant to sulfamethoxazole (results not shown), which may also be indicative of co-selection. Treatment with ceftiofur, a third-generation cephalosporin, was rare in this study and is rare in Sweden in general (Växa Sverige, 2012). Despite this, treating cows and calves with ceftiofur had a significant effect on the prevalence of Am^r or Ctx^r *E. coli*, respectively. Third-generation cephalosporins are considered by the World Health Organization to be critically important drugs in human medicine (World Health Organization, 2012) and use of these drugs should therefore be restricted. The results of the current study indicate that

measures to reduce the prevalence of resistance among bacteria from calves should include prudent use of antimicrobials for all cattle on the farm, not only for calves.

Besides calf feeding with VM and use of antimicrobials, other risk factors were identified for the presence of resistant *E. coli* isolates. As observed in previous studies (Hoyle et al., 2004; Edrington et al., 2012; Watson et al., 2012), resistance in *E. coli* declined with increasing age of the calf. The housing of the calf was also important for some resistance phenotypes, a link reported earlier by Short et al. (2012). It is likely that group housing enhances the spread of resistant *E. coli*. Nevertheless, when some of the calves in the pen were older than 1 mo, the prevalence of resistant *E. coli* among calves was lower in the group pens than in the single pens. This may be due to a dilution effect, with shedding of more susceptible *E. coli* by older calves. Fewer calves carried Sm^r *E. coli* and in lower numbers during October to December compared with the rest of year. A similar seasonal variation was observed in a longitudinal study for the isolation of ESBL-producing *E. coli* from adult dairy cattle, where significantly fewer ESBL-producing *E. coli* were found during November than the rest of the year (Watson et al., 2012). Whether this difference is due to seasonal variations in temperature, humidity, or other factors or occurs solely by chance remains to be determined.

As observed by de Verdier et al. (2012), large herd size was a significant predictor for the presence of resistance to multiple types of antimicrobials. Large herds are more likely than small ones to have disease problems (Gulliksen et al., 2009; Hill et al., 2009), and hence have more frequent use of antimicrobial drugs. We assume that transmission pathways are more abundant and that there is increased movement of people and animals between and within large farms compared with small farms. Finally, calves from farms where cows were milked in a milking parlor or other milking facilities (e.g., a milking carousel) had more Tc^r and Am^r *E. coli* than calves from farms with tiestall milking or AMS. Here, the milking system is probably a surrogate for other important risk factors not elucidated in this study.

The epidemiology of antimicrobial resistance in calves is clearly multifactorial. Although some factors in this study were shown to increase the prevalence of resistant bacteria, it is likely that these factors only help to maintain resistant strains that have already colonized the gastrointestinal tract. Future studies should therefore focus on finding the source for resistant *E. coli* and to further address which host-specific and environmental factors are responsible for the colonization and persistence of these strains in the gastrointestinal tract of young calves.

The secondary objective of the study was to describe the prevalence of antimicrobial-resistant *E. coli* in feces from preweaned dairy calves in Sweden. Streptomycin-resistant *E. coli* seemed to be widespread, whereas the occurrence of Nal^r and Ctx^r *E. coli* was more farm-specific. Plasmid-mediated genes conferring streptomycin-resistance (*strA* and *strB*) are widely distributed in the environment, and the high prevalence of Sm^r *E. coli* may therefore be explained by frequent gene transfer events between bacteria of different origin (Sundin and Bender, 1996). On the other hand, the shedding of Nal^r and Ctx^r *E. coli* is more likely due to certain farm characteristics or routines. In addition, Nal^r *E. coli* were more commonly shed by calves on farms in South Sweden, which could be explained by transmission of resistant clones within certain areas of Sweden. Likewise, clonal spread of fluoroquinolones-resistant *E. coli* has been observed in calves in Italy (Marchese et al., 2012).

Genes encoding ESBL and pAmpC were detected in *E. coli* from calves in this study, which was the first time such genes were confirmed for *E. coli* obtained from Swedish cattle. In 2009, none of the 256 fattened calves (6–8 mo old) screened at slaughter in Sweden carried these genes (SVARM, 2009). It is uncertain whether this is due to the difference in age of the calves sampled or whether this finding reflects an emergence of ESBL-producing *E. coli* in cattle in Sweden and globally (Livermore and Woodford, 2006).

One potential risk with the present study design is that conclusions were drawn based on the susceptibility testing of samples obtained from no more than 3 calves per farm. By sampling only a fraction of the whole population, there is always a risk that it is not representative of the whole population. However, this design has been used previously to assess the importance of different risk factors for the presence of resistant *E. coli* in feces of calves (Di Labio et al., 2007; de Verdier et al., 2012). In addition, collecting samples from more than 3 calves would have significantly decreased the willingness of farmers to participate in the study.

CONCLUSIONS

Antimicrobial-resistant *E. coli* isolates were commonly isolated from feces of preweaned dairy calves in Sweden. Feeding WM, but not WC or WT, significantly increased the likelihood of shedding resistant *E. coli* by preweaned calves. The use of certain antimicrobials for calves and cows, as well as some farm and calf characteristics, were also associated with increased shedding of *E. coli* with specific resistance traits. The presence of resistance in *E. coli* isolates from calves is clearly due to multiple factors, but minimizing the feeding of milk

from cows treated with antimicrobials during lactation should lower the prevalence of resistant *E. coli* in feces from calves.

ACKNOWLEDGMENTS

The Swedish Farmers' Foundation for Agricultural Research (Stockholm, Sweden) is acknowledged for financing the study. Participating farmers are acknowledged for contributing samples and questionnaire data. Växa Sverige (Stockholm, Sweden) is acknowledged for providing data from national databases, as well as Stefan Börjesson (National Veterinary Institute, Uppsala, Sweden) for conducting the sequencing of ESBL genes.

REFERENCES

- Aust, V., K. Knappstein, H. J. Kunz, H. Kaspar, J. Wallmann, and M. Kaske. 2013. Feeding untreated and pasteurized waste milk and bulk milk to calves: Effects on calf performance, health status and antibiotic resistance of faecal bacteria. *J. Anim. Physiol. Anim. Nutr.* (Berl.) 97:1091–1103. <http://dx.doi.org/10.1111/jpn.12019>.
- Berge, A. C. B., E. R. Atwill, and W. M. Sischo. 2005. Animal and farm influences on the dynamics of antibiotic resistance in faecal *Escherichia coli* in young dairy calves. *Prev. Vet. Med.* 69:25–38. <http://dx.doi.org/10.1016/j.prevetmed.2005.01.013>.
- Berge, A. C. B., D. A. Moore, and W. M. Sischo. 2006. Field trial evaluating the influence of prophylactic and therapeutic antimicrobial administration on antimicrobial resistance of fecal *Escherichia coli* in dairy calves. *Appl. Environ. Microbiol.* 72:3872–3878. <http://dx.doi.org/10.1128/AEM.02239-05>.
- Bridges, J. H., I. A. Dyer, and J. J. Powers. 1953. Penicillin and streptomycin affect the microflora of the intestinal tract of pigs. *J. Anim. Sci.* 12:96–101.
- Brunton, L. A., D. Duncan, N. G. Coldham, L. C. Snow, and J. R. Jones. 2012. A survey of antimicrobial usage on dairy farms and waste milk feeding practices in England and Wales. *Vet. Rec.* 171:296–302. <http://dx.doi.org/10.1136/vr.100924>.
- Brunton, L. A., H. E. Reeves, L. C. Snow, and J. R. Jones. 2014. A longitudinal field trial assessing the impact of feeding waste milk containing antibiotic residues on the prevalence of ESBL-producing *Escherichia coli* in calves. *Prev. Vet. Med.* 117:403–412. <http://dx.doi.org/10.1016/j.prevetmed.2014.08.005>.
- Call, D. R., M. A. Davis, and A. A. Sawant. 2008. Antimicrobial resistance in beef and dairy cattle production. *Anim. Health Res. Rev.* 9:159–167. <http://dx.doi.org/10.1017/S1466252308001515>.
- CLSI. 2013. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard (VET01–A4). 4th ed. Clinical and Laboratory Standards Institute (CLSI), Wayne, PA.
- de Verdier, K., A. Nyman, C. Greko, and B. Bengtsson. 2012. Antimicrobial resistance and virulence factors in *Escherichia coli* from Swedish dairy calves. *Acta Vet. Scand.* 54:2. <http://dx.doi.org/10.1186/1751-0147-54-2>.
- DeFrancesco, K. A., R. N. Cobbold, D. H. Rice, T. E. Besser, and D. D. Hancock. 2004. Antimicrobial resistance of commensal *Escherichia coli* from dairy cattle associated with recent multi-resistant salmonellosis outbreaks. *Vet. Microbiol.* 98:55–61. <http://dx.doi.org/10.1016/j.vetmic.2003.10.017>.
- Di Labio, E., G. Regula, A. Steiner, R. Miserez, A. Thomann, and U. Ledergerber. 2007. Antimicrobial resistance in bacteria from Swiss veal calves at slaughter. *Zoonoses Public Health* 54:344–352. <http://dx.doi.org/10.1111/j.1863-2378.2007.01071.x>.
- Dohoo, I., W. Martin, and H. Stryhn. 2010. *Veterinary Epidemiologic Research*. 2nd ed. VER Inc., Charlottetown, Prince Edward Island, Canada.

- Donaldson, S. C., B. A. Straley, N. V. Hegde, A. A. Sawant, C. DeRoy, and B. M. Jayarao. 2006. Molecular epidemiology of ceftiofur-resistant *Escherichia coli* isolates from dairy calves. *Appl. Environ. Microbiol.* 72:3940–3948. <http://dx.doi.org/10.1128/AEM.02770-05>.
- Dubos, R., R. W. Schaedler, and M. Stephens. 1963. The effect of antibacterial drugs on the fecal flora of mice. *J. Exp. Med.* 117:231–243. <http://dx.doi.org/10.1084/jem.117.2.231>.
- Duse, A., K. P. Waller, U. Emanuelson, H. E. Unnerstad, Y. Persson, and B. Bengtsson. 2013. Farming practices in Sweden related to feeding milk and colostrum from cows treated with antimicrobials to dairy calves. *Acta Vet. Scand.* 55:49. <http://dx.doi.org/10.1186/1751-0147-55-49>.
- Edrington, T. S., R. L. Farrow, B. H. Carter, A. Islas, G. R. Hagevoort, T. R. Callaway, R. C. Andersson, and D. J. Nisbet. 2012. Age and diet effects on fecal populations and antibiotic resistance of a multi-drug resistant *Escherichia coli* in dairy calves. *Agric. Food Anal. Bacteriol.* 2:162–174.
- European Food Safety Authority. 2011. EU summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food 2011. EFSA J. 11:3196 10.2903/j.efsa.2013.3196.
- European Union. 2003. Regulation (EC) no 1059/2003 of the European parliament and of the council of 26 May 2003 on the establishment of a common classification of territorial units for statistics (NUTS). *Off. J. L* 154:1–41.
- Gaines, S. A., L. D. Rollins, R. P. Silver, and M. Washington. 1978. Effect of low concentrations of dihydrostreptomycin on drug resistance in enteric bacteria. *Antimicrob. Agents Chemother.* 14:252–256.
- Giguère, S., J. F. Prescott, and P. M. Dowling. 2013. Antimicrobial Therapy in Veterinary Medicine. 5th ed. Wiley Blackwell, Ames, IA. 10.1002/9781118675014
- Gullberg, E., S. Cao, O. G. Berg, C. Ilbäck, L. Sandegren, D. Hughes, and D. I. Andersson. 2011. Selection of resistant bacteria at very low antibiotic concentrations. *PLoS Pathog.* 7:e1002158 <http://dx.doi.org/10.1371/journal.ppat.1002158>.
- Gulliksen, S. M., E. Jor, K. I. Lie, I. S. Hammes, T. Løken, J. Akerstedt, and O. Osterås. 2009. Enteropathogens and risk factors for diarrhea in Norwegian dairy calves. *J. Dairy Sci.* 92:5057–5066. <http://dx.doi.org/10.3168/jds.2009-2080>.
- Hill, A. E., A. L. Green, B. A. Wagner, and D. A. Dargatz. 2009. Relationship between herd size and annual prevalence of and primary antimicrobial treatments for common diseases on dairy operations in the United States. *Prev. Vet. Med.* 88:264–277. <http://dx.doi.org/10.1016/j.prevetmed.2008.12.001>.
- Houe, H., A. K. Ersbøll, and N. Toft. 2004. Introduction to Veterinary Epidemiology. 1st ed. Biofolia, Frederiksberg, Denmark.
- Hoyle, D. V., D. J. Shaw, H. I. Knight, H. C. Davison, M. C. Pearce, J. C. Low, G. J. Gunn, and M. E. J. Woolhouse. 2004. Age-related decline in carriage of ampicillin-resistant *Escherichia coli* in young calves. *Appl. Environ. Microbiol.* 70:6927–6930. <http://dx.doi.org/10.1128/AEM.70.11.6927-6930.2004>.
- Khachatryan, A. R., D. D. Hancock, T. E. Besser, and D. R. Call. 2004. Role of calf-adapted *Escherichia coli* in maintenance of antimicrobial drug resistance in dairy calves. *Appl. Environ. Microbiol.* 70:752–757. <http://dx.doi.org/10.1128/AEM.70.2.752-757.2004>.
- Livermore, D. M., and N. Woodford. 2006. The beta-lactamase threat in *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter*. *Trends Microbiol.* 14:413–420. <http://dx.doi.org/10.1016/j.tim.2006.07.008>.
- Marchese, A., E. Coppo, R. Barbieri, S. Zoppi, C. Pruzzo, F. Rossi, S. Bergagna, A. Dondo, and E. Debbia. 2012. Characterization of fluoroquinolone-resistant *Escherichia coli* causing septicemic colibacillosis in calves in Italy: Emergence of a multiresistant O78 clonal group. *Microb. Drug Resist.* 18:94–99. <http://dx.doi.org/10.1089/mdr.2011.0053>.
- Medical Products Agency. 2014. Läkemedelsfakta—Utökad sökning. Accessed Aug 28, 2014. <http://www.lakemedelsverket.se/LMF/>.
- Miller, J. M., and J. W. Wright. 1982. Spot indole test: Evaluation of four reagents. *J. Clin. Microbiol.* 15:589.
- Mollenkopf, D. F., C. Glendening, T. E. Wittum, J. A. Funk, L. A. Tragesser, and P. S. Morley. 2010. Association of dry cow therapy with the antimicrobial susceptibility of fecal coliform bacteria in dairy cows. *Prev. Vet. Med.* 96:30–35. <http://dx.doi.org/10.1016/j.prevetmed.2010.05.014>.
- Mörk, M., A. Lindberg, S. Alenius, I. Vågsholm, and A. Egenvall. 2009. Comparison between dairy cow disease incidence in data registered by farmers and in data from a disease-recording system based on veterinary reporting. *Prev. Vet. Med.* 88:298–307. <http://dx.doi.org/10.1016/j.prevetmed.2008.12.005>.
- Mörk, M. J., C. Wolff, A. Lindberg, I. Vågsholm, and A. Egenvall. 2010. Validation of a national disease recording system for dairy cattle against veterinary practice records. *Prev. Vet. Med.* 93:183–192. <http://dx.doi.org/10.1016/j.prevetmed.2009.09.016>.
- Pereira, R. V., J. D. Siler, R. C. Bicalho, and L. D. Warnick. 2014. Multiresidue screening of milk withheld for sale at dairy farms in central New York State. *J. Dairy Sci.* <http://dx.doi.org/10.3168/jds.2013-7421>. 97:1513–1519.
- Pérez-Pérez, F. J., and N. D. Hanson. 2002. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. *J. Clin. Microbiol.* 40:2153–2162. <http://dx.doi.org/10.1128/JCM.40.6.2153-2162.2002>.
- Randall, L., K. Heinrich, R. Horton, L. Brunton, M. Sharman, V. Bailey-Horne, M. Sharma, I. McLaren, N. Coldham, C. Teale, and J. Jones. 2014. Detection of antibiotic residues and association of cefquinome residues with the occurrence of extended-spectrum β -lactamase (ESBL)-producing bacteria in waste milk samples from dairy farms in England and Wales in 2011. *Res. Vet. Sci.* 96:15–24. <http://dx.doi.org/10.1016/j.rvsc.2013.10.009>.
- Sato, K., P. C. Bartlett, and M. A. Saeed. 2005. Antimicrobial susceptibility of *Escherichia coli* isolates from dairy farms using organic versus conventional production methods. *J. Am. Vet. Med. Assoc.* 226:589–594. <http://dx.doi.org/10.2460/javma.2005.226.589>.
- Scott, H. M., B. Norby, and G. H. Loneragan. 2011. Antimicrobial resistance surveillance: Bacterial prevalence estimates are not enough. *Epidemiol. Sante Anim.* 59–60:182–184.
- Short, D., R. Pereira, W. Sischo, L. Warnick, L. Jones, J. Silver, and M. A. Davis. 2012. Impact of dairy calf housing on antimicrobial resistance. Accessed May 28, 2014. <http://extension.wsu.edu/ve-textension/MART/Documents/DavisCalfHousing.pdf>.
- Sørum, H., and M. Sunde. 2001. Resistance to antibiotics in the normal flora of animals. *Vet. Res.* 32:227–241. <http://dx.doi.org/10.1051/vetres:2001121>.
- Summers, A. O. 2002. Generally overlooked fundamentals of bacterial genetics and ecology. *Clin. Infect. Dis.* 34:S85–S92. <http://dx.doi.org/10.1086/340245>.
- Sundin, G. W., and C. L. Bender. 1996. Dissemination of the *strA-strB* streptomycin-resistance genes among commensal and pathogenic bacteria from humans, animals, and plants. *Mol. Ecol.* 5:133–143. <http://dx.doi.org/10.1111/j.1365-294X.1996.tb00299.x>.
- Sundsford, A., G. S. Simonsen, B. C. Haldorsen, H. Haasheim, S.-O. Hjelmenvoll, P. Littauer, and K. H. Dahl. 2004. Genetic methods for detection of antimicrobial resistance. *APMIS* 112:815–837. <http://dx.doi.org/10.1111/j.1600-0463.2004.apm11211-1208.x>.
- SVARM. 2006. Swedish Veterinary Antimicrobial Resistance Monitoring (SVARM) 2006. National Veterinary Institute (SVA), Uppsala, Sweden.
- SVARM. 2009. Swedish Veterinary Antimicrobial Resistance Monitoring (SVARM) 2009. National Veterinary Institute (SVA), Uppsala, Sweden.
- van den Bogaard, A. E., and E. E. Stobberingh. 2000. Epidemiology of resistance to antibiotics: Links between animals and humans. *Int. J. Antimicrob. Agents* 14:327–335. [http://dx.doi.org/10.1016/S0924-8579\(00\)00145-X](http://dx.doi.org/10.1016/S0924-8579(00)00145-X).
- Växa Sverige. 2012. Animal Health 2011/2012: Annual report from the animal health section. Växa Sverige, Stockholm, Sweden.
- Vieira, A. R., S. Wu, L. B. Jensen, A. Dalsgaard, H. Houe, H. C. Wegener, D. L. F. Wong, and H.-D. Emborg. 2008. Using data on resistance prevalence per sample in the surveillance of antimicrobial resistance. *J. Antimicrob. Chemother.* 62:535–538.

- Villarroel, A., P. S. Morley, T. E. Wittum, and D. S. Bolte. 2006. Use of a simulation model to evaluate sampling strategies for characterization of antimicrobial resistance in non-type-specific *Escherichia coli* isolated from dairy cows. *Am. J. Vet. Res.* 67:951–956. <http://dx.doi.org/10.2460/ajvr.67.6.951>.
- Watson, E., S. Jeckel, L. Snow, R. Stubbs, C. Teale, H. Wearing, R. Horton, M. Toszeghy, O. Tearne, J. Ellis-Iversen, and N. Coldham. 2012. Epidemiology of extended spectrum beta-lactamase *E. coli* (CTX-M-15) on a commercial dairy farm. *Vet. Microbiol.* 154:339–346. <http://dx.doi.org/10.1016/j.vetmic.2011.07.020>.
- Wernli, D., T. Hausteine, J. Conly, Y. Carmeli, I. Kickbusch, and S. Harbarth. 2011. A call for action: the application of the international health regulations to the global threat of antimicrobial resistance. *PLoS Med.* 8:e1001022 <http://dx.doi.org/10.1371/journal.pmed.1001022>.
- Woodford, N., E. J. Fagan, and M. J. Ellington. 2006. Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum β -lactamases. *J. Antimicrob. Chemother.* 57:154–155. <http://dx.doi.org/10.1093/jac/dki412>.
- World Health Organization. 2012. Critically Important Antimicrobials for Human Medicine. 3rd rev. World Health Organization (WHO), Geneva, Switzerland.
- Wray, C., S. Furniss, and C. L. Benham. 1990. Feeding antibiotic-contaminated waste milk to calves—effects on physical performance and antibiotic sensitivity of gut flora. *Br. Vet. J.* 146:80–87. [http://dx.doi.org/10.1016/0007-1935\(90\)90080-M](http://dx.doi.org/10.1016/0007-1935(90)90080-M).
- Xu, F., U. Rösler, A. Riese, M. Baumann, J. Zhao, H. Wei, X. Liu, and K. Kreusikon. 2013. First findings on the prevalence of Extended-Spectrum β -Lactamases producing *Escherichia coli* (ESBL-producing *E. coli*) and risk factors in dairy farms in Beijing area, China. Pages 83–87 in *Proc 10th Year Anniversary of Veterinary Public Health Centre for Asia Pacific*, Chiang Mai, Thailand.