



Longitudinal study on the prevalence of extended spectrum cephalosporins-resistant *Escherichia coli* colonization in Dutch veal farms

Teresita de Jesus Bello Gonzalez^{a,*}, Francesca Marcato^{b,e}, Eduardo de Freitas Costa^c, Henry van den Brand^b, Fleur A. Hoorweg^e, Maaïke Wolthuis-Fillerup^e, Bas Engel^d, Sabine K. Schnabel^d, Cornelis G. van Reenen^e, Michael S.M. Brouwer^a

^a Department of Bacteriology, Host-Pathogen Interaction, and Diagnostics Development, Wageningen Bioveterinary Research, 8221 RA Lelystad, the Netherlands

^b Adaptation Physiology Group, Wageningen University & Research, 6700 AH Wageningen, the Netherlands

^c Department of Epidemiology, Bio-informatics and Animal Models, Wageningen Bioveterinary Research, 8221 RA Lelystad, the Netherlands

^d Biometris, Wageningen University & Research, 6700 AA Wageningen, the Netherlands

^e Wageningen Livestock Research, Wageningen University & Research, 6700 AH Wageningen, the Netherlands

ARTICLE INFO

Keywords:

Veal calves
Antimicrobial use
Extended-spectrum cephalosporin resistance
Antimicrobial resistance

ABSTRACT

A longitudinal study was performed to investigate the prevalence of Extended-Spectrum Cephalosporin-Resistant (ESC-R) *Escherichia coli* colonization in Dutch veal farms. Rectal swabs from 683 calves born in 13 Dutch dairy farms were collected one day prior to transportation to the veal farm at 14 or 28 days of age, and at 5 different time points 8 Dutch veal farms. In addition, characteristics of the calf, cows, and farm management were collected. Rectal swabs were selectively cultured for ESC-R *E. coli*. In total, 1202 ESC-R *E. coli* isolates were recovered. Overall, the prevalence of ESC-R *E. coli* increased from 24.4 % at one day prior to transportation to 57.3 % in week two after arrival of calves at the veal farm. No associations were found between the presence of ESC-R *E. coli* at the dairy or veal farm and age of transportation, sex and breed. The presence of ESC-R *E. coli* in week 6, 10, and 18 at the veal farm was positively associated with the presence of ESC-R *E. coli* in week 10, 18, and 24, respectively ($p < 0.05$). Individual antibiotic treatments applied before week 2 and 6 upon arrival to the veal farms tended to increase the ESC-R *E. coli* colonization frequency. Our results indicate that ESC-R *E. coli* colonization frequency substantially increases after arrival of calves on the veal farm. In addition to individual antibiotic treatments, it is considered likely that frequently applied batch antibiotic treatments are also implicated in the ESC-R *E. coli* colonization frequency.

1. Introduction

The role of *Escherichia coli* as commensal and pathogenic bacteria, and its association with the emergence and spread of antimicrobial resistance in humans, animals, and the environment, has become an important concern to the public health system across the world (European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC), 2018). In the livestock sector, the monitoring of antimicrobial use (AMU) is essential to control the exposure of humans and animals to resistant pathogens and, the transmission of resistant pathogens to humans and animals (Chang et al., 2015). Moreover, farm animals and their environment serve as a potential reservoir for antimicrobial-resistant bacteria, with potentially a high impact on the animal health status, livestock industries, and human

public health. Since 2009, a reduction of AMU in the Netherlands has been achieved as part of the Dutch government policy target. Likewise, the impact of this reduction on antimicrobial resistance (AMR) in food animals has been observed in several pathogenic bacteria, such as *Salmonella enterica*, *Campylobacter* spp., Shiga-toxin producing *Escherichia coli*, and in commensal bacteria, including *Escherichia coli*. The latter is being used as an indicator microorganism to explore AMR in Gram-negative bacteria in livestock (Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands (MARAN, 2021)). In the veal calf sector, a decrease in the AMU by 28.8 % has been observed since 2013, as reported by the Netherlands Veterinary Medicines Institute SdA (Veterinary Medicines Institute (SDa), 2020). Despite this reduction, still a high prevalence of AMR in white (38.1 %) and rose veal calves (19.4 %) is observed (MARAN, 2021), particularly for

* Corresponding author.

E-mail address: teresita.bellogonzalez@wur.nl (T.J.B. Gonzalez).

<https://doi.org/10.1016/j.vetmic.2022.109520>

Received 20 November 2021; Received in revised form 13 May 2022; Accepted 27 July 2022

Available online 28 July 2022

0378-1135/© 2022 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Extended-Spectrum Cephalosporin-Resistance (ESC-R) indicative of Extended Spectrum β -Lactamase (ESBLs) or Plasmid-mediated AmpC (pAmpC) production. This is particularly relevant during the rearing process of white veal calves, where antimicrobials are intensively used to treat animals at herd level (MARAN, 2021).

The presence of ESBL producing *Enterobacteriales* in farm animals has been increasingly detected in different countries since 2002 (Smet et al., 2010). Moreover, in 2013 a longitudinal study on the dynamics of ESBL/AmpC producing *E. coli* in three Dutch veal farms showed that the prevalence of ESBL/AmpC producing *E. coli* differed greatly between farms over time and was affected by the use of specific classes of antimicrobials. Particularly, significant changes were observed during the time the animals were housed in individual pens, suggesting that additional factors could play an important role in the dissemination of ESBL/AmpC producing *E. coli* across the farm (Hordijk et al., 2013). Recent evidence showed that 60.2 % of calves destined for veal production were colonized with multi-drug antibiotic-resistant bacteria, mainly *E. coli* (Gay et al., 2019), even before they arrive at the veal farm. Additionally, factors inherent to the calves, such as age of transportation to the veal farm, sex, breed, and characteristics of their dams such as parity; may play a role in the occurrence of antibiotic-resistant *E. coli* colonization in calves (Hille et al., 2014; Springer et al., 2019). These findings highlight the need for longitudinal studies to explore the prevalence of ESC-R *E. coli* colonization on veal farms, to estimate the transmission rate in the food production chain and the exposure of the human population to antibiotic-resistant bacteria (Depoorter et al., 2012; Hordijk et al., 2013; Mughini-Gras et al., 2019).

The present longitudinal study aimed to evaluate the prevalence of ESC-R *E. coli* colonization in white veal calves on the day before their transportation from the dairy farm of origin to the veal farm and during the rearing period in Dutch veal farms by cultivation approaches. Moreover, we also aimed to identify whether or not the cumulative effect of antibiotic use and colonization frequency could contribute to the ESC-R *E. coli* carriage at farm level by using statistical regression models.

2. Materials and methods

2.1. Experimental design

The experiment was carried out between March 2019 and May 2020 and was approved by the Central Committee on Animal Experiments (the Hague, the Netherlands; approval number 2017. D-0029). The

experimental design represented a matrix consisting of 13 Dutch dairy farms and 8 Dutch veal farms. A total of 683 calves born within the 13 Dutch dairy farms were assigned to one of two treatment groups based on their age at transportation (either 14 or 28 days). The actual age of calves transported at 14 days ranged between 14 and 22 days; whereas for the calves transported at 28 days ranged between 28 and 36 days of age. In both cases, the age category in both treatment groups was based on the days between their birth and the sampling moment one day prior to transportation from the dairy farm to the veal farm. Thus, for each dairy farm, calves born in the first two weeks from the start of the experiment left the dairy farm between 28 and 36 days of age and calves born in the subsequent two weeks left the dairy farm between 14 and 22 days of age. All calves born at all dairy farms within these timeframes were transported to the same veal farm on the same day (Fig. 1). On each transportation day, two transporters collected calves from the dairy farms on the same day (6 in the North and 7 dairy farms in the South of the Netherlands, respectively) and brought these directly to the same veal farm. This means that in a two-week period there were 4 deliveries from 13 dairy farms (2 deliveries in the first week with two trucks collecting calves from both the North and the South, and 2 deliveries performed by the same trucks in the second week). This design was repeated eight times, meaning that calves born at all dairy farms in a different timeframe were transported to 8 consecutive veal farms (Table 1s). At the veal farms (herd size = 1065 calves on average, only a part of the calves was included in the experiment), calves were individually housed in so-called “baby boxes” for the first three weeks after arrival at the veal farm, after which they were housed in group pens (5 or 6 calves per pen). The feeding scheme and the management of calves varied among veal farms, but all used a milk replacer and roughage from the same feed company. The calves included in the experiment were fully blended in and treated in accordance with the rest of the calves present at the veal farm. Additionally, veal farmers were unaware of the background and age of calves.

2.2. Data collection

2.2.1. Data on calf, cow, and farms characteristics

Calves' characteristics, including sex and breed, and parity of the dams of calves (parity levels: 1, 2, 3, 4–10), were recorded. Factors related to dairy farm management were also recorded, including the following information: (1) whether or not calves received colostrum from their dam; (2) housing location of calves (calf pen, igloo, group pen

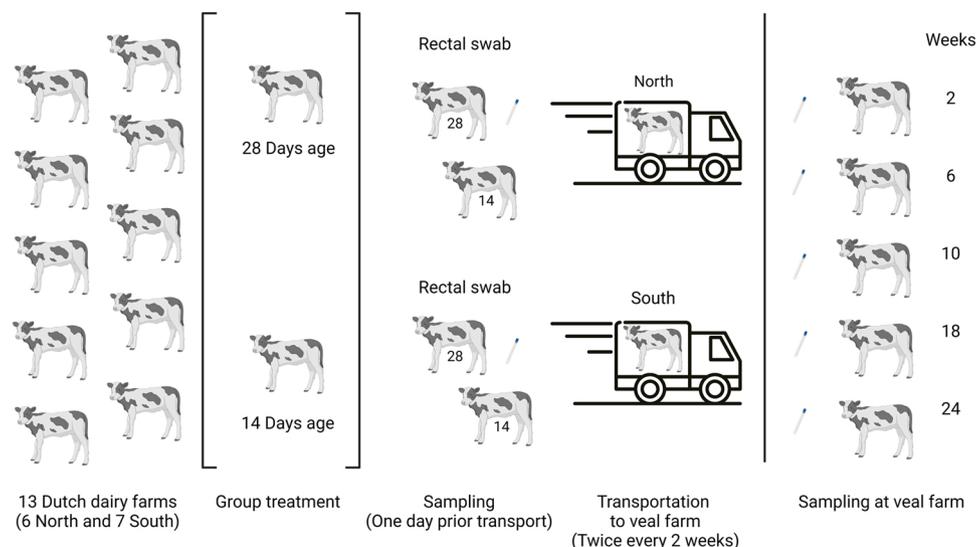


Fig. 1. Schematic representation of the experimental design. The timeframe was repeated eight times until veal farm 8 was completely filled with calves. In total this took a period of 34 consecutive weeks, which comprised almost an entire calving season.

(an average of 5 calves per pen); (3) hygiene practices after birth (disinfection or not of the navel of calves); (4) presence of other animal species on the dairy farms, since the presence of other animal species in the farm environment could also serve as a reservoir of antibiotic-resistant bacteria and contribute to their dissemination; (5) possible use of milk which may contain antibiotic residue provided to the calves; (6) management differences between the heifers for herd replacement and future veal calves.

2.2.2. Antibiotics and other medical treatments

The use of antibiotics and other medical treatments were recorded at an individual level, both at the dairy and veal farms. From this information, the frequency and class of antibiotics supplied to each animal was registered individually. For antibiotic treatments, a classification based on their mechanisms of action and chemical structure was created to group the antibiotics class into different categories: β -lactam (procaine benzylpenicillin, penicillin, ampicillin, amoxicillin), macrolides (tulathromycin, gamithromycin, tilmicosin), aminoglycoside (gentamicin, neomycin), cotrimoxazole (TMP sulfadiazine), amphenicol (florfenicol), tetracycline (doxycycline, oxytetracycline) and fluoroquinolone (flumequine).

Information on individual treatments included the following data: (1) whether or not the calf was treated with antibiotics or other medicines (this category included products, such as anti-inflammatories, multivitamins, anti-coccidiosis, with the exclusion of antibiotics); (2) single or repeated antibiotic/medical treatments; (3) age at which treatments were applied; (4) type of antibiotic or medication used. Herd treatments, were also recorded during the rearing period at the veal farm, including the type of antibiotics and other medicines (applied on all calves via the milk) and the age at which the herd treatments were applied.

2.3. Sample collection

A rectal swab (Becton Dickinson GmbH, Heidelberg, Germany) was collected from each calf at six different time points. The first sample was collected one day prior transportation to the veal farm. Consequently at the veal farm, samples were collected at 2, 6, 10, 18, and 24 weeks after arrival. (Marcato et al., 2021). Additional information regarding the health status of the calves was also retrieved during the sampling time points. The rectal swabs were labeled and linked to each animal's ear tag number and the time point of sampling. Samples were transported in a refrigerated box to the laboratory and processed within 24 h after collection for bacteriological analysis.

2.3.1. Bacterial isolation, culture, and identification of ESC-R *E. coli*

Swabs were placed into a tube containing 3 mL of buffered peptone water (BPW) (Becton Dickinson GmbH, Heidelberg, Germany) for bacterial enrichment and incubated at 37 °C for 24 h. The enriched solutions (10 μ l) were plated on a MacConkey agar plate with 1 mg/L cefotaxime and incubated at 44 °C for 24 h to isolate potential ESC-R *E. coli* strains. A single randomly picked colony from presumptive *E. coli* was streaked onto Heart Infusion Agar (HIS) (Becton Dickinson GmbH, Heidelberg, Germany) supplemented with 0.5 % sheep blood and incubated at 37 °C for 24 h for further identification by Matrix-Assisted Laser Desorption Ionization-Time of Light mass spectrometry (MALDI-TOF MS) (Bruker Daltonik, Germany).

2.4. Statistical analysis

Descriptive statistics were used to explore characteristics of the study population regarding the distribution of age, sex, and breed of calves. The antimicrobial use was described by the relative frequency of use in each dairy and veal farm, including the description of the antibiotic class.

The effect of time on the probability (frequency, prevalence) of ESC-

R. E. coli colonization was analysed with a generalized linear mixed model (McCulloch et al., 2008), employing a logit link function and a residual Bernoulli distribution. The linear predictor of the model comprised fixed effects for time points (6 levels: day prior to transportation, week 2, 6, 10, 18 and 24 after arrival of calves at the veal farm), age at transportation (two levels: 14 or 28 days), breed (three levels: Holstein Friesian, Holstein-Friesian x Belgian Blue, and other crossbreds), and sex (two levels: bulls and heifers). In addition, the linear predictor comprised random effects for veal calves, dairy farms, and veal farms; no interaction between dairy and veal farms was used due to convergence issues. All these effects were introduced on the logit scale. Random effects were assumed to be independently, normally distributed around 0 with respective variance components. Approximate maximum likelihood inference was based upon Laplacian integration, as implemented in R (R Core Team, 2018) in routine glmer from library lme4 (Bates et al., 2015).

Estimation of marginal probabilities of ESC-R *E. coli* colonization at the different time points was achieved by applying a shrinkage factor, which follows from integration over the random effects on the logit scale. Fixed effects were tested with the classic Wald test, employing an approximation by the chi-square distribution, as implemented in R routine Anova from the car library (Fox and Weisberg, 2019). This was followed up by pairwise comparisons made with a Tukey type HSD test, controlling for experiment wise error, as implemented in R routine emmeans from library emmeans (Lenth, 2016).

To assess the effect of presence or absence of ESC-R *E. coli* colonization at the dairy farm on the presence or absence of ESC-R *E. coli* colonization at the veal farm, five different generalized linear mixed models were implemented. In each model, the response was the presence or absence of ESC-R *E. coli* at a specific time point at the veal farm, while the observed presence or absence of ESC-R *E. coli* at the dairy farm was included as an explanatory variable. Other fixed effects were included for age at transportation, breed, and sex. Random effects were included for dairy farms, veal farms, and interaction between dairy farms and veal farms. The presence or absence of ESC-R *E. coli* at a specific time point at the veal farm was analysed with the presence or absence of ESC-R *E. coli* at the veal farm at the previous time point as an explanatory variable, next to the other fixed effects mentioned above. Random effects comprised effects for dairy and veal farms.

Similarly, presence or absence of ESC-R *E. coli* at a specific time point at the veal farm was analysed with the cumulative individual antibiotic treatment use until a particular time point as an explanatory variable. The random and fixed effects were the same as the ones included in the analyses are mentioned above.

3. Results

3.1. Calf and cow characteristics and dairy farm management

From the dairy farms, 54 % (n = 366) of the animals were transported to the veal farms at 14 days of age, and the remaining 46 % (n = 317) at 28 days of age. On average, 85 (range: 70–100) calves were transported to each veal farm. Out of 683 calves, 508 were males and 175 were females. In all dairy and veal farms, the frequency of female calves was approximately 25 %. With regard to breed, 37 % of the veal calves were Belgian Blue x Holstein Friesian crossbreds (BB), 30 % were Holstein Friesian (HF), 29.3 % were crossbreds other than Belgian Blue x Holstein Friesian (O), and 3.7 % non-informed. In general, the breeds were almost equally distributed across all veal farms. The average parity of the cows delivering calves for this study was 3.26, varying from 2.7 to 3.9 when considering the distribution between veal farms.

At the dairy farms, 96 % of the calves received colostrum from their own mother, while 4 % received colostrum from other cows (2.5 % from parity 2 or higher and 1.6 % from the first parity dam). The colostrum critical window was achieved for all the calves within the first \geq 4 h of life; 44 % of calves obtained colostrum directly from their dams by

sucking and the remaining 56 % were fed colostrum by the farm workers within 1–4 h after birth. Moreover, some farmers reported that the milk they provided to their surplus calves might have contained antibiotic residues. These originated from dairy farms 3, 7, 9, 10, and 11. Different locations were described for the housing of the calves, 41 % of calves were housed in a calf pen, 26 % were housed in an igloo (1 calf per igloo), 13 % stayed with their mothers in the calving pen, 14 % were housed in a calf pen during the first week after birth and then located in a group pen (5–6 per pen) and 6 % were housed first in a pen and then in an igloo. On average, 51.2 % of the calves were placed indoor, 44 % outside, and the remaining 4.8 % both indoor and outdoor at the dairy farm.

Regarding preventive health management practices, more than 70 % of the dairy farmers reported a healthy status of their farms, meaning free of bacterial and viral infections, the remaining 30 % (dairy farms 1, 6 and, 10) reported suspected cases of salmonellosis, Bovine Virus Diarrhea (BVD), and vaccination against infectious bovine rhinotracheitis (IBR). Overall, 67 % of the dairy farmers disinfected the navel of all calves after birth (time frame not specified) as a routine practice. In 42.8 % of the farms, farmers applied different managements between replacement heifers and future veal calves. Those differences were mainly related to the feeding of the animals (another type of roughage and or milk). In 20 % of the farms, no animal species other than calves and cows were present on the farm, whereas in 80 % of other farms, other animal species were present: companion animals 43 % (25 % dogs and 18 % cats), or animals and other domestic animals (57 %), including horses, sheep and/or goats.

Medical treatments, including anti-inflammatories, parasiticides, multivitamins; were provided to the animals during the rearing process. At the dairy farms, 11 % of the calves ($n = 78$) were treated with parasiticides, multivitamins, and anti-inflammatories, 62 of them (79.5 %) also received antibiotic treatments. The parasiticides were the most frequent medical treatments applied, particularly at dairy farm 7. At the veal farm, 24 % of the calves ($n = 165$) received anti-inflammatories agents, 154 of them (93 %) also received antibiotic treatments.

From two veal farms, samples were not collected at week 24 after arrival, due to a *Salmonella* outbreak amongst the animals (veal farm 2) and the outbreak of SARS2-CoV-2 (veal farm 8).

3.2. Frequency and distribution of ESC-R *E. coli*

In total, 1202 ESC-R *E. coli* isolates were recovered over time from the rectal swab samples. At the dairy farm level, we observed a high percentage of ESC-R *E. coli* (49 %) recovered in 7 farms, low percentage in 2 farms (3 %) and 4 farms with negative cultures (Fig. 1s). At the veal farm level, the highest frequency of ESC-R *E. coli* was observed in veal farms 6 and 7 over time compared to the other veal farms (Fig. 2s). The overall average percentage of ESC-R *E. coli* on the day prior to transportation was 24.4 % and it increased to 57.3 % two weeks after the arrival of calves at the veal farm (Fig. 2). The ESC-R *E. coli* colonization frequency was not affected by transportation age, breed, or sex of calves. A significant increase on ESC-R *E. coli* colonization was observed in the second week after transportation compared to all other sampling moments. Whereas in week 24, the lowest ESC-R *E. coli* colonization frequency was observed (Table 1).

3.3. ESC-R *E. coli* colonization and antibiotic use

3.3.1. Batch antibiotic treatments

Batch antibiotic treatments were recorded during the rearing period at the veal farm. The most common antibiotics administrated belong to the class of tetracyclines, including tetracycline and oxytetracycline. The second most frequent antibiotic administrated corresponded to macrolides, such as tilmicosin. Other antibiotics were administrated to particular farms: ampicillin was administrated in veal farm 3, cotrimoxazole in veal farm 5, and flumequine in veal farm 2 and 4. On

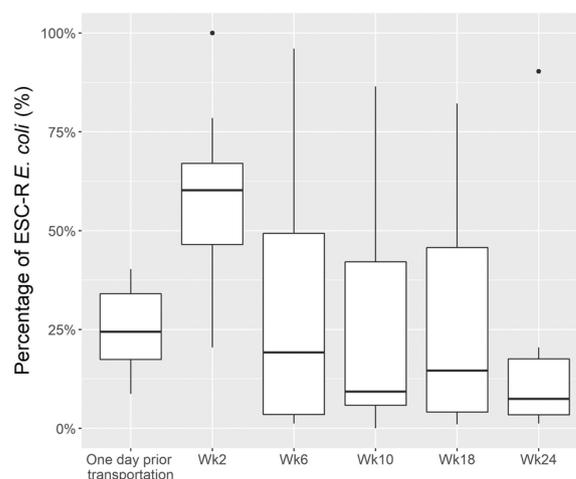


Fig. 2. Distribution of the percentage of ESC-R *E. coli* colonization detected on the day prior to transportation, and week 2, 6, 10, 18, and 24 after arrival of calves at the veal farm.

Table 1

Odds-ratio (95 % confidence intervals) and marginal ESC-R *E. coli* frequencies at different time points, ages at transportation from the dairy farm to the veal farms, breed and sex of the calves obtained by logistic regression. The “1” in each variable stands for the comparison category.

Variables	Odds-ratio (95% CI)	Overall p-value*	ESC-R <i>E. coli</i> Frequency**
Time point			<0.001
One day prior to transportation	1		25.68 % ^a
Week 2	4.28 (3.44; 5.33)		59.68 % ^b
Week 6	1.30 (1.03; 1.65)		31.07 % ^a
Week 10	1.11 (0.88; 1.40)		27.67 % ^a
Week 18	1.02 (0.81; 1.28)		26.01 % ^a
Week 24	0.57 (0.44; 0.75)		16.46 % ^c
Age at transportation			0.55
14-days	1		30.57 %
28-days	1.04 (0.91; 1.20)		37.92 %
Breed***			0.66
BB	1		30.01 %
HF	1.03 (0.86; 1.23)		30.52 %
O	1.13 (0.87; 1.48)		32.61 %
Sex			0.62
Male	1		31.47 %
Female	0.96 (0.81; 1.14)		30.57 %
Variance components†	VF(Calf): 0; DF:0.1; VF:1.45		

** Different letters mean statistical significance at 95 % confidence level.

***Breed: Belgian Blue x Holstein Friesian crossbreds (BB), Holstein Friesian (HF), crossbreds other than Belgian Blue x Holstein Friesian(O).

†DF: Dairy farm; VF: veal farm; VF(Calf):calf nested in a veal farm.

* The overall p-value was calculated by Wald test.

average over all the farms, batch antibiotic treatments were provided via the milk for 10 feedings over a period of 5 days. The majority of batch antibiotic treatments were applied within the first 6 weeks after arrival to the veal farms. The number of the batch antibiotic treatments applied per veal farm was high, on average 4.4 batch antibiotic treatments (range 3–5 treatments per veal farm).

3.4. Individual antibiotic treatments

The three main categories of antibiotics administered individually at the dairy farms included β -lactam, amphenicols, and cotrimoxazole. The average number of animals treated per dairy farm corresponded to 3 ± 1 Standard Deviation (SD). The combination of β -lactam / amphenicols was the most frequent antibiotic applied to the calves at the dairy and veal farms. The total number of calves treated individually with antibiotics at dairy and veal farms and the frequency of ESC-R *E. coli* is shown as [supplementary data \(Tables 2s and 3s\)](#).

The most frequent reason for the administration of these antibiotics at the veal farm was linked to diarrhea and respiratory diseases. In veal farm 5, a total of 33 calves were treated for at least 8 weeks due to respiratory disease. In this farm, we detected a decrease in ESC-R *E. coli* isolates from week 2 (61.3 %) to week 6 (7.3 %), where an increase of ESC-R *Klebsiella pneumoniae* isolates was detected (80 %) ([Bello Gonzalez et al., 2022](#)).

3.5. Prevalence of ESC-R *E. coli* colonization over time

3.5.1. Effect of presence or absence of ESC-R *E. coli* at the dairy farm on presence or absence of ESC-R *E. coli* at different time points at veal farms

The presence or absence of ESC-R *E. coli* observed at the dairy farm did not affect the status of the ESC-R *E. coli* at the veal farm at any time point ([Table 2](#)). The variance components were higher for the veal farms compared to dairy farms and the interaction of veal and dairy farms. Furthermore, the variance components for the veal farms decreased from week 10 until week 24 ([Table 2](#)).

3.5.2. Effect of the presence or absence of ESC-R *E. coli* at the previous time point on the presence or absence of ESC-R *E. coli* at specific time points at veal farms

The ESC-R *E. coli* presence in weeks 6, 10, and 18 was positively associated with the presence of ESC-R *E. coli* at veal farms at weeks 10, 18, and 24, respectively ([Table 3](#)). For these models the variance components for the veal farm decrease until week 18 vs week 24 and were always higher than the variance component for the dairy farm, which was zero for the model week 2 vs week 6 ([Table 3](#)).

3.5.3. Effect of the cumulative use of antibiotics per day until specific time point on the presence or absence of ESC-R *E. coli* at a specific time point at the veal farm

The effect of the cumulative antibiotics use (batch and individual antibiotic treatment) on the presence or absence of ESC-R *E. coli* at specific timepoints at the veal farms was tested. The small variation between veal farms in the number of batch antibiotic treatments administered excluded the possibility to perform an analysis on the relationship between ESC-R *E. coli* colonization frequency and the batch antibiotic treatments. For the individual antibiotic treatments, the cumulative use of individual antibiotic treatment per day seems to

Table 2

Odds-ratio (95 % confidence interval) and *p*-values of regressions regarding the effect of presence or absence of ESC-R *E. coli* at the dairy farm on presence or absence of ESC-R *E. coli* at different time points at the veal farm.

Time points	Odds-ratio (95% CI)	Overall <i>p</i> -value	Variance components*
Week 2	1.00 (0.82; 1.22)	0.98	DF:VF= 0.19; DF= 0.3; VF= 3.14
Week 6	0.88 (0.71; 1.1)	0.57	DF:VF= 0.4; DF= 0; VF= 6.8
Week 10	0.73 (0.59; 0.91)	0.18	DF:VF= 0.40; DF= 0.12; VF= 8.77
Week 18	0.75 (0.6; 0.92)	0.53	DF:VF= 0.9; DF= 0.20; VF= 6.4
Week 24	0.70 (0.53; 0.92)	0.78	DF:VF= 0.66; DF= 0; VF= 6.0

* DF: dairy farm; VF: veal farm.

Table 3

Odds-ratio (95 % confidence interval) and *p*-values of regressions regarding the effect of presence or absence of ESC-R *E. coli* at the previous time point on the presence or absence of ESC-R *E. coli* at specific time points at the veal farm.

Week comparison	Odds-ratio (95 % CI)	Overall <i>p</i> -value	Variance components*
Week 2 vs Week 6	0.91 (0.62; 1.36)	0.70	DF = 0; VF = 6.38
Week 6 vs Week 10	1.76 (1.13; 2.73)	0.011	DF = 0.24; VF = 6
Week 10 vs Week 18	3.18 (1.87; 5.38)	<0.001	DF = 0.21; VF = 3.93
Week 18 vs Week 24	3.49 (3.5; 11.8)	<0.001	DF = 0; VF = 2.9

**DF: dairy farm; VF: veal farm.

contribute to an increase of ESC-R *E. coli* colonization frequency before week 2 and before week 6 upon arrival to the veal farm, but not statistically significant at the 95 % confidence level ($p < 0.10$). A significant inverse relationship between ESC-R *E. coli* colonization frequency and individual antibiotic treatment was observed only in week 10 ($p < 0.05$) ([Table 4](#)).

4. Discussion

To our knowledge, this is the first large longitudinal study assessing the prevalence of ESC-R *E. coli* colonization in Dutch veal calves from the dairy farm of origin and throughout the rearing period. In this study, the overall prevalence of ESC-R *E. coli* at dairy farms varied between farms, resulting in farms with high prevalence (85 %), low prevalence (3 %) or completely negative. Comparable results were shown by [Gonggrijp et al. \(2016\)](#) with an estimated prevalence of 41 %. Moreover, differences have been also reported for organic dairy farms (13%) ([Santman-Berends et al., 2017](#)) compared to conventional dairy farms (41 %). A high prevalence of ESC-R *E. coli* when they are transported from the dairy farm to the veal farm may increase the likelihood for transmission of ESC-R *E. coli* on the veal farms. Similar to the dairy farms, and in line with a previous study ([Schmid et al., 2013](#)), the overall prevalence of ESC-R *E. coli* varied between veal farms; the highest prevalence (66 %) was found in veal farm 6 and 7 and the lowest prevalence (7.7 %) in veal farm 4. The prevalence of ESC-R *E. coli* colonization seems to fluctuate dynamically over time, and has increased in white veal calves in the Netherlands from 17.3 % in 2015 to 47.6 % in 2018 after which it decreased somewhat to 38.1% in 2020 ([MARAN, 2021](#)).

Since all animals included in our study were followed continuously during the rearing process, we were able to observe an overall significant increase in the frequency of ESC-R *E. coli* colonization two weeks after arrival on the veal farms (57.3 %) compared with one day prior to transportation (24.4 %) when calves were still at the dairy farm of origin. Similar to results previously obtained by [Hordijk et al. \(2013\)](#), in the current study the presence of ESC-R *E. coli* colonization decreased from week 6 until slaughter, except for veal farm 6 and 7, in which a constant high prevalence was observed over time. Our study showed that this colonization frequency was not affected by age of transportation, breed, or sex of calves, although an age-associated decrease in resistant *E. coli* colonization has been described previously ([Duse et al., 2015](#)). The effect of the factor age is strongly related to the gastrointestinal microbiota development, and other internal factors that could influence the adaptation to specific conditions at different ages ([Kha-chatryan et al., 2004](#)) and could explain why a decrease in colonization frequency after 6 weeks was observed. Another important factor that influences the frequency of resistant *E. coli* colonization is the development of the immune system. In that context, feeding colostrum provides essential nutrients and antibodies to the new born calf contributing to the passive immunity against infections ([McGuirk and Collins, 2004](#)). The majority of the calves included in our study received colostrum from the mother. However, the potential presence of antibiotic residues in the colostrum, even below the minimal selective

Table 4

Odds-ratio, ESC-R *E. coli* frequency, and p-values regarding the effect of the cumulative individual antibiotic treatments use until a specific time point on the presence or absence of ESC-R *E. coli* at a specific time point at the veal farm. The antimicrobial use refers to days use categorized in zero or larger than zero i.e., 0, ≥ 1 .

Time point (n)*	Odds-ratio (95 % CI)		ESC-R <i>E. coli</i> frequency (%)		Overall p-value	Variance component*
	0	≥ 1	0	≥ 1		
Day prior to transportation (654)	1	1.21 (0.82;1.81)	23.01	26.72	0.32	DF:8.24
Week 2 (658)	1	1.39 (0.95; 2.04)	61.13	68.70	0.08	DF:0.32 VF:3.17
Week 6 (574)	1	1.42 (0.94;2.12)	32.19	40.18	0.08	DF:0 VF:6.39
Week 10 (606)	1	0.67 (0.46;0.96)	29.72	22.01	0.02	DF:0.26 VF:8.13
Week 18 (646)	1	0.73 (0.52;1.05)	26.08	20.60	0.08	DF:0.29 VF:5.73
Week 24 (477)	1	0.79 (0.47;1.32)	20.61	16.99	0.36	DF:0.12 VF:5.54

VF: veal farm; DF: dairy farm; Time point (n): number of observations included in the model per time point.

concentration, could affect the likelihood for the selection of antibiotic resistance and contribute to the transmission and colonisation of calves, although this hypothesis needs to be confirmed. Recently, (Awoşile et al., 2017), identified multidrug resistant (MDR) ESC-R *E. coli* isolated from colostrum from dairy cows and highlighted the potential risk of colonization with MDR bacteria to the new-born during feeding.

Regarding the AMU in our study, β -lactam antibiotics were administered only as individual treatments and not as batch antibiotic treatments. However, ESC-R *E. coli* were even found on farms that did not use β -lactams (dairy farm 9 and 11), but these farms used other antibiotics or had animals that were not individually treated with antibiotics (dairy farm 4 and 13). Previous studies found that the use of non- β -lactam antibiotics can also select for ESBL resistance genes due to the presence of the resistance determinants to other antibiotics, such as tetracycline and aminoglycosides among others, in the same mobile element, most frequently plasmids (Jacoby and Sutton, 1991; Freitag et al., 2017). Consequently, the administration of individual antibiotic treatments could lead to a selective pressure to establish a pool of resistance genes in the bacterial population present in the animals as previously described (Berge et al., 2005). The genetic characterization of the ESC-R *E. coli* isolates obtained in our study is part of a follow-up study to identify the resistance genes, mobile elements and clonal spread relatedness, to reveal the epidemiological spread and transmissibility.

Furthermore, we identified a positive association for the presence of ESC-R *E. coli* in weeks 6, 10, 18 with the presence of ESC-R *E. coli* in weeks 10, 18, and 24, respectively at the veal farms. These significant positive associations emerged over time, suggesting that the colonization rate consistently persists in a specific subset of calves after six weeks. These results showed that, although the prevalence of ESC-R *E. coli* colonization in the majority of the veal farms decreased over time, the persistence effect in the resistant-*E. coli* colonization remains once the concentration of antibiotics is enough to exert the selective pressure (Gullberg et al., 2011). Possible association with other variables included in the model could not be established. We believe that additional information regarding the ecological (environmental) variables present on the farm, such as proximity of other agricultural areas, for instance pig farms (Santman-Berends et al., 2017), could provide insight into the large variability obtained between veal farms since the current model only includes animal-related variables.

In view of the high variability among dairy farms in ESC-R *E. coli* prevalence, we were interested to examine the underlying environmental and/or farm management factors involved. However, the small number of dairy farms in the current study excluded the possibility to reliably identify potential risk factors in terms of e.g. (early) rearing or management conditions on the prevalence of ESC-R *E. coli* at the dairy farm. Previous studies have reported associations between the occurrence of ESBL-producing bacteria on dairy farms and environmental factors such as the type of feeding (Kargar and Kanani, 2019), or the use

of waste milk (Brunton et al., 2012; Randall et al., 2014). Likewise, the presence of companion animals was shown to be associated with colonization and infections in dairy with extraintestinal pathogenic *E. coli* (ExPEC) carrying ESBL/AmpC genes (Bortolami et al., 2019).

Finally, we evaluated the cumulative effect of individual and batch antibiotic treatments at different time points on the presence or absence of ESC-R *E. coli* at the veal farm. Our results showed that the cumulative use of individual antibiotic treatments, seems to contribute to an increase in the ESC-R *E. coli* colonization frequency before week 2 and week 6 upon arrival at the veal farm. However, an inverse relationship was observed in week 10 and a tendency to that in week 18. This decline in the ESC-R *E. coli* colonization frequency could be associated with the impact of the antibiotic resistance fitness cost attributed to the high use of batch antibiotic treatments at the veal farms, the development of the bacterial niche and/or alteration in the ESC-R *E. coli* colonization frequency as previously reported (Björkman et al., 2000; Hoyle et al., 2004; Hordijk et al., 2013). Berge et al. (2006), showed that individual antibiotic treatments were most likely associated with an increase in transient resistant bacteria. Based on this observation, we hypothesize that in our study the initial administration of individual antibiotic treatments increases the transit resistant bacteria, particularly ESC-R *E. coli* related with the use of beta-lactam antibiotics. However, continuous administration of batch antibiotic treatments, which include other antibiotic classes, and the administration of other medicines over time, could lead to a continuous selection of resistant bacteria, in this particular case of ESC-R *E. coli*. Consequently, they could be not detected by a traditional cultivation approach. At this point, the use of any antibiotic can co-select for resistance to others, since the mobile elements can select for multidrug resistance and even genes associated with disinfectants, heavy metal tolerance, and virulence factors (Freitag et al., 2017). The high number of batch antibiotic treatments applied on the 8 veal farms involved in the current study, and the small variation between farms in the number of batch antibiotic treatments, excluded the possibility to quantitatively explore the relationship between ESC-R *E. coli* colonization and batch antibiotic treatments. We hypothesize that the administration of batch antibiotic treatments on the veal farms most likely contributed to the profound increase in ESC-R *E. coli* prevalence in week 2 after arrival to the veal farm in comparison with the day prior to transportation. It has been previously shown that feed-antibiotics (via the diet – batch treatments) were related to high levels of multidrug resistance (Berge et al., 2006). Follow-up research on a much larger number of veal farms, including farms with both high and low levels of batch antibiotic treatments use, as well as an intervention study in which the number of batch antibiotic treatments is controlled, could potentially remove the possible confounding effect and would substantiate our idea, and reveal the extent to which cumulative use of batch antibiotic treatments over time increase the ESC-R *E. coli* colonization frequency of veal calves.

5. Conclusion

Our study demonstrated that ESC-R *E. coli* colonization frequency increased after arrival of calves on the veal farm. ESC-R *E. coli* colonization was associated with the cumulative effect of the usage of individual antibiotic treatments, although batch treatment likely also played a role. The current findings highlight the importance of prudent use of antimicrobials in the livestock sector to prevent the development and selection of antimicrobial resistance.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was funded by Stichting Brancheorganisatie Kalversector, the Netherlands (SBK), ZuivelNL (the organization of the Dutch dairy sector), the Netherlands, and the Dutch Ministry of Agriculture, Nature and Food Quality, the Netherlands. Furthermore, funding was received from the European Union's Horizon 2020, Belgium, research and innovation programme through One Health EJP Project ARDIG [grant agreement number 773830] with co-funding from TKI bureau AgriFood, the Netherlands. The authors gratefully acknowledge Rimondia B.V. for providing personnel support and all dairy and veal farmers who contributed to this experiment. The authors are also very grateful to Gerald Lock, Harmen Kremers, Theo van Hattum, Henk Gunnink, Joop van der Werf, Laura Arango Carmona, Asri Wulansari, Amelia Florida Kiha, Hao Ye, Danielle Schillemans and Kelsey Veltmaat, for their help and assistance during the experiment and laboratory work. The authors would like to thank Dik Mevius and Kees Veldman for their scientific support.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.vetmic.2022.109520](https://doi.org/10.1016/j.vetmic.2022.109520).

References

- Awosile, B.B., McClure, J.T., Sanchez, J., VanLeeuwen, J., Rodriguez-Lecompte, J.C., Keefe, G., Heider, L.C., 2017. Short communication: extended-spectrum cephalosporin-resistant *Escherichia coli* in colostrum from New Brunswick, Canada, dairy cows harbor bla_{CMY-2} and bla_{TEM} resistance genes. *J. Dairy Sci.* 100. <https://doi.org/10.3168/jds.2017-12941>.
- Bates, D., Mächler, M., Bolker, B.M., Walker, S.C., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67. <https://doi.org/10.18637/jss.v067.i01>.
- Bello Gonzalez, T.D.J., Kant, A., Dijkstra, Q., Marcato, F., van Reenen, K., Veldman, K.T., Brouwer, M.S.M., 2022. Changes in Fecal Carriage of Extended-Spectrum β -Lactamase Producing Enterobacteriales in Dutch Veal Calves by Clonal Spread of *Klebsiella pneumoniae*. *Front. Microbiol.* 13, 866674 <https://doi.org/10.3389/fmicb.2022.866674>.
- Berge, A.C.B., Atwill, E.R., Sisco, W.M., 2005. Animal and farm influences on the dynamics of antibiotic resistance in faecal *Escherichia coli* in young dairy calves. *Prev. Vet. Med.* 69. <https://doi.org/10.1016/j.prevetmed.2005.01.013>.
- Berge, A.C.B., Moore, D.A., Sisco, W.M., 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. <https://doi.org/10.1128/AEM.02239-05>.
- Björkman, J., Nagaev, I., Berg, O.G., Hughes, D., Andersson, D.I., 2000. Effects of environment on compensatory mutations to ameliorate costs of antibiotic resistance. *Science* 287. <https://doi.org/10.1126/science.287.5457.1479>.
- Bortolami, A., Zendri, F., Maciuga, E.I., Wattret, A., Ellis, C., Schmidt, V., Pinchbeck, G., Timofte, D., 2019. Diversity, virulence, and clinical significance of extended-spectrum β -lactamase- and pAmpC-producing *Escherichia coli* from companion animals. *Front. Microbiol.* 10. <https://doi.org/10.3389/fmicb.2019.01260>.
- Brunton, L.A., Duncan, D., Coldham, N.G., Snow, L.C., Jones, J.R., 2012. A survey of antimicrobial usage on dairy farms and waste milk feeding practices in England and Wales. *Vet. Rec.* <https://doi.org/10.1136/vr.100924>.
- Chang, Q., Wang, W., Regev-Yochay, G., Lipsitch, M., Hanage, W.P., 2015. Antibiotics in agriculture and the risk to human health: how worried should we be? *Evolut. Appl.* 8. <https://doi.org/10.1111/eva.12185>.
- Depoorter, P., Persoons, D., Uyttendaele, M., Butaye, P., de Zutter, L., Dierick, K., Herman, L., Imberechts, H., van Huffel, X., Dewulf, J., 2012. Assessment of human exposure to 3rd generation cephalosporin resistant *E. coli* (CREC) through consumption of broiler meat in Belgium. *Int. J. Food Microbiol.* 159. <https://doi.org/10.1016/j.ijfoodmicro.2012.07.026>.
- Duse, A., Waller, K.P., Emanuelson, U., Unnerstad, H.E., Persson, Y., Bengtsson, B., 2015. Risk factors for antimicrobial resistance in fecal *Escherichia coli* from preweaned dairy calves. *J. Dairy Sci.* 98. <https://doi.org/10.3168/jds.2014-8432>.
- EFSA, E.C.D.C., 2018. Antimicrobial Resistance in Zoonotic Bacteria Still High in Humans, Animals and Food. (<https://www.efsa.europa.eu/en/press/news/180227>).
- Fox, J., Weisberg, S., 2019. An {R} Companion to Applied Regression, third ed., Sage, Thousand Oaks CA. (<https://socialsciences.mcmaster.ca/jfox/Books/Companion/>).
- Freitag, C., Michael, G.B., Kadlec, K., Hassel, M., Schwarz, S., 2017. Detection of plasmid-borne extended-spectrum β -lactamase (ESBL) genes in *Escherichia coli* isolates from bovine mastitis. *Vet. Microbiol.* 200. <https://doi.org/10.1016/j.vetmic.2016.08.010>.
- Gay, E., Bour, M., Cazeau, G., Jarrige, N., Martineau, C., Madec, J.Y., Haenni, M., 2019. Antimicrobial usages and antimicrobial resistance in commensal *Escherichia coli* from veal calves in France: evolution during the fattening process. *Front. Microbiol.* 10. <https://doi.org/10.3389/fmicb.2019.00792>.
- Gonggrijp, M.A., Santman-Berends, I.M.G.A., Heuvelink, A.E., Buter, G.J., van Schaik, G., Hage, J.J., Lam, T.J.G.M., 2016. Prevalence and risk factors for extended-spectrum β -lactamase- and AmpC-producing *Escherichia coli* in dairy farms. *J. Dairy Sci.* 99. <https://doi.org/10.3168/jds.2016-11334>.
- Gullberg, E., Cao, S., Berg, O.G., Ilbäck, C., Sandegren, L., Hughes, D., Andersson, D.I., 2011. Selection of resistant bacteria at very low antibiotic concentrations. *PLoS Pathog.* 7. <https://doi.org/10.1371/journal.ppat.1002158>.
- Hille, K., Fischer, J., Falgenhauer, L., Sharp, H., Brenner, G.M., Kadlec, K., Friese, A., Schwarz, S., Imirzalioglu, C., Kietzmann, M., von Münchhausen, C., Kreienbrock, L., 2014. On the occurrence of Extended-spectrum- and AmpC-beta-lactamase-producing *Escherichia coli* in livestock: results of selected European studies | Zum Vorkommen von Extended-Spektrum- und AmpC-Beta-Laktamase-produzierenden *Escherichia coli* in Nutztierbeständen. *Berl. Munch. Tierarztl. Wochenschr.* 127. <https://doi.org/10.2376/0005-9366-127-403>.
- Hordijk, J., Mevius, D.J., Kant, A., Bos, M.E.H., Graveland, H., Bosman, A.B., Hartskerk, C.M., Heederik, D.J.J., Wagenaar, J.A., 2013. Within-farm dynamics of ESBL/AmpC-producing *Escherichia coli* in veal calves: a longitudinal approach. *J. Antimicrob. Chemother.* 68. <https://doi.org/10.1093/jac/dkt219>.
- Hoyle, D. v, Shaw, D.J., Knight, H.I., Davison, H.C., Pearce, M.C., Low, C., Gunn, G.J., Woolhouse, M.E.J., 2004. Age-related decline in carriage of ampicillin-resistant *Escherichia coli* in young calves. *Appl. Environ. Microbiol.* 70. <https://doi.org/10.1128/AEM.70.11.6927-6930.2004>.
- Jacoby, G.A., Sutton, L., 1991. Properties of plasmids responsible for production of extended-spectrum β -lactamases. *Antimicrob. Agents Chemother.* 35. <https://doi.org/10.1128/AAC.35.1.164>.
- Kargar, S., Kanani, M., 2019. Reconstituted versus dry alfalfa hay in starter feed diets of Holstein dairy calves: effects on growth performance, nutrient digestibility, and metabolic indications of rumen development. *J. Dairy Sci.* 102. <https://doi.org/10.3168/jds.2018-15153>.
- Khachatryan, A.R., Hancock, D.D., Besser, T.E., Call, D.R., 2004. Role of calf-adapted *Escherichia coli* in maintenance of antimicrobial drug resistance in dairy calves. *Appl. Environ. Microbiol.* 70. <https://doi.org/10.1128/AEM.70.2.752-757.2004>.
- Lenth, R., 2016. Least-squares means: the R package lsmeans. *J. Stat. Softw.* v., 69. <https://doi.org/10.18637/jss.v069.i01>.
- Marcato, F., van den Brand, H., Kemp, B., Engel, B., Schnabel, S., Hoorweg, F.A., Wolthuis-Fillerup, M., van Reenen, K., 2021. Effects of transport age and calf and maternal characteristics on health and performance of veal calves. ("in press"). *J. Dairy Sci.* 2021.
- McCulloch, C.E., Searle, S.R., Neuhaus, J.M., 2008. Generalize, Linear, and Mixed Models, second ed., John Wiley.
- McGuirk, S.M., Collins, M., 2004. Managing the production, storage, and delivery of colostrum. *Vet. Clin. North Am. Food Anim. Pract.* <https://doi.org/10.1016/j.cvfa.2004.06.005>.
- Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands, 2021. (MARAN) in 2020. Lelystad: MARAN. (<https://www.wur.nl/nl/show/Nethmap-MARAN-2021>).
- Mughini-Gras, L., Dorado-García, A., van Duijkeren, E., van den Bunt, G., Dierick, C.M., Bonten, M.J.M., Bootsma, M.C.J., Schmitt, H., Hald, T., Evers, E.G., de Koeijer, A., van Pelt, W., Franz, E., Mevius, D.J., Heederik, D.J.J., 2019. Attributable sources of community-acquired carriage of *Escherichia coli* containing β -lactam antibiotic resistance genes: a population-based modelling study. *Lancet Planet. Health* 3. [https://doi.org/10.1016/S2542-5196\(19\)30130-5](https://doi.org/10.1016/S2542-5196(19)30130-5).
- Randall, L., Heinrich, K., Horton, R., Brunton, L., Sharma, M., Bailey-Horne, V., Sharma, M., McLaren, I., Coldham, N., Teale, C., Jones, J., 2014. Detection of antibiotic residues and association of ceftiofur 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. <https://doi.org/10.1016/j.rvsc.2013.10.009>.
- R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Santman-Berends, I.M.G.A., Gonggrijp, M.A., Hage, J.J., Heuvelink, A.E., Velthuis, A., Lam, T.J.G.M., van Schaik, G., 2017. Prevalence and risk factors for extended-spectrum β -lactamase or AmpC-producing *Escherichia coli* in organic dairy herds in the Netherlands. *J. Dairy Sci.* 100. <https://doi.org/10.3168/jds.2016-11839>.

- Schmid, A., Hörmansdorfer, S., Messelhäusser, U., Käsbohrer, A., Sauter-Louis, C., Mansfeld, R., 2013. Prevalence of extended-spectrum β -lactamase-producing *Escherichia coli* on Bavarian dairy and beef cattle farms. *Appl. Environ. Microbiol.* 79. <https://doi.org/10.1128/AEM.00204-13>.
- Smet, A., Martel, A., Persoons, D., Dewulf, J., Heyndrickx, M., Herman, L., Haesebrouck, F., Butaye, P., 2010. Broad-spectrum β -lactamases among Enterobacteriaceae of animal origin: molecular aspects, mobility and impact on public health. *FEMS Microbiol. Rev.* <https://doi.org/10.1111/j.1574-6976.2009.00198.x>.
- Springer, H.R., Denagamage, T.N., Fenton, G.D., Haley, B.J., van Kessel, J.A.S., Hovingh, E.P., 2019. Antimicrobial resistance in fecal *Escherichia coli* and *Salmonella enterica* from dairy calves: a systematic review. *Foodborne Pathog. Dis.* <https://doi.org/10.1089/fpd.2018.2529>.
- Veterinary Medicines Institute (SDa), 2020. Usage of Antibiotics in Agricultural Livestock in the Netherlands in 2019, Utrecht. (<https://cdn.ipulse.nl/autoriteitdie-rgeenmiddelen/userfiles/Publications/sda-rapport-usage-of-antibiotics-in-agricultural-livestock-in-2019-corr-fig5b.pdf>).