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Effect of heifer-raising practices on *E. coli* antimicrobial resistance and *Salmonella* prevalence in heifer raisers

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SUMMARY

Although cattle movement and commingling play an important role in the inter-herd transmission of pathogens, little is known about the effect of commingling of heifers at raising operations. The objective of this study was to compare the resistance of *E. coli* and prevalence of *Salmonella* from pooled faecal pats of heifers raised off-farm at multi-source raisers (MULTI) that raised heifers from at least two farms compared with on-farm raisers (HOME), with heifers from only that farm. MULTI faecal pat samples were collected from pens with animals that had arrived at the farm within the previous 2 months (AP) and from animals that would be departing the heifer raiser in 2–3 months (DP). Corresponding age sampling was conducted at HOME raisers. Odds of ampicillin resistance were 3·0 times greater in *E. coli* collected from MULTI compared to HOME raisers. *E. coli* from AP pens had significantly ($P < 0{\cdot}05$) higher odds of resistance to ampicillin, neomycin, streptomycin, and tetracycline compared to DP pens. *Salmonella* recovery was not significantly different between heifer-raising systems ($P = 0{\cdot}3$). Heifer-raising system did not have a major overall impact on selection of resistant *E. coli*, which was strongly affected by the age of the animals sampled.

Key words: Antimicrobial resistance, commingling, heifer raiser, *Salmonella*.

INTRODUCTION

According to the 2013 report by the Centers for Disease Control and Prevention (CDC) on foodborne disease outbreaks in the Unites States, non-typhoidal *Salmonella* was responsible for more than half of multistate outbreaks and was the most common cause of outbreak-related hospitalization [1]. Compared to susceptible strains, multidrug-resistant (MDR) *Salmonella* pose an increased threat to public health as observed in a 2011 multistate outbreak linked to ground beef involving 20 persons infected with *Salmonella* Typhimurium. The outbreak strain was resistant to several commonly prescribed antibiotics, which was thought to account for the increased risk of hospitalization and possible treatment failure in infected individuals [2].

In the CDC's first report on antibiotic resistance threats released in 2013, drug-resistant non-typhoidal

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Salmonella was labelled with a serious threat level, requiring prompt and sustained actions to ensure that the problem does not increase [3]. As described in the report, costs related to *Salmonella* are expected to be higher for resistant than for susceptible infections, because resistant infections are more severe, and patients are more likely to be hospitalized, and have treatment failure.

Cattle movement and commingling have been shown to have an important role in the inter-herd transmission of pathogens such as *Salmonella* [4]. A study by Adhikari *et al.* observed that the practice of raising heifers off-farm in situations where the heifers were commingled with cattle from other sources resulted in an 8·9 times higher risk for introduction of MDR *Salmonella* strains into the dairy herd ($P = 0{\cdot}001$) [5]. In this study, faecal samples were collected from the heifers after they returned to the home farm, and thus the effect of commingling of animals at the heifer raiser on the selection of MDR *Salmonella* was not directly evaluated.

Environmental survival of MDR *Salmonella* is a concern for the transmission of this pathogens in animals housed in the same environment [6]. In one study evaluating the associations between cattle-level factors and environmental samples with the isolation of *Salmonella* from dairy farms in the United States, water troughs were among the environmental locations that had a higher chance of having *Salmonella* isolated [7]. Sharing the same water trough may be an important source to increase the transmission of *Salmonella* between animals from different farms being commingled in a same pen. Commensal bacteria such as *Escherichia coli*, even if not pathogenic, can represent a hazard to animal and human health because they may serve as reservoirs for antimicrobial resistance, disseminating resistance to pathogenic bacteria through the exchange of resistance genes [8].

Currently there is a lack of information on commingling of heifers at heifer raisers as a risk factor for dissemination of MDR *Salmonella* and *E. coli*. Most studies have focused on heifers after returning to the home dairy farm and therefore lack important information that could be learned if sampling was performed at the raising facility itself. The objective of this study was to compare the resistance of *E. coli* and *Salmonella,* and the prevalence of *Salmonella* from fresh faecal pats of heifers raised off-farm at multi-source heifer raisers that raised heifers from at least two farms *vs.* on-farm with heifers from only that farm.

MATERIAL AND METHODS

Inclusion criteria for farms

Heifer raisers were eligible for inclusion in the study if they were either: (1) offsite multi-source heifer raisers (MULTI) that raised heifers from at least two farms, or (2) on-farm heifer raisers (HOME) that raised heifers from only that farm. A total of three MULTI raisers and three HOME raisers were enrolled in the study. Herds were selected from a convenience sample of commercial dairy farms within a 3-h radius of Cornell University (Ithaca, NY). All farmers answered a short questionnaire on heifer management practices on the farm.

Study design and sample collection

Each MULTI and HOME raiser was visited three times at 2- to 3-month intervals over a period of 6–9 months. This was a cross-sectional repetitive sampling study design. MULTI and HOME farms were matched based on number of heifers. The same numbers of pooled faecal samples collected from animals that had arrived at the farm within the previous 2 months (AP) and from animals that would be departing the heifer raiser in 2–3 months (DP) during the first visit were also collected during the following farm visits. Pooled faecal samples consisted of ~5 g faeces randomly collected from each of three freshly passed faecal pats from different corners of the pen floor. Sampling was conducted using the collection spoon provided in the cap of the Para-pak C & S vials (Meridian Bioscience Inc., USA). During each farm visit, half of the MULTI samples were collected from pens with AP animals and the other half from pens with DP animals. The average age of animals in pens sampled at MULTI raisers was used to select pens sampled at HOME raisers. Pooled faecal samples were collected from each age group (AP and DP), and a minimum of three pooled faecal pats and a maximum of 12 pooled faecal pats were collected from each pen. The number of pens sampled per age group per farm visit ranged from 1 to 4 pens. The estimated samples size and number of samples collected for this study was of 434 pooled faecal samples, collected from six farms during three visits ($\alpha = 0{\cdot}05$, S.D. = 0·1, power = 0·89).

Environmental samples were collected from pen floors using sterile drag swabs (four 4 × 4-inch gauze sponges saturated in double-strength skim milk (Becton Dickinson and Company, USA). During

each farm visit one environmental sample was collected from pens belonging to AP, and one environmental sample was collected from pens belonging to DP animals. Gauze sponges were pooled into one environmental sample per age group.

Bacterial isolation, culture, and identification

Each Para-pak vial containing the collected sample was streaked onto MacConkey agar plates and incubated overnight at 37 °C. Two distinct *E. coli* colonies were collected and frozen at −80 °C.

Standard bacteriological culture methods were used to isolate *Salmonella* from faecal pat samples and environmental samples. Environmental drag swabs and a swab from each faecal pat sample vial were enriched in tetrathionate broth (Difco, USA) containing iodine solution; the mixture was incubated at 42 °C for 18–24 h. After incubation, the sample-broth mixture was streaked onto Brilliant Green agar with novobiocin (Northeast Laboratory, USA) and xylose lysine tergitol 4 (XLT-4) selective media, and both plates were incubated at 37 °C for 18–24 h. Red colonies (lactose non-fermenting bacteria) on Brilliant Green agar with novobiocin and black colonies (hydrogen sulfide-producing bacteria) on XLT-4 were inoculated into Kligler iron agar slants and incubated at 37 °C for 18–24 h. XLT-4 plates without suspected colonies were reincubated at 37 °C for an additional 18–24 h before checking again for characteristic black colonies. If a Kligler iron agar slant exhibited the biochemical properties of *Salmonella*, the isolate was confirmed by slide agglutination using *Salmonella* O Antiserum Poly A-I & Vi (Becton Dickinson and Company, USA). Confirmed *Salmonella* isolates were stored in Luria–Bertani broth containing 20% glycerol at −80 °C. Additionally, select *Salmonella* isolate from each sample were sent to the National Veterinary Services Laboratories in Ames, Iowa for serotyping and confirmation of results from slide agglutination.

Antimicrobial susceptibility of *E. coli* and *Salmonella* isolates was tested using a modified National Antimicrobial Resistance Monitoring System (NARMS) panel of 12 antimicrobial drugs. Susceptibility testing test was performed using a Kirby–Bauer disk diffusion agar assay in accordance with the guidelines published by the Clinical and Laboratory Standards Institute (CLSI) and methodology previously described [9–11]. Internal quality control was performed by inclusion of *E. coli* ATCC 25922, previously determined to be pansusceptible, and a previously characterized in-house *E coli* isolate known to have the $bla_{\text{CMY-2}}$ gene and to be resistant to nine of the antimicrobial agents tested. Antimicrobial susceptibility for all isolates was assessed using the following panel: 10 μg ampicillin, 30 μg cefoxitin, 30 μg ceftiofur, 30 μg ceftriaxone, 30 μg chloramphenicol, 5 μg ciprofloxacin, 10 μg gentamicin, 30 μg nalidixic acid, 30 μg neomycin, 10 μg streptomycin, 30 μg tetracycline, and 23·75/1·25 μg trimethoprim-sulfamethoxazole. Results of the disk diffusion test for the internal quality control strains were within the anticipated standards. Susceptibility of the isolates to antimicrobial drugs was categorized as susceptible, intermediate, or resistant (SIR) by measuring the inhibition zone according to interpretive criteria and breakpoints established by CLSI guidelines [10].

Statistical analyses

Descriptive analysis of the SIR distribution of *E. coli* and *Salmonella* isolates by antimicrobial drug for each heifer-raising type was performed using PROC FREQ in SAS (SAS Institute Inc., USA). Descriptive analysis of *Salmonella*-positive sample distribution, *E. coli* resistance phenotypes, and the proportion of *E. coli* resistant to $\geqslant 3$ antimicrobial drugs was also performed using PROC FREQ. In this study, MDR was defined as having resistance to $\geqslant 3$ antimicrobial agents.

To evaluate the effects of heifer-raiser type and age group on the odds of resistant *E. coli* per pooled faecal sample for each of the 12 antimicrobial agents tested and on the odds of *Salmonella* recovery per pooled faecal pat, multivariable mixed logistic regression models were fitted to the data using the GLIMMIX procedure of SAS. The independent variables heifer-raiser type, age group, and their interaction were included in all models. A continuous variable with the number of animals per pen was also included in the model as a fixed effect. This continuous variable was dropped from all the models because it was not significant and had a minimal effect on the parameter estimates of the other variables. The effects of farm visit and pen where faecal pats were collected, which was nested within herd, were controlled for in the models as random effects. This statistical model was also used to evaluate the effects of heifer raiser and age group on the odds of MDR *E. coli*, where the only difference was that the dependent variable was the binary variable for classification of *E. coli* as resistant or not to $\geqslant 3$ antimicrobial drugs. The goodness of fit of the models was assessed with the Hosmer & Lemeshow

test using the LOGISTIC procedure of SAS. No model rejected the goodness-of-fit test. The COVTEST statement was used to test the variance of the random effect of pen nested within herds, and for all models the results indicated that this *G*-sided random effect should remain in the model.

E. coli antimicrobial drug susceptibility phenotypes (ADSPs) consisted of patterns of resistance to the antimicrobial drugs, including a pansusceptible phenotype describing isolates that were susceptible to the 12 antimicrobial agents screened for in this study. Diversity and richness of ADSPs at the pen level were estimated using version 9.1 of EstimateS software [12]. Richness was calculated using the Chao index, and diversity was estimated using the Shannon index [13]. Diversity is a measure that incorporates both the number of ADSPs in an assemblage and a measure of their relative abundance. Richness is only a measure of the total number of ADSPs in a sample, and therefore its value can be affected by sampling effort. Chao index and Shannon index were estimated by heifer-raiser type and age group at the pen level. *E. coli* ADSP similarities between pairs of pooled faecal pats were assessed using the Jaccard index, which compares the number of shared species to the total number of species in the combined assemblages [14]. Similarity indices were calculated at the pen level using EstimateS software [12]. The similarity index ranges from 0 to 1, with 0 indicating that isolates from faecal pats from a pair of pens did not share any ADSPs, and 1 indicating complete agreement of ADSPs between isolates from these two pens (all the ADSPs present in pen 1 were also present in faecal pen 2 and vice versa). The similarity indices are shown as a percentage in the results by multiplying the index by 100. The main objective of the use of these ecological measuring indices was to estimate and identify similarities between the ADSPs identified in sampled faecal pats at the pen level, comparing results within and between pens.

To determine if there was a statistical difference between *E. coli* ADSP richness, diversity, and similarity indices by heifer type and by age group, generalized linear models were fitted to the data using the GLM procedure of SAS. For richness and diversity indices, the independent variables were the heifer-raiser type, age group, and interactions. For the similarity index, the independent variable was either heifer-raiser type or age group. This was done because the similarity index is a result of the comparson of two pens, calculated in this study by using one of two different grouping factors: heifer-raiser type and age group. Two similarity datasets resulted from these two analysis. For both linear models, the effect of herd was controlled for as a random effect. Adjusted means for each index were obtained using the LSMEANS statement. For all statistical models and tests, variables were considered significant when a P value $<0{\cdot}05$ was observed.

RESULTS

Herd descriptive data

Most heifers arriving at MULTI raisers were aged between 3 and 5 months and remained on the farm until age 18–24 months. The number of animals per pen varied greatly within and between heifer raisers, ranging from 10 to 160 heifers per pen. The majority of heifers remained at MULTI raisers until 1–3 months prior to parturition. The approximate number of farms that sent heifers to the MULTI raisers A, B, and C was 14, 5, and 4, respectively. The approximate number of heifers at MULTI raisers A, B, and C was 3100, 1100, and 300, respectively. The approximate number of heifers at HOME raisers D, E, and F was 3000, 1361, and 200, respectively. The approximate age of heifers within AP pens where faecal pats were collected ranged from 3 to 6 months, while the approximate age of heifers within DP pens where faecal pats were collected ranged from 14 to 19 months. None of the MULTI farms quarantined animals arriving at the farm.

Heifer raisers were located in the following counties in central New York: Cayuga (herd E), Ontario (herd A), Seneca (herd C), Schuyler (herd F), Steuben (herd B), and Tompkins (herd D). Because farms where selected based on their proximity to Cornell University, the applicability of the results from this study to other regions must be carefully taken into consideration.

Farms participating in the study were asked questions about drug use on the farms. The most common antimicrobial drug used in MULTI farms was oxytetracycline for herds A and B, and tulathromycin for herd C. The most common antimicrobial drug used in HOME farms was oxytetracycline for herd E, and tulathromycin for herds D and F. None of the herds participating in the study had individual antimicrobial drug use records for heifers.

Antimicrobial resistance in *E. coli* isolates

A total of 1296 faecal pats were collected, resulting in a total of 432 pooled faecal samples. At the pen level, the odds of *E. coli* resistance were significantly greater

Table 1. *Effect of heifer-raiser type and age group on the odds of antimicrobial resistance in* E. coli *at the pen level while controlling for the random effect of pen (nested within herd) and farm visit*

Antimicrobial agent	%, Resistance MULTI* (429)	%, Resistance HOME† (429)	OR (95% CI) MULTI* *vs.* HOME†	*P* value	%, Resistance AP‡ (428)	%, Resistance DP§ (430)	OR (95% CI) AP‡ *vs.* DP§	*P* value
Ampicillin	11·0	6·0	3·0 (1–8)	**0·04**	13·0	4·0	3·0 (1–9)	**0·02**
Cefoxitin	2·0	3·0	2·0 (0·2–15)	0·6	4·0	1·0	1·0 (0·1–9)	0·9
Ceftiofur	1·0	0·5	2·0 (0·2–13)	0·5	1·0	0·5	2·0 (0·2–14)	0·5
Ceftriaxone	1·0	0·5	2·0 (0·5–13)	0·3	1·0	0·5	2·0 (0·5–13)	0·3
Chloramphenicol	4·0	1·0	4·0 (0·7–18)	0·1	1·0	0·5	2·0 (0·5–13)	0·3
Ciprofloxacin	0·2	0·2	1·0 (0·03–22)	0·9	4·0	1·0	1·0 (0·04–27)	0·9
Nalidixic acid	0·2	0·5	0·5 (0·04–5)	0·6	0·2	0·5	0·5 (0·2–22)	0·6
Neomycin	2·0	3·0	0·7 (0·1–4)	0·7	4·0	0·2	26·0 (2–272)	**0·007**
Streptomycin	12·0	9·0	1·0 (0·5–3)	0·6	17·0	4·0	4·0 (1–10)	**0·01**
Tetracycline	31·0	22·0	2·0 (0·7–4)	0·2	42·0	11·0	8·0 (3–19)	**<0·0001**
TMP	2·0	0·2	10·0 (0·9–115)	0·06	2·0	1·0	0·7 (0·08–7)	0·8

OR, Odds ratio; CI, confidence interval; TMP, trimethoprim-sulfamethoxazole.
* Off-farm multi-source heifer raisers (MULTI) that raised heifers from at least two farms. Number of isolates in parentheses.
† On-farm heifer raisers (HOME) with heifers from only that farm. Number of isolates in parentheses.
‡ AP: isolates from pooled faecal pats collected from pens with animals that had arrived at the farm within the previous 2 months.
§ DP: isolates from pooled faecal pats collected from pens with animals that would be departing the heifer raiser in 2–3 months.

in MULTI compared to HOME raisers for ampicillin only ($P = 0{\cdot}04$) (Table 1). Odds of *E. coli* resistance to ⩾3 antimicrobial drugs did not differ significantly by heifer-raiser type ($P = 0{\cdot}2$) (Table 2). Odds of *E. coli* resistance were significantly greater in AP pens compared to DP pens for ampicillin ($P = 0{\cdot}02$), neomycin ($P = 0{\cdot}007$), streptomycin ($P = 0{\cdot}01$), and tetracycline ($P < 0{\cdot}001$) (Table 1). Odds of *E. coli* resistance to ⩾3 antimicrobial drugs were significantly greater in AP pens compared to DP pens ($P = 0{\cdot}005$) (Table 2). No significant difference was observed between the interaction term of type of heifer raiser and type of pen for any of the antimicrobial agents tested.

Table 2. *Effect of heifer-raiser type and age group on the odds of* E. coli *resistance to ⩾3 antimicrobial drugs (MDR) at the pen level while controlling for the random effect of pen (nested within herd) and farm visit*

Factor	MDR % (*n*)	OR (95% CI)	*P* value
Heifer raiser			0·2
HOME* (*n* = 429)	5·6 (24)	0·4 (0·1–1·5)	
MULTI† (*n* = 429)	8·6 (37)	Ref. (n.a.)	
Age group			**0·005**
AP‡ (*n* = 428)	11·9 (51)	6·2 (1·7–22·7)	
DP§ (*n* = 430)	2·3 (10)	Ref. (n.a.)	

OR, Odds ratio; CI, confidence interval; n.a., not applicable.
* On-farm heifer raisers (HOME) with heifers from only that farm. Number of isolates in parentheses.
† Off-farm multi-source heifer raisers (MULTI) that raised heifers from at least 2 farms. Number of isolates in parentheses.
‡ AP: isolates from pooled faecal pats collected from pens with animals that had arrived at the farm within the previous 2 months.
§ DP: isolates from pooled faecal pats collected from pens with animals that would be departing the heifer raiser in 2–3 months.

Distribution of *E. coli* ADSPs

Of the 429 *E. coli* isolates from HOME pooled faecal pats, 75% were pansusceptible and 5·6% were MDR. Of the 429 isolates from MULTI pooled faecal pats, 64·3% were pansusceptible and 8·6% were MDR. The most common resistance phenotype observed in *E. coli* was tetracycline for both HOME (10·7%) and MULTI isolates (16·7%). The ranking of the most common antimicrobial resistance phenotypes for each heifer-raiser type by age group is shown in Table 3.

The mean Chao richness index for *E. coli* ADSP was significantly different between heifer-raiser types, with HOME isolates having a mean index of 3·7 [95% confidence interval (CI) 1·8–5·6] and MULTI isolates having a mean index of 7·7 (95% CI 5·6–10). The mean

Table 3. *Ranking of the most common* E. coli *antimicrobial resistance phenotypes (ARPs) for each heifer-raiser type by age group.*

Antimicrobial resistance phenotypes	HOME rank‡	MULTI rank§	HOME‡ %, *n*	MULTI§ %, *n*
AP* (n_{HOME} = 214 and n_{MULTI} = 214)				
TET	1	1	15·0 (32)	27·1 (6)
STR-TET	2	2	4·5 (10)	6·5 (28)
AMP-FOX-STR-TET	3	None	4·2 (9)	0·0 (0)
AMP-TET	9	3	0·9 (2)	3·7 (21)
Pansusceptible			63·5 (136)	43·4 (93)
DP† (n_{HOME} = 215 and n_{MULTI} = 215)				
TET	1	1	6·5 (14)	6·5 (14)
STR-TET	2	3	2·8 (6)	1·9 (4)
AMP	3	2	0·5 (1)	1·4 (3)
Pansusceptible			87·9 (189)	85·1 (183)

AMP, Ampicillin; FOX, cefoxitin; STR, streptomycin; TET, tetracycline.
* AP: isolates from pooled faecal pats collected from pens with animals that had arrived at the farm within the previous 2 months. Number of isolates (*n*) for each heifer-raiser type in parentheses.
† DP: isolates from pooled faecal pats collected from pens with animals that would be departing the heifer raiser in 2–3 months. Number of isolates (*n*) for each heifer-raiser type in parentheses.
‡ Ranking or percent of ARP for isolates from pooled faecal pats from on-farm heifer raisers (HOME) with heifers from only that farm.
§ Ranking or percent of ARP for isolates from pooled faecal pats from off-farm multi-source heifer raisers (MULTI) that raised heifers from at least two farms.

Shannon diversity index and the Jaccard similarity index were not significantly different between HOME and MULTI isolates (Table 4). The mean Shannon diversity index for *E. coli* ADSPs was significantly different between age groups, with an index of 1·1 (95% CI 0·8–1·4) for AP isolates and an index of 0·5 (95% CI 0·2–0·8) for DP isolates. The mean Chao richness index and the Jaccard similarity index were not significantly different between AP and DP isolates (Table 4).

Distribution of *Salmonella* serovars and their resistance phenotypes

Among the 434 faecal pat samples collected, 39 (9%) were positive for *Salmonella*. Of these, 31 *Salmonella* isolates were from HOME and eight *Salmonella* isolates were from MULTI farms. Most of the *Salmonella* isolated from faecal pats (36/39) belonged to serovar Cerro. Of the 36 environmental samples collected, five (14%) were positive for *Salmonella*. Of these, three *Salmonella* isolates were from HOME and two *Salmonella* isolates were from MULTI farms. All environmental *Salmonella* isolated during the study belonged to serovar Cerro. Faecal and environmental *Salmonella* serovar distribution by heifer-raiser type and age group is displayed in Table 5 and Supplementary Table S1. No significant association was observed between either heifer-raiser type ($P = 0·3$) or age group ($P = 1·0$) on the odds of having a *Salmonella*-positive culture from pooled faecal pat samples.

All *Salmonella* environmental isolates were pansusceptible to the drugs tested. A total of four *Salmonella* isolates from pooled faecal pats were resistant to at least one drug. Of these, two were Cerro isolates, with one being resistant to only tetracycline (isolated from a HOME raiser) while the other was resistant to ampicillin-cefoxitin-ceftiofur-chloramphenicol-streptomycin-tetracycline (isolated from a MULTI raiser). The remaining resistant *Salmonella* were serovar Dublin and had the same resistant phenotype as the MDR Cerro (both isolated from a MULTI raiser). One MDR Dublin and the only recovered MDR Cerro were isolated from the same MULTI raiser.

DISCUSSION

Antimicrobial resistance in *E. coli* isolates and drug susceptibility phenotypes

E. coli isolates from multi-source heifer raisers were more likely to be resistant to ampicillin than isolates from on-farm heifer raisers with heifers from only that farm (Table 1). No significant differences were observed between the two heifer-raiser types for the remaining drugs tested. Our hypothesis was that

Table 4. *Mean richness, diversity, and biotic similarity of* E. coli *antimicrobial drug susceptibility phenotypes at the pen-level by heifer raiser and age group. Values in parentheses are the 95% confidence intervals of the least squares mean*

	Raiser		Age group	
Description	HOME*	MULTI†	AP‡	DP§
Richness				
Chao index	**3·7 (1·8–5·6)**	**7·7 (5·6–10)**	6·1 (4·5–7·7)	5·4 (3·5–7·3)
Diversity				
Shannon index	0·6 (0·2–1·0)	1·0 (0·5–1·4)	**1·1 (0·8–1·4)**	**0·5 (0·2–0·8)**
Similarity				
Jaccard index	50% (36–62)	36% (23–49)	38% (24–51)	46% (31–60)

* On-farm heifer raisers (HOME) with heifers from only that farm.
† Off-farm multi-source heifer raisers (MULTI) that raised heifers from at least two farms.
‡ AP: solates from pooled faecal pats collected from pens with animals that had arrived at the farm within the previous 2 months.
§ DP: isolates from pooled faecal pats collected from pens with animals that would be departing the heifer raiser in 2–3 months.

commingling of heifers at MULTI raisers would provide a mechanism for transfer of MDR *E. coli* and *Salmonella* between animals from different farms (horizontal transmission). The overall lack of a significant difference in resistance of *E. coli* between MULTI and HOME raisers suggests that either commingling heifers from different farms in this region does not increase the prevalence and abundance of different resistant *E. coli* in heifer faecal pats, or that the environment is not a major source for dissemination of resistant *E. coli* in heifer raisers. Because MULTI raisers had a significantly higher mean richness index for *E. coli* ADSPs at the pen level, we cannot rule out that the introduction of animals from different farms of origin is not a potential source for the spread of resistance to different antimicrobial drugs (Table 4). However, the lack of a significant difference in the diversity index for *E. coli* between raiser types indicates that although there is a greater diversity of ADSPs in MULTI raisers, they are not present in high numbers. This suggests that the commingling of heifers from different farms may have a minor role in the dissemination of different resistance phenotypes in *E. coli*.

Independent of the heifer-raiser type, a significant difference in *E. coli* antimicrobial resistance was observed when comparing isolates from AP (age range 3–6 months) and DP (age range 14–19 months) faecal pats, with respect to ampicillin, neomycin, streptomycin, and tetracycline resistance. Similar findings were observed in a study by Khachatryan *et al.*, in which *E. coli* isolates from the faeces of heifers aged 3–6 months had a higher percentage of resistance compared to heifers aged $\geqslant$7 months for ampicillin (14·5% *vs.* 5·9%), tetracycline (35·7% *vs.* 17%), and streptomycin (26·4% *vs.* 10·9%) [15]. In addition to increased resistance to individual antimicrobial drugs, *E. coli* isolates from AP pens were more likely to be resistant to $\geqslant$3 antimicrobial drugs compared to isolates from DP pens (Table 2). The influence of age on antimicrobial resistance in cattle has been suggested to be a consequence of the undeveloped enteric microflora in younger animals, which could result in higher colonization by resistant bacteria. The assumption behind this thought is that as the indigenous microflora matures, there is an increase in the degree of protection against colonization by bacteria with a higher fitness cost, such as antimicrobial-resistant bacteria and pathogenic enteric bacteria, resulting in a decreased prevalence of resistant bacteria [16, 17]. This is supported by our results which showed a significantly higher diversity index for *E. coli* ADSPs from AP animals, which suggests younger heifers (AP) are less resistant to the establishment of different *E. coli* ADSPs within the gut microbiota (Table 4). This can further be confirmed by a significantly greater proportion of MDR *E. coli* in AP compared to DP animals, indicating lower microbiota resilience to invasion by bacteria with a probable higher fitness cost (Table 2).

Some additional factors that could contribute to a change in the gastrointestinal microbiota of calves and results in increased shedding of resistant *E. coli* includes stress from transportation and potential

Table 5. *Distribution of* Salmonella *serovars from faecal pats and environmental samples by heifer raiser and age group*

Serovar	No. (%)*
Heifer raiser	
HOME†	
Pooled faecal pats ($n = 31$)	
Liverpool	1 (3·2)
Cerro	30 (96·7)
Environment ($n = 3$)	
Cerro	3 (100)
MULTI‡	
Pooled faecal pats ($n = 8$)	
Dublin	2 (25)
Cerro	6 (75)
Environment ($n = 2$)	
Cerro	2 (100)
Age group	
AP§	
Pooled faecal pats ($n = 12$)	
Liverpool	1 (8·3)
Dublin	2 (16·6)
Cerro	9 (75)
Environment ($n = 2$)	
Cerro	2 (100)
DP‖	
Pooled faecal pats ($n = 27$)	
Cerro	27 (100)
Environment ($n = 3$)	
Cerro	3 (100)

* Number or percent of samples that cultured positive for *Salmonella* and that belonged to the referred serotype.
† On-farm heifer raisers (HOME) with heifers from only that farm.
‡ Off-farm multi-source heifer raisers (MULTI) that raised heifers from at least two farms.
§ AP: isolates from pooled faecal pats collected from pens with animals that had arrived at the farm within the previous 2 months.
‖ DP: Isolates from pooled faecal pats collected from pens with animals that would be departing the heifer raiser in 2–3 months.

changes in diet regimen. Stress from transportation of cattle, including handling of animals, and feed and water withholding, have been shown to result in the increased shedding of *E. coli* O157:H7 [18]. The cause for this has been thought to be related to changes in the microbial ecological dynamic in the gastrointestinal tract of cattle, favouring the growth and shedding of these bacterial populations [19]. Upon arrival at the heifer raiser, the introduction of calves into pens with unfamiliar animals could also result in stress to the animals, which could affect the shedding patterns of these animals [20]. Although all calves arriving at the heifer raiser were already weaned, another stress to the gastrointestinal microbiota could be a slight change in diet. In our study we compared animals arriving at multi-source heifer raisers with animals of a similar age at home farms, and many of the burdens resulting in stress to animals arriving at the heifer raisers were not experienced by calves in the home farms. Furthermore, because we did not observe any significant difference in the odds ratio of antimicrobial resistance between *E. coli* isolates from pooled faecal pats in AP of both MULTI and HOME raisers, the stresses mentioned above may not have played a major role in the higher prevalence of antimicrobial resistant *E. coli* in pooled faecal pats from AP compared to DP animals.

None of the farms sampled had a consistent record-keeping of individual antimicrobial drug use records for heifers, and the lack of this information is a limitation for the study. The most common justification was that antimicrobial drugs were infrequently used for this age group, and when it was used it was not recorded. The United States Department of Agriculture (USDA) National Health Monitoring System (NAHMS) last report on dairy heifer raisers (2012) presented data on antibiotic use in heifer raisers. The age of animals in the AP and DP pens, respectively, corresponded to animals referred to as weaned heifers and pregnant heifers in the report. For weaned heifers, the most common reason for treatment with drugs was respiratory disease, for which 11% of affected animals received an antibiotic treatment. According to the report, pregnant heifers were infrequently affected or treated for disease. The most common reason for treatment of pregnant heifers with drugs was respiratory disease, for which 1·2% of affected animals were treated with an antibiotic [21].

Higher resistance prevalence in younger animals has been observed in various studies that compared resistance between pre-weaned calves at different ages, and between calves and cows [22, 23]. Our findings indicate that when heifers raised at multi-source raisers return to the home farm, they pose a lower risk for the transmission of antimicrobial resistance on the home farm than when they went to the heifer raiser. Because of the significant correlation between young animals and a higher prevalence of resistance, housing calves and young heifers in a facility apart from the rest of the herd could perhaps decrease the hazard for dissemination of resistance on the home farm.

More studies are needed to further investigate and measure the impact that young cattle have on the spread of antimicrobial resistance to older animals on the farm.

Distribution of *Salmonella* serovars and their resistance phenotypes

Our study did not show an increase in faecal pats testing positive for *Salmonella* associated with commingling at multi-source heifer raising operations. In a study by Adhikari *et al.* conducted at 59 commercial dairy farms, history of off-farm heifer raising, including contract heifer raising with commingling of cattle from other farms, was significantly associated with the introduction of new MDR *Salmonella* strains on the farm [5]. The lack of an association between raising animals at multi-source heifer raisers and prevalence of MDR *Salmonella* in our study may be explained by the fact that in our study the prevalence of *Salmonella* was measured at the raiser prior to the return of heifers to the home farm. A study conducted by Edrington *et al.* at a single heifer-raiser facility observed findings similar to ours, where commingling of calves from multiple farms at a heifer feedlot did not serve as a major source of *Salmonella* transmission back to the dairy farm [24]. These authors suggested that 24-month-old heifers have a lower *Salmonella* prevalence than calves, and since this is the age when heifers are returning to the home farm, it is unlikely that they represent a major source of *Salmonella* when they return to the home dairy. Furthermore, they concluded that calves and cattle in the sick or fresh pens should be the primary concern regarding MDR *Salmonella*. In our study we also observed that younger heifers in AP pens had a higher prevalence of samples positive for non-Cerro *Salmonella* isolates compared to older heifers in DP pens. Another possible explanation for the observed low dissemination of *Salmonella* at MULTI raisers could be a low prevalence of *Salmonella* in the heifers that were sent to the heifer raiser participating in the study; if more animals shedding *Salmonella* were sent to the heifer raiser, a more noticeable spread of the pathogen to animals from other farms might have been observed.

Independent of heifer-raiser type or if samples were collected from faecal pats or the environment, Cerro was the most common *Salmonella* serovar. Similar findings have been observed by other studies performed at dairy herds in the northeastern Unites States [25, 26]. A recent study conducting a genomic characterization of *S.* Cerro from dairy cattle suggested that the increase in prevalence of *S.* Cerro is probably caused by a highly clonal subpopulation, which is characterized by unique genomic deletions that may indicate adaptation to specific ecological niches and possibly reduced virulence in some hosts [27]. The findings from this genomic study could also help explain why although *S.* Cerro is commonly isolated from cattle, its role in causing clinical disease in cattle remains uncertain [28].

In summary, heifer-raising system did not have a major overall impact on selection of resistant *E. coli*. Younger heifers recently arrived at the heifer raiser had a significantly higher prevalence of MDR *E. coli* and resistance to ampicillin, neomycin, streptomycin and tetracycline compared to older heifers soon to return to the home farm. Prevalence of non-Cerro *Salmonella* was low on faecal pat and environmental samples, and no significant effect of heifer-raiser type or age group was apparent. The most prevalent serovar for both faecal pat and environmental samples was *S.* Cerro.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0950268815000357.

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DECLARATION OF INTEREST

None.

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