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Antimicrobial susceptibility in *E. coli* and Pasteurellaceae at the beginning and at the end of the fattening process in veal calves: Comparing 'outdoor veal calf' and conventional operations

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ABSTRACT

Animal husbandry requires practical measures to limit antimicrobial resistance (AMR). Therefore, a novel management and housing concept for veal calf fattening was implemented on 19 intervention farms (IF) and evaluated regarding its effects on AMR in *Escherichia (E.) coli*, *Pasteurella (P.) multocida* and *Mannheimia (M.) haemolytica* in comparison with 19 conventional control farms (CF). Treatment intensity (−80%) and mortality (−50%) were significantly lower in IF than in CF, however, production parameters did not differ significantly between groups. Rectal and nasopharyngeal swabs were taken at the beginning and the end of the fattening period. Susceptibility testing by determination of the minimum inhibitory concentration was performed on 5420 isolates. The presence of AMR was described as prevalence of resistant isolates (%), by calculating the Antimicrobial Resistance Index (ARI: number of resistance of one isolate to single drugs/total number of drugs tested), by the occurrence of pansusceptible isolates (susceptible to all tested drugs, ARI=0), and by calculating the prevalence of multidrug (≥3) resistant isolates (MDR). Before slaughter, odds for carrying pansusceptible *E. coli* were higher in IF than in CF (+65%, p=0.022), whereas ARI was lower (−16%, p=0.003), and MDR isolates were less prevalent (−65%, p=0.001). For *P. multocida*, odds for carrying pansusceptible isolates were higher in IF before slaughter compared to CF (+990%, p=0.009). No differences between IF and CF were seen regarding the prevalence of pansuceptible *M. haemolytica*. These findings indicate that easy-to-implement measures to improve calf management can lead to a limitation of AMR in Swiss veal fattening farms.

1. Introduction

Impaired animal health and high antimicrobial resistance (AMR) rates are important concerns in the veal industry (Catry et al., 2005; Rérat et al., 2012; Schönecker et al., 2019). To control disease, antimicrobials are frequently administered as metaphylactic or therapeutic treatment (Dorado-García et al., 2016; Lava et al., 2016; Schnyder et al., 2019), which increases the risk of selection of bacteria resistant to antimicrobials (WHO, 2018). These resistant bacteria represent a growing threat for human and animal health. Measures that can demonstrably lead to a reduction not only of antimicrobial use (AMU) but also of AMR are therefore urgently needed in animal production systems.

Measuring the effect of interventions on susceptibility of indicator as well as clinically relevant bacteria in field studies is more challenging than assessing their effect on AMU or production parameters. This is, on one hand, due to the labor and cost intensive process of collecting and analyzing appropriate samples. On the other hand, evaluating and interpretating susceptibility test results to assess e.g. the effects of measures to improve management in veal calf operations can be done by several means, of which all have advantages and inconveniences. The choice of indicators of antimicrobial susceptibility may make answering certain questions impossible. For instance, reporting pansusceptibility describes the prevalence of fully susceptible isolates but does not detect any details of resistance, e.g. whether isolates show resistance to few vs many tested drugs. The Antimicrobial Resistance Index (ARI, i.e. the number of resistances of one isolate to tested drugs divided by the total

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number of tested drugs) reports precise numeric values (between 0.00 and 1.00) but does not differentiate between resistant isolates with relatively low and high minimum inhibitory concentrations (MIC) to a given drug (Hinton et al., 1985). Multidrug resistance (MDR) describes the occurrence of resistance to three or more antimicrobial classes, but cannot distinguish between pansusceptibility and resistance to less than three classes (Magiorakos et al., 2012).

A novel management and housing concept implemented in the frame of the intervention study 'outdoor veal calf' was shown to be associated with reduced AMU, reduced mortality, unchanged average daily weight gain, and increased animal welfare (Becker et al., 2020; Moser et al., 2020).

The present study aimed at describing the prevalence of resistant bacteria isolated from the rectum (*Escherichia (E.) coli*) and the nasopharynx (*Pasteurella (P.) multocida* and *Mannheimia (M.) haemolytica*) of veal calves, and the effect of the novel management and housing concept on the susceptibility of these three bacterial species. Fecal *E. coli* have repeatedly been shown to be a suitable indicator organism for AMR (Berge et al., 2006; EFSA and ECDC, 2019). Pasteurellaceae isolated from the calves' nasopharynx were selected to investigate AMR in bacteria which are commensals of the respiratory tract but can also act as pathogens (Amat et al., 2019), causing pneumonia, by far the leading indication for antimicrobial treatments in Swiss veal calves (Lava et al., 2016; Schnyder et al., 2019). We hypothesized that the novel 'outdoor veal calf' concept would be associated with lower AMR at the end of the fattening period. To test this hypothesis, AMR in bacteria isolated from calves fattened in the 'outdoor veal calf' system at the beginning and at the end of the fattening period was compared to AMR in calves from traditional farms by determining and comparing pansusceptibility, ARI and MDR.

2. Material and methods

2.1. Farm and animal characteristics

Data of the present prospective longitudinal study were collected within the framework of the 'outdoor veal calf' study where a novel management and housing system for veal calves was tested (Becker et al., 2020). In the present study, the factor of influence on the calves' bacterial community was considered to be the implementation of that system. Briefly, two populations of veal calves were fattened in two different systems in Swiss veal fattening farms: 900 calves were fattened on 19 intervention farms (IF) under standardized conditions of the novel 'outdoor veal calf' concept, including direct transportation of purchased calves by the owner in a private trailer from the birth farm (source dairy) to the fattening farm without contact with other animals, a quarantine period of at least three weeks in individual hutches for all calves (those born on the farm and purchased calves), vaccination against calf pneumonia (Rispoval intranasal®, Zoetis, Delémont, Switzerland), and finally fattening in groups not exceeding ten calves of similar weight in group hutches with permanent access to a roofed and straw-bedded outdoor pen. Material to implement the new system (hutches, feeding devices) was provided to the farmers for the duration of the study to ensure homogeneous implementation of the concept.

For comparison purposes, data on 1005 calves fattened in 19 control farms (CF) of similar size, situated in the same region as IF, and operating under a label certifying improved animal welfare and sustainability standards were collected in parallel. Standards included, among others, feeding with fresh milk, space per animal above legal standards and access to an unroofed outside pen (IP-SUISSE, 2019).

The purchase rate was high in both farm types (IF: 62.7% ± 11.1; CF: 70.8% ± 18.1, p=0.137) and calves originated from a comparable number of birth farms (per 10 purchased calves: IF: 2.3 ± 1.1; CF: 3.0 ± 1.9, p=0.189). Calves were purchased at a mean age of 33.0 ± 12.0 days (IF) and 30.1 ± 13.2 days (CF, p=0.311, Wilcoxon rank sum test) and slaughtered after a mean fattening duration of 121.2 ± 25.0 days (IF) and 116.0 ± 26.1 days (CF, p=0.603, Wilcoxon rank sum test). After leaving the fattening groups, all calves were directly brought to slaughter. Each farm (IF and CF) was visited and data as well as samples were collected once monthly during one year. Extensive data on animal and farm level were collected (including birth date and place, ear tag number, date of transport and distance to the fattening farm if applicable, sex, breed, signs of disease, antimicrobial treatments, feed and bedding consumption, date and place of slaughter, carcass weight and quality). Comparative analysis showed a 5.3-fold lower treatment incidence in IF compared to CF (5.9 ± 6.5 vs. 31.5 ± 27.4 days under antimicrobial treatment per animal and year; p<0.001, Wilcoxon rank sum test). Treatment incidence was calculated using a method published by the European Medicines Agency (EMA), which provides standardized defined daily doses for treatment per kilo live weight of calves for the commonly used antimicrobial drugs in Europe. The unit of the treatment incidence is days under treatment per animal-year (EMA, 2013, 2016). This method has been adopted by various countries and its compatibility with Swiss treatment recommendations has been shown (Becker and Meylan, 2021). Mortality was halved in IF (3.1% ± 2.3 vs. 6.3% ± 4.9 in CF, p=0.020, Wilcoxon rank sum test), whereas no difference was seen in average daily gain (1.29 ± 0.17 kg/day in IF vs. 1.35 ± 0.16 kg/day in CF, p=0.244, Wilcoxon rank sum test) (Becker et al., 2020). Furthermore, animal welfare, including signs of disease during the fattening period and signs of pneumonia assessed at slaughter on a subset of the calves, was significantly better in IF than in CF (Becker et al., 2020; Moser et al., 2020).

2.2. Nasopharyngeal and rectal swab sample collection

Nasopharyngeal and rectal swabs were taken from each calf at two different time points, regardless of the presence or absence of signs of respiratory disease. As far as possible, monthly farm visits were arranged in such a way that own and purchased calves were sampled shortly after birth or purchase, respectively (first sampling time, T1). At the end of the fattening period (second sampling time, T2), calves were sampled shortly before leaving the farm for slaughter. The sampling procedure of a single calf always included both rectal and nasopharyngeal swabbing. For practical reasons, not all enrolled calves could be sampled twice, due e.g. to sudden death, euthanasia, or early slaughter. For sampling, calves were restrained manually or by means of a headlock and halter. For *E. coli* isolation, a sterile swab (BD BBL CultureSwab, Becton Dickinson AG, Basel, Switzerland) was inserted into the rectum and immediately placed into transportation medium suitable for Enterobacteriaceae (DeltaSwab Cary Blair, deltalab, Barcelona, Spain). For isolation of *P. multocida* and *M. haemolytica*, one nostril was randomly chosen, cleaned and disinfected using gauze swabs (Provet AG/ Henry Schein Animal Health, Lyssach, Switzerland) soaked in 70% propylalcohol (F25-A Feinsprit 2% MEK, Alcosuisse AG, Bern, Switzerland). Sterile swabs (COPAN Italia SpA, Brescia, Italy) were inserted into one nostril to swab the nasopharyngeal epithelium, and samples were placed in liquid Amies transportation medium (Axonlab SwabAX, Axon Lab AG, Baden, Switzerland) (Catry et al., 2005; Schönecker et al., 2019). Samples were transported to the laboratory at room temperature on the day of collection and processed within 24 h. All procedures were approved by the competent Committee for Animal Welfare and Protection (authorization number BE 71/16).

2.3. Isolation and identification of E. coli and Pasteurellaceae

Rectal swabs were spread onto selective agar BROLAC (Thermo Fisher Scientific, Waltham, USA) for the isolation of *E. coli* and nasopharyngeal swabs onto Pasteurella selective agar (Thermo Scientific Oxoid, Reinach, Switzerland) for the isolation of Pasteurellaceae, and the plates were incubated at 37 °C for 24 h. One single colony per plate or species was selected randomly for species identification with Matrix Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF)

(Microflex LT, Bruker Daltonics GmbH, Bremen, Germany). Colonies identified as *E. coli, P. multocida* and *M. haemolytica* were purified on trypticase soy agar plates containing 5% sheep blood (TSA-SB; Becton, Dickinson and Company, New Jersey, USA) and incubated at 37 °C for 24 h. Identification was confirmed by a second MALDI-TOF and the isolates were frozen in 30% glycerol stocks at − 80 °C until further analyses.

2.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility was tested by determination of MIC of antimicrobials in cation-adjusted Mueller-Hinton broth using EUVSEC Sensititre® plates for *E. coli* (Thermo Fisher Scientific, Waltham, USA), and in cation-adjusted Mueller-Hinton broth supplemented with 5% of laked horse blood using BOPOF6 Sensititre® plates for Pasteurellaceae according the manufacturer's instructions (Thermo Fischer Scientific, Waltham, USA). Based on the MIC values, *E. coli* isolates were classified either as 'resistant' to the tested drug or not according to the European Clinical Breakpoints (CB) published by the Committee on Antimicrobial Susceptibility Testing (EUCAST, 2022) or, if none was available for a drug, following the recommendations of the Clinical and Laboratory Standards Institute (CLSI). Isolates of *P. multocida* and *M. haemolytica* were classified as resistant or not based on CB published by the CLSI (CLSI, 2021). Whenever the term 'AMR' is used regarding data of the present study, it refers to isolates classified as resistant. All tested antimicrobials and breakpoints are listed in detail in Tables 1 and 2 and supplementary material S2. The prevalence of AMR was calculated for T1 and T2 as the percentage of resistant isolates for each bacterial species and each antimicrobial drug separately.

For drugs for which no defined CB by CLSI or EUCAST were available, the MIC_{50} und MIC_{90} were determined. The $MIC_{50(90)}$ depicts the concentration of a drug at which at least 50% (90%) of the tested bacterial population are inhibited.

2.5. Data management

For each isolate of *E. coli*, *P. multocida* and *M. haemolytica*, ARI and MDR were determined. For ARI calculation, the number of drugs an isolate was resistant to was divided by the total number of drugs tested (Hinton et al., 1985; Catry et al., 2016). Thus, values of ARI ranged from 0 (susceptible to all tested drugs, hereafter also named 'pansusceptibility') to 1 (resistant to all tested drugs). Isolates were classified as MDR if resistance to at least one drug was observed in at least three different antimicrobial classes (Magiorakos et al., 2012; Sweeney et al., 2018).

2.6. Statistical analyses

Statistical analyses were performed using 'R' Version 3.5.1 (R Core Team; package lme4). The level of significance was set as α=0.05. The isolation rates between IF and CF were compared with the Pearson's chi-squared test. Mean intervals between T1 and T2 of IF and CF were compared using Wilcoxon rank-sum test. To analyze whether the prevalence of resistant bacteria differed between farm type (IF vs. CF) and swabbing time point (T1 vs T2), we assessed the number of resistances each isolate exhibited. Pansusceptible *E. coli* isolates (ARI =0) were frequent. This was taken into account by building a zero-inflated model, followed by a second model describing the extent of AMR, in case ARI> 0. Models were built as follows: first, we analyzed the effect of farm type (IF/CF), swabbing time point (T1/T2), and the interaction term between the two variables as explanatory variables on presence or

Table 1
Results of susceptibility testing for 3376 intestinal *Escherichia coli* isolates from Swiss veal calves. Antimicrobials tested, corresponding interpretive criteria and prevalence[a] (%) of resistant isolates from 38 veal farms at the beginning (T1, n=1794) and at the end (T2, n=1582) of the fattening period (in decreasing order of prevalence of resistant isolates).

Antimicrobial drug	Antimicrobial class	Test range (µg/ml)	Threshold value[b]	Time point (T) of swabbing	Range		Median	Mean	Prevalence of resistant isolates per time point	Overall prevalence of resistant isolates
					Min.	Max.				
Tetracycline	Tetracyclines	2–64	R ≥ 16	1	21.1	84.6	56.8	57.9	59.3	55.6
				2	0	86.4	50.0	49.1	51.4	
Sulfamethoxazole	Sulfonamides	8–1024	R ≥ 512	1	21.1	82.7	60.9	59.0	60.0	55.5
				2	3.6	94.4	49.2	47.9	50.5	
Ampicillin	Penicillins	1–64	R ≥ 16[c]	1	18.2	76.7	51.3	49.6	51.1	45.3
				2	3.6	92.6	34.6	36.0	38.9	
Trimethoprim	Diamino-pyrimidines	0.25–32	R ≥ 8[c]	1	4.0	56.5	24.9	26.6	28.5	26.0
				2	0	83.3	14.2	20.8	23.2	
Chloramphenicol	Phenicols	8–128	R ≥ 8[c]	1	2.0	50.0	22.9	24.3	25.3	23.3
				2	0	61.4	17.3	19.9	21.2	
Gentamicin	Aminoglycosides	0.5–32	R ≥ 4[c]	1	0	53.1	14.9	15.7	16.6	15.1
				2	0	87.0	3.3	11.6	13.4	
Nalidixic Acid	Fluroquinolones	4–128	R ≥ 32	1	0	28.2	8.5	10.5	10.9	8.3
				2	0	28.6	3.3	5.0	5.4	
Ciprofloxacin	Fluroquinolones	0.03–8	R ≥ 1[c]	1	0	21.8	3.1	5.1	5.4	3.4
				2	0	11.8	0	1.2	1.2	
Tigecycline	Glycylcyclines	0.25–8	R ≥ 1[c]	1	0	10.9	1.5	2.0	2.2	2.1
				2	0	10.5	0	1.8	2.1	
Cefotaxime	3rd generation cephalosporines	0.25–4	R ≥ 4[c]	1	0	10.0	0	0.8	0.7	0.6
				2	0	15.2	0	0.6	0.4	
Ceftazidime		0.5–8	R ≥ 8[c]	1	0	2.3	0	0.1	0.2	0.2
				2	0	3.0	0	0.2	0.2	
Meropenem	Carbapenems	0.06–16	R ≥ 4[c]	1	0	0	0	0	0	0
				2	0	0	0	0	0	
Colistin	Polymyxines	1–16	R ≥ 4[c]	1	0	0	0	0	0	0
				2	0	0	0	0	0	

[a] Values of range, median and mean of % of resistant isolates per farm as well as prevalences (in %) of resistant isolates over all farms are indicated for the 38 participating farms (19 intervention farms, IF, and 19 control farms, CF).

[b] Clinical and Laboratory Standards Institute (CLSI): Performance Standards for Antimicrobial Susceptibility Testing; 31st ed. M100 (ISBN 978-1-68440-104-8), Clinical and Laboratory Standards Institute, USA, 2021. R=resistant.

[c] The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 12.0, 2022. http://www.eucast.org

Table 2
Results of susceptibility testing for 1594 *Pasteurella multocida* isolates from the nasopharynx of Swiss veal calves. Antimicrobials tested, corresponding interpretive criteria and prevalence[1] (%) of resistant isolates from 38 veal farms at the beginning (T1, n=535) and at the end (T2, n=1059) of the fattening period (in decreasing order of prevalence of resistant isolates).

Antimicrobial drug	Corresponding antimicrobial class	Test range (µg/ml)	Clinical Break-point (CLSI)[2]	Time point of swabbing	Range		Median	Mean	Prevalence of resistant isolates per time point	Overall prevalence of resistant isolates
					Min.	Max.				
Oxytetracycline	Tetracyclines	0.5–8	R ≥ 8	1	0	100	77.7	63.0	67.3	71.9
				2	0	100	86.2	69.5	74.2	
Spectinomycin	Aminoglycosides	8–64	R ≥ 128[4]	1	0	100	60.0	49.0	55.1	57.1
				2	0	100	56.2	53.1	58.1	
Danofloxacin	Fluroquinolones	0.12–1	R ≥ 1	1	0	35.0	0	1.9	2.2	1.9
				2	0	25.9	0	1.4	1.8	
Penicillin	Penicillins	0.12–8	R ≥ 1	1	0	28.6	0	1.5	2.0	1.4
				2	0	11.5	0	0.8	1.1	
Tulathromycin	Macrolides	1–32	R ≥ 64	1	0	40.0	0	1.1	2.9	1.4
				2	0	22.2	0	0.6	0.6	
Florfenicol	Phenicols	0.25–8	R ≥ 8	1	0	0	0	0	0	0.1
				2	0	2.9	0	0.1	0.1	
Enrofloxacin	Fluroquinolones	0.12–2	R ≥ 2	1	0	0	0	0	0	0
				2	0	0	0	0	0	
Ceftiofur	3rd generation cephalosporines	0.25–8	R ≥ 8	1	0	0	0	0	0	0
				2	0	0	0	0	0	

See Table 1 for legends.

absence of pansusceptibilty (ARI =0vs. ARI >0). To this end, we specified presence and absence of resistances as dependent binary variable in a logistic regression model with the explanatory variables and their interaction term. Calf nested within farm was added as random effect term to account for dependence among calves of the same farm and repeated measuring of the same calves between time points. Results on the presence of pansusceptibility are presented as odds ratios for the odds to find pansusceptible isolates among the farm types or time points. Second, we modeled the effect of the explanatory variables for all *E. coli* isolates where ARI> 0 using a Poisson regression. Variables were specified as in the previous (zero-inflated) model and animal nested within farm was used as random effect term. Results are presented as ratios (percent difference) between the numbers of resistances.

Due to the rare occurrences of resistances in isolates of *P. multocida* and *M. haemolytica*, modeling of the number of resistances was not feasible. Therefore, for *P. multocida* and *M. haemolytica*, the presence of pansusceptibility was analyzed statistically (i.e. ARI=0 vs. ARI>0) using the same statistical approach as for *E. coli*.

To assess differences in the presence of MDR in *E. coli* between the two farm types and the two swabbing time points, we used MDR as dependent binary variable in a logistic regression, and farm type (IF/CF), swabbing time point (T1/T2), as well as the interaction term between the two variables as explanatory variables. To account for dependence of MDR between time points within farms, animal nested within farm was added as random effect. Results on the presence of MDR are presented as odds ratios for the odds to find MDR isolates among the farm types or time points. Because the prevalence of MDR among isolates of *P. multocida* and *M. haemolytica* was very low, the presence of MDR was not analyzed statistically. Because model outputs in R only provide the effects of variables for the respective reference level of each factor, we additionally extracted contrasts to obtain the effect of each variable for the non-reference levels. The resulting pairwise contrasts were adjusted for multiple comparisons using Tukey's method (Tukey, 1949).

3. Results

Of the 1905 calves enrolled in the 'outdoor veal calf' study, 1892 calves (99.3%) were swabbed. For each time point, calves were sampled at similar ages in both farm types (T1: IF 38.5 ± 5.6 days of age, CF 39.9 ± 7.2, p=0.512; T2: IF 136.3 ± 13.3, CF 130.0 ± 8.5, p=0.212), indicating a mean interval between T1 and T2 of 97.8 days and 90.1 days in IF and CF, respectively.

The results of susceptibility testing to different classes of antimicrobials are shown in Tables 1 and 2 and Tables S2 and S3.

The number of obtained swab samples and isolation rates (%) are shown for each organism separately, overall and stratified by time point and farm type in Table S1. Isolation rates of Pasteurellaceae were higher in CF than in IF, overall and at T1, as well as at T2 for *P. multocida* but not *M. haemolytica*.

Tables 1 and 2 as well as S2 show the results of susceptibility testing on drug level, the test range of each drug and the prevalence of resistant isolates for *E. coli*, *P. multocida* and *M. haemolytica*, respectively. For *E. coli*, prevalences of resistant isolates ranged from high (tetracycline 55.6%, sulfamethoxazole 55.5%) to zero (meropenem, colistin). A similar range was observed for *P. multocida*, with prevalences of resistant isolates over 70% for oxytetracycline (71.9%) and at or close to zero for florfenicol (0.1%) and ceftiofur (0%), respectively. The highest overall prevalence of resistant isolates in *M. haemolytica* was 16.7% for oxytetracycline while it was zero for tulathromycin and for ceftiofur. Results of MIC determination given as MIC_{50} und MIC_{90} for drugs without defined CB by EUCAST or CLSI are shown in Table S3. An increase in MIC_{50} over the fattening process (i.e. over time from T1 to T2) was observed for azithromycin (*E. coli*), as well as for clindamycin and tiamulin (*M. haemolytica*). The MIC_{50} and MIC_{90} values for the remaining 17 tested drugs did not change over time. The MIC_{90} values were higher than MIC_{50} values for 9 drugs. The $MIC_{50/90}$ were above the tested concentrations for several drugs and time points for *P. multocida* and *M. haemolytica*.

The odds to find pansusceptible *E. coli* isolates (ARI=0) did not differ significantly between farm types at T1 (29% higher odds in IF, p=0.244) but were significantly higher in IF than CF at T2 (65% higher odds, p=0.022; Tables 3 and 4, Fig. 1-A). Concomitantly, IF had significantly lower odds for pansusceptibility at T1 compared to T2 (34% lower odds, p<0.001), whereas the odds for pansusceptibility in CF did not differ significantly between time points (15% lower odds at T1 than at T2, p=0.117). In *E. coli* isolates with ARI> 0 (i.e. resistance to at least one of the thirteen tested drugs was present), ARI did not differ significantly between farm types at T1 (5% lower ARI in IF, p=0.320). In contrast, it differed at T2 as *E. coli* isolates showed a significantly lower ARI in IF than CF (−16%, p=0.003, Fig. 1-B). When ARI> 0, ARI values decreased in a more pronounced way between T1 and T2 in IF than in CF (IF: 23% lower ARI at T2 than at T1, p<0.001; CF: 9% lower ARI, p=0.004).

For *P. multocida* isolates, we found significantly higher odds for pansusceptibility in IF than CF at both time points (T1: 940% higher, p=0.012; T2: 990% higher, p=0.009). We found no significant

Table 3
Results of the mixed regression models on absence of resistance (i.e. pansusceptibility, ARI[a]=0) in *Escherichia (E.) coli*, *Pasteurella (P.) multocida* and *Mannheimia (M.) haemolytica* isolated from rectal and deep nasopharyngeal swab samples of 1892 calves in 38 veal farms in Switzerland. Additionally, for *E. coli,* the number of drugs the respective bacteria exhibits resistance to (if ARI>0), and multidrug resistance (MDR[b]) were analyzed. References are values for intervention farms (IF) at the beginning of the fattening period (T1[c]). Model estimates are presented as odds ratios with lower and upper levels of the 95% confidence intervals (CI, in brackets).

Bacterial species		Parameter	Odds ratio (Upper/lower 95% CI)	z value	p-value[d]
E. coli (n=3376 isolates)	Presence of pansusceptibility (mixed logistic regression)	Intercept	0.54 (0.40/−0.73)	-3.96	< 0.001
		T2	1.52 (1.23/1.87)	3.93	< 0.001
		CF[e]	0.78 (0.51/1.19)	-1.17	0.244
		Interaction T2/CF	0.78 (0.58/1.05)	-1.65	0.100
	Number of resistances (mixed poisson regression)	Intercept	3.58 (3.31/3.87)	31.76	< 0.001
		T2	0.81 (0.76/−0.87)	-5.69	< 0.001
		CF	1.06 (0.95/1.18)	1.00	0.320
		Interaction T2/CF	1.13 (1.03/1.24)	2.60	0.010
	MDR (mixed logistic regression)	Intercept	0.81 (0.58/1.13)	-1.22	0.221
		T2	0.41 (0.33/0.52)	-7.92	< 0.001
		CF	1.51 (0.94/2.41)	1.71	0.087
		Interaction T2/CF	1.88 (1.40/2.52)	4.21	< 0.001
P. multocida (n=1594)	Presence of pansusceptibility (mixed logistic regression)	Intercept	0.97 (0.27/3.55)	-0.04	0.967
		T2	0.60 (0.33/1.11)	-1.63	0.102
		CF	0.10 (0.02/0.60)	-2.50	0.012
		Interaction T2/CF	0.95 (0.44/2.06)	-0.12	0.903
M. haemolytica (n=450 isolates)	Presence of pansusceptibility (mixed logistic regression)	(Intercept)	9.01 (2.30/35.40)	3.15	0.002
		T2	1.54 (0.57/4.21)	0.85	0.397
		CF	0.99 (0.17/5.92)	-0.01	0.989
		Interaction T2/CF	0.88 (0.25/3.10)	-0.20	0.840

[a] Antimicrobial Resistance Index (ARI) = number of drugs an isolate exhibited resistance to divided by the total number of drugs tested (Hinton et al., 1985; Catry et al., 2016)
[b] Isolates were classified as multidrug resistant (MDR) if resistance to at least one drug was observed in at least three different antimicrobial classes (Magiorakos et al., 2012; Sweeney et al., 2018)
[c] T1: swab collection at the beginning of the fattening period, T2: swab collection at the end of the fattening period.
[d] p-values were estimated using the Satterthwaite method.
[e] CF: control farms.

difference in the odds for pansusceptibility between the two time points for IF (66% higher odds at T2, p=0.102), however, *P. multocida* isolates from CF had 74% higher odds for pansusceptibility at T2 compared to T1 (p=0.025, Fig. 1-D). No significant differences between farm types or time points were found for *M. haemolytica* (Fig. 1-E). On five farms, MDR *P. multocida* were isolated (2 IF, 3 CF), and on two farms MDR *M. haemolytica* were isolated (both CF). Thus, no inferential statistics were performed for MDR for these bacteria.

When calves were swabbed at the beginning of the fattening period (T1), the odds for MDR *E. coli* in IF were 34% lower than those for CF, but not significantly so (p=0.087; Table 4, Fig. 1-C). At the end of the fattening period (T2), the risk for MDR *E. coli* was significantly lower in IF than in CF (by 65%, p<0.001). The odds for MDR *E. coli* were significantly lower at T2 compared to T1 in both farm types. This effect was more pronounced in IF than in CF (IF: 142% lower odds, p<0.001, CF: 28% lower odds, p=0.013).

4. Discussion

Restructuring management and housing of veal calves according to the concept 'outdoor veal calf' had a quantifiable effect on AMR. At the end of the fattening period, calves fattened according to the novel concept carried bacteria with significantly reduced AMR in comparison to conventionally fattened calves.

Although a few other studies with similar numbers of swabs collected from calves are available (Alexander et al., 2013; Schönecker et al., 2019), most study reports provide data on considerably less isolates (Snyder et al., 2017; Timsit et al., 2017). The present study includes a high number of *E. coli* and Pasteurellaceae isolates from veal calves at the beginning and at the end of the fattening period, which allows for describing and comparing antimicrobial susceptibility between these two time points. In this study, AMR in *E. coli* and Pasteurellaceae were found to be widespread in Swiss veal calves and AMR rates to several commonly used drugs were alarmingly high.

This study design allowed for demonstrating lower AMR levels in IF compared to CF as well as a decrease in AMR values of *E. coli* during the fattening period in IF but not in CF. Given the significant interaction term for the different change in *E. coli* ARI over time between farm types, we conclude the stronger decrease in *E. coli* AMR over time in IF to be practically relevant. Similarly, the decrease in MDR over time was significantly stronger in IF than CF, as indicated by the significant interaction term. Our analysis did not yield a conclusive statement on an actual difference between farm types regarding the prevalence of pansusceptible *E. coli* isolates at T2 only. It is conceivable that farm type has a more direct or pronounced effect on the number of resistances than on the mere presence or absence of resistances. Previous studies reporting the prevalence of AMR of rectal *E. coli* in veal calves showed that AMR decreases slightly over the course of the fattening period in a consistent way and for almost all drugs tested (Hoyle et al., 2004; Catry et al., 2016; Gay et al., 2019). The present study confirmed this observation. It is possible that younger calves have a higher prevalence of AMR because they receive more antimicrobial treatments than older ones (Lava et al., 2016). The stronger decrease of resistant *E. coli* in IF compared to CF over time may be due to the observed lower AMU in IF (Becker et al., 2020). The IF management system is associated with treatment incidence, however, investigations of direct associations between antimicrobial use and AMR were deliberately not the focus of this study, as we primarily wanted to investigate the effects of the management system as a whole (i.e. on farm level) on bacterial susceptibility to antimicrobials. Correspondingly, associations of AMU with AMR on calf level regardless of the management system have also been investigated and will be reported elsewhere.

The observed lower levels of ARI and MDR at the end of the fattening period for *E. coli* and the more pronounced decrease in IF compared to CF underline that relatively simple modifications of animal husbandry can be associated with lower prevalence of resistant bacteria. This is

Table 4
Extracted contrasts from the analyses on the absence of resistance (i.e. pansusceptibility, ARI[a]=0) in *Escherichia (E.) coli*, *Pasteurella (P.) multocida* and *Mannheimia (M.) haemolytica* isolated from rectal and deep nasopharyngeal swab samples of 1892 calves in 38 veal farms in Switzerland. Additionally, for *E. coli*, contrasts were extracted for the number of drugs the respective bacteria exhibits resistance to (i.e. if ARI>0), as well as the presence of multi-drug resistance (MDR[b]). Contrasts are given for the comparison between farm types per time point (T1[c], T2) and between time points per farm type. Odds ratios and ratios are provided with lower and upper levels of the 95% confidence intervals (CI, in brackets).

		Contrast	**Odds ratio ± CI**	**z-value**	**p-value**[d]
		IF vs CF[e] (T1)	1.29 (0.84/1.98)	1.17	0.244
	Presence of pan-susceptibility	IF vs CF (T2)	1.65 (1.07/2.55)	2.28	0.022
		T1 vs T2 (IF)	0.66 (0.54/0.81)	-3.93	< 0.001
		T1 vs T2 (CF)	0.85 (0.69/1.04)	-1.57	0.117
		Contrast	**Ratio ± CI**	**z-value**	**p-value**
		IF vs CF (T1)	0.95 (0.85/1.06)	-1.00	0.320
***E. coli* (n=3376)**	**Number of resistances if ARI>0**	IF vs CF (T2)	0.84 (0.75/0.94)	-2.97	0.003
		T1 vs T2 (IF)	1.23 (1.15/1.32)	5.69	< 0.001
		T1 vs T2 (CF)	1.09 (1.03/1.15)	2.88	0.004
		Contrast	**Odds ratio ± CI**	**z-value**	**p-value**
		IF vs CF (T1)	0.66 (0.42/1.06)	-1.71	0.087
	Presence of MDR	IF vs CF (T2)	0.35 (0.22/0.57)	-4.25	< 0.001
		T1 vs T2 (IF)	2.42 (1.94/3.01)	7.92	< 0.001
		T1 vs T2 (CF)	1.28 (1.05/1.56)	2.49	0.013
		Contrast	**Odds ratio ± CI**	**t-value**	**p-value**
		IF vs CF (T1)	10.4 (1.66/64.9)	2.50	0.012
***P. multocida* (n=1594)**	**Presence of pan-susceptibility**	IF vs CF (T2)	10.9 (1.83/64.9)	2.63	0.009
		T1 vs T2 (IF)	1.66 (0.90/3.06)	1.63	0.102
		T1 vs T2 (CF)	1.74 (1.07/2.84)	2.24	0.025
		Contrast	**Odds ratio ± SE**	**t-value**	**p-value**
		IF vs CF (T1)	1.01 (0.17/6.07)	0.01	0.989
***M. haemolytica* (n=450)**	**Presence of pan-susceptibility**	IF vs CF (T2)	1.15 (0.23/5.75)	0.17	0.862
		T1 vs T2 (IF)	0.65 (0.24/1.77)	-0.85	0.397
		T1 vs T2 (CF)	0.74 (0.34/1.59)	-0.78	0.437

[a] Antimicrobial resistance Index (ARI) = number of drugs an isolate exhibited resistance to divided by the total number of drugs tested (Hinton et al., 1985; Catry et al., 2016)
[b] Isolates were classified as multidrug resistant (MDR) if resistance to at least one drug was observed in at least three different antimicrobial classes (Magiorakos et al., 2012; Sweeney et al., 2018)
[c] T1: swab collection at the beginning of the fattening period, T2: swab collection at the end of the fattening period.
[d] p-values were estimated using the Satterthwaite method.
[e] CF: control farms.

beneficial for the calves and for the farmers, who are also less exposed to resistant bacteria, as well as for the downstream supply chain. At slaughter, lower levels of resistant *E. coli* in the gut flora of the slaughtered animals may be associated with a lower risk of contamination of carcasses with resistant bacteria. Restructuring animal husbandry in such way may contribute to lessen the public health threat of AMR.

In Pasteurellaceae, the power was insufficient to detect changes in ARI between T1 and T2 due to the smaller number of isolates and the lower prevalence of resistant isolates. This effect was even stronger for *M. haemolytica* than for *P. multocida* due to the lower isolation rate. Also, besides a generally lower number of isolates, Pasteurellaceae are not permanently present in the body and may be less exposed to antimicrobial treatments than *E. coli*. At T1, more pansusceptible isolates of *P. multocida* were found in IF than in CF. This may be due to the different management regarding purchase (IF: direct sourcing of calves from regional farms without mixing calves from various farms, CF: sourcing calves from livestock dealers), as purchased calves on IF were in contact with fewer (potentially treated) calves before arrival on the fattening farm. Diseased and treated calves may have a higher likelihood for carrying resistant *P. multocida*. Also, sellers may refrain from offering diseased or treated calves in the frame of direct purchase in order to maintain a good working relationship with the buyer.

At the end of the fattening period, the prevalence of pansusceptible *P. multocida* was distinctly higher in IF than CF. The higher odds of finding pansusceptible isolated of *P. multocida* at T2 compared to T1 was not significant in IF (+66%, p=0.102), yet significant in CF (+74%, p=0.025). In practice, this differences are probably neither relevant in IF nor in CF and may be due to a much higher isolation rate in CF (Table S1, Fig. 1-D).

The higher prevalence of *P. multocida* in comparison to *M. haemolytica* in the nasopharynx of young fattening cattle was in line with other studies (Catry et al., 2005; Timsit et al., 2017). However, comparison of isolation rates with these studies must be interpreted with caution as different study designs were applied. Comparable AMR figures have been previously reported for *E. coli* in veal calves in Switzerland (tetracycline 41.2–66%, sulfamethoxazole 64–64.9%, ampicillin 38.7–54%) (Di Labio et al., 2007; ANRESIS, 2018; Schönecker et al., 2019). For both Pasteurellaceae under study, even higher overall AMR rates have been reported recently in Switzerland for tetracyclines (94% in *P. multocida*, 27% in *M. haemolytica*), penicillin (42% and 52%, respectively) and spectinomycin (81% in *P. multocida*) (Schönecker et al., 2019). Internationally, AMR in *E. coli* has been reported to be generally higher while showing similar patterns for many drugs, for example high AMR for tetracycline (60–95%) and ampicillin (58–93%) (Catry et al., 2016; Callens et al., 2018; Gay et al., 2019). For Pasteurellaceae, higher overall AMR rates were reported for enrofloxacin (11–37%) but not for tetracyclines (39–43%) by Catry et al. (2016) in Belgium. The differences of AMR between countries may be due to varying laboratory procedures, but also to different treatment habits such as generalized group treatment upon arrival (Catry et al., 2016) and health status of the herd at the time of sampling.

Fluoroquinolones and 3rd generation cephalosporines are highest priority critically important antimicrobials. In the present study, AMR to fluoroquinolones of *E. coli* and Pasteurellaceae was found to be lower compared to other studies in other countries, where the prevalence of AMR was reported to be 21–79% (Catry et al., 2016; Callens et al., 2018; Gay et al., 2019). Higher prevalence of AMR to fluoroquinolones in Pasteurellaceae in comparison to the present study were also reported in Switzerland by Schönecker et al. (2019) (26–36% in *P. multocida* and 14–18% in *M. haemloytica*). Different use of clinical breakpoints when

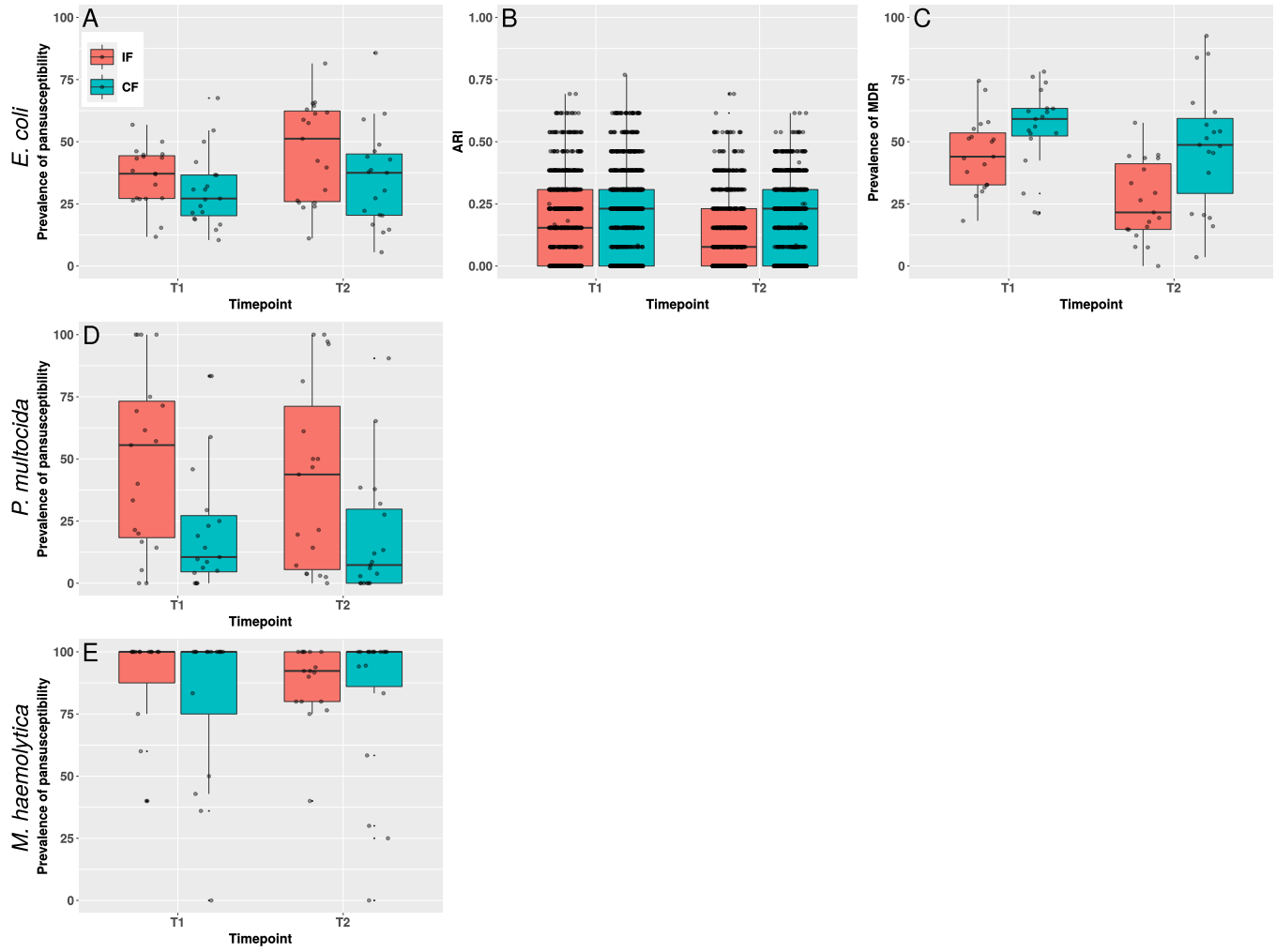

Fig. 1. Farm-level comparison of the prevalence of pansusceptibility, Antimicrobial Resistance Index (ARI[a]) and prevalence of multidrug resistant isolates (MDR[b]) between farm types (intervention farms, IF, vs control farms, CF) and time points (beginning vs end of fattening period, T1 vs T2). For *Escherichia (E.) coli* isolates, prevalence of pansusceptible isolates (plot A), ARI (plot B) and the prevalence of MDR isolates (plot C) is shown. For *Pasteurella (P.) multocida* and *Mannheimia (M.) haemolytica*, the prevalence of pansusceptibility is shown (plots D and E), [a]ARI=number of drugs an isolate exhibited resistance to divided by the total number of drugs tested (Hinton et al., 1985; Catry et al., 2016), [b]Isolates were classified as MDR if resistance to at least one drug was observed in at least three different antimicrobial classes (Magiorakos et al., 2012; Sweeney et al., 2018).

reporting results of susceptibility testing makes comparison difficult and comparisons must be interpreted with care. The prevalence of AMR of 3rd generation cephalosporines was low, which is in line with the low use reported by several authors (Lava et al., 2016; Schönecker et al., 2019). The prevalence of tigecycline-resistant *E. coli* isolates was only 2.1%, but given the ban for its use in food animals in Switzerland and the high importance of tigecycline to treat complicated skin infection in humans, emergence of such resistance characteristics has to be monitored closely (Sun et al., 2019). No AMR was observed for meropenem and colistin, which are not used in calves in Switzerland.

For seven drugs, MIC_{50} values of the three organisms under study were lower than MIC_{90}, thus the existence of two bacterial populations, of which one exhibits reduced antimicrobial susceptibility, can be suspected (EUCAST, 2022).

A limitation of the study is the selection of the participating farmers, who could not be selected and assigned to the study groups randomly for practical reasons. Farmers of both groups had to be motivated and willing to provide detailed records on treatments, slaughter receipts, working habits, among others, and to assist in swab sampling the calves on at least twelve consecutive monthly visits. Calves that were followed throughout the study year represented approximatively 2% of the nationwide annual production of veal calves (Proviande, 2019). We conclude that our results regarding prevalence of resistant isolates upon entry in the fattening period can be generalized to other Swiss veal farms, especially those with similar purchase rate and similar annual production. In addition to this, purchased calves originated from more than 500 different source dairies (data not shown). Therefore, the prevalence of resistant isolates at the beginning of the fattening period may also be interpreted as an estimate of the AMR situation on Swiss dairies selling calves to veal operations.

Resistance rates for the three organisms under study were lower in comparison with international figures. However, prevalences of resistant isolates between 50%– 70% for certain drugs are alarming and likely reflect widespread use of common and highly critically important antimicrobials in Swiss veal farms.

5. Conclusion

A straightforward approach including risk factor analysis and subsequent implementation of targeted measures in the frame of a novel management and housing concept led to a lower prevalence of resistant rectal *E. coli* and *P. multocida* in the nasopharynx of veal calves at the end of the fattening period compared to control farms. In the frame of the 'outdoor veal calf' concept, animal husbandry was reorganized in a way

that allowed not only for decreased mortality, improved animal health, and unchanged average daily weight gains, but also for lower prevalence of resistant isolates at the end of the fattening period. Although alarming levels of resistant isolates were observed, including to critically important antimicrobials of highest priority, the present results show that it is possible to influence AMR prevalence through the implementation of relatively simple management measures in veal farms.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetmic.2022.109419.

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