



Exploring potential risk factors of antimicrobial use in beef cattle

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ABSTRACT

Livestock species are major contributors to the increase of antimicrobial (AM) resistance which is a worldwide concern for both human and animal health. The over-use of AM is widely acknowledged, however, unlike pigs, poultry and dairy cattle, knowledge on potential risk factors affecting AM usage (AMU) in beef industry is limited. Hence, this study aimed to investigate the impact of farm, breed, sex and season of arrival of purchased beef cattle on AMU in Italian beef cattle. Data on 1063 batches were collected from January 2016 to April 2019 from specialised beef fattening farms located in the north of Italy. Information on breed, sex, date of arrival, performance traits and AM agents used on farm was collected, and the treatment incidence 100 (TI100) indexes per batch were calculated using the defined daily dose animal estimated according to Italian summaries of product characteristics. Factors affecting TI100 indexes were investigated using a cross-classified multilevel model. Farms largely differed in terms of AMU. Males had greater AMU than females ($P < 0.001$), likely due to their higher susceptibility to disease. Statistically significant differences were observed between seasons of arrival with summer and spring having lower TI100 indexes than winter and autumn ($P < 0.001$). Indeed, winter is commonly linked to an increase in respiratory diseases in beef cattle. Finally, the TI100it indexes tended to be different among breeds with Blonde d'Aquitaine and Limousine having greater AMU compared to the other breeds. Results of this study provided valuable information on potential risk factors of AMU in beef production which may be useful to address its reduction. For instance, the development of tailored management strategies for specific breeds, targeted approaches to improve the health of males as well as greater care towards batches purchased in winter are possible advice to implement on-farm for a more responsible AM stewardship.

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Implications

Strategies to reduce antimicrobial use are pivotal in the livestock industry due to concerns of public opinion over the problem of antimicrobial resistance. Defining potential risk factors affecting antimicrobial use in beef cattle may help to outline new on-farm strategies for more judicious use of medications. Results from this study demonstrated that breed, sex, season and farm are important factors associated with the use of antimicrobials. Hence, we suggested that simple actions applied on-farm such as greater care towards animals purchased in winter or tailored management strategies towards certain breeds, may lead to a reduction of antimicrobials in the beef industry.

Introduction

Since their discovery in the late 1940s, antimicrobials (AM) have been considered essential tools to tackle infectious diseases thus

providing a positive impact on animal production (Pagel and Gautier, 2012). However, the increase of AM resistance (AMR), likely associated with widespread use and abuse of AM in both humans and animals (World Health Organization, 2014), highlights the need for new strategies to foster a more prudent use of medications. Hence, accuracy in providing data on AM use (AMU) is pivotal to identifying potential drivers of an inappropriate AMU and to developing efficient strategies for its reduction (Pinto Ferreira, 2017).

Full harmonisation of methods to record and quantify AMU in food-producing species and European Union (EU) countries is still ongoing. So far, one of the most accredited technical unit to measure AMU is the defined daily dose animal (Pinto Ferreira, 2017), which is fundamental to calculate the treatment incidence 100 (TI100), an indicator used to assess the frequency of treatments (Timmerman et al., 2006; AACTING, 2019).

Beef production is the third-largest meat industry in the EU, behind pig and poultry, and Italy is the fourth major producer of beef meat in EU (Hocquette et al., 2018) with the northern area as the main supplier of specialised fattening farms (Gallo et al., 2014). In addition, cattle and pigs are known for the large use of AM and Italy is the second EU country

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in terms of AM sales in the livestock industry (European Medicines Agency, 2019). Consequently, animal production is generally blamed as a major contributor to the increase of AMR (Chantziaras et al., 2014) suggesting the need for more in-depth knowledge on drivers of AMU.

Potential risk factors affecting AMU have been presented in several studies and results showed that issues surrounding farming systems, herd management, biosecurity, population density, veterinary advice and external pressures were linked to AMU (McDougall et al., 2017; Postma et al., 2017). Currently, data are mainly available for pigs, poultry and dairy cattle whereas only a few studies explored potential contributing factors of AMU in beef production. For instance, Lava et al. (2016a) identified the duration of the fattening period, quarantine and feeding areas as factors significantly associated with AMU in veal calves, while Hommerich et al. (2019) reported a significant positive impact of farm size on treatment frequency. To the best of our knowledge, factors such as breed and sex, which are well-known for their strong impact on performance traits, risk of mortality and quality of animal products (Pesonen et al., 2012; Visentin et al., 2016) have never been explored before as potential sources of variation of AMU in beef cattle.

Therefore, this study aimed to assess the effect of farm, breed, sex and season of arrival of purchased cattle on AMU in Italian beef cattle. An in-depth understanding of such a relationship may help to outline preventive on-farm strategies to optimise animal health and welfare by following a better AM stewardship.

Material and methods

Data collection and calculation of the treatment incidence 100

Data were collected from January 2016 to April 2019 in specialised fattening farms of Veneto region (north-east of Italy) by a cooperative of beef producers (AZoVe – Cittadella, Italy). The diet was based on a total mixed ration with a high proportion of concentrates, mineral and vitamin supplementations and different proportions of feedstuffs according to fattening stage, breed and gender. Corn silage was the main component of the total mixed ration while wheat straw and soybean meal were the main sources of long fibres and proteins, respectively. Occasionally, dry or pressed ensiled sugar beet pulps were used as non-starchy energy feeds.

The initial dataset accounted for 1487 batches with information on farm, breed, sex, number of animals, number of deaths, date and BW at the beginning and at the end of the fattening cycle, and AM treatments. A batch was meant as a group of animals that were sorted according to their BW, breed and sex before their arrival at the fattening farm. Each batch entered the fattening farm on the same day and was subjected to the same conditions during the fattening period, whose length was equal for the entire batch. Mortality rate, the average percentage of shrink, percentage of animals treated, average daily gain and length of the fattening cycle (days) per batch were calculated. Data collected in 2019 were removed from the dataset as they were only available until April. Moreover, batches with missing data on BW at the beginning and/or at the end of the fattening cycle, and on the percentage of shrink were discarded from the dataset as well as single-breed farms and breeds with less than 20 batches. The final dataset included 1063 batches from 25 farms and six beef breeds: Charolaise (CHR), Limousine (LIM), Blonde d'Aquitaine (BDA), French crossbred (FRC), Irish crossbred (IRC) and Italian crossbred (ITC). Specifically, FRC, IRC and ITC indicated the result of crossbreeding with unknown breeds.

Information on veterinary medicinal products (VMP, $n = 33$) used on farms and containing AM was also available and a total of 155 074 treatments were administered to the animals of the 1063 batches. A DDDA for Italy (DDDAit) was assigned to each active ingredient (AI) with AM activity of those VMP. The DDDAit represents the dose (mg)

of the AI administered per kg of BW per day and was estimated using Italian summaries of product characteristics. When dosages were provided as an interval, the average value was used (e.g. 10–30 mg/kg per day, DDDAit = 20 mg/kg per day). These were established during the development of the ClassyFarm integrated monitoring system (www.classyfarm.it) of the Italian Ministry of Health. The DDDAit was used to calculate an index called TI100 for Italy (TI100it) which quantifies the treatment frequency. This index allows for better monitoring of AMU in livestock farming as it is more consistent with the measurement system used in human medicine (Timmerman et al., 2006). The following formula (modified from Timmerman et al., 2006) was used to calculate the TI100it per each VMP (AACTING, 2019):

$$TI100it = \frac{\text{amount of AI administered per batch (mg)}}{\text{DDDAit (mg/kg/day)} \times \text{animals at risk} \times \text{standard weight (kg)} \times \text{days at risk}} \times 100$$

where 'animals at risk' is the total number of animals of the batch, 'standard weight' is the standard BW of animals at treatment (400 kg) and 'days at risk' is the standard number of days of the fattening cycle (230 days). The TI100it values of each VMP were then summed to obtain the total TI100it per batch.

In addition to TI100it, another index was calculated using only those VMP classified as 'Highest Priority Critically Important Antimicrobials' (HPCIA) by the World Health Organization and named as HPCIA TI100it.

Further details on original data and calculation of TI100 indexes can be retrieved from Diana et al. (2020).

Statistical analysis

Data were analysed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Batch was considered the analytical unit. Data were tested for normality by means of the Shapiro–Wilk test, skewness and kurtosis and visual inspection of the normal plot. Descriptive statistics of the number of animals, mortality rate, percentage of animals treated, performance traits, percentage of shrink and TI100 indexes per breed, sex and their interaction were calculated. Descriptive statistics of TI100 indexes by year and season were also calculated. Data were not normally distributed. A cross-classified multilevel model with gamma distribution and log link function in GLIMMIX procedure of SAS was used. Sex, year and season of arrival of purchased beef cattle were included as fixed effects in the final model used to analyse TI100it and HPCIA TI100it. Initial BW was included in the model as linear covariate while intercepts of farm and breed were modelled as random effects. To build the final model, a step-wise forward selection process was used for both TI100 indexes. Percentage of shrink, days spent in the fattening cycle, BW gain, number of animals per batch and the interaction between year and season were also tested but they were removed from the final model because not significant. Goodness-of-fit was evaluated by checking Akaike's Information Criterion and Bayesian Information Criterion of each step of model building, and the one with smaller values was selected. We then used the covariance parameter estimates to compute the intraclass correlation coefficient (ICC) which provided information on how much variation of TI100 indexes was accounted for by farm and breed effects. Results of fixed effects are presented as least squares means \pm SE, those for the covariate are presented as regression coefficient \pm SE while results of random effects are presented as estimates \pm SE. A Tukey–Kramer adjustment was used to account for multiple *post hoc* comparisons. The criterion for statistical significance was established at $P < 0.05$ and statistical trend was set at $0.05 < P < 0.10$. Further details on model building and validation are available in Supplementary Material S1.

Table 1
Descriptive statistics of the number of animals, percentage of animals treated, performance traits and mortality rate per batch (n = 1063) by breed¹ in beef cattle.

Variable	Breed	Batches (n)	Mean	SD	Median	Q1-Q3 ²
Animals per batch (n)	BDA	21	34.5	10.8	36.0	26-39
	CHR	624	67.1	38.3	55.0	35-95
	FRC	66	60.2	27.8	56.0	36-84
	IRC	25	51.1	11.6	56.0	53-58
	ITC	67	43.6	16.8	39.0	31-60
	LIM	260	54.1	30.1	50.0	30-72
Animals treated (%)	BDA	21	89.9	19.2	100.0	94.4-100
	CHR	624	57.7	38.7	48.9	19.7-100
	FRC	66	61.2	41.1	93.2	20.0-100
	IRC	25	53.9	39.2	30.0	19.6-100
	ITC	67	50.5	40.7	29.7	13.1-100
	LIM	260	90.9	23.6	100.0	100-100
Initial BW (kg)	BDA	21	267.5	12.1	267.0	259-271
	CHR	624	382.4	50.6	391.0	334-406
	FRC	66	375.8	30.4	385.0	369-393
	IRC	25	401.1	16.1	402.0	393-408
	ITC	67	270.6	33.9	266.0	249-297
	LIM	260	288.2	11.8	288.0	282-294
Final BW (kg)	BDA	21	518.3	29.9	526.0	519-531
	CHR	624	671.2	82.0	709.0	570-732
	FRC	66	673.0	78.3	705.0	664-719
	IRC	25	720.4	24.8	718.0	710-731
	ITC	67	578.7	67.8	604.0	503-631
	LIM	260	553.9	45.4	569.0	524-583
Average daily gain (kg/day)	BDA	21	1.15	0.15	1.18	1.12-1.24
	CHR	624	1.44	0.29	1.53	1.23-1.65
	FRC	66	1.38	0.23	1.40	1.31-1.50
	IRC	25	1.64	0.16	1.61	1.56-1.69
	ITC	67	1.26	0.22	1.32	1.07-1.43
	LIM	260	1.34	0.23	1.42	1.19-1.49
Mortality rate (%)	BDA	21	1.27	2.56	0.00	0.00-2.32
	CHR	624	0.63	1.27	0.00	0.00-0.88
	FRC	66	0.42	1.10	0.00	0.00-0.00
	IRC	25	0.54	0.94	0.00	0.00-1.67
	ITC	67	0.63	1.34	0.00	0.00-0.00
	LIM	260	0.92	2.01	0.00	0.00-1.15

¹ BDA = Blonde d'Aquitaine, CHR = Charolaise, FRC = French crossbred, IRC = Irish crossbred, ITC = Italian crossbred, LIM = Limousine.

² Q1-Q3 = Interquartile range.

Results

Production data and animals treated

Large variability was observed for production data among breeds (Table 1). The greatest BW at the beginning and at the end of the fattening cycle was reported for IRC (401.1 and 720.4 kg, respectively) while

Table 2
Descriptive statistics of the number of animals, percentage of animals treated, performance traits and mortality rate per batch (n = 1063) by sex in beef cattle.

Sex	Variable	Mean	SD	Median	Q1-Q3 ¹
Female (306 batches)	Animals per batch (n)	40.1	22.9	35.0	27.0-49.0
	Animals treated (%)	58.0	41.4	56.0	14.7-100
	Initial BW (kg)	306.8	28.1	314.0	286.0-328.0
	Final BW (kg)	534.2	37.3	543.9	503.9-560.5
	Average daily gain (kg/day)	1.06	0.19	1.05	0.94-1.19
	Mortality rate (%)	0.73	1.68	0.00	0.00-0.00
Male (757 batches)	Animals per batch (n)	69.4	35.4	60.0	40.0-92.0
	Animals treated (%)	69.4	36.8	100.0	29.7-100
	Initial BW (kg)	367.6	63.5	388.9	295.9-403.4
	Final BW (kg)	675.6	72.6	707.6	593.9-730.0
	Average daily gain (kg/day)	1.53	0.17	1.54	1.43-1.65
	Mortality rate (%)	0.69	1.44	0.00	0.00-0.92

¹ Q1-Q3 = Interquartile range.

the lowest for BDA (267.5 and 518.3 kg, respectively). The batch size ranged from 34.5 (BDA) to 67.1 heads (CHR) and the percentage of animals treated per batch ranged from 50.5% (ITC) to 90.9% (LIM). Production data and animals treated differed also between females and males with the latter having higher BW at the beginning (367.6 vs 306.8 kg) and at the end of the fattening cycle (675.6 vs 534.2 kg) and greater percentage of animals treated (69.4 vs 58.0%) than females (Table 2). Finally, descriptive statistics of production data and animals treated by breed and sex are presented in Supplementary Tables S1 and S2.

Association between antimicrobial use and farm

Median of TI100it per batch was 1.79 (IQR: 0.70-3.12) whereas median of HPCIA TI100it was 0.86 (IQR: 0.21-2.07). Both TI100 indexes varied among farms. Specifically, TI100it ranged from 0.17 to 4.62 whereas HPCIA TI100it ranged from 0.03 to 3.99. There were statistically significant differences among farms for both TI100it (0.138 ± 0.047; P = 0.002; Table 3) and HPCIA TI100it (0.235 ± 0.094; P = 0.006; Table 3) indicating a substantive variability of AMU among farms. Results of the ICC showed that farm accounted for 23 and 21% of the variability of TI100it and HPCIA TI100it, respectively.

Table 3
Estimates and SE of breed¹ and farm for treatment incidence 100 (TI100) indexes² obtained from the batches of beef cattle included in the final analysis (n = 1063).

		TI100it		P-values	HPCIA TI100it		P-values			
		Estimate	SE		Estimate	SE				
Breed	Intercept	0.129	0.086	= 0.06	0.252	0.181	= 0.08			
	BDA	0.412 [^]	0.222		0.433	0.361				
	CHR	0.076	0.163		0.216	0.241				
	FRC	-0.026	0.177		-0.014	0.256				
	IRC	-0.152	0.204		-0.586 [^]	0.336				
	ITC	-0.637 [*]	0.186		-0.674 [*]	0.272				
	LIM	0.277 [^]	0.169		0.526 [*]	0.253				
	Farm	Intercept	0.138		0.047	= 0.002		0.235	0.094	= 0.006
		F1	0.268 [*]		0.131			-0.282	0.220	
		F2	-0.559 [*]		0.193			-0.563 [*]	0.288	
F3		-0.199	0.125	-0.317	0.223					
F4		0.193	0.138	0.362	0.239					
F5		0.173	0.157	0.475 [*]	0.245					
F6		0.251 [^]	0.134	0.452 [*]	0.215					
F7		-0.408 [*]	0.130	-0.337	0.263					
F8		0.143	0.150	0.299	0.258					
F9		-0.258	0.195	-0.063	0.276					
F10		-0.465 [*]	0.123	-0.504 [*]	0.261					
F11		0.245 [^]	0.133	-0.083	0.241					
F12		0.246	0.157	-0.527 [^]	0.306					
F13		0.036	0.222	-0.515	0.352					
F14		0.052	0.149	-0.049	0.251					
F15		0.311 [*]	0.125	0.582 [*]	0.226					
F16	0.611 [*]	0.123	0.883 [*]	0.225						
F17	-0.595 [*]	0.189	-0.190	0.304						
F18	-0.261 [*]	0.128	0.041	0.248						
F19	-0.049	0.120	-0.126	0.209						
F20	-0.615 [*]	0.171	-0.427	0.292						
F21	0.072	0.135	-0.187	0.247						
F22	-0.049	0.288	0.196	0.385						
F23	-0.117	0.109	-0.258	0.216						
F24	0.476 [*]	0.244	0.675 [*]	0.332						
F25	0.449 [*]	0.143	0.372	0.239						

Statistically different from the intercept = *P < 0.05; [^]0.10 < P < 0.05.

¹ BDA = Blonde d'Aquitaine, CHR = Charolaise, FRC = French crossbred, IRC = Irish crossbred, ITC = Italian crossbred, LIM = Limousine.

² TI100it = treatment incidence 100 for Italy, calculated using the defined daily dose animal for Italy based on Italian guidelines of dosage obtained from the Italian database (www.classyfarm.it); HPCIA = Highest Priority Critically Important Antimicrobials.

Table 4
Descriptive statistics of treatment incidence 100 (TI100) indexes¹ per batch ($n = 1063$) by sex and by breed² in beef cattle.

		TI100it				HPCIA TI100it			
		Mean	SD	Median	Q1–Q3 ³	Mean	SD	Median	Q1–Q3 ³
Sex	Female	1.36	1.28	1.02	0.37–1.75	0.83	0.99	0.46	0.12–1.18
	Male	2.46	1.88	2.23	0.94–3.43	1.62	1.65	1.21	0.26–2.51
Breed	BDA	2.79	1.49	2.19	1.72–3.58	1.97	1.15	1.53	1.38–2.19
	CHR	1.85	1.65	1.39	0.56–2.73	1.13	1.37	0.53	0.16–1.57
	FRC	2.22	2.05	1.90	0.49–3.42	1.24	1.42	0.68	0.18–1.77
	IRC	1.70	1.55	1.33	0.59–2.42	0.36	0.35	0.26	0.12–0.47
	ITC	1.07	0.95	0.80	0.30–1.56	0.48	0.79	0.21	0.05–0.55
	LIM	3.13	1.88	2.89	1.83–3.89	2.35	1.69	1.96	1.22–3.24

¹ TI100it = treatment incidence 100 for Italy, calculated using the defined daily dose animal for Italy based on Italian guidelines of dosage obtained from the Italian database (www.classyfarm.it); HPCIA = Highest Priority Critically Important Antimicrobials.

² BDA = Blonde d'Aquitaine, CHR = Charolaise, FRC = French crossbred, IRC = Irish crossbred, ITC = Italian crossbred, LIM = Limousine.

³ Q1–Q3 = Interquartile range.

Table 5

Least squares means (LS mean) and SE of sex, season of arrival of purchased beef cattle and year, and regression coefficient and SE of initial BW (kg) for treatment incidence 100 (TI100) indexes¹ obtained from the batches of beef cattle included in the final analysis ($n = 1063$).

		TI100it		HPCIA TI100it		P-values
		LS mean	SE	LS mean	SE	
Sex	Female	1.07 ^a	0.20	0.53 ^a	0.14	<0.001
	Male	2.01 ^b	0.35	1.07 ^b	0.27	
Season	Winter	1.82 ^a	0.32	0.95 ^a	0.24	<0.001
	Autumn	1.95 ^a	0.35	1.08 ^a	0.27	
	Spring	1.16 ^b	0.21	0.53 ^b	0.14	
	Summer	1.13 ^b	0.20	0.59 ^b	0.15	
Year	2016	1.56 ^a	0.28	0.85 ^a	0.22	<0.05
	2017	1.50 ^a	0.27	0.70 ^b	0.18	
	2018	1.35 ^b	0.24	0.72 ^b	0.18	
Initial BW (kg) [^]		−0.002 ± 0.001 [*]		−0.003 ± 0.001 [*]		<0.05

^{a,b}Different superscripts within trait and effect differ significantly from each other ($P < 0.05$).

[^]Results for continuous covariate presented as regression coefficient ± SE; ^{*} $P < 0.05$.

¹ TI100it = treatment incidence 100 for Italy, calculated using the defined daily dose animal for Italy based on Italian guidelines of dosage obtained from the Italian database (www.classyfarm.it); HPCIA = Highest Priority Critically Important Antimicrobials.

Associations between antimicrobial use and breed, sex, season and year

Descriptive statistics of TI100 indexes by breed and by sex are presented in Table 4 while those of TI100 indexes by breed and sex and by year and season are presented in Supplementary Tables S3 and S4, respectively. The TI100it tended to be different among breeds (0.129 ± 0.086 ; $P = 0.06$) with BDA having greater AMU than all other breeds followed by LIM (Table 3). Similar results were observed for the HPCIA TI100it that tended to be different among breeds (0.252 ± 0.181 ; $P = 0.08$) but with LIM having greater AMU than all other breeds followed by BDA (Table 3). Results of the ICC showed that breed accounted for 10% and 12% of the variability of TI100it and HPCIA TI100it, respectively.

Males had greater TI100it and HPCIA TI100it than females ($P < 0.001$; Table 5). Both TI100it ($P = 0.05$) and HPCIA TI100it ($P = 0.01$) differed among years with a reduction of AMU over time (Table 5). Statistically significant differences were also detected for the TI100it and the HPCIA TI100it among seasons of arrival of purchased cattle ($P < 0.001$). Specifically, animals purchased during summer and spring had lower AMU than those purchased in winter and autumn while no evidence for season differences was observed between autumn and winter and between spring and summer ($P > 0.05$; Table 5).

Discussion

A preliminary screening on AMU for the Italian beef scenario was presented by Diana et al. (2020) who reported differences of AMU among years and AM classes. In the present study, we aimed to investigate the impact of breed, sex and season of purchased animals on AMU since, to the best of our knowledge, information on potential risk factors affecting AMU in beef production is still limited. Gaining knowledge on predictors that may explain a greater risk of treatment is crucial to propose efficient strategies for better AM stewardship. To investigate the aforesaid effects, we decided to use the DDDAit metric instead of the EMA's defined daily doses (DDDvet) because, as explained in Diana et al. (2020), the latter was not available for more than 25% of AM used in Italy. The advantage is that TI100 indexes based on DDDAit provide a more representative scenario of AMU at the national level while still allowing a reliable comparison among EU countries due to the presence in the formula of standard units (i.e. BW and days at risk). Indeed, as reported in our previous study (Diana et al., 2020), when the TI100it was calculated by removing those AI for which DDDvet was not available, a positive significant relationship was observed between TI100it and TI100vet (i.e. the TI100 calculated based on DDDvet), highlighting that differences in AMU between them were minimal. This result confirms the reliability of employing the DDDAit to assess the AMU, and justifies the use of the TI100it indexes in the current study to check whether the predictors had an impact on AMU.

Effect of farm

A substantive variation of AMU was observed among farms. This result emphasised the need of defining benchmark data in beef production to monitor and evaluate the status of AMU on farms. However, these findings also suggest that other aspects such as peculiar characteristics of each farm, may play an important role in the overall AMU. The impact of farm has been somewhat identified and/or suggested, although not fully investigated, by Bos et al. (2013) who reported a large variation of AMU among farms within different livestock categories/species (veal, pig and poultry). In their study on cattle, Carmo et al. (2017) suggested 'farming conditions' as a potential explanation for differences observed on AMU between Switzerland and Denmark. Hence, distinctive farm-factors such as management practices, farm location, welfare standards, feeding strategies and veterinary advice are likely to affect AMU in cattle (Lava et al., 2016a and 2016b; McDougall et al., 2017) as reported for other food-producing sectors. For instance, improved biosecurity in pig farms was linked to lower levels of AMU (Postma et al., 2017) and the implementation of tailored welfare-friendly measures contributed to the reduction of AMU (Raasch et al., 2020). The role of farmers may also be a key-component to explain farms' variability with regards to AMU. For instance, this may be due

to the treatment strategies that farmers are used to applying on-farm which are likely influenced by both the veterinary–farmer relationship and farmers' belief about AMU and the associated risk of AMR. Jones et al. (2015) reported that 64% of dairy farmers identified cost-reduction as the most important reason to justify a reduction in AMU while only 15% of them considered a decrease of AM an important goal to achieve given the risk of AMR. The participants also declared that the most reliable source of information on AMU was the category of veterinarians. Indeed, *Vissschers et al. (2014)* reported an association between lower AMU and veterinarian consultation in pig farms, highlighting the importance of an appropriate veterinary–farmer relationship where veterinarians should promote alternative strategies to reduce AMU and provide guidelines for a responsible AM stewardship. Hence, further investigations are needed to evaluate representative farm-factors in beef production.

Effect of breed

Data related to AMU in beef production are still poorly available, especially at the breed level. Our study investigated the effect of six beef breeds (i.e. three purebreds and three crossbreds) on AMU. Albeit of a marginal significance, differences between breeds were observed for the frequency of AM treatment. Specifically, BDA and LIM tended to have greater AMU than other beef breeds. It is important to highlight that the observational nature of our data did not allow to account for other potential variables likely linked to the breed-level effect, thus possibly explaining the minimal significance reported in the current study. Our findings showed that batches with lower initial BW statistically increased their chance of being treated. A common pattern observed for the two breeds at higher risk of treatment was the average BW. Indeed, BDA and LIM had the lowest initial and final BW among all breeds. This is in line with other studies where mainly LIM was slaughtered at lower BW than CHR or other beef breeds (*Albertí et al., 2008; Gallo et al., 2014*). Specifically, even if in the present study LIM was recorded as the third breed with the lowest initial BW after BDA and ITC, at sale it became the second breed with the lowest BW after BDA. Therefore, the aforesaid findings may help to explain the higher likelihood of AMU reported for BDA and LIM. We know from the literature that beef breeds with low average BW may be at greater risk of mortality and increased susceptibility to disease, thus making them more likely to be treated with AM. In their review, *Taylor et al. (2010)* emphasised that lighter beef calves were more likely to develop bovine respiratory disease (BRD) than heavier calves. Descriptive data also helps to support our findings because BDA and LIM were the breeds with the highest mortality rate (1.27 and 0.92%, respectively) and the highest percentage of animals treated per batch (89.9 and 90.9%, respectively) likely due to BRD which is the most detrimental health problem in beef production and primary cause of mortality (*Edwards, 2010*). Lastly, the documented lower incidence of BRD for crossbreds compared to purebreds (*Snowder et al., 2005*) may contribute to explain the higher AMU observed in BDA and LIM. Our results are in line with this assumption as the three crossbreds of the current study were those with lower AMU among all breeds.

Breed is commonly identified as a source of variation in food-producing species including beef cattle. Its impact on production traits such as carcass weight and feed intake, BW, carcass weight and feed intake, is well-known and it is the main genetic aspect affecting animal products such as milk and meat quality (*Pesonen et al., 2012; Penasa et al., 2014*). For instance, *Pesonen et al. (2012)* reported differences in carcass weight and meat quality between LIM, Aberdeen Angus and their crossbreds. Studies on beef cattle also showed an association between breed and risk of mortality. For example, *Lava et al. (2016a)* reported that beef breeds were at lower risk of mortality than dairy and dual-purpose breeds. However, the same study reported an increased risk of treatment in beef than dairy and dual-purpose breeds. In contrast, *Hommerich et al. (2019)* reported a higher AMU in pig and dairy

than beef production. The opposite results observed in the aforesaid studies would suggest that AMU may be affected, among other factors, by the type of beef breed considered. This is supported by our findings where certain breeds tended to have greater AMU than others, thus emphasising the need to further investigate the impact of breed and other associated variables on AMU within each food-producing species.

Effect of sex

Both TI100 indexes were statistically higher in males than females. According to the literature, males have a higher incidence of disease, especially to BRD, than females and are more prone to other infections, for instance due to castration procedures, albeit this was not the case in the current study (*Taylor et al., 2010; Magrin et al., 2020*). This in turn may increase their risk of being treated. Indeed, castration is considered a source of stress making animals more susceptible to diseases due to its detrimental effect on their immune system response (*Carroll and Forsberg, 2007*). Whereas, respiratory disease has been reported as the major reason for AMU in beef cattle (*Braut et al., 2019; Diana et al., 2020*) thus supporting the high TI100 values found in our study for males. Descriptive data also revealed that the average batch size for males was almost double than for females (69.4 vs 40.1 heads). This may increase the exposure to infectious diseases due to the large number of animals sharing the same space and the consequent higher chance of spreading pathogens (*Woolums et al., 2013; Hommerich et al., 2019*). Studies made at herd level considered herd size as a cause of disease persistence (*Brooks-Pollock and Keeling, 2009*). *Woolums et al. (2013)* specified that a potential predictor of BRD in beef calves was the number of animals in the herd. This in turn would increase the likelihood of AMU, as seen in *Lava et al. (2016b)* who reported that treatments were associated with BRD risk.

Effect of season of arrival of purchased beef cattle

Statistically significant differences were observed among seasons of arrival of purchased cattle for the frequency of AM treatment. Overall, purchasing beef cattle during cold seasons (i.e. winter and autumn) increased the risk of being treated compared to summer and spring. This pattern was similar for both TI100 indexes. Winter and autumn are commonly associated with a higher incidence of respiratory and gastrointestinal diseases and mortality rate (*Gay and Barnouin, 2009*). Moreover, previous studies showed that these diseases were among the major reasons of AMU in beef cattle (*Braut et al., 2019; Diana et al., 2020*). This may help to explain our findings and the association observed between season and AMU.

Conclusion

Results of the present study highlighted that farm, breed, sex and season of arrival of purchased cattle may be helpful to explain the variability of AMU, since statistically significant differences were observed for both TI100 indexes for the studied factors. This information can be used to address the upcoming research in beef production for the development of new strategies and a more responsible AMU. For instance, we recommend targeted management strategies aiming to improve the health of males and to assist specific beef breeds, as well as greater care towards batches purchased during the winter season, as possible preventive actions to reduce AMU in beef production. In addition, given the substantive impact of farm on AMU, future research should also investigate other farm-distinctive factors such as management system, standards of biosecurity and welfare indicators to better clarifying the variability of AMU among farms. However, it is worth highlighting that some limitations surrounding observational studies may arise such as the difficulty in controlling potential confounding factors or datasets with missing values. Indeed, retrospective data may only provide part of the story, as for the present study where we aimed to

provide a first view on potential factors to be considered as drivers of AMU in future investigations. Thus, future longitudinal controlled studies are needed to further support the current findings.

Supplementary materials

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.animal.2020.100091>.

Ethics approval

This study was approved by the Ethical Committee for the Care and Use of Experimental Animals of the University of Padova, Italy (approval no. 74/2018).

Data and model availability statement

None of the data were deposited in an official repository due to their sensitive nature.

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Declaration of interest

The authors declare that they have no conflict of interests.

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