Effect of commingling animals at sorting facilities on performances and antibiotic use in beef cattle

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ABSTRACT

The misuse of antibiotics in the animal sector is the main driver of antibiotic resistance. More than 80% of Italian beef cattle imported from France are Charolais, which undergo a commingling procedure before reaching Italian fattening farms. The aim of this study was to evaluate the effect of commingling Charolais cattle in France on antibiotic use (AMU) and animals' performances in Italy. A total of 19,756 young bulls from 449 batches transported to Italy between 2016 and 2018 were considered. Carcase weight (CW), slaughter age (SA), antibiotic treatments, vaccinations information and the French department of origin were available for each animal. Also, treatment incidence 100 (TI100it) and average daily carcase gain (ADCG) were calculated. Three classes of commingling were assigned to each animal according to the French department of origin. A linear mixed model was used to investigate sources of variation of CW, SA, ADCG and TI100it. Respiratory diseases were the main reasons for treatment and macrolides were the most used class of antibiotics. The TI100it decreased by 11% from 2016 to 2018 (p < .05). Animals that went through the highest level of commingling had lighter CW, lower ADCG and greater TI100it than animals subjected to lower commingling (p < .05). Younger and highly commingled animals had higher TI100it (p < .001) than older animals equally commingled. This first-of-its-kind study showed that commingling procedures increase the risk of AMU and affect the performances of Charolais cattle.

HIGHLIGHTS

- Commingling procedures increase the use of antibiotics and penalise animals’ performances.
- Younger and highly commingled animals are at greater risk of being treated.
- Reducing commingling levels can be an effective strategy to reduce antibiotic use in beef cattle.

Introduction

Antibiotics are used to tackle infectious diseases in the livestock sector. However, antibiotic use (AMU) is the main driver of antibiotic resistance (Chantziaras et al. 2014) which jeopardises human and animal health alike. The livestock sector is generally blamed as one of the major consumers of antibiotics (Chantziaras et al. 2014) and consequently one of the main contributors to antibiotic resistance. Thus, a more judicious AMU and concrete strategy to investigate possible sources of variation are needed (Delabouglise et al. 2017; Diana et al. 2020; Santinello et al. 2020).

Beef production is the third-largest meat industry in the EU with Italy as the fourth main producer among EU countries (Hocquette et al. 2018). Approximately two-thirds of Italian beef fattening farms are located in the North-East of the country and animals are mainly imported from France (Cozzi et al. 2009; Gallo et al. 2014). Imported animals go through environmental stressors that may promote the onset of diseases with a potential effect on AMU. An example is the practice of commingling the animals from different areas of origin to create homogeneous batches (Herve et al. 2020) and the long transportation prior to reaching the fattening farms (Fike and Spire 2006; Earley et al. 2017). Loerch and Fluharty (2000) suggested that commingling animals from different origins in the same fattening farms lead to a disruption...
of the hierarchy which is stressful for the animals. Indeed, a study conducted by Step et al. (2008) showed that steers from a single source ranch were less likely to be treated for Bovine Respiratory Disease (BRD) compared to multiple-source steers. Long transportation to the fattening farm is also considered a source of stress (Schwartzkopf-Genswein et al. 2007; Herve et al. 2020) that may lead to an impairment of the animals’ immune system with a consequent reduction of performance and a less effective response to diseases (Van Engen and Coetzee 2018). Therefore, the practice of commingling, the group size and the distance to reach the fattening farms are considered potential promoters of diseases such as the BRD (Hay et al. 2014).

The BRD is the most significant health problem in the beef cattle industry (Callan and Garry 2002; Duff and Galyean 2007) and it is well-known for its detrimental effects on animal performance (Cusack et al. 2007; Schneider et al. 2009; Delaboulge et al. 2017). The risk of developing BRD increases during the first days after the arrival of the animals to the new farm (Hulbert et al. 2011), likely due to the stress generated by commingling procedures applied at collection centres (Callan and Garry 2002; Duff and Galyean 2007; Herve et al. 2020).

Although most studies showed the negative effects of commingling procedures on the performance, health and behaviour of the animals (Mounier et al. 2005, 2006; Step et al. 2008; Herve et al. 2020), some researchers did not highlight the impact of commingling on animals’ performances. For instance, Wiegand et al. (2020) did not observe any detrimental impact on the welfare and performance of beef heifers of different origins in small pens (4 heifers per pen), even if higher AMU to treat BRD was observed in pens with heifers of multiple origins. The same authors suggested considering large pens and group sizes typical of commercial fattening farms in future studies.

Little is still known about the effects of the commingling procedure on the health and performances of animals in commercial farming conditions, especially with regards to AMU. Therefore, the aim of this study was to investigate the effect of commingling Charolais young bulls from different origins in France on AMU during the fattening phase in Italian beef farms.

**Materials and methods**

**Italian specialised beef fattening system**

Charolais young bulls are the main imported category of beef cattle in the Veneto region (Gallo et al. 2014) and the trade relationship with France is considered an efficient way to produce meat in Italy (Berton et al. 2017). In France, animals are generally reared on cow/calf farms at pasture until an average age of 11–12 months (Herve et al. 2020). Then, they are transported to collection centres and commingled from multiple French departments of origin to facilitate the sorting of batches that will be sold to Italian fatteners. Usually, animals are grouped into homogeneous batches with the ultimate goal of obtaining homogeneous carcases (Mounier et al. 2005). Each batch consists of animals of the same breed, gender and similar BW, that arrive at the Italian fattening farm on the same day. The average batch size represents approximately the loading capacity of a truck (i.e. 30–40 animals). The typical diet supplied in Italian fattening farms is a total mixed ration with on average 60% concentrates and 40% forages, and different proportions of mineral and vitamin supplementations to reach the desired slaughter weight 6–7 months after animals’ arrival to the fattening farm, at an age comprised between 20 and 22 months. The diet was similar across all farms involved in the study (Table 1).

During the transition from a pasture-based to an intensive confined system, the animals are subjected to several stressors such as the new housing system, and the change of temperature and humidity typical of the Po Valley that may threaten the overall health and welfare of the animals. In general, Italian fatteners carry out two fattening cycles per year with a housing system characterised by closed or open barns with multiple pens. The flooring system consists of a fully slatted or concrete floor with straw bedding which is substituted throughout the fattening cycle (Cozzi et al. 2009).

Table 1. Average characteristics of the diet provided to Charolais young bulls.

| Diet composition |  |
|------------------|--|
| Total ingestion, kg | 16.5 |
| DM, kg | 9.9 |
| ME, UFV¹ | 10.0 |
| PDI, g | 966.6 |
| PDIN, g | 890.4 |
| Concentrates, % | 63.7 |
| Forages, % | 36.3 |
| Chemical composition, % |  |
| Moisture | 39.9 |
| CP | 13.4 |
| EE | 3.9 |
| CF | 14.2 |
| Ash | 4.9 |
| NDF | 32.1 |
| Starch | 33.9 |

¹ UFV: Unité fourrage viande (INRA)

Note. The values were calculated as average of the diet of each farm involved in the trial. Abbreviations: DM: dry matter; ME: metabolisable energy; PDI: protein digestible in the intestine; PDIN: true protein absorbable in the intestine when N is limiting in the rumen; CP: crude protein; EE: ether extract; CF: crude fibre; NDF: neutral detergent fibre.
Study design and data curation

The study was carried out using data on Charolais young bulls collected between 2016 and 2018 by AZoVe, a cooperative of beef producers located in the North-East of Italy (Cittadella, Italy). Specifically, information on animal ID, date of birth, fattening farm, start and end of the fattening cycle, entry BW (available as mean BW of the batch, in kg), carcase weight at slaughter (CW, kg), number of parenteral treatments administered to the animals, date of treatment, reasons of administration (respiratory diseases, locomotor disorders, gastrointestinal diseases and others), amount of antibiotic administered per each parenteral treatment (mL) and number/type of vaccines were available. Animals with incomplete information on AMU and performance traits were discarded from the dataset. Other variables such as age at the beginning of the fattening period, slaughter age (SA, days), duration of the fattening cycle (days) and average daily carcase gain \( \text{ADCG} = \frac{\text{CW (kg)}}{\text{duration of the fattening cycle (days)}} \) were calculated. The Animal ID allowed to retrieve the French department of origin of the animals and the number of French farms that provided animals to each batch. The map of the French departments which originated the animals is depicted in Figure 1.

To avoid potential biases, batches with less than 10 animals were removed from the dataset because they were considered not representative of the French procedure of commingling. Indeed, most of those batches contained only 1 animal. Similarly, we retained only batches with animals from 3 to 8 French departments since 90% of the data fell within this range. Furthermore, 76 animals were removed from the dataset because their CW was outside the range mean ± 3 standard deviations. After editing the data as above, 19,756 Charolais young bulls from 449 batches remained for statistical analysis.

Quantification of antibiotic use

A total of 57 veterinary medicinal products (VMP) that contained antibiotics were used in the 3-year period of the study. A defined daily dose animal for Italy (DDDAit) was assigned to each medical active ingredient (MAI) with the antibiotic activity of those VMP. A DDDAit is the dose (mg) of the MAI administered per kg of BW per day. In particular, the DDDAit was established during the development of the ClassyFarm integrated monitoring system (www.classyfarm.it) of the Italian Ministry of Health and was based on Italian summaries of VMP characteristics. Six out of 57 VMP were removed from the final dataset because the DDDAit of their MAI was not available. Then, an index called treatment incidence 100 for Italy (TI100it) was calculated for each VMP at the animal level using the DDDAit as a metric. Currently, this index is the most
reliable tool to quantify the frequency of antibiotic treatments (Timmerman et al. 2006; AACTING-network 2019) and allows a better comparison of AMU among countries. The following formula, modified from Timmerman et al. (2006), was used:

\[
\text{TI100it} = \frac{\text{Amount of MAI administered per animal (mg)}}{\frac{\text{DDDAit (mg/kg/day)}}{\text{Standard BW (kg)}} \times \text{Standard days at risk}} \times 100,
\]

where ‘standard BW’ is the average expected BW of the animal at treatment (400 kg) and ‘days at risk’ is the standard number of days of the fattening cycle (230 days). The use of standard measures in the formula is the common suggested practice in AMU studies as reported in the practical guidelines developed by the AACTING-network (2019). This decision has been proposed not only for feasibility reasons – the actual BW would require collecting a relevant amount of data which is not always feasible to apply, especially in commercial farms – but also to simplify and allow for comparisons among countries and food-producing species, which is one of the ultimate aims of AMU monitoring programs (AACTING-network 2019). Indeed, although the actual measures would provide a more accurate estimate, the former would reduce the opportunity of comparing AMU data beyond the national level. The TI100it of all VMP were summed up to obtain a total TI100it per animal. If a VMP had two MAI, both were considered in the calculation of the DDDAit as two different treatments. A TI100it of zero was attributed to animals that did not receive any treatments. The number of animals that received at least one treatment during the fattening cycle, the percentage of parenteral treatments according to the reason of administration, the percentage of parenteral treatments per class of antibiotics and the prevalence of the most used vaccines were calculated.

Statistical analysis

Since AMU was calculated at the individual level, the experimental unit of the analysis was the animal. Carcase weight, SA and ADCG were tested for normality by checking skewness and kurtosis, and by visual inspection of their distribution, and all of them were normally distributed. To investigate the effect of the commingling procedure on these traits, each batch was allocated to 1 of 3 classes of commingling, according to the number of French departments of origin that composed the batch. Specifically, low (3–4 departments; \( n = 7151 \) animals), medium (5–6 departments; \( n = 6905 \) animals) and high (7–8 departments; \( n = 5700 \) animals) classes of commingling were defined. Sources of variation of CW, SA and ADCG were investigated using a linear mixed model through the MIXED procedure of SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA). The model accounted for the fixed effects of class of commingling, month of arrival, year of arrival, age of bull at arrival to the fattening farm (3 classes, according to mean age ± 0.5 standard deviations; young: age at arrival <291 days; medium: age at arrival between 291 and 355 days; old: age at arrival >355 days) and the interaction between the class of commingling and age at arrival to the fattening farm. Fattening farm and the residual were the random effects.

The TI100it was not normally distributed and thus a generalised linear mixed model with gamma distribution and log link function in GLIMMIX procedure of SAS was used for the analysis. Since the TI100it was always positive and skewed near the ordinate axis, the gamma distribution was assumed for TI100it in statistical analysis. A constant of \(+3\) was added to the TI100it to avoid an overestimation of the index due to the high number of zeros (animals not treated) and the difficulty of modelling the log. The model accounted for the same fixed and random effects described for CW, SA and ADCG. The goodness of fit of each model was evaluated through Akaike’s Information Criterion and Bayesian Information Criterion.

Results are presented as least squares means ± standard error for CW, SA, ADCG and TI100it. Tukey–Kramer post-hoc adjustment was used for multiple comparisons of least squares means of the fixed effects. The level of statistical significance was set at \( p < .05 \).

Results

Descriptive statistics

Overall, animals of 137, 162 and 150 batches arrived at 20 Italian specialised beef fattening farms in 2016, 2017 and 2018, respectively. Descriptive statistics of
performance traits and other characteristics are presented in Table 2. One hundred and sixty-five animals out of 19,756 died before the end of the fattening cycle. On average, a batch consisted of 44 animals from 5 French departments and 18 French farms of origin.

On average, 55.3% of animals per batch received at least one treatment during the fattening cycle. Specifically, 11,473 animals out of 19,756 received at least one parenteral treatment. Respiratory diseases were the main reasons for treatment (63.7%) followed by locomotor disorders (26.2%), gastrointestinal diseases (2.5%) and others, such as horn fracture and urogenital diseases (7.6%). The most used class of antibiotics was macrolides (23.2%), followed by aminoglycosides (18.2%), penicillins (13.9%), aminopenicillins (12.5%), anphenichols (10.0%), fluoroquinolones (7.7%), tetracycline (6.5%), sulphonamides (4.5%), cephalosporins of 3rd and 4th generation (1.8%) and lincosamides (1.7%). Four hundred thirteen animals out of 19,756 were not vaccinated in Italy whereas 98% of them received a polyvalent vaccine for bovine viral diarrhoea and bovine respiratory syndrome viruses. The Ti100it, before the addition of +3, ranged from 0.00 to 45.37 (mean: 2.08 ± 2.92).

Effects of year of arrival, month of arrival, commingling and age at arrival on performance traits and AMU

The effects of year of arrival, class of commingling and age at arrival are reported in Table 3. The Ti100it decreased from 2016 to 2018 (p < .05). Animals in the high class of commingling had lower CW and ADCG and greater Ti100it than animals subjected to lower commingling (p < .05). Younger animals reached lower CW and ADCG and higher Ti100it than older animals (p < .05).

The effect of the month of arrival on SA, CW, ADCG and Ti100it is depicted in Figure 2. The Ti100it was greater from September to February and ADCG was lower from September to May (p < .05).

The least-squares means of performance traits and Ti100it for the interaction between the class of commingling and age at arrival are presented in Figure 3. The interaction was significant for all traits (p < .05). As expected, younger and highly commingled animals had lower CW, ADCG and SA but higher Ti100it than older animals equally commingled (p < .05).

Discussion

Effects of age, month and year at arrival, and commingling procedure on performance traits

On average, Charolais young bulls were imported at 10–11 months of age at greater BW compared to other French breeds such as Limousin, in agreement with Gallo et al. (2014). During summer, CW decreased likely due to the high temperature and humidity typical of the Po Valley whereby affecting animal performance (Mazzenga et al. 2006). In general, a reduction in performance was observed in young animals. It is likely that an immature immune system, caused by the young age at arrival combined with the stress generated by long transportation, may have affected the animals’ growth. Indeed, young animals subjected to the aforesaid procedures might become more susceptible to pathogens with a consequent reduction in their performances (Herve et al. 2020).

Animals that went through lower commingling in France had significantly lower SA and greater ADCG compared with animals that went through high commingling, suggesting that the performances are

Table 2. Descriptive statistics of the edited dataset of Charolais bulls.

| Item                        | Mean  | SD   | Minimum | Maximum |
|-----------------------------|-------|------|---------|---------|
| Batch level                 |       |      |         |         |
| Number of animals           | 44.37 | 22.45| 10.00   | 173.00  |
| Treated animals, %          | 55.28 | 37.12| 0.00    | 100.00  |
| Number of French departments of origin | 5.04  | 1.59 | 3.00    | 8.00    |
| Number of French farms of origin | 18.55 | 8.81 | 4.00    | 58.00   |
| BW at arrival to the fattening farm, kg \(a\) | 429.80| 33.09| 350.17  | 544.75  |
| Mortality, %\(b\)           | 0.87  | 2.21 | 0.00    | 30.00   |
| Individual level            |       |      |         |         |
| Age at arrival to the fattening farm, days | 323.05| 64.20| 120.00  | 580.00  |
| Slaughter age, days         | 518.24| 60.97| 360.00  | 752.00  |
| Length of the fattening cycle, days | 195.19| 22.22| 77.00   | 362.00  |
| Carcase weight, kg          | 439.11| 33.13| 331.04  | 545.57  |
| Average daily carcase gain, kg/day \(c\) | 2.27  | 0.30 | 1.02    | 5.21    |

\(a\)BW at arrival was available at batch level, because it was not possible to weight individually the animals.
\(b\)Mortality rate was calculated as mean per batch.
\(c\)Calculated as the ratio of carcase weight to the length of the fattening cycle.

Abbreviations. SD: standard deviation; BW: body weight.
affected by the level of commingling applied during batches formation. The mortality rate was in line with the results of Rumor et al. (2015) who observed that Charolais young bulls were more susceptible to diseases compared to other French breeds. Those authors also argued that genetic selection of specialised French beef breeds may affect their capacity of reacting to stress and to adapt to Italian intensive fattening conditions, thus making them more susceptible to diseases and increasing their likelihood of being treated with antibiotics. Our results may support this hypothesis. In fact, the percentage of animals that received at least one parenteral antibiotic treatment per batch was high (55.3%) and showed large variability (0–100%). Unfortunately, it was not possible to assess if the higher percentage of treated animals was due to the breed or other factors such as farm management, transport conditions or veterinary advice.

**Effects of age, month and year at arrival, and of commingling procedure on AMU**

The Ti100it decreased from 2016 to 2018 by 11%, likely due to the enhancement of management practices and animal welfare standards in Italian fattening farms. Indeed, in the last years, the Italian National Reference Centre for Animal Welfare (CReNBA, Brescia, Italy) has established the Italian protocol for the assessment of dairy cow welfare in loose housing systems (Bertocchi et al. 2018) as part of the ClassyFarm monitoring scheme. In addition, the cooperative of beef producers that provided the data (AZoVe, Cittadella, Italy) was involved in a project that aimed to reduce antibiotics in beef cattle through a more judicious antibiotic stewardship (Diana et al. 2020).

The Ti100it varied across seasons, with higher values during the coldest months of the year (September to February). Cusack et al. (2007) suggested that weather conditions can predispose animals to the development of BRD. In particular, those authors reported a correlation between minimum daily temperature and the occurrence of BRD with a peak of incidence in autumn and early winter. Similar results were observed in previous studies (Santinello et al. 2020; Diana et al. 2021).

The procedure of commingling to create homogeneous batches in terms of gender, breed and BW is quite common (Mounier et al. 2005; Herve et al. 2020; Morel-Journel et al. 2021). Commingling is applied after weaning when young animals with different pathogenic backgrounds are joined together, thus increasing the possibility to develop diseases and generate stress (Callan and Garry 2002; Duff and Galyean 2007). Also, this procedure may be a source of stress for the animals because it induces the formation of a new hierarchy and thus an initial aggressive behaviour.

Although we did not find any statistical difference between medium and high classes of age, younger animals had higher Ti100it than medium-class animals, suggesting that their immune system was less capable to deal with environmental stressors and pathogens.
The farms involved in the study used a similar veterinary protocol because they belonged to the same cooperative of beef producers. Therefore, most of the animals received at least one vaccine for the BRD upon their arrival in Italy. Despite the vaccination programme against BRD, the most important reason for antibiotic treatment was for this disease, followed by locomotor disorders, in agreement with Diana et al. (2020). It is likely that the vaccinations supplied to the animals at their arrival to the fattening farms were not enough to protect them against BRD, especially in the case of Charolais young bulls commingled from multiple origins (Herve et al. 2020). Assié et al. (2009) reported that vaccinating the animals against BRD at their arrival is questionable because the incidence of respiratory diseases during the first 6 weeks of the fattening cycle was higher for vaccinated young bulls. Therefore, a pre-commingling vaccination programme before reaching the collection centres in France could be a strategy to reduce the spread of the BRD and other diseases, but the appropriate timing for vaccination should be investigated. Indeed, studies showed that vaccinations administered to the calves during the first months after birth may be affected by the transmission of antibodies through the colostrum of the dams (Fulton et al. 2004). This may interfere with the immune system response given by the vaccine and result in a lack of efficacy.

The BRD is a multifactorial disease that occurs when environmental stressors like the long transportation distance and infectious agents are combined (Callan and Garry 2002). This assumption is confirmed by our findings: the more the animals were commingled in France the more the T1100it increased, and this was mainly due to BRD. The interaction between the class of age and class of commingling showed that both factors are crucial to predicting AMU; in fact, young and highly commingled animals were those at greater risk of being treated.

Factors such as pre-transport rearing conditions, age of cattle, duration of the journey and loading and unloading procedures contribute to the development of diseases during transportation (Hulbert et al. 2011; Earley et al. 2017; Van Engen and Coetzee 2018; Morel-Journel et al. 2021). In the current study, data on transportation were not available. We acknowledge its potential importance to predict animal performance and AMU, especially in the first weeks of the fattening cycle (Morel-Journel et al. 2021), whereby we suggest considering this aspect for future studies.

Finally, the diet supplied to the animals can have affected the findings of the present study. Italian beef fattening farms are characterised by a diet with high energy content provided by concentrates (Gallo et al. 2014). This may increase the risk of developing antibiotic resistance due to the enhanced growth and

Figure 2. Least squares means and standard error (SE) of (a) slaughter age, (b) treatment incidence 100 for Italy (T1100it), (c) carcase weight and (d) average daily carcase gain for the effect of month of arrival of Charolais young bulls (n = 19,756). Means with different letters differ significantly according to Tukey–Kramer post-hoc adjustment (p < .05).

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Figure 3. Least squares means and standard error (SE) of (a) slaughter age, (b) treatment incidence 100 for Italy (TI100it), (c) carcase weight and (d) average daily carcase gain for the interaction between class of commingling (low, medium, high) and class of age at arrival (young, medium, old) of Charolais young bulls \( (n = 19,756) \). *Means with different letters differ significantly according to Tukey–Kramer post-hoc adjustment \( (p < .05) \).
reproduction of pathogens into the rumen (Auffret et al. 2017). In addition, a diet rich in concentrates can lead to acidicotic events and thus to locomotor disorders (Compiani et al. 2014) such as lameness and interdigital phlegmon. Before moving to Italy, animals were fed in pasture and the change of energy content in their diet may have promoted acidicotic events. The diet provided to Charolais young bulls changed according to the fattening phase and the management applied on-farm. Good optimisation of the feed ration at the arrival to the farm combined with a gradual transition towards the energy-rich diet, are applied in Veneto beef herds to prevent acidicotic events and the associated risk of developing locomotor disorders (Compiani et al. 2014). Whereas, in the last part of the fattening cycle there is an increase in energy intake characterised by a forage-concentrate ratio of 30–70%.

Due to the observational nature of the present study, it is important to highlight that the commingling categories were unbalanced in terms of the number of animals. This may have led to potential biases. Nevertheless, the 3-year period of the study, the large sample size (19,756 animals from 20 farms), and appropriate modelling of the data succeeded to overcome this limitation.

Conclusions

Commingling of animals may increase the risk of AMU in Charolais beef cattle and thus this practice is a potential source of variation of AMU. Our findings suggest that a reduction of commingling is an effective strategy to reduce AMU in beef cattle. However, it is worth noting that a decrease in the number of departments of origin is difficult to achieve in French production systems, where several small farms supply animals to the collection centres. Therefore, preventive strategies to face the spread of pathogens during commingling procedures and transportation of the animals, such as vaccination programmes applied on cow/calf farms, should be promoted. Indeed, purchasing animals already vaccinated before the creation of the batches can be of help, although the proper time to vaccinate the animals is still questionable. Finally, the creation of a French dataset with AMU data collected at the animal level to be integrated with the Italian data for a more holistic view should be pursued.

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Ethical approval

Animal Care and Use Committee approval was not obtained for this study because the data were from an existing database. The analysed records were registered by AZoVe (Cittadella, Italy) from 2016 to 2018. The authors did not have direct control over the care of the animals included in this study.

Author contributions

Conceptualisation, MS, MDM, AD, NR, JFH, MP; Methodology, MS, MDM, AD, NR, MP; Formal Analysis, MS, AD, MP; Data Curation, MS, AD; Writing-Original Draft Preparation, MS, AD, MP; Writing-Review & Editing, MS, MDM, AD, JFH, MP; Supervision, MDM, JFH, MP.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

Restrictions apply to the availability of the data, which were used under licence for this study. Data are available from the authors with the permission of AZoVe (Cittadella, Italy).

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