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Large-size fattening calves' lots fed with automatic milk feeders may have an increased risk for *Mycoplasma bovis* infection spread and for antibiotic use

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ABSTRACT

Bovine respiratory disease is the leading user of antibiotics (**AB**) in calf production. *Mycoplasma* (***M.***) *bovis* could lead to greater use of AB as it is a persistent and AB resistant causative agent for respiratory diseases. Two cross-sectional studies were set up to assess the effects of lot size and feeding system on *M. bovis* infection and the effects of *M. bovis* seroconversion, lot size and feeding system on AB use in calves' feedlots. Twenty-six lots in 22 fattening farms were monitored for 41–81 days, from all-in entry of calves until three consecutive weeks without using any collective antibiotics. *M. bovis* spread was estimated by measuring seroconversion at entry and at the end of study period in 10–15 calves randomly sampled in each lot. All AB treatments used in the meanwhile were recorded. The lots were selected according to feeding system, *i.e.* individual bucket (n = 7) *vs.* automated milk feeder (**AMF**, n = 19), and lot size (30–519 calves), less than 50 calves (n = 9) *vs.* more than 50 calves (n = 17). Statistical analysis was performed using multivariable generalised linear models with fattening farms as random effect. *M. bovis* spread increased with lot size (odds ratio (**OR**) 2.9[1.4; 5.8] per two-fold increase in lot size). This proportion of seroconverted calves was lower in bucket-fed lots compared to lots fed with the AMF using a shared nipple (OR = 0.03[0.003; 0.41]). The main risk factor for AB use was the lot size, with an increase of 1.5[0.94; 1.98] treatments per two-fold increase in lot size. For same size lots, the use of bucket can decrease AB consumption by up to 1.03[−2.18; 0.14] treatments per calf compared to AMF. Analysis of the association between seroconversion to *M. bovis* and AB use was inconclusive. We found that bucket feeding in small-size lots, *i.e.* up to a maximum of 50 calves in the same space, limits seroconversion to *M. bovis* and enables lower use of AB in veal calf production.

Implications

We address here the influence of size lot and feeding methods, via individual bucket or collective automated milk feeders, on *Mycoplasma bovis* infection spread among veal calves lot, this mycoplasma being a major component of calves' respiratory disease. Risk for calf-to-calf transmission of *Mycoplasma bovis* is limited in small-sized, bucket-fed lots. Reducing the lot size in a same room also reduces the antibiotic use.

Introduction

Veal calf fattening is a hugely important sector of cattle farming in Europe, particularly in France where 29% of the total European veal production is slaughtered (Jarrige et al., 2017). French production is mainly organised in all-in all-out systems with calves commingled from their herds of origin by mixing them in homogenised lots through a sorting centre. They are usually introduced at 2–6 weeks old, put into batches completed within 1–7 days

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(Pardon et al., 2013, Mounaix et al., 2007). Calves are housed either in individual boxes for a maximum of six weeks (Directive 97/2/EC) before being gathered into groups of 6–8 animals by removing the fence separations or directly into pens housing 15–75 individuals (Jarrige et al., 2018). In the first case, the milk is delivered in individual boxes by buckets with or without a nipple. In the second case, milk is delivered to the pen by automated milk feeder (**AMF**) systems with multiple shared nipples. During the first weeks of feeding, farm managers use substantial amount of antibiotics (**AB**), reaching on average 8.5 treatments per calf in 2013–2014 in France, equivalent to an average daily dose of 152 per 1 000 calves, (Jarrige et al., 2017). Since then, the AB use has been reduced throughout Europe veal production. In Belgium and Netherlands, the other two main veal producers, the average daily dose per 1 000 calves decreased from 164 to 90 in the period between 2007–2009 and 2014–2016 (Bokma et al., 2019).

Respiratory diseases are the most frequent in the veal calf sector, where they account for almost 75% of AB treatments (Lava et al., 2016b; Pardon et al., 2012a). Among the pathogens involved, *Mycoplasma* (***M.***) *bovis* is highly prevalent (65–100% of calves) in calf feedlots and appears early (5–21 days after entry) in disease onset (Arcangioli et al., 2008; Pardon et al., 2011). It is detected in both acute and recurrent or chronic respiratory diseases (Thomas et al., 2002), and its presence has been linked to increased use of AB (Tschopp et al., 2001; White et al., 2010). *M. bovis* is naturally resistant to sulphonamides, streptomycin and beta-lactams, which are three AB widely used for prophylaxis and curative treatments in calves (Gauthier-Bouchardon et al., 2014; Pardon et al., 2012b). Furthermore, *M. bovis* was shown to have acquired resistance to most AB in France, including the first-line AB used for respiratory diseases, such as tetracyclines and macrolides (Gauthier-Bouchardon et al., 2014). Intrinsic and acquired resistance may explain the persistence of *M. bovis* after AB treatments, in chronic cases or at necropsy (Thomas et al., 2002). Preventive control of this bacterium would consequently help reduce AB use in veal calf feedlots, as recommended by the European Union and endorsed in France via the EcoAntibio plan launched in 2011 (Ministère de l'Agriculture et de l'Alimentation).

In the absence of commercial vaccines in Europe, preventive control mainly relies on biosecurity measures. Measures of all-in all-out management, disinfection between lots and lot separation are usual routine in this sector. They often prove inadequate, as animals are mixed from a wide range of herds of origin, after stressful transport by truck (Callan and Garry, 2002; Lava et al., 2016a). Risk factor studies could contribute to improve these control measures, provided that they test simple and realistic hypotheses. Given that the presence of a *M. bovis*-seropositive calf at entry was identified as a risk for diffusion (Tschopp et al., 2001) and that *M. bovis* seroprevalence was estimated at 2–3% of veal calves entering fattening units in France (Arcangioli et al., 2008), allocating calves into small-size lots should be effective in reducing *M. bovis* infection. In contrast, mixing more than 50 calves increases the risk of introducing seropositive calves and hence the risk of transmission (Callan and Garry, 2002).

Controlling the speed of infection spread is another option to limit *M. bovis* infection by allowing the immune system to strengthen after stressful allotment conditions. Respiratory routes are major ways of contamination, but oral transmission of *M. bovis* has also been demonstrated in both pneumonia and otitis (Maunsell et al., 2012). Consequently, shared feeder nipples could contribute to rapid *M. bovis* spread, especially in pens with AMF where 12–30 calves use the same nipple.

Two cross-sectional studies were set up during the winter seasons of 2014, 2017 and 2018 to assess (i) the effects of lot size and feeding system on *M. bovis* infection, estimated by rate of seroconversion and (ii) the effects of *M. bovis* infection (*i.e.* rate of seroconversion), lot size and feeding system on AB use.

Material and methods

Fattening farms and lots

The study population was made of calves for veal meat production or weaning lots for beef production, mixed in different size lots, either in Rhône-Alpes region (seven weaning lots, year 2014) or in Pays de Loire and Bretagne region (19 veal calf lots, years 2017 and 2018). A lot was a batch of calves entered in a same building during a maximum three days period of time from their herd of origin. Lots of veal and weaning calves shared common characteristics fulfilling our inclusion criteria: they were fattened in specialised fattening farms, gathering each year several lots of 2–5 week-old calves, mixed by age and weight in a sorting centre, and managed as all-in all-out systems. All lots were studied during the winter season (December to March).

A total of 26 calf lots in 22 fattening farms were studied. Each lot was identified by a short letter code (Table 1).

Milk distribution was either individual by means of buckets or collective via AMF with shared nipple(s). Bucket-fed calves were housed individually for 3–6 weeks on slatted floors, separated by removable tubes allowing visual and nose contacts, constituting small pens of 6–8 calves after the tubes were removed (6–8 weeks later, Directive 97/2/EC). The AMF lots were composed of 15–70 calves' pens, generally on straw bedding.

Lots were homogenised by weight and age in the sorting centre and the mean weight of lot's calves was provided at lot constitution (Table 1).

Sampled animals and serological analyses

Ten to thirty calves were randomly sampled within each lot, whatever its size. If, within a lot, the animals were allotted in different rooms or pens, then we first selected one or two rooms or pens and then choose an equal number of calves within the selected room(s) or pen(s).

Blood was collected from calves on the day of entry into lots, and at the end of the study period. Sera were stored at −20 °C until analysis.

Infection spread was assessed by seroconversion rate. Seroconversion was assessed from paired sera using *M. bovis* diagnostic kit Bio K302 (Bio-X diagnostics, Belgium), hereafter named BioX, and ID Screen *Mycoplasma bovis* Indirect (ID.Vet, France), hereafter named ID Screen. Both tests were used as they were shown to have different performances. Despite its lower sensitivity, compared to the ID Screen test (Andersson et al., 2019), the BioX kit was considered more relevant to evaluate infection rate than ID Screen that detected "exposure rate" (Petersen et al., 2018; 2020). The paired sera from the same calf and lot were analysed at the same time, on the same plate, and results were interpreted as per the manufacturers' recommendations. Briefly, a calf seroconverted when it changed from seronegative at entry to seropositive at the end of the study period using the interpretation criteria provided with the kits. Entry sera with discrepancies between the two kits were re-tested to confirm the results.

Study period, treatment recording and data definitions

The study period corresponded to the period of time between two serological samplings, until weaning for weaning lots (57–62 days after entry), and, for veal lots, after 4 weeks without

Table 1
Description and follow-up data summary for the 26 calves' lots included in the study.

Lot	Feeding system[1]	Calves' number	Lot Type[2]	Entry[3] weight	Age at entry[3]	1st tt Day[4]	Study Time[5]	nAB[6]	TI*$_{ADD}$[7]	BioX Kit test SC[8]	BioX Kit test Nsero[9]	BioX Kit test SeroIn[10]	ID Screen kit test SC[8]	ID Screen kit test Nsero[9]	ID Screen kit test SeroIn[10]
AP	AMF	220	V	53	15	3	74	6.58	209	9	9	N	9	9	N
CA	AMF	109	V	45	15	6	81	5.31	184	6	9	N	9	9	N
CA	AMF	110	V	45	15	7	75	6.05	194	6	8	Y	9	9	Y
CAM	B	384	V	52	25	14	54	4.11	194	9	9	Y	10	10	N
CAS	B	276	V	50	26	15	58	6.84	225	27	29	Y	30	30	N
Esp1	AMF	60	W	55	15	17	64	2.40	93	8	15	N	11	11	N
FO	B	220	V	51	20	14	56	4.09	189	5	10	N	10	10	N
Gi	AMF	130	V	78	35	16	58	4.48	245	2	10	N	7	10	N
HA	B	108	V	75	33	10	46	3.68	239	3	10	N	9	10	N
LE	B	128	V	66	31	14	68	5.20	213	0	10	N	10	10	N
LEP	AMF	519	V	45	NA	8	61	7.18	29	6	9	Y	10	10	N
MA	B	135	V	48	NA	6	65	4.30	173	1	7	Y	7	9	N
ME	AMF	207	V	42	26	7	59	5.19	206	4	9	Y	10	10	N
NE	AMF	183	V	54	21	6	71	7.74	312	8	10	N	10	10	N
OL	B	147	V	55	NA	21	75	3.49	135	5	9	N	9	9	N
Vei	AMF	70	W	51	15	14	57	3.43	205	12	15	N	8	8	N
Esp2	AMF	30	W	52	15	18	64	1.27	49	0	14	N	0	10	N
Esp3	AMF	40	W	50	15	21	64	3.38	129	2	15	N	0	13	N
GU	AMF	38	V	57	31	none	53	0.00	0	1	9	N	0	9	N
HO	AMF	41	V	60	30	5	61	2.12	99	0	10	N	10	10	N
Mon	AMF	40	W	57	15	12	57	1.10	51	1	15	N	0	15	N
PE	AMF	50	V	60	33	11	41	2.21	12	4	10	N	10	10	N
RO	AMF	31	V	58	31	9	54	1.35	71	6	9	N	9	9	Y
Ver1	AMF	40	W	54	15	9	47	3.13	167	2	14	Y	13	15	N
Ver2	AMF	40	W	56	15	9	47	3.18	172	11	15	N	14	14	N
PER	AMF	80	V	48	NA	NA	47	NA	NA	8	9	Y	8	8	Y

[1] Feeding system: Milk feeding delivery system: automatic milk feeder (AMF) or bucket (B).
[2] Lot type: lots of calves intended to fatten calves for bulls (weaning calves W) or for veal calves (V).
[3] Entry weight, average in kg after mixing in the sorting centre; Age at entry: average age of sampled calves at introduction, in days.
[4] 1st tt day: number of days between introduction and the first collective treatment for Bovine respiratory Disease.
[5] Study time: duration of study period in days.
[6] nAB: number of antibiotic treatments per calf including collective and individual ones.
[7] TI*$_{ADD}$: proxy for treatment incidence at average daily dose during the study period, per 1 000 calves.
[8] SC: number of sampled calves that seroconverted to *Mycoplasma bovis*, BioX with BioX kit, ID Screen with ID.Vet kit.
[9] Nsero: number of paired sera analysed for seroconversion, which considers only seronegative calves at entry with a second sampling (i.e. dead calves were removed); NA: Not applicable.
[10] SeroIn: presence of at least one seropositive calf at entry. Y for Yes there was at least one calf seropositive at entry, N = No, there was no calf detected seropositive at entry in the sampled calves.

implementation of any collective AB treatment. All treatments were done upon prescription and recorded by farmers, who gave us full access to the data.

In every lot, we defined the number of AB treatments per calf during the study period (noted ***nAB***) as the number of AB treatments initiated per calf in the i^{th} lot, as in Jarrige et al. (2017). It was calculated as the sum of all specialties containing at least one AB administered at the prescribed daily dose with the s^{th} antibiotic specialty at both individual and collective levels, as in equation (1):

$$nAB_{s,i} = \frac{nABcoll_{s,i} \times Ncalves_i + nABind_{s,i}}{Ncalves_i}$$
$$nAB_i = \sum_{s=1}^{Nsi} nAB_{s,i} \quad (1)$$

where Ns_i is number of antibiotic specialties used in the i^{th} lot, $Ncalves_i$ is number of calves in the i^{th} lot, $nABind_{i,s}$ is number of individual treatments initiated with the s^{th} antibiotic specialty in the i^{th} lot, and $nABcoll_{i,s}$ is number of collective treatments initiated with the s^{th} antibiotic specialty in the i^{th} lot.

A collective treatment was recorded for all the calves present at the time of treatment, but excluding oral colistin treatment, as it is not indicated for respiratory disease and is unable to pass the gastrointestinal barrier. Administration of a pharmaceutical specialty was considered as one treatment even if it contained two antibiotics. This was different from Jarrige et al. (2017) but was chosen in order to homogenise the different veterinarian practices.

For the purpose of comparison with other studies, for each lot we calculated $TI^*_{ADD,i}$, a proxy of the treatment incidence of average used daily dose animal ($TI_{ADD,i}$) defined by Pardon et al. (2012b) as follows. Formula of $TI^*_{ADD,i}$ is presented in equation (2) and explained in supplementary material S1,

$$TI^*_{ADDi} = \left(\sum_{s=1}^{Nsi} nAB_{s,i} \times TD_s(\text{day})\right) \times \frac{MWF_i(\text{kg})}{StT_i(\text{day}) \times 164(\text{kg})} \times 1000 \quad (2)$$

where TD is the duration of each treatment, MWF_i is the weight of the calves of the lot at mid-study time and StT is the duration of the study period for the i^{th} lot .

Statistical analyses

Statistical analyses were performed using R 4.0.3 software (R Foundation for Statistical Computing, Vienna, Austria), with the packages lme4 (Bates et al., 2015), lmerTest (Kuznetsova et al., 2017), influence.ME (Nieuwenhuis et al., 2012) and emmeans (Lenth, 2019). Complete specification of the models is provided in part B of the supplementary material.

The numbers of animals per lot were included in the models on a centred log 2 scale. In the output of the model, the intercept corresponds to a lot with a log-transformed lot size of 0, equivalent to a lot of 95 calves. Any increase (or decrease) of one unit of the log-

transformed lot size corresponded to a multiplication (or division) by two of the "true" lot size.

In all models, we included fattening farms as a random effect considering that different lots from a same farm could share common and unaccounted characteristics that could have an influence on seroconversion rates or antibiotic use.

To assess the risk factors for seroconversion to *M. bovis* in lots, we built two independent multivariable logistic regression models (one for each serological test) to predict the number of seroconverted calves out of the number of non-seropositive calves at entry (Nsero in Table 1) in each lot. Fixed effects were feeding system, transformed lot size, duration of the study period for each lot (in days), presence/absence of a seropositive calf at entry into the lot, type of lot (weaning or veal), number of antibiotic treatments during study period in the lot (*nAB*), and feeding system × lot size interaction.

To assess the risk factors for increased antibiotic consumption in each lot, we built two multivariable linear regression models with fattening farm as random effect, one using number of antibiotics per calves (*nAB*) and the other using the proxy for treatment incidence of average daily doses animal (TI^*_{ADD}). Fixed effects included in both complete models were feeding system, lot size, presence/absence of a seropositive calf at the entry into the lot, type of lot (weaning or veal), proportion of seroconverted calves in the lot.

In all three models, in order to obtain a parsimonious model, we used a backward selection procedure based on Akaike information criterion (Burnham & Anderson, 2002) to exclude all non-significant variables except the one required to fulfil the two main objectives of this study (feeding system, transformed lot size and presence/absence of a seropositive calf at entry for the seroconversion model, and feeding system, transformed lot size and the proportion of seroconverted calves in the lot for both antibiotic models). We checked both linear models for homoscedasticity and random distribution of residuals, and we assessed the correct fit of the logistic regression using Pearson's residual analysis. Influence analysis was performed by calculating the Cook's distance of all data points (see supplementary material for details). All parameters were null-checked using a Wald test and considered significant at a *P*-value <0.05. Calibration of the mixed logistic regression model was assessed by examining calibration at the weak and moderate senses (Van Calster et al., 2019). We also assessed the influence of the nature of the lots (weaning versus veal lots) by re-running models without the weaning lots.

Results

Description of the dataset

We followed 3420 calves allocated in seven weaning lots and 19 veal calf lots. Lot size varied from 30 to 519 calves introduced as one batch (Table 1): nine lots counted fewer than 50 calves (considered as small lots hereafter) and the other 17 lots included more than 50 calves (large lots). Feed management was AMF (shared nipples) in 19 lots (nine small and 10 large) and individual buckets in seven lots (only large ones; Table 1).

On average, calves were 22 days old (SD = 7.2 days) and weighed 55 kg (SD = 8.6 kg) at entry. Mean study period between the two serum samplings was 60 days, and median was 58 days (41–81 days), which corresponds to approximately a half-feeding time in the veal calf sector.

The proportion of *M. bovis*-seroconverted calves per lot ranged from 0% to 100% with both ELISA tests. With BioX, three lots showed no seroconversion at all and two showed 100% seroconversion, against four and 18 lots, respectively, for ID screen results. The four lots with no seroconversion with ID Screen display no or very low number of seroconverted calves with BioX (0, 1, 1, 2, respectively, Table 1). Within lots with BioX seroconversion, the mean proportion of seroconverted calves was 56.0%. Ten calves from eight different lots were seropositive at entry (seropositivity at entry was of 3.5% and 1.5% with BioX or ID Screen, respectively [for all results 3.4%; with 95% CI:[0–9.8%]). Within lots with seroconversion with ID screen, the mean proportion of seroconverted calves was 96.5%. Four calves from three different lots were ID Screen seropositive at entry, three of them were also BioX seropositive.

Mean *nAB* was 3.8 (SD = 2.1).
Mean TI^*_{ADD} was 160 (SD = 81) daily doses per 1 000 calves for the study time.

Statistical analysis

Risk factors for seroconversion to M. bovis

Because of the marked "positive or negative" seroconversion pattern of lots obtained with ID Screen (18 lots showed 100% seroconversion) and because of the limited number of lots, the study has a low statistical power to model the infection spread rate using this test. Consequently, we only present here the results obtained using the BioX results. All results obtained with ID Screen, including analyses for AB use, are presented in supplementary Tables S1–S3. Random effect estimate at the farm level is presented in the supplementary material (section B.IV.a).

After the backward selection procedure, the variables remaining in the logistic model for seroconversion with BioX were lot size, feeding system and their interaction (Table 2). The proportion of seroconverted calves increased with doubling size of the lot (odds ratio (**OR**) 2.9[1.4; 5.8]; $P < 0.01$). Bucket-fed lots had a lower proportion of seroconverted calves than AMF-fed lots (OR 0.03[0.003; 0.41]; $P < 0.01$), but their proportion of seroconverted calves increased more with the lot size than in AMF-fed lot with an OR of 38[4; 407] ($P < 0.05$). In lots above 200 calves, both AMF and bucket-fed lots showed similar level of seroconversion using BioX (Table 2, Fig. 1). Seropositivity at entry had no significant effect in our model (Table 2). Analyses obtained from data without the weaning calves' lots were similar but also with wider confidence intervals than the one obtained with all lots. Supplementary Tables S4 displayed the results obtained from those data with BioX.

Antibiotic use

This analysis was conducted on 25 out of the 26 lots, as one large lot (PER in Table 1) had only partial data on antimicrobial use. After the backward selection procedure, only lot size and feeding system remained in the model. We also analysed seroconversion as a potential explanatory variable in both nAB and TI^*_{ADD}

Table 2
Final multivariable logistic regression model for *Mycoplasma bovis* seroconversion on calves using BioX kit results. Odds ratio for feeding system, lot size, interaction of these two variables and seropositivity of calves at entry for *Mycoplasma bovis* seroconversion using BioX Elisa kit.

Factor	Odds ratio	95% CI[1]	*P*-value
Bucket feeding	0.03	[0.003; 0.41]	0.007
Two-fold increase of the lot size	2.9	[1.4; 5.8]	0.002
Two-fold increase of the lot size in bucket-fed lots	38	[4; 407]	0.032
Seropositive calves at entry	0.8	[0.22; 2.95]	0.761

The reference population to calculate odds ratios is composed of Automated Milk fed lots with a size of 95 calves. In this reference population, the proportion of seroconversion is estimated to be 0.566.
[1] 95% confidence intervals.

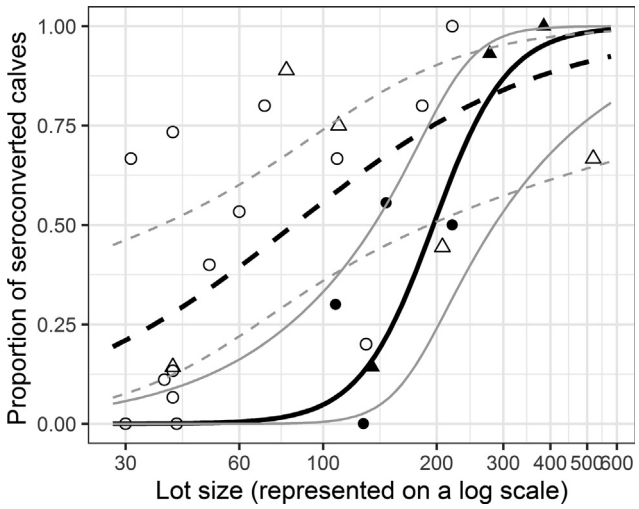

Fig. 1. Proportion of *Mycoplasma bovis*-seroconverted calves (as estimated using BioX Elisa) plotted as a function of lot size represented on a log scale. White data points are Automated milking (AMF) fed lots, and black data points are bucket-fed lots. Triangles are lots with a seropositive calf at entry, and circles are lots without any seropositive calf at entry. The solid and dashed black lines correspond to average predicted proportions of seroconversion in bucket-fed and AMF-fed lots, respectively. Variation intervals for models are between solid and dashed grey lines.

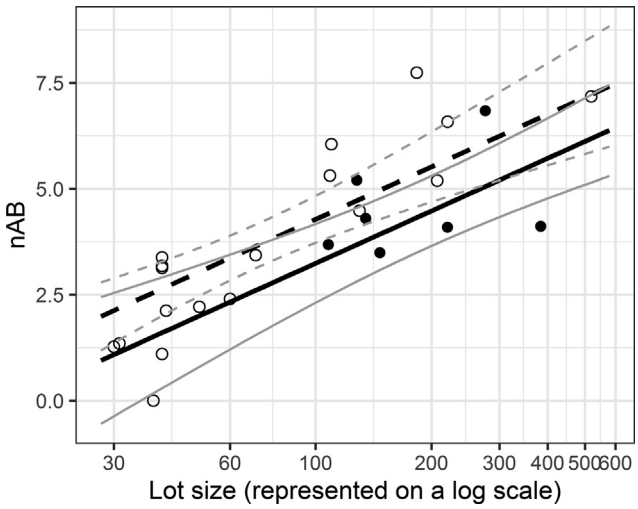

Fig. 2. Number of antibiotic treatments used per calf (nAB) as a function of lot size (in log 2 scale) and feeding system. Black points are bucket-fed lots and white points are Automated Milk (AMF) fed lots. The solid and dashed black lines plot the average estimated number of antibiotic treatments per calf in individual bucket and AMF-fed lots, respectively. Variation intervals for models are solid and dashed grey lines, respectively, for bucket and AMF-fed lots.

models. Outputs are presented with BioX seroconversion results (Table 3 and Fig. 2 for nAB; Table 4 and Fig. 3 for TI^{*}_{ADD}), and for ID Screen in Supplementary Tables S2 and S3. Random effect estimates at the lot and farm level are presented in the supplementary material (B.IV.b and B.IV.c for nAB and $TI^{*}_{ADD,}$ respectively).

Irrespective of feeding system, *nAB* increased by 1.5[0.94; 1.98] per two-fold increase of the lot size ($P < 0.001$). At a fixed sample size, *nAB* was lower by 1.03[−2.18; 0.14] treatments ($P = 0.077$) in bucket-fed lots. *M. bovis* seroconversion showed a very limited association with AB use, not statistically significant, with an increase of 0.05[−0.1; 0.2] or 0.09[−0.04; 0.2] treatment per calf by 10% increase of the apparent seroconversion rate observed using BioX and ID Screen, respectively.

TI^{*}_{ADD} increased by 51[27; 75] ($P < 0.001$) per 1 000 calves per two-fold increase of the lot size (Fig. 3). Bucket feeding presented a similar tendency to be associated with TI^{*}_{ADD} decrease (−27[−80; 25]).

Results obtained using data without the weaning calves' lots were similar but with wider confidence intervals than the one obtained with all lots (Supplementary Tables S5 and S6).

Table 3
Results of the multivariable linear mixed-effects model of the number of antibiotic (AB) treatments administered per calf (nAB), obtained using BioX Elisa *Mycoplasma bovis* seroconversion results.

Factor	nAB variation compared to the reference population	95% CI[4]	*P*-value
Milk distribution by bucket[1]	−1.03	[−2.18; 0.14]	0.077
Two-fold increase of the lot size[2]	1.5	[0.94; 1.98]	<0.001
Ten per cent increase of the seroconversion[3] (BioX)	0.05	[−0.12; 0.22]	0.498

[1] Feeding system.
[2] Lot size.
[3] *Mycoplasma bovis* seroconversion (using BioX seroconversion kit results) effect estimate.
[4] 95% confidence intervals and *P*-values for the number of AB administered per calf in calves' lot. The reference population to calculate the modification of AB use is composed of Automated Milk fed lots with a size 95 calves and with no seroconversion observed. In this reference population, nAB is 4.2 [95%CI: 3.2; 5.1] treatment per calf.

Table 4
Results of the multivariable linear mixed-effects model of the incidence of treatments at average daily dose per 1 000 calves (TI^{*}_{ADD}), obtained using BioX Elisa *Mycoplasma bovis* seroconversion results.

Risk factor	Size effect estimate	95% CI[4]	*P*-value
Milk distribution by bucket[1]	−27	[−80; 25]	0.301
Two-fold increase of the lot size[2]	51	[27; 75]	<0.001
Ten per cent increase of the seroconversion[3] (BioX)	0.3	[−7; 9]	0.934

[1] Feeding system.
[2] Lot size.
[3] *Mycoplasma bovis* seroconversion (using BioX seroconversion kit results) effect estimate.
[4] 95% confidence intervals and *P*-values for treatment incidence per 1 000 calves, obtained using BioX seroconversion results. The reference population to calculate the evolution of TI^{*}_{ADD} is composed of Automated Milk fed lots with a size 95 calves, with no seroconversion observed. In this reference population, TI^{*}_{ADD} is 177 (95%CI [137; 217]) per 1 000 calves.

Discussion

A total of 26 calf lots (nine small and 17 large, including 19 AMF-fed and seven bucket-fed) were monitored for *M. bovis* seroconversion and antimicrobial treatments over 41–81 days after entry. Weaning and veal calf lots were managed similarly (mixing, all-in all-out, AB treatment at entry, etc.). In weaning lots, raised on milk for the study period, first treatment for bovine respiratory disease was administered at the same time than in veal calves lots (Table 1). Including weaning lots did not show any impact on the study results as the lot-type was not conserved in the final model and results without the weaning lots were rather similar, albeit with wider confidence intervals. Hence, we concluded that includ-

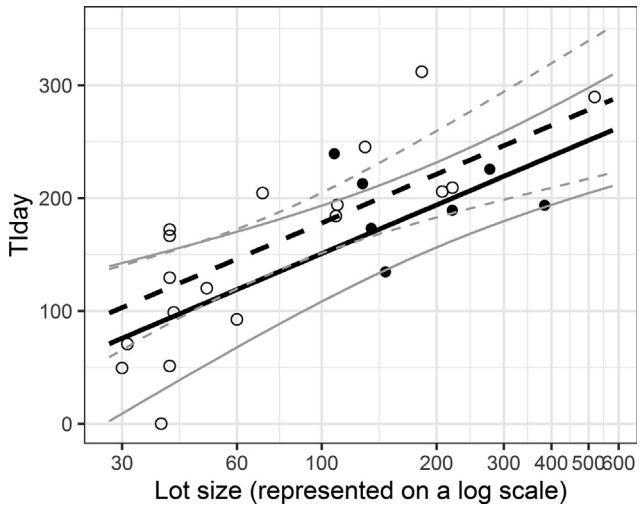

Fig. 3. Treatment incidence at average daily dose (TI^*_{ADD}) per 1 000 calves as a function of lot size (in log 2 scale) and feeding system. Black points are bucket-fed lots and white points are Automated Milk (AMF) fed lots. The solid and dashed black lines plot the average treatment incidence in individual bucket and AMF-fed lots, respectively. Variation intervals for models are solid and dashed grey lines, respectively, for bucket and AMF-fed lots.

ing weaning lots did not bias our results but, on the contrary, it increased the statistical power of our study.

In the original study design, we intended to test seroconversion using BioX only, which was the only one commercially available at that time. However, a recent study demonstrated a better sensitivity and specificity of the new ID Screen, and we decided to include both tests (Andersson et al., 2019). With our sample set, ID Screen gave a "positive/negative" seroconversion pattern which limits the interest of a logistic regression with the aim of explaining the proportion of calves that seroconverted. In contrast, the BioX Elisa test results included almost all intermediate results between 0 and 100% seroconversion rates. It was previously hypothesised that BioX might not be appropriate for serological diagnosis in calves aged less than 2 months because of its low sensitivity (Petersen et al., 2018). In our case, this potential low-sensitivity bias did not seem a problem as we had eleven lots with a seroconversion rate above 66% using BioX.

We further hypothesised that the two serological tests might detect different serological conditions, which are exposure to *M. bovis* for ID Screen and the "clinical disease" or "past-infection" for BioX. This could explain both the agreement between the two tests in lots where all exposed calves developed an infection or a clinical disease and the discrepancy between the two tests in lots where only a part of exposed calves developed an infection or a clinical illness. This discrepancy in "diagnostic accuracy" was already questioned in the precedent study of Petersen et al. (2018) and in a field study on cows with history of *M. bovis* disease but without any clinical signs at sampling time (Petersen et al., 2020). As we wanted to focus more on the clinical impact of *M. bovis* than its diffusion capacity, we hypothesised that BioX was more appropriate for our study about risk factors for clinical infection caused by *M. bovis*, although it has a lower sensitivity for detecting exposure only. The relevance of the identified risk factors, from a biological perspective, somehow confirmed our initial hypothesis.

We found a meaningful association between lot size and *M. bovis* infection, with an OR of 2.9 (P = 0.002). As we had no small-sized bucket-fed lot, we were unable to assess the role of this feeding system on *M. bovis* disease's spread in small lots. Nevertheless, bucket-fed calves displayed less seroconversion than AMF-fed ones, with an OR of 0.03 for same lot size. However, in bucket-fed lots, the seroconversion rate increased strongly above 40–60 calves with an OR of 38 per doubling increase of the lot size (Fig. 1). The fact that seroconversion rate was higher in AMF-fed lots than in individual bucket-fed lots strengthens our initial hypothesis that oral-route may enhance *M. bovis* contamination. Oral-route *M. bovis* contamination has previously been suspected through shared access to water between different pens (Shibrowski et al., 2018), and may result from biofilm formation on nipples, as previously described for managers (Piccinini et al., 2015). Consequently, individual bucket feeding in boxes may slow the spread of *M. bovis* by limiting the risk to aerosols only. As the seroconversion increased more with the lot size in bucket than in AMF-fed lots, this slowdown effect disappeared at above 200 calves in a same batch (Fig. 1). The AMF system has already been reported as a risk factor for incidence of bovine respiratory disease (**BRD**) in calf lots (Brscic et al., 2012, Mounaix et al., 2007). It would be of interest to assess if it also enhances the diffusion of other respiratory pathogens.

Seropositivity at entry was low with both tests. This may indicate either a poor sensitivity of both tests in very young calves or a true low seroprevalence at entry. This last hypothesis is consistent with previous results on veal calves in eastern France using another serological Elisa test and was interpreted as colostral seropositivity (Arcangioli et al., 2008). Absence of a significant effect of seropositivity at entry on the seroconversion rate (eight lots using BioX) might be partly explained by colostral transfer. This differs from the results of Tschopp et al. (2001) who have shown that the presence of a seropositive calf at entry to the feedlot increased the risk for *M. bovis* seroconversion (OR = 2.02). However, their observations concerned very small-sized veal units (fewer than 20 veal calves), not represented in our study population. In fact, in our study, with around 2% of seropositivity at entry and because the majority of the included lots are composed of more than 50 calves, almost all lots might have included at least one seropositive calve at entry even if we did not find it in our random sample of ten to thirty calves per lot. Consequently, we could also posit that a population composed of larger feedlots are probably all submitted to the same risk factor that impede us to detect this effect.

It is important to note that the use of antibiotics did not appear to influence the spread of *M. bovis*. However, unlike other bacterial agents implicated in BRD, *M. bovis* is multiresistant, and so the systematic use of metaphylaxis might facilitate its persistence.

We also monitored how AB use was influenced by lot size, feeding systems, and seroconversion to *M. bovis*. During the study period, collective AB treatments all targeted BRD outbreaks, except for the first treatment with colistin or sulphonamides at entry, which mainly targeted diarrhoea. BRD outbreaks are generally known to occur during the first weeks of fattening, which accounts for nearly the two-thirds of AB treatments (Jarrige et al., 2017; Lava et al., 2016b; Pardon et al., 2011). In our dataset, only one lot did not receive any collective AB treatment during the study period (38 veal calves, 42 days, lot GU). The mean number of AB administered was 3.8 (SD = 2.1) treatments per calf, 4.8 if we account for the first day's metaphylactic treatment oriented against gastrointestinal diseases, which is less than previously estimated in France (8.5) (Jarrige et al., 2017). To compare our results with more studies, we calculated $TI^*_{ADD,}$ a proxy of TI_{ADD} (Jarrige et al., 2017; Pardon et al., 2012b). The average TI^*_{ADD} was 160 per 1 000 calves considering the average 60 days' study period at beginning of the fattening time that encompasses the majority of treatments and represents 66–75% of total AB use (Fertner et al., 2016; Jarrige et al., 2017; Pardon et al., 2012b). If we fit it to a complete production cycle, *i.e.* a mean of 160 days including the period with less AB use (Jarrige et al., 2017), the mean TI^*_{ADD} would approximate 90

per 1 000 calves. Compared to results of French farms for 2013–2014 (152 per 1 000 calves; Jarrige et al., 2017), this confirms the decline in AB use on French veal calves (Chanteperdrix et al., 2018).

In this study, the lot size demonstrated to be the most important risk factor associated with AB use (1.5 AB treatment per calf or, in TI^*_{ADD}, 51/1 000 calves per day treated in addition per two-fold increase of lot size; $P < 0.001$). This was not surprising, as group size, or herd size, are almost always found to be risk factors for morbidity, mortality and BRD in veal calves (Brscic et al., 2012; Lava et al., 2016a; 2016b). A majority of studies that analysed the AB use have also found herd size/group size to be a risk factor, even if there are slight differences in the effect patterns found. For example, in a recent study conducted on all the veal feedlots from one practice, herd size had an influence on AB during the univariate analyses, but not in the multivariate analysis which found only a season effect (Bokma et al., 2019). This season factor was fixed in our study, which was performed entirely during the winter months from December to March.

The AMF feeding method also seemed to increase the use of AB (Figs. 2 and 3) with bucket feeding reducing by one treatment the nAB for a same size lot, ($P \approx 0.05$ for nAB). This influence had already been suggested for AB use or heifer morbidity in dairies as in veal calf herds (Brscic et al., 2012, Curtis et al., 2016, Schnyder et al., 2019). The feeding method may have had a confounding effect with pen size as, once opening the fences, the bucket-fed pens were systematically of smaller size (*i.e.* a maximum 10 *vs* at least 30 calves in AMF-fed pen), but our results support the final model in Schnyder et al. (2019) that retained only the number of calves per drinking nipple and not the number of calves per rearing unit.

In our study, *M. bovis* seroconversion explained only a small part of AB use compared to the lot size (0.05 additional treatment per calf). As, the lot size also increases the probability of seroconversion we could not exclude a confounding effect of this factor, hiding the actual role of *M. bovis* seroconversion towards AB used. This confounding factor could also explain the discrepancy with the study of Tschopp et al. (2001) which found a 2.3-fold higher risk for AB use when calves seroconverted to *M. bovis* without accounting for the lot size.

To conclude, calf feedlots should be less exposed to *M. bovis* infection spread if the animals are separated into small-sized lots, fed in different rooms using buckets instead of shared nipples (AMF), each managed as separate units with substantial biosecurity measures. In very large lots (more than 200 animals), feeding system (bucket or AMF) no longer has influence on spread due to the overwhelming effect of multiple potential introductions of *M. bovis* carriers. It would be usefully informative to test whether these conclusions also hold for other BRD pathogens, *i.e.* viruses or *Pasteurellaceae*. Likewise, small units, ideally in starting individual boxes with individual buckets (to limit contamination through the feeding device), should be preferred in order to limit the use of antibiotics.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2021.100397.

Ethics approval

Fattening farms' owners gave formal consent to the study and provided access to treatment records and to the calves for sampling purposes. The study was approved by the VetAgro Sup Ethics Committees in Animal Testing (No.1947).

Data and model availability statement

The raw data are available upon request. The model is available in the supplementary materials only.

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Declaration of interest

None.

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