ABSTRACT

Quick thoracic ultrasonography (qTUS) is increasingly used as an on-farm method to diagnose clinical and subclinical pneumonia in dairy calves. The primary objective of this prospective cohort study was to describe dynamics of lung consolidation in a purchase-dependent production system for male dairy calves in relation to antimicrobial therapy and respiratory diagnostics. In addition, we studied the association of cured and uncured pneumonia with average daily gain (ADG) and cold carcass weight (CCW). The third objective was to determine the effects of arriving with lung consolidation on the probability of developing chronic unresponsive pneumonia and reduced performance. A total of 295 male dairy calves were intensively followed by qTUS and clinical scoring on 7 strategic occasions (wk 1, 2, 3, 4, 6, 8, and 12) during the production cycle. Of the calves, 17.6% (52/295) arrived with a lung consolidation ≥1 cm. At the first outbreak of respiratory disease (wk 1 after arrival), this incidence had risen to 30.8%. Initial therapy with tulathromycin and subsequently doxycycline appeared ineffective, resulting in an increase to 43.8% of calves having pneumonia in wk 4. At the start of the first outbreak (wk 1), the majority (86.8%) of the pneumonia cases were subclinical. At wk 4, the outbreak became more clinical, and treatment with amoxicillin resulted in a cure risk of 52.7%. Culture and nanopore sequencing diagnostics on nonendoscopic broncho-alveolar lavage (nBAL) samples identified bovine respiratory syncytial virus and Mycoplasma bovis as the dominant agents in the first outbreak. The isolated M. bovis strain showed mutations associated with macrolide resistance. The second outbreak was characterized by a Pasteurella multocida superinfection and isolation of multiple M. bovis strains from nBAL diagnostic testing. Evaluated over the complete observation period, 83.4% of the calves developed consolidations ≥1 cm on qTUS. Of these calves, 53.9% (135/246) were cured by antimicrobial therapy. Chronic pneumonia (≥30 subsequent days of pneumonia) was seen in 13.9% of the animals (n = 41). Calves with uncured or chronic pneumonia had a lower ADG (992 ± 174 g/d and 930 ± 146 g/d, respectively) compared with calves that never developed pneumonia (ADG = 1,103 ± 156 g/d). In contrast, calves that did fully cure trended toward a lower ADG than calves that never developed pneumonia, but differences were no longer significant. Also, the effect of uncured pneumonia was no longer significant for CCW. Calves with lung consolidation upon arrival had a lower ADG (992 ± 174 g/d vs. 1,045 ± 159 g/d, respectively) compared with calves without consolidation upon arrival. Animals with chronic pneumonia, in turn, had a lower CCW than animals without chronic pneumonia (10.3 ± 4.4 kg; 95% confidence interval: 1.6–19.1 kg). This study documents the consequences of subclinical pneumonia upon arrival and pneumonia developed later in the production cycle on production outcomes in a veal calf setting. Both qTUS and nBAL diagnostics provide important information, offering potential for better control and prevention of bovine respiratory disease in dairy calves.

Key words: bovine respiratory disease, thoracic ultrasound, Mycoplasma bovis, bovine respiratory syncytial virus, economics
INTRODUCTION

Respiratory tract infections, especially when resulting in pneumonia, are a leading health concern in both female and male dairy calves worldwide. They have major consequences for production, animal welfare, and antimicrobial use (Pardon et al., 2012a; Windeyer et al., 2014; Bokma et al., 2020a). For every female calf, a male calf is born that, in North America and Europe, is transported to either the veal or dairy beef industry. The destination of these surplus calves is an ever-increasing concern for the dairy industry as a whole because of consumer concerns related to animal welfare and intensive antimicrobial use (Pardon et al., 2012b; Murphy et al., 2017; Creutzinger et al., 2021; Roadknight et al., 2021). For economic reasons, the level of care for male dairy calves at the dairy farm of origin appears to be lower than that for female calves. This results in a lower BW and higher prevalence of failed transfer of passive immunity, 2 factors associated with a higher risk for bovine respiratory disease (BRD) in the veal facility (Brscic et al., 2012; Pardon et al., 2015; Renaud et al., 2020). What percentage of male calves arrive in the veal of dairy beef facility with pneumonia (and how this affects their performance) is largely undocumented.

In recent years, lung ultrasonography became available as an on-farm tool, allowing differentiation of pneumonia from upper respiratory tract infections (URT; Ollivett and Buczinski, 2016; van Leenen et al., 2020a; Jourquin et al., 2021). To make lung ultrasonography feasible in practice, a more rapid, standardized technique with a short learning curve for inexperienced operators was developed: quick thoracic lung ultrasonography (qTUS; Pardon, 2019; Jourquin et al., 2021). The use of qTUS makes lung ultrasonography achievable in large groups of animals and holds great potential both for scientific research and practical applications. In purchase-dependent production systems, like the veal or dairy beef industry, *Mycoplasma bovis* is the leading primary pathogen, with a herd prevalence of almost 100% (Caswell et al., 2010; Pardon et al., 2011; Castillo-Alcala et al., 2012). Antimicrobial treatment of *M. bovis* is often problematic because of both natural and acquired resistance, resulting in unresponsive, chronic pneumonia with serious consequences for production and animal welfare (Pardon et al., 2012b; Bokma et al., 2020c; Masmeijer et al., 2021). Knowledge on the prevalence of subclinical pneumonia in relation to clinical pneumonia, on cure risk in response to antimicrobial treatment, and on the effects of uncured pneumonia on growth and carcass weight, are essential in order to revise current management practices in the veal and dairy beef sector. This is particularly important for current antimicrobial strategies.

Therefore, the first objective of this study was to describe the dynamics of lung consolidation in veal calves in the first half of the production cycle in respect to clinical signs and antimicrobial therapy. The second objective was to determine the influence of cured and uncured pneumonia on ADG and cold carcass weights (CCW). The third objective was to determine the correlation between calves arriving with lung consolidations on development of chronic pneumonia, ADG, and CCW.

MATERIALS AND METHODS

Study Design, Animals, and Housing

A prospective cohort study was conducted involving 295 male Holstein Friesian calves, aged 14 to 21 d during one production cycle in a commercial veal farm located in the province of Antwerp, Belgium. Calves were derived from different Belgian dairy farms that aimed to sell male dairy calves that are of no value for their own production. The study period ran between January and August 2021, with ultrasonographic follow-up during the first 3 mo of the production cycle. Sample size of the study was based on the available herd size, including 295 animals housed in the same airspace. The available sample size was large enough to detect a 50 g/d difference in ADG (expected SD of 150 g/d) and an 8-kg difference in CCW (expected SD of 25 kg) between animals with and without pneumonia with 95% confidence and 80% power.

The study stable was 1 of 2 rosé veal calf stables present on the farm. Calves arrived in 3 separate batches within 1 wk. For the first 6 wk, calves were housed on slatted floors in individual calf boxes, separated by wired fences. After 6 wk, the wired fences were removed, leaving the calves housed in 56 pens consisting of 5 to 6 animals each. During the individual phase in the first 6 wk, calves were fed from individual drinking buckets. During group housing, feeding took place from a common feeding trough. All calves received 2.2 L (500 g of solids) of commercially available milk replacer (18% CP and 18% crude fat) on a DM basis, twice a day. This amount increased during the next 7 mo of feeding to 7 L twice a day. In this study, milk replacer was fed throughout the entire production cycle. In addition, calf muesli was provided (25 g/d gradually increasing to 3 kg/d after 7 mo; CP = 14%; crude fat = 4.5%). The trial protocol was approved by the Ethical committee of the Faculty of Veterinary Medicine and Bioengineering from Ghent University under license EC2020–092.
Clinical Scoring and Production Monitoring

Clinical detection of BRD was done using the California (Davis) score chart (BRD3) by Love et al. (2014). For each calf, the following clinical parameters were evaluated: respiration quality (dyspnea or tachypnea if difficulties breathing or breathing frequency >44 breaths per minute; 2 points), rectal temperature (fever if >39.2°C, 2 points), coughing (none, 0 points; induced or spontaneous, 2 points), nasal discharge (none, 0 points; any, 4 points), ocular discharge (none; any, 2 points), and ear position (normal or ear flick or head shake or ear droop; 5 points). For all clinical parameters that were absent, a score of 0 points was given. For each individual calf, a final score was determined by adding up the respective points. Calves having a total score ≥5 were considered clinically ill (Love et al., 2014). Calves were weighed at arrival and after 12 wk on a calf scale with 0.1-kg accuracy (Basules Robbe). Animals were brought to the slaughterhouse at 8 to 9 mo of age, after 33 wk on the veal facility. After slaughter, CCW was provided by the abattoir at precision of 0.1 kg.

qTUS

Thoracic ultrasound was performed using the UGent qTUS technique, with 70% isopropyl alcohol as a transducing agent and an ultrasound device with a 7.5-MHz linear probe (KXVET5200, Kaixin; Pardon, 2019; Jourquin et al., 2021). During the scan, as soon as any abnormality (e.g., comet tail artifact, pleural irregularity, consolidation) was seen, the probe was halted at that position and gently moved cranio-caudally and back to expand the area of visualization. Consolidation depth was measured in a dorso-ventral plane using the grid on the screen of the ultrasound as a reference. Case definitions of pneumonia were based on consolidation depth. Consolidations ≥1 cm were defined as lobular pneumonia and those ≥3 cm as lobar pneumonia. All other findings (comet tail artifacts, consolidations <1 cm, and pleural effusion) were noted for each scanned quadrant of the lungs (right caudal, right cranial, left caudal, and left cranial).

Both clinical examination and qTUS were performed on all animals in wk 1 to 6, 8, and 12. Due to labor intensity, in wk 7, 9, 10, and 11, only calves with consolidations ≥1 cm on the previous observation were scanned to allow calculation of the duration of pneumonic episodes. In this study, emphasis was put on the first period after arrival because outbreaks of pneumonia are typically expected in this period (Pardon et al., 2012a). Durations of pneumonia episodes were calculated using the dates on which scanning was performed. Any time qTUS was performed on a calf, the clinical status of the animal was also determined using the BRD3 system as described above. The combination of qTUS and clinical scoring allowed classification of the animals into 1 of 4 groups: (1) healthy (no clinical signs, no consolidation on ultrasound), (2) subclinical pneumonia (no clinical signs, but consolidations ≥1 cm on ultrasound), (3) clinical pneumonia (both clinical signs and consolidations ≥1 cm), and (4) URT (clinical signs, but no lesions on ultrasound) (Ollivett and Buczinski, 2016; van Leenen et al., 2020b). The aim of this study was to observe a production cycle without interfering with normal management and veterinary practices. Therefore, clinical and ultrasonographic findings were not shared with either the farmer or the local veterinarian. All treatments, both individual and group treatments, were left at the discretion of the local veterinarian and collected in a software package. For each treatment, the product, dosing regimen, route of administration, and therapy length were recorded.

Laboratory Diagnosis

Respiratory sampling was done on 2 occasions: at the start of the first clinical outbreak of respiratory disease (wk 2) and in wk 6 when a second outbreak occurred. An outbreak was defined by the time points at which the veterinarian considered clinical signs of BRD to be present in 10 to 15% of the animals and decided antimicrobial group treatment was necessary. Each time, samples were taken immediately before antimicrobial group therapy was initiated from calves that had not been treated individually before the group treatment. On both occasions, a small-volume (30 mL of saline) nonendoscopic broncho-alveolar lavage (nBAL) was performed in 5 standing, unsedated calves by a veterinarian using a sterilized catheter as previously described (Van Driessche et al., 2017). Also, at the start of the first group treatment (wk 2), 10 deep nasopharyngeal swabs (DNS) were taken as described previously (Van Driessche et al., 2017). On both occasions, all sampled animals were selected based on ultrasonographic findings (consolidations ≥1 cm). One of the calves that was sampled in wk 2 was also sampled in wk 6.

On the first sampling occasion, metagenomic sequencing using nanopore sequencing was performed on a pooled nBAL sample of 5 animals with ultrasonographic consolidations ≥1 cm to identify viral pathogens and M. bovis (Theuns et al., 2018; Bokma et al., 2021a). No metagenomic sequencing was performed on nBAL samples from the second sampling occasion, excluding detection of viral pathogens at this time. To identify involved bacteria, all DNS and nBAL samples were inoculated directly on Columbia agar supplemented with 5% sheep blood (blood agar; Oxoid) or on M.
bovis selective indicative agar (Bokma et al., 2020b), or both. Blood agar plates were incubated for 24 to 48 h and M. bovis selective indicative agar plates for 10 d at 37°C in a 5% CO₂ enriched atmosphere. Bacteria from blood agar were identified by MALDI-TOF MS (Bruker Daltonik GmbH), and M. bovis was identified based on lipase activity, as described previously (Bokma et al., 2020b). When present, M. bovis was isolated and cultured in broth for additional strain typing. Determination of Belgian genomic clusters of M. bovis isolates was done using SNP analysis following previously described methods (Bokma et al., 2020b).

Antimicrobial resistance for administered antimicrobials was, when possible, determined by disk diffusion for Pasteurellaceae and by microdilution for M. bovis (Timsit et al., 2017; Bokma et al., 2020c). Clinical breakpoints for Pasteurella multocida were used according to the Clinical and Laboratory Standards Institute (CLSI) standards (CLSI, 2018) and epidemiological cutoffs for M. bovis (Bokma et al., 2020c). In addition, the genome of M. bovis strains involved in both the first and second outbreaks were fully sequenced using nanopore sequencing and screened for the presence of known point mutations previously associated with antimicrobial resistance against tetracyclines, macrolides, and fluoroquinolones (Vereecke et al., 2020; Bokma et al., 2021b).

Data and Statistical Analyses

All data were entered in a spreadsheet (Excel, Microsoft Corp.) and transferred to both SPSS statistics version 27.0. (IBM Corp.) and SAS 9.4 (SAS Institute Inc.). Graphs were made using GraphPad Prism (version 9.1.1 for Mac, GraphPad Software; www.graphpad.com).

Average daily gain was calculated by dividing the difference in BW between arrival and 12 wk in production by the number of days on farm. Pneumonia was defined as the presence of consolidated lung tissue ≥1 cm on qTUS. Calves with no consolidations or consolidations <1 cm were defined as healthy. Animals with pneumonia were considered cured when consolidated lung tissue regressed to <1 cm in depth (i.e., lung reaeration; Jourquin et al., 2021). Similarly, cure risk was defined as the proportion of calves that showed lung reaeration of a previously consolidated lung area over all calves with a lung consolidation. For each calf, the duration of each episode of pneumonia was determined by calculating the number of days between the date when a consolidation was found and the date when the lung had reaerated to <1 cm in depth in all quadrants. For calves still showing consolidations at the end of the observation period, the final examination date was used to calculate the duration of disease. Calves having 30 or more subsequent days of pneumonia were defined as having chronic, unresponsive pneumonia. A categorical variable “cure status” was made based on the presence of consolidations ≥1 cm and whether an animal was cured at the end of the ultrasonographical follow-up period. This “cure” variable consisted of the following categories: healthy (i.e., never developed a lung consolidation in the follow-up period), cured (i.e., developed or arrived with lung consolidation, which reaerated within the follow-up period), and uncured (i.e., developed or arrived with a lung consolidation which did not fully reaerate). Calves were only considered cured when, after reaeration, no lesions were found on any of the subsequent ultrasonographic examinations. When calves relapsed or had a new infection, they were only considered cured if they no longer showed consolidations ≥1 cm in wk 12.

To determine the effect of cure status on ADG and CCW, 2 mixed models were built (PROC MIXED) with the respective outcomes. Both ADG and CCW were checked for normal distribution by visual assessment of the histogram and Q-Q plots. Arrival batch and pen were included as random intercepts in each model to account for clustering. Bonferroni corrections were used for multiple comparisons. Model fit was evaluated using the −2log-likelihood. Normal distribution was evaluated by inspection of the residuals. In addition, using the same modeling procedure, the effects of having chronic, unresponsive pneumonia and arriving with pneumonia on ADG and CCW were determined. Potential confounding by lesion size on ADG and CCW was accounted for by linear mixed-model analysis, one-way ANOVA, and inspection of boxplots.

To determine the association between arriving with a lung consolidation and the risk of developing chronic pneumonia, a general linear model with binomial distribution and logit link function with Wald’s statistics for type 3 contrasts was used (PROC GLIMMIX). Pen and arrival batch were added as random factors to account for clustering. Model fit was evaluated by the Hosmer-Lemeshow test. Significance was set at $P < 0.05$.

RESULTS

Animals and Observations Upon Arrival

All calves arrived in 3 batches within 1 wk. On the day of arrival of each batch, qTUS, clinical scoring, and weighing were performed in all animals. Lung consolidations ≥1 cm were detected in 17.8% of animals in batch 1 (28/158), 14.5% in batch 2 (8/55), and 19.5% in batch 3 (16/82). Overall, 13.2% had consolidations of 1 to 3 cm in depth and 4.4% had 1 or more con-
solidations ≥3 cm, resulting in a total of 17.6% of the animals (52/295) with ultrasonographically confirmed pneumonia on arrival. Average (± SD) BW on arrival was 51.7 ± 6.5 kg (range: 39–79 kg). Characteristics of each batch are shown in Table 1. Calves from batch 1 had a lower BW than calves from batches 2 and 3 ($P < 0.001$). No significant difference in BW was found between healthy animals and animals with pneumonia on arrival ($P = 0.6$). Clinical signs of respiratory disease and elevated temperatures (>39.2°C) were present in 4.7% (14/295) and 10.8% (32/295) of the animals on the day of arrival, respectively. Clinical signs of respiratory disease were more frequently seen in animals from batch 1, resulting in a higher number of animals with a BRD3 score in batch 1 compared with animals in batches 2 and 3 ($P = 0.009$). At the end of the trial, carcass weights of 208 calves were recorded at the slaughterhouse. The other 80 calves were taken to an overseas slaughterhouse and were not included in carcass weight analyses.

Of animals with a consolidation ≥1 cm upon arrival, only 13.5% (7/52) also displayed clear clinical signs of respiratory disease, making subclinical pneumonia (88.5%) the most important form in which pneumonia was expressed at that time (46/52). Combining clinical scoring and qTUS, 79.6% of the animals were categorized as healthy, 15.3% had subclinical pneumonia, 2.4% had clinical pneumonia, and 2.4% had URT disease.

**Dynamics of Pneumonia in Relation to Antimicrobial Therapy**

Figure 1 displays a temporal overview of clinical signs and pneumonia as determined by qTUS. Elevated temperatures were highest in the first 2 wk after arrival and declined after the first treatment. The prevalence of clinical signs of BRD at arrival was 4.7% and peaked at 10.2% and 11.6% in wk 3 and 4, respectively. Altogether, 83.4% (246/295) of the calves developed pneumonia within the 12-wk observation period, of which the majority remained subclinical (69.1%; 170/246). Clinical pneumonia was seen in 25.8% (76/295) of the animals, 4.4% (13/295) only developed URT, and 12.2% (36/295) remained healthy. The incidence of ultrasonographic pneumonia increased in the first week after arrival and peaked in wk 4 at 43.8% (Figure 1). A gradual decline in disease prevalence of pneumonia was seen from wk 4 to 6, after which qTUS-confirmed pneumonia declined rapidly to 26.1% in wk 8. From wk 8 onward, the incidence of pneumonia increased again, reaching 38.5% at the end of the study period (wk 12). Seven animals died (2.4%), of which 2 had pneumonia on qTUS at the time of death and 1 showed signs of abomasal ulceration on postmortem examination. The other 4 animals did not have consolidations around the time of death, but no further diagnostics (e.g., postmortem examination) were performed. Both animals with pneumonia that died had arrived on the farm with consolidations ≥1 cm and, at the time of death, still had consolidations of 1.5 and >3 cm, respectively. On average, episodes of ultrasonographically visible pneumonia lasted 14.3 d in this study (SD = 18.2 d; range: 0–73 d; median = 9 d).

Five metaphylactic treatments were initiated in the observation period (first 12 wk). On the day of arrival of the third batch, a first metaphylactic treatment with tulathromycin (TUL) was done by injecting each calf intramuscularly with 450 mg of TUL (Draxxin, Zoetis). Other group treatments consisted of oral treatments with 0.5 g of doxycycline (DOX) twice daily (Doxyveto

| Variable | Batch 1 | Batch 2 | Batch 3 | Herd |
|----------|--------|--------|--------|-----|
| Number in group | 158 | 55 | 82 | 295 |
| California Davis score (BRD3) (%) | 8.2$^a$ | 1.8$^b$ | 0$^b$ | 4.7 |
| Clinical signs of BRD (%) | 32.2 | 29.0 | 23.2 | 29.2 |
| Fever (%) | 12.0 | 9.1 | 9.8 | 10.8 |
| Lung consolidation 1–3 cm (%) | 12.0 | 10.9 | 18.2 | 13.6 |
| Lung consolidation ≥3 cm (%) | 6.3 | 3.6 | 1.2 | 4.4 |
| Pneumonia (%) | 18.4 | 14.5 | 19.5 | 18.0 |
| Arrival weight (kg) | 48.5 ± 4.7$^a$ | 54.2 ± 6.2$^b$ | 56.0 ± 6.3$^b$ | 51.7 ± 6.5 |
| ADG after 12 wk (g/d) | 1,033.7 ± 139.8 | 999.5 ± 124.2 | 1,078.5 ± 199.7 | 1,039.3 ± 160.4 |
| Production outcomes | | | | |
| Number in group | 139 | 39 | 30 | 208 |
| Carcass weight (kg) | 182.6 ± 23.1 | 181.1 ± 21.3 | 172.7 ± 32.5 | 180.9 ± 24.5 |

$^a,b$Mean values within a row with different superscripts differ significantly ($P < 0.05$).

1Bovine respiratory disease.

2Fever was defined as rectal temperature >39.2°C.

3Mean± SD.
Table 2. Overview of metaphylactic treatments and their estimated effects on 295 veal calves over the first 12 wk using quick thoracic ultrasonography (qTUS) to confirm pneumonia

| Treatment     | Week of initiation | Treatment length (d) | Pneumonia at start of treatment (%) | Cure risk (%) | Pneumonia after treatment (%) | Animals at risk |
|---------------|--------------------|----------------------|-------------------------------------|---------------|------------------------------|-----------------|
| Tulathromycin | 2                  | 7                    | 30.8 (n = 91)                       | 29.7          | 43.3 (n = 127)               | 295             |
| Doxycycline   | 3                  | 12                   | 27.5 (n = 81)                       | 42.0          | 43.8 (n = 128)               | 295             |
| Amoxicillin   | 6                  | 6                    | 38.4 (n = 112)                      | 52.7          | 18.9 (n = 55)                | 292             |
| Florfenicol   | 7                  | 4                    | 18.9 (n = 55)                       | 20.0          | 26.1 (n = 76)                | 291             |
| Doxycycline   | 9                  | 5                    | 15.9 (n = 46)                       | 10.8          | 14.2 (n = 41)                | 289             |

1Cure risks were based on the proportion of animals no longer having consolidations ≥1 cm after treatment. Cure risk of both tulathromycin and doxycycline should be interpreted with care as there was an overlap of 5 d in both treatments.

50% Pulvis, VMD), 1.5 g of amoxicillin twice daily (Dokamox 80%, Emdoka), and 1 g of florfenicol (Flordofen 100 mg/mL, Dopharma). For each antimicrobial treatment, therapy length, cure rate, and overall therapy effects are provided in Table 2. Cure risk was determined solely on animals having consolidations ≥1 cm at the start of the treatment, whereas the proportion of pneumonia after treatment was determined using all animals at risk for pneumonia at that time. Because the observed clinical response of the animals on the first TUL treatment was unsatisfactory according to the veterinarian, oral DOX treatment was started 2 d after initiation. Due to the long-acting activity of TUL, the animals remained under coverage for 7 d. Cure risks were generally very low and after wk 7 declined to 20% or less (Table 2).

**Laboratory Diagnosis**

An overview of all pathogens identified in the respiratory samples taken at the start of outbreak 1 (wk 1) and 2 (wk 6) is given in Table 3. In the nBAL samples collected during outbreak 1, before TUL treatment was initiated, bovine respiratory syncytial virus (BRSV), *M. bovis*, and *Mycoplasma dispar* were detected by means of metagenomic nanopore sequencing on a pooled sample of 5 animals with consolidations ≥1 cm. Bacterial cultures on selective indicative agar were...
positive for \( M. \) bovis in 4 of 5 nBAL samples and 6 of 10 DNS samples. In addition, \( T. \) pyogenes, \( P. \) multocida, and \( G. \) anatis were detected on blood agar in 1 case each. All 3 samples that were positive on blood agar also contained \( M. \) bovis at this time.

In wk 6, no metagenomic sequencing was done. Nevertheless, all bacterial cultures from the nBAL samples taken at this time (wk 6) showed pure cultures of \( P. \) multocida on blood agar, and 4 of 5 samples were positive for \( M. \) bovis on selective indicative agar. Strain typing and antimicrobial resistance determination of \( M. \) bovis, by means of whole-genome sequencing, was performed on samples from wk 2 (B2 and B3) and wk 6 (B6 and B10), respectively.

In Tables 4 and 5, results of phenotypic and molecular antimicrobial susceptibility testing (MIC with microbroth dilution determination and genetic markers from \( M. \) bovis whole genomes, respectively) of \( M. \) bovis are shown. For \( M. \) bovis, acquired resistance was found for gamithromycin (\( \text{GAM} \)), tilmicosin (\( \text{TIL} \)), and tylosin (\( \text{TYL} \)). Antimicrobial susceptibility of 2 \( P. \) multocida isolates obtained in wk 6 was tested by disk diffusion and indicated antimicrobial resistance against TUL, DOX, and tetracycline.

Phylogenomic analysis identified the 2 \( M. \) bovis strains isolated in wk 2 (B2 and B3) as part of Belgian genomic cluster I and II, whereas the strains isolated in wk 6 (B6 and B10) were part of Belgian genomic clusters II and IV (Table 5; Bokma et al., 2020d). In addition, the genome was screened for previously described point mutations associated with antimicrobial resistance in \( M. \) bovis (Bokma et al., 2021b). In both isolates from wk 2, a G748 mutation and a A2058 mutation in the 23S rRNA gene, associated with antimicrobial resistance against TIL and GAM/TYL, respectively, were found. In one of the isolates (B2), a Gln93His (RplV) and T1199C mutation on both alleles of the 16S rRNA gene were identified, possibly linked to resistance to GAM/TYL and oxytetracycline, respectively (Hata et al., 2019; Bokma et al., 2021b). In 1 of the 2 isolates collected in wk 6 (B10), again, mutations in both alleles of the 23S rRNA G748 and 23S rRNA A2058 gene were found. The G748 and Gln93His mutations were also found in an isolate obtained from the B6 nBAL sample taken in wk 6.

**Effects on ADG and CCW**

After 12 wk, average BW was 134.9 kg (SD = 14.7 kg; range: 95.6–199.4 kg), resulting in an ADG of 1.035 kg/d (SD = 0.160 kg/d; range: 0.550–1.640 kg/d) in this period. Average CCW was 180.9 kg (SD = 24.5 kg; range: 63.9–239.7 kg). Animals with pneumonia at ≥1 time point (n = 246), both cured and uncured by
the end of the observation period, had an 83 g/d lower ADG compared with animals that never developed pneumonia within the observation period (SD = 29 g/d; 95% CI: 34–133 g/d; \( P = 0.001 \)). Carcass weight was not different between healthy animals and animals with an episode of pneumonia (95% CI: −3.5 to 10.0 kg; \( P = 0.3 \)). After 12 wk, ADG of uncured animals (992 g/d; SD = 173 g/d; range: 500–1,400 g/d) was lower than that of calves that remained healthy (1,103 g/d; SD = 156 g/d; range: 800–1,600 g/d; \( P < 0.001 \)) and calves that were cured (1,046 g/d; SD = 131 g/d; range: 700–1,500 g/d; \( P = 0.02 \)) within the observation period. In contrast, ADG of cured animals was not different from that of animals that remained healthy (95% CI: −6 to 122 g/d; \( P = 0.09 \)), but a trend was observed. Cold carcass weight did not differ between healthy, cured, and uncured animals (\( P = 1 \)). A visual representation of the differences in ADG is presented in Figure 2a.

Mixed model analysis and inspections of the boxplots did not indicate confounding effects of lesion size on ADG (\( P = 0.3 \)) or CCW (\( P = 0.1 \)). One-way ANOVA did not indicate any associations between lesion size and ADG or CCW (\( P = 1 \)).

Animals with chronic pneumonia (≥30 subsequent days of pneumonia) showed a reduction in ADG of 172 g/d (SD = 33 g/d; 95% CI: 94–254 g/d) and 107 g/d (SD = 27 g/d; 95% CI: 43–173 g/d) compared with healthy animals (\( P < 0.001 \)) and animals with shorter episodes of pneumonia (<30 d; \( P < 0.001 \)), respectively. Also, shorter episodes of pneumonia resulted in a reduction in ADG of 65 g/d (SD = 25 g/d; 95% CI: 6–126 g/d; \( P = 0.03 \)) compared with healthy animals.

For CCW, both linear mixed-model analysis and one-way ANOVA indicated a difference of 10.3 kg (SD = 4.4 kg; 95% CI: 1.6–19.1 kg; \( P = 0.02 \)) when comparing CCW of chronically ill calves (172.3 kg; SD = 33.2 kg; range: 63.9–231.7 kg) with calves with <30 subsequent days of pneumonia (182.7 kg; SD = 21.9 kg; range: 95.8–239.7 kg). Differences in ADG and CCW between healthy animals, animals with shorter episodes of pneumonia (<30 d), and chronically affected calves (≥30 d) are presented in Figure 2b.

### Table 4. Minimum inhibitory concentration values of isolated *Mycoplasma bovis* sampled on wk 2 and 6 of a veal calf production cycle (295 Belgian calves)

| Item                  | Sample type | GAM | TIL | TYL | TIA | ENRO | FFC | GEN | DOX | OTC |
|-----------------------|-------------|-----|-----|-----|-----|------|-----|-----|-----|-----|
| **Sampling wk 2**     |             |     |     |     |     |      |     |     |     |     |
| B2²                   | BAL³        | >128| >128| >32 | 0.5 | 0.5  | 2   | 4   | 2   | 8   |
| B3                    | BAL         | >128| >128| >32 | 0.25| 0.25 | 2   | 4   | 2   | 8   |
| **Sampling wk 6**     |             |     |     |     |     |      |     |     |     |     |
| B6²                   | BAL         | >64 | >128| 16  | 0.06| 0.12 | 1   | 1   | 0.5 | 2   |
| B10                   | BAL         | >128| >128| >32 | >2  | 0.5  | 4   | 2   | 1   | 4   |
| ECOFF⁴                | >64         | ND  | >32 | >0.5| >1  | >16  | >16 | >4  | >8  |

1GAM = gamithromycin; TIL = tilmicosin; TYL = tylosin; TIA = tiamulin; ENRO = enrofloxacin; FFC = florfenicol; GEN = gentamycin; DOX = doxycycline; OTC = oxytetracycline.
2Different samples taken from the same animal at a different time within the observation period.
3Broncho-alveolar lavage.
4Epidemiological cutoff values, based on the visual estimation method as previously described (Bokma et al., 2020c; 2021b)
5ND = not determined.

### Table 5. Overview of genomic mutations, previously linked to in vitro antimicrobial resistance, present in *Mycoplasma bovis* strains isolated in wk 1 and wk 6 of a veal calf production cycle (Bokma et al., 2020d, 2021b)

| Item                  | Belgian genomic cluster | 23S rRNA gene | RplV | 16S rRNA gene |
|-----------------------|-------------------------|---------------|------|---------------|
|                       |                         | G748A allele 1 and 2 | A2058G allele 1 and 2 | Gln93His | T1199C allele 1 and 2 |
| **Sampling wk 2**     |                         | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present |
| B2²                   | I                       | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present |
| B3                    | II                      | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present |
| **Sampling wk 6**     |                         | Present | Not present | Present | Present | Present | Present | Present | Present | Present | Present |
| B6²                   | IV                      | Present | Not present | Present | Present | Present | Present | Present | Present | Present | Present |
| B10                   | II                      | Present | Present | Present | Present | Present | Present | Present | Present | Present | Present |

¹Nucleotide and AA positions are labeled according to classic *Escherichia coli* numbering.
²Different samples taken from the same animal at a different time within the observation period.
Of the 295 calves, 52 had consolidations upon arrival (17.6%), of which 88.5% of cases were subclinical (46/52). The ADG of healthy animals on arrival was 1,045 g/d (SD = 159 g/d; range: 1,025–1,066 g/d), whereas animals with ultrasonographic-confirmed pneumonia upon arrival had an ADG of 981 g/d (SD = 159 g/d; range: 938–1,029 g/d), resulting in a difference of 64 g/d (SD = 25 g/d; 95% CI: 15–113 g/d; \( P = 0.01 \)) between groups. Of the 52 animals with pneumonia on arrival, 88.5% (46/52) were subclinical pneumonia, and 11.5% (6/52) were confirmed by lung ultrasonography.

**Effects of Pneumonia at Arrival on Chronicity of Disease and Performance**

Figure 2. (A) Average daily gain in the first 12 wk of production in 295 male dairy calves, stratified by pneumonia cure status as determined by lung ultrasonography; (B) differences in ADG and cold carcass weights (CCW) between healthy animals, animals with <30 consecutive days of thoracic ultrasound-confirmed pneumonia, and chronically infected animals. Chronic pneumonia is defined as the presence of consolidated lung tissue (≥1 cm) for 30 consecutive days or more. The middle box represents the interquartile range (mid 50%), the line marks the median, the whiskers represent the 5th to 95th percentiles, and the symbols (dots) are all values outside of the 5th to 95th percentile range. Significant difference between cure status groups: * \( P < 0.05 \), ** \( P < 0.001 \).
arrival, 17 developed chronic pneumonia, resulting in 4.2 times higher odds (95% CI: 2.1–8.6; \( P < 0.001 \)) of developing chronic unresponsive pneumonia compared with calves without pneumonia upon arrival.

**DISCUSSION**

In this study, we applied qTUS and nBAL diagnostics to visualize and better understand what happens at the lung level in a veal calf production cycle under standard antimicrobial management. Several interesting observations were made, which may contribute to future design and evaluation of preventive and therapeutic management in calves with respiratory tract diseases.

First, lung ultrasonography confirmed that the incidence of pneumonia in a typical veal farm is particularly high and, importantly, mainly subclinical in nature. At the peak of respiratory disease, only 11.6% of the animals showed clinical signs, whereas almost 50% had pneumonia. This observation is very similar to the high prevalence of subclinical pneumonia seen in dairy farms in the study region (van Leenen et al., 2020b). Presence of a consolidation on TUS has been linked to a decrease in daily growth; therefore, not treating these animals will result in an important welfare issue and significant losses in the veal industry (Binvieres et al., 2020). In the veal sector, veterinarians often use a daily incidence of 5 to 10% of the animals with clinical disease as the criterion for group antimicrobial treatment (Berman et al., 2019). The present study showed that the incidence of clinical BRD is almost consistently around 5%. Whether a much larger proportion of animals would have developed clinical pneumonia if group antimicrobial treatment had not been initiated is unknown. Noting that the incidence of pneumonia continued to increase despite clinical improvement after DOX treatment, we can question whether treatment decisions based only on clinical signs and not on qTUS should be considered rational.

Second, cure risks of antimicrobial treatment with different antimicrobials were generally very poor. For TUL, the clinical effect appeared insufficient, after which the veterinarian decided to switch to DOX only 2 d after first treatment. There are several possible explanations for the observed therapeutic failure in this early outbreak. First, BRSV was detected at that time, which is a well-recognized primary pathogen with the potential to induce severe interstitial pneumonia and mortality (Brodersen, 2010; Pardon et al., 2020). Antimicrobials are ineffective against viruses and, as in humans, self-cure of the lung, assisted by anti-inflammatory therapy, is likely the general rule (Smith et al., 2020). However, to the best of the our knowledge, no studies have documented self-cure of viral pneumonia in cattle. Moreover, in the first BRD peak, \( M. \ bovis \) was detected in all sampled animals, whereas different *Pasteurellaceae* species were only found in nBAL samples from 2 animals. The identified \( M. \ bovis \) strains both had acquired antimicrobial resistance as well as mutations coding for antimicrobial resistance against macrolides. Subsequently, even with DOX therapy, the prevalence of pneumonia did not decrease as expected. In both cases, ineffective therapy could have been caused by a combination of multiple reasons. Given the incomplete reference framework for antimicrobial resistance determination for \( M. \ bovis \) (no clinical breakpoints and no standardization for MIC methodology), we can only speculate about a potential role of antimicrobial resistance in this outbreak. Third, underdosing may have played a role, because a standard volume was used in a group of animals with a relatively wide range of BW. Especially for oral dosing, diseased animals may consume less milk, resulting in underdosing (Borderas et al., 2009; Cramer et al., 2020). Fourth, male dairy calves are exposed to a variety of stressors during transportation. Therefore, they may have a suppressed immune system when they arrive at the veal farm (Masmeijer et al., 2021). Because all antimicrobials used in the first outbreak were bacteriostatic, and \( M. \ bovis \) is known for its ability to evade the immune system of the host, this may have contributed to therapy failure (Buchenu et al., 2010).

In the second outbreak, clinical and ultrasonographical cure was only reached when amoxicillin was given. Although this study was not a randomized clinical trial, it is remarkable to see that, in the second outbreak, all nBAL samples had pure cultures of *P. multocida* and \( M. \ bovis \), consistent with superinfection with a bacterial opportunist on the initial \( M. \ bovis \) or BRSV (co)infection. *Mycoplasma bovis* is naturally resistant to amoxicillin, and has been described as a predisposing factor for secondary infections with *Pasteurellaceae*, whereas the isolated *P. multocida* strain was sensitive (Biriki et al., 2015). Hence, it is likely that superinfection played an important role in the pneumonia observed in the second outbreak. The nBAL diagnostics, as done in this study, may be useful to guide antimicrobial therapy in the future. In the present study, we detected \( M. \ bovis \) in both the first and second outbreaks. It is unknown how long \( M. \ bovis \) strains persist in the respiratory tract, even after antimicrobial treatment. Nevertheless, based on genetic analysis, a third strain (Belgian genomic cluster IV) was found in the second outbreak. This third strain may have been present in the studied stable or may have been introduced by new batches of calves that arrived around wk 6 in the second stable on the farm that was not part of the study. Two strains of \( M. \ bovis \) were detected in one calf that was sampled...
in each outbreak. Because only one *M. bovis* strain was sequenced from each sample, it is currently unclear whether reinfection of the same animal with a different strain occurred or whether both strains were already present in this animal.

A third interesting observation in this study was that male dairy calves exposed to *M. bovis* and BRSV could fully reaerate on qTUS and, when fully healed, negative effects on ADG were no longer detectable. This is an important observation because it suggests that reaeration can ensure that an animal is cured and decreased performance may be avoided, while adapting treatment length to the individual animal (Jourquin et al., 2021). In fact, even though the effect of lesion size was not included in the analysis, by using qTUS, our results suggest that unresponsive pneumonia is a major reason for production loss and that all efforts need to be made to maximize cure in the first weeks after arrival. *Mycoplasma bovis* undoubtedly plays a role in this chronic, unresponsive pneumonia, but to prevent an *M. bovis* infection from becoming chronic, likely multiple risk factors, such as stress or concurrent viral infections, need to be controlled (Pardon et al., 2012a; Lion et al., 2021).

This study showed that about one-fifth of calves arrived with a lung consolidation. These calves were more likely to develop chronic, unresponsive pneumonia, eventually resulting in reduced performance. Potentially, the fact that neonatal calves cope less well with an *M. bovis* infection is an explanatory factor (Chase et al., 2008; Askar et al., 2021). Under the circumstances of this study, we clearly demonstrated that calves arriving with pneumonia at the veal facility should be prevented if possible. Because prevention of infection is virtually impossible in the current veal calf setting, early detection and management of these animals could result in reduced spread of disease, less chronic disease, and potentially reduced antimicrobial use.

In this study, the use of qTUS made it possible to intensively follow a substantial number of animals in a structured way. This should be a stimulus for both dairy and veal producers to use qTUS to take better care of male calves for the sake of the individual animals and for the production system as a whole. The qTUS technique, requiring little time to perform, allowed for frequent monitoring of a large group of animals. This finding is of use for future studies and shows the potential of using this technique at strategic time points in different cattle production systems. Potential moments to perform a checkup of the respiratory status in calf rearing or fattening facilities could be, for example, upon arrival, during an outbreak of respiratory disease, or when therapy evaluation is desired. Although the qTUS technique has been shown to be of great value for respiratory management in the veal, dairy, and beef industry as a whole, further research should be done to validate the technique. Lung ultrasonography has been proven to be the most reliable tool compared with pathology, and qTUS has been described to have a relatively short learning curve, in which a reasonable sensitivity and specificity can be achieved (Pardon, 2019). Nevertheless, future research could improve validation of this technique in practitioners and, given that there currently is no gold standard, against gross pathology.

The study itself, as it was performed on a commercial veal facility, was subject to several limitations. First, there was a noteworthy loss of data because several calves were sent overseas for slaughter. Because we were unable to obtain carcass weights from this slaughterhouse and results of weighing can differ between slaughterhouses, analyses on the effects of pneumonia on carcass weight were performed only on the calves that were slaughtered in the same Belgian abattoir. Although data were partially lost, 208 of 288 carcass weights were available for analysis, resulting in sufficient power to provide statistically significant results. Also, although selection of the calves that were sent overseas was solely based on logistical considerations, we cannot guarantee this did not affect the results of this study. Second, in this production cycle, although effects of long-lasting pneumonia on performance were clear, we were not able to identify differences in CCW between animals that were cured from pneumonia and animals still showing consolidations at the end of the study period. To what extent this was due to animals recovering after the end of observation period is unknown. In future trials, postmortem examination of the lungs in the abattoir would not only add to our knowledge of the number of animals reaching cure before slaughter but would also allow more specific analysis of the effects of unresponsive pneumonia on carcass weight. Third, because the trial was very labor intensive to perform, qTUS was performed by 3 operators. Although this renders the trial more prone to observational bias, recent literature has shown that the interobserver agreement of thoracic ultrasound is higher than that of clinical scoring, for example (Buczinski et al., 2018; De Cremer et al., 2018). However, all 3 operators were trained equally, via lessons on image recognition and interpretation as well as hands-on practical sessions under supervision of trained operators. All 3 operators had more than 1 yr of field experience with qTUS. Therefore, we believe observer bias was as limited as reasonably achievable for this study type. Fourth, multiple studies have been reported the sensitivity of TUS to range from 79.4 to 100% using Bayesian latent class statistics and also necropsy, and research has shown that identification
The incidence of pneumonia, especially subclinical pneumonia, in veal calf facilities was much higher than previously expected, mainly because 69.1% of the cases remained subclinical. Under the conditions of this study, substantial therapy failure was observed for different antimicrobial group treatments. In the studied population, animals with pneumonia had reduced ADG, but appeared to be able to catch up in growth compared with healthy animals when cure was achieved before 12 wk in production. Chronic unresponsive pneumonia is frequently encountered and, in this study, was a major reason for production loss. Calves arriving with a lung consolidation had higher odds to develop chronic unresponsive pneumonia, resulting in lower ADG and CCW. The use of qTUS and nBAL diagnostics in a large calf-rearing facility holds multiple opportunities for practical application, such as risk classification and management of calves upon arrival, early disease detection, and therapy evaluation.

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