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Noninferiority trial in veal calves on the efficacy of oxytetracycline and florfenicol treatment for pneumonia guided by quick thoracic ultrasound

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ABSTRACT

Purchase dependent calf rearing systems, such as the white veal industry, systematically rely on antimicrobial mass medication (metaphylaxis) to counter respiratory tract infections. Despite mounting criticism, the industry fears that without metaphylaxis, mortality would drastically increase. This randomized clinical trial aimed to compare the efficacy of a quick thoracic ultrasonography (qTUS) individualized treatment length between oxytetracycline (OTC) and florfenicol (FF). Regression of maximum consolidation depth <1 cm was used as a criterion for cure and to stop antimicrobial treatment. Additionally, the study assessed the associations of consolidation depth at treatment initiation with cure and treatment duration. The trial involved 320 veal calves, randomly assigned into one of 2 groups: one receiving OTC (n = 160) and the other FF (n = 160) on d 1 (2-d metaphylaxis). Clinical scoring and qTUS were done on d 1 and every 48 h for a 10-d period. After d 1, only calves with consolidations ≥1cm were given further treatment. On each time point, maximum consolidation depth was used to categorize calves into 4 qTUS categories: healthy (no consolidation), mild pneumonia (consolidation <1 cm), moderate pneumonia (consolidation 1–3 cm) and severe pneumonia (consolidation ≥3 cm). Cure, treatment duration and the number of antimicrobial dosages (NAD) were compared between treatment groups. In addition, pathogen identification and antimicrobial susceptibility testing was performed on isolates from nonendoscopic broncho-alveolar lavage fluid. On d 1, 30.0% (96/320) of the calves had consolidation ≥1cm, which increased to 50.9% (162/318) by d 9. After single metaphylactic treatment, cure was 20.9% (9/43) and 20.9% (9/43) in the OTC and FF group, respectively. Calves with severe pneumonia had lower odds to be cured after first treatment than calves with moderate pneumonia (odds ratio = 0.17; 95% CI: 0.04–0.63). By d 9, final cure of the initial cases was 27.9% in both the OTC and FF groups. In both groups, cure was similar at all observation points. Overall, final cure of all calves with either moderate or severe pneumonia during the trial was 41.2% (52/102) and 19.0% (12/63), respectively. Median (Med) treatment duration was 4 d (interquartile range [IQR] = 2–6; minimum [Min] = 2; maximum [Max] = 8) and was similar in both treatment groups. Treatment duration for calves with moderate pneumonia (Med = 6; IQR = 4–6; Min = 2; Max = 8) was lower than the median treatment duration of calves with severe pneumonia (Med = 8; IQR = 4–8; Min = 2; Max = 8). When compared with calves with mild pneumonia on d 1, calves with moderate and severe pneumonia had significantly longer treatment durations. In this study, cure was low and not different between both antimicrobials. Categorizing calves based on consolidation depth appears useful as both cure and treatment duration were different for the mild, moderate, and severe groups.

Key words: bovine respiratory disease, thoracic ultrasound, rational antimicrobial use, precision medicine, *Mycoplasmopsis bovis*

INTRODUCTION

Respiratory tract infections in calves continue to have major negative consequences for production, animal welfare and antimicrobial use in all calf rearing systems worldwide (Pardon et al., 2012; Windeyer et al., 2014; Bokma et al., 2019; Dubrovsky et al., 2020). However, in recent years, thoracic ultrasound (**TUS**) and novel laboratory diagnostic tests have become available to rapidly diagnose pneumonia and identify etiological agents, respectively (Buczinski and Pardon, 2020). Thoracic ultrasound can reliably identify calves with (sub)clinical pneumonia and differentiate clinical pneumonia from upper respiratory tract infection (**URTi**), allowing more rational use of antimicrobials (Ollivett et al., 2015; Ol-

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livett and Buczinski, 2016). In addition, both in humans and more recently also in calves, TUS was successfully used as a tool to evaluate treatment efficacy, using lung reaeration of a previously consolidated area as a cure criterion (Bouhemad et al., 2010; Bello and Blanco, 2019; Jourquin et al., 2022).

Due to risk factors inherent to the sector and hurdles to systematically apply these new advancements, the industries who are still struggling the most with respiratory disease are those in which purchase, transport and commingling of young calves is standard practice, such as white veal, dairy beef, and, to a lesser extent, dairy calf rearing (Karle et al., 2019; Masmeijer et al., 2021; Renaud and Pardon, 2022). In veal production systems, mixed infections, consisting of viruses, *Pasteurellaceae* and *Mycoplasmopsis* (formerly *Mycoplasma*) bovis are common, making the sector rely heavily on antimicrobial group treatments to control bovine respiratory disease (**BRD**; Pardon et al., 2011; Soehnlen et al., 2012; Antonis et al., 2022). In Europe, feed directives prohibit prophylactic use of antimicrobials (Regulation (EU) 2019/6 and 2019/4). Yet, metaphylaxis, defined as administration of a medicinal product to a group of animals after a diagnosis of clinical disease in part of the group has been established, with the aim of treating the clinically sick animals and controlling the spread of disease to animals in close contact and at risk and which may already be subclinically infected, is currently still allowed under high-risk conditions, such as the veal industry (Regulation (EU) 2019/6 and 2019/4). From both a welfare (i.e., reduced short-term morbidity and painful injections) and ease of administration point of view, these systems habitually opt for oral group medication instead of parenteral treatment (Baptiste and Kyvsgaard, 2017; Word et al., 2020). Nonetheless, recent studies have shown higher degrees of antimicrobial resistance (**AMR**) after oral treatments and have increasingly questioned the benefits of metaphylaxis (Zaheer et al., 2013; Simoneit et al., 2015; Baptiste and Kyvsgaard, 2017; Cazer et al., 2020). If both an overall decrease in usage and restriction in the range of antimicrobials is warranted, the most effective method likely consists of a shift toward individualized (parenteral) treatment with first-choice antimicrobials. To this date however, clinical trials evaluating treatment efficacy in veal calves appear to be lacking. The few existing randomized clinical trials use only clinical definitions for BRD, did not use TUS to assess cure, or only evaluated metaphylaxis protocols with second line antimicrobials (macrolides; Rérat et al., 2012; Berman et al., 2017). In addition, when dealing with highly contagious bacterial pathogens such as *M. bovis* (involved in almost every respiratory outbreak in veal calves), providing oral mass medication is often still considered essential to control disease (Pardon et al., 2011; Buczinski and Pardon, 2020; Lora et al., 2022). Also, because there is no consensus on optimal treatment duration, and effects of disease severity on healing time are unknown, a standard treatment duration remains the default option (Apley, 2015). Yet, even with metaphylaxis, individualizing follow-up treatment based on quick thoracic ultrasound (**qTUS**) has been successful in reducing antimicrobial usage by >50% during a single-pathogen *M. bovis* outbreak in a commercial beef herd, using both oxytetracycline (**OTC**) and florfenicol (**FF**) (Jourquin et al., 2022). Still, the effectiveness of this approach, using 2 first-choice empirical treatments recommended by the European Medicines Agency and the World Health Organization, remains uncertain in the challenging veal calf industry (De Briyne et al., 2014; Murphy et al., 2017; WHO, 2017).

Therefore, the primary objective of this study was to compare the effect of a qTUS based individualized treatment with either OTC or FF on cure and required treatment duration, using regression to <1 cm in consolidation depth as cure criterion. The secondary objective was to relate 3 qTUS categories of pneumonia severity (mild, moderate, and severe) with cure and treatment duration. Finally, this study aimed to gain insight into the pathogens involved and their potential influence on antimicrobial efficacy using both culture and whole genome sequencing (**WGS**).

MATERIALS AND METHODS

Study Design and Sample Size

A triple blinded, randomized controlled clinical trial on the effects of OTC and FF for treatment of pneumonia was conducted on a commercial veal calf farm housing 320 male Holstein Friesian calves, aged 14 to 21 d. The study was conducted between January and March 2024 on a farm located in the province of Limburg (Belgium), and consisted out of 2 stages. First, the randomized clinical trial itself was performed in the first 21 d after arrival. In this period, clinical and ultrasonographic follow-up, using the Wisconsin score chart and qTUS technique, were performed on the day of arrival of each batch, at the outbreak of respiratory disease (d 1 of the trial) and subsequently every other day for a 10-d period after the outbreak identification (trial period; McGuirk and Peek, 2014; Pardon, 2019). Figure 1 provides an overview of the study setup, and more detail on the clinical scoring and qTUS methods is described in the following sections. During the trial period, as is described further, antimicrobial treatment was guided by qTUS findings. After the trial period, an oral treatment with doxycycline twice daily (Doxyveto 50% Pulvis, VMD, Arendonk, Belgium) was given to all calves for 11 consecutive

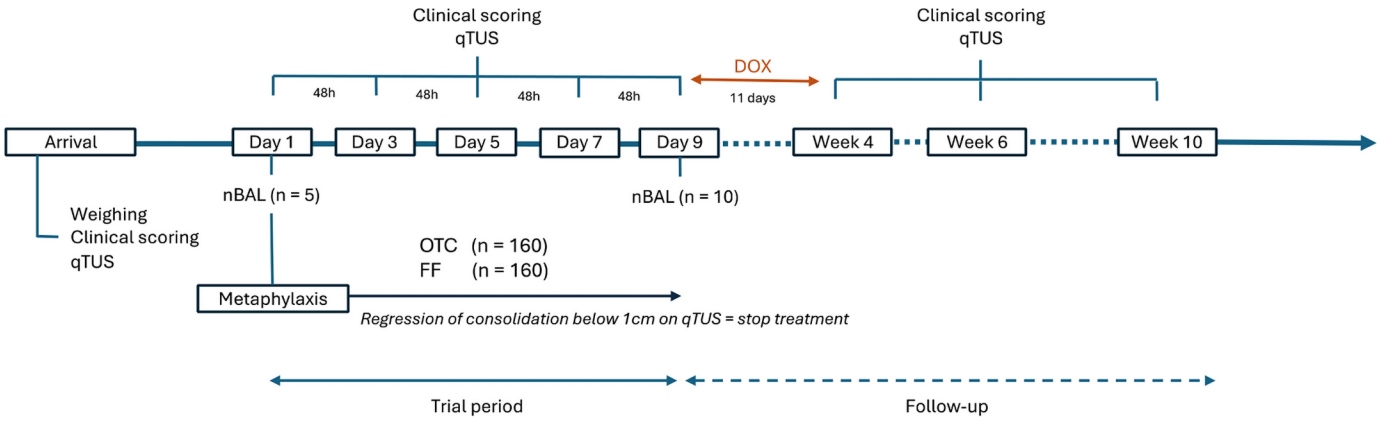

Figure 1. Temporal overview of a randomized clinical trial setup on the efficacy of oxytetracycline and florfenicol for treatment of pneumonia in veal calves, using regression of consolidation size to <1 cm as a cure criterion. qTUS = quick thoracic ultrasound; nBAL = nonendoscopic bronchoalveolar lavage; OTC = oxytetracycline; FF = florfenicol; DOX = doxycycline.

days, and follow-up was done on predetermined points in wk 4, wk 6, and wk 10 of the production cycle. At each of these time points in the follow-up period, clinical scoring and qTUS were performed again on all calves, but no interventions were performed based on either qTUS or clinical score. Based on an observational study in a similar setting, we assumed ~50% of the calves would develop pneumonia, defined by the presence of a consolidation ≥1 cm on qTUS, within the first weeks after arrival (Jourquin et al., 2023b). With a noninferiority margin of 10% considered as clinically relevant, the available sample size of 80 animals with pneumonia per treatment group allowed detection of a difference in cure up to 10 percentage points between the 2 antimicrobial groups with 80% power and an α level of <0.05. The trial protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine and Bioengineering from Ghent University under license number EC 2023–073. Authors declare human ethics approval was not needed for this study.

Animals and Housing

Before the trial, 320 male Holstein Friesian calves from different Flemish dairy farms were collected at the distribution center, weighed, and transported to the veal facility where the trial was performed. The trial was conducted in 1 of the 2 barns on the farm, which consisted of 3 interconnected areas. These areas held 280 calves (area 1), 40 calves (area 2), and 210 calves (area 3), respectively. The first 2 areas were separated by a door and a wall that was partly open, allowing open airflow between the areas. The third area was fully separated from the other areas by a sealed wall and closed doors. Only calves placed in areas 1 and 2 were included in the trial. All calves from area 3 were excluded. Calves that were included in the study arrived in 3 batches over 3 consecutive days, while calves destined for area 3 arrived 1 wk later and had no contact with the trial calves. The barn areas included in this study consisted of 4 compartments housing 70 calves each (area 1; 280 calves) and one compartment containing 40 animals (area 2; 40 calves). Compartments were separated by a partly sealed wall, but with open airflow between them. Upon arrival, animals were housed in individual calf pens on slatted floors separated by wired fences, according to European standards (Pardon et al., 2014). After 3 wk, fences were removed, leaving the calves in 64 pens consisting of 5 calves each. During the individual housing, calves were fed from individual drinking buckets. During group housing, milk and feed was provided through a common feeding trough. All calves received 2.2 L (500 g solids) of commercially available milk replacer (21% CP and 19% crude fat [**CF**] on a DM basis), 2 times per day. Additionally, calf muesli was provided (25 g/d at start, gradually increasing to 3 kg/d after 7 mo; CP = 14%; CF = 4.5%).

Clinical Scoring, qTUS, and Follow-Up

At all observation points, calves were clinically assessed using the Wisconsin respiratory scoring chart (McGuirk and Peek, 2014). For each calf, the following clinical parameters were evaluated: rectal temperature (°C), cough (both spontaneous and induced), ocular discharge, nasal discharge, and ear flick or head tilt. For each parameter, a score ranging from 0 to 3 was given based on the severity of presentation. Calves with a total score ≥5 and at least 2 clinical parameters with a score ≥2 were considered clinically ill and were labeled as clinical BRD.

For detection of pneumonia, TUS was performed using the UGhent qTUS technique, relying on anatomical landmarks to make sure the entire lung is visualized (Jourquin et al., 2022, 2024). During the trial, scanning was performed by the same 3 experienced operators that had received extensive training and had over 3 yr of experience. To ensure image quality, a 98% isopropyl solution was used as a transducing agent, and scanning was performed with a standard ultrasound device with a 7.5 MHz linear probe (manual ultrasound scanner V1, Kaixin, China). During the scan, as soon as an abnormality (e.g., comet tail artifacts, pleural irregularity, pleural effusion, or consolidation) was seen, the probe was halted in that position and gently moved cranio-caudally and back to expand the area of visualization. Consolidation depth was measured in the dorso-ventral plane using the grid on the screen of the ultrasound as a reference. For each scanned quadrant (right caudal, right cranial, left caudal, and left cranial), the most severe qTUS finding was noted. A description of the lung lobes that are described in each quadrant can be found elsewhere (Pardon, 2019; Jourquin et al., 2024). Definitions for pneumonia were based on maximum consolidation depth, creating the following qTUS categories: healthy (no consolidation), mild pneumonia (consolidation <1cm), moderate pneumonia (consolidation 1–3 cm), and severe pneumonia (consolidation ≥3cm). Combination of clinical scoring and qTUS allowed a second classification of the calves into 1 of 4 groups: healthy (no consolidation on ultrasound and no clinical BRD), clinical pneumonia (consolidation ≥1cm and clinical signs of BRD), subclinical pneumonia (consolidation ≥1cm, but no clinical signs of BRD) or URTi (clinical signs of respiratory disease, but no lesions on qTUS; van Leenen et al., 2020).

Randomization Process and Treatment Protocol

Before transportation to the veal facility, all calves were weighed at the distribution center. Next, within 24 h of arrival at the veal facility, all calves were clinically (Wisconsin score) and ultrasonographically (qTUS) evaluated by the 3 trained operators. In the following period, the local veterinarian visually assessed the calves every 48 h for clinical signs associated with respiratory disease (cough, tachypnea, dyspnea, ear drop, head tilt, and reduced feed intake or activity), while determining the rectal temperature of several calves at their own discretion. This routine was repeated until the local veterinarian considered 10% to 15% of the calves to be clinically ill and deemed antimicrobial group treatment to be necessary. This day was defined as the outbreak of respiratory disease (d 1 of trial period), at which point the research team operators performed clinical and ultrasonographic evaluation on all calves. Immediately after this evaluation, the randomization process was started.

Randomization, ensuring unbiased allocation of subjects to different treatment groups, was performed by developing a custom Python script (Python 3.10), using the train_test_split () function from the scikit-learn library. The dataset was randomly split into 2 equal subsets, with a 50:50 ratio, to create balanced experimental groups. Arrival batch, pen, pre-arrival weight, presence of consolidation ≥1 cm upon arrival and presence of consolidation ≥1 cm at the time of the outbreak were added into the script. Finally, following treatment groups with evenly distributed weights, calves with consolidation ≥1 cm on arrival and pneumonia prevalence at the outbreak were created: oxytetracycline group (OTC group) and florfenicol group (FF group). Randomization on batch and pen level ensured equal distribution of the treatment groups over each pen.

On the day of the outbreak (d 1), after randomization was completed, the treatment protocol was initiated. At this point, metaphylactic treatment was performed in all 320 calves, adapting dosing regimen to the average BW of the calves on arrival. Calves in the OTC group (n = 160) received oxytetracycline (Engemycine 10% LA, MSD Animal health, Boxmeer, the Netherlands) at a dosage of 20 mg/kg, corresponding to an i.m. injection of 10 mL. In the FF group (n = 160), calves were treated with FF (Nuflor 300, MSD Animal health, Boxmeer, the Netherlands) at a dosage of 20 mg/kg, equal to an i.m. injection of 3.5 mL. Sequence generation and administration of the antimicrobials were done by external veterinarians, leaving the trial blinded to all observers performing clinical examination or qTUS throughout the entire trial. No externally visible marks were present on the animals to distinguish treatment groups. Once allocated to a treatment group, calves were treated with the same antimicrobial as the previous treatment at the same dose for as long as a lung consolidation ≥1 cm was present throughout the trial. Both drugs were administered via i.m. injection every 48 h, according to manufacturer's recommendations. During the first 3 d of the trial period, calves also received oral sodium salicylate at 0.5 g/kg milk (sodium salicyl 80% WSP; Dopharma; Raamsdonksveer, the Netherlands). From d 1 onward, calves were scanned every other day, only retreating animals with lung consolidation ≥1 cm. Cure was defined by regression of maximum consolidation depth below 1 cm. At this point, treatment was discontinued. Calves where regression of consolidation depth <1 cm was seen, but consolidation ≥1cm reappeared within in the trial period were considered as a relapse and treatment was restarted using the same treatment regimen as before (OTC or FF).

In the period between arrival and start of the trial period (outbreak), the local veterinarian was asked to only

treat animals with severe clinical signs of disease (e.g., depression, dyspnea, fever, and reduced milk intake). These calves were treated with a 5-d parenteral treatment with 15 mg/kg lincomycin and spectinomycin (Emdactilin 50/100 mg/mL, Emdoka, Hoogstraten, Belgium) in combination with penicillin (PENI-kel, Kelan v, Hoogstraten, Belgium). All calves that were treated individually before the outbreak occurred were registered and accounted for in the analyses. All individual treatments were stopped when the trial period started. During the trial, ethical endpoints were defined based on the clinical presentation of the calves. At every scanning point, in addition to the clinical scoring performed by the research team, the local veterinarian assessed the calves visually for severe clinical signs of disease (fever, depression, dyspnea, and reduced milk intake). Calves that showed signs of severe respiratory disease were treated first according to their allocated treatment group, but if no improvement was seen after 48 h, a rescue was performed with a single s.c. injection of long-acting tulathromycin (Draxxin 100 mg/mL, Zoetis, Zaventem, Belgium) and dexamethasone intravenously (Dexaject 2 mg/mL, Dopharma, Raamsdonksveer, the Netherlands). After a rescue, the treatment protocol with either florfenicol or oxytetracycline was continued if consolidation ≥1 cm remained. Individual treatments by the local veterinarian and their indication (e.g., respiratory disease or diarrhea) were passed down to the research team and calves where rescue treatment was performed during the trial were excluded from cure analyses.

Laboratory Diagnosis

Identification of the pathogens present at the beginning and end of the trial period was done by sampling a total of 15 calves on 2 separate time points. The first samples were taken when the outbreak of BRD was first identified, before treatment was initiated (d 7 after arrival, 5 samples). On this time point, a calf from each compartment was conveniently selected based on the presence of clearly defined lung consolidation ≥1 cm. Next, 10 animals were sampled at the end of the trial period (d 9). At this point, 5 animals were randomly selected, using the Excel RAND function, from calves with consolidation ≥1 cm that did not have a consolidation ≥1 cm on any of the previous observation points (d 1, 3, 5, 7, and 9). At the same time, 5 other calves were randomly selected, using the same RAND function, out of the calves that had a consolidation ≥3 cm at all previous observation points (d 1, 3, 5, 7, and 9). In all calves, respiratory sampling was done using nonendoscopic broncho-alveolar lavage (**nBAL**), performed on unsedated calves using a sterilized catheter, as previously described (Van Driessche et al., 2017; Pardon and Buczinski, 2020).

At each sampling point, the nBAL samples from each group of 5 calves were pooled and sequenced using nanopore sequencing to identify all involved viral pathogens and *Mycoplasma* spp. (Theuns et al., 2018; Bokma et al., 2021b). To identify bacterial infections, individual nBAL samples were sent to an external accredited laboratory (DGZ Vlaanderen, Flanders, Belgium), and were directly inoculated on Colombia agar supplemented with 5% sheep blood (blood agar; Oxoïd, Hampshire, UK) and on *M. bovis* selective indicative agar (Bokma et al., 2020b). Bacteria detected on blood agar were identified by MALDI-TOF MS (Brüker Daltonik GmbH, Bremen, Germany) and *M. bovis* was identified based on lipase activity, as described previously (Bokma et al., 2020b). At the external laboratory, disk diffusion was used to test antimicrobial resistance in all Pasteurellaceae that were isolated. The antimicrobials tested were amoxycillin, amoxicillin-clavulanic acid, cefalexin, cefquinome, ceftiofur, doxycycline, enrofloxacin, florfenicol, flumequine, gamithromycin, kanamycin, marbofloxacin, penicillin, trimethoprim sulfonamides, tetracycline, Spectinomycin, tulathromycin, tildipirosin, gamithromycin, and tilmicosin. The clinical breakpoints used were based on the recommendations of references except tildipirosin and gamithromycin, which were assessed using the suppliers' recommendations (Mast Group, Liverpool, UK; Humphries et al., 2018; Amara et al., 2024).

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., 2020c). When possible, the genome of the involved strains was fully sequenced using nanopore sequencing and screened for the presence of known point mutations previously associated with antimicrobial resistance against enrofloxacin, gamithromycin, tylosin, tilmicosin, tetracycline, spectinomycin, and gentamycin (Vereecke et al., 2020; Bokma et al., 2021a).

Data and Statistical Analyses

All collected data were saved in a spreadsheet (Excel, Microsoft Inc.) and transferred to SAS enterprise guide 9 (SAS Institute Inc., Cary, NC) and SPSS statistics Version 29.0. (IBM Corp., Armonk, NY) for analysis. The individual calf was defined as the experimental unit. Primary outcomes of interest were cure (0 = not cured, 1 = cured) and treatment duration (continuous days). An overview of the definitions used for analyses and outcomes is provided in Table 1. At each time point, determination of cure was done using the proportion of calves that showed regression of consolidation size <1 cm in depth, over the total number of calves with pneumonia (consolidation ≥1 cm) at the previous scanning point. Final cure was

Table 1. Definitions and outcomes used for determination of treatment efficacy for respiratory disease in veal calves

Term	Definition[1]
Pneumonia	Presence of a lung consolidation ≥1 cm on thoracic ultrasound
Initial cases	Calves that had a consolidation ≥1 cm at the time of the outbreak (d 1)
New cases	Animals that developed pneumonia between start (d 1) and end of the trial period (d 9) but, had no consolidation ≥1 cm on d 1
All cases	All calves that developed pneumonia during the trial period and were treated with either FF or OTC
Cure	Regression of maximum consolidation depth from ≥1 cm to less than 1 cm
Treatment duration	The total number of days a calf was under antimicrobial treatment, determined by the number of injections with either FF or OTC it received during the trial period
Number of antimicrobial dosages	The total number of antimicrobial daily dosages that was given to each calf, including rescue treatments, based on the long-acting effects of the administered antimicrobials
Relapse	Reappearance of a consolidation ≥1 cm in calves where regression below 1 cm was previously seen
Treatment failure	Calves with consolidation ≥1 cm on d 1 where no regression of maximum consolidation depth below 1 cm was seen on any time point of the trial period
Clinical BRD	Calves with a total Wisconsin score ≥5, with at least 2 parameters scoring ≥2

[1]FF = florfenicol; OTC = oxytetracycline.

determined by the proportion of calves that were cured on qTUS at the end of the trial period (d 9). For each objective, analyses were performed separately on both the initial cases and all cases. Statistical analyses were performed without disclosure of the treatment groups.

For the primary objective, to determine differences in cure between FF and OTC, multiple general linear models with binomial distribution and logit link function with Wald's statistics for type 3 contrasts were created (PROC GLIMMIX). For each scanning point in the trial period, overall treatment effect was based on the Chi-squared test (Wald test). For cure, 2 models were created: one containing only the initial cases from the outbreak (to evaluate efficacy of a 2-d metaphylaxis), and another containing all calves that developed pneumonia over the course of the trial period for which treatment effects could be assessed (all cases). In addition to treatment group, arrival batch, arrival status (consolidation ≥ 1cm) and arrival weight, the presence of clinical signs at the time of treatment, compartment (1–5) and individual treatment before the trial period (0 = no individual treatment before the trial period, 1 = individual treatment before the trial period) were included in the model building procedure to identify potential confounding effects on cure. All variables with $P < 0.20$ in the univariable analysis were maintained for construction of the multivariable model. The multivariable model was constructed in a backward stepwise fashion, excluding factors with the highest *P*-value until all *P*-values were <0.05. Parameters were checked for confounding and model fit was evaluated by the Hosmer-Lemeshow test. Similar models were created to assess the probability of developing pneumonia after initial metaphylaxis (new cases) and the risk of treatment failure in both treatment groups. Finally, a Cox proportional hazards model was constructed with cure as a binary outcome (0 or 1), using treatment group and qTUS category at time of treatment as predictors. The time variable was defined as the number of days between first observation with pneumonia and discontinuation of treatment either when regression to <1 cm was seen, or the trial period ended. Right censoring was done at d 9 after the outbreak, at which point qTUS-guided treatment was stopped. Two separate Cox proportional hazard models were created: one containing all calves that developed pneumonia during the follow-up period, and another containing only the initial cases that had pneumonia at the outbreak of respiratory disease (d 1). For calves that were cured upon treatment, but relapsed during the trial period, healing time was based on the number of days it took for healing to occur the first time. In each model, the same parameters as described for the logistic regression modeling procedure were included.

Treatment duration was calculated by adding the number of days each calf was under treatment, including retreatments when consolidation ≥1 cm reappeared after initial cure. Considering the long-acting effect of both OTC and FF (48 h = per injection), each injection corresponded to a treatment duration of 2 d and 2 dosages (number of antimicrobial dosages, **NAD**). For the NAD, if parallel treatment (rescue) was performed by the local veterinarian, the NAD accounting for this treatment, as determined by the respective long-acting factor, were added (tulathromycin = 9 NAD). To determine the effect of OTC and FF treatment on treatment duration and total NAD, first these outcomes were checked for normal distribution and homoscedasticity by visual inspection of the histogram and quantile-quantile plots. When no normal distribution was present, nonparametric tests were used. Differences in treatment duration and NAD between treatment groups were assessed using the Mann-Whitney U test. One-sample *t*-testing was used to compare the NAD of ultrasound-guided treatment to a hypothetical 10-d metaphylaxis.

For the secondary objective (association between qTUS category and cure), 2 similar multiple general linear models with binomial distribution and logit link function with Wald's statistics for type 3 contrasts were created (PROC

Table 2. Overview of clinical characteristics, lung ultrasound findings, and arrival weight of 320 veal calves, stratified by treatment group at the outbreak of respiratory disease[1]

Variable	OTC	FF	Herd	*P*-value
Number in group	160	160	320	—
Positive Wisconsin score, %	11.3	10.6	10.9	1.000
Consolidation ≥1 cm	30.6	29.4	30.0	0.90
Mild pneumonia (consolidation <1 cm), %	5.6	13.8	9.7	0.02
Moderate pneumonia (consolidation 1–3 cm), %	16.9	17.5	17.2	0.65
Severe pneumonia (consolidation ≥3 cm), %	13.8	11.9	12.8	1.000
Arrival weight, kg (SD)	49 (5)	49 (5)	49 (5)	0.52

[1]OTC = oxytetracycline; FF = florfenicol.

GLIMMIX). Similar to the methods for objective 1, the effects of the different qTUS categories on treatment duration and NAD were evaluated using Kruskal-Wallis pairwise comparisons with Bonferroni correction for multiple tests. In all models, significance was set at $P < 0.05$. Finally, 2 separate Cox proportional hazard models were created, similar to the methods for objective 1.

RESULTS

Animals and Arrival Observations

Upon arrival, consolidation ≥1 cm was found in 6.3% (4/64) of the calves in batch 1, 9.9% (23/233) in batch 2, and 17.4% (4/23) in batch 3, resulting in a total of 9.7% (31/320) calves diagnosed with pneumonia upon arrival. Based on maximum consolidation depth on arrival, 85.0% (272/320) of the calves were classified as healthy (no consolidation), 5.3% (17/320) had mild pneumonia (consolidation <1 cm), 5.6% (18/320) had moderate pneumonia (consolidation 1–3 cm), and 4.1% (13/320) had severe pneumonia (consolidation ≥3 cm). Average BW on arrival was 49 kg (±5 SD; Minimum [**Min**] = 34 kg; Maximum [**Max**] = 78 kg). In batch 3, average BW was 5.7 kg (95% CI = 8.0–3.3; $P < 0.001$) and 7.4 kg (95% CI = 9.5–5.3; $P < 0.001$) lower than BW of calves from batch 1 and batch 2, respectively. Compared with batch 1, the average BW of calves in batch 2 was 1.8 kg (95% CI = 3.1–0.39; $P = 0.01$) higher. Of the calves arriving with consolidation ≥1 cm, only 19% (6/31) were also labeled with clinical BRD. Thus, of the 31 animals diagnosed with pneumonia on arrival, 25 (81%) had subclinical pneumonia. At arrival, combining clinical scoring and qTUS, 89.4% (286/320) of the animals were categorized as healthy, 7.8% (25/320) as subclinical pneumonia, 1.9% (6/320) as clinical pneumonia, and 0.9% (3/320) as URTi.

Outbreak Characteristics and Dynamics of Pneumonia

One week after arrival of the first batch, the local veterinarian deemed antimicrobial group treatment to be necessary. At this point, clinical scoring found that 10.9% (35/320) of the calves had clinical BRD (positive Wisconsin score) and consolidation ≥1 cm was found in 30.0% (96/320) of the animals. This point was labeled as the outbreak (d 1) and calves were randomly assigned to the OTC or FF group following the randomization process described above. An overview of the clinical characteristics, qTUS category on d 1, and arrival weight at the time of the outbreak is provided in Table 2. At the start of the outbreak, based on the combination of clinical scoring and qTUS, 66.9% (214/320) of the calves were healthy, 22.5% (71/320) had subclinical pneumonia, 7.8% (25/320) had clinical pneumonia and 3.8% (10/320) had URTi. Thus, of the calves diagnosed with pneumonia (consolidation ≥1 cm) at the start of the outbreak, 74.0% (71/96) had subclinical pneumonia.

Figure 2 gives a temporal overview of the prevalence of clinical BRD (positive Wisconsin score) and qTUS-defined pneumonia categories at each time point. Over the course of the trial period, the incidence of consolidation ≥1 cm progressively increased, reaching a prevalence of 50.9% (162/318) on d 9. At this point severe pneumonia was seen in 32.1% (102/318) of the calves and the trial period was ended. Prevalence of pneumonia was similar in both treatment groups at all observation points ($P > 0.05$). Over the trial period, 68.8% (220/320) of calves were observed with consolidation ≥1 cm on at least 1 occasion, with similar prevalences in both OTC (71.9%) and FF groups (65.6%; $P = 0.28$). The odds of having a consolidation ≥1 cm were similar in both antimicrobial groups at all observation points ($P > 0.31$).

Immediately following the trial period, upon request of the local veterinarian, antimicrobial group treatment was initiated consisting of oral treatment with doxycycline twice daily (Doxyveto 50% Pulvis, VMD, Arendonk, Belgium) for 11 consecutive days. After doxycycline treatment, with a cure of 46.5% (74/159) compared with the end of the trial period (d 9) and 28 new cases that developed during treatment, the prevalence of pneumonia had decreased to 35.8% (113/316). In wk 6 after arrival (d 38 after outbreak), consolidation ≥1 cm was found in 24.7% (78/316) of the calves. Three days before the

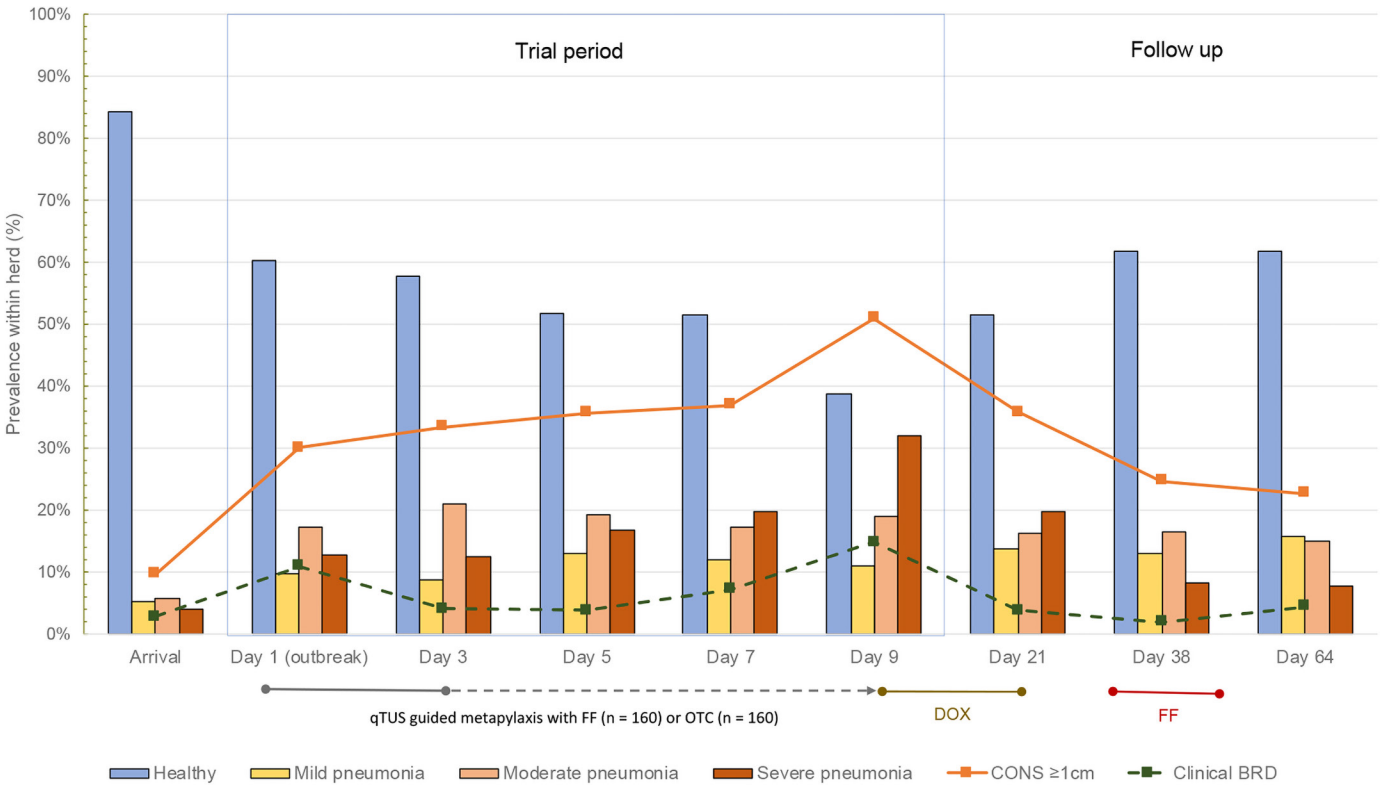

Figure 2. Overview of quick thoracic ultrasound (qTUS) defined pneumonia and clinical signs of respiratory disease in 320 veal calves over a 10-wk period, with a focus on the first 21 d after arrival. Healthy = no consolidation; mild pneumonia = consolidation <1 cm; moderate pneumonia = consolidation 1–3 cm; severe pneumonia = consolidation ≥3 cm; CONS = consolidation; clinical BRD = positive Wisconsin score; DOX = doxycycline; FF = florfenicol; OTC = oxytetracycline. Animals arrived at the veal facility 7 d before the trial was started. On d 1, a 2-d metaphylactic treatment was given on d 1 and follow-up was done every 48 h, only treating calves with pneumonia at that time. The days provided on the x-axis are calculated from d 1.

scanning point in wk 6, at the discretion of the local veterinarian, oral antimicrobial group treatment with 10 mg/kg (Flordofen 100 mg/mL, Dopharma) twice daily was started for 10 consecutive days. Because no observations were made immediately before or after this treatment, no treatment effects could be determined. Finally, in wk 10 of the production cycle, 61.8% (194/314) of the calves were healthy, 15.6% (49/314) had mild pneumonia, 15.0% (47/314) had moderate pneumonia and 7.6% (24/314) had severe pneumonia. By wk 10, the final cure of the initial cases from the outbreak (d 1) was 68.8% (64/93). In total, rescue treatment was performed on 23 calves during the trial, 12 in the OTC group and 11 in the FF group. These calves were excluded from all the following cure analyses.

Effects of Antimicrobial Treatment Group on Cure

Effects of Treatment Group on Cure of the Initial Cases. For the cases with pneumonia at the outbreak where no rescue treatment was performed, cure after the 2-d metaphylactic treatment was 20.9% (18/86). At this point, cure was 20.9% (9/43) and 20.9% (9/43) in the OTC and FF group, respectively. On d 5, 18.6% (8/43) and 27.9% (12/43) of the calves with pneumonia at the outbreak were cured in the OTC group and FF group, respectively ($P = 0.44$). Seven days after the first treatment, overall cure of these initial cases was 34.9% (30/86), again with equal cure in both the OTC (34.9%) and FF (34.9%) groups ($P = 1.0$). Finally, after 9 d, cure of the initial cases at the outbreak had decreased to 27.9% in both the OTC group and in the FF group, resulting in a final cure of 27.9% (24/86) for the initial cases at the outbreak. In Figure 3, an overview is provided of the overall lung health of all 320 calves, separated by treatment group.

Univariable logistic regression models with outcome cure on d 1 to 7 indicated that having pneumonia on arrival, compartment and qTUS category were suitable for inclusion in the multivariable models ($P < 0.2$). After backward stepwise selection, in the final models, next to treatment group as a forced variable, only qTUS category at the outbreak and compartment were maintained ($P < 0.016$). However, at none of the observation points was

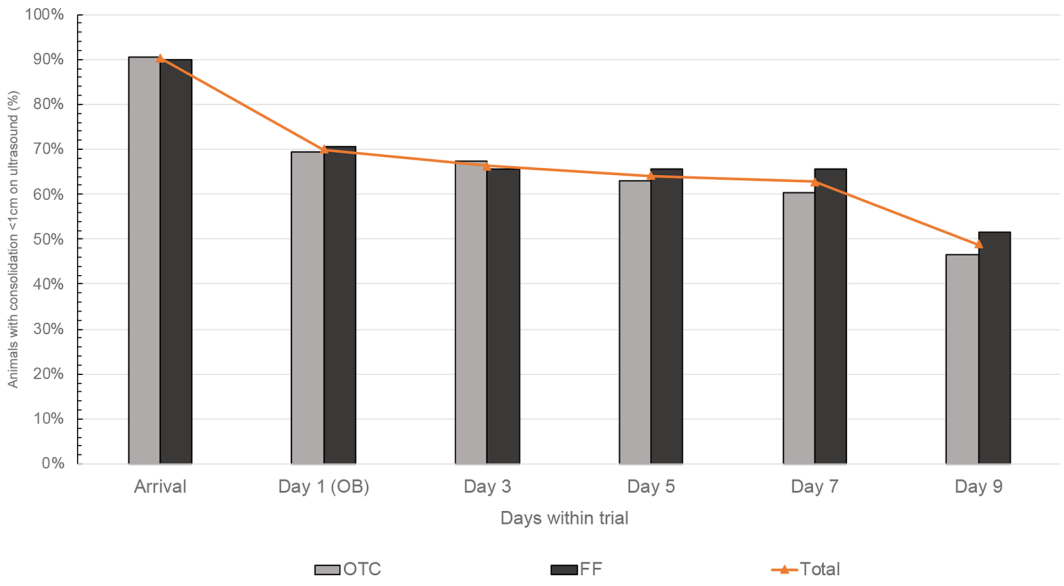

Figure 3. Overview of ultrasonographic lung health of 320 veal calves during the first 16 d after arrival at the facility. On d 1, all calves were treated with oxytetracycline (n = 160) or florfenicol (n = 160). Over the next 9 d, only calves with pneumonia were treated, using regression of consolidation to <1 cm as a criterion to stop antimicrobial treatment. OTC = oxytetracycline; FF = florfenicol; OB = outbreak.

there a significant difference in the odds of cure between the OTC and FF groups ($P > 0.22$). Final cure on d 9 was also similar when comparing the FF group to the OTC group (odds ratio [**OR**] = 0.93; 95% CI: 0.34–2.5; $P = 0.89$). Compartment no longer had a significant effect on the odds to cure on d 7 and d 9, but was kept into the model to account for clustering.

Effects of Treatment Group on All Cases. Over the trial period, including calves that had pneumonia at the start of the outbreak, a total of 183 calves had pneumonia on at least one time point between outbreak and d 7. When excluding calves that received additional treatment with tulathromycin (rescue), 166 calves remained that were only treated with OTC or FF during the trial and treatment effects could be assessed. For these calves, cure after first (re)treatment was 32.6% (28/86) and 37.5% (30/80) in the OTC and FF groups, respectively ($P = 0.52$). By d 9, the final cure of the surviving animals was 32.7% (54/165), and was similar in the OTC (31.8%) and FF (33.8%) groups ($P = 0.87$).

For all calves that developed consolidation between d 1 and d 7 and were included in the analyses, (n = 166), univariable logistic regression models with outcome cure on d 9 indicated that compartment ($P = 0.14$) and qTUS category (moderate or severe) at first observation with consolidation ≥1 cm ($P = 0.004$) needed to be included in the multivariable model building procedure. After backward stepwise selection, only treatment group and qTUS category at first observation with pneumonia were included in the final model. However, the odds of cure did not differ between the OTC and the FF groups (OR = 0.98; 95% CI: 0.50–1.92; $P = 0.95$). Cox regression analysis, containing treatment group and qTUS category at time of treatment, showed a similar hazard ratio (**HR**) for cure when comparing the FF group to the OTC group, when considering all animals that developed pneumonia during the trial (HR = 0.99; 95% CI; 0.58–1.68; $P = 0.96$) as when considering the initial 86 cases at the start of the outbreak (HR = 1.1; 95% CI: 0.47–2.3; $P = 0.90$).

Effects of Ultrasonographic Categories on Cure

Effects of qTUS Category on Cure of the Initial Cases. Next to treatment group, this study also assessed the associations between qTUS category (mild, moderate, or severe) at the time of treatment initiation and cure. For the initial cases at the outbreak that were included (n = 86), the cure for calves with moderate or severe pneumonia at the outbreak are provided in Table 3. After the single metaphylactic treatment, 69.2% (18/26) of the calves with mild pneumonia had fully reaerated lungs, and 3.8% (1/26) still had mild pneumonia. Despite antimicrobial treatment, 26.9% (7/26) had worsened to moderate pneumonia. None of the calves with mild pneumonia at the time of metaphylaxis evolved to severe pneumonia. In contrast, 9.2% (17/184) and 1.6% (3/184) of the calves that were healthy at the outbreak developed moderate and severe pneumonia despite metaphylactic treatment, respectively. After metaphylaxis on d 1, only calves with consolidation ≥1 cm were retreated. In Figure 4, the transitions between the qTUS categories are represented for each observation point. The odds of cure after 2-d

Table 3. Effect of qTUS category on cure in veal calves with either moderate or severe pneumonia at the time of treatment initiation[1]

Variable	Moderate pneumonia	Severe pneumonia	Total	*P*-value
Number at outbreak	48	38	86	—
Cure d 3, %	33.3	5.3	20.9	0.001
Cure d 5, %	33.3	10.8	23.3	0.02
Cure d 7, %	50.0	15.8	34.9	0.001
Cure d 9, %	37.5	15.8	27.9	0.03

[1]Moderate pneumonia = consolidation 1–3 cm; Severe pneumonia = consolidation ≥3 cm; Cure = regression of consolidation depth below 1 cm.

metaphylaxis were much lower for calves with severe pneumonia at the outbreak than for calves with moderate pneumonia on d 1 (OR = 0.17; 95% CI: 0.044–0.63; P = 0.008). For the initial cases, odds for cure by d 9 were also decreased for calves with severe pneumonia on d 1 when compared with calves with moderate pneumonia at that time (OR = 0.31; 95% CI: 0.11–0.89; P = 0.03).

Effects of qTUS Category on Cure of All Cases. Using the qTUS category found on the first observation with consolidation ≥1 cm as a predictor for cure, effectiveness of subsequent treatments of all 166 calves that developed pneumonia between d 1 and d 7, and were not treated with a rescue treatment is provided in Table 4. In total, when considering all included calves that developed consolidation ≥1 cm before d 9, final cure on d 9 was 41.2% (52/102) and 19.0% (12/63) for calves with moderate and severe pneumonia at the time of their first (re)treatment, respectively (P = 0.004). Binary logistic regression models for cure, containing qTUS category and treatment group, indicated that odds to cure within the trial period for calves with severe pneumonia were only 34% of those with moderate pneumonia at first treatment (OR = 0.34; 95% CI: 0.16–0.71; P = 0.004). Further, Cox regression analysis indicated that the hazard of cure within the trial period was significantly lower for animals with severe pneumonia at the start of treatment (HR = 0.37; 95% CI: 0.19–0.70; P = 0.002).

Treatment Duration and Antimicrobial Use

Treatment duration was calculated based on the number of treatments with either OTC or FF given to each calf, including cases where treatment was restarted. Because a single metaphylactic treatment was given on d 1 (outbreak), minimal treatment duration was set at 2 d for each calf. For both OTC and FF, this corresponded

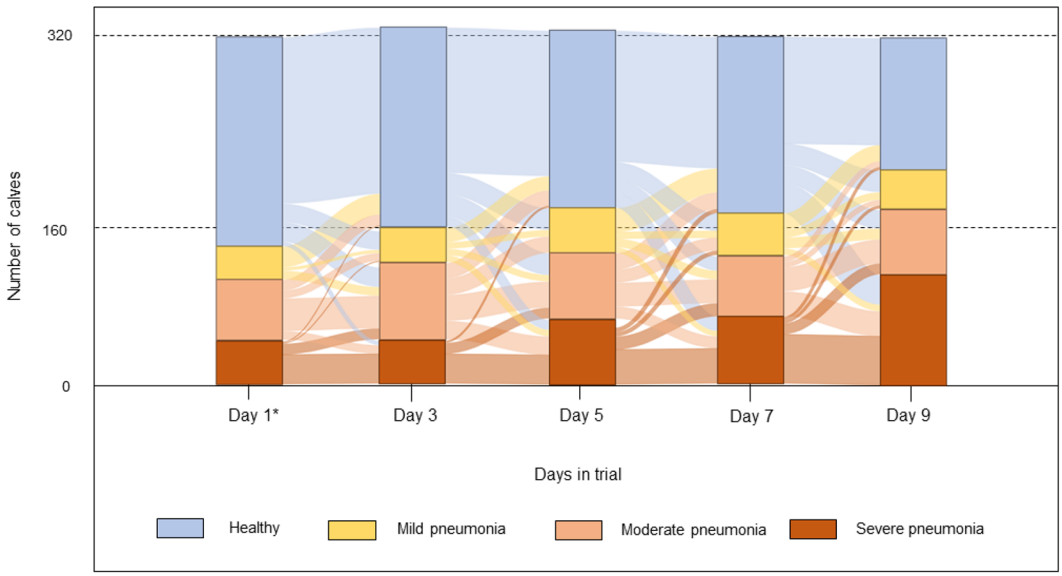

Figure 4. An alluvial plot showing the transitions in quick thoracic ultrasound (qTUS) scores over time. The vertical columns represent each visit, each time with a 48-h interval. The colors of each block correlate to a qTUS score depicted at the bottom of the figure. Individual calves are represented by individual lines passing between visit blocks. Calves can be tracked over the study period with the qTUS score at each point. Healthy = no consolidation; mild pneumonia = consolidation <1 cm; moderate pneumonia = consolidation 1–3 cm; severe pneumonia = consolidation ≥3 cm. *On d 1, metaphylactic treatment was performed on all calves. On all subsequent days, only calves with moderate or severe pneumonia were treated.

Table 4. Effect of qTUS category on the likelihood of cure in veal calves with either moderate or severe pneumonia, stratified number of consecutive treatments[1]

Item	No. treated	Moderate pneumonia, n (%)	Severe pneumonia, n (%)	Total cure, n (%)	No. of calves with pneumonia after treatment	*P*-value
qTUS start	166	102	63	—	—	—
Cure after 1st treatment	165	46 (45.1)	11 (17.4)	57 (34.9)	108	<0.001
Cure after 2nd treatment	90	12 (24.0)	1 (2.5)	13 (14.4)	77	0.005
Cure after 3rd treatment	68	11 (37.9)	3 (7.7)	14 (20.6)	54	0.002
Cure after 4th treatment	45	1 (6.7)	0 (0.0)	1 (2.2)	44	0.3
Final cure	165	42 (41.2)	12 (19.0)	54 (32.7)	111	0.004

[1]Moderate pneumonia = consolidation 1–3 cm; Severe pneumonia = consolidation ≥3 cm; Cure = regression of consolidation depth below 1 cm.

to 2 antimicrobial doses (NAD = 2). During the trial, 23 calves were treated individually by the local veterinarian with tulathromycin, corresponding to 9 antimicrobial dosages (NAD = 9). Analysis for treatment duration were performed excluding the 23 calves where rescue was performed. Over the entire herd (n = 297), median treatment duration was 4 d (interquartile range [**IQR**] = 2–6; Min = 2; Max = 8). Treatment duration was similar in both treatment groups ($P = 0.59$).

Median treatment duration of all calves with a consolidation ≥1 cm at the outbreak was 8 d (IQR = 6–8; Min = 2; Max = 8). In Table 5, an overview of the median treatment duration is given, stratified by the qTUS category to which calves were assigned on d 1 (outbreak) and using the calves that were healthy on d 1 as a reference group. When compared with calves with mild pneumonia at the outbreak, calves with moderate ($P = 0.01$) and severe pneumonia ($P < 0.001$) had significantly longer treatment durations. Treatment duration between calves with moderate and severe pneumonia at the outbreak was not different ($P = 0.10$). In contrast, median treatment duration for calves with moderate pneumonia (Med = 6; IQR = 4–6; Min = 2; Max = 8) at their first observation with pneumonia was lower than the median treatment duration of calves with severe pneumonia (Med = 8; IQR = 4–8; Min = 2; Max = 8) at that time ($P = 0.004$).

In total, including the 23 individual treatments performed by the local veterinarian, the NAD given throughout the trial period was 1,525. The median NAD was 4 d (IQR = 2–6; Min = 2; Max = 17) and was similar in the OTC and FF groups ($P = 0.57$). In addition, the total NAD was significantly higher for calves with severe pneumonia when compared with calves with moderate pneumonia at their first observation with consolidation ≥ 1cm ($P = 0.03$). Compared with a hypothetical 10-d metaphylactic treatment (NAD = 3,200), a qTUS-guided approach resulted in a reduction of 52.3% in NAD used ($P < 0.001$).

New Cases, Treatment Failure, and Relapse

Apart from the 96 calves that had pneumonia at the start of the outbreak, an additional 124 (38.9%) calves developed pneumonia between d 1 (start outbreak) and d 9 of the trial period. These calves were labeled as "new cases" of pneumonia. Despite metaphylactic treatment at the outbreak, 31 calves (13.8%) still developed pneumonia between d 1 and d 3, with similar occurrences in the OTC (12.6%) and FF (15.0%) groups ($P = 0.70$). Over the entire trial period, the proportion of new cases was similar in both treatment groups ($P = 0.36$). Of these new cases, 86 were detected on d 3, 5, or 7, and were treated following the protocol either with OTC or FF. For these calves, treatment efficacy could be assessed. An overview of the differences in cure of the initial cases, the new cases that were seen before d 9 and all pneumonic cases combined is provided in Table 6. On d 9, 60 calves with pneumonia were observed that did not have pneu-

Table 5. Effect of qTUS categories and antimicrobial treatment group on treatment duration of veal calves with oxytetracycline or florfenicol[1]

qTUS category at outbreak	No. in group	Median	IQR	Min–Max	*P*-value
Healthy (referent)	184	2	2–4	2–8	—
Mild pneumonia	26	4	2–4	2–8	0.31
Moderate pneumonia	48	6	4–8	2–8	<0.001
Severe pneumonia	39	8	8–8	2–8	<0.001
Treatment group					
Oxytetracycline (referent)	148	4	2–6	2–8	
Florfenicol	149	4	2–6	2–8	0.59

[1]Healthy = no consolidation; mild pneumonia = consolidation <1 cm; moderate pneumonia = consolidation 1–3 cm; severe pneumonia = consolidation ≥3 cm.

Table 6. Effects of antimicrobial treatment group on occurrence of new cases, cure, relapse, and treatment failure in veal calves treated with either oxytetracycline or florfenicol based on ultrasonographic findings[1]

Item	No. in group	OTC	FF	Total	*P*-value
New cases, %	124	41.5	36.3	38.9	0.36
Cure initial cases, %	96	27.1	25.5	26.3	1.0
Cure new cases, %	86	34.8	37.5	36.0	0.83
Cure all cases, %	183	30.9	31.0	30.9	1.0
Relapse, %	44	12.5	15.0	13.8	0.63
Treatment failure, %	48	15.1	15.0	15.0	1.0

[1]OTC = oxytetracycline; FF = florfenicol.

monia on d 7. At this point, the trial period ended, and doxycycline treatment was started. Of these 60 new cases that developed between d 7 and 9, 68.3% (41/60) were cured after doxycycline treatment.

Of the 96 calves that had pneumonia on d 1, 50.0% (48/96) were observed with pneumonia on each subsequent scanning point during the trial. These cases were labeled as "treatment failure." When considering the effects of consolidation depth at the outbreak, treatment failure was observed in 29.1% (16/55) of the calves with moderate pneumonia and 80.0% (32/40) of the calves with severe pneumonia at that time ($P < 0.001$). Odds for treatment failure were 9.75 times higher (95% CI: 3.7–25.7; $P < 0.001$) for animals with severe pneumonia at the outbreak when compared with animals with moderate pneumonia at that time. Of the calves with treatment failure during the trial, 20.8% (10/48) were cured after doxycycline treatment. By wk 10, 57.7% (27/47) of the surviving calves with initial treatment failure were cured.

In 44 calves, consolidation ≥1 cm reappeared in calves where cure was previously observed. These calves were labeled as "relapse" cases and had an occurrence of 21.1% (20/95) and 27.3% (24/88) in the OTC group and FF group, respectively ($P = 0.36$). When comparing calves that had fully reaerated lungs after a first treatment (n = 34) to calves where consolidation regressed to <1 cm in depth but no full reaeration was seen (n = 22), after 4 d, consolidation ≥1 cm reappeared in 23.5% (8/34) and 63.6% (14/22) of these calves, respectively ($P = 0.01$). Compared with calves that fully reaerated after first treatment, calves that still had mild pneumonia when treatment was stopped had 3.15 times higher odds to relapse within the trial period (95% CI: 1.09–9.10; $P = 0.04$). An overview of the dynamics of qTUS categories over the trial period is shown in Figure 4.

Mortality

In total, 6 (1.9%) calves died within the first 10 wk after arrival, of which 2 occurred within the trial period. Calves that died during the trial period each originated from a different treatment group. The calf from the OTC group developed severe pneumonia on all 4 quadrants between arrival and outbreak and died within 48 h after receiving its first treatment with OTC. The calf from the FF group developed pneumonia between d 3 and d 5 and did not respond to antimicrobial treatment. Both calves that died during doxycycline treatment had pneumonia on d 9, and each originated from a different treatment group. The 2 calves that died between wk 6 and wk 10 did not have pneumonia at their previous observation point and were noted as peritonitis cases by the local veterinarian. Because mortality was low and equal in both treatment groups, no sensitivity analysis was performed.

Pathogens

On both sampling points, a combination of *M. bovis*, respiratory viruses and *Pasteurellaceae* were found. A detailed overview of all pathogens identified in the trial period and their respective sampled calf characteristics are given in Table 7. At the outbreak, antimicrobial susceptibility testing showed 2/4 *Pasteurella multocida* isolates with in vitro resistance to both tetracyclines and spectinomycin. Both *P. multocida* isolates with OTC resistance originated from a different treatment group. The other 2 were susceptible to both tetracycline and florfenicol, with one isolate showing resistance to penicillin. All isolates were susceptible to florfenicol. All *Mannheimia haemolytica* isolates that were found at this point showed in vitro susceptibility to all tested antimicrobials. On d 9, for the calves with treatment failure, tetracycline resistance was detected in both *P. multocida* isolates, and both isolates originated from calves in the OTC group. Additionally, in vitro resistance against doxycycline, gamithromycin and penicillin were detected. In the new cases, antimicrobial resistance against penicillin was found in all sampled calves with *P. multocida* (n = 4). No resistance was found for any of the other tested antimicrobials.

Phylogenomic analysis after nano sequencing identified the *M. bovis* strain found at the outbreak as part of Belgian genomic cluster I. On d 9, both from the new cases and the cases with treatment failure, each time a strain from both Belgian genomic clusters I and IV were identified (Bokma et al., 2020c). In all isolates, a G748

Table 7. Results of etiological respiratory diagnostics by bacterial culture and nano sequencing of nonendoscopic broncho-alveolar lavage samples from 15 veal calves on 2 sampling points during the first 4 wk of the production cycle

Nr.	Group[1]	Category	Blood agar	Selective indicative agar	Metagenomic analysis (pooled sample)
Outbreak (d 1)					
B1	FF	Clinical	*Mannheimia haemolytica*	Negative	Bovine coronavirus, influenza D virus, *Mycoplasma dispar*, *Mycoplasma bovis*, *Pasteurella* sp.
B2	FF	Clinical	*Mannheimia* sp., *Pasteurella multocida*	*M. bovis*	
B3	OTC	Clinical	*Mannheimia* sp., *P. multocida*, *Streptococcus gallolyticus*	Negative	
B4	FF	Clinical	*Mannheimia haemolytica*, *P. multocida*	Negative	
B5	OTC	Subclinical	*P. multocida*	*M. bovis*[2] (cluster I)	
Day 9: New cases					
B6	OTC	Clinical	*P. multocida*	*M. bovis*[2] (cluster I)	Bovine rhinitis A virus, *Mannheimia* sp., *M. dispar*, *Mycoplasma arginini*, *M. bovis*, *Pasteurella* sp., *Ureaplasma* sp.
B7	FF	Subclinical	*P. multocida*	*M. bovis*	
B8	OTC	Subclinical	*P. multocida*, *Trueperella pyogenes*	*M. bovis*[2] (cluster IV)	
B9	FF	Subclinical	*Escherichia coli*, *Mannheimia glucosida*, *Strep. gallolyticus*	*M. bovis*	
B10	FF	Subclinical	*P. multocida*, *S. gallolyticus*, *T. pyogenes*	*M. bovis*	
Day 9: Treatment failure					
B11	FF	Subclinical	*E. coli*, *Gallibacterium anatis*, *Strep. gallolyticus*	*M. bovis*	Bovine respiratory syncytial virus, bovine coronavirus, bovine rhinitis A virus, *M. dispar*, *M. bovis*
B12	OTC	Subclinical	*P. multocida*	*M. bovis*	
B13	OTC	Subclinical	Negative	*M. bovis*[2] (cluster IV)	
B14	OTC	Subclinical	*S. gallolyticus*, *Streptococcus pluranimalium*	*M. bovis*	
B15	OTC	Subclinical	*P. multocida*	*M. bovis*[2] (cluster I)	

[1]OTC = oxytetracycline; FF = florfenicol.
[2]These *M. bovis* strains were phylogenomically analyzed and categorized into Belgian genomic clusters following previously described methods (Bokma et al., 2020c).

mutation in the 23S rRNA gene was found, previously associated with resistance against tilmicosin (Sato et al., 2017; Bokma et al., 2021a). In addition, both A965T and A967T mutations, associated with tetracycline resistance were found in all isolates (Amram et al., 2015; Hata et al., 2019). Next, in one isolate, the Gln93His mutation in the *rplV* gene was found, which has been associated with resistance against gamithromycin and tylosin (Bokma et al., 2021a). No other mutations that have previously been associated with antimicrobial resistance were found. A more detailed overview of the antimicrobial susceptibility test results for each Pasteurellaceae isolate and WGS results from the *M. bovis* strains are provided in Supplemental File S1, sheet 1 and 2 (see Notes), respectively.

DISCUSSION

The present study can be considered pioneering for multiple reasons. First, to the authors' knowledge, it is the first peer-reviewed report on a clinical trial that evaluated the efficacy of 2 first-choice antimicrobials, using injectables and a TUS based definition of pneumonia, in veal calves. Second, this is the first trial in veal calves where treatment length for each individual calf was tailored to the lung ultrasound findings. One other trial used ultrasound to evaluate the efficacy of macrolides in veal calves, but TUS was only used as a tool to detect consolidation (Berman et al., 2017). Lastly, this is the first trial that investigated the associations between consolidation depth and cure, linking the qTUS classifications of pneumonia (mild, moderate, severe) with cure and required treatment length. This information is not available for any other lung ultrasound scoring system.

The primary objective of this study was to investigate the effects of parenteral treatment with oxytetracycline or florfenicol in a high-risk environment for BRD, using regression of maximal consolidation depth to <1cm on qTUS as a criterion to stop antimicrobial treatment. For this objective, 2 interesting findings were made. First, no differences in cure were observed between oxytetracycline and florfenicol at any time point. Second, cure in this trial was remarkably low, especially when compared with reported treatment effects of oral doxycycline in 2 previous studies in Belgian veal calves (Jourquin et al., 2023a; b). At the time of this study, no other trials exist evaluating injectable first-choice antimicrobials in veal calves. Additionally, in other sectors, determination of treatment efficacy is mostly determined based on a variation of clinical definitions and do not incorporate ultrasound findings (DeDonder and Apley, 2015a; O'Connor et al., 2016). Nevertheless, despite the common perception that second and third choice products are more effective, a meta-analysis of existing clinical trials showed no evidence supporting superior efficacy of a specific an-

timicrobial class, with the exception of oxytetracycline performing worse than other classes (O'Connor et al., 2016). In this regard, only one other study compared the efficacy of oxytetracycline and florfenicol in a natural outbreak of *M. bovis* in beef calves, using qTUS to guide treatment duration. In that trial, apart from a slightly higher health ratio in the FF group after a first injection, final cure for oxytetracycline and florfenicol was similar, which is in line with our results. Despite observing similar effects between the antimicrobials, cure in the current trial was much lower than the findings on that commercial beef farm, which already reported a cure > 90% after 4 d of treatment (Jourquin et al., 2022). There are different reasons why cure in the current trial was so low. First, as is confirmed by the laboratory results from the start of the trial period, bovine coronavirus (**BCoV**), bovine influenza D virus and bovine respiratory syncytial virus (**BRSV**) were circulating during the trial period and were likely causing disease while being that would not have been influenced by antimicrobial treatment. However, despite being performed under highly comparable circumstances (similar time of treatment initiation, similar housing and feeding and similar pathogens involved), a previous trial in veal calves reported a cure just over 50% after oral doxycycline treatment (Jourquin et al., 2023a). A second possible reason is the study protocol itself. Because of the qTUS-guided treatment, calves only received antimicrobial treatment as long as they had a consolidation ≥ 1cm on qTUS. This contrasts with the usual protocol, consisting of metaphylactic treatment of all calves at risk for 7 to 10 consecutive days. Although this approach is increasingly criticized, providing a 'blanket therapy' might be useful to prevent massive spread and bacterial superinfection during the stages of the production cycle where viruses and *M. bovis* play an important role (Pardon et al., 2011; Antonis et al., 2022). In situations where there is a high risk of BRD, full metaphylactic treatment could be useful to subdue the clonal spread of (primary) bacterial pathogens such as *M. bovis*, and has been described to be more beneficial for animal welfare than individual treatment (Word et al., 2020). In the present study, a cure of 45.7% was seen upon doxycycline treatment after the trial, which is more similar to previously reported effects. Still, because the differences in this study are only observational, there is an urgent need to make a direct comparison between oral doxycycline treatment and parenteral treatment with first-choice antimicrobials. Third, the role of antimicrobial resistance should be considered. Despite phenotypical antimicrobial resistance to both oxytetracycline and florfenicol being reportedly low in Belgian *M. bovis* isolates, WGS did indicate mutations previously associated with elevated minimum inhibitory concentration values for OTC (Hata et al., 2019; Bokma et al., 2020a). However, since mutations associated with in vivo resistance against florfenicol are still unknown, and cure was equally low in both groups, it is difficult to give a clinical interpretation to these results. For the other bacterial pathogens, no in vitro resistance against florfenicol was found in any of the *P. multocida* or *Mannheimia haemolytica* isolates taken at the outbreak, while oxytetracycline resistance was found in 2 *P. multocida* isolates, each originating from a calf in a different treatment group. Therefore, it is possible that AMR selection contributed to the low cure rates in the OTC group, but its role in the FF group is more difficult to assess (Bürki et al., 2015; Kudirkiene et al., 2021). In general, given that there currently is no consensus on clinical breakpoints for florfenicol and oxytetracycline using disk diffusion, no definitive conclusions can be drawn from this study. Fourth, the dose that was administered might have influenced treatment efficacy. All calves were treated based on the average body weight upon arrival, leading to potential underdosing of heavy animals and overdosing lighter animals later in the trial (DeDonder and Apley, 2015b). Although this could have affected treatment efficacy, this study found no associations between arrival weight and cure. Finally, antimicrobial treatment was stopped when maximal consolidation depth regressed to < 1 cm, while previous studies used full lung reaeration as a cure criterion (Jourquin et al., 2022). Although the current study was conducted under more extreme conditions, the number of new cases and relapses is remarkably high. In this study, when compared with full reaeration, the relapse rate was more than doubled when reaeration was incomplete. Both in human and veterinary medicine, regression of consolidation size has been used as a sign of healing, but little is known on the capacity for self-cure of lung lesions (Bouhemad et al., 2010; Bello and Blanco, 2019). In a veal calf setting, the findings of this study suggest that regression to <1 cm is insufficient as a cure criterion. Although still disputed for FF, both antimicrobials rely on the host immune system to eliminate the infection (bacteriostatic; Trif et al., 2023). However, under these extreme conditions, with high stress, severe disease and multiple factors compromising the immune system, it is likely that these drugs are not as effective as they would be in other production systems (WHO, 2016; Becker et al., 2020). Whether self-cure of mild pneumonia is more feasible in other production systems has yet to be investigated.

A second finding in the present study is that the qTUS categorization based on maximum consolidation depth was associated with cure and treatment duration. Different studies exist where TUS lesion scoring is performed to assess disease severity, but currently no studies have assessed the effects of disease severity on cure (Ollivett and Buczinski, 2016; Rhodes et al., 2021). Under the

conditions of this study, cure decreased substantially with each increase in qTUS category, and calves with severe pneumonia responded poorly to treatment. As a consequence, both treatment duration and NAD increased with consolidation depth, categorized with the qTUS scoring system. When compared with a similar approach on conventional farms, where no new cases or relapses were seen, and treatment failure was only observed in one animal, the number of new cases, relapses and treatment failures in the present study should not overlooked (Jourquin et al., 2022). The number of new cases in this trial was also substantially higher than what has been described during metaphylaxis in an observational study in veal calves (Jourquin et al., 2023a). Possible reasons for these differences are already described earlier. However, the laboratory findings in this study provide interesting insights. The finding that *M. bovis* was omnipresent in all 10 samples taken at the end of the trial suggests that rapid horizontal transmission occurred, despite antimicrobial treatment (Timsit et al., 2012). Pneumonia caused by *M. bovis* is known to be poorly responsive to antimicrobial treatment and can lead to chronic infection, which could explain the low cure (Dudek and Szacawa, 2020). However, nanopore sequencing found both BRSV and BCoV in calves that never cured over the entire trial period, while no noteworthy viruses were found in the group of calves that developed pneumonia for the first time at the very end of the trial period. For BRSV, clinical recovery is usually reported within 10 d, and postmortem investigations mostly describe multilocular consolidations (Makoschey and Berge, 2021). Although only 5 calves were sampled in each group, this is an important finding because it could suggest that the lung lesions found in these uncured calves were, at least partly, of viral origin, and thus remained unaffected by antimicrobial treatment. In addition, even after an additional 11 d of doxycycline treatment, cure of these initial cases of treatment failure was poor (20.8%), whereas almost 70% of the new cases on d 9 were cured after doxycycline treatment. Currently, little is known about the persistence of infection in consolidated lung tissue after viral infection. During most viral infections, the virus causes direct damage to the lung tissue, immediately activating wound-healing response. However, as is also described for COVID-19 in human medicine, viruses can induce persistent lung damage (Huang and Tang, 2021; Makoschey and Berge, 2021). Therefore, with the methods used in this study, we can only speculate whether these chronic lesions still had an active infectious component, albeit as a secondary bacterial infection. However, because *M. bovis* was present in all samples, and the isolated strains appeared to be genetically similar, it is likely that clonal spread of this primary pathogen was involved in the disease process.

Because the trial was performed on a commercial veal facility using multiple qTUS operators, the study had several limitations. First, the current trial setup does not allow definitive conclusions to be made on the degrees of possible self-cure using different cure criteria. Subdividing calves into mild, moderate, and severe pneumonia is an accessible way to quickly categorize without the need to individually measure each consolidation. However, consolidation depths around or just below 1 cm might be more prone to be classified differently between operators, or by the same operator on a subsequent scanning occasion. Although good interrater agreement has been described for interpretation of TUS images, interobserver variation can be substantial when performed by novice users (Buczinski et al., 2018; Jourquin et al., 2024). However, the 2 of the 3 qTUS operators in this trial were highly experienced and had an almost perfect agreement when tested (kappa >0.9), while the third received the same training and had equal experience. Nevertheless, because of the reasons mentioned above, the definition of relapse should be interpreted with care, especially because TUS on itself does not allow differentiation between incomplete elimination of the pathogen or a new infection (Porter et al., 2021). A second limitation in this study was that, because metaphylactic treatment was performed at the outbreak, animals that only developed pneumonia later in the trial period had already received a single dose of OTC or FF on d 1. For these calves, it is possible that the dynamics of disease and effects of a second treatment were influenced by this initial treatment. Because new cases were observed and treated within 48 h, the higher cure can potentially be explained by the disease being tackled at an earlier stage in the process. Also, antimicrobial resistance selection could have played a role in these calves. Third, calves also received sodium salicylate during the first 3 d of the outbreak, likely masking clinical signs of disease during this time. Furthermore, despite contradictory and inconsistent effects of nonsteroidal anti-inflammatory drugs (**NSAID**), an effect of NSAID use on development of consolidation cannot be excluded (Francoz et al., 2012). However, because sodium salicylate was given to both treatment groups for only a short duration, we believe that the implications on our primary objective were limited. Finally, for ethical and practical reasons, no positive control group was used in this study, making it impossible to make a direct comparison to the usual protocol using oral doxycycline.

CONCLUSIONS

Under the conditions of this study, in a challenging veal calf setting, qTUS-guided treatment using OTC or FF resulted in disappointingly low cure rates for both

antimicrobials. A 2-d metaphylactic treatment resulted in low ultrasonographic cure, decreasing with consolidation depth at time of treatment initiation. Cure, treatment duration, and antimicrobial use were all significantly different between calves with mild, moderate, and severe pneumonia. Using a consolidation depth of 1 cm to initiate treatment seems appropriate, but lung reaeration likely must be complete for it to be a reliable cure criterion when *M. bovis* is involved. The presented study protocol, combining qTUS and nBAL diagnostics, offers major benefits for the interpretation of randomized clinical trials.

NOTES

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Nonstandard abbreviations used: AMR = antimicrobial resistance; BCoV = bovine coronavirus; BRD = bovine respiratory disease; bRSV = bovine respiratory syncytial virus; CF = crude fat; DOX = doxycycline; FF = florfenicol; HR = hazard ratio; IQR = interquartile range; Max = maximum; Min = minimum; nBAL = nonendoscopic broncho-alveolar lavage; NAD = number of antimicrobial dosages; NSAID = nonsteroidal anti-inflammatory drugs; OB = outbreak; OR = odds ratio; OTC = oxytetracycline; qTUS = quick thoracic ultrasound; TUS = thoracic ultrasound; URTi = upper respiratory tract infection; WGS = whole genome sequencing.

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