Effect of on-arrival bovine respiratory disease vaccination on ultrasound-confirmed pneumonia and production parameters in male dairy calves: A randomized clinical trial

Stan Jourquin,[1]* ● Thomas Lowie,[1] ● Florian Debruyne,[1] ● Laurens Chantillon,[1] ● Justine Clinquart,[1] ●
Mathilde L. Pas,[1] Randy Boone,[2] Geert Hoflack,[3] Geert Vertenten,[3] ● Bart Sustronck,[3] ● and Bart Pardon[1] ●
[1]Department of Internal Medicine, Reproduction and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133,
9820 Merelbeke, Belgium
[2]Veterinary Practice Venhei, Geelsebaan 95-97, 2460 Kasterlee, Belgium
[3]MSD Animal Health Benelux, Lynx Binnenhof 5, 1200 Brussels, Belgium

ABSTRACT

The high degree of commingling and accumulation of stressors during and after transport makes prevention of bovine respiratory disease (BRD) extremely challenging in the veal and dairy beef industry. Upon arrival, vaccination for agents involved in BRD is practically most achievable, but its efficacy under such conditions in dairy veal calves is unknown. Given the high prevalence of subclinical pneumonia in these settings, the primary objective of the present study was to determine the effect of 2 vaccination protocols administered upon arrival against bovine respiratory syncytial virus (BRSV), bovine parainfluenza type 3 virus (BPI-3), and *Mannheimia haemolytica* on clinical BRD and lung ultrasonographic findings in dairy veal calves. In addition, the effects of vaccination on average daily live weight gain and cold carcass weight were determined. In this randomized clinical trial, 443 male dairy calves were assigned to one of 3 groups: a negative, placebo-controlled group (n = 151), a vaccination group with 2 subcutaneous injections 4 wk apart with an inactivated vaccine containing BRSV, BPI-3, and *M. haemolytica* (parenteral [PE] group; n = 149) and a second vaccination group receiving an intranasal live-attenuated vaccine containing BRSV and BPI-3 and 2 subcutaneous vaccinations with the same inactivated vaccine as the PE vaccination group (intranasal–parenteral [IN-PE] group; n = 143). Clinical scoring and quick thoracic ultrasonography (qTUS) were performed on all calves on arrival (wk 0), at the peak of respiratory disease (outbreak; wk 1), at the end of the first antimicrobial group treatment (wk 3), and at a long-term evaluation point (wk 10). Culture and nanopore sequencing on nonendoscopic bronchoalveolar lavage (nBAL) samples were used to identify pathogens involved in the outbreak. Upon arrival, 15.1% of the calves had lung consolidation ≥1cm and incidence quickly rose to 42.8% during the outbreak. In both the PE and IN-PE group, the odds of pneumonia in wk 10 were reduced by 62% (odds ratio [OR] = 0.38; 95% confidence interval [CI] = 0.23–0.64) and 41% (OR = 0.59; 95% CI = 0.37–0.96), respectively. Short-term cure rate (50.3%), as determined immediately after the first group antimicrobial treatment, was not influenced by vaccination. In contrast, long-term cure rate, determined at wk 10, was affected by vaccination with higher cure in the PE group compared with the control group (69.4% vs. 51.2%; OR = 2.2; 95% CI = 1.1–5.0). Average daily gain in the first 10 wk of production was not affected by vaccination. Vaccination resulted in an increase in cold carcass weight of 3.5 and 4.3 kg in the PE (95% CI = −0.9–7.9) and IN-PE group (95% CI = −0.17–8.7), respectively. In conclusion, under the conditions of the present study, vaccination upon arrival resulted in a reduced prevalence of pneumonia at wk 10 of production, likely caused both by an improved cure rate of secondary infections and a reduced incidence of new cases between outbreak and long-term evaluation. The present protocol, using qTUS for pneumonia detection and nBAL diagnostics for pathogen identification adds a new dimension to randomized clinical trials on respiratory disease in calves.

Key words: bovine respiratory disease, vaccination, thoracic ultrasound, bovine respiratory syncytial virus, *Mannheimia haemolytica*

INTRODUCTION

Despite years of research advancing laboratory and in-field diagnostics, respiratory tract infections (bovine respiratory disease [**BRD**]), especially those resulting in pneumonia, continue to be a leading cause of morbidity, mortality, economic losses, and antimicro-

bial use in all cattle industries worldwide (Pardon et al., 2012; Windeyer et al., 2014; Karle et al., 2019). Prevention of infection is the most sustainable way to reduce their effect, and vaccination against respiratory viruses and bacteria is regarded as one of the most important preventative measures for BRD (O'Connor et al., 2019). Apart from a few trials evaluating vaccination for respiratory disease in young dairy calves, randomized clinical trials evaluating vaccine efficacy under field conditions are almost exclusively done in the North American feedlot industry (Windeyer et al., 2012; Ollivett et al., 2018a; O'Connor et al., 2019). The prevalence of clinical and subclinical pneumonia is substantial in female dairy calves in conventional dairy farms (van Leenen et al., 2020). However, the highest burden of BRD is seen in production systems that purchase, transport, and commingle young male dairy calves, namely the veal calf and dairy beef industries (Masmeijer et al., 2021; Renaud and Pardon, 2022). Good standards of vaccination prescribe that animals should be fully vaccinated before exposure to the pathogen occurs (Richeson and Falkner, 2020). In the feedlot industry, preconditioning programs with vaccination of calves on the farms of origin before transport to the feedlots, have been shown to successfully reduce BRD incidence (Stilwell et al., 2008). The same approach of vaccination of calf or dams on the dairy farms of origin could be followed for male dairy calves. However, for logistic and especially economic reasons (i.e., low value of the calves), this is not commonly applied (Renaud and Pardon, 2022). Currently, the most accessible way for vaccination in veal or dairy beef farms is vaccination upon arrival. Efficacy of this vaccination strategy is however questionable because exposure to pathogens and stressors are maximal on arrival. Consistent with this, a meta-analysis in feedlot calves found little compelling evidence that respiratory vaccines administered upon arrival reduced BRD incidence in the first months after arrival (O'Connor et al., 2019).

In recent years, several novel technologies, such as thoracic ultrasound (**TUS**) and rapid laboratory diagnostic techniques, have become available for on farm diagnosis of pneumonia and etiological agents, respectively (Pardon and Buczinski, 2020). TUS detects lung consolidation and makes it possible to differentiate both clinical and subclinical pneumonia from upper respiratory tract inflammation/infection (Ollivett and Buczinski, 2016; van Leenen et al., 2020). Recent studies showed that any single clinical sign or scoring system combining multiple clinical signs are inferior to detect pneumonia as compared with TUS (Buczinski et al., 2015; Lowie et al., 2022). These findings shed a new light on the usefulness of clinical BRD outcome to evaluate vaccine efficacy. Next to prevention of pneu-

monia, vaccination may also improve cure rate, which can be visualized by TUS by means of a reaeration criterion (Jourquin et al., 2022). A second shortcoming of almost all BRD field trials is that they do not provide evidence that natural infection with the target pathogen of the vaccine occurred during the trial. In veal calf farms, especially in winter, all known BRD pathogens are mostly present, with a leading role of *Mycoplasma bovis* (Pardon et al., 2011). The nonendoscopic bronchoalveolar lavage (nBAL) offers a practical method of lower airway sampling and the combination of nanopore sequencing with bacterial culture ensures the broadest detection of pathogens possible and an interpretable result (Pardon and Buczinski, 2020; Bokma et al., 2021).

To the authors knowledge, no peer-reviewed randomized clinical trials on the efficacy of vaccination upon arrival to reduce BRD incidence are available for the veal calf and dairy beef sectors. Additionally, the combination of TUS and laboratory diagnosis have previously not been used for evaluation of vaccine efficacy for BRD. Therefore, the primary objective of this study was to determine the effect of 2 vaccination protocols administered upon arrival against viruses and *Mannheimia haemolytica* on clinical signs of BRD, pneumonia, and cure of pneumonia as determined by lung ultrasonography. In addition, the effects of vaccination on ADG and cold carcass weight (**CCW**) were determined.

MATERIALS AND METHODS

Study Design and Sample Size

A triple blinded, placebo-controlled randomized clinical trial was conducted on a commercial veal calf farm involving 444 male Holstein Friesian calves, aged 14 to 21 d, over the course of one production cycle. The trial period ran between January and September 2022 on a farm located in the province of Antwerp, Belgium. Clinical and ultrasonographic follow up by means of quick thoracic ultrasonography (**qTUS**) was performed on 4 strategic occasions during the first 10 wk of the production cycle: upon arrival at the facility, at the start of the first metaphylactic treatment for respiratory disease, at the end of the group treatment, and during wk 10 of the production cycle. After 33 wk, carcass weights were collected in the abattoir. Figure 1 gives an overview of the study setup. Sample size calculations were made based on the primary outcome, namely pneumonia incidence as determined by qTUS (lung consolidation ≥ 1 cm). Based on a previous observational study in a similar setting, we assumed that approximately 40% of the calves would develop pneu-
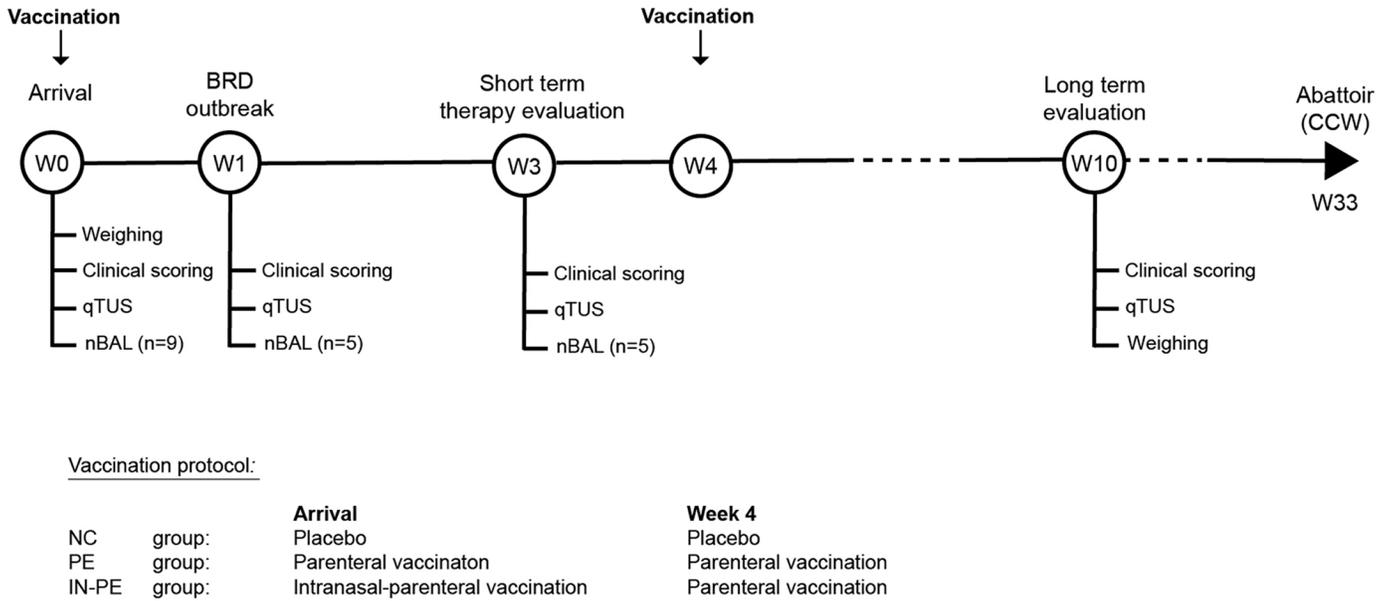
Figure 1. Temporal overview of a randomized clinical trial setup on the effects of vaccination of male dairy calves in a high-risk environment for development of pneumonia. W = week; qTUS = quick thoracic ultrasound; nBAL = nonendoscopic bronchoalveolar lavage; CCW = cold carcass weight; NC = negative control group; PE = parenteral group; IN-PE = intranasal–parenteral group.

monia (Jourquin et al., 2023). With a sample size of 150 calves in each group the study had a power of 97% to demonstrate a 50% reduction of pneumonia incidence in vaccinated animals compared with nonvaccinated animals. The trial protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine and Bioengineering from Ghent University under license EC 2021–070. Authors declare human ethics approval was not needed for this study.

Animals and Housing

The trial barn was one of 2 veal calf barns present on the farm. Calves arrived in 3 separate batches over a 3-d period. In the first 6 wk after arrival, calves were housed in individual calf pens on slatted floors, separated by wire fencing. After 6 wk, the fences were removed, leaving the calves in 64 group pens containing 2 to 8 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 solids) of commercially available milk replacer (21% CP and 19% crude fat [**CF**]) on a DM base, 2 times a day. This amount increased during the next 7 mo of feeding to 7 L twice per day (1,590 g of solids). In this study, milk replacer was fed throughout the entire production cycle. In addition, calf muesli was provided (25 g/d at start gradually increasing to 3 kg/d after 7 mo; CP = 14%; CF = 4.5%).

Randomization Process and Vaccination Protocol

Within 24 h after arrival of the last batch, calves were randomly assigned to 1 of 3 vaccination groups using block randomization. A predefined randomized spreadsheet was prepared for each arrival batch. Next, group pen, arrival weight (kg), and presence of consolidation ≥1cm on qTUS on arrival were added to a spreadsheet (Microsoft, Excel) and calves were randomized using the excel Aselect function. The following vaccination groups with evenly distributed weights and pneumonia incidence were created: negative control (**NC**) group, parenteral (**PE**) group, and intranasal–parenteral (**IN-PE**) group. A detailed description of each vaccination protocol is provided below. Randomization on pen-level ensured equal distribution of the vaccines over each pen. The vaccination protocol was initiated one day after arrival of the last batch. The vaccine was stored and used according to the manufacturer's recommendations. Sequence generation and implementation of vaccines and placebos were done by external participants, leaving the trial blinded to all observers performing clinical examination, qTUS or weighing, caregivers, and the herd veterinarian throughout the entire trial. After the trial, statistical analyses were performed without disclosure of the vaccine groups.

PE Group. One day after arrival, calves in the PE group received a subcutaneous injection (5 mL) of an inactivated vaccine containing bovine respiratory syncytial virus (**BRSV**), bovine parainfluenza type

3 virus (**BPI-3**), Mannheimia haemolytica type A1 and A6 (Bovilis Bovipast RSP, MSD Animal Health, Boxmeer, The Netherlands) following datasheet recommendations. At the same time, these calves (PE group) also received an intranasal placebo (2 mL) of sterile isotonic saline fluid (0.9% NaCl, B. Braun, Melsungen, Germany) using the commercially available vaccine dispenser of the tested intranasal vaccine. Four weeks later, a second subcutaneous injection (5 mL) of the same inactivated vaccine was administered.

IN-PE Group. For the IN-PE group, on-arrival, these animals received both an intranasal dose (2 mL) of a multivalent live vaccine protecting against BRSV and BPI-3 (Bovilis INtranasal RSP Live, MSD Animal Health, Boxmeer, The Netherlands) and a subcutaneous injection (5 mL) of an inactivated vaccine protecting against BRSV, BPI-3, *Mannheimia haemolytica* type A1 and A6 (Bovilis Bovipast RSP, MSD Animal Health, Boxmeer, The Netherlands). In this clinical trial both vaccines were administered on the same day for investigational purposes. Again, 4 wk later, all calves from the IN-PE group received a subcutaneous injection (5 mL) of the inactivated vaccine mentioned above.

NC Group. Lastly, calves from the NC group were administered both an intranasal placebo (2 mL) of sterile isotonic saline fluid (0.9% NaCl, B. Braun, Melsungen, Germany) and a subcutaneous placebo injection (5 mL) of sterile isotonic saline fluid (0.9% NaCl, B. Braun, Melsungen, Germany) upon arrival. Four weeks later, a second subcutaneous placebo injection (5 mL) of the same sterile isotonic saline fluid was administered.

Clinical Scoring, qTUS, and Production

At each of the 4 measurement points, clinical scoring was performed on all calves using the Wisconsin respiratory scoring chart (McGuirk and Peek, 2014). For each calf, a score was assigned based on the presence of the following clinical parameters: rectal temperature, cough, nasal discharge, ocular discharge, and ear flick or head tilt. For each clinical parameter, a score ranging from 0 to 3 was given based on its clinical presentation. Calves with a Wisconsin score ≥5 were labeled as clinical BRD. Definition of pneumonia was based solely on qTUS findings.

For detection of pneumonia, TUS was performed using the UGent qTUS technique, by means of a standard ultrasound device with a 7.5 MHz linear probe (Manual ultrasound scanner V1, Kaixin, China) and 98% isopropyl as transducing agent, as previously described (Pardon, 2019; Jourquin et al., 2022). While scanning, the probe was stopped as soon as any abnormality was

seen (e.g., comet tail artifacts, pleural irregularities, consolidation, pleuritis, and so on) and gently tilted cranio-caudally and back to maximize the area of visualization. Consolidation depth was measured in the dorsoventral plane using the grid on the ultrasound screen as a reference. Case definition of pneumonia was defined by consolidation depth. In this study, consolidation with a depth of 1 to 3 cm were defined as pneumonia and ≥3 cm was defined as severe (lobar) pneumonia. For each scanned quadrant of the lungs (right caudal, left caudal, right cranial, and left cranial), the most severe qTUS finding (including comet tail artifacts [B-lines], maximum consolidation depth [cm] and pleural effusion) was noted. A description of the anatomical lung lobes that are visualized within each of the 4 quadrants is described elsewhere (Pardon et al., 2019) On all 4 occasions, both qTUS and clinical scoring were performed following the procedures described above. A combination of clinical scoring and qTUS allowed classification of the calves into one of 4 groups: healthy (no lung lesions 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 upper respiratory tract infection (clinical signs of respiratory disease, but no lesions on qTUS; Ollivett and Buczinski, 2016; van Leenen et al., 2020).

For production outcomes, calves were weighed on arrival and after 10 wk in production on a calf weigh scale with 0.1 kg accuracy (Bascules Robbe, Torhout, Belgium). After 33 wk at the veal facility, calves were brought to the slaughterhouse. After slaughter, CCW was provided by the abattoir with a precision of 0.1 kg.

Treatment Protocols

In this study, an outbreak of respiratory disease was defined by the point in time where the herd veterinarian considered clinical signs of BRD to be present in 10 −15% of the herd and deemed antimicrobial group treatment necessary. At this point, at the peak of respiratory disease, a first metaphylactic treatment was initiated consisting of oral treatment with doxycycline twice daily (Doxyveto 50% Pulvis, VMD, Arendonk, Belgium) for 12 consecutive days. Duration of treatment was based on the protocols used at the local practice and dosing was done following leaflet recommendations. In wk 7, all animals were treated orally with florfenicol (Flordofen OS 100mg/mL, Dopharma, Raamsdonksveer, The Netherlands) for 10 consecutive days. In total, antimicrobial group treatments for BRD were given on 2 occasions during the observation period, resulting in 22 d of metaphylactic treatment during the 10-wk trial period.

After each scanning occasion, the farm's usual veterinarian was provided a list of animals with consolidation ≥1cm. These animals received a 5-d parenteral treatment with lincosamides and spectinomycin (Emdactilin 50/100 mg/mL, Emdoka, Hoogstraten, Belgium) in combination with penicillin (PENI-kel, Kelan v, Hoogstraten, Belgium), according to the standard protocol used at the local practice. In addition, the producer or the herd veterinarian monitored the calves daily for signs of disease (e.g., clinical signs of respiratory disease, apathy, reduced milk intake, and so on) and selected animals for individual treatment at their discretion, using the antimicrobial protocol mentioned above.

Laboratory Diagnosis

At the time of arrival, approximately 10 mL of whole blood was collected from the jugular vein into a sterile blood collection tube (Vacutest, Kima, Arzergrande, Italy) and stored at 4°C until further processing. Blood samples were centrifuged at $922 \times g$ for 10 min at approximately 20°C. For each calf, the concentration of serum IgG was determined by quantitative ELISA (Bio K420, MonoScreen QuantELISA Immunoglobulin Easy, Bio-X Diagnostics, Rochefort, Belgium). Above mentioned analyses were all done at an accredited laboratory (Centre for Diagnostic Solutions, MSD Animal Health, Boxmeer, The Netherlands) using optimized settings for bovine serum samples. Previously executed quality control procedures were within quality assurance specifications. The cutoff value for failure of transfer of passive immunity was set at serum IgG <7.5 g/L (Pardon et al., 2015). After serum IgG determination, a post hoc analysis was performed to check whether IgG concentrations were equally distributed among all 3 vaccination groups.

Identification of pathogens present throughout this production cycle was done by sampling a total of 19 calves, separated over 3 occasions: upon arrival of each batch (d 0; 4, 2, and 3 samples from batch 1, 2, and 3, respectively), at the peak of the first outbreak of respiratory disease (d 7 after arrival; 5 samples) and again at the short-term therapy evaluation point, when the first metaphylactic treatment was stopped (d 21; 5 samples). On these occasions, respiratory sampling using nonendoscopic bronchoalveolar lavage (nBAL) was performed on, unsedated calves, using a sterilized catheter as previously described (Van Driessche et al., 2017; Pardon and Buczinski, 2020). Each time, calves were conveniently selected based on the presence of clearly defined lung consolidation ≥ 1cm on qTUS. Both animals with clinical and subclinical pneumonia were included. An overview of the different sampling occasions is depicted in Figure 1. From each sampling occasion, a pooled nBAL sample of 4 to 5 animals was sequenced using nanopore sequencing to identify all involved viral pathogens and *Mycoplasma bovis* (Theuns et al., 2018; Bokma et al., 2021). To identify bacterial infections, individual nBAL samples were inoculated directly on Colombia agar supplemented with 5% sheep blood (blood agar; Oxoïd, Hampshire, UK) and on *Mycoplasma bovis* selective indicative agar (Bokma et al., 2020). Bacteria detected on blood agar were identified by MALDI-TOF MS (Brüker Daltonik GmbH, Bremen, Germany) and *Mycoplasma bovis* was identified based on lipase activity, as described previously (Bokma et al., 2020).

Data and Statistical Analyses

Collected data were saved in a spreadsheet (Excel, Microsoft Inc.) and transferred to SAS enterprise guide 9 (SAS Institute Inc., Cary, NC) SPSS statistics Version 27.0. (IBM Corp., Armonk, NY) for further analysis. Calculation of ADG was done by dividing the difference in BW between arrival and the individual calf weight in wk 10 by the number of days in the production facility. Next, a second analysis on ADG at the end of the production cycle was performed using the estimated live weight, calculated by dividing the CCW by an industry-standard factor for carcass yield (i.e., 0.63).

For analysis, pneumonia was defined as presence of lung consolidation ≥1 cm on qTUS. Animals with consolidation <1 cm were considered healthy. Calves with pneumonia were considered cured when reaeration of previously consolidated lung tissue occurred (Jourquin et al., 2022). At each time point, determination of cure rate was done using the proportion of calves that showed lung reaeration on qTUS, meaning that lung consolidation either fully reaerated or regressed to <1 cm in depth, over the total number of calves with pneumonia (consolidation ≥1 cm) at the previous scanning occasion. Short-term cure was defined as the number of animals with pneumonia at the time of the outbreak (**W1**), which no longer had consolidation ≥1 cm after treatment (**W3**). At wk 10, to include new cases of pneumonia that developed during metaphylaxis, long-term cure was defined by the number of calves that showed reaeration in calves that had pneumonia at either the outbreak (W1) or short-term evaluation point (W3). Calves that had pneumonia both at the time of therapy evaluation and long-term evaluation were considered chronic. Further, short-term new cases were defined by animals that developed pneumonia during metaphylaxis, in between outbreak and short-term therapy evaluation. Long-term new cases were labeled based on the new pneumonic cases that were detected

Table 1. Overview of clinical characteristics, thoracic ultrasound findings, and production parameters of 443 male dairy calves, stratified by vaccination group upon arrival at the veal facility[1]

Variable	NC	PE	IN-PE	Herd	P-value
Number in group	151	149	143	443	—
Wisconsin score ≥5 (%)	4.6	3.4	2.8	3.6	0.11
Fever[2] (%)	5.3	4.9	7.4	5.9	0.62
Lung consolidation 1–3 cm (%)	9.9	10.5	12.8	11	0.70
Lung consolidation ≥3 cm (%)	3.9	4.9	3.3	4.1	0.80
Pneumonia (%)	13.8	15.4	16.1	15.1	0.85
serum IgG <7.5 g/L (%)	17.9	17.6	13.3	16.3	0.50
Arrival weight (kg [SD])	52.0 (6.0)	51.6 (5.7)	53.3 (7.6)	52.3 (6.7)	0.59

[1]NC = negative control group; PE = parenteral group; IN-PE = intranasal–parenteral group.

[2]Fever was defined as rectal temperature >39.3°C.

in wk 10 in calves that did not have pneumonia at both outbreak and short-term-evaluation.

Next to the qTUS findings, clinical BRD was defined solely by having a Wisconsin score ≥5. To determine the effect of vaccination on development of clinical BRD, qTUS confirmed pneumonia, cure rate, and the number of new cases, 3 separate models were created: one at the outbreak of respiratory disease, a second at the time of therapy evaluation and a last one on the long-term evaluation point (**W10**). The 3 models each consisted of a general linear model with binomial distribution and logit link function with Wald's statistics for type 3 contrasts (PROC GLIMMIX). Model fit was evaluated using the Hosmer-Lemeshow test. Significance was set at $P < 0.05$.

To determine effects on ADG and CCW, mixed linear models were built (PROC MIXED). Both ADG and CCW were checked for normal distribution by visual assessment of the Histogram and Q-Q plots. Arrival batch was included as random intercept 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, the same modeling procedure was used to assess differences in IgG levels between the 3 groups. Significance was set at $P < 0.05$. In some instances, to increase power, analyses were performed using 2 groups, combining the PE and IN-PE group into following groups: nonvaccinated (n = 151) and vaccinated animals (n = 290).

RESULTS

Animals and Arrival Situation

Upon arrival, consolidation ≥1 cm were detected in 12.2% (5/41) of the calves from batch 1, 14.4% (29/201) in batch 2 and 16.3% (33/202) in batch 3. In total, 15.1% (67/444) of the calves arrived with pneu-

monia (consolidation ≥1 cm) on qTUS and pneumonia was severe (consolidation ≥3 cm) in 4.1% (18/444) of the cases. Overall, average BW on arrival was 52.3 kg (SD = 6.5 kg; Range (R) = 37–77 kg). Average arrival weight of calves from batch 1 was 3.2 kg (95% CI = 0.98–5.4 kg; $P = 0.005$) and 2.4 kg (95% CI = 0.23–4.6 kg; $P = 0.03$) higher than calves from batch 2 and 3, respectively. Arrival weight from animals in batch 2 (51.7 kg; SD = 6.5 kg; R = 39–76 kg) did not differ from batch 3 (52.4 kg; SD = 6.8 kg; R = 37–77 kg; $P = 0.76$). Characteristics of each separate vaccination group upon arrival, including IgG status, are shown in Table 1. At the end of the trial, post hoc analyses revealed IgG status to be similar in all 3 vaccination groups ($P = 0.5$).

In total, 7 (1.6%) of the enrolled calves died over the course of the production cycle (W0–W33), 5 of which occurred within the first 10 wk of production. One animal (NC group) died within 24h after arrival, before any vaccinations were given, and was excluded from further analyses.

Pathogen Detection

As displayed in Figure 1, nBAL samples were taken upon arrival of the first and last batch (W0), at the time of the outbreak (W1) and at the short-term therapy evaluation (W3). An overview of all pathogens identified in the respiratory samples taken over the course of the trial period is given in Table 2, whereas pathogens that are most relevant to development of BRD are further described below. On all sampling occasions, calves were randomly selected based on the presence of consolidation ≥1 cm on qTUS (n = 67). Upon arrival, bacterial cultures were positive for *M. bovis* in 2/9 nBAL samples and blood agar detected *P. multocida*, *Trueperella pyogenes*, and *Bibersteinia trehalosi* in 4, 1, and 1 of the 9 nBAL samples, respectively. Further, metagenomic nanopore sequencing identified genome segments of bovine corona virus (BCoV), BPI-3, bovine

adenovirus 3 and *M. bovis* in a pooled sample from the nBALs taken upon arrival. In the nBAL samples (n = 5) collected at the start of the outbreak (W1), right before metaphylactic treatment was initiated, BRSV, bovine coronavirus (BCoV) and *M. bovis* were detected by means of metagenomic nanopore sequencing. Bacterial cultures on selective indicative agar were positive for *Mycoplasma bovis* in all 5 samples taken at that time. In addition, on blood agar, *Pasteurella multocida* was found in 2 samples and *Histophilus somni* in 1 sample taken at the BRD outbreak. At short-term evaluation (W3), *M. bovis* was present on selective indicative agar in 4/5 samples. *Trueperella pyogenes* and *P. multocida* were found in 3 and 2 samples, respectively. One sample contained both *T. pyogenes* and *Escherichia coli*. One sample was negative on both blood culture and selective indicative agar. Again, metagenomic nanopore sequencing was performed on a pooled sample of the 5 nBAL samples taken at that time, but only *M. bovis* was detected as a primary cause of respiratory disease.

Effects of Vaccination on Clinical BRD

In Figure 2 a temporal overview of the prevalence of clinical BRD (Wisconsin score $\geq$5) and qTUS determined pneumonia at each time point is given, stratified by vaccination group. On arrival, prevalence of clinical BRD was 3.6% (16/443) and rose to 15.8% (70/443) in the first week. At this point, the first metaphylactic treatment was initiated consisting of oral treatment with doxycycline for 12 consecutive days according to the previously described protocol. After treatment, clinical BRD was lowest in the control group (2.7%), but no statistically significant differences were found between the groups ($P = 0.9$). At the long-term evaluation point (W10), although prevalence of BRD in the vaccinated group (2.1%) was lower than in the unvaccinated animals (5.0%), differences were not statistically significant ($P = 0.16$). Also, when comparing vaccinated to nonvaccinated animals, binary logistic regression did not indicate any differences in the odds of developing clinical signs of BRD at the time of the outbreak (OR = 1.1; 95% CI = 0.6–1.9; $P = 0.7$), short-term evaluation (OR = 1.2; 95% CI = 0.4–3.9; $P = 0.8$) nor at the long-term evaluation point (OR = 0.4; 95% CI = 0.1–1.6; $P = 0.2$).

Effects of Vaccination on qTUS Confirmed Pneumonia

When utilizing qTUS, in the first week after arrival, prevalence of pneumonia quickly rose from 15.1% to 42.8% (190/443; Figure 2). Consolidation $\geq$1 cm were found in 32.4% (142/438) and 31.9% (139/436) of the calves at the short- and long-term evaluation points, respectively. Over the trial period, pneumonia developed in 70.4% of calves in the NC group, 67.8% in the PE group and 68.5% in the IN-PE group, resulting in a total of 68.9% (306/443) of the animals having pneumonia on at least one of the evaluation points during this trial.

For each scanning occasion, the risk of developing pneumonia was assessed for the 3 vaccination groups. At the time of the outbreak of respiratory disease (W1), no significant differences in the risk of pneumonia were found between vaccinated and nonvaccinated animals ($P = 0.65$). At short-term therapy evaluation (W3), after a 12-d oral treatment with doxycycline, the odds of having pneumonia were lower in the IN-PE group when compared with the PE group (OR = 0.58; 95% CI = 0.36–0.96; $P = 0.03$), but no statistically significant effects of vaccination were found when comparing the IN-PE group (OR = 0.76; 95% CI = 0.46–1.3; $P = 0.29$), nor when comparing the PE group (OR = 1.3; 95%CI = 0.81–2.1; $P = 0.28$) to the NC group at this time. In contrast, at wk 10, the odds of having pneumonia were lower for animals that were vaccinated when compared with nonvaccinated animals (OR = 0.48; 95% CI = 0.32–0.73; $P < 0.001$) and effects were most pronounced when comparing the NC group to the PE group, with a reduction of 62.0% (OR = 0.38; 95% CI = 0.23–0.64; $P < 0.001$). Additionally, the odds of having long-term pneumonia were also reduced by 41% (OR = 0.59; 95%CI = 0.37–0.96; $P = 0.03$) for animals from the IN-PE group when compared with animals from the NC group. In contrast, no differences were found between the PE- and the IN-PE group ($P = 0.11$).

Calves arriving with a consolidation $\geq$1 cm had 1.9 (95% CI = 1.1–3.3; $P = 0.02$) and 2.2 (95% CI = 1.3–3.8; $P = 0.005$) times higher odds of also having pneumonia at the time of outbreak (W1) and therapy evaluation (W3), respectively. The effects of arriving with pneumonia were no longer present at the long-term evaluation point (OR = 1.4; 95% CI = 0.8–2.4; $P = 0.2$). As well as ultrasonographic parameters on arrival, arrival weight, and serum IgG levels were assessed for their influence on development of pneumonia, but no statistically significant associations were found.

Effects of Vaccination on Cure

In this study, cure was defined by reaeration of previously consolidated lung tissue ($\geq$1 cm in depth) on qTUS. Short-term cure was defined by the number of animals with pneumonia at the time of the outbreak (W1), which no longer had a consolidation $\geq$1 cm after first metaphylactic treatment (W3). Short-term

Table 2. Results of etiological respiratory diagnostics by bacterial culture and nanosequencing of nonendoscopic bronchoalveolar lavage samples from 19 veal calves on 3 sampling occasions during the first 10 wk of the production cycle

Sample number	Vaccine group[1]	Pn category[2]	Blood agar	Selective indicative agar	Metagenomic analysis (pooled sample)
Arrival (W0)					
Arrival batch 1					
B1	NC	Cl. Pn	Negative	Mycoplasma bovis	Bovine rhinitis B virus, Mycoplasma dispar, and Ureaplasma spp.
B2	PE	Subcl. Pn	Pasteurella multocida	Negative	
B3	IN-PE	Subcl. Pn	Pasteurella multocida	Negative	
B4	NC	Cl. Pn	Negative	Negative	
Arrival batch 2 and 3					
B5	—[3]	Subcl. Pn	Pasteurella multocida and Trueperella pyogenes	Negative	Bovine coronavirus, bovine parainfluenza type 3 virus, bovine adenovirus 3, bovine rhinitis virus 2, and Mycoplasma bovis
B6	PE	Subcl. Pn	Pasteurella multocida	Negative	
B7	PE	Subcl. Pn	Bibersteinia trehalosi	Negative	
B8	IN-PE	Subcl. Pn	Negative	Negative	
B9	NC	Cl. Pn	Negative	M. bovis	
Outbreak of respiratory disease (W1)					
B10	PE	Cl. Pn	Negative	M. bovis	Bovine coronavirus, bovine respiratory syncytial virus, Mycoplasma bovis, and Mycoplasma dispar
B11	IN-PE	Cl. Pn	Pasteurella multocida	M. bovis	
B12	PE	Subcl. Pn	Pasteurella multocida	M. bovis	
B13	IN-PE	Cl. Pn	Negative	M. bovis	
B14	IN-PE	Cl. Pn	Histophilus somni	M. bovis	
Short-term therapy evaluation (W3)					
B15	IN-PE	Subcl. Pn	Pasteurella multocida	M. bovis	Bovine astrovirus, bovine rhinitis A virus, Mycoplasma bovis, Mycoplasma bovirhinis, and Mycoplasma dispar
B16	IN-PE	Healthy[4]	Trueperella pyogenes and Escherichia coli	M. bovis	
B17	PE	Subcl. Pn	Trueperella pyogenes	M. bovis	
B18	PE	Subcl. Pn	Pasteurella multocida and Trueperella pyogenes	M. bovis	
B19	NC	Subcl. Pn	Negative	Negative	

[1]NC = negative control; PE = parenteral; IN-PE = intranasal–parenteral.

[2]Pn = pneumonia; Subcl. Pn = subclinical pneumonia; Cl. Pn = clinical pneumonia.

[3]Sample number B5 was taken from a calf that died within 24h after arrival and could not be assigned to a vaccination group.

[4]Sample B16 was taken from an animal that had ultrasound confirmed pneumonia upon arrival at the veal facility, but had reaerated lungs on further scanning occasions.
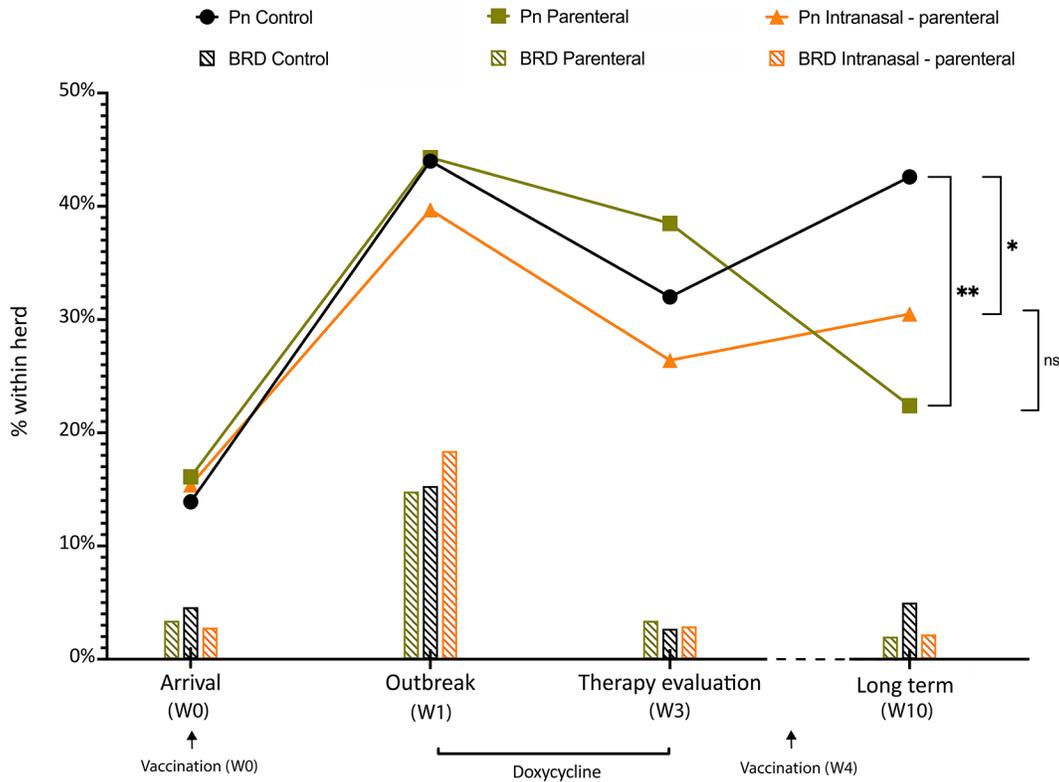
Figure 2. Overview of quick thoracic ultrasound (qTUS) confirmed pneumonia (Pn) and clinical bovine respiratory disease (BRD) in 443 male dairy calves over a 10-wk period, using consolidation ≥1 cm on lung ultrasonography as case definition of pneumonia. Clinical BRD was defined using the Wisconsin respiratory scoring chart. At the long-term evaluation point, clinical scoring was only performed on 292 animals. * Indicates a statistically significant difference with a $P < 0.05$ ** Indicates a statistically significant difference with a $P < 0.001$. ns = a nonsignificant difference between groups.

cure rates were 47.8% (32/67), 42.4% (28/66) and 62.5% (35/56) in the NC group, the PE group and the IN-PE group, respectively. When compared with the NC group, no difference in short-term cure was found for the PE group (OR = 0.82; 95%CI: 0.40–1.7; $P = 0.6$), but for the IN-PE group, a trend was observed (OR = 2.02; 95%CI: 0.96–4.35; $P = 0.07$). Moreover, when comparing the PE to the IN-PE group, odds of short-term cure were higher in the IN-PE group (OR = 2.3; 95% CI = 1.1–4.7; $P = 0.03$). In total, short-term cure rate for all enrolled calves was 50.3% (95/189).

At wk 10, to include new cases of pneumonia that developed during metaphylaxis, long-term cure was defined by the number of calves that showed reaeration in calves that had pneumonia at either the outbreak (W1) or short-term evaluation point (W3). Overall, when only considering these pneumonic animals at the time of the outbreak or short-term evaluation, a final cure rate of 61.5% (147/239) was reached in wk 10. For each vaccination group a visual representation of the differences in development and cure on short- and long-term evaluation is given in Figure 3. Compared with the

NC group, odds for long-term cure were significantly higher in the PE group (OR = 2.2; 95% CI = 1.1–4.1; $P = 0.02$).

Next to cure, Figure 3 also displays the rates of new pneumonic cases that developed at the short- and long-term evaluation points for each vaccination group. At short-term evaluation, the number of new cases were similar between all 3 groups ($P > 0.05$). In contrast, at wk 10, long-term new cases were detected in 36.2% (25/69), 11.3% (7/62), and 24.6% (17/69) of the calves in the NC, the PE, and the IN-PE group, respectively. When compared with the NC group, the odds of developing long-term new cases decreased by 77.6% (OR = 0.22; 95% CI = 0.09–0.57; $P = 0.002$) in the PE group. In the IN-PE group the odds of developing long-term new cases also decreased compared with the control calves but this difference was not significant (OR = 0.58; 95% CI = 0.28–1.2; $P = 0.14$).

Effects on Growth and Carcass Weight

In Table 3, an overview of production outcomes is given for each vaccination group. After 10 wk in pro-
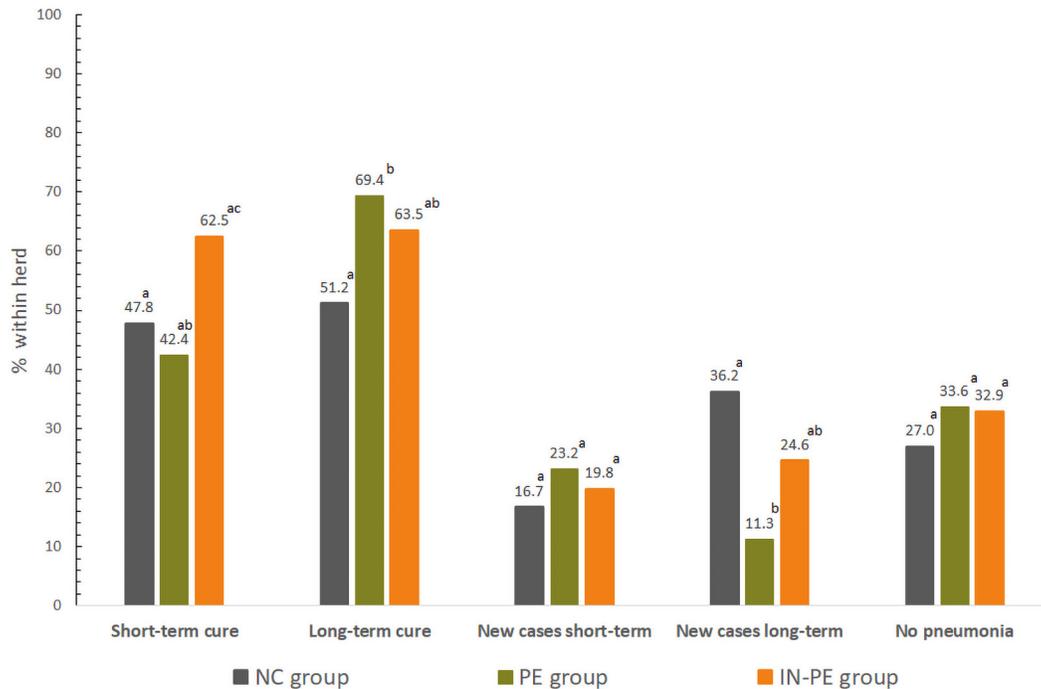
Figure 3. Differences in the development of new cases of pneumonia and cure rate in male dairy calves based on different vaccination protocols. NC = negative control; PE = parenteral; IN-PE = intranasal–parenteral. Different letters indicate a significant difference (P < 0.05) in the incidence of the event occurring between groups.

duction, BW of the enrolled animals averaged 114.1 kg (SD = 13.4 kg; R = 78.8–153.5 kg), resulting in an ADG over the first 10 wk of production of 0.888 kg/d (SD = 0.145 kg/d; R = 0.446–1.289 kg/d). Average CCW was 184.2 kg (SD = 20.3 kg; R = 87.6–250.9 kg), giving an ADG over the complete production cycle of 1.146 kg/d (SD = 0.144 kg/d ; R = 0.44–1.634 kg/d). For production analyses, CCW observations that were more than 3 times the interquartile range below the lower first quartile or 3 times above the upper third quartile were considered extreme outliers and were removed. In total, 3 extreme outliers were removed, and 7 animals died before slaughter, leaving 434 animals for production analysis.

After 10 wk, ADG of the NC group (0.877 kg/d ; SD = 0.154 kg/d ; R = 0.446–1.297 kg/d) was no different from vaccinated animals (0.894 kg/d ; SD = 0.139 kg/d ; R = 0.480–1.289 kg/d ; P = 0.3). Evaluated over the whole production cycle, ADG was 0.030 kg/d higher in vaccinated animals compared with the NC group (95%CI = 0.00–0.06 kg/d ; P = 0.03).

For CCW, both linear mixed model analysis and One-way ANOVA analysis indicated a lower CCW of 5.1 kg in animals with ≥1 episode of pneumonia compared with animals who remained healthy throughout the trial (SD = 2.1 kg; 95%CI: 1.0–9.3 kg; P = 0.02).

Regarding vaccination, CCW averaged 3.85 kg higher in vaccinated animals compared with the NC group (95% CI = 0.1–7.6 kg; P = 0.05). Compared with the NC group, CCW was 3.5 kg and 4.3 kg higher in the PE group (95% CI = −0.9–7.9; P = 0.1) and IN-PE group (95% CI = −0.17–8.7; P = 0.06), respectively.

Next to vaccination, a positive qTUS score (consolidation ≥1 cm) or clinical BRD (Wisconsin score ≥5) in wk 10 and low arrival weight also influenced production parameters. Animals with pneumonia at the long-term evaluation point (W10), had a 4.37 kg (95% CI: 0.68–8.1 kg; P = 0.02) lower CCW than animals that did not show lung consolidation at that point. Calves with consolidation ≥3 cm in wk 10 had a 12.5 kg lower CCW than animals with smaller or no consolidation at that point (SD = 2.5 kg; 95% CI: = 7.8–17.5 kg; P < 0.001). Also, calves with pneumonia in wk 10 had an ADG that averaged 0.076 kg/d (95% CI = 0.05–0.11 kg/d ; P < 0.001) lower after 10 wk compared with healthy animals without lung consolidation. In Figure 4, differences in ADG and CCW are presented based on qTUS findings in wk 10. Next to qTUS confirmed pneumonia, calves with clinical signs of BRD in wk 10 had a 14.2kg (95% CI = 1.6–26.8kg; P = 0.03) lower CCW than animals without clinical signs of BRD at that time.

Table 3. Overview of health and production characteristics of 434 dairy veal calves randomly assigned to 1 of 3 vaccination groups

Parameter	NC group (n = 146)			PE group (n = 147)			IN-PE group (n = 141)		
	Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum
Arrival weight (kg)	52.1 ± 6.2[a]	37.0	74.0	52.7 ± 6.8[a]	38.0	77.0	52.1 ± 6.5[a]	38.0	76.0
Weight wk 10 (kg)	113.2 ± 13.8[a]	78.8	150.2	115.4 ± 14.3[a]	80.8	151.2	113.8 ± 12.0[a]	87.8	153.5
Final live weight[1] (kg)	289.5 ± 32.9[a]	159.4	398.3	294.3 ± 29.1[a]	225.2	364.9	293.5 ± 34.8[a]	139.0	370.2
Cold carcass weight (kg)	181.9 ± 20.0[a]	100.4	219.5	185.4 ± 18.3[a]	141.9	229.9	186.2 ± 21.9[a]	118.4	233.2
ADG arrival to wk 10 (kg/d)	0.877 ± 0.154[a]	0.446	1.289	0.901 ± 0.145[a]	0.489	1.235	0.888 ± 0.133[a]	0.480	1.289
ADG arrival to slaughter (kg/d)	1.128 ± 0.144[a]	0.446	1.390	1.154 ± 0.128[ab]	0.854	1.502	1.161 ± 0.134[b]	0.685	1.534

[a,b]Different letters indicate a significant difference ($P < 0.05$) between groups.

[1]Final live weight was calculated by dividing the final cold carcass weight by factor 0.63.

DISCUSSION

This study is pioneering work for 2 reasons. First, to the authors knowledge it is the first peer-reviewed report on a trial that investigated the efficacy of on-arrival vaccination to reduce pneumonia in veal calves, one of the industries facing the biggest challenge to rationally reduce antimicrobial use. Second, and even more importantly, it is the first study to use thoracic ultrasonography as a reliable method to detect pneumonia while also using nanosequencing and culture on nBAL samples to identify the presence of respiratory pathogens. Another study implemented TUS to diagnose pneumonia in a vaccination trial in dairy calves, but no pathogen identification was performed (Ollivett et al., 2018b).

The goal of vaccination upon arrival is to protect calves against viral infections (BRSV and BPI-3) which are notoriously present in all production types where commingling occurs (Pardon et al., 2011). Even in these high-risk environments, vaccination might reduce the consequences of viral infections, resulting in reduced damage to the respiratory tract and subsequently a lower probability of superinfections with *Pasteurellaceae* and *Mycoplasma bovis* (Zhang et al., 2019; Masset et al., 2020; Pardon and Buczinski, 2020). The importance of these viral infections has been illustrated in this study as BPI-3 and BCoV were detected upon arrival and both BRSV and BCoV were circulating one week after arrival, meaning that all 3 primary viral agents were present immediately before and during the outbreak of respiratory disease (Pardon et al., 2011; Studer et al., 2021). Unfortunately, the genome segments of the BRSV detected by metagenomic nanopore sequencing were insufficient to determine whether it was a wild-type strain, the vaccine strain or a combination of both. Therefore, it is possible that the positive BRSV signal might have been a false-positive result brought about by the intranasal vaccination with a live vaccine, as has been described for PCR results earlier and can last up to 21 d postvaccination (Belloc et al., 2009; Walz et al., 2017). Belloc et al. (2009) demonstrated the presence of the vaccine strain in nasopharyngeal swabs shortly after intranasal vaccination. In our study however, nBAL samples were collected. Whether or not vaccine virus can be detected in nBAL samples collected shortly after application of a live viral vaccine remains unknown, but seems less likely (Makoschey B., personal communication). The fact that BPI-3 virus, also present in the live vaccine, was detected shortly before the first metaphylactic treatment, but no longer at the time of the outbreak is not in favor of a false-positive nanopore sequencing result. Moreover, the trial was done at the time of year
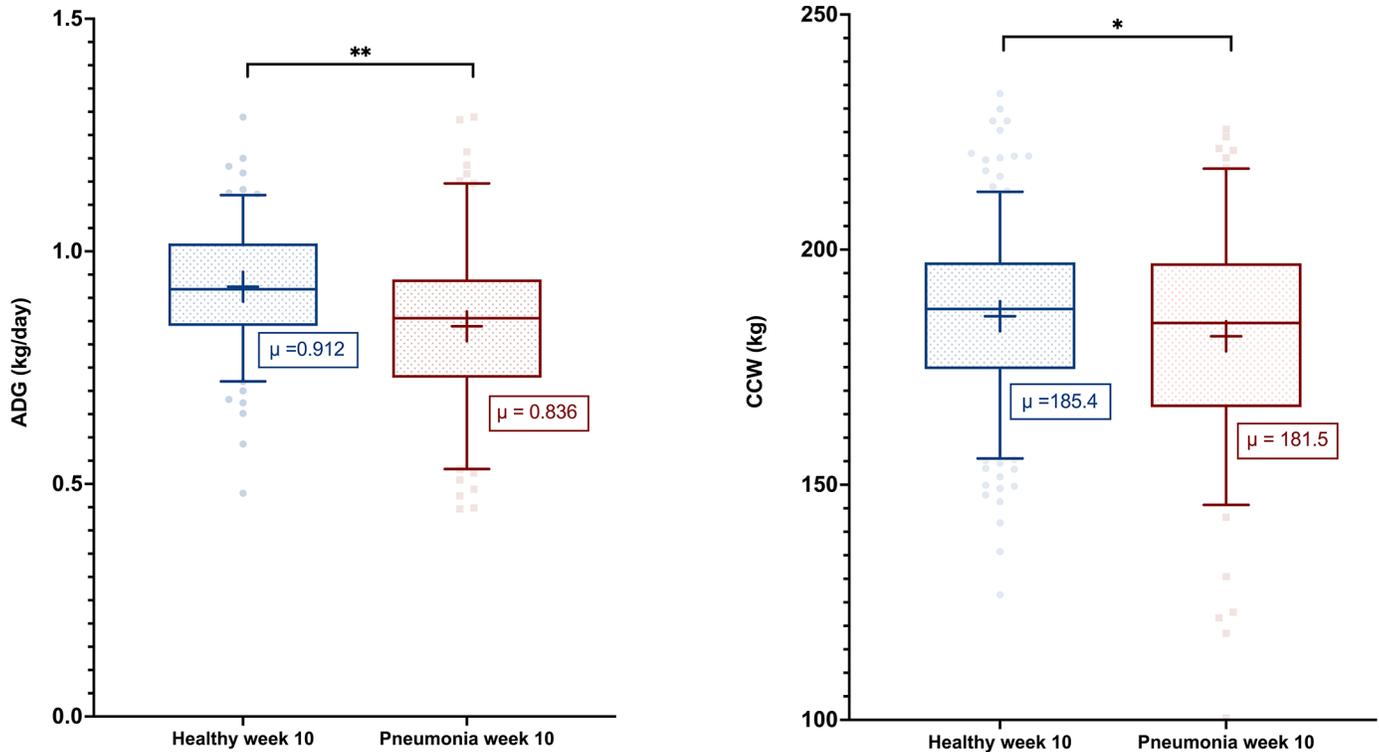
Figure 4. Average daily gain and cold carcass weight of 434 male dairy calves, stratified by qTUS findings in wk 10 of the production cycle. Average daily growth was calculated based on the difference in arrival weight and weight in wk 10 of production. The middle box represents the interquartile range (middle 50%), the horizontal line marks the media, the plus sign marks the average, whiskers represent the 5th to 95th percentiles, and dots are values outside of the 5th to 95th percentile. * Indicates a statistically significant difference with $P < 0.05$ ** Indicates a statistically significant difference with $P < 0.001$. CCW = cold carcass weight; qTUS = quick thoracic ultrasound.

that BRSV infections peak, hence the high probability that the virus was involved in the outbreak (Pardon et al., 2020). Although in the past, the use of a single dose of the inactivated vaccine has demonstrated partial protection against a challenge with BRSV, in general, a second dose of inactivated vaccine is required for a solid protection to be induced (van der Sluijs et al., 2010). In an attempt to induce an earlier onset of immunity, the second vaccination protocol (IN-PE group) included an intranasal vaccination with a live vaccine, but this could not prevent the outbreak from occurring. Ideally, this intranasal vaccination should be administered earlier to the calves before transportation to induce (partial) protection before commingling occurs. Short-term effects of viral vaccination may also have been masked by the fact that *M. bovis* and coronavirus infections were spreading rapidly at that time. Nevertheless, intranasal vaccination did result in a slightly better short-term cure rate compared with parenteral vaccination and could therefore have resulted in less severe consequences, such as *M. bovis* infections, secondary to the viral challenge at the time of the outbreak (Mosier, 2014).

The main finding in this study is that vaccination, both with the PE and IN-PE protocol, reduced the incidence of lung ultrasonographically confirmed pneumonia at wk 10 of production. Subsequent findings indicate that this reduction of pneumonia at wk 10 is likely to be caused by a combination of improved long-term cure rate and reduced incidence of new cases between outbreak (W1) and the long-term evaluation point (W10). These findings could be of economically of importance given that calves which still demonstrated pneumonia at wk 10 of production had a significantly lower carcass weight. Apparently, both vaccination protocols reduced the odds of developing chronic, unresponsive pneumonia, and the subsequent lower carcass weights, which could be economically relevant improvements. While viral pneumonia is known to be a major cause of disease in the first weeks after arrival, once these viruses recede and the animals enter the fattening stage, acute respiratory disease is mostly associated with an interaction between *M. bovis* and *Pasteurellaceae*, and more specifically, *M. haemolytica* (Pardon et al., 2020; Valeris-Chacin et al., 2022). Hence, a likely explanation is that the *M. haemolytica* component of

the inactivated vaccine offers protection against bacterial superinfection with *Pasteurellaceae.*

A third observation is that vaccination upon arrival did not result in reduced risk for BRD related clinical signs at any time. When comparing with the few studies evaluating vaccine efficacy on the expression of clinical signs (clinical BRD) in the same age category, the present study's findings are in line with earlier work (O'Connor et al., 2019). In a beef herd, Van Donkersgoed et al. (1994) only found a trend toward less clinical BRD when using a live-attenuated vaccine. In dairy calves, 2 available multiple herd studies, with a total of over 2000 calves enrolled, did not find an effect of vaccination with parenteral live-attenuated vaccines against BRSV, BPI-3, and *M. haemolytica* among other pathogens on clinical BRD (Aubry et al., 2001; Windeyer et al., 2012). Considering the current literature and the results of our study, it raises the question whether evaluation of vaccination, and even treatment in general, based on clinical BRD is sufficiently reliable. TUS has proven the importance of subclinical pneumonia and its effects on both animal welfare and production (Ollivett et al., 2015; Jourquin et al., 2023). More importantly, using qTUS, this study found associations between vaccination and development of pneumonia and even cure, whereas definitions based on clinical BRD again did not show any effects. To the authors knowledge, only one other study used TUS to evaluate vaccine efficacy, in dairy heifers up to 12 wk of age. The findings in this study are in line with the results of the present study, indicating vaccination to be positively associated with TUS findings and ADG, but not with clinical BRD (Ollivett et al., 2018a). In addition to respiratory sampling, another novelty in this study compared with the previous study, is that lung reaeration was used to evaluate cure. Lung reaeration on TUS has been described as an objective and reliable cure criterion (Jourquin et al. 2022). Our finding that vaccination influenced cure rates, while clinical BRD remained unchanged, emphasizes the usefulness of TUS to evaluate treatment effects and opens the way for future trials to use this criterion to evaluate effects of different BRD management strategies.

Fourth, this study demonstrates that the absence of pneumonia on qTUS in wk 10 was related to both a higher ADG in the first 10 wk and a higher CCW, meaning that prevention of long-term pneumonia is key in improving both welfare and production. In turn, vaccination was able to reduce the occurrence of pneumonia at wk 10 and therefore, vaccination should have a positive effect on production, as was seen in a previous study in Danish calf rearing operations (Martin et al., 2022) and confirmed in our study. To convince producers to vaccinate, positive effects on economic outcomes are important, but in this study only a borderline significant increase in CCW of 3.85kg in vaccinated animals was found. The applied sample size was not defined in function of production results resulting in insufficient power to detect a significant effect of separate vaccination groups. Although not significant, the mean increases in CCW of 3.48kg and 4.28kg for the PE and IN-PE group, respectively, would economically represent a return of investment in vaccine use.

In the current study, *Mycoplasma bovis* was a leading pathogen, rapidly spreading within 1 to 2 wk after arrival and persisting after group treatment, as is often observed in previous similar studies (Pardon et al., 2011; Jourquin et al., 2023). The short- and long-term cure rate (50.3 and 61.5%, respectively) appeared to be low, compared with a recent study showing >95% cure in an acute *M. bovis* outbreak in beef calves (Jourquin et al., 2022). There are different reasons why treatment effects are so different. In the beef calves study, not only was *M. bovis* the only primary pathogen found (no viral coinfection), but treatment duration was adapted to qTUS findings. Also, treatment itself was performed per injection instead of orally, decreasing the odds of underdosing due to differences in BW and feed intake (Borderas et al., 2009; Cramer et al., 2020). Further, in the beef calves study antimicrobial susceptibillity testing was done ensuring the *M.bovis* strain isolated was susceptible to the antimicrobials used. In contrast, in veal herds, it is known that multiple *M.bovis* strains with different susceptibillity patterns can coexist within one herd (Jourquin et al., 2023). Combining this with the significant viral challenge that was present at the time of treatment, one could argue that, within the current veal production methods, where viral coinfections are a major challenge and oral treatments are still common, a cure rate >60% might be unrealistic. Despite antimicrobial mass medication in this industry, cure rates are still low resulting in suboptimal production and animal welfare issues. Hence, efforts should be made to either prevent the damage inflicted by viral infections using vaccination and to individualize antimicrobial treatment and management based on qTUS findings rather than clinical signs.

Performing a well conducted clinical trial to evaluate the effects of vaccination on calf respiratory disease is a huge challenge for different reasons. The practical challenge was scanning a large number of calves in a reliable way. Perfoming qTUS on a large number of calves is labor-intensive, but the protocol turned out to be adapted for this need, both because of the methodology of the qTUS technique itself and because of the 4 strategical observation points in time that were chosen, all of which proved to be very informative. Application of this protocol, or a variation, has the potential to

be applied for different needs in different production types. For example, the qTUS protocol allows evaluation of the health status of recently purchased calves, early detection of pneumonia before or immediately after grouping animals on dairy or beef farms, to evaluate treatment length and efficacy and even to help predict production results (e.g., qTUS on wk 10 in veal calves). Therefore, this protocol has the potential to greatly contribute to rationalizing antimicrobial use, individualizing treatment based on the combination of qTUS and nBAL findings, and aid the industry in moving away from metaphylaxis.

The study itself, because it was performed on a commercial veal facility, had several limitations. The interpretive issues of the nanopore sequencing results are discussed above. Also, for safety and economic reasons we were not able to determine live weight just before slaughter. Hence, we needed to estimate live weight using a fixed factor, commonly used in the Belgian veal industry, for carcass yield percentage. Again, for practical reasons (slurry removal during observation), clinical scoring could not be done on all calves at the long-term evaluation point, leading to the loss of clinical data of 139 animals enrolled. Therefore, we cannot exclude whether both vaccination protocols would have affected clinical signs at wk 10. Further, we used the ≥ 1 cm lung consolidation depth cutoff point in the analysis. As other studies have suggested using a threshold of ≥ 3cm, and only evaluating caudal lobes to define active pneumonia, it could be argued that using the 1cm threshold might lead to overdiagnosis of pneumonia (Berman et al., 2019). However, multiple studies using Bayesian class modeling have shown that both sensitivity and specificity of using the 1 cm threshold are very comparable to the 3 cm threshold (Buczinski et al., 2014, 2015). Further, this study, as well as previous studies, have linked consolidation ≥ 1 cm to a decrease in production, indicating consolidation of this size to have a significant effect on the growth and wellbeing of these animals (Teixeira et al., 2017; Jourquin et al., 2023). Therefore, we believe both using the ≥ 1 cm cutoff value as well as including the cranial lobes to be the most accurate way for early detection of pneumonia and evaluating factors influencing development and progression of pneumonia in calves.

Finally, although the studied situation, being male dairy calves for veal production, is very different from conventional, closed herd dairy heifer calf raising, we argue that these study results are meaningful for the latter system as well. Afterall, in the present study we showed vaccine efficacy in very extreme conditions, with immunosuppressed calves, vaccination at the time of pathogen exposure and the dominant presence of *M. bovis* for which no vaccination was possible.

CONCLUSIONS

Under the conditions of this study, in a challenging veal calf setting, BRSV, PI-3, and *Mannheimia haemolytica* vaccination, both intranasal and parenteral, resulted in a reduced risk of long-term pneumonia, improved long-term cure, and reduced the incidence of new pneumonia cases between wk 3 and 10. In addition, vaccinated calves had a numerically higher carcass weight. These vaccination effects likely result from a reduced probability of a severe opportunistic bacterial superinfection, leading to lower odds for chronic pneumonia which is associated with carcass weight loss. The use of qTUS and nBAL diagnostics on strategic time points offers great potential to improve the ability of randomized clinical trials to evaluate preventative and curative measures for BRD.

ACKNOWLEDGMENTS

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ORCIDS

Stan Jourquin ⓘ https://orcid.org/0000-0002-8488-8494
Thomas Lowie ⓘ https://orcid.org/0000-0002-9877-3786
Florian Debruyne ⓘ https://orcid.org/0000-0002-2036-2565
Laurens Chantillon ⓘ https://orcid.org/0000-0002-9795-0699
Justine Clinquart ⓘ https://orcid.org/0000-0001-5870-5394
Geert Vertenten ⓘ https://orcid.org/0000-0002-3993-8616
Bart Sustronck ⓘ https://orcid.org/0000-0003-4978-6721
Bart Pardon ⓘ https://orcid.org/0000-0003-1026-8433