Growth performance and hematological changes of weaned beef calves diagnosed with respiratory disease using respiratory scoring and thoracic ultrasonography

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Conflict of Interest Statement

The authors declare that they have no competing interests.

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ABSTRACT

This study investigated (i) the effect of clinical bovine respiratory disease (BRD) and associated lung consolidations on growth performance and hematological profiles of recently weaned beef calves and (ii) the relationship between clinical respiratory signs and lung consolidation detected by thoracic ultrasonography (TUS). One hundred and fifty-three weaned beef calves (209 (SD; 35.8) days old and 306 (SD; 26.3) kg, at arrival) purchased and transported from auction markets were accommodated indoors in concrete slatted floor pens. Calves were weighed weekly from arrival until d 28 and on d 65 post-arrival. Assessment of BRD and blood sample collection for hematological profiles were performed on scheduled days (at arrival, on d 7, 14 and 28) and on other days upon BRD diagnosis. Animals were assessed for BRD using a total clinical respiratory score (CRS) of five clinical signs (rectal temperature, ear position, cough, nasal secretion and eye secretion with each ranging from normal (0) to abnormal (3)), and TUS scores (normal (0) to lung consolidation ≥ 1 cm^2 (2)). Based on CRS, 35% of calves were CRS+ (CRS ≥5) and 65% were CRS- (CRS <5). Although no lung consolidations (TUS-) were detected at arrival, 34% of calves developed lung consolidation (≥ 1 cm^2) (TUS+) during the first 28 d post-arrival. Only fever (>39.6^{o}C) and nasal discharge were weakly associated (*r* 0.19, *P* <0.05) with lung consolidation. On the day of BRD detection, neutrophil number and neutrophil:lymphocyte ratio was 58% and 73% greater, respectively, in BRD calves with lung consolidation compared to healthy calves. From d 0 to 65, calf ADG did not differ (*P* >0.05) between CRS+ and CRS- calves, but was 0.09 kg/d lower (*P* <0.05) for TUS+ compared to TUS- calves. Calves classified as BRD (CRS+TUS ≥5) with lung consolidation had lower (*P* <0.05) ADG from arrival until d 28 than healthy calves and BRD calves without lung consolidation (0.11 ± 0.10 *vs.* 0.53 ± 0.07 *vs.* 0.57

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± 0.10 kg/d, respectively); however, no differences in ADG were observed from d 0 to 65. Conventional methods to diagnose BRD failed to detect calves with lung lesions. Thoracic ultrasonography is a useful tool to detect lung lesions and its implementation in combination with CRS should provide a more accurate and early diagnosis of BRD, which is fundamental to successful treatment, animal welfare and growth performance.

Key words

Bovine respiratory disease, cattle, growth performance, hematology, thoracic ultrasonography.

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List of abbreviations:

ADG, average daily gain

BRD, bovine respiratory disease

BRD-con, bovine respiratory disease with lung consolidation

BRD-no-con, bovine respiratory disease without lung consolidation

CRS, clinical respiratory score

K_3EDTA, K_3Ethylene diamine tetra acetic acid

N:L, neutrophil:lymphocyte ratio

NSAID, Nonsteroidal anti-inflammatory drugs

RBC, red blood cell

sBRD, sub-clinical BRD

T, time

TUS, thoracic ultrasonography

WBC, white blood cell

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1. Introduction

Bovine respiratory disease (BRD) defines a complex, multifactorial syndrome, in which the interaction between infectious etiological agents (bacterial, virus), external stressors and the animal's immune system influence the development of the disease (Panciera and Confer, 2010). It is the most prevalent disease of recently-weaned feedlot cattle in Ireland (Murray et al., 2017) and internationally (Delabouglise et al., 2017; Hay et al., 2017; Wilson et al., 2017), and causes substantial economic losses due to decreased animal performance, higher mortality rates and increased costs associated with treatment (Cernicchiaro et al., 2013; Blakebrough-Hall et al., 2020) as well as negatively impacting animal welfare (Wolfger et al., 2015a). The temporal proximity between various stressors in feedlot cattle, such as weaning, transport, housing and dietary change results in immunosuppression, thus predisposing the respiratory tract to colonization by infectious pathogens (Lynch et al., 2010; Earley et al., 2017).

Despite the existence of numerous tools such as clinical respiratory scoring systems (Love et al., 2014; McGuirk and Peek, 2014; Maier et al., 2019), and automated monitoring systems of behavior to detect animals affected with BRD (Wolfger et al., 2015a), its diagnosis remains a challenge due to the lack of a gold standard diagnostic method ante-mortem (Wolfger et al., 2015b). Thoracic ultrasonography (TUS) is being used as a rapid, non-invasive, on-site diagnostic and prognostic tool for finding lung abnormalities (Ollivett and Buczinski, 2016). Detection of lung abnormalities ante-mortem using TUS has been correlated with findings post-mortem using necropsy (Abutarbush et al., 2012; Baruch et al., 2019), and TUS is reported to have greater accuracy for BRD diagnosis compared to conventional methods such as auscultation or clinical scoring criteria (Buczinski et al., 2015; Buczinski et al., 2016). Furthermore, this methodology requires only minimal training and skills, and has good inter-rater agreement (Buczinski et al., 2018).

Although BRD cannot be diagnosed solely based on hematological profiles, changes in the hemogram may provide useful information for the diagnosis, monitoring and prognosis of BRD

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(Richeson et al., 2013; Roland et al., 2014). The presence of ≥ 4% of neutrophils in the bronchoalveolar lavage fluid of Holstein calves has been related with the presence of lung consolidation (Ollivett et al., 2015). Automated blood cell counters are readily accessible nowadays in veterinary practice for diagnosing and monitoring systemic diseases.

Bovine respiratory disease diagnosis in feedlots is mainly performed through subjective evaluation of clinical respiratory signs (Sanderson et al., 2008); however, not all BRD-affected cattle show clinical respiratory signs resulting in sub-clinical BRD (sBRD) cases going undetected (Thompson et al., 2006; Murray et al., 2017). Animals affected with sub-clinical BRD also have a lower economic return at slaughter compared to healthy animals (Thompson et al., 2006; Blakebrough-Hall et al., 2020). In pre-weaned dairy calves, incorporation of TUS for diagnosing BRD led to the detection of calves with lung consolidation and these animals had reduced average daily gain (ADG) compared to calves without lung consolidation (Cramer and Ollivett, 2019). Few studies with backgrounding indoor feedlot cattle have assessed the relationship between clinical respiratory signs and lung consolidation detected by TUS or have evaluated the impact of BRD diagnosed using both TUS and respiratory signs on animal growth performance and hematology.

Therefore, the objective of this experiment was to investigate (i) the effect of clinical BRD and associated lung consolidations on growth performance and hematological profiles of recently-weaned beef calves and (ii) the relationship between clinical respiratory signs and lung consolidations detected by TUS.

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2. Material and methods

2.1 Ethical approval

All animals procedures performed in this study were reviewed and approved by the Teagasc Animal Ethics Committee (TAEC-221-2019) and were conducted under experimental license from the Health Products Regulatory Authority, Dublin, Ireland (AE19132-P101) in accordance with the protection of animals used for scientific purposes i.e. Directive 2010/63/EU and S.I. No. 543 of 2012, as amended by S.I. No. 434 of 2013 and S.I. No. 174 of 2014.

2.2 Animals management

One-hundred and fifty-three (209 (SD; 35.8) days old and 306 (SD; 26.3) kg, at arrival) spring-born weaned "suckler" beef male (n=79) and female (n=74) calves were used. Forty-seven were sired by early-maturing breeds (Aberdeen Angus and Hereford) and 106 were late-maturing breeds (Charolais and Limousin). Animals were purchased through auction markets (n=10) during October 2019, over five consecutive days, and were transported by road (transport duration, 1 to 2.5 h) in five different groups to the research centre at Teagasc, Grange (longitude 6°65 W; latitude 53°52 N). At arrival (day -1), calves were housed and penned on concrete slatted floors (n=32) in groups of five according to group and sex, with an individual space allowance of 2.9 m^2 per animal. During the study, the mean indoor and outdoor temperature was 5.5°C (range 0 to 11°C) and 6.1 (range 1 to 12°C), respectively; the ambient outdoor relative humidity ranged from 72 to 97.5%.

Grass silage (in vitro dry matter digestibility 690 (SD; 18.0) g/kg) was offered ad libitum once daily and each animal received 60 g of a mineral and vitamin supplement applied on the forage. The objective was to grow the animals at a moderate ADG during this indoor winter period (backgrounding) and subsequently avail of compensatory growth during the grazing season (McGee et al., 2014). All animals had free access to clean, fresh water. Animals were vaccinated 24 h post-arrival (day 0) against bovine respiratory syncytial virus, parainfluenza-3-virus, *Mannheimia haemolytica* A1 (Bovilis Bovipast RSP, MSD Animal Health), bovine herpesvirus type 1 (Bovilis IBR

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marker live, MSD Animal Health) and against clostridial diseases using toxoids (Covexin 10, Zoetis). Animals were dewormed at d 21 post-arrival with a subcutaneous injection of ivermectin solution (Closamectin, Norbrook). Male calves were castrated on d 30-35 by a veterinarian using standard Burdizzo castration management procedures with pain relief (administration of local anaesthesia and of non-steroidal anti-inflammatory drug prior to castration). The study duration was 65 days.

2.3 Experimental design

The experimental design of the study is detailed in Fig. 1. Individual bodyweight was measured on days 0, 7, 14, 21, 28 and 65 post-arrival, using a calibrated platform scales (Tru-Test XR3000, load bars XHD 10000, Aukland, New Zealand) placed in the hydraulic squeeze chute (Titan, O'Donovan Engineering, Ireland).

Clinical and TUS assessment of individual calves was performed on days 0, 7, 14 and 28 post-arrival at pen-side and in the hydraulic squeeze chute. All calves were subjected to clinical respiratory assessment, using the clinical respiratory score (CRS) developed in the University of Wisconsin (McGuirk and Peek, 2014), and TUS by the same trained research veterinarian. At the same time as clinical respiratory assessment, whole blood samples were collected by jugular venipuncture into a 4ml K_3Ethylene diamine tetra acetic acid (K_3EDTA) tubes (Vacuette; Cruinn Diagnostics, Dublin, Ireland) for hematological analysis within 1 to 2 h of blood collection. Additionally, from d 0 to 28 calves were visually checked by the research veterinarian once daily, and those with ≥ 1 visual sign included in the CRS or showing a depressed appearance were temporarily moved from their home pen to the handling chute and subjected to a complete clinical assessment and TUS. Calves continued to be observed on a daily basis by technical research personnel from day 28 until the end of the study.

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2.4 Clinical respiratory score and thoracic ultrasonography assessment.

The Wisconsin CRS classifies rectal temperature, presence of cough, appearance of nasal and eye discharges, and ear position with scores ranging from normal (score 0) to very abnormal (score 3) (McGuirk and Peek, 2014), and the sum of all scores of each clinical sign evaluated form the CRS. Thoracic ultrasonography was performed using an 8 MHz Wi-Fi linear probe (Tecnoscan SR-1C, Imporvet, Spain) at a maximal depth of 10 cm, and gain of 50 dB. Hair on the thorax was clipped with electric clippers and isopropyl alcohol (70%) was used as a transducing agent before performing TUS, as described by Ollivett and Buczinski, 2016. Thoracic ultrasonography was performed on the left and right sides of the thorax. Since the 1st to 3rd intercostal spaces could not be scanned in animals of this size, particularly when restrained in a chute, only the 4th, 5th, and 6th intercostal spaces were examined as these are the locations where lung consolidation has been associated with negative outcomes in feedlot cattle (Thompson et al., 2006; Rademacher et al., 2014). The appearance of the lungs in the thoracic ultrasonograms was classified according to a three point scale (Fig. 2). Aerated lungs without any sign of consolidation or comet tail artefacts were classified as TUS=0. The ultrasonogram of the lungs with a TUS=0 is characterized by the visualization of the pleura as a hyperechoic line with echogenic lines parallel and below the pleural line representing reverberation artefacts. Lungs with one or more comet tail artefacts were classified as TUS=1. Comet tail artefacts were visualized as bright echogenic lines perpendicular to the pleural line. Lungs with any consolidation ≥ 1 cm^2 were classified as TUS=2. Consolidation is visualized as a hypoechoic area of varying size with an echogenicity similar to that of liver parenchyma, developing in some cases fluid bronchograms. Visualization of normal lungs and presence of comet tails by TUS were annotated on-site while a 10-second loop of ultrasound footage was stored when consolidation was visualized in order to evaluate its size off-line by the same researcher after the study period was complete. For measuring consolidation size, squares in the screen representing 1 cm^2 each, were used.

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Three classifications according to CRS, TUS scores and combination of them were made retrospectively (Fig. 3). Calves with a CRS ≥ 5 were CRS + and calves with a CRS < 5 were CRS -. Thoracic ultrasonography classification was determined by the presence (TUS +) or absence (TUS -) of lung consolidation ≥ 1 cm^2. The combined classification determined clinical status of animals and was used to determine the need for antibiotic and NSAID (Nonsteroidal anti-inflammatory drugs) therapy. Calves with a sum of both scores (CRS+TUS) ≥ 5 at any time between 1 and 28 d post-arrival were diagnosed as having clinical BRD (with or without lung consolidation, BRD-no-con and BRD-con respectively). Once diagnosed, these animals were weighed, blood sampled and, subsequently, treated with a subcutaneous dose of 2.5 mg/kg tulathromycin (Draxxin, Zoetis) and an intravenous dose of 0.5 mg/kg meloxicam (Metacam, Boehringer Ingelheim). Calves with a sum of both scores (CRS+TUS) < 5 and no lung consolidation detected during 28 d post-arrival were considered healthy. Calves that developed lung consolidation (≥ 1cm^2) and a combined score (CRS+TUS) < 5 were considered sBRD, did not receive veterinary medication unless they had a combined score ≥ 5, and were thereby classified as BRD. Calves with a combined score (CRS+TUS) ≥ 5 at least 7 d after initial drug medication were considered relapses and were re-treated with the same drugs. A matched healthy control calf corresponding to each individual BRD case was selected retrospectively from the nearest pen to compare hematology variables at the same sample times and ADG prior and after treatment.

2.5 Hematological analysis

Unclotted K_3EDTA whole blood samples were analyzed using an ADVIA hematology analyzer (ADVIA 2120; Bayer Healthcare, Siemens, UK) equipped with software for bovine blood. White blood cell (WBC) count, total neutrophil, lymphocyte, monocyte, basophil, red blood cell (RBC), platelet numbers, hemoglobin concentration and hematocrit percentage were evaluated. Three time (T) points were selected to analyze the hematological profile. The blood samples obtained 7 d prior to BRD detection are represented by T1, T2 represents the samples collected on the day of BRD diagnosis and treatment, and T3 those obtained 7 d post BRD detection.

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2.6 Data management and statistical analyses.

Statistical analyses were conducted using SAS v.9.4 software (SAS Institute, Inc.). The experimental unit was the individual animal. The outcomes of interest were the hematology variables and ADG. Calf ADG was determined for different periods: d 0 to d 28 (ADG1), d 28 to d 65 (ADG2) and d 0 to d 65 (ADG-overall). Additionally, ADG prior to detection and treatment of BRD was calculated from d 1 to the day of BRD diagnosis (ADG-PRE), and ADG-POST was calculated from BRD diagnosis to d 65. Calf ADG -PRE and -POST was calculated retrospectively in matched healthy control animals that were weighed on the same days as the BRD cases. Categorical explanatory variables included clinical status obtained from combined classification (Treatment classifier (healthy, sBRD, BRD-con and BRD-no-con)) and categorical dichotomous explanatory variables included CRS classification (+/-) and TUS classification (+/-). Three calves (1 sBRD and 2 BRD-con) were removed from the study after d 28 due to injury not related to treatment classification; their ADG and blood data are included from period 1 only. Data were checked for normality and homogeneity of variance by histograms, q–q plots, and formal statistical tests as part of the UNIVARIATE procedure of SAS. Data that were not normally distributed were transformed by raising the variable to the power of lambda. The appropriate λ value was obtained by conducting a Box-Cox transformation analysis using the TRANSREG procedure of SAS. Data subjected to transformation were used to calculate P-values. Associations between explanatory variables and the outcomes of interest were evaluated using mixed-effects linear models (PROC MIXED). Group was included as random effect and age, sex and breed as fixed effects. There was no effect of age (P >0.05) and it was excluded from the model. Hematological variables were analysed according to combined classification at T1 (Pre-BRD detection), T2 (BRD detection), and T3 (7 d post-BRD). Values of healthy matched controls were used corresponding with T1, T2 and T3 of BRD calves. As 6 sBRD calves were detected at d 28, they were not evaluated 7 d post-BRD so that T3 was not analyzed in sBRD calves. Number ($\times 10^3$ cells/µL) of WBC, neutrophils, lymphocytes, basophils, monocytes, eosinophils, RBC, platelets, neutrophil:lymphocyte ratio (N:L), concentration of hemoglobin and hematocrit percentage were analysed using repeated measures mixed models

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(PROC MIXED) where time-point defined the repeated measure. A Tukey adjustment was used to correct for multiple testing. Multivariable linear regression models (PROC GLM with MANOVA) for ADG1, 2 and overall were conducted including CRS and TUS variables. To evaluate the prevalence of BRD disorders, the clinical parameters included in the CRS and the TUS were used. Scores recorded at d 0, 7, 14 and 28 were expressed as percentages (PROC FREQ) of calves that showed each abnormal clinical disorder. Cough, nasal, eye and ear score were considered abnormal when ≥ 1. Rectal temperature score of 3 was considered abnormal (fever, >39.6ºC). Lung consolidation ≥ 1cm^2 (TUS=2) was considered abnormal compared with absence of lung consolidation (TUS=0) or presence of comet tails (TUS=1). Association between clinical disorders and presence of lung consolidation recorded at d 7, 14 and 28 were evaluated by calculating correlation coefficients between variables using Pearson correlation (PROC CORR). Data were considered statistically significant when P <0.05. A statistical trend was considered when P-values ranged from 0.05 to 0.10. Least square means (Lsmeans) are reported with a standard error.

3. Results.

3.1 BRD incidence using different diagnostic methods.

Using CRS classification, 35% (54/153) and 65% (99/153) of calves were classified as CRS + and CRS -, respectively (Table 1). Although no lung consolidation was detected at arrival, 34% (52/153) calves developed lung consolidation ≥ 1 cm^2 (TUS +) and 66% (101/153) were TUS- in the 28 d post-arrival based on TUS classification. Using the combined classification, sixty-eight calves (44.4%) were classified as BRD cases, of which 30 BRD-no-con and 38 BRD-con. Fourteen (9.2%) calves were classified as sBRD and 71 (46.4%) were healthy. Of 68 BRD cases, 8 (12%) calves relapsed once after the first BRD medication and no calves required any further BRD medication. Fifty percent (34/68) of BRD cases were detected within the first 7 d post-arrival and 81% (55/68) within the first 14 d (Fig. 4). Lung consolidations were not found in 60.5% (23/38) of BRD calves post-antibiotic treatment.

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Conversely, lung consolidation was detected in all subsequent evaluation of sBRD calves diagnosed before d 28. No further cases of BRD were detected after day 28.

3.2 Prevalence of clinical disorders and associations

Descriptive statistics for BRD associated disorders recorded on d 0, 7, 14 and 28 are reported in Table 2. A relatively low prevalence (< 10%) of cough, eye discharge and ear drooping was observed. The greatest percentages of calves with fever were observed on d 7 and 14 (28.1 and 26.8%, respectively). The percentage of calves that showed nasal discharge on d 0 (31.4%) and 7 (59.5%) decreased to 9% and 10% by d 14 and 28, respectively. No lung consolidations were detected by TUS on d 0, however by d 28, 18% of calves were TUS+. Fever was correlated (P <0.05) with presence of lung consolidation ≥ 1cm^2 on d 7 (Table 3). There was no correlation between percentage of calves with clinical respiratory signs and lung consolidation at d 14 and 28 post-arrival (P >0.05).

3.3 Hematological variables

There was no effect of treatment (P >0.05) prior to the development of BRD (T1) on any of the hematological variables except for platelet cell number (Table 4). There was a treatment × time interaction (P <0.05) for neutrophil number, N:L, basophil and platelet numbers. On the day of BRD detection (T2), neutrophil number was greater (P <0.05) in BRD-con calves than in healthy and sBRD calves, with BRD-no-con intermediate (P >0.05), whereas post-BRD (T3) neutrophil number was greater (P <0.05) in BRD-con calves than in healthy calves, with BRD-no-con calves intermediate (P >0.05). Neutrophil number was greater (P <0.05) in BRD-con calves at T2 compared to T1. Neutrophil:lymphocyte ratio was greater (P <0.05) in BRD-con calves compared to healthy and sBRD calves and tended to be greater (P =0.07) in BRD-no-con calves than in healthy calves at T2. At T3, N:L was greater (P <0.05) in BRD-con calves than in healthy calves without difference (P >0.05) when compared with BRD-no-con calves. Neutrophil:lymphocyte ratio was greater (P <0.05) in BRD-con at

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T2 and T3 compared to T1. Basophil number was lower (P <0.05) in BRD-con compared to sBRD calves at T2 and no differences were found (P >0.05) with healthy or BRD-no-con calves. Basophil and platelet numbers (P <0.05) were greater at T3 compared to T1 and T2 in BRD-con calves. At T1, platelet number was greater (P <0.05) in sBRD calves than in healthy calves while there was no difference (P >0.05) with BRD-con or BRD-no-con calves. At T3, platelet number was greater (P <0.05) in BRD-con calves than in healthy calves and were not different (P >0.05) when compared with BRD-no-con calves.

There was no treatment × time interaction (P >0.05) for WBC, eosinophil and RBC numbers, hemoglobin concentration and hematocrit percentage. No differences (P >0.05) in lymphocyte and monocyte numbers were found between treatment groups. Eosinophil numbers tended to be lower (P =0.09) in BRD calves with lung consolidation compared to sBRD calves. White blood cell number was greater, while RBC number, hemoglobin concentration and hematocrit percentage were lower (P <0.05) in BRD-con calves compared to healthy calves while no differences (P >0.05) were found when values were compared to sBRD and BRD-no-con calves.

3.4 Growth performance

Least squares means for ADG according to clinical classification of calves are presented in Table 5. The ADG1 of calves classified as BRD-con was 0.42 and 0.46 kg lower (P <0.05) than healthy calves and BRD-no-con calves, respectively; however, no difference (P >0.05) was found between healthy, sBRD, BRD-no-con and BRD-con calves in ADG2 or ADG-overall. Regarding ADG prior to and after BRD treatment, BRD-no-con and BRD-con calves had a lower (P <0.05) ADG-PRE compared to healthy calves, whereas ADG-POST was greater (P <0.05) for BRD-no-con and BRD-con calves than healthy calves.

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Multivariable linear regression evaluating the association between ADG and CRS status as well as TUS status is presented in Table 6. Although there was no difference (*P* >0.05) in calf ADG according to CRS status, ADG1 was 0.28 kg lower and ADG-overall was 0.09 kg lower (P <0.05) in TUS+ compared to TUS- calves.

4. Discussion

Currently, there is no 'gold standard' ante-mortem method for diagnosing BRD meaning that delayed and under-detection of BRD is a significant problem in feedlot cattle (Wolfger et al., 2015b; Blakebrough-Hall et al., 2020). In this study the evaluation of clinical respiratory signs, which is widely used as a BRD diagnostic method in feedlots (Sanderson et al., 2008; Leruste et al., 2012), was used in conjunction with TUS, a measure of the presence of lung consolidation. The TUS method is a novel technique that can be used for early-detection of BRD but is rarely used in feedlot studies to date (Abutarbush et al., 2012; Timsit et al., 2019).

The incidence of BRD based on evaluation of solely clinical respiratory signs obtained in the current experiment (35%) is intermediate to the range in values (15-53%) reported for feedlot studies (Wittum et al., 1996; Thompson et al., 2006; Fulton et al., 2009; Ball et al., 2019; Blakebrough-Hall et al., 2020). In feedlot studies, the incidence of BRD based on clinical respiratory signs is much lower than the prevalence of lung lesions at slaughter. For example, Thompson et al. (2006) reported that 22.6% of animals had clinical BRD whereas 42.8% had lung lesions at slaughter, and Blakebrough-Hall et al. (2020) reported that 17% of animals showed clinical respiratory signs, whereas 68% had lung lesions at slaughter. This underlines the importance of including TUS to detect lung lesions as not all BRD affected animals are diagnosed when evaluated using only clinical respiratory signs during their productive life.

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When comparing CRS and TUS in the current study, 28% of calves classified as CRS- had lung consolidation, whereas 56% of calves classified as CRS+ had no lung consolidation detected by TUS. Similarly, Abutarbush et al. (2012) evaluating BRD cases and healthy controls in feedlot cattle found that 16% of healthy controls had lung consolidation, whereas 72% of BRD cases had no lung consolidation detected by TUS. Given that cranial lung lobes could not be evaluated in this study due to the size of animals, it is possible that more calves classified as CRS- could have had lung consolidation. Using the combined classification (CRS+TUS) in this study, 54% of calves had BRD or sBRD, which implied a detection of 18% (28/153) additional calves that would have not been detected using CRS alone. Similarly, when Thompson et al. (2006) used the combined definition of clinical BRD (treated animals) and sub-clinical BRD (never treated but with lesions at slaughter), they obtained a BRD incidence of 53%. Thus, the combination of CRS with TUS used in this study has shown that both methods are necessary to provide a better classification of BRD cases (calves that show clinical signs without evidence of lung consolidation detectable by TUS, calves with lung consolidation detected by TUS without evidence of clinical signs, and calves with both clinical signs and lung consolidation).

Interestingly, in this study 60.5% (22/38) of lung consolidation detected in treated BRD-con calves were not detected in the ultrasound evaluation at d 28. However, lung consolidation was present in sBRD calves that were evaluated at least twice, suggesting that consolidation could have responded to treatment in some of the BRD-con treated calves. Similarly in feedlot cattle, (Abutarbush et al., 2012) found that 49% of lung consolidations were not detected in subsequent evaluations by TUS in BRD animals after receiving treatment. Lung tissue is reported to have extensive capacity to repair and regenerate damaged cells after injury or disease both in humans and mice (Herriges and Morrisey, 2014; Zacharias et al., 2018), though more investigation is needed in this regard in cattle.

In the current study, the greatest incidence of BRD cases occurred within the first 14 d post-arrival. Previous authors have reported that BRD incidence was greatest in the first week after arrival to the

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feedlot and decreased subsequently (Sanderson et al., 2008). Management practices conducted at feedlots such as transport or commingling predispose calves to BRD development in an early stage after entry (Taylor et al., 2010). In the present study, although calves were visually checked once daily during 28 d post-arrival to detect clinical respiratory signs, a greater number of BRD cases (53/82) were detected on the four days when the combined score was used (d 0, 7, 14 and 28) than on the intervening days when solely CRS was used (29/82 calves detected). Thus, evaluation of feedlot calves using CRS with TUS detected a greater number of BRD affected animals at the early stage of the infection (i.d. 7 to 14 d).

In the present study, the most prevalent clinical disorders associated with BRD were nasal discharge at d 0 and 7 post-arrival and fever at d 7 and 14 post-arrival. The rest of the clinical signs had a low prevalence during the first 28 d. The poor correlations between calf clinical signs and the simultaneous presence of lung consolidation detected by TUS are in agreement with (Leruste et al., 2012), who found weak correlations (r_{sp} from 0.16 to 0.40) between clinical signs of BRD and moderate to severe lung consolidation at slaughter in feedlot cattle. Therefore, clinical signs are not accurate indicators of lung consolidation, thereby making it necessary to use TUS to detect lung consolidations ante-mortem. Due to the limitation of TUS as a routinely diagnostic tool, particularly in large high throughput facilities, advances in TUS-related technology in the future may facilitate an early detection of lung consolidation in cattle.

The greatest changes in the haematology profile were observed in neutrophil number and N:L. Calves that were classified as BRD-con had a 52% and 92% increase in neutrophil number and N:L, respectively, the day that they were diagnosed with BRD compared with the day of arrival, when they were healthy. This increase is substantially greater than the values reported in beef calves (neutrophil, 5%; N:L, 12%, increase) following a combination of standard post-weaning management practices including, housing and adapting to a new diet (Lynch et al., 2011). Moreover, BRD-con calves had greater number of neutrophils (58%) and N:L ratio (73%) compared to healthy calves on

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the day of BRD diagnosis and post-BRD (73% and 72% greater, respectively). Ollivett et al. (2015) reported a greater percentage of neutrophils (14.0%) in the bronchoalveolar lavage fluid of Holstein calves with lung consolidation compared to calves with completely normal lungs (1.2%). However, neutrophil number in sBRD calves in this study did not differ from the neutrophil number in healthy calves. This could indicate that not only lung consolidation but clinical disease symptoms are necessary to detect differences in neutrophil number. The neutrophil and N:L profiles are in agreement with previous studies, where higher values have been reported in calves affected by BRD compared to healthy calves (Burciaga-Robles et al., 2010; Lindholm-Perry et al., 2018). Previous authors reported low eosinophil and high RBC numbers in blood samples at arrival to facilities as indicators of calves at a greater risk of developing clinical signs of BRD (Richeson et al., 2013). In the present study at arrival, eosinophil numbers tended to be lower in BRD-con calves compared to sBRD calves. However, BRD-con calves had lower RBC number than healthy calves. Thus, changes in blood neutrophil number and N:L could be useful indicators of respiratory disease in calves that develop lung consolidation following natural infection.

Use of thoracic ultrasonography is more common in studies of pre-weaned dairy heifers. Recent experiments show that compared to pre-weaned dairy heifers without lung consolidation, those exhibiting consolidation had reduced (0.12 kg) ADG during the pre-weaning period (Cramer and Ollivett, 2019), produced 525 kg less milk in their first-lactation (Dunn et al., 2018), and had a higher probability of being culled by the end of first lactation (Teixeira et al., 2017). In contrast, there is limited information relating TUS findings with growth outcomes of feedlot calves. Abutarbush et al. (2012) did not find an association between lung consolidation detected by TUS in feedlot cattle and growth performance; however, they only performed TUS on one side of the thorax using a 3.5 MHz sectorial probe and they evaluated frozen images, which could have affected the diagnostic accuracy (Rademacher et al., 2014).

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In the current study, calves with lung consolidation detected at least once by TUS, regardless of their CRS status, had a reduction of growth rate by 28.1% (0.23 vs. 0.32 kg/d) during the first 65 d post-arrival compared to those without lung consolidation. Timsit et al. (2019) reported that severity of lung consolidation as measured using TUS in feedlot cattle was negatively related with ADG (-34 g/cm lung consolidation depth) and was associated with a higher risk of BRD relapse after first BRD antibiotic treatment (Odd ratio, 1.337/cm lung consolidation depth). Moreover, studies have reported the impact of lung lesions identified at slaughter on growth performance of feedlot cattle; compared to animals without lung lesions during the late fattening period, those with severe lung lesions had a reduction of growth rate by 5.3% (1.67 vs 1.58 kg/d) (Thompson et al., 2006) and 16.7% (1.8 vs 1.5 kg/d) (Blakebrough-Hall et al., 2020).

Considering the combined classification in the present study, BRD-con calves had reduced ADG during the first 28 days post-arrival compared to healthy and BRD-no-con calves. However, whether the day of BRD diagnosis and treatment is considered, BRD-no-con and BRD-con calves (clinically-ill calves) had lower ADG than healthy calves prior to BRD treatment with calves having increased ADG above healthy calves after BRD treatment. In the present study, it is recognized that the ADG measurement duration is short. Additionally, the absolute growth rates are relatively low as the animals were on a 'back-grounding' phase in order to minimize feed costs and exploit subsequent compensatory growth at pasture during the following grazing season (McGee et al., 2014). The changes in ADG may be attributed to reduced gut-fill at the time of BRD diagnosis due to the lower intake of sick calves compared to healthy calves (Wolfger et al., 2015a), resulting in an increased apparent weight gain post-treatment when appetite resumed. Previous authors have reported dairy calves reduced their visits to the feeder during 3 days prior to BRD detection and tended to reduce those visits 7 days post BRD detection (Johnston et al., 2016). In beef feedlot calves, mean meal intake and frequency of visits to the feeder were reduced 7 days before BRD detection (Wolfger et al., 2015a). On the other hand, animals could have experienced a physiological compensatory growth after a period of reduced intake caused by BRD (Hornick et al., 2000). Therefore, a low intake

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caused by BRD may have triggered a compensatory growth which may have led to BRD calves growing faster after BRD treatment so that, eventually, no difference in ADG was found from d 0 to 65 between calves diagnosed as BRD and healthy calves. Moreover, a success of BRD treatment could have caused a recovery of growth in BRD calves. In this study, sBRD calves, which were not treated, had a numerically lower ADG than healthy and BRD calves in all growth periods evaluated. However, these differences were not statistically significant, which could be due to the low number (n=14) of sBRD calves present in this study. Thompson et al. 2006 reported that BRD feedlot cattle that were treated tended to grow faster in the finishing period than those with sub-clinical disease that were not treated. Further research should be directed towards the evaluation of compensatory growth and effect of antibiotic treatment in calves diagnosed as having BRD using both CRS and TUS.

5. Conclusion

A greater number of BRD affected calves were detected using TUS and CRS when compared with CRS alone. Moreover, an increased N:L ratio could be a useful indicator of respiratory disease in calves which develop lung consolidation. The detection of lung consolidation ante mortem can only be confirmed using TUS and this is important since TUS+ calves had lower growth performance than TUS– calves while no differences in ADG were observed between CRS+ and CRS- calves. The current detection of BRD in feedlots through CRS alone may lead to calves going undetected with lung consolidation due to the weak correlation between clinical signs and lung consolidation. Accordingly, TUS could be implemented in feedlots to detect BRD-associated lung consolidation during the first weeks post-entry, when animals are at greater risk of developing BRD. Future research should focus on the evolution of lung consolidation after therapy and its effect on growth performance as well as exploiting the recent advances in sequencing technologies to characterize the microbiome and virome associated with the development of BRD in this cattle population.

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Figure legends

Fig 1. Experimental design. Timeline representing the experimental design from arrival of animals to the Research Centre facilities (d -1) until the last weight was measured (d 65). The bovine respiratory disease (BRD) evaluation was performed during the first 28 d of study. Average daily gain (ADG) was measured in three periods: from d 0 to d 28 (ADG-1), from d 28 to d 65 (ADG-2), and from d 0 to d 65 (ADG-overall). BS, blood sampling; CRS, clinical respiratory score; TUS, thoracic ultrasonography.

Fig 2. Ultrasonograms of the three thoracic ultrasound score classification. Echo of lung tissue is observed in the bottom part of each ultrasonogram, separated from the intercostal muscles by the pleura. Two comet tails in TUS=1 are indicated with arrows and lung consolidations in TUS=2 are marked with stars. Each square delimited by green lines indicates 1 cm^2. ICM, intercostal muscles; P, pleura; TUS, thoracic ultrasonography score.

Fig 3. Criteria for clinical respiratory score classification, thoracic ultrasonography score classification and combined classification. The sum of rectal temperature, nasal, eye, cough and ear scores form the clinical respiratory score (CRS) (McGuirk and Peek, 2014). Calves with CRS < 5 were considered CRS -, calves with CRS ≥ 5 were considered CRS +. Thoracic ultrasonography score (TUS) represent the appearance of lungs. Calves without lung consolidation (TUS = 0 or 1) were considered TUS -, calves with lung consolidation (TUS = 2) were considered TUS +. Combined classification used both CRS and TUS. Calves with CRS + TUS < 5 without lung consolidation were considered healthy; calves with CRS + TUS < 5 with lung consolidation were considered sBRD; calves with CRS + TUS ≥ 5 without lung consolidation were considered BRD-no-con and calves with CRS + TUS ≥ 5 with lung consolidation were considered BRD-con. [1]BRD-no-con, bovine respiratory disease without lung

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consolidation; [2]BRD-con, bovine respiratory disease with lung consolidation; CRS, clinical respiratory score; TUS, thoracic ultrasonography score.

Fig 4. Distribution of bovine respiratory disease detection. Representation of number of bovine respiratory disease (Nº BRD) cases (X axis) detected each day (Y axis) from arrival (d -1) until d 28. Fifty per cent of cases were detected within the first 7 d while 80% within the first 14 d post-arrival.

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Table 1. Distribution of 153 weaned beef calves according their clinical respiratory and thoracic ultrasonography status.

	CRS status[1]	
TUS status[2]	**-**	**+**
No Consolidation	71	30
Consolidation	28	24

[1]Clinical respiratory score from McGuirk et al. (2014). Calves were considered CRS + when the score was ≥ 5

[2]Thoracic ultrasonography. Consolidation was defined as ≥ 1 cm^2

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Table 2. Prevalence (%) of clinical signs associated with bovine respiratory disease in 153 weaned beef calves evaluated at four stages within the 28 first days post-arrival.

% Clinical scores[1]:	**Day 0**	**Day 7**	**Day 14**	**Day 28**
Fever (> 39.6ºC)	9.2	28.1	26.8	9.2
Cough	1.3	7.8	5.9	6.5
Nasal discharge	31.4	59.5	9.15	9.8
Eye discharge	2.6	9.8	2.6	7.2
Ear drooping	0	2.6	2.6	0.7
TUS evaluation:				
% Consolidation ≥ 1cm^{2*}	0	9.8	19.0	17.7

[1]Percentage of calves with evidence of each clinical sign associated with bovine respiratory disease.

[*]Percentage of calves with lung consolidation ≥ 1cm^2

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Table 3. Pearson correlation coefficients between clinical signs associated with respiratory disease and lung consolidation detected by ultrasonography simultaneously in 153 recently weaned beef calves.

Clinical scores[1]:	Lung consolidation ≥ 1cm^2		
	TUS 7	**TUS14**	**TUS28**
Fever (> 39.6ºC)	0.19*	0.13	0.04
Cough	0.07	0.09	0.02
Nasal discharge	-0.13	-0.04	-0.10
Eye discharge	-0.03	0.02	0.00
Ear drooping	0.30	0.02	-0.04

[1]Clinical sign associated with bovine respiratory disease.

[2]Lung consolidation ≥ 1cm^2 detected by thoracic ultrasonography (TUS) on day 7, 14 and 28.

*P-value < 0.05

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Table 4. Hematology variables (Lsmean with pooled SE) in 153 weaned beef calves according combined classification at three sampling timepoints.

Hematology parameter	Combined classification[1]	Time 1[2]	Time 2[3]	Time 3[4]	SE	Treatment[5] (Trt)	Time (T)	Trt × T
White blood cells ($\times 10^3$ cells/µL)	Healthy	10.4	9.7	9.1	0.34	0.0037	0.3366	0.4437
	sBRD	10.3	10.1	-	0.78			
	BRD-no-con	10.8	10.4	10.1	0.51			
	BRD-con	10.7	10.8	11.2	0.45			
Neutrophils (N) ($\times 10^3$ cells/µL)	Healthy	3.1	2.6^{x}	2.2^{x}	0.26	0.001	0.1136	0.0018
	sBRD	2.7	2.1^{x}	-	0.56			
	BRD-no-con	2.9	3.8^{xy}	2.6^{xy}	0.38			
	BRD-con	2.7^{a}	$4.1^{b,y}$	$3.8^{ab,y}$	0.33			
Lymphocytes (L) ($\times 10^3$ cells/µL)	Healthy	6.9	6.6	6.5	0.21	0.1952	0.0062	0.1241
	sBRD	7.1	7.4	7.6	0.48			
	BRD-no-con	7.6	6.3	7.0	0.32			
	BRD-con	7.6	6.3	6.9	0.28			
N:L	Healthy	0.46	0.40^{x}	0.36^{x}	0.05	0.0002	0.0448	0.0004
	sBRD	0.39	0.31^{x}	-	0.10			
	BRD-no-con	0.42	0.63^{xy}	0.41^{xy}	0.07			
	BRD-con	0.36^{a}	$0.69^{b,y}$	$0.62^{b,y}$	0.06			
Basophils ($\times 10^3$ cells/µL)	Healthy	0.12	0.12^{xy}	0.12	0.006	0.0334	0.0029	0.0196
	sBRD	0.13	0.14^{x}	0.15	0.013			
	BRD-no-con	0.12	0.09^{y}	0.13	0.009			
	BRD-con	0.12^{a}	$0.11^{a,x}$	0.14^{b}	0.00			

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			[y]		7			
Monocytes (×10^3 cells/µL)	Healthy	0.45	0.41	0.37	0.023	0.1376	0.1685	0.7377
	sBRD	0.44	0.49	-	0.053			
	BRD-no-con	0.46	0.46	0.40	0.035			
	BRD-con	0.48	0.44	0.46	0.031			
Eosinophils (×10^3 cells/µL)	Healthy	0.27	0.22	0.25	0.035	0.0935	0.3718	0.1847
	sBRD	0.21	0.42	0.33	0.082			
	BRD-no-con	0.24	0.16	0.33	0.055			
	BRD-con	0.17	0.17	0.21	0.048			
Red blood cells (×10^6 cells/µL)	Healthy	10.7	10.6	10.5	0.16	0.001	0.1131	0.7346
	sBRD	10.6	10.4	-	0.37			
	BRD-no-con	10.5	10.1	10.6	0.24			
	BRD-con	10.3	9.6	9.9	0.21			
Hemoglobin (g/dL)	Healthy	13.8	13.5	13.5	0.21	0.0072	0.0206	0.8926
	sBRD	13.6	13.3	-	0.44			
	BRD-no-con	13.6	12.9	13.2	0.30			
	BRD-con	13.5	12.6	12.9	0.27			
Hematocrit (%)	Healthy	36.2	35.3	34.9	0.55	0.0107	0.0058	0.8440
	sBRD	35.8	34.7	-	1.19			
	BRD-no-con	35.6	33.5	34.6	0.80			
	BRD-con	35.4	33.0	33.5	0.71			
Platelets	Healthy	503.3[x]	541.3	556.1	22.1	<.0001	0.01	0.02

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(×10^3 cells/µL)				[x]		32	81
	sBRD	692.8[y]	648.5	-	51.5		
	BRD-no-con	560.4[x y]	528.0	609.6[xy]	33.9		
	BRD-con	540.2[a ,xy]	539.6[a]	714.7[b,y]	29.5		

[1]Classification based on the combination of clinical respiratory score an thoracic ultrasonography score. Healthy (n=68), sBRD (n=14), BRD-no-con (n=38), BRD-con (n=30). A matched healthy control calf corresponding to each individual BRD case was selected retrospectively from the nearest pen to compare haematology variables at the same sample times.

[2]Seven days before BRD detection

[3]Day of BRD diagnosis

[4]Seven days of BRD detection

[5] Treatment (TRT) refers to classification groups (Healthy, sBRD, BRD-no-con, BRD-con).

N:L, neutrophil to lymphocyte ratio; SE, standard error.

[a,b,c] Within rows, Lsmeans differ from pre-BRD baseline by $P \leq 0.05$.

[x,y,z] Within columns, Lsmeans differ between treatments by $P \leq 0.05$.

Lsmeans with the same letter are not significantly different.

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Table 5. Least squares mean (SE) of average daily gain (kg) calculated in different periods.

Clinical status[1]	n	ADG1[2]	ADG2[3]	ADG-overall[4]	ADG-PRE[5]	ADG-POST[6]
Healthy	71	0.53[a] (0.07)	0.17 (0.04)	0.32 (0.03)	0.16[a] (0.10)	0.26[a] (0.04)
sBRD[7]	14	0.45[ab] (0.14)	0.09 (0.09)	0.23 (0.07)	-	-
BRD-no-con[8]	30	0.57[a] (0.10)	0.22 (0.07)	0.37 (0.05)	-0.26[b] (0.15)	0.48[b] (0.06)
BRD-con[9]	38	0.11[b] (0.10)	0.30 (0.06)	0.26 (0.05)	-0.44[b] (0.13)	0.44[b] (0.06)

[1]A matched healthy control calf corresponding to each individual BRD case was selected retrospectively from the nearest pen to compare ADG prior and after treatment at the same times.

[2]Average daily gain calculated from d 0 until d 28

[3]Average daily gain calculated from d 28 until d 65

[4]Average daily gain calculated from d 0 until d 65

[5]Average daily gain calculated from d 0 until the day of BRD diagnosis and treatment

[6]Average daily gain calculated from the day of BRD diagnosis and treatment to d 65

[7]Calves diagnosed as sBRD: CRS+TUS < 5 and lung consolidation ≥ 1cm^2

[8]Calves diagnosed with BRD (CRS+TUS ≥ 5) without lung consolidation

[9]Calves diagnosed with BRD (CRS+TUS ≥ 5) with lung consolidation ≥ 1cm^2

[a,b] Within columns, Lsmeans differ between treatments by $P \leq 0.05$.

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Table 6. Multivariable linear regression model for ADG1, ADG2 and ADG-overall[1] in 153 weaned beef calves according to their clinical respiratory score status[2] and thoracic ultrasonography status[3].

Variable	ADG1			ADG2			ADG-overall		
	Estimate ADG (kg/d)	SE	*P-value*	Estimate ADG (kg/d)	SE	*P-value*	Estimate ADG (kg/d)	SE	*P-value*
Intercept	0.54	0.055	<.0001	0.17	0.035	<.0001	0.32	0.027	<.0001
CRS status:									
-	Referent			Referent			Referent		
+	0.01	0.088	0.9029	0.03	0.057	0.6316	0.02	0.043	0.5745
TUS status:									
-	Referent			Referent			Referent		
+	-0.28	0.088	0.0016	0.05	0.057	0.4028	-0.09	0.043	0.0346

[1]Average daily gains calculated with individual bodyweights (kg) from d 0 to d 28 (ADG1), from day 28 to d 65 (ADG2), and from day 0 to d 65 (ADG-overall).

[2]Classification based on the clinical respiratory score developed by McGuirk and Peak, (2014). Calves with CRS ≥ 5 were CRS +.

[3]Classification based on the thoracic ultrasound evaluation. Animals with lung consolidation ≥ 1 cm^2 in at least one ultrasound evaluation were TUS +.

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Figure 1

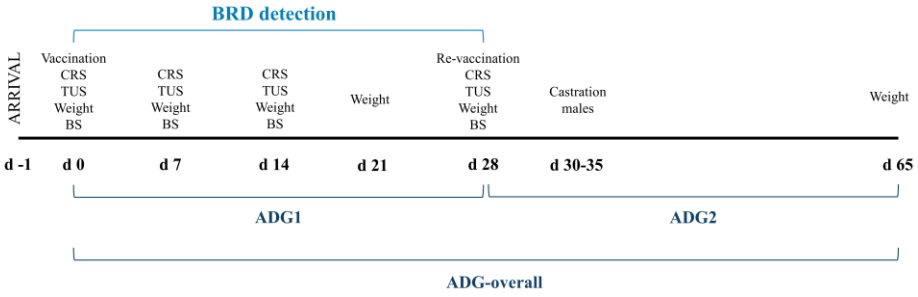

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Figure 2

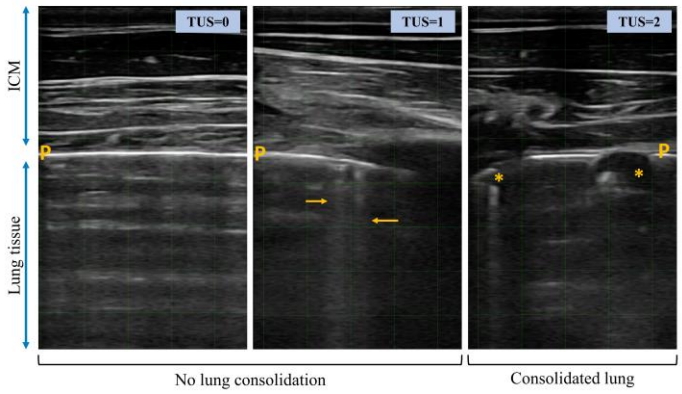

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Figure 3

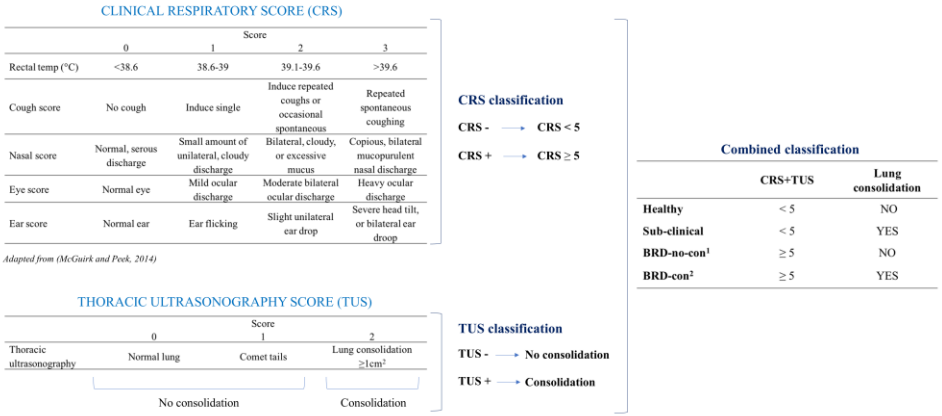

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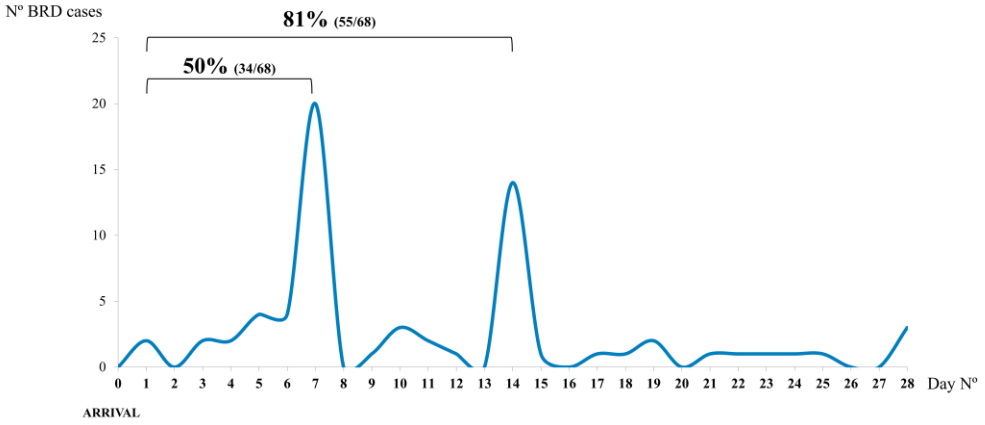
Figure 4



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