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(Leiter: Univ.-Prof. Dr.med.vet. Marc Drillich Dip. ECBHM Dip. ECAR)

Diagnosis of bovine respiratory tract disease in weaned calves by use of ultrasonography and measurement of arterial oxygen saturation by pulse oximetry and blood gas analysis

Diagnostik respiratorischer Erkrankungen beim Kalb durch Ultraschall und Messung der arteriellen Sauerstoffsättigung mit Pulsoximeter und Blutgasanalyse

Diplomarbeit

Veterinärmedizinische Universität Wien

vorgelegt von

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Widmung

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Content

1 Background

Bovine respiratory disease (BRD) is a multifactorial disease of great importance in cattle industry. An accurate diagnosis in life animals is still difficult. A delayed diagnosis may result in prolonged use of antibiotics, a high recurrence rate, and the development of chronic lung injury, pulmonary abscessation, ear infections, and endemic herd respiratory disease (MCGUIRK a. PEEK, 2014). Pneumonia is one of the most common reasons for disease-related death in calves and heifers. The consequences are financial losses due to diagnosis, treatment, and rearing and replacement costs (MCGUIRK, 2008). Apart from the immediate consequences, several studies show that BRD has a negative impact on future performance. In feedlot cattle decreased daily weight gains were associated with pulmonary lesions (WITTUM et al., 1996). Cows diagnosed with lung consolidation in early age produced significantly lower amounts of milk in the first lactation (DUNN et al., 2018), and there is evidence that heifers with lung lesions have a lower hazard of pregnancy (TEIXEIRA et al., 2017). Thus, rapid and early detection of diseased calves is crucial to avoid animal suffering and economic losses.

Usually, BRD diagnosis is based on clinical examinations. Producers identify sick calves by observing abnormal clinical signs and behaviour such as depression, loss of appetite, abnormal breathing, and fever (BUCZINSKI et al., 2014). McGUIRK and PEEK (2014) developed a standardised scoring system, which can be implemented on farms for regular screening for respiratory disease. This Wisconsin Score consists of four parameters: rectal temperature, cough, nasal discharge, ocular discharge, and ear position. This score can be adapted for the use in groups. In grouped animals categories scored are nasal discharge, ocular discharge, ear or head tilt, and coughing. Each parameter can be rated from 0 (normal) to 3 (severely abnormal). Calves with a total respiratory score ≥ 5 or that have two or more clinical parameters with score 2 or 3 are considered to have respiratory disease (MCGUIRK a. PEEK, 2014). These cases have to be further diagnosed. The most common further diagnosis is done by a clinical examination including measuring rectal temperature and thoracic auscultation. However, sensitivity for clinical signs and auscultation is low (LERUSTE et al., 2012; BUCZINSKI et al., 2014). Moreover it must be

kept in mind, that auscultation is highly dependent on the operator as demonstrated by a poor interrater reliability (PARDON et al., 2019).

Ultrasonography is a tool that could increase sensitivity and specificity in the diagnosis of BRD and lead to better disease classification. Lung lesions diagnosed by ultrasonography are highly correlated with post mortem findings (REINHOLD et al., 2002; JUNG a. BOSTEDT, 2004; OLLIVETT et al., 2015). Consequently, different authors examined fast on-farm ultrasonography techniques with a rectal probe performed without prior clipping of the hair (BUCZINSKI et al., 2013; OLLIVETT a. BUCZINSKI, 2016; CREMER et al., 2018). These methods are feasible and applicable on farm and have a high inter- and intraobserver agreement.

A relatively new approach in our study is the use of pulse oximetry as a tool for detection of BRD. Pulse oximetry is a non-invasive, easy-to-use method to measure and monitor the oxygen saturation in blood and the pulse rate. Technically it is based on illuminating the skin and measuring the changes between oxygenated and deoxygenated haemoglobin. The technique is widely used in critical care medicine and in anaestesiology in human medicine, as well as in small animals and horses (CHAFFIN et al., 1996; MATTHEWS et al., 2003; JUBRAN, 2015). So far, few studies using pulse oximetry were performed in cattle. Mainly evaluating the use in newborn calves (COGHE et al., 1999; BIRGIT SEELBACH-GÖBEL et al., 1999; UYSTEPRUYST et al., 2000; KANZ et al., 2020), but also in BRD (BARUCH et al., 2019).

In contrast to pulse oximetry, blood gas analysis is an invasive method to measure oxygen saturation in animals. Blood gas values provide information about the lung function, hence can aid in estimation of the severity of the disease and in determining prognosis. In calves, arterial blood can be drawn from the caudal auricular artery (MUYLLE et al., 1996; SILVA et al., 2017).

The objective of this study was to compare a fast screening and a clinical score with thoracic ultrasonography, pulse oximetry, and blood gas analysis for diagnosis of BRD. Moreover, the study aimed to evaluate the practicability of these methods for on-farm evaluation of postweaned calves. The hypotheses of the study were that the presented methods are well feasible and reproducible in a farm setting. Moreover, we hypothesised that

further methods such as pulse oximetry and arterial blood gas analysis correlate with ultrasonography and the clinical scores and provide information on the severeness of the disease.

2 Materials and Methods

2.1 Population of calves

The study procedures were approved by the national Slovakian authorities and noted by the Institutional Ethics and Animal Welfare Committee of the University of Veterinary Medicine, Vienna. The study was conducted in December 2019 on a commercial Slovakian dairy farm in four to five month old Holstein Friesian heifer calves. The calves were born and raised on the farm. During the milk feeding period they were kept in single calf hutches and after weaning in group boxes. Each Monday, calves were moved from the group boxes to a separate stable for young stock and housed in groups of approximately 30 calves. We took samples from calves in these barns. Five by five heifers were selected by chance and restrained in headlocks.

2.2 Clinical score and clinical examination:

Calves were clinically scored based on a scoring system from the University of Wisconsin (MCGUIRK a. PEEK, 2014). The parameters included cough score (CS), nasal discharge (ND), ocular discharge (OD), and head and ear carriage (EHD). Afterwards we measured rectal temperature (T), counted the respiratory rate (RR) in a minute, and assessed the presence of dyspnea (DYS). Furthermore, we performed a bilateral thoracic auscultation for the presence of abnormal lung sounds (AUS). Abnormal lung sounds were characterised as F sound, strong F sound, and crackles. The examination was done according to the guidelines of clinical propaedeutics in Vienna (BAUMGARTNER a. WITTEK, 2018) The scoring categories are presented in Table 1. The scores of CS, ND, OD, and EHD were summarized to one score (McGuirk Score; MCG). Calves with a MCG ≥ 5 were considered as 'at risk' for BRD. To summarise all clinical findings, we generated a general clinical score (GCS), including MCG ('at risk' = 1, 'not at risk' = 0), T, RR, DYS, and AUS. Therefore, the variables T, RR, DYS, and AUS were also categorised in a binary variable ('healthy' = 0, 'diseased' = 1). Calves with a GCS > 3 were classified as diseased.

Tab. 1. Description of the scoring systems used in the study (scoring system of the University of Wisconsin (MCG; McGUIRK a. PEEK, 2014) and general clinical score (GCS)). Cough score (CS), nasal discharge (ND), ear and head carriage (EHD), and ocular discharge (OD) were summarised in the McGuirk Score (MCG). If the MCG was ≥ 5 the calf was classified as diseased (S). A classification for temperature (T), respiratory rate (RR), dyspnea (DYS), and auscultation (AUS) was applied.

CS	ND	EHD	OD	T	RR	DYS	AUS
0) no	0) normal	0) normal	0) normal	°C	Frequency/min	0) normal	0) normal
1) induced	1) cloudy	1) flick	1) small			1) moderate	1) F sound
2) spontan-eous	2) mucosal	2) unilateral	2) moderate			2) severe	2) strong F sound
a) one	3) muco-purulent	3) bilateral	3) heavy				3) crackels
b) repeated							
c) multiple							
		MCG					
H		< 5		≤ 39.5	< 30	0	0, 1
S		≥ 5		> 39.5	> 30	1, 2	2, 3

2.3 Ultrasonography

Bilateral thoracic ultrasonography was performed with a 7.5 MHz linear probe (Easi-Scan GO, IMV Imaging, USA), set at a depth of 7 cm. The operator wore a wireless head-mounted viewing device (BUG:GO, IMV Imaging, USA). The ultrasound scanner was connected via wifi to an app (IMV Go Scan App, IMV Imaging, USA) installed on an Android tablet (Samsung Galaxy Tab A, Samsung, South Corea). The operator was a trained and supervised veterinary student.

Ultrasonographic examinations were performed as described by OLLIVETT et al. (2016). First, 70 % isopropyl alcohol was flushed on the area of interest and the probe was directly applied to the surface without prior clipping. The operator stood at one side of the calf reaching with the hand over the dorsum to the opposite side. After positioning the probe parallel to the ribs within each intercostal space (ICS), it was moved ventrally until specific

ultrasonographic landmarks were identified. In this way, the probe was moved in cranial direction to examine each ICS up to the right 1st or left 2nd ICS. The cranial aspect of the left and right lung was examined with the transducer between the forelimb and the cranial ventral thoracic body wall. When a hyperechoic line with reverberation artefact was present, the peripheral lung tissue was considered normal. A vertical hyperechoic line emanating from the pleural surface indicated pleural roughening or comet-tailing artefacts. Lung lesions (also referred to as consolidated lung or nonaerated lung) were characterised by their hypoechoic appearance and the lack of both the bright white band at the pleural interface and reverberation artefact. The lesions were documented according to their location within the ICS and their size as measured by the ventral-dorsal distance on the ultrasonography screen. Findings were classified according to a scoring system (OLLIVETT a. BUCZINSKI, 2016).

2.4 Pulse Oximetry

A portable pulse oximetry device was used (Radius-7, Masimo, Switzerland). We measured the oxygen saturation (SO_2) on two different sites with different clips: a reusable animal transflectance sensor (M-LNCS TF-I AH, Masimo, Switzerland) was placed with the flat hand on the ventral side of the tail root. A reusable animal multisite sensor (M-LNCS YI AH, Masimo, Switzerland) was clipped on the ventral aspect of the labia with the sensor aligned to the mucous membrane. Duration of measurement was between 30 and 60 seconds. The values were taken, as soon as an uniformly, stable waveform appeared. Factors that could have an impact on the feasibility like movement of the calf or pigmentation of the vulva or skin were noted.

2.5 Blood gas analysis

Arterial blood samples were drawn from the intermediate branch of the caudal auricular artery. Depending on accessibility we used either the right or the left ear. The hair on the dorsal surface of the pinna was removed with an electric clipper and disinfected with alcohol. The artery was made visible by compressing the top of the ear with one hand, while the other hand handled the syringe. Samples were taken with a 23 G (0.64 x 25 mm) needle and collected into a 1 ml heparinized arterial blood collection syringe. For blood gas analysis we used a portable handheld analyser (VetScan® i-STAT®1, Abaxis Inc., USA) with disposable, single-use cartridges (i-STAT CG4+, Abbot, USA). The blood was transported within a

minute to the device, that was placed in an air-conditioned side room. The following parameters were measured: pH, partial pressure of CO_2 (PCO_2), partial pressure of oxygen (PaO_2), bicarbonate (HCO_3), and lactate (LAC). Base excess (BE) and oxygen saturation (SaO_2) were calculated by the device.

2.6 Statistical analysis

Sample sizes was calculated using G*Power (effect size = 0.6, α = 0.05, power = 0.95; version 3.1.9.2, University of Kiel, Germany). Further statistical evaluations were performed using SPSS Statistics for Windows (version 23.0; IBM Deutschland GmbH, Ehningen, Germany) and Microsoft Excel for Microsoft 365 MSO (version 16.0.13127.21624, Microsoft Corporation, Redmond, WA). The level of significance was set at $P < 0.05$ for all statistical tests. The descriptive statistics were summarised as median and 25 % and 75 % interquartile ranges (IQR) for the clinical parameters and blood gas values. Statistical frequencies were illustrated for clinical parameters, MCG, and US. To compare the clinical findings with ultrasound findings and the measurements of the pulse oximeter as well as with the results from the blood gas analyser Spearman's rho (ρ) correlation coefficients were calculated for all the data. To visualise associations clinical scores as well as MCG were plotted against each other and against blood gas values. Individual categories of clinical scores in relation to T were visualised as box-and-whisker plots showing the median and the interquartile (midspread) range (boxes containing 50 % of all values). The whiskers (representing the 25 and 75 percentiles) and the extreme data points.

3 Results

Overall, 60 heifer calves were recruited for this study and all of them could be included in statistical analysis. Clinical examination revealed that 43 calves (28.3 %) had a rectal temperature above 39.5°C; the median temperature was 39.1°C (IQR 38.8;39.5). The cough score was elevated (> 0) in 35 animals (58.3 %). Furthermore, 39 animals (65.0 %) had an abnormal nasal discharge (Score 1 to 3) and 49 (76.7 %) had abnormal ocular discharge (Score 1 to 3). Abnormal ear and head carriage (Score 1 to 3) was recognized in 22 calves (36.7 %). Dyspnea was present in 33 animals (moderate n = 29, 48.3 % and severe n = 4, 6.7 %). Overall, 57 (95.0 %) animals had a respiratory rate higher than 30. Lung auscultation revealed a normal sound or F-sound in 44 (73.3 %), a strong F-sound in 15 (25.0 %), and crackles in one animal (1.7 %).

Results of the McGuirk Score are summarised in Fig. 1. If we categorised the variable in 'not at risk' (MCG < 5) and 'at risk' (MCG ≥ 5), 38 (63.3 %) animals would not be at risk and 22 (36.7 %) would be at risk. Of these 22 animals at risk, 18 were classified as diseased using the GCS. Another nine diseased animals (15 %) by GCS were not recognised by the MCG.

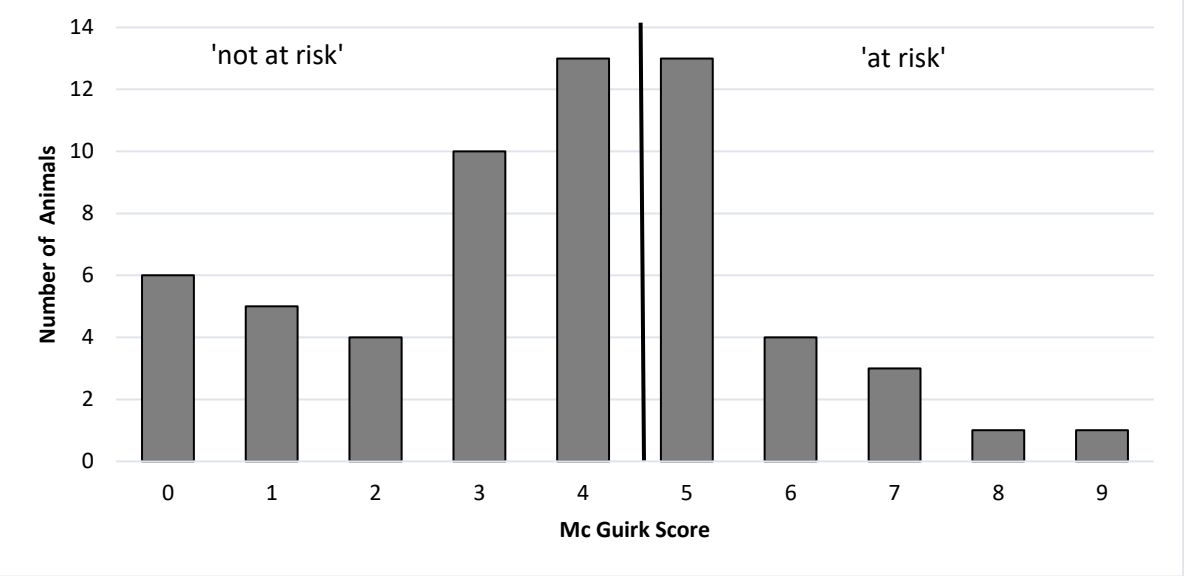

Fig. 1. Distribution (number of animals) among the nine McGuirk Score categories. These categories were summarised in a binary variable with a score < 5 'not at risk' and ≥ 5 'at risk'.

McGuirk Scores correlated significantly with T ($r = 0.57$; $P < 0.001$). With higher MCG, T increased (Fig. 2). This was also recognised for each of the individual parameters, that are included in MCG (CS, ND, EHD; Fig. 3). Furthermore, RR, DYS, and AUS correlated significantly with an increased T. Calves with dyspnea had higher CS, OD, MCG, and AUS scores.

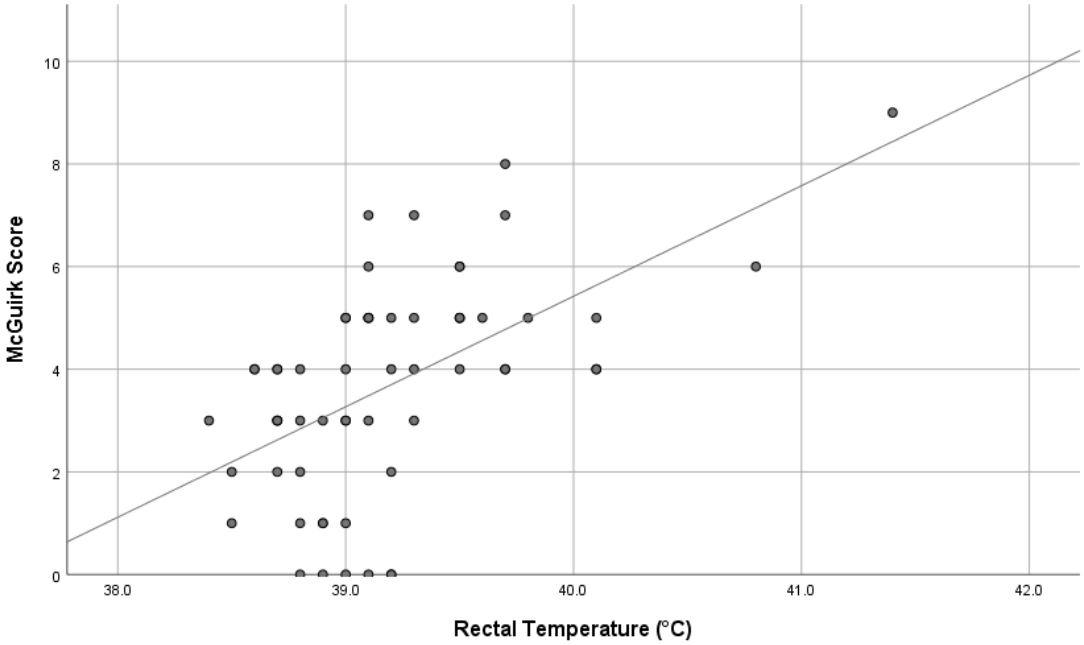

Fig. 2. Relation between McGuirk Score and rectal temperature. McGuirk Score was plotted in relation to the rectal temperature (trend line $R^2 = 0.333$). Higher rectal temperatures were significantly related to higher McGuirk scores ($P < 0.001$).

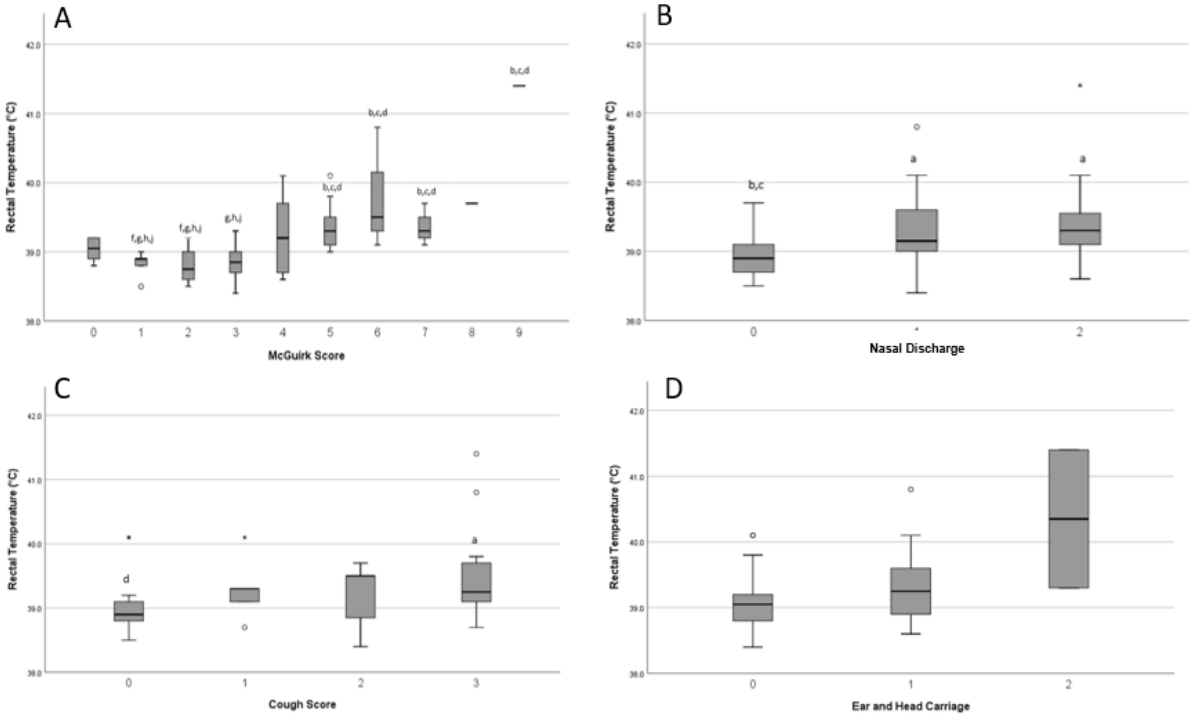

Fig. 3. Boxplots of McGuirk Score (A), nasal discharge (B), cough score (C), ear and head carriage (D) in relation to rectal temperature. Data are visualised as box-and-whisker plots showing the median and the interquartile (midspread) range (boxes containing 50% of all values). The whiskers (representing the 25 and 75 percentiles) and the extreme data points. Letters above boxes indicate significant differences between the categories.

Thoracic ultrasonographic scores are summarised in Tab. 2. Overall, 63.3 % (n = 38) of the calves had a score of 0 to 2, 28.3 % (n = 17) a score of 3, 10.0 % (n = 6) a score of 4, and the remaining 5.0 % (n = 3) were classified with the highest score (5). Ultrasound scores did not correlate with clinical findings, blood gas parameters, or pulse oximetry results.

Tab. 2. Results of thoracic ultrasonographic examinations. Findings were categorised in six scores (0 to 5) according to OLLIVETT a.BUCZINSKI (2016).

Score	Number of animals	Percentage of animals
0	6	10.0
1	15	25.0
2	17	28.3
3	13	21.7
4	6	10.0
5	3	5.0
Total	60	100.0

The median SO_2 measured by pulse oximetry at the tail was 100 (IQR 97;100) and the median SO_2 measured in the vulva was 95 (IQR 94;97). Pulse oximetry (SO_2-results) did not correlate between the two locations tail and vulva (r = -0.044, P = 0.741). Overall, in 14 of the 60 animals (23.3 %) we had problems measuring SO_2 at the tail. Mainly (7/60; 11.7 %) due to excessive moving, in 4 animals the pulsatile signal showed an erratic waveform (6.7 %), and in three animals (5.0 %) the device indicated a low perfusion. At the vulva the signal was poor in two animals (3.3 %), because one animal had a pigmented vulva and one animal had a low perfusion. In all other animals no problems were recorded. No significant correlations could be revealed between SO_2-results from the tail and vulva, and the clinical parameters, blood gas, and ultrasound results.

Significant negative correlations were found between PaO_2 and MCG (r = -0.271; P = 0.037). The PaO_2 correlated with T (r = -0.307; P = 0.017; Fig. 4) and DYS (r = -0.403; P = 0.001). The PaO_2 decreased with increasing T, DYS, and MCG. When individual dyspnea scores were plotted against PaO_2, significant differences between score two and three could be revealed. Negative correlations were also noted between DYS and SaO_2 (r = -0.324, P = 0.012). The PCO_2 levels correlated positively with EHD (r = 0.263; P = 0.042) and negatively with T (r = -0.265; P = 0.041; Fig. 5).

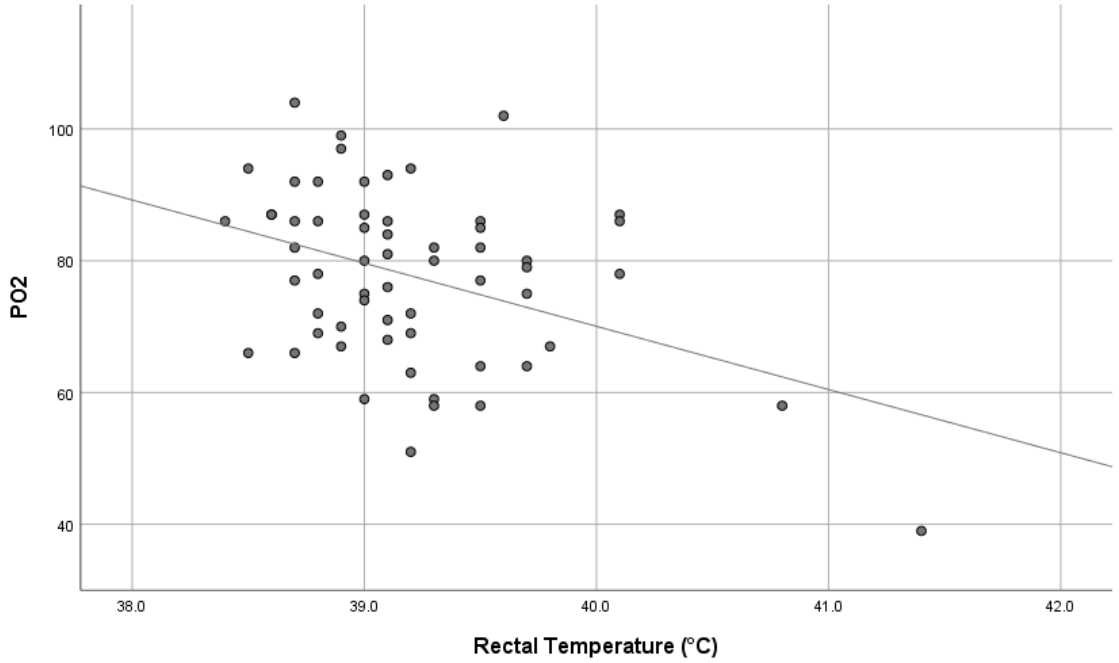

Fig. 4. Relation between rectal temperature and partial pressure of O_2 in blood gas (PaO_2). Rectal temperature was plotted in relation to partial pressure of O_2 in blood gas (PaO_2) and a trend line was set (R^2 = 0.157, P = 0.017).

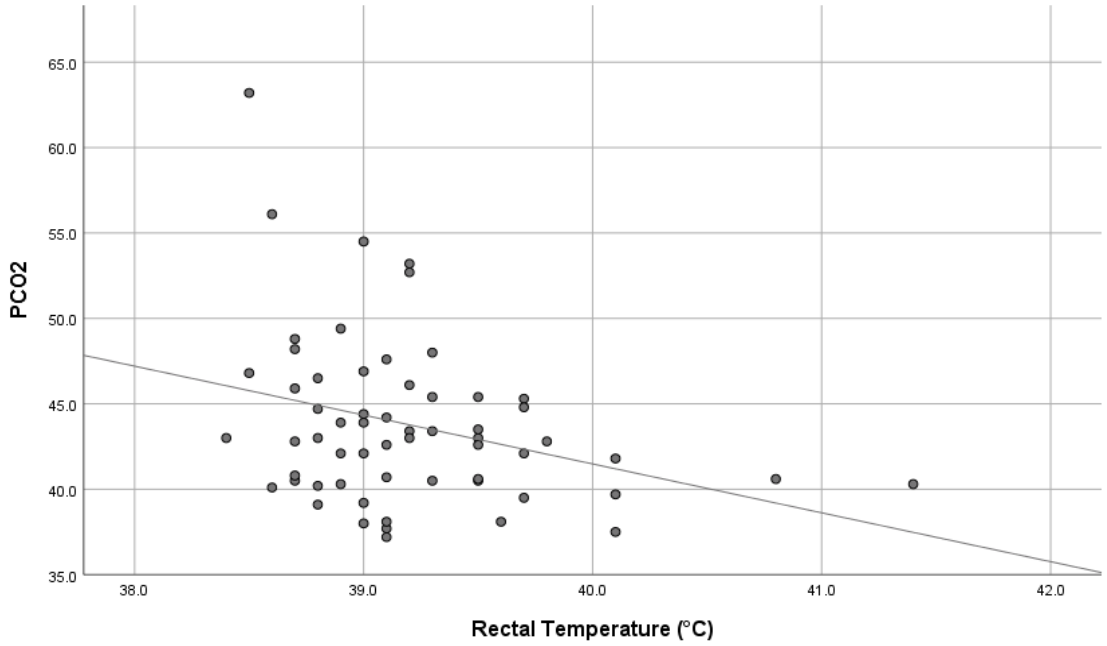

Fig. 5. Relation between rectal temperature and partial pressure of CO_2 in blood gas (PCO_2). Rectal temperature was plotted in relation to blood gas PCO_2 and a trend line was set (R^2 = 0.101, P = 0.041).

Correlations were also examined for the GCS. The GCS correlated with PaO_2 (r = 0.373, P = 0.003), BE (r = 0.273, P = 0.035), and HCO_3 (r = 0.298, P = 0.021). Significant differences in PCO_2, PaO_2, HCO_3, and BE could be revealed between calves categorised as diseased and healthy according to the GCS (Tab. 3).

Tab. 3. Differences in blood gas parameters between healthy and diseased calves (classified by the general clinical score). Medians (with 25% und 75% interquartile range; IQR), and P-values are presented.

Parameter	Healthy	Diseased	P-value
	Median (IQR)		
pH	7.49 (7.46;7.51)	7.49 (7.46;7.52)	0.619
PCO_2	43.9 (41.5;47.8)	41.8 (39.5;44.2)	0.008
PaO_2	82.0 (70.5-89.5)	77 (64.0-84.0)	0.046
HCO_3	34.0 (32.5;36.3)	32.1 (30.7;33.7)	0.004
BE	10.0 (9.0;13.0)	9.0 (7.0;11.0)	0.007

4 Discussion

This study aimed to evaluate the accuracy and practicability of different screening methods to detect or confirm the presence of BRD in calves under farm conditions. Our study population have been four to five months old calves, representing an age group which is greatly affected by BRD (KLEIN-JÖBSTL et al., 2015; DUBROVSKY et al., 2019). The common method to detect BRD is the examination for clinical signs. Different scoring systems have been implemented as standardised observation schemes for respiratory disease. In the current study we used a respiratory scoring system from the University of Wisconsin (MCGUIRK a. PEEK, 2014). We applied the modified version of the scheme for the use in group pens. To our knowledge, data are available on accuracy of the individual score, but not for the group pen score (OLLIVETT et al., 2011; LOVE et al., 2014; BUCZINSKI et al., 2014; BUCZINSKI, FECTEAU et al., 2018). General weaknesses of the score are poor interobserver agreements especially in unexperienced observers (BUCZINSKI et al., 2016), that the linear four-point score leads to the assumption that each 1-unit increase has the same effect on disease risk, and the absence of specific weighting between clinical signs indicating that each point means the same alteration in severity (MAIER et al., 2019).

Overall, 36.7 % of the examined calves have been classified as 'at risk' using MCG. The GCS (taking further parameters like rectal temperature, respiratory rate, dyspnea, and lung auscultation into account) classified 45.0 % of the calves as diseased. Of all the calves positive in GCS, 15.0 % were not noticed by the MCG. It must be considered that GCS included parameters (like rectal temperature, respiratory rate), that can also be altered due to other reasons (e.g. other infections or heat stress). One difference between MCG and GCS is the inclusion of rectal temperature in the latter. Although MCG and rectal temperature correlated well, approximately 10 % of animals with elevated rectal temperature (> 39.5°C) would not have been further examined by using MCG. Nevertheless, an easy and fast method to control animals in groups daily on farm without restraining is needed. Measuring rectal temperature requires animal restraining and handling and is thus time consuming. Adding rectal temperature, however as a confirmatory step for active infection seems reasonable (LERUSTE et al., 2012; GRISSETT et al., 2015). As in the present study animals could not be examined post mortem, we do not have a confirmation, if animals with an

elevated rectal temperature really suffered from respiratory tract disease or possibly any other disease, although we controlled this clinically.

In our study, significant associations were found between MCG and RR. This is interesting, as the value of RR in diagnosis of BRD is controversial. In a scoping review on clinical signs of BRD in cattle increased RRs were only reported in less than half of the included studies (FERRARO et al., 2021). The increased RR is not specific for BRD and can also be observed in association with heat stress (GALÁN et al., 2018) and other nonspecific stressors such as pain and exercise. Difficult or labored breathing (dyspnea), however, is often correlated with severe disease (MAIER et al., 2019).This resembles the findings in our study, where elevated dyspnea scores positively correlated with T, CS, OD, MCG, and AUS. Presence of dyspnea as a sign for severe disease was also reflected in decreased PaO_2 values. Furthermore, lung auscultation was performed as a part of the clinical examination. We detected significant associations between abnormal lung sounds and increased T and elevated DYS scores, but could not find associations between AUS and US. This may be because of the poor ability of AUS to detect lung consolidation as reported by BUCZINSKI et al. (2014) and PARDON et al. (2019). It has to be taken into account that lung auscultation was performed as a single confirmation test in these studies. As a part of a complete clinical examination diagnostic accuracy of AUS is likely higher (PARDON et al., 2019).

Ultrasonography was performed by a relatively unexperienced operator. This condition can be disregarded, given the fact, that interobserver agreement even for novice operators is high (BUCZINSKI et al., 2013; CREMER et al., 2018; BUCZINSKI, BUATHIER et al., 2018), The technique used, was described by OLLIVETT a. BUCZINSKI (2016). We would confirm the practicability of this technique for on-farm use. If enough alcohol was applied, ultrasound probe connection worked well without shaving. It might only be problematic with winter coat, when more alcohol will be needed to establish a good connection and wet hair might lead to hypothermia. The approach is described as suitable for scanning a group of calves with the goal to examine the most affected parts of the lung (cranial aspect of the right cranial lung lobe, the right middle lung lobe, and the caudal aspect of the left cranial lung lobe). Extensive documentation and subsequent work was required to analyse the ultrasonography lesions and categorise them in the six point score. CRAMER and OLLIVETT (2019) tried to simplify this ultrasonographic scoring system and came to the result, that identifying the presence or absence of lung consolidation without differentiation of the extent of changes.is sufficient for

detection of diseased lungs. Moreover they defined a cutpoint for estimating the risk for poor future performance (CRAMER a. OLLIVETT, 2019).

Clinical findings did not correlate with ultrasonographic findings in our study. The number of examined animals in our study was with 60 low and only few were classified with high ultrasound scores. It is possible that the majority of calves in our study were mainly affected by mild disease caused by viral agents. Regular screening of nasal swabs in weaned calves on farm for viruses and bacteria were positive for Bovine Corona Virus (20 %), *Pasteurella multocida* (6 %), and *Mannheimia haemolytica* (6 %). The number of animals with increased temperature, which can be a sign of the early stage of BRD would support this hypothesis. Furthermore, it must be considered that ultrasonographic findings and clinical signs reflect different manifestations and causes of BRD. It has been proven that the value of clinical examination is limited since it fails to detect calves with subclinical-BRD (LERUSTE et al., 2012; BUCZINSKI et al., 2014; OLLIVETT et al., 2015; CRAMER a. OLLIVETT, 2019; CUEVAS-GÓMEZ et al., 2021). Subclinical lung lesions however have a negative impact on preweaning growth (CUEVAS-GÓMEZ et al., 2021). Given the long-term effects of lung consolidation (e.g. reduced growth, impaired fertility) it is crucial to identify calves with subclinical-BRD (WITTUM et al., 1996; TEIXEIRA et al., 2017; DUNN et al., 2018; CUEVAS-GÓMEZ et al., 2021). Implication of a combination of ultrasonography and MCG, rather than using each test as a single screening test may therefore prove useful for more accurate detection of BRD and distinction in BRD subtypes to identify sick calves that are at risk for poor performance (OLLIVETT a. BUCZINSKI, 2016; DUNN et al., 2018; CRAMER a. OLLIVETT, 2019; BERMAN et al., 2019; CUEVAS-GÓMEZ et al., 2021). Further studies are necessary to elucidate this.

Pulse oximeter measures the oxygen saturation in the blood and the heart rate. Different sites for application of the sensor are reported in ruminants: the tail, the nasal septum, the genital mucosa of females, and the interdigital space in calves during birth (COGHE et al., 1999; UYSTEPRUYST et al., 2000; KANZ et al., 2018; KANZ et al., 2020). When compared to the oxygen saturation measured with a blood gas analyser in the study of COGHE et al. (1999), the accordance was best when the probe was attached to the tail. In the preparation phase of the present study we tried to apply the described sites in weaned calves. Measurements in the interdigital space were not feasible in our study population, because the calves were standing and reacted defensive when touching their legs. Furthermore, we

were not able to fixate a probe to the nasal septum due to excessive head movement. Therefore, we decided to use the lip of the vulva and the ventral site of the proximal part of the tail. General limitations of pulse oximetry are well described. Low perfusion state, pigmentation, vasoconstriction, hypothermia as well as improper probe placement are important causes of insufficient signal strength. Motion or ambient light interference can lead to signal artefacts (UYSTEPRUYST et al., 2000). Insufficient signal or artefacts were either notified by the device sending an alarm signal or were recognised by observing an erratic wave form. In our sample the vulva turned out to be easy-accessible and well tolerated once the clip was attached. Even though Holstein calves are partly black coloured, pigmentation did not affect the measurements with one exception. In contrast, tail measurements were difficult to perform. We noted a high occurrence of motion artefacts due to defensive reactions. The ventral site of the tail was often pigmented or contaminated with faeces, resulting in poor signal quality. These experiences are not consistent with findings of other studies. One explanation might be the fact that we placed a transflectance sensor with the flat hand under the tail. Whereas in another study a clip was placed on a washed and shaved tail and covered with rubber (UYSTEPRUYST et al., 2000). The erratic results obtained from tail measurements might be an explanation for lack of correlation between the SO_2 results obtained from the tail and vulva. Another explanation can be the fact, that measurements did not take place at the exact same point of time. Different underlying technical principles of transmission sensor and transreflectance sensor also have an impact on response time and precision (JUBRAN, 2015). Pulse oximetry did not correlate with clinical or ultrasound scores. In our sample few animals were classified with high ultrasound scores, thus few had great amounts of consolidated lung tissue. This might be a reason for lacking associations between clinic and US and pulse oximetry. In a challenge study BARUCH et al. (2019) did not observe significant changes in the mean levels of oxygen saturation during the viral phase, where lung lesions were not present. But in a later stage (bacterial phase) as the amount of consolidated lung increased, levels of oxygen saturation decreased (BARUCH et al., 2019).

In contrast to pulse oximetry, arterial blood gas analysis is an invasive, difficult to perform, and expensive technique which can only be used by skilled persons, usually not under field conditions. These conditions would disapprove blood gas analysis as a screening method for calves. However, it provides objective and immediate information about the oxygenation of haemoglobin on the metabolic and respiratory acid-base problems and

serves as a tool to estimate the severity of lung tissue damage (PROULX, 1999). Thus, we used blood gas analysis as reference method for pulse oximetry and to compare with clinical findings and US. In field settings we were confronted with complications in performing blood gas analysis. We experienced extreme defensive movements when restraining the heifer calves and taking blood samples. Another difficulty was to maintain the temperature optimum required by the device, as temperatures were low in the stables. Interestingly, blood gas and pulse oximetry results did not correlate in our study. This is surprising, as other authors report good correlations between the two methods. They resume, that there is a small bias between oxygen saturation in pulse oximeter and in blood gas. Also, they postulate that pulse oximetry is a relatively accurate method, even though it tends to underestimate the higher values and overestimate the lower values. (COGHE et al., 1999; UYSTEPRUYST et al., 2000). In a study in calves during birth the same devices were evaluated for their accuracy and revealed a correlation coefficient of 93.8 %. But the author stressed that the results are only valid for calm calves that are lying down (KANZ et al., 2018). Another factor might also explain the different results in the present study. The two measurement methods were not carried out simultaneously, but blood samples were drawn after a stressful situation. Further investigations under clinical conditions would be required to evaluate pulse oximetry for the use in calves in the field.

Clinical signs were also set in relation to arterial blood gas analysis. The results showed marked relationships between the clinical picture and $PaCO_2$ and PaO_2 levels. PaO_2 levels decreased with elevated temperatures and MCG. Hypoxemia can be caused by disturbances in ventilation, pulmonary diffusion, pulmonary haemodynamics, or ventilation-perfusion mismatching in the lungs of diseased animals (REINHOLD, 1997).

Interestingly, we recorded decreasing values of $PaCO_2$ in calves with elevated temperatures. Usually, lower $PaCO_2$ values in calves with respiratory disease are expected due to higher respiratory rates and hyperventilation (COLLIE, 1992). HELENA et al. (2015) reported increasing values of $PaCO_2$ only in calves with severe clinical symptoms of BRD. Thus, an explanation might be that calves in our study population were not severely enough affected to cause an increase in $PaCO_2$. Another interesting aspect is the finding that enhanced respiration rate and panting lead to greater loss of CO_2 via pulmonary ventilation (WANG et al., 2020). Probably high RR in the majority of the examined calves in our study had the same effect, thus leading to lower $PaCO_2$ values.

In conclusion, establishing a fast and accurate screening test for BRD in calves remains challenging. This study evaluated conventional and newer methods. Assessment for BRD based on clinical signs is a good tool, but detection rate depends on the parameters included in the examination scheme. Ultrasonography provides further information regarding the type of BRD and aids in determining a prognosis. Implication of a combination of ultrasonography and clinical scores seems to be promising. Pulse oximetry turned out to be well feasible in the vulva in postweaned calves. But further studies under clinical settings are required to investigate the accuracy and benefit for diagnosis of BRD in the field.

5 Abstract

5.1 Background

Bovine respiratory disease (BRD) is one of the most common and most important diseases in pre- and postweaned calves. It has both, short- and long-term impact on the calves health and future performance and consequently, negative economic effects. Thus, rapid and accurate screening methods are required for implementation of health programmes on farm. This study aimed to compare different screening methods and to evaluate their practicability. The methods evaluated were a clinical score modified for the use in group pens from the University of Wisconsin (MCGUIRK a. PEEK, 2014), a fast thoracic ultrasonography (OLLIVETT a. BUCZINSKI, 2016), and pulse oximetry.

5.2 Materials and Methods

The study was conducted on a commercial Slovakian dairy farm in a group of sixty heifer calves aged four to five months. The calves were examined according to a standardised scoring scheme adapted for group pens (MCG, MCGUIRK a. PEEK, 2014). The scheme included the parameters coughing, nasal discharge, ocular discharge, and ear and head carriage. Additionally, further clinical parameters were assessed and summarised to a general clinical score (GCS): rectal temperature (T), respiratory rate (RR), dyspnea (DYS), and presence of abnormal lung sounds (AUS). Fast thoracic ultrasonography was performed according to a recently described protocol for on-farm use (OLLIVETT a. BUCZINSKI, 2016). The findings were categorised in a six-point score. Oxygen saturation of haemoglobin was measured with two different pulse oximetry sensors at the tail and in the vulva. As a reference method for pulse oximetry arterial blood gas samples were analysed with a portable blood gas analyser. For all results Spearman correlations with a level of significance of < 0.05 were calculated.

5.3 Results

Of the 22 animals categorised as 'at risk' using MCG, 18 were classified as diseased by the GCS. Another nine diseased animals detected by GCS were not recognised by the MCG. Significant correlations were noted between MCG and T (r = 0.579) and between DYS (r = 0.435) and AUS (r = 0.360). Increased T was linked to DYS (r = 0.349), RR (r = 0.422), and AUS (r = 0.456). Rectal temperature was also associated with blood gas results. Increasing T correlated with decreasing partial pressure of oxygen (PaO_2; r = -0.307) and partial pressure of CO_2 (r = -0.265), both measured in arterial blood. Similar alterations were found between MCG and PaO_2 (r = -0.271). No significant correlations could be seen between pulse oximetry in the vulva and at the tail (r = -0.044, P = 0.741) and between pulse oximetry and blood gas analysis. A lower rate of errors and better acceptance by the calves were observed when pulse oximetry was performed in the vulva compared to the tail. Ultrasonographic findings did not correlate with other results.

5.4 Conclusion

This study confirms that scoring systems based on clinical examination are potentially good tools to assess the presence of BRD. However, depending on the parameters included the detection rate varies. Ultrasonography provides further information and could be implemented in a combination with clinical scores. Pulse oximetry is well feasible in the vulva in postweaned heifer calves but needs to be further investigated for accuracy and diagnostic validation.

5.5 Keywords

Bovine respiratory disease, thoracic ultrasonography, pulse oximetry, calves, respiratory score, screening methods

6 Deutsche Zusammenfassung

Enzootische Bronchopneumonie (EB) ist eine der bedeutendsten und häufigsten Erkrankungen unter Kälbern und jungen Rindern. Die Erkrankung hat sowohl kurz- als auch langfristige Auswirkungen auf die Kälbergesundheit und die Leistungsfähigkeit und ist von großer ökonomischer Relevanz. Daher sollten schnelle und akkurate Screening Methoden für den Einsatz am Betrieb zur Verfügung stehen, die eine einfache Diagnosestellung der EB ermöglichen. Diese Studie hatte zum Ziel verschiedene Screening Methoden für den Einsatz am Betrieb zu evaluieren, zu vergleichen und auf ihre Praktikabilität zu prüfen. Ein klinischer Score der Universität von Wisconsin, modifiziert für den Einsatz in Gruppen, eine schnelle Ultraschalltechnik und erstmals auch die Messung der Sauerstoffsättigung mittels Pulsoximeter im Vergleich zur arteriellen Blutgasanalyse.

Die Studie wurde auf einem konventionellen slowakischen Milchviehbetrieb an 60 vier bis fünf Monate alten Kuhkälbern durchgeführt. Die Kälber wurden anhand eines an Gruppenbuchten angepassten, standardisierten Scoring Schemas beurteilt. In dem Schema waren die Parameter Husten, Augenausfluss, Nasenausfluss und Kopf- und Ohrenhaltung enthalten. Zudem wurde eine klinische Untersuchung mit Messung der inneren Körpertemperatur (T), Beurteilung der Atemfrequenz (RR) und des Atemtypus (DYS) sowie einer Lungenauskultation zur Erfassung pathologischer Lungengeräusche (AUS) durchgeführt. Diese weiteren Parameter wurden unter einem generellen klinischen Score (GCS) zusammengefasst. Danach wurde die Lunge der Kälber mittels einer schnellen Ultraschalltechnik auf das Vorhandensein von Läsion untersucht. Die Läsionen wurden anhand eines sechs Punkte Scores nach ihrem Schweregrad eingestuft. Die Sauerstoffsättigung wurde mit zwei unterschiedlichen Pulsoximeter Sensoren an der Ventralseite des Schwanzes und der Vulva gemessen. Als Referenzmethode wurden arterielle Blutproben mit einem portablen Blutgasmessgerät untersucht. Die Werte wurden untereinander mittels Spearman Korrelation auf einem Signifikanzniveau von $P < 0,05$ auf Zusammenhänge untersucht.

Von den 60 Kälbern wurden 22 im MCG als „at risk“ eingestuft. Von diesen 22 Kälbern wurden 18 im GCS nicht als krank klassifiziert. Dagegen wurden neun im GCS kranke Tiere nicht vom MCG erkannt. Signifikante Korrelationen wurden jeweils zwischen MCG und den Parametern T ($r = 0,579$), DYS ($r = 0,435$) und AUS ($r = 0,360$) festgestellt. Mit steigender T

waren auch DYS (r = 0,349), RR (r = 0,422) und AUS (r = 0,456) Scores erhöht. Die innere Körpertemperatur wies Zusammenhänge mit den Blutgasen auf: mit ansteigender Temperatur fiel der Partialdruck von O_2 (r =- 0,307) und CO_2 (r = -0,265). Negative Korrelationen wurden auch für MCG und den O_2 Partialdruck gefunden (r = -0,271). Keine signifikanten Zusammenhänge konnten hingegen zwischen den Pulsoximeter Messungen in der Vulva und an der Schwanzunterseite festgestellt werden (r = -0,044). Auch zwischen Pulsoximeter und Blutgasen wurden keine Korrelationen gefunden. An der Vulva traten während der Messungen weniger Fehlermeldungen auf und diese Stelle wies allgemein eine bessere Akzeptanz bei den Kälbern auf. Die Ultraschall Befunde korrelierten nicht mit den anderen Ergebnissen.

Diese Studie bestätigt, dass Scoring Systeme basierend auf klinischen Parametern einen großen Wert für die Erkennung von EB haben. Allerdings ist die Rate der als krank identifizierten Kälber abhängig von den im Score inkludierten Parametern. Ultraschalluntersuchungen der Lunge können zusätzliche Informationen geben und könnten in einer Kombination mit klinischen Scores von Nutzen sein. Die Pulsoximeter Messungen an der Vulva erwiesen sich in dieser Studie als gut durchführbar. Weitere Untersuchungen sind notwendig, um die Genauigkeit und den zusätzlichen diagnostischen Wert dieser Methode zu erfassen.

7 List of abbreviations

BRD	bovine respiratory disease
CS	cough score
ND	nasal discharge
EHD	ear and head carriage
T	rectal temperature
RR	respiratory rate
DYS	dyspnea
AUS	auscultation
MCG	McGuirk score
GCS	general clinical score
ICS	intercostal space
US	ultrasound score
SO_2	oxygen saturation
SO_2T	oxygen saturation measured by pulse oximetry at the tail
SO_2V	oxygen saturation measured by pulse oximetry in the vulva
PCO_2	partial pressure of CO_2, measured in arterial blood
PaO_2	partial pressure of oxygen measured in arterial blood
LAC	lactate measured in arterial blood
HCO_3	bicarbonate measured in arterial blood
BE	base excess calculated in arterial blood
SaO_2	oxygen saturation calculated in arterial blood
H	healthy
S	diseased

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9 List of tables and figures

Supplemental material

10 Supplemental material

Tab. 1. Spearman correlation coefficients (cc[1]) for all clinical parameters, pulse oximetry, blood gas analysis, and ultrasonography results.

		CS	ND	EHD	OD	MCG	T	RR	DYS	AUS	SO_2T	SO_2V	PH	PCO_2	PaO2	BE	HCO_3	SaO_2	LAC	US
CS	cc[1]	1.00	0.14	0.24	0.01	**0.72**	**0.35**	0.10	**0.37**	**0.34**	0.01	0.00	0.03	-0.18	-0.22	-0.19	-0.20	-0.15	-0.02	0.17
	sig		0.28	0.06	0.97	**0.00**	**0.01**	0.43	**0.00**	**0.01**	0.92	0.99	0.81	0.17	0.09	0.15	0.13	0.26	0.85	0.20
ND	cc[1]	0.14	1.00	0.10	**0.28**	**0.57**	**0.42**	0.13	0.20	0.17	0.05	-0.21	0.03	0.08	-0.25	0.07	0.08	-0.21	0.11	0.09
	sig	0.28		0.45	**0.03**	**0.00**	**0.00**	0.31	0.12	0.20	0.73	0.11	0.83	0.53	0.05	0.59	0.52	0.11	0.42	0.50
EHD	cc[1]	0.24	0.10	1.00	**0.30**	**0.53**	**0.26**	0.25	0.17	0.02	0.01	-0.15	0.09	**-0.26**	-0.11	-0.20	-0.21	-0.02	-0.04	0.00
	sig	0.06	0.45		**0.02**	**0.00**	**0.04**	0.06	0.21	0.88	0.93	0.25	0.51	**0.04**	0.40	0.13	0.10	0.85	0.79	0.97
OD	cc[1]	0.01	.278*	.300*	1.00	**0.53**	0.25	0.01	**0.28**	**0.27**	0.01	-0.10	0.06	-0.15	-0.06	-0.04	-0.05	-0.02	0.13	-0.14
	sig	0.97	0.03	0.02		**0.00**	0.05	0.92	**0.03**	**0.03**	0.92	0.46	0.64	0.26	0.64	0.79	0.72	0.89	0.34	0.28
MCG	cc[1]	**0.72**	**0.57**	**0.53**	**0.53**	1.00	**0.57**	0.18	**0.44**	**0.36**	0.05	-0.14	0.08	-0.25	**-0.27**	-0.18	-0.19	-0.17	0.13	0.04
	sig	**0.00**	**0.00**	**0.00**	**0.00**		**0.00**	0.16	**0.00**	**0.00**	0.73	0.29	0.54	0.06	**0.04**	0.17	0.14	0.20	0.31	0.77
T	cc[1]	**0.35**	**0.42**	**0.26**	0.25	**0.57**	1.00	**0.42**	**0.35**	**0.46**	0.01	-0.16	0.12	**-0.27**	**-0.31**	-0.19	-0.21	-0.18	0.17	0.15
	sig	**0.01**	**0.00**	**0.04**	0.05	**0.00**		**0.00**	**0.01**	**0.00**	0.94	0.23	0.34	**0.04**	**0.02**	0.15	0.10	0.17	0.20	0.25
RR	cc[1]	0.10	0.13	0.25	0.01	0.18	**0.42**	1.00	0.17	0.11	0.02	-0.03	0.04	-0.19	-0.24	-0.23	-0.25	-0.14	-0.04	0.13
	sig	0.43	0.31	0.06	0.92	0.16	**0.00**		0.19	0.39	0.86	0.80	0.76	0.15	0.07	0.08	0.06	0.29	0.79	0.34
DYS	cc[1]	**0.37**	0.20	0.17	.280*	**0.44**	**0.35**	0.17	1.00	**0.67**	0.06	-0.21	0.06	-0.14	**-0.40**	-0.22	-0.25	**-0.32**	0.23	0.24
	sig	**0.00**	0.12	0.21	0.03	**0.00**	**0.01**	0.19		**0.00**	0.64	0.10	0.63	0.29	**0.00**	0.10	0.06	**0.01**	0.08	0.06
AUS	cc[1]	**0.34**	0.17	0.02	**0.27**	**0.36**	**0.46**	0.11	**0.67**	1.00	0.05	-0.08	0.04	-0.20	-0.18	-0.24	**-0.26**	-0.13	0.24	0.18
	sig	**0.01**	0.20	0.88	**0.03**	**0.00**	**0.00**	0.39	**0.00**		0.71	0.52	0.75	0.12	0.18	0.06	**0.04**	0.33	0.06	0.16
SO_2T	cc[1]	0.01	0.05	0.01	0.01	0.05	0.01	0.02	0.06	0.05	1.00	-0.04	0.05	0.07	0.03	0.08	0.10	0.06	-0.16	-0.09
	sig	0.92	0.73	0.93	0.92	0.73	0.94	0.86	0.64	0.71		0.74	0.69	0.59	0.82	0.55	0.44	0.63	0.22	0.52
SO_2V	cc[1]	0.00	-0.21	-0.15	-0.10	-0.14	-0.16	-0.03	-0.21	-0.08	-0.04	1.00	-0.18	0.16	0.10	0.03	0.03	0.04	0.06	0.14
	sig	0.99	0.11	0.25	0.46	0.29	0.23	0.80	0.10	0.52	0.74		0.17	0.23	0.45	0.83	0.81	0.76	0.66	0.28
PH	cc[1]	0.03	0.03	0.09	0.06	0.08	0.12	0.04	0.06	0.04	0.05	-0.18	1.00	**-0.42**	**0.33**	**0.50**	**0.37**	**0.52**	-0.07	0.04
	sig	0.81	0.83	0.51	0.64	0.54	0.34	0.76	0.63	0.75	0.69	0.17		**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	0.57	0.76
PCO_2	cc[1]	-0.18	0.08	**-0.26**	-0.15	-0.25	**-0.27**	-0.19	-0.14	-0.20	0.07	0.16	**-0.42**	1.00	**-0.41**	**0.44**	**0.56**	**-0.49**	**-0.33**	-0.03
	sig	0.17	0.53	**0.04**	0.26	0.06	**0.04**	0.15	0.29	0.12	0.59	0.23	**0.00**		**0.00**	**0.00**	**0.00**	**0.00**	**0.01**	0.83
PaO2	cc[1]	-0.22	-0.25	-0.11	-0.06	**-0.27**	**-0.31**	-0.24	**-0.40**	-0.18	0.03	0.10	**0.33**	**-0.41**	1.00	-0.02	-0.07	**0.95**	-0.04	-0.08
	sig	0.09	0.05	0.40	0.64	**0.04**	**0.02**	0.07	**0.00**	0.18	0.82	0.45	**0.01**	**0.00**		0.88	0.59	**0.00**	0.74	0.56
BE	cc[1]	-0.19	0.07	-0.20	-0.04	-0.18	-0.19	-0.23	-0.22	-0.24	0.08	0.03	**0.50**	**0.44**	-0.02	1.00	**0.98**	0.06	**-0.26**	-0.07
	sig	0.15	0.59	0.13	0.79	0.17	0.15	0.08	0.10	0.06	0.55	0.83	**0.00**	**0.00**	0.88		**0.00**	0.63	**0.04**	0.60
HCO_3	cc[1]	-0.20	0.08	-0.21	-0.05	-0.19	-0.21	-0.25	-0.25	**-0.26**	0.10	0.03	**0.37**	**0.56**	-0.07	**0.98**	1.00	-0.01	**-0.31**	-0.10
	sig	0.13	0.52	0.10	0.72	0.14	0.10	0.06	0.06	**0.04**	0.44	0.81	**0.00**	**0.00**	0.59	**0.00**		0.92	**0.02**	0.43
SaO_2	cc[1]	-0.15	-0.21	-0.02	-0.02	-0.17	-0.18	-0.14	**-0.32**	-0.13	0.06	0.04	**0.52**	**-0.49**	**0.95**	0.06	-0.01	1.00	-0.07	-0.09
	sig	0.26	0.11	0.85	0.89	0.20	0.17	0.29	**0.01**	0.33	0.63	0.76	**0.00**	**0.00**	**0.00**	0.63	0.92		0.58	0.51
LAC	cc[1]	-0.02	0.11	-0.04	0.13	0.13	0.17	-0.04	0.23	0.24	-0.16	0.06	-0.07	**-0.33**	-0.04	**-0.26**	**-0.31**	-0.07	1.00	0.19
	sig	0.85	0.42	0.79	0.34	0.31	0.20	0.79	0.08	0.06	0.22	0.66	0.57	**0.01**	0.74	**0.04**	**0.02**	0.58		0.14
US	cc[1]	0.17	0.09	0.00	-0.14	0.04	0.15	0.13	0.24	0.18	-0.09	0.14	0.04	-0.03	-0.08	-0.07	-0.10	-0.09	0.19	1.00
	sig	0.20	0.50	0.97	0.28	0.77	0.25	0.34	0.06	0.16	0.52	0.28	0.76	0.83	0.56	0.60	0.43	0.51	0.14	

CS = cough score
ND = nasal discharge
EHD = ear and head carriage
OD = ocular discharge
MCG = McGuirk Score
T = rectal temperature
RR = respiratory rate
DYS = dyspnea
AUS = auscultation
SO_2T = oxygen saturation measured by pulse oximetry in tail
SO_2V = oxygen saturation measured by pulse oximetry in vulva
PCO_2 = partial pressure of CO_2, measured in arterial blood gas analysis
PaO_2 = partial pressure of oxygen measured in arterial blood gas analysis
BE = base excess measured in arterial blood gas analysis
SaO_2= oxygen saturation measured in arterial blood gas analysis
LAC = lactate measured in arterial blood gas analysis
US = ultrasound score