Calf welfare monitored using physiologically based precision livestock farming technology during different sampling techniques of the respiratory tract

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Abstract

In recent years respiratory tract sampling techniques are more frequently used in calves to rationalize antimicrobial use and target respiratory health care towards the pathogens involved. This study's objective was to compare the effects on animal welfare of the three most commonly used respiratory tract sampling techniques in calves, namely deep nasopharyngeal swabbing (DNS), non-endoscopic bronchoalveolar lavage (nBAL), and transtracheal wash (TTW). The metabolic energy for mental performance, based on heart rate and movement data, and other frequently used heart rate variability parameters were determined as a measure for animal welfare. A crossover study was conducted, including five male Holstein-Friesian calves. Individually housed calves were equipped with a combined heart rate and accelerometer sensor which transmitted data to a top view camera. Five sessions were organized, and calves were randomly assigned to one of the five test groups (control (= no animal handling), fixation, DNS, nBAL, TTW). Heart rate variability parameters (mean heart rate, standard deviation of N-N intervals (SDNN), root mean square of successive differences of N-N intervals (RMSSD), ratio of the low frequency (0.04-0.15 Hz) to the high frequency (0.15–0.4 Hz) (LF/HF ratio), triangular index, SD1 and SD2) were calculated and analyzed using mixed models with calf as random factor. All sampling techniques (DNS, nBAL, TTW) and fixation resulted in an increased mean heart rate compared to the control event. SDNN and SD2 were significantly higher in nBAL and TTW compared to the control. Triangular index showed a discrepancy between TTW, DNS and control. In conclusion, using non-invasively measured heart rate variability parameters showed inconsistent changes. No uniform conclusion could be made based on heart rate variability parameters frequently used in humans for stress monitoring in calves. For the mental component algorithm, it seems that the TTW induced the largest variation in the HR mental component dynamics compared individually per calf with the fixation and control moments. DNS and nBAL seem to induce a smaller and similar variation.

Keywords: heart rate variability, stress monitoring, respiratory sampling techniques, PLF

Introduction

Bovine respiratory disease (BRD) is one of the most common causes of morbidity and mortality in all types of calves (Pardon et al., 2012). It's a leading concern for economic losses (Yazdanbakhsh et al., 2017), hampered animal welfare and antibiotic resistance selection by intensive antimicrobial use (Mitrenga et al., 2020). In the contemporary context with increasing pressure on antimicrobial use, animal welfare and sustainable production, the interest in respiratory disease diagnostics to identify the causative pathogen substantially increased (Van Driessche et al., 2017). Diagnostic test results are not only used to rationalise antimicrobial use, but also to tailor on farm interventions towards the identified pathogen. The most frequently used sampling techniques are deep nasopharyngeal swab (DNS), non-endoscopic broncho-alveolar lavage (nBAL) and transtracheal wash (TTW) (Van Driessche et al., 2019). Depending on the technique, sampling of the respiratory tract is more or less invasive and a potential cause of both pain and stress for the animal. A consequence of the COVID-19 pandemic is that almost every cattle product consumer nowadays is aware of the adverse effects of deep nasopharyngeal swabbing. Therefore, the cattle industry and its veterinarians cannot justify the use of pain- and stressful methods for sampling. Therefore, there is an urgent need on information on how stressful the commonly used techniques are and whether the induced stress is of such an extend that alleviating measures like local anaesthesia or sedation should be required. By the knowledge of the authors, no studies, comparing the stress induction between different respiratory tract sampling techniques are available in calves or any other animal species.

Nowadays stress in calves is mostly measured using momentary methods, such as by invasive blood sampling or non-invasive collection of saliva, to determine cortisol concentration(Kovács et al., 2021). Both are potentially stressful as animal handling is required. An optimal measurement system is continuous and does not require animal handling. In human medicine, heart rate and heart rate variability (HRV) is used to measure stress continuous and non-invasive. Some of these HRV parameters, in particular LF/HF ratio and triangular index, are documented to be meaningful predictors of stress (Vavrinsky et al., 2021) More recently, an algorithm (Mindstretch, BioRiCS, Louvain, Belgium) was developed in humans which uses combined accelerometer data and heart rate to estimate the metabolic component which reflects mental stress. This algorithm is validated for humans and commercially available. The objective of the present study was to compare the effect of three sampling techniques of the respiratory tract (DNS, nBAL and TTW) on stress-indicators based on heart rate variability parameters and the Mindstretch algorithm in calves.

Materials and methods

Study design, animals and housing

A crossover study design was conducted on five male Holstein-Friesian calves, aging thirty-four to forty-three days at the beginning of the trial. All calves came from the same dairy farm. Calves were housed in a research stable at Ghent University for the duration of the experiment. After arrival, the calves underwent a two-week habituation period. The calves were individually housed in a straw bedded pen, with visual contact with other calves. They were fed 3 L of commercially available milk replacer twice a day from individual drinking buckets. Additionally the calves had add libitum access to hay, water and concentrates. Each day the health condition of the calves was assessed visually and rectal temperature was taken twice a day, during milk feeding. The trial protocol was approved by the Ethical committee of the Faculty of Veterinary Medicine and Bioengineering from Ghent University under license EC2020-087.

Sensor system

The sensor system consisted of a (for humans) commercially available Movesense active sensor (Movesense Ltd, Finland, Vantaa) and an in-house developed gateway with a build-in camera module based on the ESP-32 CAM board. The sensor collects heart rate, electrocardiogram (128 Hz), nine degrees of freedom inertial measurement unit (26 Hz) and temperature data. The sensor is attached to a shaved area at the left side of the thorax just behind the shoulder with the Movesense strap (Movesense Ltd, Finland, Vantaa). The gateway was placed above each pen, so the camera recorded a top view of each individual calf which was later used for annotating the data.

Sampling techniques

Based on the crossover design five sampling sessions were organized, and calves were randomly assigned to one of the five test groups (negative control, fixation for two minutes, DNS sampling, nBAL sampling, TTW sampling). During fixation, the calf was approached by one person and secured in the corner of the pen. The head of the calf was held up so that the position was as similar as possible compared to the other handling events. This position was held for two minutes. To take a deep nasal swab, a nasal swab was inserted into the ventral meatus of the nasal cavity up to the level of the ventro-medial corner of the eye.

The swab was rotated a couple of times after which it was removed. For nBAL and TTW the calf was restrained with two people. For the nBAL the nostrils were disinfected with isopropanol (99%) before inserting a custom-made polyethylene bronchoalveolar catheter (Van Driessche et al., 2019). Next the bronchoalveolar catheter was guided under finger control into the ventral nasal passage up to the larynx. Then the catheter was blindly introduced into the trachea until the wedge position was reached. Next, 30 mL of sterile physiological 0.9% NaCl solution was injected and immediately aspirated. Finally, the catheter was removed and the calf was released. For the TTW sampling, the tracheal region at the level of the neck was shaved, rubbed (with hibitane solution 5%), scrubbed (with isopropanol 99%), and locally anesthetized with 3 mL procaine hydrochloride (4%) per subcutaneous injection with a 21G needle. The trachea was secured with one hand and with the other hand a central venous catheter (Centracath, Vygon, Ecouen, France) for human use was inserted through the skin into the tracheal lumen. The catheter was advanced until the wedge position was reached, and 30 mL of physiological 0.9% NaCl solution was injected and instantly aspirated. After aspiration, the catheter was removed and the calf was released. For blood sampling all the calves received a permanent intrajugular vein catheter before the experiment (MILA international, Kentucky, Florence, US). Blood samples were collected at -1h, 0h, +1h, +5h and +24h relative to the respiratory tract sampling technique.

Labelling data and feature extraction

All of the events (control, fixation, DNS, nBAL, TTW, blood sampling, feeding, rectal temperaturing) were noted with the corresponding begin and end time, derived from the timestamp on the video recordings. The annotation of the sensor data and feature extraction (based on the hrv-analysis library, by Robin Champseix) were executed in Python.

Mindstretch algorithm

The Mindstrech algorithm is owned by the BioRiCS company so the labeled data from each calf was sent there for further analysis. An individual analysis based on the labelled data was performed for each calf. The algorithm adapts and evolves differently according to the individual data for each calf and provides a value for the variation of the heart rate mental component, where it is assumed that the higher the value is, the more stressful the operation is. When applying the technology on humans who are doing different physical activities like playing soccer, driving a race car or walking, the algorithm must be tuned, as the correlation between the specific movement and heart rate response will change. In this study, we have used the measured heart rate and movement for each calf's labelled activities to tune the algorithm from humans to calves in a similar manner. Important to mention is that each calf is by default in a different level of the heart rate mental component and the Mindstretch algorithm had adapted its parameters differently to each calf. For this reason, an average or standard deviation for the different calves from the same test can't be taken in consideration.

Statistical analysis

Parameters were calculated for each and calve and event in Python. The following parameters were calculated out of the heart rate: mean heart rate, SDNN, RMSSD, LF/HF ratio, triangular index, SD1 and SD2. Statistics was done in SAS Enterprise Guide 8 (SAS Institute, Inc, Cary, North Carolina). A linear mixed model was used to determine any difference in mean heart, SDNN, RMSSD, LF/HF ratio, triangular index, SD1 and SD2 between the different sampling techniques (control, fixation, DNS, nBAL, TTW). The dependent variable was the parameter of interest during the different sampling events and the predictor was the sampling procedure. Calf was included in each model as random factor, to account for physiological variation between different individuals. All the different events were pairwise compared. A similar model approach was also used for a wider variety of handlings as there are milk feeding and blood sampling. Results with $P<0.05$ were considered significant. The outcome variables were checked for a normal distribution. The LF/HF ratio did not complied with this assumption, after a log(x+1) transformation a normal distribution was reached. Compliance with the assumptions of linear regression was checked by inspecting the normal distribution of the residuals.

Results and discussion

Mean heart rate, SDNN, triangular index and SD2 all increased significantly from the control in at least one of the stressors compared to the control event (DNS, nBAL, TTW).

A significant difference for the mean heart rate (Figure 1) was found between each of the handling events (fixation, DNS, nBAL and TTW) compared to the control handling ($P<0.05$). As already shown in humans the mean heart rate could be used as a stress indicator when an increase of nine beats per minute could be detected (Pakhomov et al., 2020). This may be an indication that some sort of stress response is triggered during the different sampling events (DNS, nBAL, TTW) and fixation.

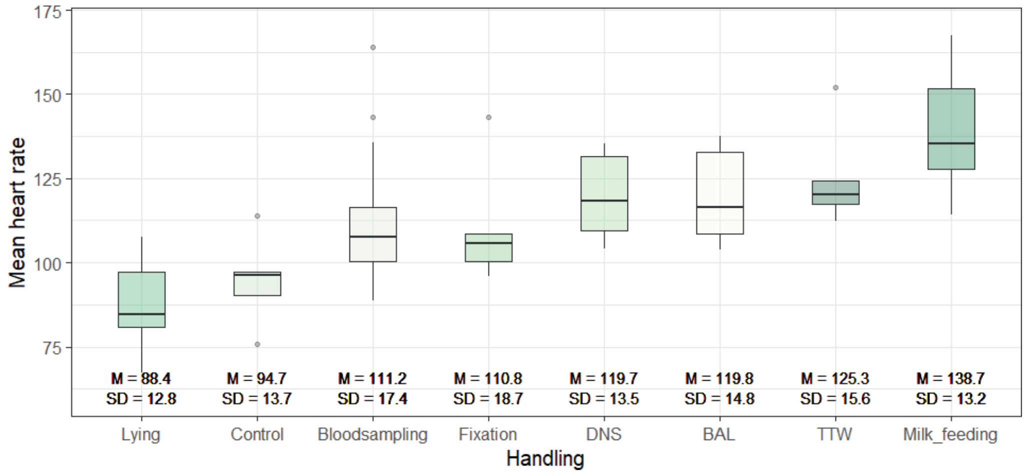

Figure 1: Mean heart rate during methods of respiratory tract sampling, as compared to lying, catheterized blood sampling and milk feeding in five dairy calves

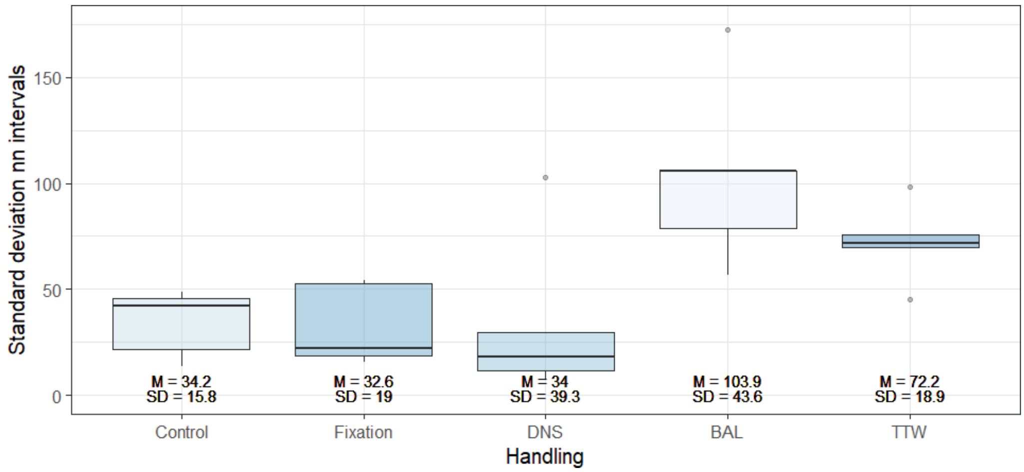

Figure 2: Standard deviation of N-N intervals during the different methods for respiratory tract sampling in five dairy calves

No significant differences could be observed, for RMSSD, between the control event and the other handling techniques (fixation, DNS, nBAL and TTW). In contrast, for SDNN a significant discrepancy was detected during nBAL and TTW compared to the control handling (Figure 2).

For the LF/HF ratio no significant differences could be detected between the control event and the other sampling events (fixation, DNS, nBAL, TTW). This model was based on the $\log\left(\frac{LF}{HF} ratio + 1\right)$ transformation, due to non-normality. For the Point care plot features no significant discrepancies were detected for the SD1, in contrast to the SD2 where a significant difference could be demonstrated between the nBAL (*P*-value = 0.0008), TTW (*P*-value = 0.0383) and the control event. Frequency and Point care plot features studies have shown that the LF/HF ratio was more accurate in stress detection than cortisol in humans (Hong et al., 2010). No changes in the LF/HF ratio, RMSDD and SD1 between the different sampling techniques and the control could be detected, possibly indicating the absence of a severe stressor. Also, extrapolation of observations from human medicine should be done with care, as calves may express stress through other (heart rate variability) parameters. Another possible explanation may be hardware associated. It has already been shown in humans that the method of R-R interval detection may have a large influence on heart rate variability parameters, e.g. heart rate variability parameters derived from photoplethysmography and electrocardiography signals (Jeyhani et al., 2015).

For the triangular index, a significant difference was found for TTW (*P*-value = 0.0259) and DNS (*P*-value = 0.0271). The triangular index is shown to be very accurate in stress monitoring in humans and could be used as an independent predictor of cardiovascular and all-cause mortality in a cohort of patients with atrial fibrillation. Our study demonstrates a difference during the DNS and TTW compared to the control, which may be indicative for an elevated stress level during those two sampling techniques (Hämmerle et al., 2020). The triangular index is influenced with the duration on which it is calculated. Thus this parameter may be less reliable with short-term DNS.

Next to exploring non-invasive stress monitoring methods, the main aim of the present study was to identify which of the three most commonly used respiratory sampling techniques would be most stressful. No uniform conclusion could be made based on the heart rate and heart rate variability parameters, however discrepancies between the different events could be noticed.

For the mean heart rate a dissimilarity was demonstrated between TTW and fixation. No differences could be detected between DNS, nBAL and TTW, which could be a sign that entering the nose or the trachea of the calf induce a similar stress response. However if we introduce milk feeding to the model, a significant difference in mean heart rate could be shown between the feeding event and all of the other events (control, fixation, DNS, nBAL and TTW). From this we conclude that in the current experiment it was impossible to distinguish between positive and negative experiences based upon mean heart rate alone. Because an even higher increase of the mean heart rate was noted during milk feeding time, which is considered a positive event (Figure 1). No inter-handling discrepancies could be detected for RMSSD and LF/HF ratio, while for SDNN a significant difference was observed when comparing TTW and nBAL versus DNS and fixation. The same observations were found for the triangular index, which may be an indication that TTW and nBAL are more stressful than fixation and DNS. Anyway, the remark, mentioned above, that the triangular index is affected by the duration of the handling must be kept in consideration. For the point care features the SD1 shows a discrepancy when BAL was tested versus DNS and fixation, indicating that BAL was more stressful than DNS and fixation. SD2 differed when mutually comparing nBAL and TTW with all of the other handlings (fixation, DNS, nBAL, TTW). Which may lead to the consideration that BAL and TTW are perceived as more stressful, with respect to the other handlings.

Table 1: Maximal variation of the heart rate mental component during the different events and the subsequent blood draw. The tests are deep nasopharyngeal swabbing (DNS), the non-endoscopic bronchoalveolar lavage (nBAL), the transtracheal wash (TTW), just performing a fixation and a selected control moment.

Calf	*DNS*	*TTW*	*nBAL*	*Fixation*	*Control*
1	3.3	NA	2.6	0.3	1.4
2	0.7	3.2	2.1	NA	NA
3	NA	16	1.5	0.5	NA

The Mindstretch algorithm could only return a value for the maximal variation of the heart rate mental component during the test and the subsequent blood sampling for three out of the five calves (Table1). The missing results are due to missing data around the moment of sampling, this makes that once the data is back, the algorithm still needs some time to adapt to the current status of the calf and the estimated results for the heart rate component are distorted. First of all is it worth mentioning that this is a preliminary analysis to evaluate the potential of using the heart rate mental component as an indicator of the stress induced by the performance of the different respiratory tract sampling techniques (DNS, nBAL, TTW). Based on those results, the highest values could be detected during the TTW compared to the fixation and the control event (Figure 3). The increase is followed next by the nBAL and DNS, both showing an approximately similar increase. Which may indicate that TTW is the most stressful sampling technique, this strokes with the feelings of the observers. However, more calves must be included.

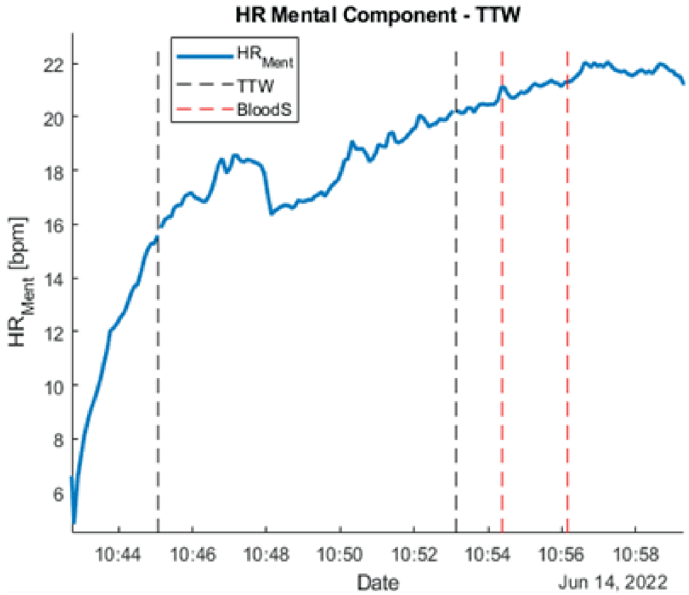

Figure 3: Example of the evolution of the heart rate mental component (HR_{Ment}) in beats per minute (bpm) estimated right before, during and right after the TTW test and blood draw from calf 3. The vertical black and red dashed lines indicate start and end of test and blood sampling, respectively.

Conclusions

In the present study, of all heart rate and heart rate variability parameters studied, mean heart rate, SDNN, triangular index and SD2 showed some potential for non-invasive stress monitoring in calves. Overall, with the studied parameters, the stressful nature of three sampling techniques appeared to be similar, regardless of the marked differences in invasiveness or length of the procedure. Based on this research no uniform statement could be made regarding the stress levels during the different sampling techniques, based on heart rate variability parameters commonly used in human stress monitoring. However, if we keep count of the discrepancies per handling for the different parameters compared to the control, TTW followed by nBAL showed the most significant differences. Based on the Mindstretch algorithm we may have an indication that TTW is the most stressful procedure, but further research with a higher number of animals and finetuning of the algorithm is needed.

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