Real-time and video-recorded cattle pain assessment: clinical application and reliability

Rubia Mitalli Tomacheuski (✉ rmtomach@ncsu.edu)
North Carolina State University

Alice Rodrigues Oliveira
Federal University of Bahia

Pedro Henrique Esteves Trindade
North Carolina State University

Magdiel Lopez-Soriano
North Carolina State University

Victoria Rocha Merenda
North Carolina State University

Stelio Loureiro Luna
São Paulo State University (Unesp)

Monique D Pairis-Garcia
North Carolina State University

Article

Keywords:

Posted Date: August 16th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3179421/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Bovine pain assessment relies on validated behavioral scales related to normal and pain-related behaviors. This study aimed to investigate the reliability and applicability of real-time and video-recorded cattle pain assessment, and to compare its agreement. Ten Nelore and Nine Angus bulls underwent general anesthesia and surgical castration. Three-minute real-time observations and simultaneous videos were recorded at -48 h (M0), preoperative (M1), after surgery (M2), after rescue analgesia and at 24 h (M4). Animals received morphine, dipyrone and flunixin meglumine after surgical castration. Two trained evaluators assessed real-time (n = 95) and video-recorded time-points (n = 95) using the Unesp-Botucatu Cattle Pain Scale (UCAPS). Both assessment methods inferred 'very good' reliability (≥ 0.81) with minimal bias, however, video-recorded assessment (4.33 ± 2.84) demonstrated slightly higher scores compared to real-time (3.08 ± 2.84). The results from this study suggest the UCAPS can be used in both real-time and video-recorded to assess pain and guide analgesic therapy in cattle.

Introduction

Society is increasingly demanding better husbandry practices and welfare for food-producing animals. In this regard, managing pain is a core principle to guarantee basic animal welfare. However, pain assessment, recognition and treatment are a significant challenge in livestock industries given limited educational resources guiding veterinarians on appropriate pain management and inaccessibility of products safe for food producing animals, such as cattle.

Pain assessment in animals is typically performed by evaluating changes to whole body and/or facial behavioral expression. For either approach, pain assessment instruments must be validated for use, robust and flexible enough to be applied across different study designs and must be species-specific utilizing behaviors typical to the repertoire of the species. Specific to bovine, several pain scales can be found in the literature; however, these scales differ regarding study design use and validation process.

The Unesp-Botucatu Cattle Pain Scale (UCAPS) developed for beef cattle is considered the most robust cattle specific tool due to its high strength of evidence. The UCAPS was developed and validated using pre-recorded videos in which observers assessed pain post-hoc. However, relying on video-recordings is a limiting factor for assessing pain in cattle given veterinarians and farmers have limited access and financial capabilities to install video equipment. In addition, assessing pain via video delays intervention opportunities to provide analgesic intervention to cattle in pain. Hence, exploring opportunities to implement and validate the UCAPS for real-time assessment is needed. Therefore, this study aimed to investigate the reliability and the agreement of real-time and video-recorded cattle pain assessment using the UCAPS. Our hypothesis suggests that the reliability and the agreement of real-time assessment is not different than video-recorded assessment.
Results

The UCAPS presented an overdispersion of zeros based on the histogram and Cameron and Trivedi's test (Lambda t-test score = 4.687 and p < 0.00001), therefore a multilevel zero-inflated Poisson model based on Bayesian information criterion (BIC) was used.

The post-hoc test for the UCAPS scores inferred similar trends over-time (M2 > M3/M4 > M = M1; Fig. 1A) regardless of the assessment method. Total pain scores obtained by video-recordings were greater than the total pain scores obtained by real-time assessment (Fig. 1B) at time-points M0, M1, M3 and M4 (Fig. 1C).

Table 1 depicts variables composing the UCAPS scale, the total scores for real-time and video-recorded behaviors, the statistical model implemented and the need for rescue analgesia. Video-recording assessments had higher means for locomotion, interactive behavior, and miscellaneous behavior when compared to real-time assessment (Table 1).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Real-time</th>
<th>Video-recorded</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locomotion</td>
<td>0.51 ± 0.66 b</td>
<td>0.71 ± 0.75 a</td>
<td>MGLM</td>
</tr>
<tr>
<td>Interactive behavior</td>
<td>0.39 ± 0.57 b</td>
<td>0.58 ± 0.69 a</td>
<td>MGLM</td>
</tr>
<tr>
<td>Activity</td>
<td>1.06 ± 0.96</td>
<td>1.25 ± 0.94</td>
<td>MGLM</td>
</tr>
<tr>
<td>Appetite</td>
<td>0.70 ± 0.90</td>
<td>0.90 ± 0.97</td>
<td>MGLM</td>
</tr>
<tr>
<td>Miscellaneous behavior</td>
<td>0.56 ± 0.74 b</td>
<td>0.90 ± 0.82 a</td>
<td>MGLM</td>
</tr>
<tr>
<td>Rescue analgesia</td>
<td>0.29 ± 0.46</td>
<td>0.42 ± 0.50</td>
<td>MBLM</td>
</tr>
<tr>
<td>UCAPS total score</td>
<td>3.08 ± 2.84 b</td>
<td>4.33 ± 2.84 a</td>
<td>MZIBN</td>
</tr>
</tbody>
</table>

MGLM: multilevel generalized linear model adjusted by Poisson distribution; MBLM: multilevel binomial logistic model; MZIBN: multilevel zero-inflated negative binomial model. Bold is highlighting P< 0.05.

Figure 2 compares the real-time and video-recorded assessment of the Unesp-Botucatu Cattle Pain Scale (UCAPS) using Bland-Altman plots. The limit of agreement (LoA) was between -6.24 and 3.74, with a bias of -1.25 and a Lin's concordance correlation coefficient (CCC) of 0.53 (Fig. 2).

Figure 2. **Bland-Altman plots comparing real-time and video-recorded assessment of the Unesp-Botucatu Cattle Pain Scale (UCAPS)**. LoA, limit of agreement; CI, 95% confidence interval; solid line represents the bias; dashed line represents the lower and upper LoA; dotted lines represent the 95% confidence interval; CCC, Lin's concordance correlation coefficient; green line is the simple linear model.
Table 2 depicts the percentages of LoA agreement and disagreement between real-time and video-recorded assessments using the Unesp-Botucatu Cattle Pain Scale. There was a low percentage of perfect agreement between assessment methodologies (Table 2).

<table>
<thead>
<tr>
<th>Types</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfect agreement</td>
<td>23.15</td>
</tr>
<tr>
<td>Difference within the LoA</td>
<td>70.52</td>
</tr>
<tr>
<td>Difference beyond the LoA</td>
<td>06.33</td>
</tr>
</tbody>
</table>

LoA: limit of agreement based on Bland-Altman test.

The slope coefficient of the mean between the two assessment methodologies ($\beta = 0.0010$) was not significant ($P = 0.9888$; Table S2), suggesting no proportional bias. The model showed homoscedasticity according to the Breusch Pagan test ($X^2 = 0.2940; P = 0.5876$).

Table 3 shows the reliability of real-time and video-recorded assessment for Unesp-Botucatu cattle pain scale (UCAPS) and need for rescue analgesia. The agreement between real-time versus video-recorded pain assessment for rescue analgesia was significant. The need for rescue analgesia was good for real-time assessment and reasonable for video-recorded assessments (Table 3).

Table 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>Method</th>
<th>Coefficient</th>
<th>Estimate</th>
<th>CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCAPS</td>
<td>Real-time</td>
<td>ICC</td>
<td>0.90</td>
<td>0.85–0.94</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>UCAPS</td>
<td>Video-recorded</td>
<td>ICC</td>
<td>0.81</td>
<td>0.71–0.87</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Rescue Analgesia</td>
<td>Real-time</td>
<td>Weighted Kappa</td>
<td>0.70</td>
<td>0.53–0.84</td>
<td>NA</td>
</tr>
<tr>
<td>Rescue Analgesia</td>
<td>Video-recorded</td>
<td>Weighted Kappa</td>
<td>0.48</td>
<td>0.28–0.66</td>
<td>NA</td>
</tr>
</tbody>
</table>

Table 4 depicts the agreement on need for rescue analgesia between the two assessment methods using UCAPS. The reliability of UCAPS was very good for both assessment methodologies.
Table 4

Agreement on need for rescue analgesia between the two assessment methods for UCAPS. $X^2 = 45.46$, df = 1, $P$-value < 0.0001.

<table>
<thead>
<tr>
<th></th>
<th>Video-recorded</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Analgesia not indicated</td>
<td>Analgesia indicated</td>
<td></td>
</tr>
<tr>
<td><strong>Real-time</strong></td>
<td>99</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Analgesia not indicated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analgesia indicated</td>
<td>11</td>
<td>45</td>
<td></td>
</tr>
</tbody>
</table>
### Table 4

**Unesp-Botucatu Cattle Pain Scale (UCAPS)**

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Locomotion</strong></td>
<td>(0) Walking with no obviously abnormal gait.</td>
</tr>
<tr>
<td></td>
<td>(1) Walking with restriction, may be with hunched back and/or short steps.</td>
</tr>
<tr>
<td></td>
<td>(2) Reluctant to stand up, standing up with difficulty or not walking.</td>
</tr>
<tr>
<td>**Interactive</td>
<td>(0) Active; attention to tactile and/or visual and/or audible environmental stimuli; when near other animals, can interact with and/or accompany the group.</td>
</tr>
<tr>
<td>behaviour**</td>
<td>(1) Apathetic: may remain close to other animals but interacts little when stimulated.</td>
</tr>
<tr>
<td></td>
<td>(2) Apathetic: may be isolated or may not accompany the other animals; does not react to tactile, visual and/or audible environmental stimuli.</td>
</tr>
<tr>
<td><strong>Activity</strong></td>
<td>(0) Moves normally.</td>
</tr>
<tr>
<td></td>
<td>(1) Restless, moves more than normal or lies down and stands up with frequency.</td>
</tr>
<tr>
<td></td>
<td>(2) Moves less frequently in the pasture or only when stimulated.</td>
</tr>
<tr>
<td><strong>Appetite</strong></td>
<td>(0) Normorexia and/or rumination.</td>
</tr>
<tr>
<td></td>
<td>(1) Hyporexia.</td>
</tr>
<tr>
<td></td>
<td>(2) Anorexia.</td>
</tr>
<tr>
<td>**Miscellaneous</td>
<td>Wagging the tail abruptly and repeatedly.</td>
</tr>
<tr>
<td>behaviours**</td>
<td>Licking the affected area.</td>
</tr>
<tr>
<td></td>
<td>Moves and arches the back when in standing posture.</td>
</tr>
<tr>
<td></td>
<td>Kicking/foot stamping.</td>
</tr>
<tr>
<td></td>
<td>Hind limbs extended caudally when in standing posture.</td>
</tr>
<tr>
<td></td>
<td>Head below the line of spinal column.</td>
</tr>
<tr>
<td></td>
<td>Lying down in ventral recumbency with full or partial extension of one or both hind limbs.</td>
</tr>
<tr>
<td></td>
<td>Lying down with the head on/close to the ground.</td>
</tr>
<tr>
<td></td>
<td>Extends the neck and body forward when lying in ventral recumbency.</td>
</tr>
<tr>
<td></td>
<td>(0) All of the above-described behaviours are absent.</td>
</tr>
<tr>
<td></td>
<td>(1) Presence of 1 of the behaviours described above.</td>
</tr>
<tr>
<td></td>
<td>(2) Presence of 2 or more of the behaviours described above.</td>
</tr>
</tbody>
</table>
Discussion

Pain assessment in bovine is challenging. The implementation of a reliable and applicable pain scoring instrument that may be used with video-recordings or real-time assessment is crucial to improve pain management in cattle. This is the first study confirming the applicability of the Unesp-Botucatu Cattle Pain Scale (UCAPS) by video-recordings and validating the UCAPS by real-time assessment in a clinical setting. Even though perfect agreement between the real-time and video-recorded assessments methods was low, the UCAPS demonstrated a high reliability between assessment methods with minor bias and a narrow limit of agreement.

The similarity between trends over time (moments post-castration) by UCAPS scores confirmed that the instrument was able to detect and differentiate painful and no-painful states by both methods of assessment. Similarly to previous studies using UCAPS\textsuperscript{11,15,22}, there were differences in the total pain scores and in some of the scale variables (i.e., locomotion, interactive behavior, and miscellaneous behavior) when comparing real-time to video-recorded methods. These results could be explained by the fact that the evaluators were not masked to the time-points during real-time assessment, which may have influenced their scoring. Nonetheless, no differences between the assessment methods were found in M2 (the most painful state right after the surgical procedure) which reinforces that UCAPS is able to detect pain-related behaviors regardless of the assessment method used.

Similarly, to studies in cats, rats and mouse\textsuperscript{23–25}, the Bland-Altman for repeated measures methods demonstrated a narrow limit of agreement and a minor bias between both methods of pain assessment, reinforcing the sufficient agreement between assessment methods. From a bovine-specific perspective, this study is beneficial not only to veterinarians and technicians that can apply the UCAPS in real-time, but also to researchers and laboratory animal technicians that aim to assess pain and intervene in experiments involving cattle.

The UCAPS reliability was very good for real-time and video-recorded pain assessment methods which demonstrated that the UCAPS is a reliable instrument to be implemented. Even though the need for rescue analgesia had good reliability for the real-time and only moderate reliability for video-recorded pain assessment, these findings were still superior to previous studies in cattle and sheep\textsuperscript{11,17}. These results could be explained by the fact that evaluators were aware of the time-points during real-time, which increased the likelihood of assigning higher scores during post-castration moments.

In conclusion, real-time assessment using UCAPS may be implemented by veterinarians and producers from a clinical perspective to improve pain diagnosis and pain management in cattle with similar reliability to that of video-recorded assessment.

Limitations

This study confirms that UCAPS is a reliable tool that may be used for video-recording or real-time assessment. Even though this study had the limitation of observers being aware of time-points and
clinical state of the animals, the pain assessment provided by the UCAPS was still consistent and reliable. Additional limitations include the restricted number of evaluators and the fact that all evaluators were female. Previous research has shown that female observers tend to assign higher pain scores to during pain assessments. Even though the gender of the observers might have overestimated the final pain scores, this possible confounding effect would have been applied to both video- and real-time assessments, which does not impact the interpretation of the results. Furthermore, a recent study suggested that the use of three evaluators is ideal in pain assessments. Therefore, future research evaluating pain assessment tools should include a larger sample size of observers from both genders. Finally, given that the evaluators were not masked to time-points in real-time, the scoring in real-time assessment could have been overestimated.

**Methods**

**Ethical statement**

The study was approved by the University of São Paulo State—Unesp School of Veterinary Medicine and Animal Science Ethical Committee for the Use of Animals in Research (Approval number, 0147/2018) and performed in accordance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching, COSMIN and ARRIVE guidelines and recommendations. Bulls enrolled in the study were part of larger experiment, which contributes to one of the four R’s of animal experimentation.

**Animals and surgical procedure**

Ten *Bos indicus*, Nelore breed (451 kg ± 41 kg) and nine *Bos taurus*, Angus breed (264 kg ± 24 kg; mean ± SD), age 19 to 24 months were purchased from two private farms, transported and maintained separately in two groups (Nelore and Angus) at the Experimental Farm Lageado – FMVZ/Unesp. They were housed outdoors in two separate paddocks, had *ad libitum* access to water and were fed with hay and grain. They were acclimatized to this site for one month before the start of the experiment. After this period, they were transported to the FMVZ/Unesp veterinary hospital in groups of three to four animals per week, where they were maintained under similar conditions receiving the same food and water *ad libitum*. The animals had a varied acclimatization time at the FMVZ/Unesp veterinary hospital, according to the order and date of the procedure for each animal. The first animal of the week had the shortest acclimatization time (2h to 12h) before being separated for fasting, and the other two animals of each week had a longer acclimatization time (24h to 72h). After the end of the experiments, the animals were kept at the Experimental Farm Lageado - FMVZ/Unesp for two months for fattening and then sent for humane slaughter.
These bulls were selected for a study assessing testicular warming and after completion of the sampling all bulls underwent surgical castration. At the FMVZ/Unesp veterinary hospital, each animal was individually fasted for water and food for 24 and 48 hours, respectively, before the procedure. Prior to castration, a physical examination was performed and bulls were fasted for water and feed for 24 and 48 h, respectively. Bulls underwent general anesthesia using xylazine (0.05 mg/kg, Xilazin, Syntec do Brasil Ltda, Santana do Parnaíba SP, Brazil) administered intravenously (IV) and induced with ketamine (2.5 mg/kg, Dopalen, Ceova Saúde Animal Ltda, Paulínea, SP, Brazil) and diazepam (0.05 mg/kg, Compaz, Cristália, São Paulo, SP, Brazil) IV. Anesthesia plane was maintained with isoflurane (Isoforine, Cristália, São Paulo, SP, Brazil) in oxygen (15 L/min) using a large animal anaesthetic machine (Model 2800C, Mallard Medical, Redding, CA, USA). Flunixin meglumine (1.1 mg/kg, Banamine, MSD Saúde Animal, Cruzeiro, SP, Brazil) was administered intramuscularly (IM), and xylazine (0.05 mg/kg diluted to a volume of 20 mL with saline 0.9%) was administered epidurally at the level of the sacrococcygeal intervertebral space to alleviate peri-operative and operative pain. Once cattle reached a stage 3 steady anesthetic plane, a bilateral scrotal incision was made using a scalpel blade and both testicles were completely removed. All cattle received morphine immediately post-castration (0h; 0.1 mg/kg IM), and flunixin meglumine (1.1 mg/kg) was administrated IM at 24, 48 and 72h post-castration.

Pain Assessment

The primary pain assessment tool used in this study was the UCAPS (Table 4). This tool is currently used in research and published in open-access journals under the Creative Commons license (http://creativecommons.org/licenses/by/4.0).

Pain behaviour was assessed continuously for three consecutive minutes at five time-points (Fig 3).

Behavioral assessment methodologies

Cattle pain was assessed using the UCAPS via two behavioral methodologies:

1) Real-time assessment

Two veterinarians with experience in pain assessment (A.R.O. and R.M.T.) performed all real-time evaluations at each of the aforementioned time-points. Before starting the assessment, the evaluators received training and guidelines about how to use the UCAPS. Two training sessions were performed. The first training session consisted of an introduction and overview of the UCAPS. Videos were reviewed that exemplified behaviors for each item and observers discussed each video (https://animalpain.org/en/boisdor-en/). The second training session was conducted in which observers assessed twenty videos of pre and postoperative time-points. Each video was assessed, and scores were compared between the observers and discussed if results varied significantly.

For the real-time data collection, evaluators stood within 1 meter from the paddock fence where animals were allocated to observe them. Evaluators did not talk to each other and minimized any movement during the assessment. After observing, the evaluators completed data collection in the following sequence: a) ‘Would you provide rescue analgesia according to your clinical experience?’ If yes, mark ‘1’,...
if no, mark ‘0’; and b) UCAPS scoring (Table S3). Real-time data collected occurred between March 18th and April 29th of 2019. Evaluators were aware of the time-points and procedure when scoring in real-time.

2) Video-recorded assessment

Video was recorded using a high-definition video camera (Canon PowerShot SX50 HS, Oita, Japan) placed outside the outdoor pen, 1-2 meters from the fence, using a camera tripod. A total of 95 videos (3min in duration) were collected at the same time that animals were assessed using the real-time methodology. The video-recorded pain assessment was conducted more than six months after the real-time assessment by the same evaluators (A.R.O. and R.M.T.). Evaluators were masked to time-points, and order of observation was randomized. Upon watching the video, evaluators completed data collection in the same order as real-time assessment. Evaluators assessed videos a maximum of 1h a day to avoid fatigue. Video analysis occurred from November 22nd to December 22nd, 2019.

Statistical Analysis

Data were analyzed using R software within the integrated RStudio environment (Version 4.1.0; 2021-06-29; RStudio, Inc., Boston, MA, USA). The functions and packages used were presented in the format ‘package::function’ corresponding to the computer programming language in R. For all tests, a significance of 5% was considered. All figures were created with a color palette distinguishable by colorblind people (ggplot2::scale_colour_viridis_d).

Modeling was conducted to compare real-time versus video-recorded pain assessments, both considering other effects of experimental design. The histogram plot (stats::hist) and Cameron and Trivedi’s test (overdisp::overdisp) proved the overdispersion (excess of zeros) in UCAPS, requiring a zero-inflated model. Zero-inflated models combine logistic and count distributions in the fixed effects of the same model for a better fit of the data. Then, a multilevel zero-inflated poisson model (glmmTMB::glmmTMB) was identified as the best fit compared with other models according to Bayesian information criterion (stats::BIC). The UCAPS was used as the response variable, while evaluator, and interaction between moments and assessment methods were used as explanatory variables in the model count component (Poisson distribution). Assessment methods were included as explanatory variables in the model logistic component (Bernoulli distribution). Cattle was included as a random effect of the model. In addition, behavioral items from the UCAPS were used as a multilevel generalized linear model adjusted by Poisson distribution (lme4::glmer). For rescue analgesia, a multilevel binomial logistic model (lme4::glmer) with the same fixed effects of the model count component and random effects described previously was used. For all models, the Bonferroni was used to adjust the multiple comparisons to the post-hoc test (lsmeans::lsmeans and multcomp::cld).

Bland-Altman test for repeated measures and Lin's concordance correlation coefficient (CCC) (SimplyAgree::agree_reps) were used to verify the agreement of UCAPS assessed in real-time and video-recorded methodology. A simple linear regression (stats::lm) was conducted to analyze the proportion bias between both assessment methods. Proportional bias represents an increase in the difference between the methods evaluated at higher or lower UCAPS. Then, the difference of UCAPS between the
two assessment methods was used as a response variable and the mean of UCAPS between the two methods was used as an explanatory variable. Heteroskedasticity was tested by Breusch Pagan test (olsrr::ols_test_breusch_pagan).

Intraclass correlation coefficient (ICC), two-way random effects model, type agreement multiple evaluators/measurements, and its 95% confidence interval (CI) (irr::icc) was used to evaluate the inter-rater reliability of the UCAPS. The weighted kappa and its CI (biostatUZH::confIntKappa) was used to investigate the inter-rater reliability of the rescue analgesia.

Chi-square test (stats::chisq.test) was applied to analyze the relationship of the rescue analgesia between real-time and video-recorded assessment.

All data analyzed during this study are included in this article in its supplementary information files.

References


**Figures**
Figure 1

Plots of Unesp-Botucatu cattle pain scale (UCAPS) comparing time-points (A), assessment methods (B), interaction between moments and assessment methods (C). Different lowercase letters (a>b) indicate statistical difference between the moments, while different capital letters (A>B) indicate difference between the assessment methods according to the multilevel zero-inflated Poisson model.
Figure 2

Bland-Altman plots comparing real-time and video-recorded assessment of the Unesp-Botucatu Cattle Pain Scale (UCAPS). LoA, limit of agreement; CI, 95% confidence interval; solid line represents the bias; dashed line represents the lower and upper LoA; dotted lines represent the 95% confidence interval; CCC, Lin’s concordance correlation coefficient; green line is the simple linear model.
**Figure 3**

Timeline of the time-points used for the video-recorded and real-time pain assessment using the Unesp-Botucatu Cattle Pain Scale (UCAPS). Video recording was performed during 3 minutes at each time-point.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- Data.xlsx
- Supplementarymaterial.docx