RESEARCH REPORT

ANIMAL WELFARE

Title: Validation of scan sampling techniques for pain behaviors in castrated piglets – **NPB #19-065**

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Date Submitted: 9/30/2020

Industry Summary: Pigs in the USA are routinely castrated without any pain relief. Castration is a painful procedure that results in short and long-term physiological and behavioral deviations of the piglet. Alleviating pain is important from a welfare perspective as it falls under one of the five freedoms of animal welfare. However, there are currently no FDA approved products specifically labelled to manage pig pain in the US. To gain FDA approval, a product must be shown to reduce pain in a target species utilizing validated methods of pain assessment. Behavior is a common measure used to assess pain but no behavioral sampling methodologies used to quantify castration pain have been validated. Therefore, the objectives of this study were to validate two different behavioral methodologies (scan sampling and time-interval sampling) to quantify piglet pain after castration. A total of 39 (scan sample) and 16 (time-interval sample) Yorkshire-Landrace × Duroc male piglets (five days of age) were surgically castrated using a scalpel blade. Behaviour frequency and duration of each piglet were collected continuously 24 priors to castration and 1, 8 and 24 hours post-castration. This continuous data was compared to scan sampling intervals (2-min, 3-min, 5-min, 10-min and 15-min) and time sampling intervals (5-min, 10-min, 15-min, 20-min, 30-min and 45-min) to determine if the sampling method accurately reflected true duration and frequency for each behavior using previously established criteria. No scan sampling interval provided accurate estimates for any pain behaviors while the 45-min time interval provided accurate estimates for the affiliative interactions, sitting, walking, huddled up, prostrated, scratching, spasms, and trembling behaviors. Results from this study suggest that scan sampling is not an appropriate method to evaluate pain behavior in piglets but a 45 min time sampling method can be used to investigate piglet behavior post-castration. The results from this study lay the ground-work in establishing validated methodologies. Future research and drug approval trials will likely need to utilize a multimodal approach to effectively and accurately evaluate pain behavior associated with castration.

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Key Findings:

- Surgical castration is a painful procedure routinely performed on piglets
- Pain behaviors associated with castration include decreased standing, nursing and walking, and increased trembling, scratching and tail wagging
- No study has validated the accuracy of scan sampling and time sampling methodogolies used to quantify pain behavior in piglets
- No scan sampling interval provided accurate estimates for any pain behaviors while the 45-min time interval provided accurate estimates for the affiliative interactions, sitting, walking, huddled up, prostrated, scratching, spasms, and trembling behaviors.
- Future research and drug approval trials will likely need to utilize a multimodal approach to effectively and accurately evaluate pain behavior associated with castration.

Keywords: animal welfare, behaviour, castration, pain, swine, validation

Scientific Abstract:

Scan sampling methodology

Surgical castration is a painful procedure that is routinely performed without pain relief on commercial pig (Sus scrofa domesticus) farms. Previous research has focused on quantifying piglet pain response through behaviours. However, to date, behavioural sampling methodologies used to quantify pain associated with castration have not been validated. Therefore, the objective of this study was to validate scan sampling methodologies (2-min, 3-min, 5-min, 10-min and 15-min intervals) to quantify piglet pain responses expressed by castrated piglets' behaviour. A total of 39 Yorkshire-Landrace × Duroc male piglets (five days of age) were surgically castrated using a scalpel blade. Behaviour frequency and duration (scratching, spasms, stiffness, tail wagging and trembling) of each piglet were continuously collected for the first 15 min of the following hours relative to castration (–24, 1–8 and 24). To determine if the sampling interval accurately reflected true duration and frequency for each behaviour, as determined by continuous observation, criteria previously utilised from other behavioural validation studies were used: coefficient of determination above 0.9, slope not statistically different from one and intercept not statistically different from zero. No scan sampling interval provided accurate estimates for any behavioural indicators of pain. The results of this study suggest that continuous sampling is the most appropriate methodology to fully capture behaviour specific to pain associated with castration. Using validated behavioural methodologies in future research can assist in the development of objective, science-based protocols for managing pig pain.

Time interval sampling methodology

Surgical castration is a painful procedure routinely performed on piglets. Behavioural deviations post-castration include decreased standing, nursing and walking, and increased trembling, scratching and tail wagging. However, no study has validated the accuracy of time sampling intervals (TS) in which behaviors are continuously scored at defined intervals compared to continuous recording (CR) on piglet castration pain. Therefore, the objective of this study was to validate the accuracy of six time sampling methods (5, 10, 15, 20, 30, and 45 min) to quantify piglet behaviour post-castration when compared to CR for 1-hour post-castration. Sixteen Yorkshire-Landrace x Duroc piglets were surgically castrated. Frequency and duration of maintenance and pain behaviours for each piglet were CR for one-hour post-castration. Subsequently, CR data was divided into data subsets for each defined interval and the true proportion and frequency for each behaviour was tested using a generalized linear mixed model and linear regression analysis (Coefficient of determination >0.9, slope not different from 1 and intercept not different from 0). For the generalized linear mixed model, 30 and 45 min time sampling methods were not different when compared with CR for all behaviours. For the linear regression analysis, affiliative interaction, sitting, walking, huddled up, prostrated, scratching, spasms, and trembling behaviours met the pairwise comparison accuracy criteria at the 45 min time sampling. Results from this study indicate that a 45 min time sampling method may be the optimal time point to investigate piglet behaviour post-castration dependent upon the behaviour of interest and experimental design setup.This should be a scientific description limited to one page in length to describe your project and its results.

Introduction:

Scan sampling methodology

Pain is a clinically important condition that adversely affects animal welfare (Mellor 2016; Yeates 2016). Animals in pain experience a negative mental state and, when left uncontrolled, pain can result in deleterious consequences to the animal's physical health and productivity (Hellebrekers 2000; Telles et al 2016). Veterinarians in the United States have an ethical obligation and must take an oath to eliminate or alleviate pain when necessary, including pain as a result of standard husbandry practices, such as castration. Physical castration is a painful procedure commonly performed on commercial swine farms in North America during the first week of life (Dzikamunhenga et al 2014; O'Connor et al 2014). From a behavioural standpoint, castration has been scientifically quantified by either observing deviations in the pig's (Sus scrofa domesticus) normal behavioural repertoire (eg lying, locomotion, nursing; Weary et al 2006) or identifying an increased frequency or expression of behavioural indicators of pain specific to castration (eg prostrated, scratching, trembling; Hay et al 2003). Behaviour has been used extensively in the literature to quantify pain response and determine efficacy of pain mitigation strategies (ie pharmaceutical intervention). In fact, literature evaluating behaviours induced by pain associated with castration have supported the development of standards and recommendations for castration pain management on an international scale (Primary Industries Standing Committee 2008; European Commission 2010; American Veterinary Medical Association 2013; National Farm Animal Care Council 2014; National Pork Board 2018; New Zealand Government 2018).

However, behavioural methodologies used to quantify piglet pain responses to castration vary dramatically (Table 1). This is particularly noteworthy when evaluating previous studies that have used the same behaviours to quantify pain associated with castration as in the present study. In addition, no studies to date have validated the accuracy of behavioural methodologies used in previous work. When assessing animal behaviour, scientists select methods based on a variety of factors, including, but not limited to, sample size, behaviour of interest and total observation time (Hepworth & Hamilton 2001; Lendvai et al 2015). The continuous sampling methodology is considered the most accurate for behaviour data collection as the individual animal is observed for the entirety of the observation period (Altmann 1974; Czerwinski et al 2017). This results in a complete data set that includes total frequency and duration for each behaviour of interest (Lehner 1992). However, time and labour constraints limit the use of this methodology, particularly in studies with large populations. To mitigate this, scan sampling, a methodology in which behaviours are recorded at selected time-points, allows scientists to increase throughput and minimise labour requirements (Martin & Bateson 1993). However, if these alternatives are to be used to quantify behavioural indicators of pain associated with castration and support on-farm recommendations, validating the accuracy of each methodology is critical. Therefore, the objective of this study was to validate behavioural methodologies using five different scan sampling intervals (ie 2-min, 3-min, 5-min, 10-min, and 15-min) compared to continuous sampling in castrated piglets.

Time interval sampling methodology

In the United States (U.S.) alone, approximately 94 million pigs are castrated annually to prevent unwanted breeding (Hansson et al 2011), reduce aggression (Rydhmer et al 2010) and improve meat quality (Lundstrom et al 2009; Povod et al 2019). This routine husbandry procedure is painful as identified by changes to piglet physiological and behavioural responses (Prunier et al 2006; Moya et al 2008; Van Beirendonck et al 2011; Yun et al 2019). However, in the U.S., pain mitigation is not commonly administered to piglets undergoing castration (Hannson et al 2011; Sutherland et al 2012). Furthermore, there are currently no Food and Drug Administration (FDA) approved drugs specifically labelled for pain management in swine to date (Bates et al 2014; Wagner et al 2020).

One criteria that must be met to achieve FDA approval is that the animal drug must be effective for its intended use as stated on the product label. As deviations from normal behaviour are important indicators to assess pain (Anil et al 2005), behavioural analysis is a critical component for pain assessment in animals (Weary et al 2006). However, methods used to quantify these behavioural deviations vary greatly and have not been validated (O'Connor et al 2014; Ison et al 2016). Although continuous recording (CR) is considered the "gold standard" given it captures an animal's entire behavioural repertoire, time and labor constraints limit the use of this methodology (Mitlöhner et al 2001; Ross et al 2019). Time sampling, defined as periodic recording and continuous scoring of a behaviour at defined intervals (Arnold-Meeks & McGlone, 1986; Mitlöhner et al 2001; Madruga et al 2017) may be a viable alternative to CR. Although behavioural methodologies have been validated in sheep (Pullin et al 2017a,b), beef cattle (Mitlöhner et al 2001; Madruga et al 2017), dairy cattle (Miller-Cushon & DeVries, 2011; Chen et al 2016) and poultry (Daigle & Siegford, 2014; Kristensen et al 2007), only four studies to date have validated such methodologies in swine (Whalin et al 2016; Arnold-Meeks & McGlone, 1986; Bowden et al 2008, Park et al 2020). Of these studies, none have validated the accuracy of time sampling methods to detect changes in castrated piglets' behaviour post-procedure. Utilizing non-validated methodologies poses a risk for inaccurate data collection and lead to inaccurate conclusions related to drug efficacy specific to castration pain. Therefore, the objective of this study was to validate the accuracy of six time sampling methods (5, 10, 15, 20, 30, and 45 min) to quantify piglet behaviour post-castration when compared to CR for 1-hour post-castration.

Objectives:

1. Validate the accuracy of five different behavioral sampling methodologies (i.e. 5-min, 10-min, 15-min, 20-min and 25-min intervals) when compared to a continuous sampling methodology for pain behaviors expressed post castration in piglets.

2. Develop and validate a behavioral pain scale in piglets undergoing surgical castration.

Materials & Methods:

Scan sampling methodology

Data for the present study were derived from a portion of a larger data set (Viscardi & Turner 2018a,b, 2019) and behavioural data were not collected for the primary purposes of addressing the objectives of the present study. All animal use and procedures were approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol #3350). This institution is registered under the Animals for Research Act of Ontario (1990) and holds a Good Animal Practice certificate issued by the Canadian Council on Animal Care.

Study animals and housing

A total of 39 Yorkshire-Landrace × Duroc male piglets from 32 litters (aged five days old and weighing a mean [± SEM] of 2.20 [± 0.38] kg and with an average litter size of 13) were used in this study across three separate trials. Piglet number was based on exclusion criteria that disqualified data from any piglet that had been provided with an analgesic drug intervention. All piglets, regardless of weight, were included in the original study and no crossfostering was implemented. Sow breed was selected since it represented the most common genetic profile used commercially in Canada and the United States and piglet age was selected for five days as it also represented typical commercial production standards. Sows and piglets were housed in farrowing rooms at the University of Guelph Arkell Swine Research Station. Within each room, sows were housed in farrowing pens (floor space: 1.8 × 2.4 m [length × width]; farrowing crate: 0.8 × 2.3 m). Farrowing crates were utilised as these are the most commonly available systems used in Canada and the US. Light was provided daily between the hours of 0700 and 2100h. Creep areas for piglets were heated to approximately 30–35ºC using a heating pad or lamp. Sows had ad libitum access to feed (their diet met or exceeded National Research Council [NRC 2012] nutrient requirements for lactating sows) beginning four days after farrowing and ad libitum access to one water nipple.

Behavioural measurements

Behavioural collection was conducted as described by Viscardi et al (2017), Viscardi and Turner (2018a,b, 2019). Video was recorded pre-procedure for 1 h using a high definition video camera (JVC GZ-E200 full HD Everio Camcorder, Yokohama, Japan) mounted on a tripod outside the farrowing pens. The positioning of the camera was unable to provide complete coverage of the entire pen for the trial. On average, 10% of the time, behaviours were unable to be recorded due to piglets being out of view. Immediately post-castration, piglets were recorded continuously for 8 h, and again for 1 h, 24 h post-procedure. Behavioural pain responses for each piglet were scored continuously for the first 15 min of every hour, utilising an ethogram adapted from Hay et al (2003) (Table 2). A total of 150 min of behaviour was analysed for each piglet. All continuous behaviour data were collected by six trained observers using the Observer XT programme (Version 12.0, Noldus Information Technology, Wageningen, The Netherlands). Videos were randomised and assigned to observers who were masked to time-point. Inter-observer reliability was assessed at three time-points during the behaviour-scoring period in which all participants scored the same piglet in a video and an intra-class correlation coefficient (ICC) was calculated. All inter-observer reliability tests throughout the trial produced an ICC above 0.9, indicating excellent agreement between scorers and no significant drift throughout the scoring period.

Sampling methods

Utilising the continuous sampling data set, video data were divided into 1-min intervals. These intervals were used to extrapolate five scan sampling data sets (ie 2-min, 3-min, 5-min, 10-min, 15-min). For the continuous sampling method, the data were first collected by watching the piglets continuously, then, the summation of counts and the average proportion of time spent performing each behaviour were calculated. The 2-min scan used every second 1-min interval to calculate the total occurrence and average proportion of time that the behaviour was performed. The 3-min, 5-min, 10-min and 15-min scan used every third, fifth, tenth and fifteenth 1-min interval, respectively. For each methodology, all sampling began at the 0-min interval.

Time interval sampling methodology

Animal use and procedures were approved by North Carolina State University Animal Care Committee (Animal Utilization Protocol #19-796).

Study animals and housing

Sixteen Yorkshire-Landrace x Duroc male piglets from four litters (five days old, 1.62 ± 0.26 kg BW) were used in this study. Twenty-four hours following birth, piglets were enrolled in the study, weighed and individually identified using a permanent marker on the back that was visible on camera. All male piglets, regardless of weight, were included in the study. Sow breed was selected as it represented the most common genetics used commercially in the US and piglet age at castration (5 days) was selected based on typical commercial production standards.

Sows and piglets were housed in one farrowing room at North Carolina State University (Raleigh, North Carolina, USA). Within each room, sows were housed in farrowing pens (floor space: 0.8 x 2.3 m [length x width]; farrowing crate: 0.8 x 2.3 m). Temperature in the farrowing room was maintained at 22º ± 1.0º C. Farrowing crates were chosen as these are the most commonly available systems used in the US. Light was provided daily from 0630 to 1630 h. Creep areas for piglets were heated to ~30-35°C using heating lamps. Sows had ad libitum access to one water nipple and ad libitum access to feed (their diet met or exceeded National Research Council [NRC, 2012] nutrient requirements for lactating sows) beginning the day they farrowed.

Processing procedure

On the day of castration, male piglets within a litter were separated from their littermates and placed into a transport cart. Piglets were picked up, held by the hind legs, and placed under the arm of the castrator to minimize piglet movement. Using a 'two vertical incision' approach, piglets were surgically castrated by one-trained personnel. Briefly, a 2.5-3.8 cm incision was made over each testicle and the spermatic cords were torn to remove the testicles from the body (no spermatic cords were visible after castration). All castrations were performed between 0800-1000 h on the same day. Piglets were returned to the farrowing crate immediately post-castration and had complete access to the sow.

Behavioral data collection

Video was recorded using high definition video cameras (AMCREST IP3M-943B; Houston, TX, USA) mounted and angled above the farrowing crates. A total of 60 minutes of video was recorded and analyzed one-hour post-castration for each piglet. All behaviours (Table 1) were analyzed from pre-recorded video continuously by two trained observers using the Observer XT program (Version 14.0: Noldus Information Technology, Wageningen, Netherlands). Inter-observer reliability was assessed prior to data collection. Briefly, both observers scored the same piglet at three time-points during the behaviour-scoring period to calculate an intra-class correlation coefficient (ICC). Inter-observer reliability testing resulted in an ICC above 0.9 for all time-points, indicating excellent agreement between observers and no significant drift throughout the scoring period. Camera position inhibited complete coverage of the entire pen. Duration of time in which piglets were 'not in view' has been included in the percentage of time performing each behaviour to accurately estimate the percentage (100% in total) and total counts (number of times it occurred).

Sampling methods

For the continuous sampling method, the data were first collected by watching the piglets continuously, then, the summation of counts and the average percentage of time spent performing each behaviour were calculated. Following this, the continuous data set was subdivided into the first 5, 10, 15, 20, 30, and 45 min as individual time intervals. For each methodology, all sampling began at the 0-min of the hour.

Statistical analysis

Behavioural data were analyzed using SAS software version 9.4 (v9.4; SAS Institute Inc. Cary, NC, USA). To determine if the time sampling method accurately reflected true proportion and frequency for each behaviour, as determined by CR, two statistical tests were performed.

A Generalized Linear Mixed Model (PROC GLIMMIX) procedure in SAS (v9.4; SAS Institute Inc. Cary, NC, USA) was used to determine differences between behaviours in time sampling methods (5 min, 10 min, 15 min, 20 min, 30 min, 45 min) and CR. The model included time sampling method, piglet, and pen as fixed effects and piglet within pen was included as a random effect. The means for each sampling technique were compared to the 1-min interval using contrast statements. A sampling interval was considered adequate if it was not statistically different from the CR.

To evaluate the accuracy and bias of each time sampling method, a linear regression analysis (PROC REG) was conducted. For each behavior, pairwise comparisons were made between the behavioral estimates from each time sampling method (5 min, 10 min, 15 min, 20 min, 30 min, and 45 min) and the CR. A tested sampling interval considered to accurately estimate the behavior if the following three criteria were met: 1) the coefficient of determination (R2) was > than 0.90, 2) the intercept did not differ from 0 ($P > 0.05$), and 3) the slope did not differ from 1 ($P > 0.05$).

Results:

Scan sampling methodology

No behavioural indicators of pain met all three criteria for any of the scan sampling methodologies investigated in the present study when compared with continuous sampling (Tables 3 and 4). For durations, behavioural indicators of pain including tail wagging and trembling achieved an R2 above 0.90 for 2-min scan sampling interval. However, a slope not different from one was not achieved for either behaviour and an intercept not different from zero was only achieved for trembling. For bouts, trembling was the only behaviour that achieved an R2 above 0.90 for 2-min scan sampling interval. However, neither a slope not different from one nor an intercept not different from zero was achieved. In addition to the assessment of accuracy, duration and bouts for each behaviour by methodology can be found in Table 5

Time interval sampling methodology

Least square means of data obtained by CR and data generated using the time sampling methods expressed in duration of time and total counts are presented on Table 2 and 3, respectively. All maintenance and pain behaviour durations were not different from CR when using the 30 min and 45 min time sampling method ($P > 0.05$). In addition, all maintenance behaviours, except standing and nursing, were not different from CR when using the 20 min time sampling method ($P > 0.05$). Lying, sitting and aggression were not different from CR when using the 15 min, 5 min, and any time sampling method, respectively ($P < 0.05$; Table 2). Similarly, for pain behaviours, all time sampling method under 20 minutes demonstrated different durations when compared to CR with the exception of huddled up that was only different when using a 5 min time sampling ($P < 0.05$; Table 2).

Maintenance behaviour counts for sitting, nursing and aggression were not different from CR when using the 15 min, 45 min and 45 min time sampling method respectively ($P > 0.05$; Table 3). All pain behaviour counts were not different from CR when using the 45 min time sampling method ($P > 0.05$). Prostrated and trembling pain behaviours were not different from CR when using the 30 min time sampling method and spasms were not different from CR for any time sampling methods ($P > 0.05$).

For the linear regression analysis, walking and affiliative interaction maintenance behaviours duration and prostrated and huddled up pain behaviours met all accuracy criteria using the 45 min time sampling method when compared with CR (Table 4). The counts for the maintenance behaviour sitting and pain behaviours huddled up, spasms, trembling and scratching met all accuracy criteria using the 45 min time sampling when compared to CR (Table 5). In addition, the counts for spasms met all accuracy criteria for all the time sampling methods greater than 5 minutes (Table 5).

Discussion:

Scan sampling methodology

Throughout the previous 20 years, research has focused on identifying alternative management practices to reduce or eliminate pain associated with castration in piglets (for a thorough review of this topic, see Sutherland 2015). Behaviour is a common metric used to quantify pain associated with castration (von Borell et al 2009) and has been utilised to support the development of pain management protocols implemented on an international scale (Viscardi & Turner 2018a). However, no prior studies have validated the accuracy of these behavioural sampling methodologies used to quantify pain associated with castration. Therefore, the objective of the present study was to validate the use of five scan sampling methodologies, compared with continuous sampling methodology, to assess behavioural indicators of pain in castrated piglets. In this study, no scan sampling methodology was effective for quantifying piglet pain responses through behaviour specific to piglet castration, which is likely due to the nature of the behaviours being evaluated. Behavioural indicators of pain for pigs are categorised as behaviours that are typically short in duration with variable frequency (ie event behaviour; Roughan & Flecknell 2003; Tardin et al 2014; Nielsen et al 2019). Event behaviours are often missed when the observation period is limited and continuous sampling is not utilised (Lehner 1987; Pullin et al 2017b; Ross et al 2019; Studd et al 2019). In this study, behavioural indicators of pain were brief in duration, averaging less than 5 s. Short duration behaviours are difficult to detect utilising a scan sampling methodology as the opportunity for behavioural observation is limited. This often results in the underestimation of behaviours, as was demonstrated by the present study. Previous validation studies in poultry (Daigle & Siegford 2014; Ross et al 2019), sheep (Pullin et al 2017a,b) and beef (Mitlohner et al 2001; Madruga et al 2017) demonstrated similar issues when evaluating short duration behaviours, such as preening, drinking and oral manipulation.

In addition to the short duration of these behaviours, the piglets in the present study spent less than 5% of their total time budget expressing behavioural indicators of pain. These results are not unique to this study, as previous work has demonstrated small proportions of total time budgets dedicated to expressing pain (6%: Hansson et al 2011; 8, 6% Viscardi & Turner 2018a, 2019). Infrequent behaviour patterns, such as these, are difficult to accurately assess through scan sampling (Martin & Bateson 2012; Ross et al 2019). Thus, utilising this sampling methodology for short duration, low frequency behavioural patterns yielded poor accuracy and continuous sampling is, therefore, ideal. In contrast, studies that evaluate state behaviours (ie behaviours which occur over a quantifiable time-period; Crews et al 2002; Malachowski & Dugger 2018) are more successful in utilising scan sampling methodologies as the behaviour duration and frequency are consistent enough to be observed periodically. For example,scan sampling methodology validations have been successful in swine, sheep, dairy and beef cattle when evaluating lying behaviour (swine: 15-min scan, Whalin et al 2016; sheep: 20-min scan, Pullin et al 2017a,b; dairy: 30-min scan, Chen et al 2016; beef: 15-min scan, Mitlohner et al 2001, and 30-min scan, Madruga et al 2017). This is also true for sitting and standing behaviours in mature swine (15-min scan, Whalin et al 2016). In addition to the challenge of behavioural indicators of pain being short in duration and infrequent in occurrence, the present study also did not utilise a fully exhaustive ethogram. Two fundamental behavioural indicators of pain that were not evaluated include huddled up, defined as 'lying with at least three legs tucked under body' and prostrated, defined as 'awake, standing or sitting, motionless with head down, lower than shoulder level' (Hay et al 2003). These behaviours are commonly evaluated in piglet castration studies (Hay et al 2003; Llamas Moya et al 2008; Yun et al 2019) with 14 studies to date integrating huddled up and prostrate into the ethogram to assess pain associated with castration (Table 6). Future studies should not only include the additional behaviours of huddled up and prostrated but also include normal behaviours representative of the piglet's behavioural repertoire. The present study also did not include typical behaviours present in a piglet's behavioural repertoire, such as nursing, activity and inactivity. Although these behaviours are less specific to pain, deviations in the frequency and duration of these behaviours are often associated with painful experiences (Kluviers-Poodt et al 2013; de Oliveira et al 2014; Whalin et al 2016). Additionally, maintenance behaviours are often longer in duration compared with behavioural indicators of pain (Rault & Lay et al 2011; Kluviers-Poodt et al 2013; McGlone et al 2016; Sutherland et al 2017; Viscardi et al 2017), potentially facilitating more accurate detection of these behaviours using scan sampling. The results from this study suggest behavioural indicators of pain specific to pain associated with castration should be evaluated using a continuous sampling methodology. Future work validating behavioural methodologies specific to pain associated with castration should utilise a more comprehensive ethogram and the authors suggest the inclusion of common, species-specific maintenance behaviours.

Time interval sampling methodology

Pain management strategies in castrated piglets have become an important area of focus in animal welfare science (as reviewed by Sutherland 2015; Dzikamunhenga et al 2014). Behaviour has been used as a tool to indicate pain post-castration within the initial hours following processing (von Borell et al 2009). Deviations in these behaviours have been assessed to develop guidelines for mitigating pain using pharmaceutical interventions in Canada and the European Union (Viscardi et al 2018). However, methodologies used to assess these behavioural deviations vary greatly (Dzikamunhenga et al 2014; O'Connor et al 2014) and only one study to date has validated the accuracy of behavioural methodologies to quantify castration pain in piglets (Park et al 2020). Therefore, the objective of this study was to evaluate the accuracy of behavioural time sampling methods compared to CR in castrated pigs for one-hour post procedure.

The results of this study demonstrate time sampling methods can be used to accurately evaluate piglet behaviour post-castration. These results are similar to previous work conducted by Arnold-Meeks and McGlone (1986) that demonstrated that a 20 min time sampling method was accurate in assessing multiple pig behaviours (i.e. attacking, feeding, drinking, lying, moving, and standing behaviours). The results of the present study are also similar to other work validating time sampling methods in multiple species including feedlot cattle, beef heifers and layers (10 min/h for 2 h, Mitlöhner et al 2001; 10 min/h for 15 h, Madruga et al 2017; Daigle & Siegford, 2014, respectively).

The results from this study suggest that when implementing criteria for the linear regression analysis, the accuracy of time sampling methods was dependent upon the behaviour of interest. Only three maintenance behaviours included in this study's ethogram met the analysis criteria at the 45 min time sampling method. No studies to date have validated the use of time sampling methods specific to swine, therefore our results cannot be compared. Nevertheless, in contrast to our work, research conducted in feedlot cattle (Mitlöhner et al 2001), beef heifers (Madruga et al 2017) and layers (Daigle & Siegford, 2014) demonstrated that time sampling methodologies were not accurate to assess some behaviours of interest utilizing similar statistical models.

All pain behaviours assessed in this study met all the accuracy criteria at the 45 min time sampling method with the exception of tail wagging. Work conducted by Park et al. (2020) evaluated pain behaviour with scan sampling methodologies and concluded that no scan sampling methodology was accurate in detecting pain behaviours. Differences between the results from the Park study (2020) and this current study are likely due to inherent features of castration pain behaviour given behaviours associated with castration are short in duration and infrequent (Park et al 2020; Hanson et al 2011; Viscardi & Turner 2019). Given this, time sampling methods may be more appropriate and accurate when evaluating castration pain in comparison to scan sampling.

This work, in conjunction with previous validation studies, that a 45 min time sampling method may be the most realistic methodology to be used for investigating piglet behaviour post-castration. However, this implication is likely dependent upon the behaviour of interest and experimental design setup. In future work, in order to capture all behaviours of interest, scientists may choose to utilize a multimodal approach by selecting different sampling techniques for evaluating behaviour (Daigle & Siegford, 2014).

Figures and Tables

Scan sampling methodology

Table 1 Behavioural sampling techniques for castration pain studies utilising one or more of the pain-associated behaviours defined in the present study ethogram.

Literature cited, first author (year)	Sampling interval (min)	Days observed (post-castration)	Approximate h observed per day
Scan sampling			
Burkemper (2019)	5	3	5
Davis (2017)	60	1	4
Gottardo (2016)	1	1	1
Hansson (2011)	10	1	1
Hay (2003)	10	5	3
Kluivers-Poodt (2013)	12	5	6
McGlone (2016)	15	1	2
Llamas Moya (2008)	3	4	4
Rault (2011)	5	3	4
Sutherland (2010)	1	1	3
Sutherland (2012)	1	1	1
Sutherland (2017)	1	1	3
Continuous sampling			
Hay (2003)	–	4	6
Hug (2018)	–	1	0.17
Van Birendonck (2011)	–	8	0.33
Viscardi (2017, 2018a,b, 2019)	–	1	2
Yun (2019)	–	2	0.5

Table 2 Castration pain ethogram.

Behaviour	Definition
Scratching	Rubbing the rump against the floor, pen walls or littermates
Spasms	Quick and involuntary contractions of the muscle
Stiffness	Lying with extended and tensed legs
Tail wagging	Tail movement from side-to-side (or up and down)
Trembling	Shivering, as with cold

Adapted from Viscardi *et al* (2017), Viscardi and Turner (2018a,b, 2019) and Hay et *al* (2003).

Table 3 R^2, slope P-value and intercept P-value for behavioural indicators of pain durations at each scan sampling interval*.

Behaviour	2-min	3-min	5-min	10-min	15-min
Scratching					
R^2	0.87	0.58	0.52	0.41	0.15
Slope	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Intercept	0.10	< 0.01	< 0.01	< 0.01	< 0.01
Spasm					
R^2	0.78	0.46	0.35	0.12	0.10
Slope	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Intercept	0.02	< 0.01	< 0.01	< 0.01	< 0.01
Stiffness					
R^2	0.72	0.67	0.53	0.38	0.29
Slope	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Intercept	0.02	< 0.01	< 0.01	< 0.01	< 0.01
Tail wagging					
R^2	0.91	0.80	0.67	0.49	0.32
Slope	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Intercept	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Trembling					
R^2	0.91	0.48	0.62	0.16	0.46
Slope	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Intercept	0.09	< 0.01	< 0.01	< 0.01	< 0.01

* The sampling intervals were considered accurate if they met three criteria: $R^2 \geq 0.9$, slope was not different from one ($P > 0.05$), and intercept was not different from zero ($P > 0.05$; Chen *et al* 2016).

Table 4 R^2, slope *P*-value and intercept *P*-value for behavioural indicators of pain bouts at each scan sampling interval*.

Behaviour	2-min	3-min	5-min	10-min	15-min
Scratching					
R^2	0.84	0.68	0.53	0.24	0.14
Slope	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Intercept	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Spasm					
R^2	0.82	0.39	0.54	0.24	0.11
Slope	0.01	< 0.01	< 0.01	< 0.01	< 0.01
Intercept	0.01	< 0.01	< 0.01	< 0.01	< 0.01
Stiffness					
R^2	0.83	0.69	0.69	0.38	0.25
Slope	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Intercept	0.04	< 0.01	0.04	< 0.01	< 0.01
Tail wagging					
R^2	0.88	0.78	0.63	0.46	0.30
Slope	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Intercept	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Trembling					
R^2	0.94	0.80	0.69	0.28	0.34
Slope	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Intercept	0.04	< 0.01	0.08	< 0.01	< 0.01

* The sampling intervals were considered accurate if they met three criteria: $R^2 \geq 0.9$, slope was not different from one ($P > 0.05$), and intercept was not different from zero ($P > 0.05$; Chen *et al* 2016).

Table 5 Mean values (± SEM) of duration percentage and frequency for each behavioural indicator of pain performed by castrated piglets across the entire behavioural data collection period.

Behaviour	Continuous	2-min	3-min	5-min	10-min	15-min
Scratching						
Duration	0.16 (± 0.04)	0.16 (± 0.04)	0.14 (± 0.05)	0.13 (± 0.04)	0.15 (± 0.06)	0.09 (± 0.04)
Bouts	3.56 (± 0.83)	1.95 (± 0.46)	1.08 (± 0.30)	0.77 (± 0.23)	0.38 (± 0.15)	0.28 (± 0.12)
Spasms						
Duration	0.10 (± 0.02)	0.09 (± 0.02)	0.05 (± 0.02)	0.08 (± 0.02)	0.03 (± 0.02)	0.05 (± 0.02)
Bouts	3.13 (± 0.75)	1.44 (± 0.38)	0.62 (± 0.17)	0.67 (± 0.24)	0.23 (± 0.10)	0.15 (± 0.07)
Stiffness						
Duration	0.33 (± 0.08)	0.25 (± 0.06)	0.40 (± 0.10)	0.39 (± 0.10)	0.50 (± 0.15)	0.34 (± 0.11)
Bouts	4.77 (± 1.08)	2.21 (± 0.54)	1.64 (± 0.38)	1.51 (± 0.34)	0.85 (± 0.21)	0.72 (± 0.21)
Tail wagging						
Duration	3.77 (± 0.51)	3.64 (± 0.51)	4.13 (± 0.61)	3.76 (± 0.51)	4.63 (± 0.71)	3.41 (± 0.61)
Bouts	73.13 (± 5.85)	38.26 (± 3.23)	25.97 (± 2.29)	19.54 (± 1.83)	12.08 (± 1.33)	9.33 (± 1.12)
Trembling						
Duration	0.22 (± 0.07)	0.24 (± 0.08)	0.11 (± 0.05)	0.25 (± 0.09)	0.12 (± 0.08)	0.32 (± 0.13)
Bouts	4.49 (± 1.57)	2.90 (± 1.07)	1.03 (± 0.44)	1.05 (± 0.37)	0.28 (± 0.11)	0.64 (± 0.24)

Table 6 Pain-associated behaviours included in piglet pain castration studies utilising one or more of the behavioural indicators of pain defined in the present study ethogram.

Literature cited, first author (year)	Huddled up	Prostrated	Scratching	Spasms	Stiffness	Tail wagging	Trembling
Burkemper (2019)	×	×	×	–	–	×	×
Davis (2017)	–	×	–	–	–	–	×
Gottardo (2016)	–	–	×	–	–	×	×
Hansson (2011)	×	×	×	×	×	–	×
Hay (2003)	×	×	×	×	×	×	×
Hug (2018)	–	–	×	–	–	–	×
Keita (2010)	–	×	–	–	–	×	×
Kluviers-Poodt (2013)	×	×	×	×	×	×	×
Llamas Moya (2008)	×	–	×	×	–	–	×
McGlone (2016)	–	×	–	–	–	–	×
Rault (2011)	×	×	×	×	×	×	×
Sutherland (2010)	×	–	×	–	–	–	–
Sutherland (2011)	×	–	×	–	–	–	–
Sutherland (2017)	×	–	×	–	–	–	–
Viscardi (2017)	–	–	×	×	×	×	×
Viscardi (2018a,b)	–	–	×	×	×	×	×
Viscardi (2019)	–	–	×	×	×	×	×
Yun (2019)	–	×	×	–	–	×	–

× Study used pain-associated behaviour;
– Study did not use pain-associated behaviour.

Time interval sampling methodology

Table 1. Piglet post-castration ethogram

Behaviour	Definition
Maintenance	
Affiliative interaction	Snout is in contact with littermate. Characterized by performing small chewing movements with mouth.
Aggression	Direct physical contact with another animal involving head butts, pushing, biting or chasing. Characterized by rapid and continuous succession
Lie down	Motionless; lying down with body weight supported by side or belly and legs outward, away from belly.
Nursing on sow	Snout (nose/mouth) in contact with the sow's udder, leaning against it and/or teat in mouth. Characterized by vigorous and rhythmic head movements.
Stand inactive	Motionless; weight supported by four legs with head above shoulder level
Sit	Motionless; body weight supported by hind-quarters with head above shoulder level
Walk	Moving forward one leg at a time
Pain	
Huddled up	Lying with at least three legs tucked under body
Prostrated	Awake, standing or sitting, motionless with head down, lower than shoulder level
Scratching	Rubbing the rump against the floor, pen walls or littermates
Spasms	Quick and involuntary contractions of the leg muscles while standing
Tail wagging	Tail movement from side to side (or up and down). Characterized by quick succession of movements.
Trembling	Shivering entire body, as with cold
Not in view	Piglet is unable to be seen on video. Piglet may be blocked by sow or out of picture.

Adapted from Hay et al 2003, Sutherland et al 2012, and Viscardi & Turner 2019.

Table 2. Least squares means of piglets behaviours measured with continuous recording (CR) and different time sampling methods, expressed in percentage of time over 1 h post-castration.

		Time sampling							
Behaviours	CR[1]	5 min	10 min	15 min	20 min	30 min	45 min	SEM	*P*-value
Maintenance									
Affiliative Interaction	0.78	5.15*	3.16*	2.12*	1.59	1.10	0.74	0.693	0.001
Aggression	0.01	0.00	0.05	0.04	0.03	0.02	0.01	0.297	0.430
Lying	42.48	17.35*	28.32*	37.63	42.54	44.09	45.34	6.146	0.001
Nursing	14.07	2.92*	1.65*	2.14*	2.04*	9.17	15.15	2.076	0.001
Standing	8.91	36.31*	26.59*	21.14*	17.04*	12.52	8.71	3.954	0.001
Sitting	1.26	1.29	3.76*	2.91*	2.34	1.98	1.42	0.701	0.003
Walking	4.96	10.46*	8.51*	7.35*	6.55	6.35	4.93	1.228	0.001
Pain									
Huddled up	16.85	3.79*	13.66	16.28	19.72	18.89	17.59	0.693	0.001
Prostrated	3.64	21.35*	13.47*	9.74*	7.66	5.54	3.85	2.096	0.001
Not in view	7.03	1.40*	0.85*	0.67*	0.50*	0.33*	2.25*	0.604	0.001

[1]Means in rows with the superscript * differ statistically ($P < 0.05$) from CR.

Table 3. Least squares means of piglets behaviours measured with continuous recording (CR) and different time sampling methods, expressed in total counts over 1 h post-castration.

Behaviours	CR[1]	Time sampling 5 min	10 min	15 min	20 min	30 min	45 min	SEM	*P*-value
Maintenance									
Affiliative Interaction	4.62	2.75*	3.44*	3.44*	3.50*	3.81*	3.81*	0.905	0.001
Aggression	0.13	0.00	0.06	0.13	0.13	0.13	0.13	0.108	0.430
Lying	11.75	0.56*	2.19*	3.69*	5.00*	6.75*	9.69*	0.740	0.001
Nursing	4.44	0.13*	0.19*	0.31*	0.44*	1.81*	3.75	0.335	0.001
Standing	0.38	0.00*	0.06*	0.13	0.19	0.31	0.38	0.173	0.080
Sitting	30.44	9.50*	14.00*	17.00*	18.63*	22.88*	24.69*	3.273	0.001
Walking	29.00	7.31*	11.00*	14.00*	15.50*	21.25*	23.69*	3.058	0.001
Pain									
Huddled up	5.06	0.31*	1.19*	1.75*	2.44*	3.50*	4.25	0.615	0.001
Prostrated	14.24	6.47*	8.59*	9.35*	10.06*	12.06	1.29	2.446	0.001
Scratching	0.13	0.00	0.00	0.00	0.00	0.00	0.13	0.069	0.043
Spasms	0.81	0.25*	0.69	0.69	0.69	0.75	0.81	0.423	0.049
Tail Wagging	20.63	2.94*	5.25*	7.38*	8.63*	12.31*	15.75	4.028	0.001
Trembling	6.31	0.00*	0.31*	0.44*	1.69*	3.75	5.56	1.561	0.001
Not in view	2.63	0.31*	0.44*	0.69*	0.69*	0.69*	1.06*	0.287*	0.001

[1]Means in rows with the superscript * differ statistically ($P < 0.05$) from CR.

Table 4. R^2, slope *P*-value and intercept *P*-value for piglets behaviours expressed in percentage of duration over 1 h post-castration procedure at each time sampling.

	Time Sampling					
Behaviours	5 min	10 min	15 min	20 min	30 min	45 min
Maintenance						
Affiliative Interaction						
R^2	0.72	0.89	0.89	0.89	0.91	0.91
Slope	<0.01	<0.01	<0.01	<0.01	<0.01	0.99
Intercept	0.44	0.78	0.78	0.77	0.84	0.84
Aggression						
R^2	0.00	1.00	1.00	1.00	1.00	1.00
Slope	.	<0.01	<0.01	<0.01	<0.01	<0.01
Intercept	0.33	.	.	.	.	.
Lying						
R^2	0.08	0.38	0.53	0.54	0.77	0.95
Slope	0.01	0.03	<0.01	<0.01	<0.01	0.02
Intercept	<0.01	<0.01	<0.01	<0.01	0.02	0.22
Nursing						
R^2	<0.01	0.01	0.01	0.02	0.27	0.86
Slope	<0.01	0.01	0.01	0.02	0.02	<0.01
Intercept	<0.01	<0.01	<0.01	<0.01	<0.01	0.04
Standing						
R^2	0.28	0.50	0.55	0.74	0.87	0.84
Slope	<0.01	<0.01	<0.01	<0.01	<0.01	0.31
Intercept	0.02	0.02	0.05	0.09	0.30	0.31
Sitting						
R^2	0.06	0.65	0.66	0.65	0.97	0.98
Slope	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Intercept	<0.01	0.06	0.08	0.12	0.13	0.25

Table 5. R^2, slope *P*-value and intercept *P*-value for piglets behaviours expressed in total counts over 1 h post-castration procedure at each time sampling.

	Time sampling					
Behaviours	5 min	10 min	15 min	20 min	30 min	45 min
Maintenance						
Affiliative						
Interaction						
R^2	0.79	0.92	0.92	0.92	0.95	0.95
Slope	0.03	0.01	0.02	0.01	<0.01	<0.01
Intercept	0.61	0.78	0.78	0.91	0.58	0.58
Aggression						
R^2	0.00	1.00	1.00	1.00	1.00	1.00
Slope	.	<0.01	.	.	.	.
Intercept	0.33	.	.	.	.	.
Lying						
R^2	0.01	0.33	0.44	0.56	0.59	0.81
Slope	0.75	0.49	0.62	0.79	0.67	0.38
Intercept	<0.01	<0.01	<0.01	<0.01	<0.01	0.02
Nursing						
R^2	<0.01	0.04	0.09	0.11	0.24	0.84
Slope	0.59	0.94	0.94	0.56	0.67	0.49
Intercept	<0.01	<0.01	<0.01	<0.01	<0.01	0.05
Standing						
R^2	0.37	0.55	0.56	0.73	0.97	0.94
Slope	0.16	0.07	0.10	0.04	<0.01	<0.01
Intercept	0.20	0.56	0.87	0.83	0.42	0.14
Sitting						
R^2	0.00	0.47	1.00	0.89	0.96	1.00
Slope	.	0.04	<0.01	<0.01	0.04	.
Intercept	0.16	0.33	.	0.66	0.79	.