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Validation of behavioural sampling techniques for 20-25 kg pigs during transport

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HIGHLIGHTS

- The first observations of the behaviour of weaner pigs during transport.
- Validating the interval of instantaneous scan sampling for recording body postures.
- Validating shorter continuous recording sessions for recording aggression.
- Lying can be sampled appropriately at 15 min intervals or less.
- Aggression can, with caution, be sampled appropriately at 40 min/h.

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ABSTRACT

Monitoring behaviour during transport is challenging due to the design of vehicles and often limited deck height. The aim of the present study was to validate behavioural sampling techniques during transport. This study used data from four journeys, constituting a subset of 32 experimental journeys in a commercial vehicle transporting weaners of 20–25 kg for 23 h. In each journey, behaviour was observed in two compartments holding pigs, each with a deck height of either 60 cm or 90 cm.

Body posture (upright, lying, sitting) was recorded using instantaneous scan sampling at 1 min intervals (considered 'true' occurrence). Based on these, less frequent sampling intervals were created (5, 10, 15, 30, and 60 min) and the resulting percentages of behaviour per hour were compared. Occurrences of inter-pig aggressive events were recorded using continuous sampling. It was investigated whether the occurrence of aggression when observed during selected recording sessions per hour (20, 30 and 40 min/h) differed from observing the whole hour throughout the journey (the 'true' occurrence).

Two methods were used to compare sampling techniques. First, estimates of body posture and aggression were pairwise compared with the 'true' occurrence using regression metrics (RMSE, MAE, R^2). The results showed that instantaneous scan sampling of body posture at 15 min intervals or less did reflect the 'true' values, whereas for aggression use of shorter recording sessions of 20 min/h generally did not reflect the 'true' values.

Secondly, similar results were achieved by using mixed effects logistic regression. These models showed that sampling every 30 min differed from the 'true' values, whereas scanning in 5-, 15- or 60 min intervals did not. Compared with sampling the whole hour, the total duration of aggression was significantly underestimated in all three recording sessions (20, 30 and 40 min/h), while the total frequency of aggression was only significantly underestimated in 20 min/h recording session.

Based on results of this study, the use of instantaneous scan sampling intervals of 15 min or less is considered valid for analyses of body postures during long distance transport of 20–25 kg pigs. Due to the sporadic occurrence of aggressive interactions, continuous sampling using recording sessions of 20 min cannot be recommended. Using longer recording sessions of 30 or 40 min/h should be approached with caution dependent on the specific scientific context.

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1. Introduction

Every year, around 34 million pigs are transported between EU member states, with a growing proportion (77 % in 2021 (European Court of Auditors, 2023)) consisting of weaner pigs weighing approximately 30 kg. These pigs are transported for further production (Dahl-Pedersen and Herskin, 2023). Animal transport is a source of public attention due to potential welfare concerns. Yet, the scientific attention is still limited, especially when considering behaviour during transport and for other pig categories than slaughter pigs (EFSA AHAW Panel, 2022). Pigs of approximately 30 kg are usually transported in multi-deck vehicles of 4 or 5 decks resulting in deck heights of approximately 70 cm and 60 cm, respectively (Herskin et al., 2022). Video monitoring inside multi-deck vehicles transporting pigs is therefore challenging, and the use of power cables for camera instalments is problematic in combination with the hydraulic lifting/lowering of the decks at loading/unloading. Advancements in camera tech, battery capacity, and 3D-printed camera structures have enabled the development of a quick-mount, quick-dismount camera setup (under 60 s) that can be used to monitor behaviour of weaner pigs during transport.

Although often omitted due to time constraints, it is good practice to validate the behavioural sampling technique before using it on a larger scale as done by for example Park et al. (2020) - especially in an alternative setting like during transport. In general continuous sampling is considered most accurate for behavioural data collection (Altmann, 1974). It is well known that instantaneous scan sampling is an efficient technique to record behavioural states e.g., lying and standing (Chen et al., 2016; Pullin et al., 2017). The shorter the interval between scans the more accurate the recording can be, but also more time demanding (Bateson and Martin, 2021). While instantaneous scan sampling is suitable for behavioural states, the method is known to be less suitable for recording discrete events of short duration or rare events, as these behaviours are less likely to occur at the instant of the sampling point e.g., play behaviour (Franchi et al., 2022) or post-weaning drinking behaviour (Kobek-Kjeldager et al., 2021). For such behaviours, continuous sampling should thus be used instead. However, sampling whole periods may be impossible due to time constraints and shorter recording sessions are therefore often selected (Donaldson et al., 2002; Li et al., 2008; van Staaveren et al., 2015; Wickham et al., 2022), based on the expectation or knowledge of the chosen recording session being representative for the whole period of interest.

A recording session of 40 min to observe aggression during the first hour after on-farm mixing of four unfamiliar pigs (9.4 ± 0.25 kg) had an R^2 of 0.9, but less than 0.9 when sampling 20, 10, or 5 min/h (Arnold-Meeks and McGlone, 1986). Similarly, in male piglets during the first hour after castration, behaviours (affiliative interaction, sitting, walking, huddled up, prostrated, scratching, spasms, and trembling) recorded at a 45 min session per hour, but not shorter (5, 10, 15, 20, 30 min), met three predetermined criteria (Robles et al., 2021). Thus, for discrete events of short duration, recording sessions of shorter duration - while still being representative - are possible, but often a relatively large proportion of the period needs to be recorded to give valid estimates (Arnold-Meeks and McGlone, 1986; Robles et al., 2021). The same is likely to be the case during transport. Under farm settings, instantaneous scan sampling of states like body posture has been found to be valid at intervals of 15–30 min or more dense in pigs (Whalin et al., 2016), sheep (Pullin et al., 2017) and dairy cattle (Chen et al., 2016). It is unknown whether similar sampling intervals will be valid during transport, where the movement of the vehicle may result in animals shifting posture more often. On a long journey (defined in European legislation as more than 8 h (Council Regulation (EC) No 1/2005)), it is possible that the occurrence of the behavioural state 'being upright' and the event 'aggressive interaction' either fit a diurnal pattern corresponding to the occurrence of the behaviours on farm, or are limited to specific periods such as for example the initial hours after departure. However, until now, to our knowledge, no studies have examined behavioural sampling techniques during animal transport. The aim of this study was to examine the validity of the different intervals of instantaneous scan sampling on detection of body posture (upright, lying or sitting) and the effects of the length of recording sessions of continuous sampling per hour on the ability to detect the level of aggression during 23 h journeys of 20–25 kg pigs in a commercial animal transport vehicle.

2. Materials and methods

2.1. Experimental design

This methodology study focused on data from a subset of four experimental journeys conducted in Denmark, selected from a larger study consisting of 16 23 h journeys (Herskin et al., 2022). The primary objective of the larger study was to investigate the effects of deck height on behaviour and ventilation measures. The results pertaining to deck heights are reported separately (Herskin et al., 2022). Two video monitored compartments per journey were utilized in the present study with a deck height of 60 cm located in the middle compartment of the trailer and 90 cm located in the rear compartment of the lorry. The currently used commercial deck heights are 60 and 70 cm depending on the number of decks on the vehicle (i.e., 60 cm if five decks and 70 cm if four decks). The larger study was commissioned by the Ministry of Food, Agriculture and Fisheries of Denmark to provide scientific evidence for a potential change in legislation regarding deck heights. Thus, it was not known from the beginning of the present study, which deck height would be considered legal in the near future. The deck heights of 60 and 90 cm were therefore selected to cover the minimum and maximum visibility ranges observed in the larger study.

For practical reasons, due to vertical space and standardisation of ventilation panels in the deck height treatments, only deck 1 and 3 from below held pigs (Fig. 1). To balance potential effects of the location in the vehicle (1st vs. 3rd deck), within lorry and trailer, the placing of the experimental decks was randomly rotated between four different combinations of locations.

The four journeys were selected from 23 h journeys conducted between June and September 2021. The departure time from the herd was approximately 12 noon and arrival at an assembly barn was at approximately 11 in the morning the following day. The experimental journeys took place in Jutland, Denmark and mainly on highway to simulate commercial conditions.

2.2. Animals, housing, vehicle and driver

The observed pigs were crossbred Duroc × (Landrace × Yorkshire) and consisted of a total of 221 pigs with an estimated average weight of 23.8 ± 2.5 kg (min-max: 18.8–32.6) based on weighing at least 32 randomly selected pigs per load. The pigs were approximately 8–12 weeks old at the time of the study. All the pigs originated from the same private supplier and had been housed in pens of 9 m^2 with a mixed flooring composition of approximately 50 % slatted and 50 % solid floor. Before transport, the average stocking density was 22.5 ± 5.8 pigs per pen (min-max: 11–33) resulting in 2.5 ± 0.6 m^2/pig. The pigs had been provided ad libitum access to pelleted commercial feed until the time of departure. Each pen was equipped with one feeder, allowing approximately four pigs to eat simultaneously. Consequently, no systematic pre-transport fasting was implemented. The pigs were herded directly from their home pen to the transport vehicle via a covered pig trailer used as ramp outside the barn. For the video-monitored compartments, the pigs were mixed in a standardised manner by combining pigs from three randomly selected neighbouring pens, with 10, 10, and 7–9 pigs from each respective pen.

Each of the video-monitored compartments held 27–29 pigs and the stocking density followed the maximum allowed density by legislation for long journeys (> 8 h) determined by the average weight of the load of pigs (0.199 m^2/pig for 24 kg pigs) (Bek nr. 26 13/01/2020). The average

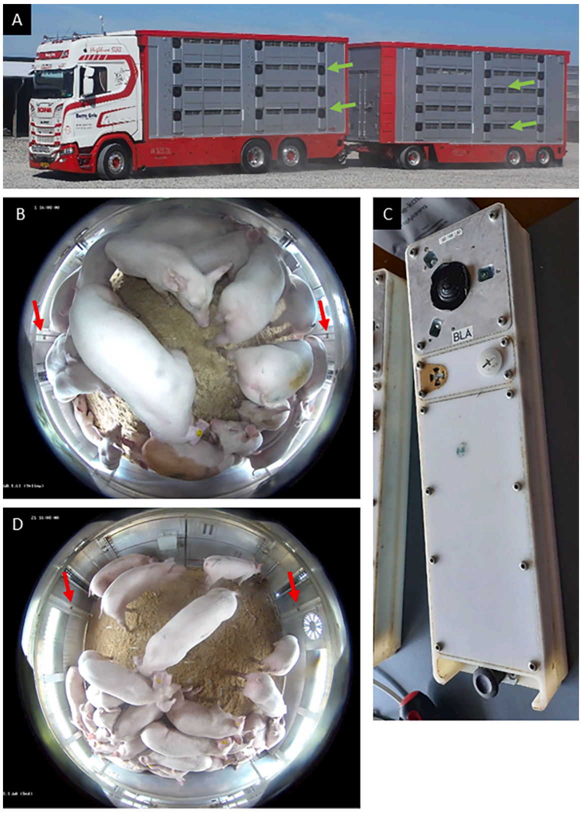

Fig. 1. (A) The 4-deck lorry and 5-deck trailer used to study the validity of behavioural sampling techniques on 20–25 kg pigs during 23 h transport. The green arrows indicate the video-monitored compartments on deck 1 and 3, in the rear compartments of the lorry and the middle compartment of the trailer, respectively. The deck heights in the lorry were 70 cm (commercial condition) and 90 cm (experimental condition) and the deck heights in the trailer were 60 cm (commercial condition) and 80 cm (experimental condition). Panel (B) and (D) are still photos from the video recordings on June 22nd 2021 at 4 in the afternoon from the 60 and 90 cm deck height, respectively. Only deck heights of 60 and 90 cm were analysed in the current study. The red arrows indicate the drinking nipples. Panel (C) Customised camera with fish-eye lens used for video-monitoring in each of the four compartments.

pig weight per load was estimated by weighing at least 32 pigs from the randomly selected pens supplying pigs for the video monitored compartments before loading (at least four pigs from each of eight pens).

One vehicle, a 4-deck lorry with a 5-deck trailer (Finkl, EURO light-Kombi Liner XL; Finkl GmbH, Gersthofen, Germany) was used (Fig. 1). The vehicle was certified for long-distance transport of pigs and equipped with adjustable shutter openings to provide natural ventilation. Additionally, it had SPAL/FAZ fans (Type VA11-BP12/C-29S, 950 m^3/h, Spal Vertrieb, Germany) for mechanical ventilation. To comply with legislation concerning long journeys (Council Regulation (EC) No 1/2005), the vehicle carried water provided via drinking nipples, protruding approximately 1 cm from the side wall of the vehicle. The video monitored compartments had at least one nipple on each side wall (see Fig. 1 panel B and D). As bedding material, 40 kg of sawdust (no. SK111, Agroform A/S, Agerskov, Denmark) was supplied per deck in the summer months, and 60 kg sawdust per deck in the winter months. The vehicle was washed with water and soap (Pre-Max DK, Klartek ApS, Denmark) and subsequently disinfected (Lerasept® Forte, Stockmeier Chemie, Germany) between journeys.

The speed and position of the vehicle were recorded every 5 min using the LinkItAll GPS system 617 (Carrier Transicold Scandinavia A/S, Padborg, Denmark). Departure was determined as the first instance after loading when the vehicle's speed exceeded 0 km/h, as indicated by the logged speed and position. Arrival was defined as the time when the vehicle reached the destination, and its speed was logged as 0 km/h.

The main driver for all 16 23 h journeys was the daily driver of the vehicle. To comply with European legislation (Council Regulation No. 561/2006) on driving time, two drivers were needed for the 23 h journeys.

2.3. Behavioural recordings

Video cameras were mounted approximately in the centre of the ceiling in the rear compartments of the lorry and the middle compartments in the trailer. The video equipment was customised to withstand pig manipulation and to cover the entire compartment area (Fig. 1). The entire compartment was visible for all deck heights, but the field of view could be obstructed by pigs in upright position, especially at the 60 cm deck height.

The cameras used were equipped with fish-eye lenses and comprised both electronic and lens components (HikVision DS-2CD2955FWD-I, Hikvision Headquarters, Hangzhou, China). Each camera body incorporated three white LED diodes, emitting 7.3 lm of light each. These lights remained turned on throughout the entire journey, ensuring sufficient illumination for video recordings even during dark hours.

The videos were used to record the following body postures: upright, lying, sitting and undetermined (refer to the ethogram in Table 1) using instantaneous scan sampling at 1 min intervals (Bateson and Martin, 2021). Bouts of inter-pig aggression were recorded using continuous sampling from departure to arrival (Table 1).

2.4. Statistical analysis

Data were analysed in R v. 4.0.3 (R Core Team, 2022) at a significance level of 0.05. Adjusted *P*-values from post-hoc analysis will be referred to as P_{adj}. Plots were made using ggplot v. 3.4.0.

2.4.1. Criteria for evaluating regression models

Regression metrics can be used to test the validity of sampling rules (Daigle and Siegford, 2014; Chen et al., 2016; Park et al., 2020; Robles et al., 2021). Common metrics are a coefficient of determination R^2 (a value closer to 1 is better), the mean absolute error (MAE) and the root mean squared error (RMSE), where smaller values indicate a better prediction for the latter two metrics (Encyclopedia of Machine Learning, 2010; Hodson, 2022). The acceptance criteria for such regression metrics, i.e., the threshold for being valid, will depend on the specific study aim.

The following criteria based on linear regression have been commonly used, given the residuals are normally distributed: (1) $R^2 \geq 0.9$, (2) the slope does not significantly differ from one ($P > 0.05$), and (3) that the intercept does not differ significantly from zero ($P > 0.05$) (Daigle and Siegford, 2014; Chen et al., 2016; Park et al., 2020; Robles

Table 1
Ethogram for weaners of 20–25 kg during transport for 23 h.

Behaviour	Definition
Upright[1]	The pig's body is supported by legs. The pig can be standing on or over another pig with one or more legs.
Sitting[1]	The pig's body is supported by the hind quarters and stretched front legs. The head is not resting on another pig.
Lying[1]	The pig's body is not supported by legs. The head can be resting on another pig.
Undetermied[1]	Not possible to distinguish whether the pig is upright, lying or sitting.
Aggression[2]	One pig pushing and/or biting at least one other pig for at least 2 s. The agonistic interaction ends when at least 2 s without pushing, or biting has passed. The bouts are scored as either reciprocal (the receiver pushed or bit back) or non-reciprocal (the receiver did not push or bit back).

[1] Instantaneous scan sampling at 1 min intervals.
[2] Continuous sampling.

et al., 2021).

Beyond linear regression, which includes models like logistic regression, there is a wide array of potential R^2 values to consider (Magee, 1990). Selecting the most appropriate one can be challenging due to the absence of a clear-cut criterion. Additionally, determining when an R^2 value can be deemed "satisfactory" depends heavily on the specific context. Interpreting R^2 from logistic regression can be especially challenging for does accustomed to seeing linear regression values as they usually take a much lower range (Shtatland et al., 2002).

2.4.2. Variables of body posture and aggression

The average proportion per hour of pigs in each body posture was calculated based on the 1 min sampling interval (referred to as the 'true' average proportion). Next, for less frequent sampling intervals, average proportions were calculated (5, 10, 15, 30 and 60 min) in order to compare the estimated average proportions of body postures per hour with the 'true' average proportion.

The total frequency and duration of aggression per hour were summarised based on continuous sampling of the whole journey (referred to as the 'true' frequency and duration, respectively). Next, the frequency per hour was estimated based on continuous sampling of selected shorter recording sessions (20, 30 and 40 min from the start of each hour) by multiplying the observed frequency and duration of bouts of aggression for each of the three recording sessions by 3, 2 and 1.5, respectively.

2.4.3. Relationships within sampling intervals and recording sessions

We compared the 'true' average proportion of body postures and 'true' total frequency and duration of bouts of aggression per hour to those estimated by less frequent sampling intervals and shorter recording sessions via a coefficient of determination (R^2), the mean absolute error (MAE), and the root mean squared error (RMSE) as calculated below:

$$R^2_{custom} = 1 - \sum (O_i - P_i)^2 \Big/ \sum (O_i - \overline{O})^2$$

$$RMSE = \sqrt{\sum (O_i - P_i)^2 \Big/ N}$$

$$MAE = \sum |O_i - P_i| \Big/ N$$

where P_i is the predicted value for the ith observation, O_i is the observed value for the ith observation, N is the sample size ($N = 4\ journeys \times 2\ compartments \times 23\ h = 184$) and $\overline{O} = \sum O_i / n$ is the average of the observed values.

Scatterplots were made for each sampling interval of body postures or recording session of bouts of aggression plotted against the 'true' average values per hour ($N = 184$).

2.4.4. Generalised linear mixed models

To examine the impact of sampling intervals on the estimated average proportions of body posture and of the shorter recording sessions, on total duration and frequency of bouts of aggression, we employed two types of generalised linear mixed effects models (GLMM) using glmmTMB v. 1.1.5.

A mixed effects logistic regression, specifically a binomial GLMM with a logit link function, was used to model the proportion of pigs in each body posture (upright, lying, or sitting) out of the total number of pigs in the compartment per hour.

The total frequency of bouts of aggression per hour per compartment was modelled by a log-link mixed effects negative binomial regression, with the logarithm of the recording session included as an offset parameter and using the quadratic parametrisation (nbinom2). The total number of seconds of aggression per compartment each hour was modelled by a corresponding mixed effects negative binomial regression using the linear parametrisation (nbinom1). In all three models the following categorical fixed effects were used: sampling interval (body posture: 1 min, 5 min, 10 min, 15 min, 30 min, 60 min) or recording session (aggression: 20 min/h, 30 min/h, 40 min/h, 60 min/h), hour since departure (1–23) and deck height (60 cm, 90 cm). In all models, a first order autoregressive covariance structure, AR(1), was included to account for repeated measurements from each compartment within journey. Journey was included as a random effect. Effects of deck height on measures of ventilation and natural movements will be reported elsewhere (Herskin et al., 2022). Pairwise comparisons were made between the 'true' values (as the control) and the remaining groups adjusted by the Dunnett method (using emmeans v. 1.7.4.1.). All models were assessed through graphical inspection of observed and fitted values (using vcd v. 1.4.9 and DHARMa v. 0.4.5.). Results on body posture are presented as odds ratios (OR) with 95 % confidence intervals (CI) and results of bouts of aggression are presented as rate ratios (RR) with 95 % CI. A coefficient of determination (R^2_{model}) was calculated for each model for each subset of sampling interval individually compared to the 'true' values using performance v. 0.10.8.1 (i.e., for body postures 1 min intervals vs. 5, 10, 15, 30 and 60 min, respectively and for aggression 60 min/h vs. 20, 30 and 40 min/h, respectively).

3. Results

3.1. Descriptive results

The average percentage ± SD of pigs lying per compartment per hour was 81 ± 27.1 % (median: 93 %, min-max: 0–100 %). See the estimated (and smoothed) development of lying over time for each sampling interval in Fig. 2. The average percentage of pigs being upright per hour per compartment was 12 ± 17.1 % (median 4 %, min-max: 0–96 %), the average percentage of sitting was 3 ± 5.8 % (median: 0 %, min-max: 0–55 %) and the remaining 4 ± 9.6 % (median: 0 %, min-max: 0–85 %) was scored as 'undetermined'. Boxplots of the percentage of pigs lying per hour are presented in Fig. 3 in separate panels for each sampling interval.

The median bouts of aggression were 0 per compartment per hour (min-max: 0–35, average = 3, SD = 6.6) and 137 per journey (min-max: 104–184, average = 140, SD = 37.7). Fig. 4 shows the frequency of bouts of aggression per compartment for each hour of the journey. Of note, almost no aggression was observed during night-time. Out of all the bouts of aggression per journey, the majority, representing 64 ± 19.1 %, were scored as non-reciprocal, while the remaining 36 % were categorised as reciprocal. The reciprocal aggressions had a median bout

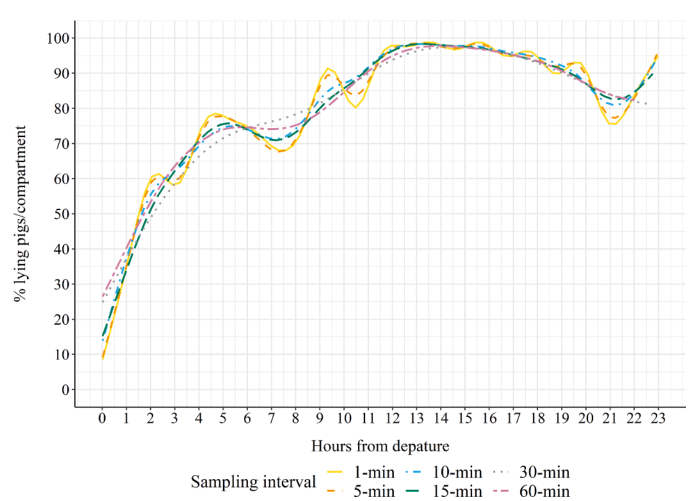

Fig. 2. The smoothed percentage of pigs lying per hour from departure to arrival for each sampling interval (1, 5, 10, 15 30 and 60 min.) during four journeys, each lasting 23 h.

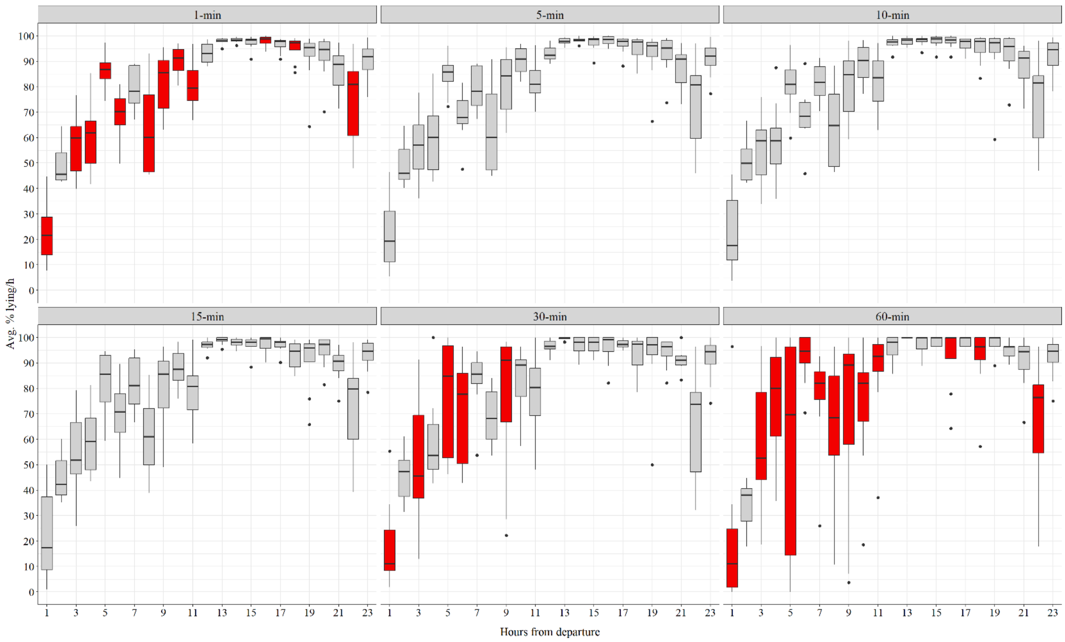

Fig. 3. The raw percentage of pigs lying per hour from departure to arrival for each sampling interval (1, 5, 10, 15, 30 and 60 min.) during four journeys, each lasting 23 h. The red filling indicates differences from the 'true' 1 min sampling of more than 10 units in the median or more than 8 units in the standard deviation.

duration of 10.7 s (min-max: 2–242.5 s) while the median bout duration for non-reciprocal aggression was 5.5 s (min-max: 2–98.0 s).

3.2. Relationships within sampling intervals and recording sessions

Scatterplots of the relationship between the sampling intervals for lying are shown in Fig. 5 together with the regression metrics: RMSE, MAE, R^2_{custom} and R^2_{model}. Summary statistics of the regression metrics for being upright and for the frequency and duration of aggression are shown in Table 2. The R^2_{custom} was equal to or above 0.9 for upright (Table 2) and lying postures (Fig. 5) with a sampling frequency of 15 min or more frequent. When data were sampled every 15 min or more frequent, for the average percentage of pigs lying per hour, the RMSE was less than 6 %, while MAE was less than 5 %.

For aggression, all the recording sessions shorter than 60 min/h had $R^2 < 0.8$ (Table 2). The highest R^2_{custom} of 0.71 was found when using recording sessions of 40 min/h. The RMSE was more than 5 bouts/h, and the MAE was more than 2 bouts/h when sampling 20 or 30 min of each hour. According to the RMSE measure, the total duration of bouts of aggression differed by nearly a minute per hour or more from the 'true' total duration, while the MAE showed an absolute error of approximately 20 s or more during the investigated shorter recording sessions (Table 2).

3.2.1. Generalised linear mixed models

The proportion of body postures, both lying and upright, per hour differed as an effect of the sampling interval ($P < 0.001$, Fig. 6), but not in a straightforward pattern. Sampling the occurrence of lying at 1 min intervals differed from 30 min ($P_{adj} = 0.001$) while it tended to differ from 60 min sampling ($P_{adj} = 0.07$), but 1 min sampling did not differ from 5 min, 10 min or 15 min sampling. Sampling pigs being upright at 1 min intervals differed from 15 min ($P_{adj} = 0.04$) and 30 min intervals ($P_{adj} < 0.001$), while it tended to differ from 60 min intervals ($P_{adj} = 0.08$). The occurrence of sitting did not differ in relation to sampling interval.

The frequency of bouts of aggression per compartment per hour differed as an effect of length of the recording session ($P = 0.05$). Comparing a recording session of 20 min/h with 60 min/h, the rate ratio was 0.72 (0.53–0.98; $P_{adj} = 0.04$), comparing 30 min/h with 60 min/h, the rate ratio was 0.78 (0.59–1.02; $P_{adj} = 0.08$), while comparing 40 min/h with 60 min/h, the rate ratio was 0.83 (0.64–1–08; $P_{adj} < 0.1$) after adjustment with the Dunnett method. Thus, a recording session of

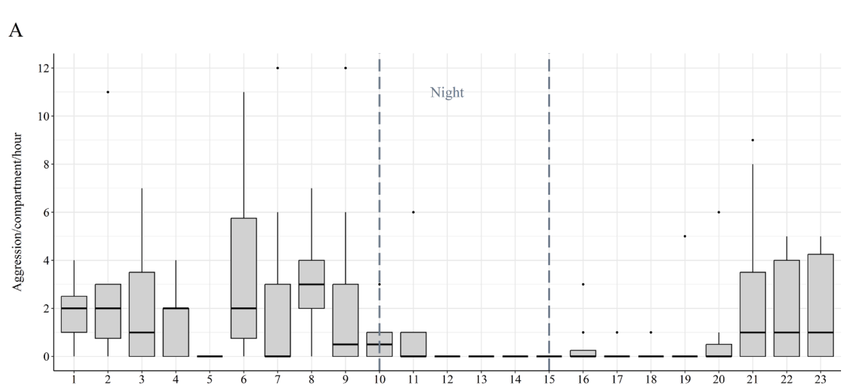

Fig. 4. Boxplot of the bouts of aggression among weaner pigs (20–25 kg) per compartment per hour for four journeys, each lasting 23 h. The dashed lines indicate approximate sunset and sunrise.

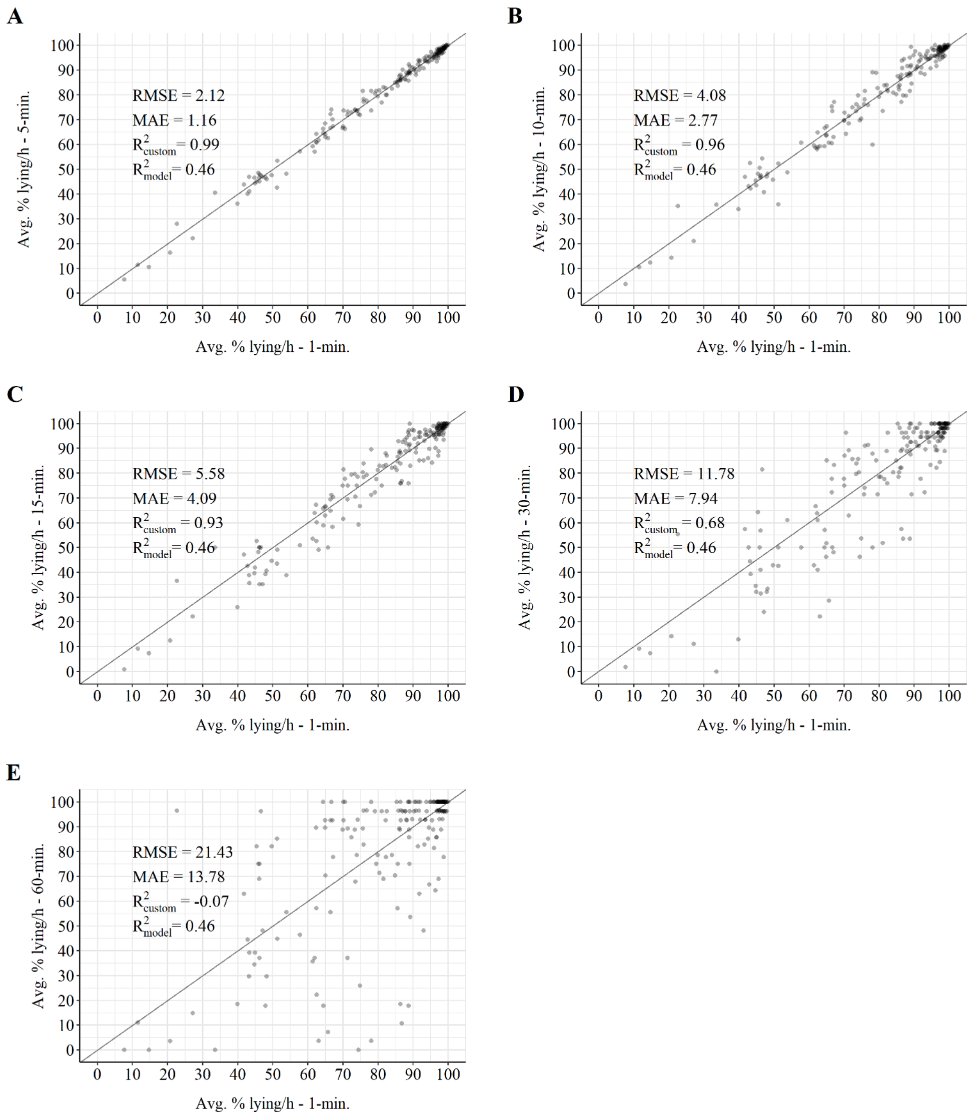

Fig. 5. Scatterplots depicting the relationship between 'true' average percentages of pigs lying per hour as determined by the 1 min sampling, and the less frequent sampling intervals by (A) 5 min, (B) 10 min, (C) 15 min, (D) 30 min, and (E) 60 min sampling. The grey reference line has an intercept of 0 and slope of 1. The colour transparency of the points indicates how many points overlap. RMSE: root mean squared error, MAE: mean absolute error, R^2_{custom}: a coefficient of determination based on a custom calculation, R^2_{model}: a coefficient of determination based on a logistic regression model.

20 min/h underestimated the frequency of bouts of aggression per hour, while 30 and 40 min/h numerically underestimated, but not significantly.

The duration of aggression per compartment per hour differed as an effect of the recording session ($P < 0.001$). Comparing sampling 20 min/h with 60 min/h, the rate ratio was 0.23 (0.15–0.33; $P_{adj} < 0.001$), comparing 30 min/h with 60 min/h, the rate ratio was 0.47 (0.35 –0.64; $P_{adj} < 0.001$), while comparing 40 min/h with 60 min/h, the rate ratio was 0.66 (0.50 – 0.87; $P_{adj} = 0.001$). Thus, the duration of aggression per hour was underestimated by all shortened recording sessions.

4. Discussion

The aim of this study was to test the validity of (1) different intervals of instantaneous scan sampling on the detection of body postures (upright, lying and sitting) as well as (2) to examine effects of shortening the duration of recording sessions on the detection of bouts and duration of aggression during transport of 20–25 kg pigs. If a less frequent or shorter duration sampling method is found valid, this means it should detect the same treatment differences as found using more frequent or longer duration sampling methods. To our knowledge, this is the first time video monitoring during transport of this weight class of pigs is being reported. Initially, the intention was also to capture drinking behaviour, but this had to be abandoned due to limited visibility of the drinking nipples located in the side wall with frequent obstruction of view caused by pigs standing upright.

4.1. Intervals for instantaneous scan sampling of body posture

Regression metrics can be used to test the validity of sampling rules (Daigle and Siegford, 2014; Chen et al., 2016; Park et al., 2020; Robles et al., 2021). In contrast to earlier studies, linear regressions could not be performed as the residuals of the present study did not comply with a normal distribution. Consequently, regression metrics (R^2_{custom}, RMSE, MAE) were used without inferring linearity. For the mean absolute error (MAE) and the root mean squared error (RMSE), smaller values indicate a better prediction. Considering the regression metrics for the percentage of pigs lying per hour, the RMSE was less than 6 %, while MAE was less than 5 % when sampling every 15 min or less frequently. In addition, the custom calculated coefficient of determination (R^2_{custom}) was

Table 2
Regression metrics comparing the 'true' average percentage of pigs being upright and the 'true' total frequency and total duration (seconds)* of bouts of aggression per compartment per hour with the estimates using less frequent sampling intervals (5, 10, 15, 30 and 60 min) or shorter recording sessions (20, 30, and 40 min/h), respectively. Metrics: a coefficient of determination (R^2) based on custom calculation (R^2_{custom}) and models (R^2_{model}), root mean squared error (RMSE) and mean absolute error (MAE).

Observational technique	Behavioural measure	R^2_{custom}	R^2_{model}	RMSE	MAE
Intervals of instantaneous scan sampling	Upright,%/h				
5 min vs. 1 min		0.93	0.39	1.31	0.93
10 min vs. 1 min		0.96	0.39	2.64	1.88
15 min vs. 1 min		0.89	0.39	4.21	2.91
30 min vs. 1 min		0.51	0.38	9.00	5.73
60 min vs. 1 min		−0.47	0.38	15.6	9.83
Recording sessions of continuous sampling					
	Frequency of aggression, /h				
40 vs. 60 min/h		0.71	0.98	3.71	1.41
30 vs. 60 min/h		0.48	0.98	5.34	2.04
20 vs. 60 min/h		0.19	0.98	6.60	2.57
	Duration of aggression, s/h				
40 vs. 60 min/h		0.61	0.97	59.9	19.6
30 vs. 60 min/h		0.43	0.96	79.3	26.8
20 vs. 60 min/h		0.49	0.96	102.9	35.0

*1 min sampling of upright and recording sessions of 60 min/h of aggression.

0.9 up until and including 15 min sampling for both lying and upright body postures. The model-based coefficient of determination (R^2_{model}) also decreased after 15 min sampling (at 30 and 60 min sampling). Take notice that the R^2_{model} was in a much smaller range than R^2_{custom} which is common for R^2 based on logistic regression (Shtatland et al., 2002). Thus, these measures indicate that 15 min sampling intervals or denser are appropriate for sampling body postures.

Regarding the logistic regression analysis, for upright behaviour, but not for lying, the 15 min sampling interval showed a significant difference compared to the 1 min sampling interval. This discrepancy can be attributed to the challenging observational environment with limited deck height. Observations were relatively easier when the majority of pigs were lying down compared to when the majority were upright – especially at 60 cm deck height. Thus, there were more instances of pigs being categorised as 'undetermined' at 60 cm than at 90 cm. Nevertheless, based on these findings we conclude that instantaneous scan sampling of activity every 15 min will be adequate in most cases – at least for lying behaviour, as has also been found for comparable behavioural states for sheep (Pullin et al., 2017), sows (Whalin et al., 2016), cattle (Chen et al., 2016) and poultry (Daigle and Siegford, 2014) while kept on farm.

For body postures, although sampling intervals up to and including 15 min provided reasonable estimates, a shorter sample interval did lead to greater accuracy in time sampled recordings (Bateson and Martin, 2021). The loss in accuracy was evident from the larger interquartile interval observed in Fig. 3, particularly in the case of 60 minute sampling. Although the same overall pattern was seen for all sampling intervals (Fig. 2) many minor peaks of activity were lost, with less frequent sampling. The emergence of computer vision technology (Wang et al., 2023), which offers automatic detection at significantly higher sampling rates than manual labelling, is expected to enhance the level of detail accessible to researchers. This includes expanding on methodological studies like the present to more efficiently label enough data to directly test if the same treatment effects are found using different sampling techniques. While the larger study by Herskin et al. (2022) did not uncover variations in body posture and aggression associated with deck height, studies like the present one, which utilized manual labelling, often face challenges of insufficient statistical power to effectively test treatment effects. Consequently, such effects were not pursued in this study. Additional sensor-based technologies, such as water-flow meters, microphones, and accelerometers, offer the capability for continuous, real-time, and objective assessment of all animals (Larsen et al., 2021) – also during transport. The advancement of such technologies can enable the quantification of drinking behaviour, which was not possible within the current setup.

4.2. Length for continuous recording session of aggression

In regard to aggressive behaviour, the highest R^2_{custom} was 0.7. Using previously used criteria for linear regression it cannot be considered

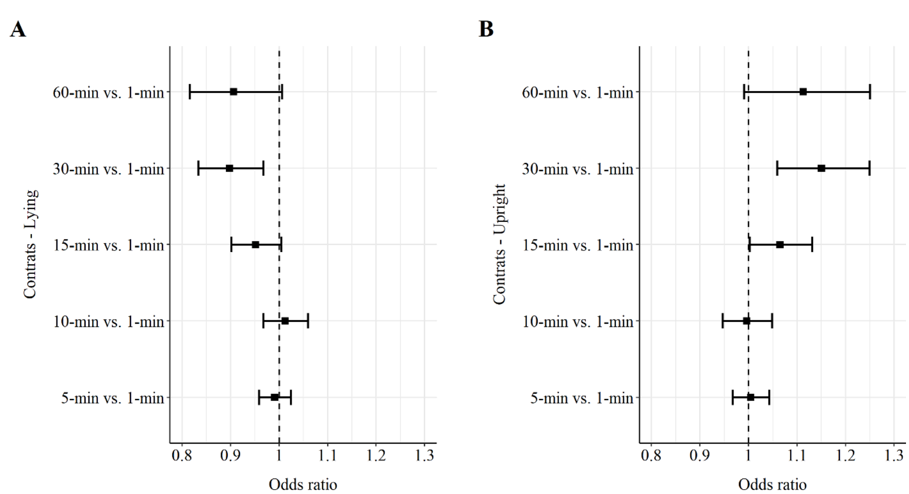

Fig. 6. Forest plot of the odds ratio ($P < 0.001$) between the different instantaneous scan sampling intervals of pigs. The square indicates the odds ratio, and the whiskers indicate the upper and lower confidence limit. (A) lying and (B) being upright during transport. Pairwise comparisons were adjusted by the Dunnett method.

appropriate to detect treatment differences ($R^2 \geq 0.9$, (Daigle and Siegford, 2014; Chen et al., 2016; Park et al., 2020; Robles et al., 2021)). Yet, in many cases $R^2 \geq 7$ can contain strong associations. It can still be useful to detect treatment differences (i.e., explaining the relationship between the predictor(s) and response variable) (Bobbitt, 2019). This means that 40 min/h recording sessions may still be appropriate depending on the study aim. However, for recording sessions of 20 min, the custom calculated coefficient was even negative for the duration of aggressive interactions, indicating that a horizontal line through the average would explain the data better. The model-based R^2 took on the same value for all recording sessions regarding the total frequency of aggression ($R^2_{model} = 0.98$), while it decreased for the duration of aggression at recording sessions of 30 and 20 min/h compared to 40 min/h. Thus, a recording session of 40 min/h can be considered appropriate depending on the expected effect size in a specific research question. The impact of these discrepancies in R^2, RSME and MAE, which compare estimated averages to the 'true' average values, should always be carefully assessed in relation to the specific research question. In certain cases, these disparities may have limited relevance while in others it may be crucial. For instance, when it comes to fulfilling specific behavioural needs such as ruminating in dairy cows (Tucker et al., 2021) or ensuring sufficient restorative sleep (Walker, 2017), even a 10 min difference in estimated lying time per hour from the true occurrence can significantly impact the interpretation of the results, thereby leading to wrongful conclusions on the welfare of the investigated animals. However, the ability to detect treatment differences may not be substantially affected by a moderately inaccurate estimate, especially when such discrepancy is much smaller than standard errors of estimates.

Regarding the logistic regression analysis, for the occurrence of aggressive behaviour, the present results suggest that continuous recording sessions of 40 min/h or more will be needed to more precisely estimate true total frequency of bouts of aggression. The total duration of aggression per hour was, however, significantly underestimated in all shorter recording sessions. The somewhat sporadic pattern of aggressive behaviour observed during the daytime is a likely explanation why recording sessions of 20 and 30 min/h were not valid. However, during the nighttime and morning, approximately from 12 to 20 h after departure (approximately from midnight to 8 in the morning), more than 90 % of the pigs were lying down, and almost no aggression was observed. Aggression was increasing after 21 h post-departure (approximately at 9 in the morning), when some of the pigs started to get up again. Although this dataset may be biased by each journey having the same departure time, these results might suggest a diurnal pattern in the occurrence of aggression, as has also been reported after on-farm mixing of pigs (Stukenborg et al., 2011). Further studies, involving journeys starting at other times of day, are needed to clarify this.

In the present study, the highest median number of bouts of aggression per hour (when 60 min recordings sessions were used) was observed 8 h after departure, and the majority of aggression occurred before this point. When weaner pigs are mixed on-farm, aggression has been reported to tend to peak within 2 h after mixing, depending on pen design features (Meese and Ewbank, 1972; Schmolke et al., 2004; Stukenborg et al., 2011). The present findings indicating that the peak occurred later during transport, maybe as a consequence of the responses of the pigs to the novel experience of being in a moving vehicle, and the extra energy demand from this, but further studies are needed to clarify this. To our knowledge, no studies have compared responses to mixing on-farm versus during transport. Studies like this, involving comparable space allowance, are needed to clarify if transport as such affects aggression.

5. Conclusion

Based on this study, reducing the continuous recording session of aggression during transport to 20 min/h compared to observing the full hour cannot be recommended, due to the sporadic pattern of the behaviour – although concentrated during the daytime. A recording session of 40 min/h can be considered appropriate – but should be approached with caution dependent on the specific scientific context. Instantaneous scan sampling intervals of 15 min or less, compared to 1 min, can be recommended to detect the occurrence of lying behaviour during 23 h transport of 20–25 kg pigs, although some details are lost.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT in order to suggest grammar/language corrections/clarifications to the text drafted by the first author. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

CRediT authorship contribution statement

Cecilie Kobek-Kjeldager: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Line D. Jensen:** Investigation, Writing – original draft, Writing – review & editing. **Leslie Foldager:** Writing – review & editing, Methodology. **Marianne Kaiser:** Writing – review & editing. **Karen Thodberg:** Writing – review & editing. **Mette S. Herskin:** Project administration, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

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