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The effects of part-time dam-contact and stepwise weaning and separation on the voluntary human approach behaviour of dairy calves

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A R T I C L E I N F O

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A B S T R A C T

Dairy calves are commonly reared without contact with their dam, which facilitates a human-animal relationship based on close human contact and feeding. Dam-contact may negatively affect calves' relationship with humans. The current study investigates the effect of dam-contact and weaning method on calves' response to humans. A total of 69 dairy calves were allocated to one of three dam-contact treatments [Control (separated from dam after 24 h), Whole-day (housed with dam for 23 h/d), and Half-day (housed with dam for 10 h/d)]. Within each treatment, calves were allocated to one of two weaning treatments [Stepwise (weaning off milk at eight weeks, dam-separation/pen change at nine weeks) or Simultaneous (weaning off milk and dam-separation/pen change simultaneously at nine weeks), i.e Control were weaned in the same manner but only the pen change was possible at the separation step, as calves were already separated from the dam]. All animals received a similar amount of human contact, except control calves who were additionally fed milk by teat bucket twice a day. Calves were tested in a random order within block using a human approach test followed by an animal approach test conducted in a 2.5 m x 10 m arena at 10 weeks of age. Stepwise-Control calves had shorter latencies to first approach the test person than Stepwise-Whole-day ($p < 0.05$, median survival time of Stepwise-Control: 11 s, Stepwise-Whole-day: 111 s and Stepwise-Half-day: 52 s). Among Simultaneous calves, no dam-contact treatment differences were detected for the latency to first approach. Similarly, Stepwise-Control calves had an odds ratio (95% CI) of 24.2 (1.6–365.9, $p < 0.05$) for coming within 1 m of the test person vs Stepwise-Whole-day calves and 12.5 (1.1–141.1, $p < 0.05$) vs Stepwise-Half-day calves. Throughout the test period Simultaneous-Control vocalised less [estimated mean no. of vocalisations (95% CI), 3.6 (2.1–6.4)] than both Simultaneous-Whole-day [18.2 (12.8–25.9), $p < 0.01$] and Simultaneous-Half-day [15.7 (11.0–22.5), $p < 0.01$] while there was no difference under Stepwise. As expected, Control approached faster and were more likely to come close to the test person than dam-reared calves, but exclusively after the stepwise weaning and separation. For calves tested one week after simultaneous weaning and separation no effect of the dam-contact treatments was found, except a higher frequency of vocalisations for dam-reared calves. This implies that controlling for the stress level related to weaning and separation from the dam is important when interpreting human-animal relationship tests, as dam-contact treatment effects appeared to be affected by high levels of weaning stress.

1. Introduction

A possible way of improving dairy cow and calf welfare is by allowing prolonged contact between a cow and her calf, referred to as dam-rearing of calves. This allows for the expression of highly motivated, natural behaviours and positive experiences such as affiliative behaviours and social play between a dam and her calf (reviewed by Meagher et al., 2019). This differs from conventional dairy calf rearing in most parts of the world where the calf is separated from the dam within 24 h of birth and reared artificially by humans.

However, there is a concern that dam-rearing leads to a low level of human contact due to the lack of human handling during milk feeding, and that this will result in calves – and subsequently cows – that have a poorer human-animal relationship (HAR) (Boivin et al., 1992; Jago et al., 1999; reviewed by: Johnsen et al., 2016; Krohn et al., 2003; Waiblinger et al., 2020). Assessment of the HAR has classically been done with 1) the human approach test (measuring mainly animal avoidance distance), and 2) the animal approach test (measuring mainly

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animal latency to first approach and time spend with the test person) (reviewed by Waiblinger et al., 2006).

Studies have shown that dam-rearing affects the HAR negatively, i.e. the HAR was judged to be more positive for artificially reared calves than for dam-reared calves (Duve et al., 2012; Mogensen et al., 1999). Dairy cows and calves should at least accept and perhaps even enjoy aspects of interactions with humans to ensure their welfare in production systems where daily handling and routine procedures such as moving the animals and milking require human contact. If animals are more fearful of humans and more difficult to handle, this can lead to stress and the animals having more negative experiences (reviewed by Mota-Rojas et al., 2020; Waiblinger et al., 2006). The difference in the HAR between dam-reared and artificially reared calves may be explained by the importance of the type of human contact for the development of the HAR and in extension hereof the association between humans and milk feeding. Indeed the positive effects on the HAR were stronger when calves visually associated milk feeding with humans than when curtains blocked any visual contact between humans and animals during milk feeding (Jago et al., 1999). Further, a more positive HAR was found in multiple studies for calves that received gentle handling [Breuer et al. (2003) (Holstein Frisian heifers, 5–14 months old); Lensink et al. (2000) (Holstein bulls, 2–21 weeks old); Lürzel et al. (2015) (Holstein-Friesian heifers and bulls, 17–86 days old)]. This effect of gentle handling, however, was not found in a study where calves were housed right next to the dam during handling, which may be a contributing factor to the more positive HAR in artificially reared calves, who experience human contact separate from their dam (Krohn et al., 2003 [Holstein Frisian heifers and bulls, tested 50–55 days old)]. It has also been shown that pair-housing results in a poorer HAR compared to individual housing in pre-waened calves, suggesting an effect of available social partners on the development of the HAR (Doyle et al., 2022).

Part-time contact has been suggested as a more feasible option for dam-contact for practical and production economic reasons (reviewed by: Johnsen et al., 2016) and would at the same time allow the calves to experience human handling and interference without the dam being present. This could possibly improve the HAR, if indeed the presence of the dam inhibits the socialisation towards humans (Krohn et al., 2003).

In the present study, we compared dam-reared calves with either whole-day or half-day contact (terminology: Sirovnik et al., 2020) with their dam (all milk supplied by the dam, for both treatments) to control calves that were separated shortly after birth and artificially reared using standard farm procedures. In regards to the two different levels of contact time between dam and calf, Boivin et al. (2009), found no difference in handleability between half-day and whole-day contact in beef calves who all received forced stroking for 5 min, 5 days per week over 3 weeks while separated from the dam. However, in the present study, we investigate the HAR of calves reared either with whole- or half-day dam-contact, in at setting where human contact involved less invasive, standard management procedures such as the provision of straw, cleaning, and filling of the feed troughs across a longer total period. For half-day calves, they would experience some of these management procedures with human presence, without the dams presence. Based on the above literature, we hypothesised control calves to approach a test person more readily during an animal approach test and allow the test person to come closer during a human approach test compared to whole-day calves and with half-day calves being intermediate.

Stepwise weaning and separation has been found to reduce the reaction to weaning and separation, which is likely due to calves not experiencing the combined stress response from two stressors at one point in time (Reviewed by Newberry and Swanson, 2008). In the present study, either a stepwise weaning and separation or a simultaneous weaning and separation strategy was applied as a second treatment. We thus expected that simultaneously weaned and separated calves would be hungrier and more affected by weaning stress at the time of testing than stepwise weaned and separated calves. To the best of our knowledge, there are no studies investigating how weaning stress and hunger affect the HAR as measured by human- or animal approach tests. On one hand, it could be that animals show faster approach behaviour and less fear of humans, if a human is regarded as a potential source of milk or social contact. On the other hand, it could be argued that hunger and weaning stress leads to calves being in a more negative affective state [e.g. negative judgement bias after weaning (Daros et al., 2014)] and thus less explorative, though interpreting inactivity in a testing setting must be done with caution (Fureix and Meagher, 2015). Based on this, we hypothesised that simultaneously weaning and separating calves would lead control calves to show a more positive HAR, as they associate humans with milk feeding, and dam-reared calves to show a more negative HAR, as they do not associate human with milk compared to calves on the stepwise weaning and separation treatment.

2. Material and methods

The authors have read the journal policy relating to animal ethics and confirm that the present study complies.

2.1. Animals, housing, and management

The study was conducted at the cattle Research facilities at Aarhus University, Foulum, Denmark, from November 2020 to May 2021. A total of 72 pure-bread Danish Holstein calves and their dams were allocated to six blocks of 12 cow-calf pairs according to calves' birth date. Within block, animals were allocated to one of three dam-contact treatments: Control, Whole-day, and Half-day (4 cow-calf-pairs per group). The treatment groups were to the best extent balanced for sex (at least one calf of each sex in each group in all treatment pens), except for two pens with only bull calves (Whole-day in Block 2 and Half-day in Block 5, i.e. a total of 29 heifer calves and 40 bull calves). The groups were also balanced for dam parity with either one or two first parity cows in each group (4 groups had two first parity and two multi parity cows (Control in Block 2 and Whole-day, Half-day and Control in Block 4), i.e. in total 50 multi parity and 22 first parity cows). Due to three disease incidences (2 calves with diarrhoea and fever and 1 cow with mastitis), data was collected on a total of 69 calves. Calves were tested in six sessions corresponding to the six blocks.

All calves were fed the dams' colostrum (4 L) via a teat bottle within 6 h after birth. A dose (1 ml Cevivit® E-Selen) of E-vitamin was added to the colostrum. From day 2 and onwards, the calves were housed in deep bedded pens with or without their dams depending on the treatment. All calves had ad libitum access to calf-starter concentrate (DLG: "Komkalv Start Valset" FEk/kg: 0.99 FEk; Raw Protein: 20%; Raw fat: 3.6%; Fiber: 5.4%; Raw Ash: 7.5%; Water; 13%), hay, water and the cows' total mixed ration (TMR; clover-grass and maize silage (64.6%) with concentrate (35.3%).

Calves experienced human contact during daily and weekly standard care procedures such as feeding, bedding replenishment, weekly weighing, and weekly experimental health checks. Half-day calves specifically experienced evening farm-procedure health checks and refilling of hay and concentrate in the calf creep during the period where their dams were not present. The calves were not disbudded during the experimental period.

2.2. Treatments

2.2.1. Whole-day and half-day

After calving, the cow and calf stayed together in the calving pen for 24 h (range: 20–36 h) to establish suckling and bonding. At the colostrum feeding within 6 h of birth, the calf's ability to suckle the dam (filled calf stomach, milk foam around calf's mouth, saliva on the dam's udder) was assessed and if there were no signs of suckling, the calf was guided to the udder, i.e., suckling was assisted. If the calf did not suckle, suckling was assisted again 6 h later. Calves not being able to suckle within the first 24 h did not enter the experiment (n = 6 out of 78 calves).

The cow-calf-pair was moved to a deep-bedded group pen (9 m x 7.5 m) for four cow-calf pairs on the same treatment. There were four treatment group pens in the experimental barn, allowing a new block to start while the proceeding block was still running. All four pens had the same, but mirrored, layout (see Fig. 1). Treatment groups were allocated to the different sides of the barn in a balanced matter, across the six blocks. There were two calf creep areas with sides of tubular metal bars, one in each back corner, of each pen; one sized 3 m x 3 m with concentrate in bowls, a hayrack, and a water cup and one sized 1.5 m x 1.5 m with concentrate in a bowl and a hayrack (see Fig. 1).

Whole-day calves were kept with their dam at all times, except for approx. 30 min (mean ± SD: 28 ± 8 min) twice a day, while the cows were away for milking in a milking parlour in an adjacent building. Half-day calves were kept with their dam, except for approx. 14 h (mean ± SD: 13 h 58 ± 8 min) during the night (from when the cows were taken out of the pen for afternoon milking (15:30 h) until they returned from morning milking (5:30 h).

Weaning treatment started at the eighth treatment week [mean age (95% CI) and mean weight (95% CI); Whole-day: 54.9 (53.1–56.6) days and 93.2 (86.3–100) kg; Half-day: 59.3 (58.2–60.5) days and 93.8 (88.4–99.2) kg]. Two randomly selected calves in both Whole-day and Half-day treatments were at this time confined in the 3 m x 3 m calf creep, closed with pen fixtures made from tubular metal bars (Stepwise). The cow and calf pair could maintain olfactory, visual and some tactile contact, but nursing was not possible, effectively weaning the calves off milk. The two remaining calves stayed in the main part of the pen with full access to their dams for another week (Simultaneous). There was one block (Block 2) were the enrolment time for the two last calves (one Whole-day and one Control) of the block was prolonged due to either twin birth or disease. For this block, the treatment weeks followed the third youngest calf. The two youngest calves were allocated to the Simultaneous weaning to allow them an extra week of milk intake, thus in Block 2 an exception from random allocation of calves to weaning treatment was made.

All dam-reared calves were permanently separated from the dams one week later, after nine weeks on the dam-contact treatment. The dams were moved to an adjacent building but within auditory reach. The calves were moved to straw-bedded weaning pens of 3 m x 3 m in the corner of the experimental barn and followed for seven days. During this period, calves were still housed with the calves from their previous groups, in groups of four.

2.2.2. Control

Control calves were managed largely according to standard farm procedure and separation from dams took place after 12–24 h.

During the first seven days after the separation from the dam, the calves were housed in individual straw-bedded pens (1.5 m x 3 m) with sides made from tubular metal bars allowing visual and tactile contact with neighbouring calves on the same treatment and within the same block. After seven days, they were grouped in groups of four (by removing partitions), resulting in a group pen for the four Control calves (3 m x 6 m) in each block.

During the first week of life Control calves were first offered 6 L/d of whole milk in two daily feedings, which was gradually increased over seven days to 8 L/d in two daily feedings. From seven days old and throughout to weaning off milk they were offered milk to satiation twice daily at 06:30 h and 17:00 h. The calves had 20 min to drink milk before any leftovers were removed (mean daily intake per calf ± SD ranged from 7.9 L ± 0.93 in the second week to 11.08 L ± 1.7 in the eighth week).

Weaning began at the eighth treatment week [mean age (95% CI) and mean weight (95% CI) 55.7 (54.4–56.9) days and 90.3 (82.7–97.9) kg]. For Control, true Stepwise weaning and separation was not possible, due to the obvious decoupling of milk and dam. However, a version of the Stepwise weaning was achieved as described below: The group pen was split into two equally sized adjacent pens (3 m x 3 m), each housing two calves. The two calves in one pair pen were randomly allocated to be stepwise weaned and separated by first removing milk, but only one week later moving the calves away from the known environment (Stepwise). The remaining two calves continued to be fed milk for one week and then weaned from milk and moved on the same day (Simultaneous). On the moving day, all four calves were moved to a common weaning pen (3 m x 3 m) in the corner at the opposite end of the experimental barn and observed for seven days post-weaning. See Fig. 2 for a graphic timeline of treatments.

2.3. Test procedures

For all calves, behavioural tests described below were performed at 10 weeks (mean age ± SD; 68.5 ± 7.0 days). After testing, the calves were moved to the main calf herd and no longer included in the

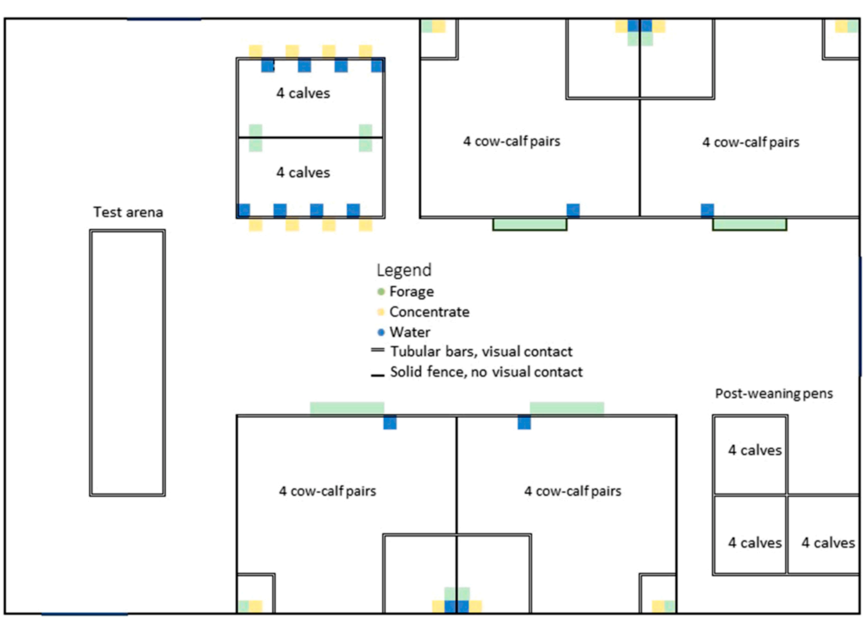

Fig. 1. Graphical illustration of the experimental barn and housing environment. Calves on the dam-contact treatments **Whole-day** and **Half-day** are housed with their dams in straw-bedded pens in groups of four cow-calf pairs. **Control** are housed in groups of four without their dam. There was room for two simultaneous blocks at a time. Calf creeps are provided in the dam-rearing treatment pens and the larger creep is used for fence-line weaning at week eight for calves on the **Stepwise** weaning and separation treatment. The weaning pens in the corner are used for all calves in a block at week nine. The location of the test arena is indicated.

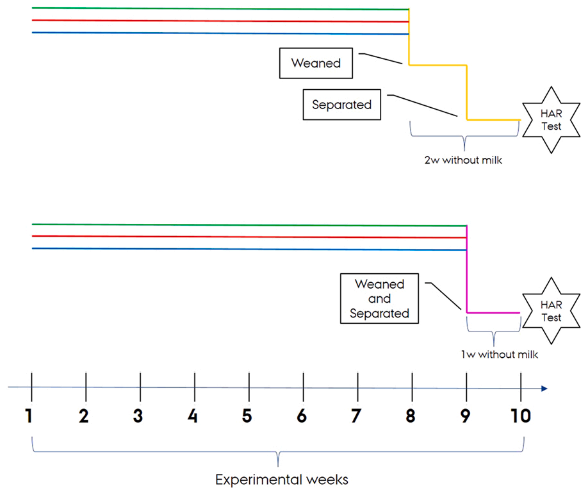

Fig. 2. Timeline of the experimental treatment of the present study. The dam-contact treatment is initiated immediately after birth (**Control**, **Whole-day**, and **Half-day**, each represented by blue, green, and red lines) while the weaning treatments start at either eight (**Stepwise**, orange) or nine (**S**imultaneous, pink) weeks of age. The 3 × 2 factorial design yields six treatment combinations. The human-animal relationship (HAR) is assessed at 10 weeks of age.

experimental study.

We measured the calves' HAR by assessing their reactions towards a test person in a human approach test (HAT) and an animal approach test (AAT), adapted from previous studies (e.g. Krohn et al., 2003). Two people, an observer and a test person, conducted the tests. The same observer did all behavioural observations, but three different test persons were included, in an unbalanced manner (Test person A: Block 1 + 2, Test person B: Block 3 + 6, Test person C: Block 4 + 5). The test people were not involved in daily management and care for the animals but assisted (in a similar degree and way) in other behavioural observations and weekly weighing. Behaviours were recorded directly by the observer. All calves were tested individually in an unfamiliar test arena placed at the far end of the experimental barn. During testing, the calf could have visual contact with the cows from other blocks if cows had their head out of the pen, but not with their pen mates or own dam. The arena consisted of ten galvanized steel fences attached to each other and enclosing a rectangular space measuring 10 m x 2.5 m (See Fig. 3).

A coloured spray marker on the floor, just outside of the test arena, was used to indicate distance increments of 1 m. The calves were tested in a random order according to a list generated before the test session. Test sessions started approximately at 12:00 h and ended before 15:30 h. The test calf was gently guided from the home pen to the arena; it was first tested in the HAT (45 ± 15 s) followed by the AAT (180 s). Before the HAT, calves were habituated to the test arena for 3 min and the calves were left undisturbed in the arena for 3 min between the two tests. Disturbances during the testing were kept at a minimum and any unforeseen disturbances were minimal but noted. The order of the tests was chosen to prioritise the test with the highest repeatability, thus

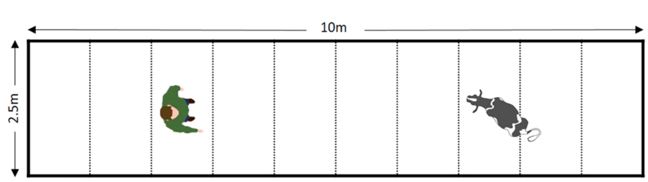

Fig. 3. Illustration of the test arena used on dairy calves for both a human approach test and an animal approach test. For both tests, the test person entered and started the test at a four-zone (equal to 4 m) distance.

starting with the HAT (Lensink et al., 2003; Waiblinger et al., 2006). The total time in the arena including habituation, testing, and pause was 8 min and 45 ± 15 s

2.3.1. *Human Approach Test*

After the three-minute habituation period, the test person entered the arena. The HAT test started once the calf was standing still and at least 1.5 m from either of the two ends of the arena (to ensure space to allow for a withdrawal) and the test person positioned at four zones distance from the calf (see Fig. 3). The test person could access the arena by moving any of the fences. In case the calf started moving, the test person re-positioned according to the calf's new location before the test was initiated. To start the test, the test person said, "Hey you, I am here" to catch the calf's attention and started to approach, one step per sec, with one arm stretched at 45 degrees angle. No abrupt or sudden moves were made. When the test person's hand was within the calf's reach, the approach was stopped. If the calf sniffed the test person's hand, the test person tried to touch the calf on the cheek. The test ended whenever the calf moved one of its forelegs backwards or when the test person touched it. After the HAT, the test person left the test arena and the calf was left alone in the arena for 2 min

Behaviours were observed continuously, and the following measures were recorded: distance from the test person at withdrawal (m, in 0.5 m increments), whether the calf sniffed the hand (yes, no), and whether the calf allowed touch by the hand (yes, no).

2.3.2. *Animal Approach Test*

After 2 min of pause, the test person re-entered the test arena. The test person again entered and positioned at a four zones distance from the calf. The test person stood motionless, gaze lowered, with one arm stretched at 45 degrees angle, and waited for the calf to approach. If the calf sniffed the test person's hand, the test person tried to touch the cheek of the calf. If the calf withdrew, the test person stayed motionless. The test lasted 3 min from when the test person was correctly positioned.

Behaviours were observed continuously, and the following measures were recorded: latency to first approach the test person (s) (more than one step in the direction of the test person), duration of time spent within 1 m of the test person (s); duration of sniffing and touching the test person (s); the total number of lines crossed. The frequency of vocalisation and defecation (n) was recorded throughout the test session from when the calf entered the arena for habituation until the AAT finished.

After the AAT finished, the calf was gently guided back to the home pen.

2.4. *Statistical analysis*

From the HAT, we analysed only the variable "avoidance distance" statistically due to low response on "sniffing the test person" and "allowing touch" (Sniffing: 7 out of 69 calves, 4 Control, 2 Half-day, 1 Whole-day; Allowing touch: 3 out of 69 calves, 1 Control, 1 Half-day, 1 Whole-day). Due to low behavioural durations the variable "latency to first approach within 1 m of the test person" from the AAT was changed into a binary variable: "calf approaching within 1 m of test person (yes/no)" and due to high collinearity with response variables "sniffing the test person" the analysis of sniffing duration was omitted.

Statistical analysis was performed in R, using RStudio (Core Team, R, 2022) and the package "glmmTMB" (Brooks et al., 2017) for generalised linear mixed models (GLMM) or Survival Analysis using "Coxme" (Therneau, 2022) for a mixed cox proportional hazards model. The choice of distribution was based on an initial visual inspection of raw data histograms and following model comparison using the residual investigation tool from the "DHARMa" package in R.

For "avoidance distance" from the HAT, the Normal Distribution was used (family = Gaussian in glmmTMB, R, treated as continuous data after inspecting residual plots). For the AAT, "latency to the first approach" was analysed using a mixed cox proportional hazards model. The binary response variable "calf approaching within 1 m of test person (yes/no)", was analysed with logistic regression (family = binomial in glmmTMB, R). Count data ("number of vocalisations", "number of defecations", and "number of lines crossed") were fitted using a Quasi-Poisson distribution (family = nbinom1 in glmmTMB, R). The model included dam-contact treatment, weaning treatment, and their interaction as fixed effects, age as covariate, and block as random effect.

Significant effects were found using the type II Wald Chi^2 test and when relevant, pairwise comparisons within each of the weaning treatments using the package emmeans in R with the Sidak adjustment.

3. Results

3.1. Human Approach Test

3.1.1. Avoidance Distance

We did not find any significant differences in avoidance distance between contact or weaning treatments. There was however a significant effect of age on the day of testing, with older calves having larger avoidance distances (slope estimate ± SE: older calves had a 4.1 ± 1.7 cm increase in avoidance distance per extra day of age, between 57 and 83 days of age, $Chi^2 = 5.58$, df = 1, $p < 0.05$).

3.2. Animal Approach Test

3.2.1. Latency to First Approach

For the latency to first approach there was a significant interaction between dam-contact treatment and weaning treatment ($Chi^2 = 11.7$, df

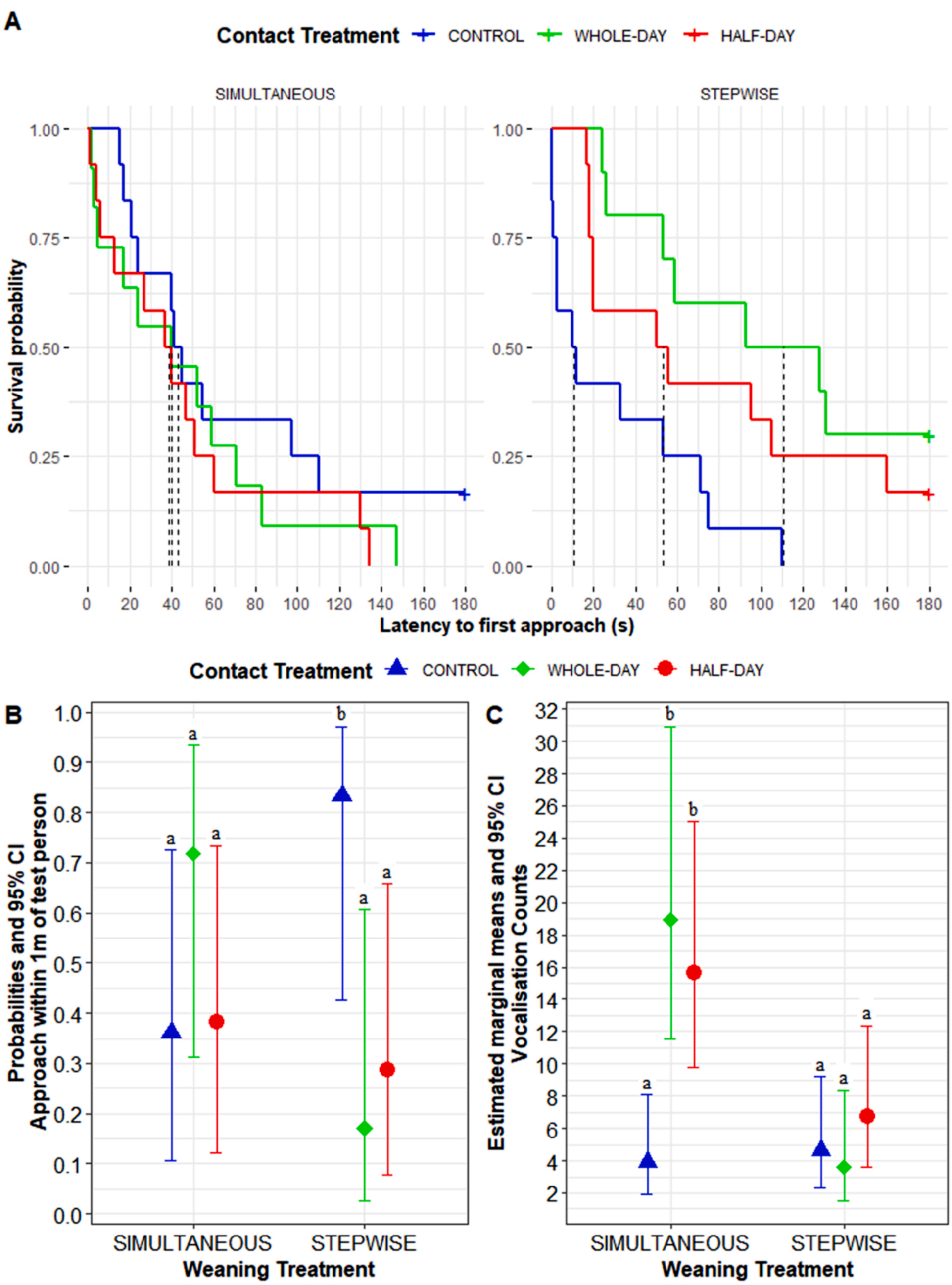

Fig. 4. Behavioural responses from an animal approach test for either artificially-reared (**Control**) or dam-reared (**Whole-day** and **Half-day**) dairy calves on one of two weaning treatments (**Stepwise** or **Simultaneous**). A) Kaplan-Meier survival curves for the latency to the first approach. Vertical, dashed lines are median survival times. Crosses on the line are were data is censored. B) Probability of approaching within 1 m of the test person and 95% CI. C) Vocalisation counts, back-transformed estimated means, and 95%CI during a 9-minute test period. Pairwise comparisons are made within each weaning treatment. Points that share a letter are not significantly different.

= 2, $p < 0.01$, see Fig. 4 A). The interaction was driven by no significant differences between dam-contact treatments among simultaneously weaned and separated calves, while there were significant differences for stepwise weaned and separated calves. Stepwise-Control had a 90.3% probability of having shorter latencies to first start approaching the test person than Stepwise-Whole-day (Hazard Ratio (95% CI): 0.106 (0.028–0.412), $p < 0.01$), and a 82.35% probability of having shorter latencies than Stepwise-Half-day (Hazard Ratio (95% CI): 0.214 (0.064–0.722), $p < 0.05$) to first start approaching the test person. There was no significant difference between Stepwise-Whole-day and Stepwise-Half-day. The median survival time (where half of the animals "at risk" had performed the behaviour and half had not) as estimated from Kaplan-Meier survival curves were respectively 11 s for Control, 53 s for Half-day and 111 s for Whole-day under Stepwise. There was no effect of calf age.

3.2.2. Approaching within 1 m of the test person

There was also a significant interaction between dam-contact treatment and weaning treatment for the probability of calves approaching within 1 m of the test person ($Chi^2 = 10.13$, df = 2, $p < 0.01$, see Fig. 4B). Within Stepwise, the odds ratio of Control for coming within one meter of the test person was 24.16 (95% CI: 1.59–365.97, t = 2.82, $p < 0.05$) vs Whole-day and 12.47 vs Half-day (95% CI: 1.10–141.07, t = 2.50, $p < 0.05$). There was no significant difference between Stepwise-Whole-day and Stepwise-Half-day. Within Simultaneous there were no significant differences between dan-contact treatments and overall, there was no effect of age.

3.2.3. Activity

For the number of lines crossed, there was an interaction between dam-contact treatment and weaning treatment [$Chi^2 = 7.69$, df = 2, $p < 0.05$, results given as back-transformed estimated mean no. of lines crossed (95% CI)] with Stepwise-Control [10.5 (6.9–15.9)] crossing significantly more lines than Stepwise-Whole-day [5.5 (3.2–9.5)] and Stepwise-Half-day [4.8 (2.7–8.2)]. There was no significant difference within simultaneously weaned and separated calves [Simultaneous-Control 7.4 (4.7–116), Simultaneous-Whole-day: 8.4 (5.1–12.6), Simultaneous-Half-day: 9.1 (6.1–14.1)]. There was no effect of calf age.

3.3. Across test period

3.3.1. Vocalisations

Vocalisations were recorded from when the calf entered the test arena until both tests were finished. There was a significant interaction between the dam-contact treatment and weaning treatment ($Chi^2 =$ 12.6, df = 2 $p < 0.001$, see Fig. 4 C). For this variable, the interaction was caused by significant differences within the simultaneous weaning and separation (Simultaneous-Whole-day and Simultaneous-Half-day were vocalising more than Simultaneous-Control), while there were no differences between dam-contact treatments for stepwise weaning and separation. Simultaneous-Whole-day and Simultaneous-Half-day were vocalising more than calves on the other treatment combinations.

Further, heifers vocalised more than bulls independent of dam-contact treatment and weaning treatment [estimated mean no. of vocalisations (95% CI) averaged across dam-contact treatment and weaning treatment: heifers: 9.02 (6.47–12.59), bulls: 5.66 (4.02–7.96), $Chi^2 = 8.21$, df = 1, $p < 0.01$]. There was no effect of age.

3.3.2. Defecations

No differences in the number of defecations were found for any of the treatments, sex, or age (mean ± SD: 0.39 ± 0.88).

4. Discussion

In the present study, we compared the human-animal relation as measured by a human approach test and an animal approach test in dairy calves with three different contact levels to their dams and undergoing two different weaning methods.

In summary, we found that exclusively under the stepwise weaning and separation treatment, control calves had shorter latency to first approach the test person and were more likely to go within one meter of the test person. Following, we found no differences among any of the dam-contact treatments on the simultaneous weaning and separation treatments, except that calves on whole-day contact and half-day contact treatment were vocalising more frequently than control calves.

4.1. The interaction between treatments

For most response variables, we found an interaction between dam-contact treatment and weaning and separation treatment. This interaction was based on differences between dam-contact treatments' HAR under Stepwise, but not under Simultaneous weaning and separation. It is highly likely, that weaning stress and/or hunger affected the results of the HAR test which is supported by the increased frequency of vocalisations observed under Simultaneous for both dam-reared treatments. High pitched vocalisations are interpreted as either hunger or reinstatement behaviour in cattle (Green et al., 2020; Johnsen et al., 2015). Calves on the simultaneous weaning and separation treatment were tested one week after weaning off milk and simultaneously being moved to a new environment (and for dam-reared, separated from the dam), whereas calves on the stepwise weaning and separation had gone two weeks without milk before testing and one week in a new environment (for dam-reared the new environment equalled being separated from the dam). The effect of weaning on ADG, and thus likely hunger levels, differs greatly from study to study depending on e.g. the age of calves, previous milk allowance and any stepwise reduction of milk allowance. Some studies report calves losing weight during the first week post-weaning (Budzynska and Weary, 2008), others no weight gain (Eckert et al., 2015), and others again show maintained ADG (Roth et al., 2008). However, e.g. Eckert et al. (2015) found calves to have regained pre-weaning ADG two weeks after weaning so based on the above studies it is likely that artificially reared calves are back to pre-weaning weight gains approx. two weeks after weaning abruptly from milk. Thus it is also likely that the weaning stress and/or hunger levels are at least less at the time of testing for Stepwise than for Simultaneous. We acknowledge that the abrupt weaning off milk applied to all calves of the present study will induce high levels of weaning stress, especially due to the high milk consumption up until weaning. The choice was made to make weaning comparable to the control group since it is difficult to reliably match a gradual step-down weaning schedule when calves have access to suckle their dams since calves with just 2 × 15 min access to suckle their cows can consume 10 L of milk a day (Fröberg et al., 2008). The higher number of vocalisations given by dam-reared calves than control calves, under Simultaneous may have several causes. It could indicate an increased hunger in dam-reared calves, a response to being separated from the dam, or a different expectation to the benefit of vocalising. Control may have had a higher intake of solid feed pre-weaning, since they had longer periods without milk access daily, leading to an easier transsission from milk to solid feed (Eckert et al., 2015; Roth et al., 2008). However, since Control, at the time of abrupt weaning, had a mean milk intake of approx. 11 L/day, which is close to the expected ad libitum intake of 10–12 L by both dam-reared and artificially reared calves (reviewed by Khan et al., 2011), we also expected Control to be hungry the week following weaning (Budzynska and Weary, 2008). Thus, it is plausible that at least some of the increased calling in dam-reared calves is not due to higher hunger levels than Control but reflects calling for the dam to be reunited with her either to reinstate the social contact, the milk resource or both. Studies separating the nutritional dependency from the dam from the social aspect does show that there is a bond beyond milk, but depending somewhat on the opportunity for full contact or only partial contact (Johnsen et al., 2018; Wenker et al., 2022), thus we cannot know whether calling is for the dam, milk or both.

In any case, the results illustrate how the timing of weaning or other similar stressful events, which may affect treatment groups differently, should be taken into account when designing studies comparing the human-animal relation using human and animal approach tests. Had we only tested and analysed data from our calves being simultaneously weaned and separated a week before testing we would not be able to confirm the previously found results of a better HAR for artificially reared calves. Completely avoiding the confounding effect of timing and weaning and separation treatments is not possible, but allowing more time after weaning and separation before testing would have allowed us to control for hunger levels between dam-contact treatments e.g. by ensuring similar average daily gain for all calves at testing. In the present study, this was not possible, due to the calves only being available for experimentation until 10 weeks of age.

For both of the HAR-related measures analysed from the AAT (latency to first approach and the probability of coming close to the test person) the lack of dam-contact treatment related differences for Simultaneous seems to be driven by a poorer HAR for Control and a better HAR for dam-reared calves, compared to Stepwise, opposite to our hypothesis. We had hypothesised that under increasing weaning stress and/or hunger, as expected under the simultaneous weaning and separation, Control would react with a shorter approach latency and more often coming close to the test person, due to the calf associating humans with milk feeding, while we had hypothesised that dam-reared calves, who do not associate humans with milk feeding, would show the opposite trend. This interaction warrants further investigation to understand the driving mechanisms. It seems the lower stress levels at the time of testing for Stepwise calves allowed the experiences of the different dam-contact treatments to influence the calves' behaviours in accordance to their HAR (Waiblinger et al., 2006).

As mentioned, when looking at Stepwise only, Control seemed to have a more positive HAR (significantly faster to approach the test person and were more likely to come up close to the test person) than dam-reared calves. This was as expected based on results from other studies (Krohn et al., 2001; Waiblinger et al., 2020; Wenker et al., 2022). Control calves had been milk fed by humans and thus had more close contact and opportunity to develop a positive association to humans. However, with regards to the effect of Half-day vs Whole-day, we did not find any significant differences. Wenker et al. (2022) compared calves with partial contact to the dam (no suckling, housed individually inside the cow pen) to full contact and a control group with a two minute HAT two weeks after weaning and found no treatment effects, although possibly due to statistical power issues as discussed by the authors. Although at present we cannot show that merely providing dam-reared calves with more experiences with humans during the first 8 weeks of life, while the dam is not present, improves the HAR, studies addressing this aspect are few and the effect of duration of cow-calf contact in dam-reared calves on HAR deserves further study.

4.2. Technical side note

A technical side note to the performance of the animal approach test is that the author regretted implementing the 'try to touch/scratch calf' after the calf had sniffed. In most cases, the movement, however gentle, by the test person led to the calf backing away and focusing its attention elsewhere, leading to very short sniffing durations, hard to analyse. This might have been avoided had the test person just remained still.

5. Conclusion

Artificially reared dairy calves showed indications of a more positive human-animal relationship with shorter latencies to first approach and a higher probability to come close to and sniff the test person, compared to dam-reared calves, when tested upon a stepwise weaning and separation period of two weeks. However, this difference was not found when testing upon a one-week simultaneous weaning and separation. Upon the one-week of simultaneous weaning and separation, dam-reared calves vocalised more during the test session, but this was the only difference between simultaneously weaned and separated calves. Overall, this implies that controlling for the hunger and/or stress level related to weaning off milk is important when interpreting human-animal relation tests, as dam-contact treatment effects were affected by high levels of weaning stress.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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