Calf- and herd-level factors associated with dairy-calf reactivity

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INTERPRETIVE SUMMARY: The quality of the human-animal relationship has profound effects on the welfare of farm animals. We measured the reactivity of calves from 30 dairy herds in response to the active approach of an experimenter. We considered management and infrastructure characteristics, in addition to calf manager and calf-level predictors. We identified that Holstein calves were more fearful. Additionally, calf managers with other jobs on the farm, no training, low job satisfaction, or more negative attitudes and behaviors, were predictive of fearful calves. Understanding the factors influencing calves' fear responses may highlight a path to improve the human-animal relationship.

Running head: Reactivity of milk-fed dairy calves

Calf and herd-level factors associated with dairy-calf reactivity

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ABSTRACT

A positive relationship between handlers and animals in farm systems is essential, since the human-animal relationship has implications for welfare and productivity. For this reason, on-farm animal welfare assessment protocols often include the behavioral response of animals to humans to measure the quality of the human-animal relationship. The existing literature has described the multifactorial nature of this relationship. The current study aimed to investigate the potential influence of farm management and infrastructure characteristics, calf manager traits, and intrinsic features of dairy calves on the human-animal relationship. To this end, an Escape Test was conducted with 698 calves on 30 dairy farms in Chile. This test measured the calf's response to the active approach of an unfamiliar human (and was scored from 0 ("fearful") to 4 ("friendly")). The explanatory variables used to predict calves' response in the Escape Test were grouped into: 1) farm management and infrastructure (e.g., calf-dam separation age, space allowance); 2) calf manager (e.g., attitudes, behavior, and background); and 3) calf (e.g., breed, sex, age). We concluded that calf managers with additional jobs on the farm, with no training, low job satisfaction, a greater proportion of negative contacts, and more negative attitudes, were predictive of fearfulness in the Escape Test. Holstein breed (compared to Holstein and Jersey crossbreeds), were associated with greater odds of fearful calves. Our study confirms the association between animals' fear and handlers' features, which can be used as a potential opportunity to select employees on a farm. Understanding the factors that influence fear responses in calves may highlight the path to improve the relationship between animals and humans.

Keywords: human-animal relationship, animal welfare, dairy calf

INTRODUCTION

Ensuring the welfare of dairy calves is challenging due to the many interdependent factors that can influence welfare status. The human-animal relationship (HAR) is a significant constituent of animal welfare at a behavioral, physiological, and productive level (Waiblinger et al., 2006; Hemsworth and Coleman, 2011); thus, a greater understanding of HAR represents a potential focal point in the improvement of animal welfare. According to Hemsworth and Coleman (2011), the quality of HAR is built upon a repertoire of relevant interactions and determined by the context in which they occur. In farm animals, this relationship is governed by the constant contact with humans who care for the animals and can be classified as positive, negative, or neutral by the animal (Hemsworth and Coleman, 2011).

The quality of HAR can be measured by observing the interactions between the farmer or other humans and the animals under their care (Hemsworth and Coleman, 2011). According to Waiblinger et al. (2006), there are 3 methods available to assess the reactivity of animals to humans: responses to handling, reactivity towards a stationary human, and reactivity towards a person in movement. The Calf Escape Test with an Unfamiliar Person (Escape Test) conducted in the home environment, falls into the final category. The Escape Test is easily performed on commercial farms, and the influence of potential confounding factors is reduced when the test is carried out in the animals' familiar environment. Results from Escape Test performed with a familiar and unfamiliar person are highly correlated, and the test also has high inter-observer and high test-retest reliability (Bokkers et al., 2009). One potential disadvantage is the potentially subjective interpretation; for example, animals that do not move might be motivated by fear or indifference (Waiblinger et al., 2006).

The behavioral responses of animals to humans are varied and depend on dynamics of human-animal interactions, with fear being the most commonly evaluated (Lensink et al., 2001; Waiblinger et al., 2006). The first and main reaction of fearful animals is the escape-avoidance behavior that can lead to

acute and chronic stress, immunosuppression and health problems (Breuer et al., 2003; Hemsworth, 2003). In the study by Munksgaard et al. (1997), lactating cows were assigned to two treatments: aversive (strike with the hand on the cow´s head) and gentle handling (offer food, gently stroking and friendly voice). After treatment cows were capable to distinguish among handlers, showing greater distances towards the aversive handler and defecating and urinating more during the aversive treatment. Negative interactions between the handler and lactating cows have previously been associated with fewer cows approaching an experimenter and increased milk cortisol concentrations (Hemsworth et al., 2000). A positive correlation has also been observed between approaching behavior and milk yield (Breuer et al., 2000).

In young animals, early interactions and fear of humans are shown to have an important impact. Lürzel et al. (2015) reported that calves petted and stroked during the first 2 wk of life showed less avoidance distance before disbudding and had greater average daily gain. Probst et al. (2012) described a long-term effect in calves that received gentle contacts in the first 3 wk of life; these calves exhibited less movements backward in a stunning box during slaughter (at 10 months), which was also associated with greater meat tenderness.

There are many factors that influence the quality of HAR (Waiblinger et al., 2003, 2006; Leruste et al., 2012; de Roches et al., 2016). Personality and attitude are believed to be the most important influencers of farmer behavior (Breuer et al., 2000; Waiblinger et al., 2002; Hanna et al., 2009). For instance, positive farmer attitudes towards dairy cattle were positively correlated with soft vocalizations and negatively correlated with negative tactile interactions (Breuer et al., 2000; Hemsworth et al., 2000). The quality of the HAR can also be influenced by factors that affect farmer attitudes, such as job satisfaction or experience (Hemsworth and Coleman, 2011). Demographic factors such as gender are shown to have an impact, with women presenting more positive behavior (Lensink et al., 2000).

Farm characteristics and management also affect the response of farm animals. Leruste et al. (2012) reported that larger herd size, less space allowance, and absence of environmental enrichment led to a higher proportion of fearful calves in an Escape Test. Individual animal characteristics are also shown to affect the HAR, and include health status (Cramer and Stanton, 2015) and temperament, which differs between breeds (Leruste et al., 2012; Grandin and Deesing, 2014). As described, HARs are multifactorial, therefore identifying influential factors and aid in the development of intervention strategies to improve the welfare of animals and the farmers who care for them.

The aim of the current study was to investigate how different categories and combinations of variables (at the farm level, personnel level, and calf level) influence the HAR on farm. To quantify the HAR, we measured calf performance in an Escape Test.

MATERIALS AND METHODS

Farm Selection

Thirty dairy farms were visited during Spring-Summer season 2017 (n=17 farms) and Autumn-Winter 2018 season (n=13 farms) in the region of Los Ríos, Chile. The selection criterion were artificial rearing of calves (separation from the dam before 7 days postpartum) and the use of group pens at some point in the preweaning period. All participant farms were convenience sampled by means of personal contacts in the industry and within the Universidad Austral de Chile. No exclusion criteria were placed upon herd size or dairy production type.

Because the participants could be sensitive to the objectives of the study, and consequently change their behavior and responses, they were only partially informed of study objectives. They were told that the study aimed to evaluate the characteristics of the artificial breeding of dairy calves in the region.

After the study was completed, all farms involved were given a report detailing the results. Farms were visited once.

Before data collection was initiated, 3 farms were visited to pilot-test the surveys and the measurements obtained from the calves and from the calf manager. The aims of the visits were: to practice and standardize the Escape Test by the experimenter; to ascertain if calf managers were able to understand the statements in the surveys; to check and practice the evaluation of the calf manager behavior. Any procedural modifications resulting from the pilot testing are specified in the subsequent sections where applicable. These farms were not included in the final dataset.

The measurements were grouped as: 1) Calf Escape Test (the outcome of interest) and 2) Explanatory variables (Management and infrastructure; Calf manager behavior, attitude and background; and Calf). To eliminate bias during the visit, measurements were carried out in the following order: Calf manager behavior, Calf manager attitudes and background, Management and infrastructure measurements, Escape Test, and Calf measurements. All tests were conducted by the same person on all farms to reduce the possible influence of different experimenters.

Calf Escape Test

The Escape Test was always completed with an unfamiliar person during the herd visit. All farms had different schedules for moving calves from the individual hutches to group pens; consequently, only the unweaned calves in group pens were considered for evaluation. On average 23.3 ± 7.3 (SD) calves per farm were evaluated (a total of n = 698 calves). The number of evaluated animals per farm depended on the total number of calves: if there were fewer than 30 calves, all were evaluated; if there were more than 30, pens were chosen on a pseudo-random basis until 30 measurements were reached. On average

(±SD), calves spent 7.5 ± 17.7 days in the individual hutches before moving to group pens. The mean number of evaluated pens per farm was 2.9 ± 1.7 and an average of 9.2 ± 6.9 calves were found per pen.

The test was carried out in the group pens and was performed only once per calf, by the same experimenter (Caucasian female, brown hair, 1.58 m high, wearing blue coveralls). The Escape Test was carried out according to Bokkers et al. (2009) by measuring the reaction of calves to the active approach of an experimenter. The Escape Test began when the experimenter entered the pen and waited 1 min for the calves to become accustomed to her presence. Next, the observer chose a calf and positioned herself at a distance of 1.5 m in front of the animal. If the calf turned its head in another direction to avoid the observer, the observer waited approximately 20 seconds. If after this, the calf did not direct its head towards the observer or showed a withdrawal response, it was scored with 0 (see below). The test had 4 stages: 1) eye contact, 2) one step towards the calf with an extended arm, 3) a second step, and 4) an attempt to touch the calf's snout. The test ended when the calf moved, even if all 4 steps were not completed. Once the first stage began, and if the calf showed no withdrawal response, the observer waited 1 second to proceed to the next stage. The behavior of the calf was scored on a 5 point scale, with a score of 0 corresponding to an animal that avoided eye contact, a score of 1 for an animal that moved after the first step taken, a score of 2 for an animal that stood still after the first step, a score of 3 for an animal that stood still after the second step, and a score of 4 for an animal that could be touched. All measurements were made in the morning after feeding, with the exception of two farms, in which visits were scheduled between 1 to 3 pm, before afternoon feeding.

Explanatory Variables

The information collected was grouped as: management and infrastructure (application of structured survey and direct measurements), calf manager behavior, attitude and background (application of structured surveys and observational measurements), and calf (direct measurements). The surveys were

administered in Spanish; thus, all subsequent descriptions provided in the Materials and Methods represent English translations.

Management and infrastructure. Information related to the management of the calves was collected by means of a face-to-face interview with the calf manager and/or the dairy farm manager. The survey contained 6 questions (Table 1). Infrastructure measurements were carried out by the experimenter and included assessments of bedding material and space allowance.

Calf manager behavior, attitude and background.

All calves included in the Escape Test were weighed using the same electronic scale (SDS model IDS 701) that was transported to the farm by the experimenter. The behavior of the calf manager weighing the calves was assessed by the experimenter during the weighing procedure, which was done approximately 1 hour before the Escape Test was performed. If more than one staff member was involved in weighing the calves, only the person in charge of the calves was observed. On 6 farms, the behavior of the calf manager could not be assessed due to the absence of this individual at the time of the visit, or engagement with other tasks, so the behavioral assessment was not recorded. During the observation, which lasted approximately 30 min, the experimenter noted the number and nature of contacts between the calf manager and the calves as the calf was moved into the scale, weighed, and then removed. Types of contacts observed between the stockperson and the calf were adapted from Lensink et al. (2000) and Waiblinger et al. (2002) and are presented in Table 2. The interactions were grouped into “positive” (talking quietly, allowing the calf to suck the fingers, petting, slow movements, gentle chasing), “neutral” (neutral talking, neutral contacts), or "negative" (talking impatiently or shouting, slapping, kicking, pushing, twisting the tail and ears, fast movements, aggressive chasing), and the proportion of positive and negative interactions was obtained in relation to the total. After the pilot

farms were visited the following contacts were added: gentle and aggressive chasing and twisting the tail and ears.

Calf manager attitudes were measured after all observations were completed. The calf managers were asked to fill out a questionnaire adapted from Lensink et al. (2000). The questionnaire was designed to infer the attitudes of the calf managers towards their calves and was divided into 3 sections (Table 3). Two calf managers were unable to complete the survey because they were non-literate.

The second survey assessed the calf manager's background and contained questions related to gender, age, educational level, years of experience, training (any training related to animal welfare or animal handling), other jobs on the farm, number of calves, and one question about job satisfaction in the form of "how much do you like working with the calves?"; the answer was given on a 7-point scale where 1 represented "not at all" and 7 represented "a lot".

Calf. Calves' health assessment was completed by a trained veterinarian, including evaluation for skin lesions, joint lesions, respiratory disease, ocular discharge and diarrhea. All evaluations were based on the Welfare Quality® protocol (2009) and the protocol of the University of Wisconsin-Madison (https://www.vetmed.wisc.edu/dms/fapm/fapmtools/8calf/calf_health_scoring_chart.pdf). The diseases and conditions considered were diarrhea, nasal discharge, cough, and lameness. Calves were classified as sick if they exhibited symptoms of any of these listed diseases or conditions. Animals that did not stand up during the Escape Test (showing signs that their general health was compromised) were excluded. Additionally, age, sex and breed of the calves were recorded.

The final list of variables collected consisted of 23 potential explanatory factors for calf performance in the Escape Test, divided into 3 categories: management and infrastructure, calf-manager factors and calf-level factors (Table 4). Season variable was included in management and infrastructure category.

All experimental procedures applied during the course of this study were discussed and approved for humans (Ord. 287) and animals (N°359) by the institutional ethics committees of Universidad Austral de Chile.

Statistical Analyses

All data were analyzed using SAS (version 9.4, SAS Institute, Cary, NC). Descriptive statistics were obtained using PROC univariate. For data categorization, presence of maternity pen, calf manager training, and others jobs on the farm were coded as binary (yes/no). Amount of milk was categorized as ≤ 4 liters/day and > 4 liters/day. Space allowance was classified according to the legal minimum space for calves in the European Union as ≤ 1.8 m^2/calf and > 1.8 m^2/calf (as in Leruste et al., 2012). Bedding was divided into presence of bedding substrate (straw) and absence of bedding substrate. Calf manager gender and calf sex were classified as female or male. Job satisfaction, which was measured on a 7-point scale, was categorized as high (score 7) or low (< 7). This categorization was made based on the distribution of data, since 60% of calf managers scored 7 (high) and 40% between 4 and 6 on the scale (low). Educational level was classified as low (incomplete primary education), medium (complete primary education and incomplete high school) and high (complete high school). Sickness was coded as healthy or sick (presence of at least one disease). Breed was classified as Holstein, Holstein crossbreed (Holstein + beef breed) or Jersey crossbreed.

Categorical Principal Components Analysis. Due to multicollinearity of individual questions pertaining to stockperson attitude, a principal components analysis (PCA) was conducted to reduce the 15 statements into a smaller set of linear combinations, for use as potential predictors in the mixed logistic regression model. Because the attitude scores were obtained on an ordinal scale (from 1 to 7), an assumption of standard PCA (that variables must be measured on an interval or ratio scale) was violated; we therefore elected to conduct a categorical PCA (Linting and Van der Kooij, 2012). A monotonic

spline transformation (of degree 2, with 2 internal knots) was applied to all ordinal variables using PROC Prinqual, and components were extracted using PROC Factor. Components were retained if their eigenvalue was ≥1. A Varimax rotation was performed for ease of interpretation.

Mixed ordinal logistic regression modeling. Results from the Escape Test were grouped into 3 categories: friendly calf (score 4), cautious calf (score 1, 2, 3) and fearful calf (score 0); this variable served as the outcome in all further analyses. First, univariable analyses (mixed ordinal logistic regression models using PROC Glimmix) were carried out for each factor at a time, controlling for pen nested within farm as a random effect. The Sattherwaite approximation for the denominator degrees of freedom was applied, which accounts for the differences in degrees of freedom between farm-level and calf-level effects. Variables with results below the p-value threshold of ≤ 0.2 were considered as potential predictors in the multivariable logistic regression model. These variables were assessed for multicollinearity using PROC corr. If a pair of variables was correlated at a threshold of $r \geq |0.6|$, the variable most strongly related to the outcome in univariable analyses would be retained. A manual backwards stepwise elimination procedure was used to obtain the final multivariable, mixed ordinal logistic regression model, with only variables significant at $p < 0.05$ retained in the final model. Biologically relevant interactions were also considered for inclusion.

RESULTS

Descriptive Statistics

The mean number of lactating cows in the visited farms was 413.2 ±268.2 (SD) (min = 50, max = 1200). The mean number of calves per farm at the time of the visit was 148.6 ±135.9 (min = 20, max = 585), with an average age of 46.4 ±23.6 days (min=10; max=144). Most of the calves evaluated were females (65.8%). The distribution of main breeds on the farms was: Holstein (33.3%); Holstein

crossbreed (26.7%); Jersey crossbreed (40%) (Table 4). Most of the farms (77.4%) maintained a feeding regime of 4 L per d per calf twice a day, while the remainder offered between 5 and 8 L. In terms of milk distribution method, 83.3% of the farms used milk bar feeders while the rest used automated systems.

Seventy-seven percent of the calf managers were male and on average the calf manager's age was 43.9 ±13.0 (min=25, max=68). According to their educational level, 38.7% of calf managers reported low educational level (incomplete primary education), 29.0% median level (complete primary education and incomplete high school), and 32.3% high level (complete high school). Calf managers had 9.3 ±7.8 (min=1, max= 30) years of experience working with calves.

On 2 farms, no calves accepted to be touched and only one farm had no calves scored as 0 (fearful). In total, 32.7% of the calves evaluated were classified as friendly, 30.8% as cautious, and 36.5% as fearful.

Categorical PCA

The 15 transformed statements from the attitude survey were reduced into 3 components based upon the eigenvalue criterion of ≥ 1.0. These 3 components collectively accounted for 72.6% of the total variance. The first component (PC1) included statements such as: "petting calves is important for the success of the farm" and "how often do you kick your calves" and was labeled as "interacting with calves". The second component (PC2) included statements such as: "calves are sensitive to kicking" and "calves are sensitive to touching" and was labelled as "sensitivity". The last component (PC3) included statements such as: "How often do you let calves suck your fingers" and "letting calves suck your fingers is important for the success of the farm" and was labelled as "positive contact". Detailed results are presented in Table 5.

268 ***Risk factor analysis***

269 The univariable results are presented in Supplemental Table S1; https://doi.org/10.3168/jds.20XX-
270 XXXXX. Of the 23 factors evaluated at the univariable stage, 17 were retained (at $P \leq 0.20$) for
271 evaluation in the final multivariable model.

272 Complete results of the final model are presented in Table 6. Breed (Holstein, Holstein crossbreed,
273 Jersey crossbreed), space allowance (≤ 1.8 m^2/calf, > 1.8 m^2/calf), other jobs on the farm (Y/N), training
274 (Y/N), job satisfaction (High, Low), attitude (positive contact (PC3)) and the proportion of calf
275 managers' positive behavior were identified to ($P< 0.05$) influence the behavior of the calves in the
276 Escape Test. In summary, the Holstein breed (compared to Holstein and Jersey crossbreeds) was
277 predictive of a fearful response in the Escape Test. A space allowance higher than 1.8 m^2/calf was
278 associated with a higher risk of observing fearful calves. Farms in which the calf manager performed
279 other jobs (besides working exclusively with the calves) was predictive of more fearful calf responses.
280 Additionally, calf managers lacking training related to calf handling and welfare, with low job
281 satisfaction, and with a low proportion of positive contacts, were predictive of more fearful calves.
282 Finally, calf managers scoring higher in the attitude component "positive contacts" were associated with
283 lower odds of observing fearful calves.

284 **DISCUSSION**

285 Our study reaffirms the association between attitudes and the quality of the human-animal
286 relationship (Des Roches et al., 2016). The "Positive contact" component of attitude of calf managers
287 (PC3), which included items such as "How often do you let calves suck your fingers" and "Letting
288 calves suck your fingers is important for the success of the farm", was associated with friendlier calves
289 in the Escape Test. The calf managers who prioritized positive contact in the attitude survey and self-

reported that they performed these behaviors frequently, had a greater proportion of calves touched by the experimenter. Lensink et al. (2000) described that calves that were stroked and allowed to suck the farmer's fingers exhibited fewer fear behaviors and interacted longer with the experimenter. This behavior appears to strengthen the human-animal bond, being considered by the calves as a positive interaction.

It is reported that underlying attitudes can predict the farmer's behavior towards cows and calves (Breuer et al., 2000; Lensink et al., 2000; Waiblinger et al., 2002). There is a consistent correlation between the farmer's behavior and animal behavior (Breuer et al., 2000; Hemsworth and Coleman, 2011). Calves in our study were friendlier (more likely to be touched in the Escape Test) if the calf manager displayed a higher percentage of positive vocal and tactile interactions (petting, gentle chasing, talking quietly, letting the calves suck their fingers, slow movements), as assessed during calf weighing. This result adds to the large number of reports that associate the behavioral response of animals with the corresponding behavior of the farmer. More positive interactions or gentle handling are linked with animals that show fewer escape or withdrawal responses (Breuer et al., 2000; Hemsworth et al., 2000; Waiblinger et al., 2002; 2003)

We detected in our study that calf managers who reported a high score in the job satisfaction question ("How much do you like working with calves"), were associated with fewer fearful calves. Job satisfaction is a highly valuable aspect for dairy farmers (Bruijnis et al., 2013), which has the potential to influence animal welfare and performance (Hemsworth and Coleman, 2011). Also, job-related aspects could affect their behavior by modifying attitudes (Maller et al., 2005; Waiblinger et al., 2006). Maller et al. (2005) reported that farmers with more positive attitudes towards handling dairy cows (such as ease of movement) were correlated with enjoying the work during milking. Coleman et al. (1998) surveyed farmers in the pig industry and found that greater job satisfaction was correlated with lower negative

attitudes towards working with pigs, and that these negative attitudes were associated with negative behavior evaluated during handling activities with breeding female pigs.

If the calf manager had other jobs on the farm besides exclusively caring for the calves, animals avoided the experimenter more. This may be associated with the fact that the calf manager, in general, was expected to spend less time with the animals if preoccupied with other farm tasks. A calf manager who has other tasks on the farm may engage in a lower level of physical or visual contacts with the animals, affecting their behavioral response in the Escape Test. This effect has been described in other studies, but in the context of herd size. That is, a larger herd size has been associated with a decreased ability to touch cows in an avoidance-distance test and with a higher proportion of calves scoring 0 in the Escape Test (Waiblinger and Menke, 1999; Leruste et al., 2012). In larger herds, farmers have less time to interact with their animals, so the frequency of contacts per animal is lower (Waiblinger and Menke, 1999). However, in the present study, no association was detected between the size of the herd and the results of the Escape Test.

In our study, we identified that calf managers with training (animal welfare or animal handling training) were associated with less-fearful calves. These results are consistent with a demonstrated effectiveness of training programs in animal production systems. For example, Hemsworth et al. (2002) reported that the application of a training program (cognitive-behavioral intervention) improved the attitudes and behavior of dairy farmers, which were, in turn, associated with shorter flight distances and better productive records in cows. Considering that most of the calf-care personnel learn their job with the help of their experienced peers (Sischo et al., 2019), there could be a link between trained calf managers and increased knowledge and translatable skills. Additionally, Stup et al. (2006) stated that continuing training was associated with return on equity (a measure of financial performance) in dairy farmers. Training farmers is associated with the acquisition of new knowledge, but also linked to higher

self-esteem, improvement of morale and job satisfaction (Stup et al., 2006; Hemsworth and Coleman, 2011). In Hemsworth and Coleman (2011) it is clear that many of the factors that can influence farmer attitudes, behavior, and subsequent behavioral response of the animals, are interrelated. Coleman et al. (1998) reported a positive relationship between positive attitudes and the willingness of the farmers to attend to training sessions and learn more about working in the industry. All these aspects have the potential to improve the quality of HAR and thus the productivity and welfare of the animals. This can be reflected in the behavioral response of the calves in the Escape Test.

In the current study, it was identified that Holstein calves were more fearful than Holstein and Jersey crossbreeds. These results are consistent with those obtained by Leruste et al. (2012), who reported that dairy breeds compared to dual breeds or crossbreeds were more likely to score 0 in the calf Escape Test. Temperament and fear of humans has been shown to be heritable, and there are differences between breeds (Hemsworth et al., 1990; Grandin and Deesing, 2014). Lanier et al. (2000) reported that dairy breeds showed more sensitivity to sound and touch than beef breeds, perhaps explaining the increased fearfulness of Holsteins in our study compared to calves crossbred with beef breeds. In contrast, Murphey et al. (1981) reported that dairy breeds showed less avoidance than beef cows, concluding that this result was due to genetic selection and constant human handling in dairy production. In this study, all visited farms implemented artificial rearing systems, with daily human contact, suggesting a potential genetic component to our results.

The only farm-level factor associated with the behavioral response of the calves in the Escape Test was the space allowance. Larger space (>1.8 m^2 / calf) in the group pens, was associated with more fearful calves. The Escape Test as administered in our study, and similar variants of this test, have been validated in previous research with both dairy cows and calves and have been determined to accurately reflect the human-animal relationship on farm (Lensink et al, 2000a; Lensink et al., 2001). These

studies, however, did not evaluate whether space allowance affected calf response, and whether the manifestation of the fear response may differ based upon the ability of the animal to withdraw from the fearful stimulus. It is difficult to interpret the increase in fearful calves in our study when higher space allowance per calf was provided. It is conceivable that immobility in the case of small space allowance was a reflection of fearfulness, as fear responses such as passive avoidance or immobility have been noted previously in animals (Forkman et al., 2007; Hemsworth and Coleman, 2011). It is equally plausible that the smaller space allowance facilitated a great frequency of human-animal interactions, leading to decreased fear responses to humans. Considering that daily chores of a calf manager include pen cleaning and calf feeding, it is certainly possible that calves are accustomed to the closest contact with their calf manager when housed in smaller spaces. The later explanation better corresponds to the model results, given that positive interactions and attitudes were associated with a higher odds of being touched, even after adjusting for space allowance. In any case, our results pertaining to space allowance are discordant with previous findings (Leruste et al., 2012), and the influence of space on calf response in the Escape Test certainly warrants further exploration.

This study was limited by several factors. One of the limitations was the impossibility of making multiple visits to the farms to corroborate (Bokkers et al., 2009) and improve the reliability of the measurements (Waiblinger et al., 2006). Additionally, we were not able to investigate if the order in which the test was performed affected the behavioral response of the animals. Another limitation was that, because feeding schedule was not recorded, it was not possible to give an exact time between feeding and performing the Escape Test. Even so, all measurements were made postprandial. Further studies may include exploring the motivations of animals towards humans during this type of test, since misreading the animal's behavior can lead to erroneous interpretations that can be critical from a welfare point of view (e.g. the misclassification of hunger as friendliness). It is worth noting that caution

382 towards humans (likely indicative of some low level of fear) is not necessary detrimental to the animal;
383 however, extreme fear towards humans is likely to compromise welfare. In order to facilitate an accurate
384 interpretation of an animal's reaction, it would also be useful to know the extent to which social
385 dynamics affect the individual response of the animal.

386 To our knowledge, few studies (with the exception of Leruste et al., 2012) have included farm,
387 farmer, and calf-level traits to evaluate the human-animal relationship by means of the Escape Test.
388 Because these different trait levels may influence each other, a single multivariable model is a useful
389 tool to evaluate the complexities of calf behavior and to provide a complete picture of motivating
390 factors. Additionally, many studies addressing similar objectives have modeled dichotomous responses
391 (percentage of touched animals or withdrawal responses) without exploring intermediate calf behaviors.
392 In the present study, we employed an ordinal logistic regression model with 3 categories (fearful,
393 cautious, friendly) to provide a more nuanced representation of calf responses in the Escape Test. While
394 there are other potential groupings that may be hypothesized to be representative of calf behavior, we
395 attempted to strike a balance between statistical parsimony and behavioral complexity through the use of
396 3 levels of the response variable. The odds ratios provided in the model (and their reciprocals) can allow
397 for the comparison of friendly calves to their cautious and fearful counterparts, in addition to a
398 comparison of fearful calves to their cautious and friendly counterparts. Moreover, many studies
399 assessing attitudes treated Likert-type scales (e.g., responses scored as 1-7) as continuous variables,
400 either as outcomes in linear regression models or as input variables in standard PCAs, or both. For
401 categorical or ordinal variables, a suitable option is the categorical PCA (Linting and Van der Kooij,
402 2012).

403 In conclusion, we determined that there are factors at the farm, calf manager, and animal level that
404 affect the behavioral response of calves in an Escape Test. As in previous studies (Hemsworth et al.,

2000, Hanna et al., 2009), our results showed the consistent relationship between characteristics of the farmer and fear of animals to humans. Identifying the intrinsic factors of farmers, which affect fear in animals, can be used as a potential opportunity to select employees on a farm, based for example on their attitudes towards working with calves. Additionally, our results suggest that calf managers may benefit from training to improve their interactions with the animals they care for. These results support and highlight the importance of calf managers' attitudes and actions in influencing the behavior of calves in dairy production systems.

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546 Table 1. Targeted area of management and scale used in the survey on calf management.

Area of management	Scale
Number of lactating cow present on the farm	open-ended question

Number of calves present on the farm	open-ended question
Are cows brought into a maternity pen to calve?	☐Yes ☐No
At what stage postpartum do you separate the calf from the dam?	open-ended question
How much time do the calves spend in the individual hutches?	open-ended question
How many liters of milk do you feed the calves?	☐≤4 liters ☐>4 liters

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548 Table 2. Positive, negative and neutral interactions assessed between the calf manager and the calves.

549 Vocal (*) and tactile (**) interactions are highlighted, and descriptions for each behavior are provided.

Contact	**Description**
Positive interactions	
Talking quietly*	Talking calmly, in a friendly tone, with a soft and low voice.
Suck the fingers**	Extending the hand to allow the calf suck the fingers.
Petting**	Stroking any part of the calf´s body.
Slow movements**	Moving and walk calmly and gently in the pen.
Gentle chasing**	Moving the calves while respecting their natural rhythm or helping them gently
Negative interactions	
Talking impatiently or shouting*	Talking with impatient, violent or loud voice to move or stop the calves.
Slapping**	Hitting or slapping the calf with moderate to high use of force.
Kicking**	Kicking the calf.
Pushing**	Pushing the calf aggressively.
Twist tail or ears**	Twisting the tail or ears to move the calf.
Fast movements**	Moving abruptly and quickly in the pen.
Aggressive chasing**	Moving the calves aggressively without respecting their natural rhythm.
Neutral interactions	
Neutral talking*	Talking without friendly or impatient tone or remaining silent.

Neutral contacts**	Putting the hand on the animal without petting or hitting it or not making tactile interactions.

Table 3. Attitude survey to the calf managers. The information presented is divided into 3 sections, each containing 5 statements and descriptions for each section are provided. The answers to the statements were given on a 7-point Likert scale.

Section	Purpose Infer attitudes towards behaving with calves from:	No of statements	Statements	Scale
1	Their beliefs regarding the sensitivity of calves to contact.	5	Do you agree with the following statements: calves are sensitive to x (pain, petting, touching, talking, kicking)	7-point scale, 1= full disagreement to 7= full agreement
2	Their beliefs regarding the importance of the calf manager behavior for the success of the farm.	5	Do you agree with the following statements: x (letting calves suck your fingers, petting, being patient with, working quietly with, talking to calves) is important for the success of the farm.	7-point scale, 1= full disagreement to 7= full agreement
3	Their own description of interactions with the calves.	5	How often do you x (pet your calves, slap calves with your hands, let calves suck your fingers, talk to your calves, kick your calves).	7-point scale, 1= never, 4= sometimes, 7= very often

Table 4. Potential explanatory variables to explain calf performance in an Escape Test. The information presented is divided into 3 sections: Management and infrastructure, calf manager-level factors (both behavioral and demographic), and calf-level factors. The table was populated by means of

560 the survey responses from 2 separate surveys (one pertaining to attitudes towards the calves and the
561 other addressing demographic information and management practices) and direct observations of the
562 calf handler and of the calves. Variables measured on a continuous scale are flagged with an '*'

Category and factors	Level	Scores
Management and infrastructure		
Season	0:Spring-summer; 1:Autumm-winter	0:17; 1:13 farms
Presence of maternity pen	0: Yes; 1: No	0:19; 1:11 farms
Calf-dam separation*		29 farms
Number of calves*		30 farms
Time in individual hutches*		30farms
Space allowance	0: ≤1.8 m^2/calf; 1: >1.8 m^2/calf	0: 191; 1:507 calves
Bedding	0: Presence bedding; 1: absence bedding	0:27; 1:2 farms
Calf manager		
Attitude*		28 calf managers
Proportion of positive behavior*		24 calf managers
Proportion of negative behavior*		24 calf managers
Gender	0: Female; 1: Male	0: 6; 1: 24 calf managers
Age*		30 calf managers
Educational level	0: Low; 1: mid; 2: high	0: 11; 1:9; 2:10 calf managers
Training	0: Yes; 1: No	0:13; 1: 15 calf managers
Job Satisfaction	0: Low; 1: High	0:11; 1:17 calf managers
Other jobs	0: Yes; 1: No	0: 22; 1: 9 calf managers
Years of experience*		30 calf managers
Calf		
Sickness	0: Healthy; 1: Sick	0:422; 1:276 calves
Sex	0: Female; 1: Male	0:451; 1:234 calves
Breed	0: Holstein; 1: Holstein crossbreed; 2: Jersey crossbreed	0:241; 1:170; 2:287 calves
Age		698 calves

563 *Continuous factors

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565 Table 5. Varimax rotated pattern for transformed attitude scores. For each retained principal
566 component we provide the eigenvalue, the percentage of variance explained, and the contribution of
567 each statement from the attitude survey. Values within the component pattern are rounded to two
568 decimal places, and contributions of ≥ |0.5| are bolded and flagged with an '*'. Results are ordered by
569 contribution within component

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Results	**Component 1: Interacting with calves**	**Component 2: Sensitivity**	**Component 3: Positive contact**
Eigenvalue	5.23	3.20	1.74
Percentage of total variance explained	37.3%	22.9%	12.4%
Contribution of each statement			
How often do you kick your calves	**-0.84***	0.17	0.02
Petting calves is important for the success of the farm	**0.83***	-0.25	0.00
How often do you pet your calves	**0.79***	0.09	0.39
Talking to calves is important for the success of the farm	**0.76***	0.45	0.22
Calves are sensitive to petting	**0.71***	0.11	**0.53***
Calves are sensitive to talking	**0.66***	**0.57***	0.36
How often do you slap your calves with your hands	**-0.60***	-0.18	0.16
Calves are sensitive to kicking	0.09	**0.90***	-0.10
Calves are sensitive to touching	-0.08	**0.81***	0.12
Working quietly with calves is important to the success of the farm	0.03	**0.79***	-0.20
How often do you talk to your calves	0.46	**0.68***	0.25
Calves feel pain	-0.10	**0.55***	-0.38
How often do you let calves suck your fingers	0.03	-0.03	**0.91***
Letting calves suck your fingers is important for the success of the farm	0.10	-0.10	**0.86***

571 Table 6. Results of the mixed ordinal logistic regression model using farm-, calf manager-, and
572 calf-level factors to predict calf response in an Escape Test. Estimates represent the slope (β) estimates
573 for the included fixed effects. Standard errors (SE), Odds ratios (OR), 95% confidence intervals for the

574 ORs (CI), and P values are also presented. Results are cumulated over the lower-ordered values (with 0
575 indicating fearful calves, 1 indicating cautious calves, and 2 indicating friendly calves)

Factor	Level	Estimate	SE	OR	95% Wald CI for OR	P value
Intercept		-0.69	0.68			
Intercept		1.31	0.68			
Breed	Holstein crossbreed	3.06	0.56	21.41	6.90, 66.34	<0.0001
	Jersey crossbreed	2.09	0.48	8.11	3.07,21.39	<0.0001
	Holstein (ref)					
Space allowance	≤ 1.8 m^2/calf	0.86	0.41	2.37	1.03,5.45	0.041
	> 1.8 m^2/calf (ref)					
Other jobs	Yes	-1.89	0.42	0.15	0.06,0.35	<0.0001
	No (ref)					
Training	Yes	1.18	0.45	3.26	1.31,8.08	0.011
	No (ref)					
Satisfaction	Low	-0.92	0.36	0.39	0.18,0.82	0.014
	High (ref)					
Attitude: positive contact		0.54	0.18	1.73	1.19,2.51	0.005
Proportion of positive interactions		0.02	0.006	1.11*	1.04,1.19	0.001

576 * Calculated based upon an increase of 5 units

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581 **APPENDIX**

582 Table S1. Results of the univariable mixed ordinal logistic regression model using farm-, calf
583 manager-, and calf-level factors to predict calf response in an Escape Test. Estimates represent the slope
584 (β) estimates for the included fixed effects. Standard errors (SE), Odds ratios (OR), 95% confidence
585 intervals for the ORs (CI), and P values are also presented. Results are cumulated over the lower-ordered
586 values (with 0 indicating fearful calves, 1 indicating cautious calves, and 2 indicating friendly calves)

Factor	Level	Estimate	SE	OR	95% CI for OR	p-value
Sick	Healthy	-0.10	0.19	1.10	0.76-1.61	0.580
	Sick (ref)					
Sex	Female	-0.44	0.22	0.64	0.41-0.98	0.044
	Male (ref)					
Calf age		0.002	0.004	1.00	0.99-1.01	0.670
Breed	Holstein	-0.06	0.43	0.94	0.39-2.24	0.006
	Holstein crossbred	1.25	0.43	3.50	1.46-8.35	
	Jersey crossbreed (ref)					
Season	Spring-summer	-0.57	0.47	0.56	0.22,1.43	0.220
	Autumn-winter (ref)					
Calf-dam separation		0.55	0.22	1.01	1.00-1.02	0.002
Number of calves in the calf barn		0.0004	0.001	1.00	0.99-1.00	0.780
Number of calves in the pen		1.57	0.31	0.95	0.90-0.99	0.03
Amount of milk	≤ 4 liters	0.81	0.46	2.26	0.90-5.68	0.080
	> 4 liters					
Days alone in hutches		0.02	0.01	1.02	0.99-1.05	0.057
Space allowance	≤ 1.8 m2/calf	1.23	0.38	3.43	1.58-7.43	0.002
	> 1.8 m2/calf (ref)					
Bed	With bed	0.84	0.61	2.31	0.67-7.91	0.170
	Without bed (ref)					
Proportion of positive interaction		0.02	0.007	1.15	1.06-1.24	0.001
Proportion of negative interactions		-0.01	0.006	0.98	0.96-0.99	0.013

Gender	Male	-1.24	0.43	0.28	0.12-0.67	0.005
	Female (ref)					
Educational level	Basic	0.39	0.43	1.48	0.61-3.58	0.290
	Intermediate	0.69	0.44	2.00	0.81-4.90	
	High (ref)					
Training	Yes	-0.93	0.37	0.39	0.18-0.82	0.014
	No (ref)					
Job satisfaction	Low	-0.60	0.40	0.55	0.24-1.23	0.140
	High (ref)					
Other jobs on the farm	Yes	-1.34	0.38	0.26	0.12-0.55	0.001
	No (ref)					
Age		0.02	0.015	1.02	0.99-1.06	0.069
Years of experience		0.04	0.02	1.04	0.99-1.10	0.099
Attitude: Interacting with calves		-0.36	0.22	0.69	0.44-1.08	0.100
Attitude: sensitivity		0.11	0.23	1.12	0.69-1.80	0.620
Attitude: positive contact		0.59	0.20	1.82	1.22-2.72	0.004

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