Reliability of sampling strategies for measuring dairy cattle welfare on commercial farms

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ABSTRACT

Our objective was to evaluate how the proportion of high-producing lactating cows sampled on each farm and the selection method affect prevalence estimates for animal-based measures. We assessed the entire high-producing pen (days in milk <100; range = 81–241 cows) on 10 California farms using measures from the Welfare Quality Protocol for Cattle. Cows were restrained in head locks and visually evaluated for body condition, dirtiness, skin alterations (hair loss, lesions, or swelling), discharge (ocular, nasal, vulvar), diarrhea, and impaired respiration. Lameness was scored upon release. Prevalence was calculated as a percentage of assessed cows. The most common conditions were dirty hindquarters ($33.5 \pm 10.7\%$, mean $\pm$ standard deviation) and lesions or swelling on the carpal joint ($34.4 \pm 7.0\%$) and hock ($26.4 \pm 16.7\%$). Diarrhea ($8.0 \pm 5.8\%$), lameness (moderate = $7.3 \pm 4.7\%$, severe = $2.2 \pm 2.2\%$), and neck ($5.8 \pm 12.6\%$), flank ($4.5 \pm 5.0\%$), or hindquarter alterations ($5.5 \pm 3.9\%$) were less common. Very fat cows, vulvar discharge, and impaired respiration were rare ($\leq 1\%$) and were excluded from further analysis. Four sampling strategies were used to generate 20 estimates for each animal-based measure. The strategies were (1) selecting every 10th, 5th, 4th, 3rd, 2nd, 2 of 3, or 3 of 4 cows at the feed bunk (7 estimates/measure); (2) randomly selecting 7 matching proportions of the pen; (3) randomly selecting cows using 3 sample size calculations from the Welfare Quality Protocol; and (4) selecting the first, middle, or final third of cows exiting the milking parlor. Estimates were compared with true values using regression analysis and were considered accurate if they met 3 criteria: the coefficient of

determination was ≥ 0.9 and the slope and intercept did not differ significantly from 1 and 0, respectively. All estimates met the slope and intercept criteria, whereas the coefficient of determination increased when more cows were sampled. All estimates were accurate for neck alterations, ocular discharge ($22.2 \pm 27.4\%$), and carpal joint hair loss ($14.1 \pm 17.4\%$). Selecting a third of the milking order or using the Welfare Quality sample size calculations failed to accurately estimate all measures simultaneously. However, all estimates were accurate when selecting at least 2 of every 3 cows locked at the feed bunk. Using restraint position at the feed bunk did not differ systematically from computer-selecting the same proportion of cows randomly, and the former may be a simpler approach for welfare assessments.

Key words: health, on-farm assessment, sampling, validation

INTRODUCTION

Welfare describes an animal's state and spans a spectrum from good to poor. Based on this concept, assessments of farm animal welfare increasingly focus on evaluating animal-based measures (e.g., Whay et al., 2003) in addition to aspects of the environment or management. Animal welfare assurance programs not only seek to accurately classify the state of the animals on a given farm, but are also faced with feasibility constraints, namely investment of time and the number of assessors.

A key issue is the appropriate number of animals to sample on a given farm, as this can affect both the accuracy of welfare classification and the costs associated with conducting the assessment. Studies on dairy cattle (Endres et al., 2014) and swine (Mullan et al., 2009) have shown some associations between the true prevalence of an animal-based measure and the sample size needed to accurately estimate it, such that conditions with low prevalence sometimes require sampling

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more animals. This means that accurately estimating low-prevalence conditions may present a feasibility challenge on larger operations, particularly given the continued global trend of farms increasing in size (reviewed by Robbins et al., 2016).

Assessment schemes have attempted to balance accuracy and feasibility in their guidelines for the number of animals to score. Among the Welfare Quality protocols, these sample size recommendations vary. Within the swine protocol (Welfare Quality, 2009b), certain measures call for different sample sizes, but the number of pigs sampled does not increase directly with farm size. For dairy cattle (Welfare Quality, 2009a), a single sample size, based on Cochran (1977), is used for all animal-based health measures on a given farm, and the number of cows sampled grows at a diminishing rate as herd size increases. To our knowledge, no work has directly evaluated the reliability of using these sample size calculations to estimate prevalence across a large selection of the animal-based measures in the Welfare Quality (2009a) protocol for dairy cattle.

A few studies have focused primarily on lameness, an important animal-based measure. Strategies have included comparing sample estimates against a tolerance range relative to the true prevalence (Main et al., 2010; Hoffman et al., 2013) or using sequential sampling based on a technique used in clinical trials (Heath et al., 2016), although the latter study assumed that the sample size calculations in the Welfare Quality (2009a) protocol represented the true prevalence. One study, however, used a more stringent linear regression approach to evaluate the sample size needed to estimate lameness and other select animal-based measures (Endres et al., 2014). Estimates were deemed accurate only if their relationship to the true prevalence had a coefficient of determination (R^2) ≥ 0.9 and the slope and intercept did not differ from 1 and 0, respectively. Our research group has used the same linear regression criteria to identify schedules for sampling cattle behavior through direct observation (Stackhouse-Lawson et al., 2015; Chen et al., 2016; Tresoldi et al., 2016) or using data loggers (Ledgerwood et al., 2010).

In addition to determining how many cows to sample, questions remain about which animals to select. The Welfare Quality (2009a) protocol for dairy cattle provides recommendations such as selecting every n th cow either in the milking order or when locked at the feed bunk, or by choosing cattle distributed throughout the home pen and engaged in various activities (e.g., standing, lying, or feeding) to approximate random sampling. In studies that benchmark measures of dairy cattle welfare across farms, a common approach is to score cows during milking (von Keyserlingk et al.,

2012), but a single assessor would be unable to observe the same cow from the front, back, and while walking to score all of the animal-based measures in the Welfare Quality (2009a) assessment.

Our objective was to evaluate how prevalence estimates for a wide range of animal-based measures are affected by the proportion of high-producing cows sampled and the method used to select them. We focused on this population because considerable interest exists in studying confined, high-producing cattle (e.g., Espejo et al., 2006; von Keyserlingk et al., 2012; Cook et al., 2016), which are particularly at risk for welfare concerns such as lameness (Barkema et al., 1994). We evaluated the accuracy of the estimates generated by sampling various proportions of cattle. In addition, we compared the strategies of choosing cows based on their position while restrained at the feed bunk, selecting ear tag numbers randomly, or assessing different cows at separate times during milking.

MATERIALS AND METHODS

Animals, Housing, and Measures

Between October and December 2013, 2 assessors visited 10 California dairy farms ($n = 10$ farms, 1,805 cows total), all of which milked cows twice daily. These farms were a convenience sample among clients of the Veterinary Medicine Teaching and Research Center of the University of California-Davis (UC Davis) School of Veterinary Medicine. On each farm, all cows in 1 pen of high-producing cows (DIM < 100) were assessed. The housing type, breed managed, herd size, and size of the high-producing pen from each farm are shown in Table 1. Each cow was identified by ear tag number and was evaluated using select measures (Table 2) from the dairy cow section of the Welfare Quality (2009a) protocol. The measures selected were all nonbehavioral ones assessed at the individual animal level. The evaluation was conducted while cows were restrained in head locks in the pen with the exception of lameness, which was scored when cows were released. One assessor recorded ear tag numbers and nasal and ocular discharge from the front of the cow. The second assessor recorded all other measures from inside of the pen, releasing 1 cow at a time from the head locks to score lameness and to gain a full side view of the next cow. Intraobserver reliability was determined using a combination of photos, video clips, and live observations (≥ 20 cows per measure), and percentage agreement was $\geq 80\%$ for all measures. Cow position along the feed bunk during restraint and the order in which they were milked were also manually recorded.

Table 1. Characteristics of 10 California dairy farms that were assessed and the number of cows randomly selected in the high-producing pen (DIM <100) using various sample sizes

Housing ¹	Breed	Pen size	Equation ²			Proportion of pen ³						
			Infinite	WQ high	WQ low	1/10	1/5	1/4	1/3	1/2	2/3	3/4
Freestall	Holstein-Friesian	241	96	69	53	24	48	60	80	121	161	181
		216	96	66	51	22	43	54	72	108	144	162
		191	96	64	50	19	38	48	64	96	127	143
		145	96	57	46	15	29	36	48	73	97	110
		144	96	57	46	14	29	36	48	72	96	108
		123	96	54	43	12	25	31	41	62	82	92
Dry lot	Holstein-Friesian	233	96	68	52	23	47	58	78	117	155	174
		227	96	67	52	23	45	57	76	114	151	170
		81	—	44	37	8	16	20	27	41	54	61
	Jersey	204	96	65	51	20	41	51	68	102	136	153

¹Housing types were either freestall barns with sand bedding or dry lot corrals with dirt surfaces.

²Sample sizes were based on the equations by Cochran (1977) used to calculate the recommendations in the Welfare Quality (2009a) protocol, all using an assumed prevalence of 50% for a given health condition and a desired precision of 10%. Infinite refers to a sample size of 96 cows, based on a 95% CI and an infinite population size, independent of actual pen size. WQ high and WQ low refer to sample sizes based on 95 and 90% CI, respectively, along with the actual pen sizes for each farm.

³Sample sizes reflected proportions of the population in the pen, with cows selected both randomly by computer and based on their positions at the feed bunk.

Methods for Selecting Cows to Sample

Ear tag numbers were selected using 4 strategies, and the individual assessment data from the selected cows were used to create a total of 20 subsets of data. The first 2 strategies were to select different proportions of the pen (Table 1; 14 subsets of data). First, to reflect how an assessor would potentially identify cows when locked at the feed bunk, 7 subsets of data were created by selecting every 10th, 5th, 4th, 3rd, 2nd, 2 out of every 3, and 3 out of every 4 cows. Second, we created 7 subsets of data reflecting the same proportions of the pen, generated using the simple random sample function within PROC SURVEYSELECT in SAS software (SAS Institute, 2009).

The third strategy (Table 1; 3 subsets of data) used the equations by Cochran (1977) to determine sample size, where p = the expected prevalence of the condition, Z = the confidence interval, d = the desired precision, and N = the population (pen) size:

$$n_{\text{inf}} = \frac{p(1-p)Z^2}{d^2}$$

$$n_{\text{fin}} = \frac{n_{\text{inf}}}{1 + (n_{\text{inf}} - 1) / N}.$$

All 3 calculations used $p = 50\%$ and $d = 10\%$. The n_{inf} calculation for an infinite population, using $Z = 1.96$ (95% CI), generated a sample size of 96 cows; on the 9 farms with sufficient pen size, this number of ear tags

was randomly selected using PROC SURVEYSELECT. The other 2 subsets were generated based on the recommendations in the Welfare Quality (2009a) protocol, which determine n_{fin} using the pen size of each farm and either $Z = 1.96$ (**WQ high**) or 1.645 (90% CI; **WQ low**).

The fourth strategy was to divide each pen into 3 equal subsets based on the order in which the cows were milked (first, middle, and final third).

Statistical Analysis

The data were compiled into 25 measures (19 individual and 6 composite), with prevalence for each calculated as a percentage of the number of cows sampled on each farm. Very fat cows, impaired respiration, and vulvar discharge were rarely observed ($\leq 1\%$ of cows on any farm; Table 2), and were thus excluded from analysis. For the remaining 22 measures (16 individual, 6 composite), we evaluated relationships between the true prevalence and the estimates generated from each subset using linear regressions (PROC REG; SAS Institute, 2009). A subset of data was determined to accurately reflect the true prevalence if it met 3 criteria: the R^2 was ≥ 0.9 , the slope did not differ significantly from 1 ($P > 0.05$), and the intercept did not differ significantly from 0 ($P > 0.05$). To control for the false discovery rate associated with conducting multiple comparisons ($m = 880$), all P -values for the slopes and intercepts were adjusted using the Benjamini and Hochberg (1995) procedure in PROC MULTTEST.

RESULTS AND DISCUSSION

Prevalence of Health Conditions

Descriptive data for the true prevalence of each measure are presented in Table 2. To place our results for average prevalence in the context of existing studies, we selected those that assessed only high-producing (or <120 DIM), loose-housed cows across multiple farms. For some measures, particularly severe lameness, the mean and maximum prevalence in our study was relatively low, perhaps because we used a convenience sample of producers who had strong relationships with UC Davis veterinarians. Nonetheless, drawing conclusions about mean prevalence among studies is challenging, as estimates can vary due to differences among populations (e.g., region, housing system, and manage-

ment factors) or inconsistencies among scoring systems, assessors, and sampling strategies.

Lameness. Total clinical lameness in our study averaged 9.5%, virtually identical to the mean of 9.6% recently reported in a sample of 184 US farms across various housing types (Adams et al., 2017). In California, the average among freestall operations was previously found to be 3 times higher (30.8% in von Keyserlingk et al., 2012). Averages in other regions ranged from 13 to 25% in the Midwest (Espejo et al., 2006; Cook et al., 2016), 15 to 28% in Canada (von Keyserlingk et al., 2012; Solano et al., 2015; King et al., 2016; Westin et al., 2016), and 55% in the northeast United States (von Keyserlingk et al., 2012). Severe lameness in our study averaged 2.2%, consistent with the means of 2.2 to 2.7% reported in several North American studies (Cook et al., 2016; King et al., 2016; Adams et al., 2017), but

Table 2. Definitions, mean prevalence¹, SD, and range across California dairy farms (n = 10) for 23 measures from the Welfare Quality (2009a) protocol

Measure	Definition	Prevalence	SD	Range
Very lean ²	Depressions around tail head and loin; prominent tail head, hip bones, spine, ribs	13.0	10.4	1.2–34.3
Very fat ²	Convex between hip bones and spine, transverse processes indiscernible, fatty tissue visible under skin	0.2	0.4	0–1.0
Any dirtiness ²	Dirtiness on any of the 3 assessed body parts	46.8	12.4	18.3–62.8
Lower hind leg	Plaques ³ of dirt above the coronary band	18.4	15.5	2.6–46.9
Hindquarter	Plaques of dirt on the upper hind leg, flank, tail, or rear (excluding udder)	33.5	10.7	16.8–48.9
Udder	Distinct plaques of dirt on udder or any dirt on and around teats	16.1	12.3	0–36.8
Total lameness ⁴	Sum of moderately and severely lame cows	9.5	6.5	1.4–16.8
Moderate	Imperfect temporal rhythm in stride creating a limp	7.3	4.7	1.4–14.2
Severe	More than one affected limb or strong reluctance to bear weight on one limb	2.2	2.2	0–5.7
Nonlimb alterations ⁵	Any hair loss, ⁶ lesion, ⁷ or swelling			
Hindquarter	Upper hind leg, tail, or rear	5.5	3.9	1.4–14.1
Flank	Flank, side, or udder	4.5	5.0	0–15.7
Neck	Neck, shoulder, or back	5.8	12.6	0–41.5
Hock alterations ⁵	Outer and inner tarsal joints nearest and furthest from the assessor, respectively	38.8	14.8	13.7–61.3
Hair loss		15.0	8.8	3.9–29.3
Lesions or swelling		26.4	16.7	6.2–60.3
Carpal joint alterations ⁵	Outer carpal joint nearest the assessor	47.3	15.8	23.5–72.7
Hair loss		14.1	17.4	0–53.7
Lesions or swelling		34.4	17.0	13.8–62.6
Any hair loss	Hair loss on at least 1 assessed body region	30.6	19.9	10.3–62.0
Any lesions or swelling	At least 1 lesion or swelling on any assessed body region	52.2	18.0	33.2–84.8
Nasal discharge	Clearly visible discharge or flow from the nostrils	19.3	14.5	0.4–47.5
Ocular discharge	Clearly visible wet or dry discharge or flow from the eye ≥ 3 cm long	22.2	27.4	0–75.7
Impaired respiration	Deep and labored or overtly difficult breathing	0.1	0.2	0–0.7
Evidence of diarrhea	Loose watery manure at least the size of a hand on both sides of the tail below the tail head	8.0	5.8	0–18.6
Vulvar discharge	Purulent effluent from vulva or plaques of pus on underside of tail	0	0	0–0

¹Percentage of cows in the high-producing pen (DIM <100).

²Body condition and dirtiness were assessed from behind and from 1 side of the cow.

³Plaques were 3-dimensional layers of dirt equivalent to either the size of a palm or half the surface area of the focal body part.

⁴Lameness was assessed from the side or from behind while cows were walking.

⁵Alterations were assessed from the same side of the cow used to score dirtiness and consisted of any hair loss, lesions, or swelling.

⁶Hair loss referred to undamaged areas of the skin with either missing hair or extensive thinning of the coat (i.e., due to parasites) ≥ 2 cm in diameter.

⁷Lesions included areas of damaged skin ≥ 2 cm in diameter in the form of a scab, wound, or dermatitis due to parasites.

lower than the 3.6% previously found in California (von Keyserlingk et al., 2012) and the 4.0 to 8.2% reported across other regions of Canada and the United States (Espejo et al., 2006; von Keyserlingk et al., 2012; Westin et al., 2016).

Skin Alterations. On average, the prevalence of hair loss was 15% on the hock and 14% on the carpal joint. These values were consistent with 2 studies reporting means of 10 to 15% on the hock (Zaffino Heyerhoff et al., 2014; Adams et al., 2017) and 13% on the carpal joint (Zaffino Heyerhoff et al., 2014). All other North American studies reported much higher averages, ranging from 38 to 76% for the hock (von Keyserlingk et al., 2012; Endres et al., 2014; Cook et al., 2016) and 47% for the carpal joint (Cook et al., 2016). However, severe alterations (lesions or swelling) were more prevalent in our study, averaging 26% on the hock and 34% on the carpal joint. In contrast, most North American studies found means of only 2 to 12% for severe hock injuries (von Keyserlingk et al., 2012; Endres et al., 2014; Cook et al., 2016; Adams et al., 2017), with the exception of one in Canada reporting 47% (Zaffino Heyerhoff et al., 2014). Likewise, although studies in Canada and the northeast United States reported means of 23 to 24% for severe carpal joint injuries (von Keyserlingk et al., 2012; Zaffino Heyerhoff et al., 2014), the averages were only 0.3 to 6% in California and Wisconsin (von Keyserlingk et al., 2012; Cook et al., 2016). Total neck alterations in our study averaged 5.8%, lower than the 8.6% previously reported in Wisconsin (Cook et al., 2016) and the 16% found in Canada (Zaffino Heyerhoff et al., 2014).

Other Conditions. Previous research found that, across Minnesota farms, only 0.1% of cows had either very high or low body condition (Endres et al., 2014). Similarly, only 0.2% of cows in our study were very fat; however, we considered a much higher proportion (13%, on average) of cows to be very lean. This difference could perhaps be explained, in part, by the timing of our assessments in autumn, as body condition can decrease as a result of heat stress (Shwartz et al., 2009). Despite different methods for assessing hygiene, the 46.8% average prevalence for any dirtiness on the lower hind leg, udder, or hindquarters was similar to the mean of 54.9% reported for dirtiness on the udder or lower hind legs on farms in Minnesota (Endres et al., 2014).

Methods for Selecting Cows to Sample

The intercept was not significantly different from 0 ($P \geq 0.31$) and the slope was not significantly different from 1 ($P > 0.18$) in any comparison, suggesting a lack

of consistent over- or under-estimation across the 10 farms in our study.

Selecting Proportions of the Pen. When a larger proportion of the pen was sampled, the number of accurately estimated measures increased (Table 3; inaccurate estimates are shown in Supplemental Table S1, <https://doi.org/10.3168/jds.2017-13611>). Selecting at least 2 of every 3 cows accurately captured the prevalence of all measures when choosing cows locked at the feed bunk and accurately estimated all but severe lameness when randomly computer-selecting ear tag numbers. The association between sample size and accuracy was driven by the increase in R^2 values when more cows were sampled (2 examples of measures with mean and range prevalence values that were relatively lower and higher, respectively, are shown in Figure 1; all relationships are shown in Supplemental Figure S1, <https://doi.org/10.3168/jds.2017-13611>). Endres et al. (2014) observed a similar pattern when assessing a narrower range of measures. Selecting an R^2 threshold is subjective, and some researchers have used lower cut-offs. For example, Miller-Cushon and DeVries (2011) considered $R^2 > 0.8$ to indicate a strong association and $R^2 > 0.7$ to represent a fairly good association because, in their data set, all relationships with $R^2 > 0.75$ also met the slope and intercept criteria. Based on the same rationale, Madruga et al. (2017) used a threshold of $R^2 > 0.76$. In the current study, however, even relationships with low R^2 values met the slope and intercept criteria. Therefore, we used a conservative threshold of $R^2 \geq 0.9$, which our research group also used in other studies to identify sampling schedules for capturing cattle behavior through observation (Stackhouse-Lawson et al., 2015; Chen et al., 2016; Tresoldi et al., 2016) or with data loggers (Ledgerwood et al., 2010).

Both within and across studies, the proportion of cows to sample appears to depend, in part, on the true prevalence of a given condition. In our study, almost all of the measures with <10% average prevalence, such as moderate and severe lameness, diarrhea, and flank or hindquarter alterations, required more cows to generate accurate estimates. When Endres et al. (2014) evaluated 52 Minnesota farms, they needed to sample only 15 or 30% of the high-producing pen to estimate total and severe lameness (24.4 and 6.0% mean prevalence), respectively. In contrast, the mean lameness prevalence in our population was much lower (9.5% total, 2.2% severe), and we needed to sample at least every third or every other cow at the feed bunk, respectively. Likewise, Endres et al. (2014) needed to sample 30% of the pen to estimate hock lesions or swelling (10.6% mean prevalence), whereas we could estimate this condition (26.4% mean prevalence) by selecting every tenth cow

Table 3. Means for both the actual prevalence of each measure and for estimates that accurately¹ captured true values when sampling cows in the high-producing pen (DIM <100) on 10 California dairy farms

Measure	True prevalence	Randomly sampling proportion of pen								Sampling every <i>n</i> th cow at the feed bunk						
		1/10	1/5	1/4	1/3	1/2	2/3	3/4	10th	5th	4th	3rd	2nd	2 of 3	3 of 4	
Very lean	13.0	13.0	—	12.6	13.0	12.1	14.1	12.5	—	—	14.0	—	12.2	13.4	12.6	
Any dirtiness ²	46.8	—	—	—	48.9	46.2	47.7	46.8	—	45.8	—	46.6	—	46.9	46.2	
Lower hind leg	18.4	18.9	—	19.7	19.6	18.5	19.4	18.7	20.7	16.8	21.8	19.1	17.8	18.0	17.3	
Hindquarter	33.5	—	33.9	—	33.7	33.2	34.0	33.4	—	32.4	—	32.5	—	34.0	33.5	
Udder	16.1	—	—	16.5	17.5	16.6	16.8	16.2	—	15.1	—	17.1	14.5	15.6	14.7	
Total lameness ²	9.5	—	—	—	—	9.4	10.2	9.6	—	—	—	9.4	9.1	9.5	9.2	
Moderate	7.3	—	—	6.4	—	7.5	8.2	7.5	—	—	—	—	6.9	7.2	6.8	
Severe	2.2	—	—	—	—	—	—	—	—	—	—	—	2.2	2.3	2.4	
Nonlimb alterations																
Hindquarter	5.5	—	—	—	—	6.1	5.0	5.1	—	—	5.7	—	—	6.0	5.5	
Flank	4.5	—	—	—	—	4.6	4.6	4.3	—	—	4.7	3.8	—	4.8	4.4	
Neck	5.8	7.6	7.0	7.0	6.3	5.9	5.9	5.6	4.3	4.0	5.6	5.5	5.6	6.0	5.9	
Hock alterations ²	38.8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Hair loss	15.0	—	—	—	—	16.4	14.7	15.0	17.0	—	—	—	14.3	14.9	15.6	
Lesions or swelling	26.4	20.9	—	24.5	24.7	27.2	26.3	26.9	27.2	24.4	25.5	23.7	26.3	27.8	26.7	
Carpal joint alterations ²	47.3	—	—	48.8	45.2	48.9	47.1	48.7	—	—	—	46.7	48.1	47.5	48.9	
Hair loss	14.1	17.2	13.8	15.6	13.1	14.1	14.6	14.0	12.9	13.0	12.6	13.5	15.1	14.4	14.6	
Lesions or swelling	34.4	—	37.7	34.2	32.9	35.9	33.5	36.0	—	35.6	—	34.4	34.6	34.4	35.6	
Any hair loss ²	30.6	—	29.3	31.1	29.7	31.2	30.3	30.1	27.7	26.3	29.2	—	30.3	31.1	31.0	
Any lesions or swelling ²	52.2	—	55.5	50.9	49.9	54.4	51.6	52.7	—	—	49.0	50.3	52.5	53.2	53.3	
Nasal discharge	19.3	—	—	17.9	17.1	21.0	19.1	18.0	—	—	19.9	19.8	19.4	19.1	19.1	
Ocular discharge	22.2	23.0	23.8	23.0	23.7	22.7	22.3	21.8	21.0	19.8	24.0	23.0	21.8	21.8	21.6	
Evidence of diarrhea	8.0	—	5.7	—	—	—	8.1	8.0	—	—	—	—	—	7.6	7.6	
Number of measures ²		6	6	10	10	14	15	15	6	8	9	10	12	16	16	
accurately estimated																

¹Using regression analysis, the prevalence estimated by each sample was compared pairwise to the true value obtained by assessing all cows in the pen. Estimates were considered accurate if they met 3 criteria: $R^2 \geq 0.9$, slope not significantly different from 1 ($P > 0.05$), and intercept not significantly different from 0 ($P > 0.05$). Inaccurate estimates are denoted with a dash and are shown in Supplemental Table S1 (<https://doi.org/10.3168/jds.2017-13611>).

²Composite measures (any dirtiness, total lameness, any hock alterations, any carpal joint alterations, any hair loss, and any lesions or swelling) were excluded from the sum of 16 individual measures that were accurately estimated when selecting samples of cows.

at the feed bunk. Drawing general conclusions about the appropriate proportion of cows to sample is challenging, not only because true prevalence varies across measures and populations, but also because the relationship between true prevalence and how many cows to sample is imperfect. For example, neck alterations were accurately estimated using only 10% of the pen despite an average prevalence of only 5.8%, and the reasons for this are unclear.

In addition to evaluating the proportion of the pen to sample, we also compared approaches for choosing

cows. To approximate selecting cows randomly, the Welfare Quality (2009a) protocol suggests identifying those engaging in different activities throughout the pen. However, a potential drawback to this approach is bias resulting from cows being selected because morbidity may make them more conspicuous. In addition, when cows are not restrained, it could be more difficult to score certain measures. An alternative is to randomly preselect ear tag numbers, but identifying specific cattle would be time consuming. A simpler solution would be to assess every n th cow while restrained at the feed bunk, and we found this approach did not differ systematically from randomly computer-selecting ear tag numbers.

Sample Sizes Based on Welfare Quality. All 3 equations accurately estimated ocular discharge, neck or flank alterations, hair loss on the carpal joint, lesions or swelling on the hock, and dirtiness on the lower hind leg (Table 4). Randomly selecting 96 cows (40 to 67% of the pen on a subset of 9 farms) generated accurate estimates for 8 additional measures, but both the WQ high (44–69 cows, 29–54% of the pen) and WQ low (37–53 cows, 22–46% of the pen) equations accurately estimated only 2 additional measures. Prevalence for hair loss on the hock and moderate lameness could not be accurately estimated by any of the equations (inaccurate estimates are shown in Supplemental Table S2, <https://doi.org/10.3168/jds.2017-13611>), and both WQ high and WQ low failed to accurately capture severe lameness or dirtiness on the hindquarters and udder.

The sampling recommendations in the Welfare Quality (2009a) protocol are based on equations (Cochran, 1977) using an expected prevalence of $p = 50\%$, a desired precision of $d = 10\%$, and confidence levels of either 90 or 95% (WQ low and high, respectively). Seemingly counter to the idea that conditions with lower prevalence may require sampling more cows, the sample size is maximized using $p = 50\%$ because it is multiplied by $1 - p$. Compared with selecting the same proportion of the pen across all farms, conducting assessments on large operations may be more practical using the WQ high and low equations because cows are added to the sample at a diminishing rate as the pen size increases. Increasing practicality involves a tradeoff with precision, however, because the number of cows to sample varies inversely with d^2 . The use of $d = 10\%$ may explain why these equations underestimated the number of cows needed to accurately estimate prevalence for many measures in our study. Nonetheless, using $d = 5\%$, as suggested by Naing et al. (2006), may be impractical, as this would increase n_{inf} by 4 fold and the number of cows in our sample by 1.5 to 2.4 fold. More work is needed to further examine the reliability of the WQ high and low equations across a larger sample with

Figure 1. The coefficient of determination (R^2) for the relationship between the true mean prevalence across 10 farms for (A) moderate lameness and (B) nasal discharge, and the prevalence estimated by sampling a subset of cows as a function of the proportion of the pen sampled. Two methods were used to determine which cows to sample: their position when locked in head gates at the feed bunk (every n th cow) or random selection of ear tag numbers. When $R^2 \geq 0.9$ (dashed line), this was considered a strong relationship between the true and estimated prevalence. Relationships for the other measures that were assessed are shown in Supplemental Figure S1 (<https://doi.org/10.3168/jds.2017-13611>).

more variable pen sizes, as well as to evaluate how farm classifications may be affected when conducting the full Welfare Quality (2009a) assessment.

Milking Order. A total of 12 measures could be accurately estimated by sampling thirds of the milking order (Table 5; inaccurate estimates are shown in Supplemental Table S3, <https://doi.org/10.3168/jds.2017-13611>) compared with 11 when selecting 1 in 3 cows using feed bunk position or randomly. Within a given third of the milking order, a maximum of 7 to 9 measures were accurately estimated, and these always included ocular discharge, neck alterations, carpal joint hair loss, and lesions or swelling on the hock. In contrast, the prevalence of moderate and total lameness, hindquarter alterations or dirtiness, and signs of diarrhea could not be estimated from any third of the milking order.

Both severe lameness and very lean cows could be estimated accurately using the middle third of the milking order, but these were under- and over-estimated

by sampling from the first or final third, respectively. Indeed, in the final third of the milking order, both moderate and severe lameness had double the prevalence compared with the earlier thirds. Others have similarly found more lame cows (Walker et al., 2008; Main et al., 2010) later in the milking order. Although previous researchers found that total lameness could be estimated within 5% of the true prevalence when sampling from the middle of the milking order (Main et al., 2010; Hoffman et al., 2013), those findings have not been replicated using a statistical comparison with farm as the experimental unit. In our study, moderate and total lameness could not be estimated using any third of the milking order.

If the goal is to accurately capture all measures, reliable prevalence estimates could not be obtained by sampling cows from separate thirds of the milking order. However, a welfare assessment focusing on only a few measures could feasibly score cows from different thirds while they are in the parlor. Hock alterations

Table 4. Means for both the true prevalence of each measure and for estimates that accurately¹ captured true values when randomly sampling² cows in the high-producing pen (DIM <100) on California dairy farms

Measure	True	Infinite	WQ high	WQ low
Very lean	13.0	13.8	13.7	—
Any dirtiness ³	46.8	46.6	45.9	—
Lower hind leg	18.4	20.0	16.8	16.9
Hindquarter	33.5	31.9	—	—
Udder	16.1	17.4	—	—
Total lameness ³	9.5	11.5	—	—
Moderate	7.3	—	—	—
Severe	2.2	2.3	—	—
Nonlimb alterations				
Hindquarter	5.5	5.8	—	—
Flank	4.5	4.7	4.7	5.4
Neck	5.8	6.1	6.4	5.5
Hock alterations ³	38.8	—	—	—
Hair loss	15.0	—	—	—
Lesions or swelling	26.4	24.8	27.4	26.2
Carpal joint alterations ³	47.3	47.5	—	45.8
Hair loss	14.1	13.3	13.8	13.1
Lesions or swelling	34.4	35.1	—	33.6
Any hair loss ³	30.6	31.6	29.2	28.2
Any lesions or swelling ³	52.2	51.2	54.0	51.7
Nasal discharge	19.3	20.7	—	16.5
Ocular discharge	22.2	24.5	20.4	22.5
Evidence of diarrhea	8.0	9.0	7.6	—
Number of measures ³ accurately estimated		14	8	8

¹Using regression analysis, the prevalence estimated by each random sample was compared pairwise to the true value obtained by assessing all cows in the pen. Estimates were considered accurate if they met 3 criteria: $R^2 \geq 0.9$, slope not significantly different from 1 ($P > 0.05$), and intercept not significantly different from 0 ($P > 0.05$). Inaccurate estimates are denoted with a dash and are shown in Supplemental Table S3 (<https://doi.org/10.3168/jds.2017-13611>).

²Sample sizes were based on the equations used to calculate the Welfare Quality (2009a) recommendations, all using an assumed prevalence of 50% for a given health condition and a desired precision of 10%. Infinite refers to a sample of 96 cows, based on a 95% CI and an infinite population size, independent of actual pen size (subset of 9 farms with ≥ 96 cows/pen). WQ high and WQ low refer to sample sizes based on 95 and 90% CI, respectively, along with the actual pen sizes on each of the 10 farms.

³Composite measures (any dirtiness, total lameness, any hock alterations, any carpal joint alterations, any hair loss, and any lesions or swelling) were excluded from the sum of 16 individual measures that were accurately estimated when sampling cows randomly.

Table 5. Means for both the true prevalence of each measure and for estimates that accurately¹ captured true values when sampling the first, middle, or last third of cows in the milking order on 10 California dairy farms

Measure	True prevalence	Third of milking order		
		First	Middle	Last
Very lean	13.0	—	13.9	—
Any dirtiness ²	46.8	—	—	—
Lower hind leg	18.4	—	17.1	16.2
Hindquarter	33.5	—	—	—
Udder	16.1	17.9	—	15.0
Total lameness ²	9.5	—	—	—
Moderate	7.3	—	—	—
Severe	2.2	—	1.5	—
Nonlimb alterations				
Hindquarter	5.5	—	—	—
Flank	4.5	4.7	—	4.9
Neck	5.8	5.5	6.9	5.2
Hock alterations ²	38.8	—	38.4	—
Hair loss	15.0	13.7	—	—
Lesions or swelling	26.4	24.2	25.1	29.5
Carpal joint alterations ²	47.3	48.1	45.9	—
Hair loss	14.1	14.1	12.8	15.6
Lesions or swelling	34.4	35.2	34.3	—
Any hair loss ²	30.6	30.6	30.6	30.7
Any lesions or swelling ²	52.2	51.2	49.3	—
Nasal discharge	19.3	20.7	17.6	—
Ocular discharge	22.2	22.1	22.5	21.9
Evidence of diarrhea	8.0	—	—	—
Number of measures ² accurately estimated		9	9	7

¹Using regression analysis, the prevalence estimated by each sample was compared pairwise to the true value obtained by assessing all cows in the pen. Estimates were considered accurate if they met 3 criteria: $R^2 \geq 0.9$, slope not significantly different from 1 ($P > 0.05$), and intercept not significantly different from 0 ($P > 0.05$). Inaccurate estimates are denoted with a dash and are shown in Supplemental Table S3 (<https://doi.org/10.3168/jds.2017-13611>).

²Composite measures (any dirtiness, total lameness, any hock alterations, any carpal joint alterations, any hair loss, and any lesions or swelling) were excluded from the sum of 16 individual measures that were accurately estimated when sampling cows from each third of the milking order.

must be scored from behind during the first third of milking for accuracy, but other measures offered some flexibility in when they could be collected. While scoring hock alterations at the beginning of milking, udder dirtiness and flank alterations could be concurrently collected. The latter measures could also be recorded during the final third of milking, at which time lower hind leg dirtiness could be added. All measures that required observing the front of the cow could be accurately estimated during either the first or middle thirds of milking. A topic for future study could be whether assessing cows in the parlor is more time consuming than doing so in the pen due to wait time during milking.

CONCLUSIONS

As the proportion of cows sampled increased, so did the R^2 value for the relationship between estimates and true prevalence, resulting in greater accuracy. In general, many low-prevalence measures required sampling more cows to generate accurate estimates. Reli-

able prevalence estimates could not be obtained for all measures by sampling cows from separate thirds of the milking order or when using the sample size calculations in the Welfare Quality (2009a) protocol. In contrast, all estimates were accurate when selecting at least 2 of every 3 cows restrained at the feed bunk. Choosing cows based on their position when restrained at the feed bunk did not differ systematically from randomly computer-selecting ear tags, and the former may be a simpler approach for on-farm welfare assessments.

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