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Cleaning procedures and cleanliness assessments of bucket milkers and suckling buckets on Japanese dairy farms

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ABSTRACT. Cleanliness of milking equipment is known to be important for the safety of dairy products and to prevent the spread of diseases among cows. We investigated the cleaning procedures of milking equipment and suckling equipment on Japanese dairy farms, and the cleanliness of bucket milkers, suckling buckets, milk receivers, and bulk tanks, using adenosine triphosphate (ATP) bioluminescence test. Bulk tanks (except one bulk tank) and milk receivers were washed by automated cleaning, but all bucket milkers and suckling buckets were washed by manual cleaning. Detergents were often not used to clean bucket milkers and suckling buckets. The $\log_{10}$ transformed relative luminescence units (LRLU) of equipment washed by manual cleaning was higher than equipment washed by automated cleaning. Clean surfaces (≤2.2 LRLU) were only observed on the bulk tank and the milk receiver. More than 50% of the LRLU of the mouthpiece, the rubber packing of claw, and the nipple of the suckling bucket were determined dirty. These results suggest that the cleanliness of the bucket milkers and the suckling buckets washed by manual cleaning was lower than that of the equipment washed by automated cleaning, and may be due to insufficient cleaning procedures.

KEY WORDS: adenosine triphosphate bioluminescence, bucket milker, cleanliness, suckling bucket

The cleanliness of milking equipment has been a major focus of research in the dairy industry due to its impact on the bacteriological quality of raw milk [2, 6, 14, 15]. The dairy industry has adopted “cleaning in place” (CIP) methods, which have solved many practical problems with cleaning equipment efficiently and economically over the years [11]. However, bacterial contamination remains a problem to be solved. Some studies have clarified the key role of biofilms on the surfaces of equipment in exacerbating the microbial contamination of dairy products [9]. Quality of washing water, detergent concentration, washing temperature, and the deteriorated state of equipment have been suggested as causes of the breakdown of appropriate cleaning and insufficient control of biofilms [13, 15].

Quantifying bacteria on equipment is a direct way to assess its cleanliness. Bacterial counts by swabbing equipment surfaces and incubating the plates for 24 to 48 hr is a typical method for evaluating the cleanliness of surfaces. However, the technique only measures the number of aerobic microorganisms and not the presence of milk residue, which is a contributing factor for bacterial growth, and the method is time-consuming [12]. Adenosine triphosphate (ATP) bioluminescence test has been shown to be a fast and easy method for investigating bacterial contamination of surfaces and assessing the efficiency of cleaning [17]. In ATP bioluminescence test, ATP is converted to adenosine monophosphate, and the intensity of light released in this reaction can indicate the amount of ATP present [17]. The method indirectly measures the number of microorganisms in a sample and results are obtained within minutes. This technique detects bacterial contamination, but also non-microbial sources of ATP. Bacterial counts should also be considered in order to precisely interpret, such as organic debris and food residues, which may indicate poor cleaning and be a source of nutrients for microbial growth [3, 4].

There are reports that the ATP bioluminescence test was used to investigate the cleanliness of the inside of bulk tanks and other

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milking equipment (teat cup, milk receivers and pipeline joint) that are performing automated cleaning [8, 12, 16, 18]. Equipment that are difficult to achieving proper cleanliness such as pipeline joints have high ATP levels, and the presence of bacteria and other organic debris in pipelines and bulk tanks is generally caused by improper cleaning programs and controls [18]. Few studies have focused on the cleanliness of bucket milkers used mainly for mastitic cows or freshly calved cows and suckling buckets used for feeding calves. Further, information on cleaning methods used on bucket milkers and suckling buckets on dairy farms is lacking, although manual cleaning is thought to be the common method. Insufficiently cleaned bucket milkers may play a role in exposing cows to infection and prolonging the recovery period. Lapses in the cleanliness of suckling buckets may increase the occurrence of diarrhea and other health issues in neonatal calves [1, 5, 7, 10]. In this study, we investigated the cleaning procedures of milking equipment and suckling equipment on dairy farms, and evaluated using ATP bioluminescence test the cleanliness of equipment washed by automated cleaning and those washed by manual cleaning, especially bucket milkers and suckling buckets.

MATERIALS AND METHODS

Research farms

The investigation was carried out on 20 dairy farms located in Kanagawa, Japan. The mean (range) number of lactating cows and calves per farm was 43 (18–69) and 6 (0–12), respectively. Of the farms, 19 had tie-stall housing and 1 had free-stall housing. Inspections took place between the morning and afternoon milking sessions.

Investigation of cleaning procedures

In this study, the following milking equipment was investigated: bucket milkers (n=20), suckling buckets (n=20), milk receivers (n=17), and bulk tanks (n=10). Responses to a questionnaire were obtained from 19 farms about the washing procedures (manual or automated) and the type and usage of detergents (alkali, acid, or fungicides) for cleaning the milking equipment. The cleaning procedure was classified as "automated cleaning" if the equipment was washed by its specified machine or an automated program and "manual cleaning" if not. The usage of each detergent was rated as "always", "occasionally", or "never". Sampling was performed on clean surfaces.

Visual assessment

Visual assessment was undertaken on the inside bottom surface of the bucket milker, inside the mouthpiece chamber of the bucket milker unit, inside the claw, the rubber packing of the claw, the inside surface of the milk receiver, the inside surface of the bulk tank, the inside surface of the suckling bucket, and inside the nipple of the suckling bucket. Each location was first visually assessed, and the area was determined as "clean" if there was no adhesion of dirt and as "dirty" if dirt was visible. Whether the surface was dry or wet was also recorded. Sampling was conducted on clean surfaces.

ATP bioluminescence test

The ATP bioluminescence test was performed on the farm using the LuciPac Pen and Lumitester PD-30 (Kikkoman Biochemifa Co., Tokyo, Japan) according to the manufacturer's instructions. The results were reported as relative luminescence units (RLU). The threshold for a "clean surface" was set at ≤150 RLU (2.2 log RLU), and the fail threshold for a "dirty surface" was set at ≥300 RLU (2.5 log RLU) [8].

The swab area and number of samples collected were as follows: the inside bottom surface of the bucket milker (30 cm^2, n=20), inside the mouthpiece chamber (24.9 cm^2, n=19), inside the claw (4 cm^2, n=17), rubber packing of the claw (4 cm^2, n=9), bottom inside surface of the suckling bucket (30 cm^2, n=20), inside the nipple of the suckling bucket (27 cm^2, n=13), the inside surface of the bulk tank (30 cm^2, n=10), and the inside surface of the milk receiver (30 cm^2, n=17). The determined RLU was divided by the swab area of each location and presented as RLU/cm^2 to compare the results between different locations. The results were also shared with the farmers on each farm. The sampling was conducted on clean surfaces.

Statistical analysis

The statistical analysis software JMP® 13 (SAS Institute Inc., Cary, NC, USA) was used to analyze the data. Acquired RLU/cm^2 was log transformed to log_{10}RLU/cm^2 (LRLU) for statistical analysis. The Wilcoxon signed-rank test was used to compare between the LRLU of the cleaning procedures (manual or automated). For comparison of LRLU among equipment, the Steel-Dwass test was performed after a one-way analysis of variance. Fisher's exact test was used to assess the relationship between the washing procedure and the results of the visual assessment. A level of significance less than 0.05 was considered statistically significant. All data are expressed as mean ± standard deviation (SD).

RESULTS

Investigation of cleaning procedures

The responses from the questionnaire interviews on cleaning procedures and detergents used are shown in Tables 1 and 2, respectively. We were unable to obtain responses from all farms. Most of the bulk tank and all of the milk receivers were washed using an automated program installed in the milking system, while one farmer washed the bulk tank manually using brushes and detergents. However, as predicted, no machine or automated program was used to wash bucket milkers or suckling buckets (Table 1).

Table 1. Results of interviews with farmers on the cleaning procedure of dairy equipment

Equipment		n	Procedure	
			Mamunal	Automated
Bucket milker	Bucket	18	18	0
	Mouthpiece	17	17	0
	Claw	15	15	0
	Rubber packing of claw	8	8	0
Suckling bucket	Bucket	19	19	0
	Nipple	12	12	0
Bulk tank		9	1	8
Milk receiver		16	0	16

Table 2. Results of interviews with farmers on the use of detergents for dairy equipment

Equipment		n	Alkali			Acid			Fungicide		
			Always	Occasionally	Never	Always	Occasionally	Never	Always	Occasionally	Never
Bucket milker	Bucket	16	7	3	6	1	6	9	3	0	13
	Mouthpiece	15	7	3	5	1	6	8	3	0	12
	Claw	13	6	2	5	0	5	8	2	0	11
	Rubber packing of claw	8	4	1	3	0	4	4	2	0	6
Suckling bucket	Bucket	18	1	1	16	0	0	18	1	1	16
	Nipple	12	1	1	10	0	0	12	1	1	10
Bulk tank[a)]		8	7	1	0	6	2	0	2	1	2
Milk receiver[b)]		15	13	1	0	3	12	0	5	0	0

a) Responses about fungicide were obtained n=5. b) Responses about alkali were obtained n=14 and responses about fungicide were obtained n=5.

Most farmers properly washed their bulk tanks and milk receivers using each type of detergent at least occasionally, but two farmers did not use fungicides for their bulk tanks at all. Washing using detergent were much poorer for bucket milkers and suckling buckets. The number of farmers using detergent properly was considerably lower; for example, only one farmer reported consistently using alkali detergent for suckling buckets (Table 2).

Visual assessment

The number and percentage of clean or dirty surfaces based on visual assessment are shown in Table 3. The visual assessments of the surface of the bulk tank and the milk receiver were all clean, whereas those of the surface of the bucket milkers and the suckling buckets were confirmed as dirty. The inside of the claw and the rubber packing of the claw were especially dirty. The visual cleanliness of all investigated surfaces showed that equipment washed by manual cleaning was dirtier than equipment washed by automated cleaning (Table 4, $P<0.05$).

Dryness was assessed as follows on the surfaces: the inside bottom surface of the bucket milker (dry, 5; wet, 14), inside the mouthpiece chamber (dry, 4; wet, 15), inside the claw (dry, 4; wet, 12), the rubber packing of the claw (dry, 4; wet, 5), the inside bottom surface of the suckling bucket (dry, 6; wet, 14), inside the nipple of the suckling bucket (dry, 5; wet, 8), inside the surface of the bulk tank (dry, 0; wet, 9), and the inside surface of the milk receiver (dry, 1; wet, 16).

ATP bioluminescence test

Figure 1 shows the comparison between the LRLU of the cleaning procedures.

The LRLU of equipment washed by manual cleaning was higher than equipment washed by automated cleaning (2.2 ± 1.5 *vs.* 0.3 ± 0.6). Figure 2 shows the LRLU at each dairy equipment sampling location. The bulk tanks had a significantly lower mean LRLU against every location of the bucket milker and suckling bucket ($P<0.05$). The milk receiver also had a significantly lower LRLU against all locations of the bucket milker and suckling bucket, except for the inside bottom surface of the bucket milker ($P<0.05$).

Table 5 shows relationship of the LRLU on each dairy equipment and the threshold. Clean surfaces (≤2.2 LRLU) were only observed on the bulk tank and the milk receiver. More than 50% of the LRLU of the mouthpiece, the rubber packing of claw, and the nipple of the suckling bucket were determined dirty.

The LRLU of the area evaluated as "clean" by visual assessment (1.3 ± 1.3 LRLU) was significantly lower than that of evaluated as "dirty" (3.3 ± 1.3 LRLU), but 28 of 92 areas assessed as "clean" by visual assessment (30.4%) had a higher LRLU than 2.2. There were no differences between the LRLU of the dryness assessment.

Table 3. Visual assessment of dairy equipment surfaces

Equipment		n	Visual assessment			
			Clean		Dirty	
			n	(%)	n	(%)
Bucket milker	Bucket	20	16	80.0	4	20.0
	Mouthpiece	19	16	84.2	3	15.8
	Claw	17	10	58.8	7	41.2
	Rubber packing of claw	9	1	11.1	8	88.9
Suckling bucket	Bucket	20	14	70.0	6	30.0
	Nipple	13	8	61.5	5	38.5
Bulk tank		10	10	100.0	0	0.0
Milk receiver		17	17	100.0	0	0.0

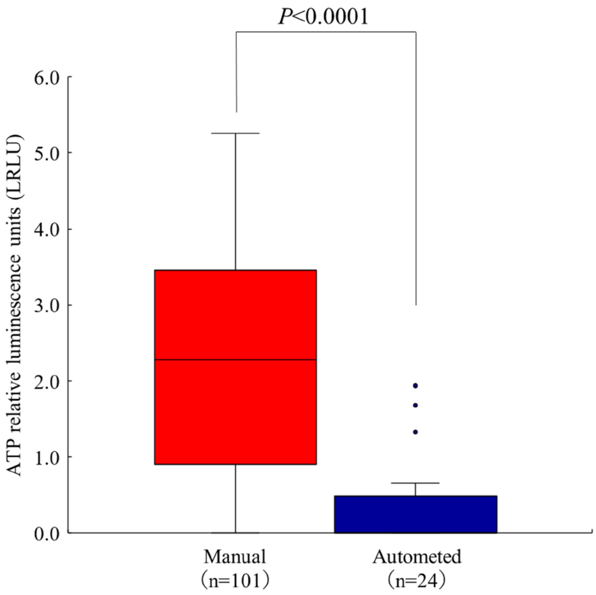

Fig. 1. Comparison between the $\log_{10}$ transformed relative luminescence units (LRLU) of the cleaning procedures.

Table 4. Visual cleanliness and cleaning procedure for all investigated surfaces

Cleaning procedure	Visual cleanliness		Total
	Clean	Dirty	
Hand	63	27	90
Machine	24	0	24
Total	87	27	114

Fisher's exact test was used to assess the relationship between the washing procedure and the results of the visual assessment ($P<0.05$).

DISCUSSION

In this study, we investigated the cleaning procedures of milking equipment and suckling equipment, and found that bucket milkers and suckling equipment were manual cleaning on all farmers investigated. In addition, the LRLU of equipment washed by manual cleaning was higher than that of equipment washed by automated cleaning, and was particularly high LRLU in parts with a structure that was difficult to wash. While the milk receivers and bulk tanks were cleaned with adequate use of detergents, cleaning practices on bucket milkers and suckling buckets were considerably less rigorous. This may be explained by the lack of an automated cleaning procedure or well-established procedure, but it can also be assumed that (1) farmers do not understand the importance of cleanliness for these pieces of equipment or (2) farmers understand the cleanliness of equipment is important, but manual cleaning leaves bacteria and other organic debris.

We did not observe any difference in LRLU between dry and wet surfaces, which corroborates the results of a previous study [8]. This result might be explained by adequate quality of the water used for washing, or the surfaces were not wet enough to affect cleanliness [8]. This finding also suggests that if the equipment surface is sufficiently clean, drying may not be necessary. Visual assessment of the equipment washed by manual cleaning revealed that one-third of the equipment was judged to be "dirty", indicating not only that washing is insufficient, but also that some farmers do not pay enough attention during and after the washing step. The significantly higher values for LRLU for dirty surfaces than clean surfaces suggest that visual inspection of surfaces with additional cleaning for visibly dirty surfaces may be an effective first step to achieving higher levels of cleanliness. However, the finding that 30.4% of the surfaces appearing to be "clean" had LRLU measurements higher than 2.2 indicates that visual inspection is not sufficient for determining cleanliness.

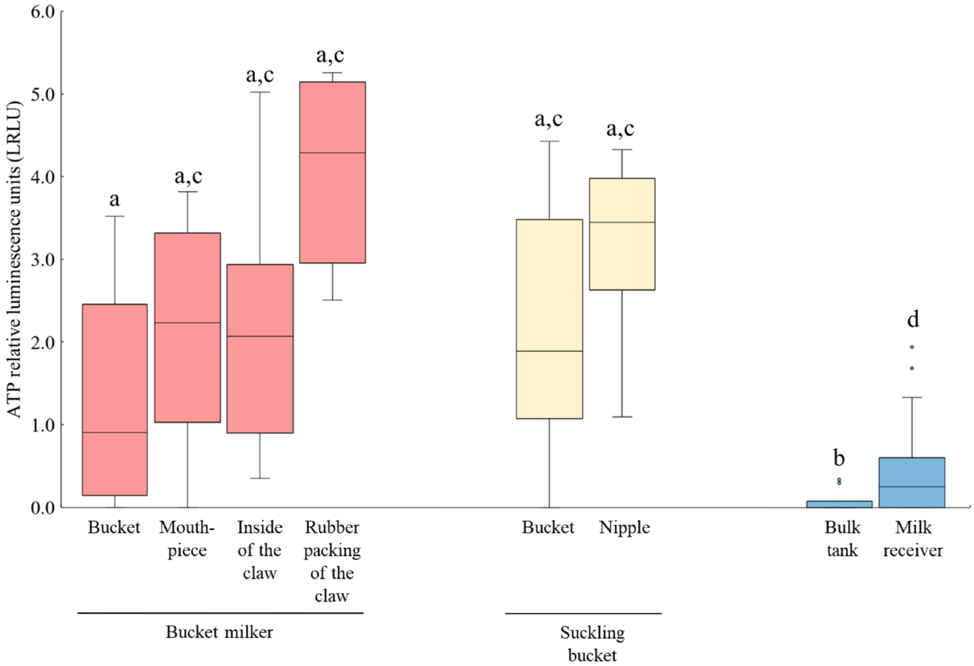

Fig. 2. The log_{10} transformed relative luminescence units (LRLU) results from adenosine triphosphate (ATP) bioluminescence test on each dairy equipment sampling location. RLU results were divided by the swab area of each location and log_{10} transformed. Significant differences are indicated by a–b and c–d (P<0.05).

Table 5. Relationship of the log_{10} transformed relative luminescence units (LRLU) on dairy equipment and the threshold

Equipment		n	Clean (≤2.2 LRLU)		Dirty (≥2.5 LRLU)	
			n	(%)	n	(%)
Bucket milker	Bucket	20	14	70.0	6	30.0
	Mouthpiece	19	9	47.4	10	52.6
	Claw	17	9	52.9	8	47.1
	Rubber packing of claw	9	0	0.0	9	100.0
Suckling bucket	Bucket	20	11	55.0	9	45.0
	Nipple	13	2	15.4	11	84.6
Bulk tank		10	10	100.0	0	0.0
Milk receiver		17	17	100.0	0	0.0

A strong positive correlation between RLU and the total number of bacteria has been reported in the literature [8, 16]. Thus, a high LRLU in the milking equipment can indicate that bacterial organisms are already growing in the milk residues and the milk/milk substitute has a poor bacteriological quality. The mean LRLU results for each sampled location were somewhat expected based on interview responses, particularly the higher values observed for bucket milkers and suckling buckets. Clearly, overall improvement is needed for those pieces of equipment, but we can also see that there are differences in the ease of cleaning each surface; the inside of the claw can be cleaned by attaching it to the milking system and running the automatic wash cycle, but the rubber packing may need to be disassembled and washed thoroughly. Further, cleaning inside the nipple of the suckling bucket can be time consuming. However, the difficulty of cleaning should not be a reason for poor cleanliness conditions; rather, these difficult-to-clean surfaces should be washed even more carefully.

The results from this study revealed that the cleanliness of bucket milkers and suckling buckets washed by manual cleaning was lower than that of the equipment washed by automated cleaning, and may be due to insufficient cleaning procedures. We conclude that cleanliness of bucket milkers and suckling buckets should be improved, and both farmers and veterinarians require a deeper knowledge on the role of cleanliness in rearing calves.

CONFLICT OF INTEREST DECLARATION. The authors declare no conflict of interest.

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