ABSTRACT

The objective of this study was to describe the cleaning practices currently used for preweaning calves on dairy farms in Quebec, Canada. In addition, the contamination of feeding equipment for preweaned calves was described using ATP luminometry (expressed as relative light units (RLU)), visual assessment and bacteriological analysis. A questionnaire was administered on 50 commercial dairy farms in Quebec, Canada, regarding the self-reported cleaning protocol used for feeding equipment of preweaned calves. During the visit, a visual score was given to the feeding equipment available at the farm. Afterward, ATP luminometer measurements were obtained using the Hygiene UltraSnap and MicroSnap swabs and the liquid rinsing technique for buckets, nipples, bottles, esophageal tube feeders (ET), the tube of automatic milk feeders (AMF), water samples, and milk replacer. An additional direct swabbing technique was performed on buckets and nipples. The fluid retrieved from the liquid rinsing technique was also used to determine the total bacterial count (TBC) and total coliform count (TCC). Based on the bacteriological analysis, optimal RLU cut-off values to determine contamination were obtained. The median (interquartile range) luminometer measurements using the UltraSnap and direct technique for buckets and nipples were 2,082 (348 – 7,410) RLU and 3,462 (462 – 7,518) RLU, respectively, and using the liquid technique for bottles, ET, AMF, water, and milk replacer were 43 (4 – 974) RLU, 15 (4 – 121) RLU, 301 (137 – 1,323) RLU, 190 (71 – 358) RLU and 94 (38 – 218) RLU, respectively. Overall, for all equipment and both techniques used, higher RLU values were obtained for the direct swabbing method compared with the liquid sampling method for both swabs used. No differences in the level of contamination were seen between the different feeding equipment used within a farm. Overall, a higher correlation with bacteriological results was noticed for ATP luminometry compared with the visual score, with a high correlation for nipples and bottles using the UltraSnap and liquid technique. Based on the classification of ‘contaminated’ (TBC ≥100,000 cfu/mL) or ‘not contaminated’ (TBC <100,000 cfu/mL), optimal ATP luminometer cut-off values for buckets, nipples, bottles, AMF, water, and milk replacer were 798 RLU, 388 RLU, 469 RLU, 282 RLU, 1,432 RLU and 93 RLU, respectively. No clear association was found between ATP measurements and the self-reported cleaning protocol. This study gave new insights into the current cleaning procedures and contamination of feeding equipment for preweaned calves on dairy farms in Quebec. In addition, ATP luminometry cut-off values could help benchmark farms regarding cleaning practices and provide customized advice, improving the overall hygiene management, and thus the health, in preweaned calves on dairy farms.

Key words: calf, milk feeding equipment, ATP bioluminescence, hygiene protocol

INTRODUCTION

Raising healthy calves is one of the key components to ensure a sustainable and productive herd in the dairy industry (Bach, 2012). Young calves are particularly susceptible to diseases, like pneumonia and diarrhea, usually caused by a deficient immune system and a high pathogen load (Murray et al., 2016). To lower pathogen load, proper management, with emphasis on good hygiene standards, is important (Klein-Jöbstl et al., 2014). It has been suggested that contamination of colostrum fed to preweaning calves can contribute to higher morbidity and mortality rates (Fecteau et al.,...
2002; Gelsinger et al., 2015). Additionally, high bacterial loads have been reported in nipples and buckets used for feeding calves, enhancing the possible spread of pathogens among calves (Heinemann et al., 2021). Therefore, not only the environment, but also the equipment used for feeding preweaned calves, has to be kept as clean as possible (Heinemann et al., 2021). Current hygiene management practices of dairy farms in Quebec, Canada, particularly related to the hygiene status of the feeding equipment, has not yet been described.

The most conventional method to assess the cleanliness of feeding equipment is visual inspection. Although this is fast and convenient, it is very subjective and lacks sensitivity to apparently clean surfaces that could be heavily contaminated (Renaud et al., 2017). A more scientifically proven and objective method is microbiological analysis. However, this method is time-consuming and cannot be performed on-farm (Renaud et al., 2017; Lindell et al., 2018). To counteract these disadvantages, ATP luminometry has recently been implemented in the farming industry (Renaud et al., 2017; Lindell et al., 2018; Seung-Won et al., 2020; Buczinski et al., 2022). This method allows for on-site assessment of cleanliness by quantifying the amount of ATP, present in every life form, into relative light units (RLU) by a chemical luminescent reaction with an enzyme, luciferase (McElroy and Deluca, 1983).

In human medicine, luminometry is commonly used in hospitals to evaluate the hygiene of surfaces (Willis et al., 2007). This method has also been proven useful in the food industry (Corbitt et al., 2000). More recently, research has been conducted to implement the luminometer in veterinary medicine. These studies show the potential of ATP luminometry as an on-farm tool to evaluate the hygiene of rubber liners (Lindell et al., 2018) and Colostrum-feeding equipment (Renaud et al., 2017; Buczinski et al., 2022). Additionally, the luminometer sampling technique has been standardized to evaluate the cleanliness of feeding equipment in preweaned calves (Chancy et al., accepted). However, ATP luminometry has not yet been tested at larger scale, so the level of intra- and inter-farm variability of RLU measurements is not known. Therefore, the objective of this study was (1) to describe the different self-reported cleaning protocols used for preweaned calves on dairy farms in Quebec, (2) to determine the variation of ATP luminometry of feeding equipment in dairy farms and to compare this method with commonly used hygiene indicators like the visual score and bacteriological analysis, (3) to determine, based on bacteriological analysis, optimal RLU cut-off values to assess contamination for the different equipment, and (4) to examine associations between luminometer measurements and the different self-reported cleaning protocols used on the farms.

MATERIALS AND METHODS

Study design and data collection

Between May and August 2021, an observational study was conducted on 50 commercial dairy farms in Quebec, Canada. Herds were selected based on their willingness to participate in the study and additionally to survey different feeding equipment. Furthermore, all selected herds are either clients of the bovine ambulatory clinic of the Faculty of Veterinary Medicine, University of Montreal or farms participating in another ongoing research project on heifer management, representing various feeding equipment. The research protocol was approved by the Animal Care Committee of the University of Montreal (20-Rech-2089). Besides common herd characteristics, a detailed self-reported questionnaire regarding the current cleaning protocol of the feeding equipment was completed on each farm. This included the frequency of cleaning and replacing feeding equipment of preweaned calves, products and utensils used for cleaning and disinfecting and the temperature of the water. The questionnaire was initially tested on 2 dairy farms to ensure its applicability and understanding before being applied on all farms.

Feeding equipment that was available on the farm and used for sampling at the time of visit consisted of buckets, nipples, bottles, esophageal tube feeders (ET) and the tube of automatic milk feeders (AMF). Additionally, water (water ponds available to calves) and milk replacer (making a 1/10 dilution) available on the farm was sampled. Before sampling, a visual score from 1 to 4 (from clean to not clean) indicating cleanliness of the surface of the equipment that comes into contact with milk was assigned to each equipment as previously described (Renaud et al., 2017; Chancy et al., accepted). No visual scoring was performed for the water or milk replacer. However, Brix measurements were taken from the undiluted milk replacer using a digital dairy refractometer (MISCO DD-1) to compare with ATP luminometry. For buckets and nipples, both a direct and liquid sampling technique were used. In order for both sampling techniques not to interfere with each other, the 2 techniques were performed on the same kind of equipment (i.e., bucket), but on a different unit (using one bucket for the direct sampling technique and another bucket on the same farm for the liquid sampling technique). For bottles, ET and AMF we only sampled using the liquid technique for practical reasons. Both sampling techniques of the different feeding equipment were executed, as previously described...
Briefly, direct sampling of the bucket was performed by swabbing one fourth of the internal bottom, the circular edge and 2 cm of the wall. For nipples, the whole inner surface was sampled by going back and forth with a swab along the entire length of the teat. When using the liquid sampling technique, 15 mL of sterile 0.9% NaCl was injected into the equipment (being a bucket, bottle, nipple or tube). The equipment was softly rotated for 5 s to ensure maximal contact with the surface and then the saline was transferred to a sterile container. Self-made videos explaining the different swabbing procedures for the direct and liquid method as for the different equipment can be viewed on YouTube following the QR code in Figure 1. Drinking water available in buckets and milk replacer were also transferred into a sterile container. Since ET and AMF can have various shapes and lengths, the obtained measurements for these tubes were standardized by the cylindric volume sampled \((\pi \times l \times r^2)\), where \(r\) is the inner radius and \(l\) the length, and reported for a tube of 100 cm of length and 0.5 cm radius (which is the dimension compatible with a relatively standard esophageal tube feeder).

For each piece of equipment and technique used, 2 different swabs, namely the UltraSnap (Surface ATP test) and MicroSnap (Coliform test), were taken as per manufacturer’s instructions (Hygiene, Camarillo, CA). UltraSnap measurements were available directly on the farm. For the MicroSnap, samples were transported for a maximum of 4 h in a cooler between 4°C and 7°C before further analysis in the laboratory where an additional incubation period of 6 h at 35°C ± 2°C was required before results were obtained. Product instructions are available at the website of Hygiena (Hygiena, 2022). All luminometry measurements were repeated twice with one Ensure luminometer (Hygiena, Camarillo, CA). All data collection was performed by the same trained operator (A.C). All measurements are shown in relative light units (RLU) per milliliter.

Bacteriological analysis was performed on the fluid retrieved from the liquid sampling technique of different feeding equipment at each farm. Additionally, microbiological analysis was conducted on a water sample and milk replacer sample from several farms. For this, samples were transported for a maximum of 4 h between 4°C and 7°C and immediately frozen at a temperature of −20°C at arrival at the ambulatory clinic of the Faculty of Veterinary Medicine. All analyses were performed at the Lactanet laboratory (Sainte-Anne-de-Bellevue, Quebec, Canada) using the 3M Petrifilm according to the manufacturer instructions (3M Canada). Briefly, a dilution of 1:1000 of the liquid was made with Butterfield Phosphate buffer. This dilution factor was chosen based on previous research of benchmarks of contamination when milk or colostrum concentration exceeds 100,000 cfu/mL for total bacterial count and 10,000 cfu/mL for coliform count (McGuirk and Collins, 2004; Morill et al., 2010). One milliliter of this dilution was placed on 2 different Petrifilm, namely the aerobic colony count Petrifilm to obtain the total bacterial count (TBC) and the coliform count Petrifilm to obtain the total coliform count (TCC). After an incubation period at 35°C ± 2°C for 48 h, the TBC could be determined by counting the red-colored colonies using a detection threshold of >1,000 and <250,000 cfu/mL. For the TCC, an incubation period of 24 h at 35°C ± 2°C was conducted and the concentration was determined by counting all red and blue-colored colonies producing gas bubbles, using a detection threshold of >1,000 and <150,000 cfu/mL.

**Statistical analysis**

All data from the questionnaires and the samples were stored in an Excel file (Excel 2016, Microsoft Corp., Redmond, WA). Analyses were performed using the open-source R software v 4.2.1 (https://www.r-project.org/). Descriptive statistics were indicated for herd characteristics, available equipment, hygiene questionnaire answers, luminometer measurements, visual score, and bacteriological analysis. Swab RLU results were all obtained by taking the mean of the 2 consecutive measurements made. The RLU results were not normally distributed and therefore are presented as median and interquartile range. The RLU values were log-transformed (using log10(RLU+1)). To compare different swabs, techniques and feeding equipment, a Kruskal-Wallis non-parametric ANOVA
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Cleaning protocol of feeding equipment for preweaned calves

The median number (range) of total animals in the herd, lactating cows, quota (the allowed amount of fat in kg per day) and annual calvings was 127.5 (50 to 800), 81 (40 to 415), 110 (48 to 500) and 105 (36 to 500), respectively. All herds were predominantly Holsteins herds except for one Jersey herd. Of the 50 farms visited, 47 had buckets, 45 farms used nipples, 39 farms had bottles and an ET and 15 had an AMF. Overall, 119 nipples, 93 buckets, 41 bottles, 39 ET and 15 AMF were sampled. The frequency of different reported cleaning procedures for the different equipment can be found in Table 1. In general, the farms showed substantial differences in hygiene procedures of the feeding equipment.

The majority of farms had 2 people involved in the cleaning process (32%), while 22%, 20%, 16% and 10% had 1, 3, 4 and 5 individuals involved, respectively. Half of the farms (51%) claimed that they unscrewed the nipples before cleaning. Different utensils were used for cleaning like a brush (68%), washcloth (2%), sponge (2%) or no utensil (28%). Very hot, hot, lukewarm and cold water was used for actual cleaning in 20%, 56%, 16% and 8% of the farms, respectively. No temperature was defined for these categories. The primary cleaning product used was dishwashing soap for 40% of the respondents, while various other soaps were used by others. Only 40% of the farms used disinfectant for the feeding equipment. The most popular product for disinfecting was sodium hypochloride (50%), followed by Pentapotassium bis(peroxymonosulphate) bis(sulfate), also known as Virkon (46%) and a combination of iodine with sodium hypochloride (4%).

A large variation between farms was noticed for the time between feeding and cleaning the equipment, ranging from 3 to 4,320 min (72h) with a median time of 30 min. Regarding the rinsing process, only 10% of the farms used a utensil, which was a brush. The temperature of the rinsing water was very hot, hot, lukewarm and cold in 18%, 12%, 32% and 38% of the farms respectively. Of the 15 farms that used an AMF, 85.5% claimed to use automatic cleaning and 68.8% used manual cleaning, of which 22% had a protocol. Different brands of AMF were used, namely 26.7% Lely (Maasshuis, The Netherlands), 20% non-specified Foerster-technik (Engen, Germany), 13.4% Holm & Laue (Westerrönfeld, Germany), 13.4% Urban (Wüsting, Germany), 13.3% Delaval (Tumba, Sweden), 6.7% GEA (Düsseldorf, Germany), and 6.7% Taxi-lait (Westerrönfeld, Germany).

ATP luminometry results

Overall RLU measurements for the different equipment, water, and milk replacer are displayed in Table 2. Regarding the different swabs, higher RLU values were found for the UltraSnap compared with the MicroSnap ($P < 0.0001$) for all the equipment and both techniques. Additionally, the direct swabbing technique obtained higher RLU measurements compared with the liquid sampling technique ($P < 0.0001$) for buckets and nipples, both with the UltraSnap and MicroSnap. Although all feeding equipment showed contamination, buckets and AMF were more contaminated compared with nipples, bottles and ET using the UltraSnap and liquid technique ($P < 0.01$, Supplementary data Figure 1). When the MicroSnap was used, buckets and AMF were more contaminated than ET ($P < 0.01$). The correlation of contamination between the different feeding equipment within a farm were examined. When using the liquid technique, a negligible to moderate correlation was found between the different feeding equipment within a farm (Figure 2). For the direct sampling technique on buckets and nipples, a low correlation of contamination was noticed for the UltraSnap ($r_s = 0.486$, 95% CI: 0.202 – 0.695; $P = 0.002$) and a negligible correlation of contamination for the MicroSnap ($r_s = 0.266$, 95% CI: −0.080 – 0.554; $P = 0.129$). In other words,
high contamination of one specific feeding equipment on a farm was not automatically correlated with a high contamination of other feeding equipment on that same farm. A negative correlation was observed between the luminometer results and Brix measurements for milk replacer using the UltraSnap ($r_s = -0.526$, 95% CI: $-0.724 – -0.248$; $P = 0.0007$) and the MicroSnap ($r_s = -0.405$, 95% CI: $-0.641 – -0.098$; $P = 0.012$).

**Association between luminometer results and the cleaning protocol**

Statistical characteristics of the association between RLU measurements and the different cleaning protocols can be found in the Supplementary data. Both different cleaning protocols as the frequency of applying different cleaning protocols were examined, all separated per equipment, swab and technique. Higher RLU values were obtained when the cleaning process was performed with cold water in comparison with lukewarm, hot or very hot water ($P < 0.01$). Using a brush during the rinsing process resulted in lower RLU measurements ($P < 0.01$). Additionally, lower RLU measurements were obtained when 3 persons were involved in the cleaning process compared with one person ($P < 0.001$).

Although some associations are statistically significant, no clear correlation could be made between ATP luminometry results and the different self-reported cleaning procedures.

**Visual scoring**

The most frequent visual score was 1 for all feeding equipment, with the exception of AMF where a visual score of 2 was mostly seen. A visual score of 4 was rarely given for equipment. When using the UltraSnap and the direct technique for buckets ($P = 0.013$) and ET ($P = 0.003$) (Figure 3), additionally an association was seen using the MicroSnap and the liquid technique ($P = 0.007$). For the other techniques, no clear correlation was seen. For the visual assessment, 17 farms showed a variation of 2 different levels for the visual assessment, 10 farms showed a variation of 3 different levels and 2 farms obtained the widest variation possible (4 different levels).

**Table 1: Characteristics of the different hygiene protocols for feeding equipment of preweaned calves among 50 dairy farms in Quebec, Canada**

<table>
<thead>
<tr>
<th></th>
<th>After every use</th>
<th>2–5 times per week</th>
<th>1 time per week</th>
<th>2 times per month</th>
<th>Every month</th>
<th>Between 2 and 8 times a year</th>
<th>Every year</th>
<th>Between 1–5 years</th>
<th>More than 5 years</th>
<th>Never</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renewal of bucket</td>
<td>6.4</td>
<td>55.3</td>
<td>36.1</td>
<td>2.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renewal of bottle</td>
<td>5.2</td>
<td>17.9</td>
<td>56.3</td>
<td>20.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renewal of AMF</td>
<td>12.5</td>
<td>43.9</td>
<td>12.5</td>
<td>25.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renewal of ET</td>
<td>4.9</td>
<td>7.3</td>
<td>26.8</td>
<td>46.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renewal of nipple</td>
<td>2.3</td>
<td>11.4</td>
<td>65.8</td>
<td>15.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unscrewing nipples</td>
<td>15.0</td>
<td>10.0</td>
<td>20.0</td>
<td>2.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cleaning bucket</td>
<td>2.3</td>
<td>11.4</td>
<td>65.8</td>
<td>15.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cleaning ET</td>
<td>4.3</td>
<td>6.4</td>
<td>12.8</td>
<td>6.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cleaning tube of AMF</td>
<td>95</td>
<td>2.3</td>
<td>2.3</td>
<td>67.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cleaning nipple</td>
<td>31.1</td>
<td>4.4</td>
<td>8.9</td>
<td>2.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cleaning milking machine</td>
<td>30.2</td>
<td>2.3</td>
<td>2.3</td>
<td>67.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manual cleaning AMF or components</td>
<td>8.3</td>
<td>9.1</td>
<td>45.5</td>
<td>18.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disinfection of the feeding equipment</td>
<td>8.3</td>
<td>4.2</td>
<td>20.8</td>
<td>8.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results are shown in percentages.
Bacteriological analysis

Bacteriological examination was performed on the liquid obtained from 41 buckets, 40 nipples, 39 bottles, 15 AMF, 45 water samples, and 44 milk replacer samples. The median (interquartile range) of TBC for buckets, nipples, bottles, AMF, water, and milk replacer was 11,000 (1,000; 221,000), 4,000 (1,000; 35,250), 1,000 (1,000; 28,500) and 17,000 (2,000; 40,000), 1,000 (999; 6,000) and 93,000 (25,001; 149,500), respectively. For the TCC, all samples were less than 1,000 except for one nipple sample with 6,000 and one bottle sample with 4,000. A small positive trend was noticed between the TBC and RLU measurements using the UltraSnap (Supplementary data), despite a low correlation for the UltraSnap (r_s = 0.442, 95% CI: 0.316 – 0.553; P < 0.0001) and a low correlation for the MicroSnap (r_s = 0.366, 95% CI: 0.231 – 0.487; P < 0.0001). The correlations between the luminometer measurements, visual score and bacteriological values per equipment are presented in Figure 4.

Cut-off values for luminometer results based on bacteriological analysis

Based on the TBC, samples could be classified as ‘contaminated’ (TBC ≥100,000 cfu/mL) or ‘not contaminated’ (TBC <100,000 cfu/mL). The ratio contaminated/not contaminated for buckets, nipples, bottles, AMF, water and milk replacer are 12/29, 5/35, 9/30, 2/13, 3/43 and 17/27, respectively. When comparing this with the RLU measurements, different cut-off values with corresponding sensitivity and specificity were obtained for the different feeding equipment, water, and milk replacer (Table 3). Additionally, the ROC curves of the different materials are displayed in Figure 5. A high area under the curve (AUC), sensitivity and specificity were obtained with the UltraSnap for buckets, nipples, bottles and water, although the category nipples had few samples.

DISCUSSION

This study described the self-reported cleaning protocols commonly used for preweaned calves on dairy farms in Quebec. Although scientific research supports the importance and relation of hygiene management practices with the overall health of preweaned calves (Bruning-Fann and Kaneene, 1992; Barrington et al., 2002; Godden, 2008; Aust et al., 2013, Barry et al., 2019), few practical guidelines are implemented. By describing these protocols, points of improvement can be identified and tackled. The standard operating procedures (SOP) for an adequate cleaning of feeding equipment.
equipment after every use are as follows: 1) disassembly of the individual parts, 2) rinse with lukewarm water until visibly clean, 3) place in hot water with detergent, 4) scrub all surfaces (in and out) with a brush, 5) rinse with hot water containing acid sanitizer and 6) drain and air dry completely (Steward et al., 2005). Overall, a large variation in the self-reported cleaning protocols was seen between different farms. Although it is recommended to clean the feeding buckets after every use (Maunsell and Donovan, 2008), only 4.3% of the farms in this study reported doing it. This is in large contrast with previous studies in Germany (Heinemann et al., 2021), Ontario, Canada (Renaud et al., 2018), Sweden (Lundborg et al., 2005) and Austria, (Klein-Jöbstl et al., 2014), where 36%, 77%, 83.3% and 97% of the farms cleaned the buckets after every use, meaning twice a day, respectively. On the other hand, this study showed better results for the ideal water temperature for cleaning, the use of detergents and the disinfection rate (Klein-Jöbstl et al., 2014; Heinemann et al., 2021) and similar results for the percentage of farms who unscrew the nipples (Heinemann et al., 2021).

Higher RLU contamination was found for buckets and AMF compared with nipples, bottles and ET, which is in accordance with previous studies using the UltraSnap on different feeding material (Buczinski et al., 2022; Chancy et al., accepted). Additionally, higher RLU values were obtained for the UltraSnap compared with the MicroSnap, as stated before (Chancy et al., accepted).

Both the liquid, as well as the direct swabbing technique, have been used in previous studies to investigate the cleanliness of feeding equipment with ATP luminometry (Renaud et al., 2017; Buczinski et al., 2022; Chancy et al., accepted). However, this study is the first to examine both techniques on the same kind of

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**Figure 2.** Correlation of RLU contamination of the different calf feeding equipment available within the farm using the liquid technique, separated by swab (UltraSnap vs MicroSnap). *P < 0.05, **P < 0.01, ***P < 0.001. AMF: automatic milk feeder line, ET: esophageal tube, RLU: relative light units.
equipment, showing higher RLU measurements for the direct swabbing technique compared with the liquid sampling technique for both buckets and nipples. It is possible that by expressing direct contact of the swab with the surface using the direct method, more organic material can be collected compared with ‘rinsing’ the material with sterile fluid as obtained by the liquid method. In order for both sampling techniques not to interfere with each other, the 2 techniques were performed on the same kind of equipment of unknown age (i.e., bucket), but on a different unit (using one bucket for the direct sampling technique and another bucket on the same farm for the liquid sampling technique). Also, higher RLU-values were obtained for the UltraSnap compared with the MicroSnap. This was expected since the MicroSnap only detects Coliform bacteria which is in contrast with the UltraSnap who detects all bacteria. Overall, a negligible to moderate correlation could be found between different feeding equipment within one farm. This could imply that no clear classification of ‘dirty’ and ‘clean’ farms can be made based on ATP luminometry performed in only one equipment in a particular farm. However, only a limited amount of equipment was used per farm since the focus of the study was sampling a large number of farms instead of a large number of equipment within a farm. Therefore, more research should be conducted to verify this observation.

A positive correlation was found between ATP luminometry and the visual score for buckets, nipples, bottles, and ET. In previous research (Heinemann et al., 2021), both the technique and swab used differed so no formal comparison can be made. However, a recent study showed similar results (Chancy et al., accepted). Although a visual score of 4 was not very common in this study, high RLU values were seen. An advantage of ATP luminometry is that contamination can be detected even when not visible with the eye, as demonstrated for buckets and bottles in this study. This shows that contamination can still be present when not visibly apparent, demonstrating the usefulness of ATP luminometry. Additionally, when using the direct swabbing technique, it can also help detect biofilms, a well-known problem on dairy farms endangering overall health (Latorre et al., 2022). A negligible to high correlation was found between bacteriological results and RLU measurements in this study, depending on the specific equipment and the swab used. Even negative correlations were seen, although very little samples were available to support this theory. These findings are supported by previous literature where the liquid

![Figure 3. Association between the visual score and RLU measurements in relative light unit (RLU) on a log scale for the different calf feeding equipment available on the dairy farms, using the UltraSnap and the liquid technique. a,b,c = different letters indicate a significant difference (P < 0.05) according to the Dunn test with Benjamini-Hochberg correction for multiple pairwise comparisons. Overall statistical difference was calculated with Kruskal-Wallis non-parametric analysis of variance. The different numbers indicate the number of samples that was used. AMF: automatic milk feeder line, ET: esophageal tube, RLU: relative light units]
technique is also used (Renaud et al., 2017; Chancy et al., accepted).

Overall, a lower correlation was found between the visual score and bacteriological results compared with RLU measurements and bacteriological results, questioning the diagnostic reliability of visual assessment (Renaud et al., 2017; Heinemann et al., 2021). Especially for AMF, where a tube is used, a low correlation between the visual score and bacteriological analysis is seen. This can be explained by the fact that the transparency of the plastic decreases over time (Baltscheit et al., 2020) and the use of chemical agents (Bohner and Bradley, 1991), giving a false impression of being dirty and contaminated. Since there is a lot of controversy in the literature regarding the relationship between visual assessment – bacteriology – ATP luminometry,

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**Figure 4.** Correlation of RLU contamination using the liquid technique and visual score with bacteriological results of the different feeding equipment, water and milk replacer. AMF: automatic milk feeder line, RLU: relative light units *P*<0.05, **P**<0.01, ***P**<0.001.

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**Table 3: The cut-off value for luminometer results with corresponding sensitivity and specificity based on the classification of ‘contaminated’ (TBC ≥100,000 cfu/mL) or ‘not contaminated’ (TBC <100,000 cfu/mL) for the different materials obtained**

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Swab</th>
<th>Number of samples</th>
<th>Optimal cut-off value (in RLU)</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bucket</td>
<td>UltraSnap</td>
<td>40</td>
<td>798</td>
<td>78.6%</td>
<td>91.7%</td>
</tr>
<tr>
<td></td>
<td>MicroSnap</td>
<td>39</td>
<td>16</td>
<td>81.5%</td>
<td>75.0%</td>
</tr>
<tr>
<td>Nipple</td>
<td>UltraSnap</td>
<td>5</td>
<td>388</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>MicroSnap</td>
<td>5</td>
<td>0</td>
<td>75%</td>
<td>100%</td>
</tr>
<tr>
<td>Bottle</td>
<td>UltraSnap</td>
<td>36</td>
<td>469</td>
<td>85.2%</td>
<td>88.9%</td>
</tr>
<tr>
<td></td>
<td>MicroSnap</td>
<td>36</td>
<td>5</td>
<td>81.5%</td>
<td>100%</td>
</tr>
<tr>
<td>AMF</td>
<td>UltraSnap</td>
<td>14</td>
<td>282</td>
<td>66.7%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>MicroSnap</td>
<td>14</td>
<td>3074</td>
<td>100%</td>
<td>50%</td>
</tr>
<tr>
<td>Water</td>
<td>UltraSnap</td>
<td>43</td>
<td>1432</td>
<td>97.5%</td>
<td>100%</td>
</tr>
<tr>
<td>Milk replacer</td>
<td>UltraSnap</td>
<td>40</td>
<td>93</td>
<td>54.5%</td>
<td>61.1%</td>
</tr>
<tr>
<td></td>
<td>MicroSnap</td>
<td>40</td>
<td>198</td>
<td>100%</td>
<td>19.7%</td>
</tr>
</tbody>
</table>
it is worth mentioning that each approach evaluates contamination from a different perspective. While the visual score neglects the non-visual contamination, which is covered by bacteriological analysis, the latter does not include other sorts of contamination such as residues associated with inadequate cleaning of milk or milk replacer traces which is then covered by ATP luminometry. Therefore, it can seem erroneous to directly compare these different hygiene indicators and try to replace one by the other. They give complementary information about the different components of feed equipment cleanliness.

From a clinical standpoint, optimal cut-off values were obtained for the different feeding equipment sampled. Since almost all bacteriological samples had a TCC of < 1,000 cfu/mL, only the TBC was used for analysis. A high area under the curve (AUC) was obtained for buckets, nipples, bottles and water. ATP luminometer thresholds have been obtained in previous studies for milking equipment (Vilar et al., 2008; Lindell et al., 2018). Also, a cut-off value has been established for feeding buckets, although different swabs were used (Heinemann et al., 2021). Another study determined cut-off values per swab by ROC analyses, but this was calculated for a combination of feeding equipment (Renaud et al., 2017), and therefore comparisons cannot be directly made. To our knowledge, this is the first study to determine optimal RLU cut-off values per feeding equipment of preweaned calves, making ATP luminometry a practical tool to quickly identify contamination. In addition to feeding equipment, RLU contamination and cut-off values were determined for

**Figure 5.** ROC curve of the RLU measurements based on the classification of ‘contaminated’ (TBC ≥100,000 cfu/mL) or ‘not contaminated’ (TBC <100,000 cfu/mL) for the different calf feeding materials obtained. AMF: automatic milk feeder line, RLU: relative light units. AUC: Area under the curve.
water and milk replacer on the farm. When comparing the RLU measurements with bacteriological analysis, a low correlation was found for water, and a negligible correlation for milk replacer, although the latter had high TBC in this study. Previous studies show a high correlation for water (Deininger and Lee, 2001) and milk (Meighan, 2014). However, a different swab was used in this study (Deininger and Lee, 2001) and the composition of milk and milk replacer is not the same (Meighan, 2014). A negative correlation was found between RLU contamination and Brix refractometer measurements for the milk replacer. At first sight one would expect a positive correlation. However, since a Brix refractometer measures the total solids concentration in milk replacer (Foren et al., 2016) which can be influenced by the CO₂ production of microorganisms in milk (Rajagopal et al., 2005), and thus both techniques measure different substrates, no clear comparison can be made.

No clear correlation was made between specific cleaning protocols and luminometer results due to several limitations in this study. First, cleaning practices have been determined by self-reporting. Therefore, we could not verify if the process mentioned by the farmer was actually the same as what is currently employed at the farm. There can be discrepancies between both, and further research should be conducted to determine the compliance with the protocols. Second, a limited amount of feeding equipment was used within one farm. When sampling only a partial (even if randomly selected) amount of buckets for example, taking into account a possible age difference of this material, it can occur that only ‘dirty’ or ‘clean’ buckets were sampled, giving a discrepancy with the general cleaning protocol of that farm. Additionally, only one measurement in time was performed on the farms. It is unknown if these cleaning protocols changed over time, or if there was a possible influence of season. To obtain a general image of contamination on the farm, several measurements with ATP luminometry need to be performed, incorporating last cleaning dates. This is also supported by previous research (Lindell et al., 2018). Lastly, to get a clear understanding of the overall condition on the farm, the health of the calves is a very good and reliable indicator which is generally associated with various other hygiene indicators (Barry et al., 2019; Bonizzi et al., 2022). Further research should therefore be conducted including the health of the calves and using different sampling periods. This would improve the understanding between hygiene management, the overall health of calves and contamination of feeding equipment.

CONCLUSIONS

This study described the current self-reported cleaning protocols for preweaned calves on dairy farms in Quebec, Canada. A large variation in cleaning protocols was found, indicating opportunities for improvement. An overview and cut-off values of ATP contamination for feeding equipment based on a large number of farms was given. This can aid in benchmarking farms regarding their overall hygiene management, leading to customized advice and improvement. No clear correlation of contamination for different feeding equipment within farms was observed. This suggests that all kinds of feeding equipment should be sampled to obtain a general perspective of the contamination level on the farm. No association between RLU contamination and the cleaning protocol was found, suggesting further research is needed.

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