Detection methods for cleanness in cattle barns

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Abstract: The suitability of microbiological dipslides and tests of general hygiene for examining cleanability of steel structures for use in cattle barns was investigated in a laboratory study. Steel is a commonly used material in barn equipment such as milk tanks and sinks. The cleanness of the steel was examined after soiling with seven typical cattle barn soils (four feeds, litter, manure and milk) and after cleaning with different detection methods including various microbiological dipslide types, protein tests, a glucose and lactose test and an ATP (adenosine triphosphate) bioluminescence method. The results were collected in a database and ranked into cleanliness classes. On surfaces, microbes were detected in the case of all other soils than milk, whereas sugars were detected only on surfaces after contamination with two of the feeds. Protein tests gave a response to all other soils than litter, and ATP was observed on all the surfaces. The clearest correlation was observed between the results of the three protein tests (r values ranging from 0.62 to 0.89, $p<0.001$). No correlations were observed between the results of aerobic microbes, protein tests and the ATP bioluminescence (r values ranged from 0.09 up to 0.47, $p<0.001$). The results of the study will help to select suitable hygiene monitoring methods for cattle barn environments.

Keywords: cattle barn, bioenvironment, hygiene, microbiological dipslides

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1 Introduction

Areas with different levels of hygiene are typical for cattle barns. Common sources of soil in these buildings are feeds, litter, manure, urine, milk and water. The hygienic condition of environmental surfaces in cattle barns is important because it may affect milk quality (De Koning et al., 2003; Hanus et al., 2004; Skrzypek, 2006; Trevisi et al., 2006; DeVries et al., 2012), animal health (Noordhuizen and Cannas da Silva, 2009; Hovinen and Pyörälä, 2011; Penev et al., 2012), animal behaviour and welfare (DeVries et al., 2012; Ito et al., 2014), safety of the personnel (Kymäläinen et al., 2009) and durability of the structures and materials of the barn (Mathiasson et al., 1991; De Belie et al., 2000). Prevention of cross-contamination and sufficiency of cleaning procedures have been recognized as a challenge in cattle barns (Kymäläinen and Kuisma, 2014), and therefore as well as studies, practical interventions focusing on hygiene and cleaning in these buildings are needed.

Different microbiological, biochemical, chemical, physical and visual methods have been used for investigating the cleanness in the context of environmental surfaces of animal buildings (Kymäläinen et al. 2009; Määttä et al., 2011). Some methods are suitable for field studies, some only for laboratory studies. In hygiene monitoring in food processing industries, e.g. microbiological methods and rapid tests of general hygiene have been used. Hygiene monitorings have been carried out for example in slaughterhouses and the meat industry (Suihko et al., 2002; Gudbjörnsdóttir et al., 2004), the fish industry (Miettinen et al., 2001), vegetable processing (Lehto et al., 2011; Kuisma et al., 2014) and cattle barns (Kymäläinen and Kuisma, 2014). Lorentzon (2005) examined the microbiology of floor bulk soil in cowsheds and De Palo et al. (2006) investigated the amount of coliforms on lying areas of freestall floorings.

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Total aerobic colony count, or heterotrophic aerobic plate count, are the most typical indicators of hygiene in the food industry (Salo et al. 2000; Carrascosa et al., 2012). Other typical microbe types are moulds, yeasts and enterobacteria. The results of an earlier study (Kymäläinen and Kuisma, 2014) showed that microbiological dipslides can be used for hygiene monitoring in cattle barn buildings. ATP bioluminescence is a rapid method, which measures the total amount of ATP in the samples (Zutter et al., 1998; Lappalainen et al., 2000; Redsven et al. 2007). It is widely used in self-monitoring and hygiene studies. Other rapid tests, such as those indicating protein or sugar, are based on colour changes.

In practical life, for example in self-monitoring, different soils on bio-environmental surfaces in the food industry and the agricultural sector are detected as mixtures. Some earlier studies have reported food debris on steel surfaces (e.g. Moore et al., 2001), but information concerning several of the various barn soils has not previously been available. The aim of this laboratory study was to examine the response of some typical cattle barn soils to different detection methods, microbiological dipslides and tests of general hygiene. The detection methods were selected in principle to be suitable for real-life conditions and field studies, and the soils (contaminants) were selected to represent different typical or potential substances present in the cattle barn environment. Steel surfaces were examined after soiling and after subsequent cleaning.

2 Materials and method

The cleanability of steel surfaces from soils (dirt) typical for cattle barn environments was examined with a laboratory method (Toiviainen-Laine et al., 2009) including soiling, cleaning with a specifically designed apparatus and detection before and after the soiling and cleaning phases. The dimensions of the soiling and cleaning areas are presented in Figure 1. Steel surface area = 8 cm × 45 cm. Two turning are as in cleaning = 4 cm × 7 cm each. Detection area = 4 cm × 23 cm.

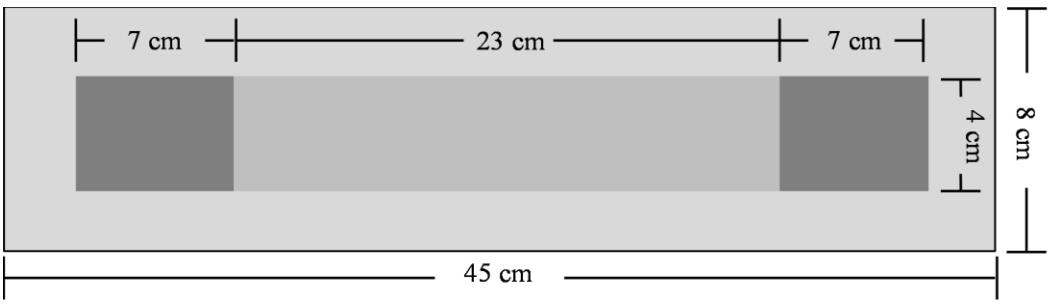


Figure 1 Soiling and cleaning areas of the steel samples.

The steel material was selected as being a common material in barn equipment such as milk tanks and sinks. Steel plates (steel type 1.4301 2K) were obtained from Outokumpu Oy, Finland. Before soiling, loose soil was removed from the steel surfaces (8 cm × 45 cm) with hot tap water (80 ℃) and a dish brush, after which the surfaces were wiped with ethanol (80% ethanol, 20% water). The topography of the steel surface was characterized by scanning electron microscopy (SEM).

Seven different model soils representing different contaminants in cattle barns were used (Table 1). These soils were selected as being possible contaminants in the barn environment. Originally only manure and milk were in liquid form. Model soil suspensions from all other soils were prepared from solid, granular or fibrous substances to allow spreading on the steel samples. The carrot juice model soil was prepared from whole, peeled carrots using a juicer (HuginVitex KP60SFK). The other model soils from non-liquid substances (peat litter, feeds 1-3) were prepared by placing 5 g of the substance into a Stomacher bag, after which 95 ml sterile saline (NaCl 9 mg/l) was added and mixed with the soil by pressing the bag

manually. The bag was then closed and set to stabilize for 30 min, after which the contents were homogenized for ten minutes (230 r/min) in a Stomacher 400 Circulator (Merck Eurolab).The homogenized model soil was poured into a sterilized decanter (V = 400 ml).

The dry matter content (Table 1) of the model soils was measured by oven drying for 24 h at 105℃. The microbiological contents (Table 2) of the model soils were measured using dipslides from Orion Diagnostica (Table 3) as in the study by Koivula et al. (2004).

Table 1 Types, origins and dry matter contents of the soils (contaminants) used in the study

Substance for model soil			Dry matter content, %	
Type	Product (ingredients)	Origin or trademark (manufacturer)	Original substance	Model soil*
Feed 1	AIV** feed	Local farm	31.0	1.4
Feed 2	Turnip rape concentrate	Rypsirouhe (Raisio Feed Ltd)	89.4	3.5
Feed 3	Horse bean (*Viciafaba*) feed from outer depository	Local farm	44.9	1.5
Feed 4	Carrot	Rainbow (Kesko Ltd)	12.6	9.2
Litter	Peat litter	Vapon Kuiviketurve (Vapo Ltd)	55.1	1.5
Manure	Slurry	Local cattle barn	10.9	10.9
Milk	Protein-enriched milk	Valio Plus™ (Valio Ltd)	11.5	11.5

* Used for soiling in the experiments. Mean of three replicates.
** AIV fodder, named according to the inventor, Artturi Ilmari Virtanen. The AIV liquid is added to green fodder to increase acidity and improve the storage of the silage particularly during long winters.

Table 2 Microbiological contents of the model soils

Model soil	Microbes, cfu/ml			
	Total aerobic microbes	Moulds	Yeasts	Enterobacteria
Feed 1 (AIV)	*	0	4.3×10^{6}	1.6×10^{4}
Feed 2 (turnip rape conc.)	4.8×10^{3}	0	1.9×10^{3}	1.4×10^{2}
Feed 3 (outer depository)	2.8×10^{2}	0	0	0
Feed 4 (carrot)	1.9×10^{4}	0	1.0×10^{3}	4.0×10^{3}
Litter	1.1×10^{6}	0	2.2×10^{6}	1.4×10^{4}
Manure	2.5×10^{6}	**	6.4×10^{1}	1.6×10^{5}
Milk	0	0	0	0

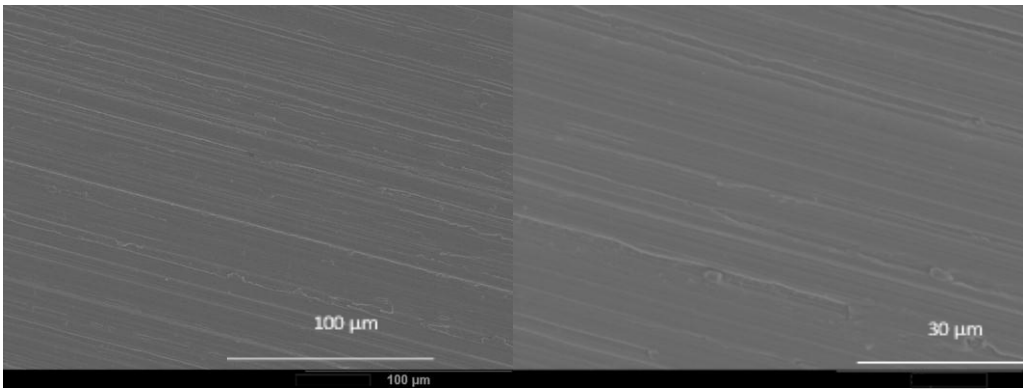
Note: * Colonies could not be counted. They were observed from the same dilutions as from litter.
** Colonies could not be counted. Many moulds were detected from the undiluted soil but not from the diluted solutions.

Thereafter, 1.0 ml of each of the model soils in liquid form was applied with a pipette to the surface and spread with an inoculation loop over the soiling area. The soil was left to dry for 24 h before cleaning.

The soiled steel materials were cleaned with an Erichsen Washability and Scrubbing Resistance Tester, model 494 (Erichsen GMBH and Co, Germany) (Pesonen-Leinonen et al., 2005; Määttä et al., 2008; Toiviainen-Laine et al., 2009). In the equipment a microfibre mop cloth (Freudenberg Household Products Oy Ab) (Pesonen-Leinonen et al., 2003; 2006; Kymäläinen et al., 2008; Määttä et al., 2008; 2010), cut into 4.5 cm × 15.0 cm pieces, was used. The cloths were pre-washed five times in a domestic drum-type washing machine at 60℃. For the cleaning experiments the cloth was moistened to 150% moisture regain (moisture regain = moisture content of the mop fibres as a percentage of the weight of the dry fibres) with purified MilliQ water (no detergent was used). The cleaning cloths were moistened using a pipette, bent double and left to stabilize for 24 h. Cleaning was carried out with three backward-and-forward cleaning movements and a pressure of approximately 1.4 kPa. A new cloth was used for each experiment. Five replicate tests were performed for all model soils.

SEM (Scanning Electron Microscopy) photomicrographs of the steel surface were obtained using a JEOL JSM-480 scanning electron microscope as in the studies by Kuisma et al., (2005) and Toiviainen-Laine et al., (2009). The photomicrographs

were taken with magnifications of 100, 500 and 1500, of which 500 and 1500 were used for the final observations of steel surfaces (Figure 2). The number of replicate samples for each magnification was five.



a) b)

Figure 2 The SEM micrographs of steel surface, magnification (a) x500 and (b) x1500

After the soiling and cleaning procedures, soil amounts on the steel surface were determined using the detection methods presented in Table 3.

Table 3 Codes and details of the detection methods

Type of the method (code)	Details and possible product name	Detection area	Manufacturer
Microbiological dipslide (M1)	Aerobic microbes, Hygicult® TPC	9.4 cm^2 per side	Orion Diagnostica, Finland
Microbiological dipslide (M2)	*Enterobacteria / *β-glucuronidase-positive organisms (e.g. *Escherichia coli*), Hygicult® E–/ß-Gur	9.4 cm^2 per side	Orion Diagnostica, Finland
Microbiological dipslide (M3)	Yeasts and moulds, Hygicult® Y&F	9.4 cm^2 per side	Orion Diagnostica, Finland
Microbiological dipslide (M4)	*Total bacteria / *enterobacteria	9.5 cm^2 per side	Labema
Microbiological dipslide (M5)	*Aerobic bacteria & *coliforms (with TTC Red Spot Dye)	9.5 cm^2 per side	Labema
Microbiological dipslide (M6)	Yeasts and moulds	9.5 cm^2 per side	Labema
Protein test (P1)	Clean Card PRO	10 cm × 10 cm	Orion Diagnostica, Finland
Protein test (P2)	PRO-Clean™	10 cm × 10 cm	Hygiena
Protein test (P3)	3M™ Clean-Trace™ Surface protein Plus	10 cm × 10 cm	Labema
Glucose and lactose (GL)	SpotCheck Plus™	10 cm × 10 cm	Hygiena
Adenosine triphosphate (ATP)	ATP bioluminescence (HY-LiTE®2)	10 cm × 10 cm	Merck KgaA, Germany

Note: *Each on one side of the dipslide. Different counts were made using the two sides of the dipslide.

One protein measurement or two dipslide measurements were carried out on one surface sample. In total, samplings were carried out with all the detection methods as many times as necessary in order to obtain five replicate results.

The microbiological sampling was performed by pressing the dipslide on the examined surface. After sampling, the dipslides were incubated for two to three days at room temperature (20℃-25℃) as in the studies by Lehto et al. (2011) and Kymäläinen and Kuisma (2014). After that the colonies were counted, or if that was not possible, they were evaluated according to the manufacturer's chart models. In the case of the microbiological dipslides not marked with * in Table 3, both sides of the slides were examined and the mean was presented as the final result. For the dipslides marked with * each result was from one side of a dipslide.

The results of the protein tests, glucose and lactose test and ATP bioluminescence were read immediately after sampling. Sampling with the protein test P1 was carried out by moistening the surface samples with water and swiping the surface with the reagent pad. The colour of the reagent pad was observed after 30 seconds and compared with the model chart. The protein tests P2 and

P3 were sampled by swabbing the surface and releasing the reagent in the test tube. If any residue containing protein was present the reagent turned purple. The more contamination was present, the quicker the colour changed to purple and the darker was the colour. Observations of the colour change were recorded. Glucose and lactose were sampled by the GL test (Table 3) by swabbing the surface and releasing the reagent in the test tube. If any residue containing glucose and/or lactose was present, the reagent turned green. ATP (adenosine triphosphate) samples were taken with sterile cotton swabs, after which the ATP bioluminescence was measured luminometrically with a HY-LiTE®2 equipment (Merck KgaA, Germany) as e.g. in the study by Lehto et al. (2011). ATP samples were taken with sterile cotton swabs. The intensity of light generated was read as relative light units (RLU). RLU is directly related to the amount of ATP and biological contamination, including microbes and organic contamination, on the surface.

Results were collected in a database. For the numerical results, means and standard deviations from five replicates were calculated.

All the microbiological results were ranked into cleanliness classes (Table 4) as in the study by Kymäläinen and Kuisma (2014). It should be noted that in the earlier study only dipslides from Orion Diagnostica were used, and the reference for the guidelines was partly taken from the instructions of that manufacturer. In the guideline by Labema the classifications would be somewhat different (for bacteria 2.5 cfu/cm^2 = very slight growth, 12 cfu/cm^2 = slight, 40 cfu/cm^2 = moderate, 100 cfu/cm^2 = heavy, and 250 cfu/cm^2 = very heavy growth; for moulds 0.4 cfu/cm^2 = slight growth, 1.6 cfu/cm^2 = moderate and 4 cfu/cm^2= heavy growth), but to allow comparison the values in Table 4 were used in this study.

Table 4 Surface hygiene guidelines for total microbes, yeasts, moulds, enterobacteria and β-glucosidase-positive bacteria

Microbial group	Classification of the results, cfu/cm^2				References
	Good	Moderate	Poor	Very poor	
Total microbes	<2	2-10	11-49	>50	Rahkio et al. (2006); Kymäläinen and Kuisma (2014)
Yeasts	<1	1-5	6-25	>25	Hakala (2001); Kymäläinen and Kuisma (2014)
Moulds	-/+ (light)	++ (moderate)	+++ (heavy)	Not included	Orion Diagnostica (2009a)
Enterobacteria	<0.1	0.1-1.1	1.2-5	>5	Orion Diagnostica (2011); Kymäläinen and Kuisma (2014)
β-glucuronidase-positive bacteria (β-GUR)	<0.1	0.1-1.1	1.2-5	>5	Orion Diagnostica (2009b); Kymäläinen and Kuisma (2014)

For ranking the ATP bioluminescence values the following scale used by Kymäläinen et al. (2009) was applied. The original scale included three classes: some organic soil or matter<500 RLU (moderate), much organic soil 500-5000 RLU (poor) and very much organic soil >5000 RLU (very poor). Because it had later been suggested that it would be practical to add a third, "good" class, having a low ATP amount of 0-50 or 0-100 RLU, the first of these definitions was added in the present study.

The non-numerical protein tests were ranked into three classes, where 0 indicated that soil was not observed, X indicated a minor amount of soil and XX a lot of soil. Based on the instructions of the tests, the result X corresponded to the situation "re-clean" ("caution"), whereas XX corresponded to "re-clean and re-test". The results of the sugar test were of the pass/fail style: 0 was recorded if contamination was not observed and X if it was observed.

Bivariate correlation analysis (Pearson's correlation coefficients, two-tailed test of significance) of the IBM SPSS statistics 22 tool was used to examine connection between the results of the different detection methods.

3 Results and discussion

The pre-cleaned steel surfaces had no detectable amounts of any of the microbe types examined in this study (Table 5). Similarly, neither protein nor sugars were detected from the steel surfaces before soiling (Table 6). Small amounts (15-23 RLU, mean 20 RLU) of ATP were detected from the pre-cleaned steel surfaces (Figure 3), but when these results were classified as presented in the Methods section, the ATP results of the pre-cleaned surface would also be ranked as good.

Table 5 Microbiological contents of the steel surfaces after soiling and cleaning

Soil	Condition of the surface examined	Detection method*										
		Aerobic microbes			Enterobacteria		β-gur	Coli-forms	Moulds*		Yeasts	
		(M1)	(M4)	(M5	(M2)	(M4)	(M2)	(M5)	(M3)	(M6)	(M3)	(M6)
-	After pre-cleaning, before soiling	0	0	0	0	0	0	0	-	-	0	0
Feed 1	After soiling	80 (0)	1.9 (0.5)	40 (0)	0	0	0	0	-	-	0.1 (0.2)	0
	After cleaning	3.4 (1.8)	4.0 (0.3)	0	0	0	0	0	-	-	0	0
Feed 2	After soiling	1.7 (0.9)	2.5 (0.4)	0.3 (0.4)	0.1 (0.2)	1.2 (1.6)	0.0 (0.1)	0.0 (0.4)	-	-	1.1 (0.8)	0.3 (0.5)
	After cleaning	0	0.2 (0.3)	0	0	0	0	0	-	-	0	0
Feed 3	After soiling	2.0 (1.0)	2.0 (0.8)	0.0 (0.0)	0	0	0	0.0 (0.0)	-	-	0	1.7 (1.0)
	After cleaning	0	0.1(0.1)	0	0	0	0	0.1 (0.2)	-	-	0	0
Feed 4	After soiling	3.7 (1.2)	5.7* (4.3)	2.0 (0.6)	4.4 (1.3)	2.0** (0.5)	3.4 (2.2)	0.5 (0.4)	-	-	2.4 (1.8)	8.2 (5.2)
	After cleaning	0.2 (0.0)	0.2 (0.2)	0.0 (0.0)	0	0.0 (0.0)	0	0	-	-	0	0
Litter	After soiling	45 (0)	12 (0)	12 (0)	0	7.5 (6.2)	0	0	-	-	29 (22)	2.5 (0)
	After cleaning	0.5 (0.3)	0.6 (1.1)	0	0	0.0 (0.0)	0	0	-	-	0.1 (0.1)	0
Manure	After soiling	45 (0)	12 (0)	12 (0)	1.3 (1.0)	2.6 (1.2)	0.7 (0.4)	0.3 (0.3)	+++ (0)	+++ (0)	29 (22)	2.5 (0)
	After cleaning	4.7 (1.3)	1.7 (0.6)	1.6 (0.7)	0.0 (0.1)	0	0	0.6 (0.5)	-	+ (+)	0	0
Milk	After soiling	0	0	0	0	0	0	0	-	-	0	0
	After cleaning	0	0	0	0	0	0	0	-	-	0	0

* Codes of the dipslides are presented in Table 3. The scale for moulds is presented in Table 4. For all other microbes the results are presented in cfu/cm^2 as means and standard deviations (± SD).
**Mean of 4 replicates

Table 6 Cleanness of the steel surfaces after soiling and cleaning as measured with the protein and sugar tests

Soil	Condition of the surface examined	Detection method (see Table 3)			
		Protein tests*			Sugar test**
		P1	P2	P3	LG
-	After pre-cleaning, before soiling	0	0	0	0
Feed 1 (AIV)	After soiling	X	X	X	0
	After cleaning	0	0	0	0
Feed 2 (turnip r.)	After soiling	XX	XX	XX	0
	After cleaning	X[1]	0	X***	0
Feed 3 (outer dep.)	After soiling	XX	XX	XX	X
	After cleaning	0	0	0	0
Feed 4 (carrot)	After soiling	XX	XX	X	X
	After cleaning	0	0	0	0
Litter	After soiling	0	0	0	0
	After cleaning	0	0	0	0
Manure	After soiling	n.a.	XX	XX	0
	After cleaning	n.a.	X	X	0
Milk	After soiling	XX	XX	XX	0
	After cleaning	XX	X	X	0

n.a. Method not applicable because of the interference of the dark colour of the soil.
*Scale for the protein tests: 0 – soil not observed, X – some indication of soil, XX – a lot of soil
**Scale for the sugar test: 0 soil not observed, X – soil observed
*** Deviation between the five replicates; variation range from 0 to X

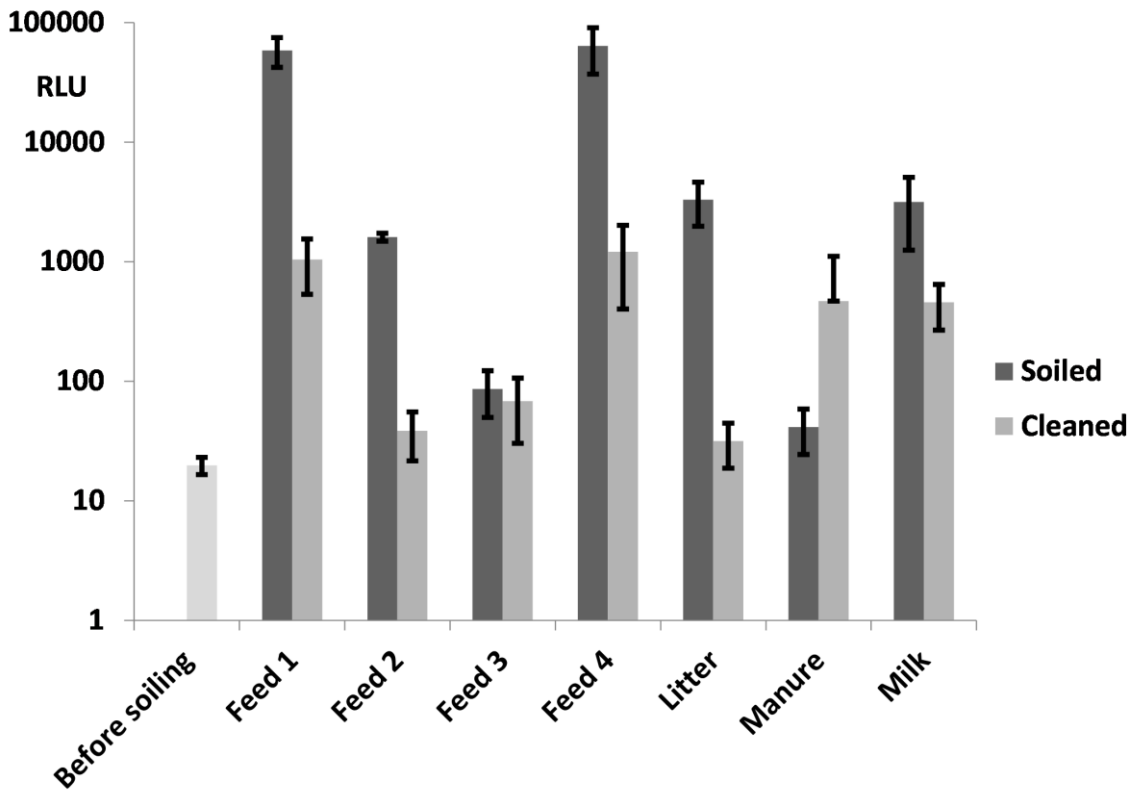


Figure 3 Cleanness of the steel surfaces after soiling and cleaning, measured with an ATP bioluminescence detection method and presented in RLU (relative light units) as means (columns) and standard deviations (±SD, bars)

The steel soiled with feed 1 (AIV feed) contained aerobic microbes both after soiling and after cleaning, and a small amount of yeasts after soiling. Two of the three tests detecting aerobic microbes showed heavy contamination after soiling (Table 5). A large number of aerobic microbes and yeasts were present in the model soil (Table 2). Enterobacteria were not detected in the surface experiments (Table 5), although the soil itself contained a high count of these bacteria (Table 2). Protein was detected after soiling but not after cleaning (Table 6). The ATP amounts were very high (mean 58400 RLU; poor according to Table 4) after soiling and moderate (1040 RLU) after cleaning (Figure 3).The AIV feed contained 32 g/kg ammoniacal nitrogen (NH_3-N), 413 g/kg soluble nitrogen, 58 g/kg lactic and formic acids, 8 g/kg volatile fatty acids and 101 g/kg sugar.

After soiling of the steel surface with the turnip rape concentrate (feed 2), all other microbe types except moulds were detected in small amounts. After cleaning only one test showed residues of aerobic microbes (Table 5). The soil contained moderate amounts of aerobic microbes, yeasts and also enterobacteria (Table 2), but as in the case of feed 1, enterobacteria were not detected in the surface measurements. After soiling a high level of protein contamination was detected with all three tests, and after cleaning two tests showed some protein residues on the surface (Table 6). The ATP amounts were high (mean 1600 RLU) after soiling but after cleaning (38 RLU) they were close to the background value (20 RLU) (Figure 3). The results were classified as poor after soiling and good after cleaning. The protein observations in the experiment are probably explained by the high protein content of the feed, which contained 40.6% crude protein, 7.05% ash, 4.28% crude fat and 24.7% NDF (neutral detergent fiber).

Feed 3 from the outer depository resulted in moderate amounts of aerobic microbes, coliforms and yeasts on the steel surface after soiling, and after cleaning

some residues of the aerobes and coliforms were still present on the steel surface (Table 5). The soil contained moderate amounts of aerobic microbes, but in contrast to the results of the soiled surface, yeasts were not observed in the soil (Table 2). Enterobacteria were not observed from the soil (Table 2) or in the surface experiments (Table 5). After soiling, a large amount of protein and some sugars were detected on the steel surface, whereas after cleaning these contaminants were not observed (Table 6). The ATP amounts were moderate both after soiling (mean 86 RLU) and after cleaning (68 RLU) (Figure 3). After soiling, the ATP amounts were the lowest of all the soils examined in the study. However, this soil was among those giving the most diversified microbial response. This feed contained 27 g/kg ammoniacal nitrogen (NH_3-N), 303 g/kg soluble nitrogen, 45 g/kg lactic and formic acids, 9 g/kg volatile fatty acids and 57 g/kg sugar.

The steel surface soiled with carrot juice (feed 4) contained all the microbe types examined except moulds. After cleaning, small amounts of aerobic microbes and enterobacteria were detected (Table 5). The soil contained clear amounts of aerobic microbes, yeasts and enterobacteria (Table 2). In the study by Määttä et al. (2013), peeled and cut/grated carrots contained 3.1-5.9 log cfu/g aerobic microbes, 2.6-3.9 log cfu/g coliform bacteria, 2.0-4.2 log cfu/g enterobacteria, 2.1-4.5 log cfu/g yeasts and a maximum of 2.2 log cfu/g moulds. In our model soil 4.3 log cfu/ml of aerobic microbes, 3.0 log cfu/ml of yeasts and 3.6 log cfu/ml of enterobacteria were detected (modified from Table 2), whereas moulds were not observed and coliforms were not examined. However, the microbial content of the carrot raw material was not examined. After soiling a high amount or at least some protein contamination with all protein tests and also sugars were observed on the steel surface, but after cleaning these contaminants were not found (Table 6). In contrast to our results, in the study by Moore et al. (2001), none of the three protein tests detected protein from carrot debris. Carrot contains only minor amounts of soluble proteins: in the study by Masih et al. (2002) the content varied between 0.5 and 0.8 mg/g (d.w.) depending on storage time. According to Butt and Sultan (2011), carrot contains 0.93 g protein per 100 g. The ATP amounts of the feed 4 (carrot) test in our study were similar to those of feed 1: very great (mean 63800 RLU) after soiling and still high (1300 RLU) after cleaning (Figure 3). Classification of these results would be very poor and poor, respectively.

Great amounts of aerobic microbes, enterobacteria (with one test), β-glucuronidase-positive organisms and yeasts were detected on the steel surface after soiling with litter. After cleaning, some residues of all these microbes were still present (Table 5). The analysis of the soil (Table 2) showed great numbers of aerobic microbes, yeasts and enterobacteria. Protein and sugars were not detected after soiling and cleaning (Table 6). Rather similarly to the results of feed 2 (turnip rape concentrate), the ATP amounts were great after soiling (3300 RLU) but close to the background value after cleaning (32 RLU) (Figure 3). Classification of these results would be poor and good, respectively.

The steel surface soiled with manure contained all the microbe types examined. After cleaning, β-gur were absent and the amount of enterobacteria was extremely small, whereas all other microbe types were still present (Table 5). The model soil contained great numbers of aerobic microbes and enterobacteria, and some yeasts and moulds (Table 2). After soiling a lot of protein and after cleaning some protein was observed on the steel surface (Table 6). The protein test P1 did not function properly after cleaning due to the dark colour of the manure soil. After soiling only low levels of ATP (41 RLU) were detected on the surface, whereas after soiling the numbers were clearly greater (467 RLU). Classification of these results would be good and moderate, respectively. If the dried soil could not be swabbed properly although the swab was moistened, but cleaning loosened the soil on the surface, a large amount of soil would detected after cleaning if it was not removed well in cleaning. However,

this could not be confirmed in our study. Related to this discussion, in the study by Moore et al. (2001) dipslides detected less bacteria from a dry surface than from a wet surface, but this reduction in sensitivity was less marked than for spread or pour plate methods. In our study the drying time of the soil was 24 h, whereas in the study by Moore et al. (2001) it was 1 h.

As an exception to all other soils in this study, soiling with milk resulted in undetectable amounts of any of the microbe types, and the situation remained the same after cleaning (Table 5). This is in accordance with the examination of the model soil, in which no microbes were observed (Table 2). Moore et al. (2001) also observed only some if any microbes on steel surface soiled with milk. According to Adams and Moss (2008), according to EU-based regulations pasteurized milk may contain less than 1 coliform/ml, and after five days of storage at 6 ℃ its count at 21 ℃ should be below 10^5cfu/ml. We selected pasteurized milk because of its consistent quality, although in the barn milk is naturally untreated. The response of the protein tests to milk was shown by Moore et al. (2001), and also in the present study a large amount of protein was detected after soiling and at least some after cleaning (Table 6). The ATP level (3160 RLU) after soiling in our study was similar to those of the litter and feed 2 (turnip rape concentrate), and after cleaning 456 RLU, which was similar to that observed with manure (Figure 3). Classification of these results according to Table 4 would be poor and moderate, respectively.

In most cases the mean numbers of aerobic microbes were rather close to each other when comparing the results obtained with different dipslide types (Table 5). When the result rows of total microbes of soiled and cleaned surfaces (Table 5) were classified according to Table 4, in 11 cases all three tests gave results belonging to the same class, whereas in three cases they did not. However, in the case of the steel surface soiled with feed 1 (AIV), the results of aerobic microbes obtained with dipslide M4 were clearly lower than those obtained with M1 and M5. With the exception of this result, when the results were classified according to Table 4, all dipslide types indicated the poor results after soiling with manure and litter. In several cases, some of the dipslides resulted in no microbes, whereas the others detected a few aerobic microbes. The correlation coefficient (r) between the results of the aerobic microbes from different manufacturers values varied from 0.45 to 0.68 ($p<0.001$).

In the case of enterobacteria, in 11 cases both tests gave similar results, belonging to the same class (Table 4), although in three cases they did not. When the results of the two tests belonged to different classes, the difference was one class/step (feeds 2 and 4 after soiling), except for the result of litter after soiling, when the difference was two classes/steps. The correlation coefficient (r) between the results of the enterobacteria tests varied between 0.64 and 0.70 ($p<0.001$). According to the manufacturer, β-gur plates are a more selective growth medium than the enterobacteria plates, and thus the β-gur plates (Table 5) indicated less contamination than those of enterobacteria. Small amounts of coliforms were detected from feed 2 (turnip rape), feed 3 (outer depository) and manure after soiling, and from feed 3 and manure after cleaning (Table 5). Coliforms belonging to the *Enterobacteriaceae* are often of intestinal origin, although some species are common in waters (Pandey et al., 1999). In the study by De Palo et al. (2006), both mats and wood shavings used as freestall beddings were contaminated with manure and contained coliforms.

In this study moulds were useful indicator microbes which were detected only from the manure soil. Both mould dipslide types gave a clear response to manure after soiling, indicating heavy soiling, whereas only one dipslide (M6) also detected a low level of moulds from these surfaces after cleaning (Table 5). However, both the dipslide results after cleaning would be classified as good (Table 4). Variation between the five replicates was observed only in the M6 results. A strong correlation between the two mould tests was observed: the r values ranged from 0.92 (both soiled and cleaned surfaces

included) to 1.0 (soiled surfaces), $p<0.001$; for the cleaned surfaces a statistical result was not obtained.

In some cases the two yeast dipslides gave clearly different results (Table 5). In nine cases the results from the two tests belonged to the same class (Table 4), whereas in five cases they did not. In two cases (feed 1 after soiling and litter after cleaning), dipslide M3 indicated some contamination, whereas dipslide M6 did not. However, according to Table 4 both these results could be ranked as good. In contrast to the previous result, in one case (feed 3 after soiling) moderate contamination was observed with M6, whereas M3 did not indicate contamination. In two cases (litter and manure after soiling), great numbers of yeasts were detected with M3 but not with M6. The results obtained with M3 were ranked as very poor (Table 4). On the other hand, in one case (feed 4 after soiling) the result of M6 was poor whereas that of M3 was moderate. According to the statistical test, the results of the two yeast tests correlated slightly with each other when they were compared including both the soiled and cleaned surfaces ($r=0.67$, $p<0.001$). However, the comparison of the soiled surfaces did not indicate correlation ($r=0.45$, $p<0.001$), while for the cleaned surfaces a statistical result was not obtained.

Kymäläinen and Kuisma (2014) reported that the dipslide methods in general were suitable for the cattle barn building environment, although the most contaminated surfaces such as floors covered with feces were not examined in that study. However, the authors suggested that surfaces with a high level of soil are not suitable for microbiological dipslide methods. Kymäläinen and Kuisma (2014) observed in a hygiene monitoring in a cattle barn building that deviations were in many cases considerable and therefore they recommended replicate samplings.

The protein tests gave a response after soiling with all other soils than litter, and with several soils also after cleaning (Table 6). Variation between the five replicates occurred very rarely, only with two protein tests in the surface after cleaning from the feed 2 soil. Some variation between the results of the three different protein tests occurred, but the difference was only one step of the scale at a time (e.g. 0 and X or X and XX, Table 6). According to the statistical analysis, the results of the three protein tests correlated with each other (r values ranged from 0.62 to 0.89, $p<0,001$). However, detection of protein after cleaning was problematic with one of the three tests, since the dark colour of the manure soil interfered with reading of the colour change on the test. Moore and Griffith (2002) reported occasional formation of unusual colour in the protein test used. Moore et al. (2001) observed in their laboratory study that the colour change in certain protein tests was sometimes difficult to interpret. Toiviainen-Laine et al. (2009) reported that a protein test was suitable in their laboratory study including bovine serum albumin as a protein soil component, which however was not included in the present study. For the protein test the results were ranked with a three-step scale. This was also the case in the study by Toiviainen-Laine et al. (2009), who also summed the results from 0-2 points (0 clean–2 very dirty). The smaller was the sum, the better was the cleanability.

The sugar test was in general not useful for most of the soils examined, since only feeds 3 (outer depository) and 4 (carrot) gave small responses after soiling. Variation was not observed within each 5 replicate set.

For the ATP results there is no established ranking scale. For ranking the ATP bioluminescence we used a three-step scale introduced by Kymäläinen et al. (2009), with the addition of a fourth class. Moore and Griffith (2002) used 500 RLU as the upper value for a clean surface, but they used a different appliance than in the present study. They grouped both the protein test and ATP results into three classes: pass, caution and fail. In a study concerning a dairy Carrascosa et al. (2012) ranked ATP results below 100 RLU as acceptable and those above that limit as unacceptable. It should be noted that the results of different ATP devices are not directly comparable with each other (Griffith et al., 1994).

The statistical test showed that the correlations were mainly weak between the different detection methods. Exceptions concerning certain methods were mentioned earlier. No correlations were observed between the results of aerobic microbes, protein tests and the ATP bioluminescence (r values ranged from 0.09 up to 0.47, $p<0.001$).

As was stated in the study by Kymäläinen and Kuisma (2014), there are no existing limit values intended specifically for cattle barns. The authors described the background of the limits presented in Table 4.

Some laboratory studies examining possible correlations between the abilities of different methods to detect certain soils were found. Moore et al. (2001) compared different protein tests with microbiological and ATP bioluminescence methods in a laboratory study. Stainless steel was soiled with serially diluted bovine serum albumen, bacteria or different food debris such as milk, chicken, carrot or tomato. None of the detection methods alone was ideal for examining all the contaminants of the study. The most sensitive protein detection tests were superior or comparable to ATP bioluminescence when detecting high-protein residues, which were milk and chicken in their study. In the study by Moore and Griffith (2002) the ATP results correlated better with the results from the protein detection test than with the microbiological tests, but the highest level of conformity was between the results of the protein test and traditional microbiology. However, there were differences between the four environments examined, namely meat, cheese, bakery and frozen meals industries. The benefits of the dipslides are convenience, simplicity of use and cost effectiveness. Some types of dipslides, e.g. the Hygicult® TPC (total microbes) and E (enterobacteria and β-GUR) dipslides used in the present study have been validated against swabbing and control plate methods and the results have been observed to be at the same level (Salo et al., 2000), but similar information is not available for all commercial products. However, in a field study in a dairy by Carrascosa et al. (2012), ATP bioluminescence detected the largest number of unacceptable surfaces, followed by the contact plates and Hygicult® dipslides.

In practice, most measurements e.g. in self-monitoring are carried out immediately after cleaning, and many of the tests are also intended for assaying cleaned surfaces. This should be taken into consideration when evaluating the current results of soiled surfaces.

Always when pressing, swabbing or other similar methods are used for collecting residues or contaminants for measurement, human factors play an important role. It is difficult to standardize the pressure and movement with which the swabs or other media are pressed or moved on the surface to be examined (Moore et al. 2001). The soil collecting capacity depends also on the size (Moore et al., 2001) and other properties of the swab, such as its material, form, surface roughness and repellency.

Previously existing information concerning monitoring of hygiene in cattle barns was very limited. The present study provided valuable information for selection of monitoring methods in practice and also for further studies.

4 Conclusions

Cleanness of steel surfaces for use in cattle barns was examined using seven different soils commonly encountered in cattle barn environments. The results showed that all the detection methods tested, namely different microbiological dipslides, protein and sugar tests and ATP bioluminescence, could be used for examining soils typically encountered in a cattle barn. However, the usefulness and width of the range, meaning the ability to react to several soils, depends on the test method and also on the aim and motivation of the study. Surfaces with a high level of soil are not suitable for microbiological dipslide methods, and due to deviations, replicate samplings are recommended. Response of the barn soils in the sugar test was limited. ATP bioluminescense is a very sensitive method. Practical considerations such as possible interference of the colour

of the soil, as e.g. in the case of some protein tests, or the time allowed to obtain the results, as e.g. in the use of the microbiological dipslides, should also be taken into account when selecting a detection method. Sampling sites must be carefully planned, and sampling should normally be done immediately after cleaning. Only the results of sampling facilities of a certain type and from the same manufacturer should be compared with each other. Evaluation and classification of the results is crucial for the interpretation of the results. There are no common limit values intended specifically for cattle barns, but values from this study and some other literature can be used as approximate guidance before setting own limits for each case.

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