Milk sampling quality of students participating in the research project ‘selective non drying off’

Hans Veenstra, 2014, University of Utrecht, Animal Health Service, Deventer

Abstract

From May 2011 until September 2012 the Animal Health Service (AHS) investigated selective non drying off with dairy cattle. Students took milk samples at the dairy farms participating in this research project. The purpose of this study was to determine the students’ quality of milk sampling. If three or more dissimilar colony types were found after bacteriological examination, this sample was characterized as a contaminated milk sample (CMS). This was seen as an erroneous sample, because the sample was most probably polluted with environmental germs. Firstly, the advances in milk sampling quality per student was determined: the first two weeks of the internship (part A) were compared with the remainder of the internship (part B). There was no significant difference, which means that there was no significant improvement or deterioration during the participation period of the students. Secondly, the percentages of CMS taken by the students were compared to the percentage of CMS in all other milk samples sent to the laboratory of the AHS. There was no significant difference in quality either. Finally, the quality of the students was compared with the milk sampling quality of the farmers participating in the research project. Students had a significant lower percentage CMS than farmers.

We can conclude that the quality of the milk sampling done by the students was sufficient. The quality did not significantly differ from the quality of samples normally submitted to the laboratory and was better than that of the farmers involved in this project.

Samenvatting

De Gezondheidsdienst voor Dieren (GD) heeft vanaf mei 2011 tot september 2012 onderzoek gedaan naar het selectief niet droogzetten van melkvee. Studenten zijn aangesteld om melkmonsters te nemen op de verschillende melkveebedrijven. Het doel van dit onderzoek is om de kwaliteit van de melkmonstername van de studenten te bepalen. Wanneer na bacteriologisch onderzoek drie of meer verschillende typen kolonies werden gevonden, werd dit monster getypeerd als gecontamineerd. Dit werd gezien als een foutief genomen monster, omdat het monster dan hoogstwaarschijnlijk is vervuild met omgevingskiemen. Allereerst werd de vooruitgang in kwaliteit per student bepaald: de eerste twee weken van de stage (deel A) zijn vergeleken met het vervolg van de stage (deel B). Er bleek geen significant verschil te zijn: er is geen significante verbetering of verslechtering tussen deze twee stagedelen. Ten tweede zijn de percentages gecontamineerde monsters van de studenten vergeleken met het percentage gecontamineerde melkmonsters ingezonden bij het laboratorium van de GD. Ook hier was er geen significant verschil in kwaliteit. Als laatste is de kwaliteit van de studenten met betrekking tot de monstername vergeleken met de kwaliteit van de veehouders. Er bleek een significant verschil te zijn:studenten hebben een lager percentage gecontamineerde monsters dan veehouders.

Hieruit kunnen we concluderen dat de kwaliteit van de studenten ten aanzien van de melkmonstername gedurende dit onderzoek voldoende is geweest. Aangezien de kwaliteit: constant is geweest en niet significant afwijkt van de kwaliteit ingezonden bij het laboratorium van de GD. Ook is de monstername kwaliteit van studenten beter dan die van de veehouders.

1. Introduction

Mastitis is of great concern for Dutch dairy farms. In the Netherlands losses, due to clinical and subclinical mastitis, varied between €17 and €198 per cow per year (Hogeveen et al., 2011). The use of antibiotics at drying off (dry cow therapy) is a proven basic measure to reduce intra-mammary infection (IMI), both to eliminate IMI already present at drying off and by preventing new IMI from occurring during the dry period (Halasa et al, 2009, Bradley and Green, 2001). However, antibiotic use creates a selective pressure on bacterial populations and contributes to development of antimicrobial resistance (Landers et al., 2012). Therefore, the dairy industry agreed upon a 70% reduction of antibiotic use in 2015. The somatic cell count (SCC) of the udder is a worldwide used parameter for subclinical mastitis. SCC is a practical tool to evaluate udder health (Schukken et al., 2003). SCC selection thresholds for IMI in the Netherlands were 150,000 cells/mL for primiparous cows and 250,000 cells/mL for multiparous cows (Windig et al, 2010). The Animal Health Service (AHS) started a research project to evaluate dry cow therapy (DCT) in cows with low SCC (primiparous cows <150,000 cells/mL and multiparous cows <250,000 cells/mL) at the last milk recording before drying off. The effect on clinical mastitis (CM), bacteriological status, SCC, and antibiotic use was evaluated when no DCT was administered in low-SCC cows. Additionally, the effect of bacteriological status and SCC at drying off on CM and SCC at 14 days postpartum (PP) was assessed (Scherpenzeel et al., 2014).

The infection status of mammary quarters is determined by microbiological culture of aseptically obtained milk samples and interpretation of the culture results. Strict adherence to aseptic sampling technique is essential, because otherwise the outcome of the culture may not represent the true infection status of the quarter (Hogan et al., 1999). It is important that the sampling method is homogenous, for accurate interpretations of SCC the milk sample should be taken immediately before milking (Olde Riekerink et al., 2007, and Wellnitz et al., 2009). The milk sample is most likely contaminated when a quarter milk sample results in the culture of three or more dissimilar colony types (Hogan et al., 1999). A contaminated milk sample (CMS) is considered to be an incorrectly taken sample and is not used as parameter for bacteriological status on quarter level. Bacterial contamination during sampling could influence various cell functions and may interfere with the interpretation of physiological effects on cell function (Vangroenweghe et al., 2001). Therefore, it is necessary to optimize sterile conditions for milk sampling.

The aim of the current study is to evaluate the milk sampling quality by students. The research question is whether the milk sampling quality by students is sufficient. This results in the following hypothesis:

“The milk sampling quality of students participating in the research project ‘selective non-drying off’ is sufficient”

2. Material and methods

2.1 Population

1640 cows at 100 different dairy farms were included in this research project and each cow was followed from the beginning of the dry period to 100 days of lactation. Milk samples were taken from each quarter of every individual cow to analyze. There were three sampling moments: at drying off (D), at freshening (K) and between 7 and 21 days of lactation (L). Students took milk samples at drying off and between 7 and 21 days of lactation. The farmer took milk samples at freshening.

Since May 2011 18 students participated in the research program to take milk samples from every cow participating in the research project. These 18 students have taken 11.209 milk samples till April 1st 2012. Bacterial analysis and cell counts were done on all samples.
In this study, milk samples are mainly taken by students. Before students participate in this research project, they are given a two week training from employees of the AHS who. These employees are experienced in taking quarter milk samples, in order to achieve a homogenous milk sampling technique along all students.

2.2 Instruments

The data are collected by the laboratory of the Animal Health Service. Each student has his or her samples submitted at the laboratory through a form per farmer. This includes the name of the sampler plus date of sampling.

The sampling method published by the Udder Health Centre Holland (UGCN) has been used in this study in order to ensure that the different samplers use the same aseptic milk sampling technique (Kirk, 2010).

Subsequently, bacteriological examination was made on the milk samples. When bacteriological examination of a milk sample resulted in a culture of three or more dissimilar colony types, the sample was most likely to be contaminated. The sample then was characterized as such. Conclusions regarding mastitis pathogens cannot be made in these samples, because it is likely that the sample is contaminated (Hogan et al, 1999).

2.3 Design

This study contains two experimental groups:

1. Students
2. Farmers

There have been several measurement points per student. Students took milk samples on a daily base. Bacteriological examination was done on all samples. The farmers were spread over the different students so that each student had his own group of farmers. Every student had multiple measurement points in different groups.
The farmers took milk samples of the cows participating in the research project within 24 hours after calving.

A total of 18 students and 100 dairy farms participated in the research project selective non drying off'. This corresponds to approximately 100 farmers who have taken samples. Exact numbers are not available, as farmers did not have to indicate who took the sample.

2.4 Procedure

Students took milk samples at drying off (D) and between 7 and 21 days of lactation (L). These samples were taken before milking, because that would give a more representative image of the udder health on quarter level (Olde Riekerink et al., 2007). The sample was taken after the following procedures:

- Udder and teat were cleaned with dry paper towels (possibly first with a wet cloth in case of an abnormal dirty udder)
- Disinfection of udder and teat with alcohol on cotton wool
- Pre-jetting for 2-3 times
- The cap of the sample tube was removed, and the cap was held with the bottom down
- The sample tube is filled three-quarters full

- Prevent dirt falling in the tube
- The cap is placed immediately at the tube

The farmers took milk samples between 0 and 24 hours after calving (K). They followed the same procedures as the students did before milk sampling. These samples were frozen by the farmer, during the next visit the student took the sample to the laboratory of the AHS.

2.5 Statistics

To evaluate the milk sampling quality of the students, the students are compared in three different manners. First, the differences of CMS taken by the students. Second, the percentage CMS of individual students compared to the CMS sent to the laboratory of the AHS. Third, the differences of CMS taken by students and farmers.

2.5.1 Differences between individual students

The internship of each student was divided into part A and part B. The percentage contaminated samples was calculated for each part and this was compared with each other. Here, the increase or decrease in the quality of milk sampling per student was calculated from Part A to Part B of the internship.

Through the paired T-test the following hypotheses have been tested:

H0: contamination rate in part A and part B of the internship are equal
H1: contamination rate in part A and part B of the internship are not equal

2.5.2 contamination rate student versus contamination rate of samples sent to the laboratory of the AHS

The percentage of contaminated samples submitted to the AHS stands at 3.8% (April 2012). The one sample t-test is done to see whether there is a difference in sample quality between the students and the people who send milk samples to the AHS(farmers and veterinarians).

H0: percentage of contaminated samples of students and percentage of contaminated samples submitted to the lab of the AHS is not significantly different

H1: percentage of contaminated samples of students and percentage of contaminated samples submitted to the lab of the AHS is significantly different.

2.5.3 Differences between student vs farmer

The total percentages of contaminated samples of each individual student are compared with the average percentage contaminated samples of all farmers (reference value). Through the one-sample t-test the following hypotheses are tested:

H0: mean percentage of contaminated samples of students and farmers are equal
H1: mean percentage of contaminated samples of students and farmers are not equal.

3. Results

The students took a total of 11.209 milk samples (Figure 1). The farmers took 5.447 samples. Milk sampling per student is divided into two parts: Part A, week 1 and 2 of the internship, and Part B, Week 3 to the end of the internship. Between these two groups the percentage of contaminated samples was plotted as a percentage of the samples taken by the student in those weeks (Figure 2).

Figure 1.Total of milk samples taken by students and the number of samples thereof which are CMS. In addition the samples and the CMS are divided in part A and part B of the internship.

Student	CMS[1]	Total[2]	CMS part A	Total part A	CMS part B	Total part B	Intern weeks
A	48	644	3	60	45	584	14
C	20	772	3	104	17	668	7
D	8	180	2	36	6	144	15
E	19	580	3	76	16	504	12
F	20	1241	1	40	19	1201	24
G	9	251	3	44	6	207	8
H	10	346	1	87	9	259	6
I	14	532	0	52	14	480	12
J	38	258	20	83	18	175	6
K	120	1251	3	123	117	1128	28
L	14	454	5	100	9	354	10
M	25	312	4	40	21	272	8
N	10	473	1	48	9	425	12
O	10	355	7	76	3	279	8
P	5	602	3	40	2	562	12
Q	21	1030	2	64	19	966	14
R	24	1006	4	72	20	934	13
S	66	1003	9	84	57	919	14
Farmers	460	5447					
AHS	19	472					
Unknown	15	503					

[1]*contaminated milk samples*
[2]*total of samples taken during the internship*

Figure 2. The total percentage of CMS per student and the division between part A and part B of the internship.

Sample	%CMS total	%CMS part A	%CMS part B
A	7,45	5	7,71
C	2,59	2,88	2,54
D	4,44	5,56	4,17
E	3,28	3,95	3,17
F	1,61	2,5	1,58
G	3,59	6,82	2,9
H	2,89	1,15	3,47
I	2,63	0	2,92
J	14,73	24,1	10,29
K	9,59	2,44	10,37
L	3,08	5	2,54
M	8,01	10	7,72
N	2,11	2,08	2,12
O	2,82	9,21	1,08
P	0,83	7,5	0,36
Q	2,04	3,13	1,97
R	2,39	5,56	2,14
S	6,58	10,71	6,2
farmer	8,45		
AHS	4,03		
Unknown	2,98		

3.1 Differences between students

The differences in milk sampling quality between students have been determined using the paired T-test. Table 1 shows that during the first two weeks (part A) the average percentage of contaminated samples (CMS) is 5.98% and that the average percentage of contaminated samples from week 3 to the end of the internship (Part B) is 4.07%. The question is whether these rates differ significantly from each other. Table 3 shows that p-value is greater than 0.05 (0.109), so the H0 is not rejected: there is no significant difference in the percentage of contaminated samples between Part A and B of the internship. This means that the quality of the milk sampling per student is not significantly improved or worsened after the 2 introduction weeks (Part A).

Table 1

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	% CMS Part A	5.977222	18	5.4415869	1.2825943
	% CMS part B	4.0694	18	3.05713	.72057

Table 2

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	%CMS part A & %CMS part B	18	.482	.043

Table 3

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	%CMS part A %CMS part B	1.9077778	4.7892030	1.1288260	-.4738368	4.2893924	1.690	17	.109

3.2 contamination rate student versus contamination rate of samples sent to the laboratory of the AHS

The differences in milk sampling quality between the students and the samples submitted to the laboratory AHS is determined by a one sample T-test. Table 4 and 5 show that the average percentage of contaminated samples of all students throughout the internship is 4.48%. The percentage of contaminated samples submitted to the AHS (3.8%) is in-between the 95% confidence interval. So the H0 is not rejected: there is no significant difference in the percentage of contaminated samples between students and the samples submitted to the laboratory of the AHS.

Table 4

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
%CMS total	18	4.481111	3.5216672	.8300649

Table 5

One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
%CMS total	5.399	17	.000	4.4811111	2.729827	6.232395

3.3 Differences between students and farmers

We investigated whether students who have taken milk samples at a daily base, have a lower percentage than the farmers (as a group), who took samples weekly. We have individual rates of CMS of the students plotted against the mean percentage of CMS of farmers (reference value). The difference in sampling quality between students and farmers is determined by a one-sample t-test. The difference between the average percentage CMS of students and the average percentage CMS of farmers (test-value) being -3.97. The P-value is less than 0.05, so H0 is rejected: there is a significant difference in the percentage contaminated samples of students and farmers. Students have a lower percentage of contaminated samples than farmers.

Table 6

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
%CMS total	18	4.481111	3.5216672	.8300649

Table 7

One-Sample Test

	Test Value = 8.45					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
%CMS total	-4.781	17	.000174	-3.9688889	-5.720173	-2.217605

4. Discussion

In the present study the milk sampling quality of students participating in the research project ‘selective non-drying off’ was determined. To determine the sampling quality, we took a contaminated sample (CMS) as an incorrectly taken sample. We examined the sampling quality in three different ways. First, we examined whether there was a significant difference between the first two weeks of the internship (part A) and the remainder of the internship (part B) within students. Second, we compared the percentage of CMS of students to CMS sent

to the laboratory of the Animal Health Service. Third, we compared the percentages of CMS of each individual student with the average CMS of all farmers.

There was no significant difference in percentages CMS between part A and B of the internship. Although the percentage of CMS was lower in part B (4,07% versus 5,98%) of the internship. We expected that there would be a significant difference, as in part A of the training you do not have perfectly mastered the sampling technique. There may be several reasons for this: students have taken too few samples in part A of the internship, making the average rate of CMS not representative. In Part A of the internship, the students were guided by employees or students who already have mastered the sampling technique, such monitoring may have led to the fact that the sampling quality was well assured. Another reason could be that the new students are incorporated very well, so there is no clear difference in quality between part A and B of the internship.

The percentage of CMS is not significantly different from the percentage of CMS submitted to the laboratory of the AHS. Samples submitted to the laboratory are usually taken by veterinarians and farmers. They have to take the samples in a proper way, so that the results will be reliable. Therefore, we expected that the sampling quality, between students and samples send to the laboratory of the AHS, would not be different.

The sampling quality of students was significantly better than the sampling quality of farmers. This is what we expected, based on experiences in the past. Farmers took an average of 20 times 4 quarter samples, students took many more samples. Experience is one thing that plays a role, but laxity of farmers is also a reason. Some farmers did not persist the rules for taking milk samples (Kirk, 2010). They did not see the point or did not have the time to do it properly. This may be a reason for this difference, however, there is assumed by the AHS that experience is the biggest factor in this story. Since participation in this survey is voluntary, and the farmers who participated in the study were generally very motivated. Farmers that did not fulfill the demands of the AHS were removed from the study.

The aseptic milk sampling technique used in this study is a manual technique (Kirk, 2010). Manual techniques are susceptible to human errors, therefore a mechanical sampling method might be preferred. However, previous study in which they evaluated three different milk sampling techniques (direct septic collection from the udder, mechanical- and manual collection)showed that bacterial contamination is significantly ($P<0.001$) higher in machine milking samples (Vangroenweghe et al., 2001). This indicates that the sampling technique used in this study is preferable to a mechanic technique.

In the current study milk samples were taken immediately before milking. A previous study stated that for the interpretation of SCC milk samples should be taken immediately before milking (Olde Riekerink et al., 2007). However, other studies stated that individual cows show dramatic variations in foremilk SCC that were not very well related to total quarter milk SCC (Wellnitz et al., 2009, Sarikaya et al., 2006). They concluded that foremilk samples are useful to detect high quarter milk SCC to recognize possibly infected quarters, only if precise cell counts are not required. This implies that the foremilk samples used in this study to determine the total quarter SCC can be deceptive.

Milk samples were considered as contaminated when a quarter milk sample resulted in the culture of three or more dissimilar colony types (Hogan et al., 1999). However, a sample still can be contaminated when less than three

dissimilar colony types are found. In a case that there are less than three dissimilar colonies found at bacterial examination, which are not from the quarter milk fraction, the sample is unfairly characterized as non-contaminated. In future studies we could perhaps compare bacteriological examination of the udder surface to bacteriological examination of the quarter milk sample to avoid such errors.

In the present study farm conditions such as hygiene of the udder and environment have not been included in this study. A previous study showed that there is a significant association between udder hair clipping and teat-end cleanliness, and that teat-end cleanliness is associated with high bacterial counts in bulk tank milk (Elmoslemany et al., 2009). Another study showed that total aerobic counts are mainly associated with cow and stall hygiene: washing the teats with water, not using pre-dip and dirty teats were risk factors (Elmoslemany et al., 2009). This highlights the importance of udder and stall hygiene on hygienic quality of the milk sample. This results might be an explanation for the difference in percentages contaminated samples between students.

On average, the milk sampling quality is sufficient. However, in the study, there are a few students who scored far above the 3.8% MF, respectively: 7.45%, 8.01%, 14.73% and 9.59%. Of these students, the quality of sampling was not sufficient. There are also students who had a lower percentage of CMS in part A of the internship compared to part B. The sampling quality of these students is reduced, or part A of the training was not representative, because of too few samples taken. Students who have taken part in this study were selected on the basis of motivation. The question is whether this is sufficient. Perhaps the quality of their actions must be reviewed before they are allowed to participate in the research through an internship. In this way you can probably skip students who cannot work properly.
The quality of the milk sampling of the students has been found sufficient. However, because of the large individual differences, more research have to be done at individual level in future studies.

5. Conclusion

The aim of the current study was to evaluate the milk sampling quality by students participating in the research project ‘selective non drying off’ of the AHS. This resulted in the following hypothesis:

“The milk sampling quality of students participating in the research project ‘selective non-drying off’ is sufficient”

There is no significant difference in both the percentage of CMS in Part A and Part B of the internship, and between the percentage of CMS samples of the students and the percentage of CMS samples sent to the laboratory of the AHS.
However, there is a significant difference in the mean percentage CMS of students and farmers: students have a lower percentage of CMS than farmers.

The hypothesis is confirmed: the milk sampling quality of the students remains the same over the whole training period, is equal to the quality of samples sent to the laboratory of the AHS, and is significantly better than the sampling quality of the farmers. It can thus be concluded that the milk sampling quality of the students participating in the research project “selective non drying off” is sufficient!

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7. References

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