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Evaluation of The Effects of Milking Hygiene and Sanitation Education on Total Bacterial and Somatic Cell Number of Bulk Tank Milk in Dairy Cattle Breeding

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ABSTRACT

Hygiene and sanitation, which are of great importance for the protection of human. Beside this quality and quantity of the milk is very important for human health. The aim of this project is to develop a training model in raw milk hygiene in Aydın province, and to increase knowledge, skills and competencies of vocational/professional groups in raw milk production. Two surveys were conducted for the farm workers to be trained within the scope of the project. Firstly, a demonstrative survey was conducted for the farms where milk hygiene training was provided. The education satisfaction survey was conducted for the people who were educated after the seminar. The surveys were prepared by the project executives and the questions were answered by face to face interview method. The milk samples of the study were collected from 157 dairy farm bulk tanks located in Aydin province, before the beginning of the project and after training courses. The milk samples were analyzed for Total Viable Count (TVC) and Somatic Cell Count (SCC). In conclusion, 11% of tank milk samples were below the norm value of 100000cfu/mL out of 157 farms. It is also seen that 27 farms reduced their SCC values below 400000cells/mL out of 56 farms with a norm value of ≥400000cells/mL after hygiene training. When the results were evaluated in general, hygiene was improved by 11% in average TVC value and 1% in SCC value after milking hygiene education.

Key words: Bulk Tank Milk, Education, Milk Hygiene, SCC, TVC.

INTRODUCTION

Milk has many important properties, for the growth and development of mammals, in the life cycle due to its fatty acids, vitamins and minerals, which are found in its structure. It also contains physiologically important components such as immunoglobulin, enzymes, enzyme inhibitors, growth hormones, antibacterial agents, protein and peptides. Milk and dairy products are considered the most important foodstuffs for living organisms (Maijala 2000; Fox and McWeeney 2003; Miller et al. 2010).

The employment created for each country is the key to the balance of rural development and agriculture, especially in terms of the value of the domestic and export markets it creates. Dairy products take an important place in these markets (Beykaya et al. 2017). About 18 million tonnes of milk are produced annually in Turkey. The 42% of produced milk reaches the consumer as raw milk. The 40% of total produced milk are processed in small enterprises and 20% in large and modern enterprises (Güzel-Seydim and Koçak 2004; TSI 2016). Aydin, where the project has been implemented, is one of Turkey's top milk producers with a national share of 2.5%, and 344 thousand cattle. It has a 4% share in Turkey-4th in the country-in culture race cattle breeding with a total of 253887 animals, 92260 milked animals and 366 tons of

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milk. There are generally mid-sized and small livestock enterprises in Aydın. In 2014, according to the registry of the Ministry of Food, Agriculture and Livestock Breeding, there were over 46 thousand companies and more than 43 thousand of which were dairy cattle enterprises (TSI 2016).

More than 75% of the milk produced by the EU countries is collected through cooperatives and other organizations then handed over to industrial establishments in cold chain and hygienic conditions (Ateş 2015). Since milk has a rapid spoiling feature, it must be kept under the special conditions until consumption. The quality of raw milk depends on the first stage of production in the enterprises (Srairi et al. 2009).

Somatic cell count (SCC) and Total Viable Count (TVC) are used as the important criteria in raw milk quality and mammary health issues (Mu´nera-Bedoya et al. 2017). Somatic cells in the milk consist of epithelial cells, large squamous cells, epithelial cell debris and non-nucleated cells, erythrocytes, plasma cells, colostrum corpuscles and leukocytes. High SCC was affected by age of the cow, age of lactation, stress, season, nutrition, observance of hygiene rules and mastitis. The levels of SCC and TVC provide valuable information on the hygienic conditions during various steps of milk production on the farm (Cedden et al. 2002; Eyduran et al. 2005; Macedo et al. 2018; Ginestreti et al. 2020). Consumers need to be able to source raw milk from farmers that have adopted a high level of good animal husbandry practices and manufacturing practices that can reduce potential food safety hazards to very low levels (Berge and Baars 2020; Singhal et al. 2020).

Sustainable Milk Hygiene Training Model for Safe Milk and Safe Future (2015-1-TR01-KA202-022316) project was funded by the European Commission under the Erasmus+ Program. The applicant organizer of project was Aydın Provincial Food Agriculture Animal Husbandry Directorate (Turkey) and the other partners were Aydın Governorship EU and External Relations Coordination Centre (Turkey), Aydın Adnan Menderes University Faculty of Veterinary Medicine (Turkey), ÖR-KOOP (Nazilli and Surrounding Agricultural Development Cooperative) (Turkey), Dairy Training Centre (The Netherlands), Asociace Soukromeho Zemedelstvi Ceske Republiky (Association of Private Farmers, Czechia).

The current situation about milk hygiene in Turkey is far behind the desired level in terms of quality of raw milk. It is now more meaningful to make safe and quality production because of the reasons such as the developing economic conditions; the level of consumer consciousness and the sharing of technological information reach the upper level. In terms of the quality and safe product, hygienic principles have become the most important criteria. In close collaboration with the relevant partners, we have planned to develop training modules, written and visual training materials, to implement series of training courses to increase awareness, knowledge, skills and competencies for raw milk hygiene.

The aim of this study was to prepare a training model for milk hygiene, to increase the expertise and skills of vocational/professional groups in the raw milk production sector and to bring raw milk production to EU standards in terms of hygiene criteria.

MATERIALS AND METHODS

Analysis of Survey Data

Two surveys were conducted for the farm workers to be trained within the scope of the project. Firstly, a demonstrative survey was conducted for the farms where milk hygiene training would be provided. The education satisfaction survey was conducted for the people who were educated after the seminar. The both surveys were prepared by the project executives and the questions were answered by face to face interview method. Before the application, the personnel who were planned to conduct the survey, the purpose of the study was explained. After the reliability test was applied to the surveys, the statistical analyses were done. The population of surveys were the dairy cattle farms of Aydin-Turkey. A survey was given to 150 enterprises selected by random sample method before training, and the satisfaction survey after training was applied to 274 people working in these enterprises. Pre-training survey consist of three sub-dimensions as; General Information about farms and milk parlor staff, information about milking practices at farms, and information related to parlor hygiene. A total of 60 questions were asked in these three sections. The post-training satisfaction survey consisted of three sub-dimensions; Training Planning and Implementation, Educators and Training results. In these factors, 32 questions were asked to participants. The items in the survey were presented as options using the Likert scale of 5, and according to the average of the responses given to the survey items, the following interval values were interpreted: 1.00-1.79: strongly disagree, 1.80-2.59: disagree, 2.80-3.39: Have no idea, 3.40-4.19: agree, 4.20-5.00: strongly agree. Quantitative analyses were conducted by using SPSS software version 22. Descriptive analyses were presented using means, standard error of means and percentages. Exploratory factor analysis using principal-components analysis was carried out as recommended by Pallant (2007). The Kaiser-Meyer-Olkin measure of sampling adequacy and Bartlett's test of sphericity confirmed. Cronbach's Alpha coefficient was used to determine the reliability of the survey.

Milk Samples

Milk samples were collected from 157 dairy farms located in Aydın Districts (Çine: 35, Efeler: 25, Germencik: 7, Karpuzlu: 26, Koçarlı: 29, Söke: 5, Kuyucak: 14, Nazilli: 16), before the beginning of the project and after training courses.

Raw milk samples (500mL) were collected from isothermal bulk tanks at a temperature lower than 5°C into the sterile bottles by specially educated technicians 3 times within 2 weeks intervals, at the beginning of the project (Sample 1 group) and after the trainings (Sample 2 group). The samples were labelled and transported in cold chain to Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology laboratory. The distribution of the total number of samples analyzed by districts was shown in Table 1.

Microbiological Examination

The milk samples collected from in bulk tank, 25mL was taken and homogenized in 225mL of peptone water

(Oxoid CM0009) using a Lab-Blender 400 Stomacher (Interscience, France) for at least two minutes in order to enumerate TVC. Decimal serial dilutions were prepared.

Total Viable Count (TVC) and Somatic Cell Count (SCC)

The prepared decimal dilutions of raw milk samples inoculated onto Plate Count Agar (Oxoid CM0325), for TVC after incubation at 30°C for 48-72 hours (Maturin and Peeler 2001). SSC of milk samples were determined by NucleoCounter® SCC-100™ (Chemometec, Denmark).

Statistical Analysis

Statistical analyses were performed using the SPSS software version 22.0. The data were presented as the mean and standard deviation (SD) for the item statistic of the survey results; while mean and standard error of mean (SE) for descriptive results.

Milk samples were grouped as ≥100000cfu/mL and <100000cfu/mL for TVC and ≥400000cells/mL and <400000cells/mL for SCC. The proportions of TVC and SCC groups presented as percentages at the beginning of the education (pre-education) and at the end of the education (post-education) of the staff. The McNemarchi-square test was used to compare these proportions between pre-education and post-education of the staff. $P<0.05$ was considered to show a statistically difference.

RESULTS

Survey Results

Cronbach's Alpha reliability values determined as a result of the reliability test on the first and second survey data collected within the scope of the study were calculated as 0.872 and 0.895, respectively. These values are above the absolute limit values suggested by Nunnally (1978), so the answers to the surveys can be said to be reliable. General survey descriptive results presented on Table 2. Majority (68%) of the survey participants was graduated from elementary school, while 25.3% graduated from secondary school and the others (4%) graduated from higher schools. Survey participants mentioned three main subjects about farm types as family farms, individual private farms and partnership private farms. Proportion of these farms were 60.7, 36.0 and 3.3%, respectively. Participant farms had six different types of barns as open air (40.0%), open air free stall (0.7%), semi-open (32.7%), semi-open free stall (23.3%), closed (0.7%) and closed free stall (2.7%). Using portable milking machine proportion was 83.3% while using fixed milking machine was 14.0% and by hand milking was 2.7%.

Table 1: Total number of samples distribution by districts

District of the farm	Farm Number	1st Sampling	2nd Sampling
Çine	35	105	105
Efeler	25	75	75
Germencik	7	21	21
Karpuzlu	26	78	78
Koçarlı	29	87	87
Söke	5	15	15
Kuyucak	14	42	42
Nazilli	16	48	48
Total number of samples analyzed		471	471

The Likert scale averages of the survey before milking hygiene education were varied between 2.16 (teat dipping was done before each milking) and 4.44 (the milking process was carried out on a regular basis). Survey items answer averages and interpretations were shown in Table 3.

Training satisfaction survey was done for 274 participants. The distribution of the participants according to the Çine, Efeler, Germencik, Karpuzlu, Koçarlı and Söke districts were 27.7, 36.5, 4.0, 18.2, 9.5, and 4.0%, respectively. Majority of the participants (94.9%) were male, while 5.1% were female. Educational status of the participants distributed as graduated from elementary school (76.3%), graduated from secondary school (20.4%) and graduated from higher school (3.3%). One-fourth (24.1%) of the participants had education before the milk hygiene education course and rest of them (75.9%) did not have any hygiene education.

The Likert scale averages of the training satisfaction survey after milking hygiene education were varied between 4.31 (the duration of the training was sufficient) and 4.70 (educators had enough communication with the participants). Survey items answer averages and interpretations were shown in Table 4.

Microbiological Results

The results of the microbiological analysis (TVC) and SCC of milk samples before and after hygiene training were given in Table 5. The data obtained according to TVC norm values for some of the farms constituting the research material were shown in Table 6. As seen in Table 6, 143 farms were found above 100000cfu/mL norms before milk hygiene education and the TVC value of 12 of the farms dropped to 100000cfu/mL after the training. At the same time, while 14 farms with <100000cfu/mL norm values before education, 9 of them exceeded 100000cfu/mL after hygiene education. In total, 17 (11%) of samples were below 100000cfu/mL norm values after the education. There were no significant difference between pre and post education TVC ratios ($P>0.05$)

The data regarding to the SCC values obtained from the farms were shown in Table 7.

As shown in Table 7, 56 farms with a norm value of ≥400000cells/mL in this study showed SCC values below 400000cells/mL in 27 farms after hygiene training, whereas 30 of 101 farms with a value of <400000cells/mL were detected above 400000cells/mL norm value. There were no statistical significant difference found between pre and post education SCC percentage changes ($P>0.05$). The distribution of TVC values obtained from districts after hygiene education were shown on Table 8. When the distribution according to the districts was examined, TVC values decreased by 65 (41.4%) indices of 157 farm milk samples.

Table 2: Survey descriptive results of the sample farms before milking hygiene education

	N	Mean±SE
Average Milk Production per cow (kg)	144	20.40±0.41
Age of the Employee	148	45.71±0.97
the number of people in the family	141	3.79±0.12
Employee number working in milking parlor	150	1.53±0.04
Milking Parlor Capacity (head/cow)	38	5.37±0.80

Table 3: Item Statistics (Before Milking Hygiene Education, n: 150)

	Mean±SD	Interpretation
Barn cleaning is done on a regular basis	3.94±0.84	Agree
The farm is well usefulness	4.03±0.93	Agree
Barn ventilation is good	4.22±0.87	strongly agree
Barn lighting is good	4.09±0.96	Agree
The cleanliness of the cows is good	4.00±0.67	Agree
The feeding of the cows are regularly held	4.31±0.78	strongly agree
water quality is given to the cows	4.09±0.99	Agree
Manure is cleaned every day	3.19±1.42	have no idea
Cow density is sufficient	4.22±0.83	strongly agree
Milking parlor cleaning is done every day	4.19±0.96	Agree
Milking parlor usability is good	3.97±1.25	Agree
Milking parlor ventilation is good	4.13±0.97	Agree
Milking parlor lightning is good	4.00±1.04	Agree
The milking process is carried out on a regular basis	4.44±0.56	strongly agree
Milking rules are applied to the milking parlor	4.25±0.71	strongly agree
Parlor employees comply with the rules of hygiene	4.06±0.75	Agree
udder and teats are controlled before each milking	4.13±1.12	Agree
Each cow's teats are cleaned before each milking	4.09±1.14	Agree
Teat dipping is done before each milking	2.16±1.46	Disagree
Teat dipping is done after each milking	2.41±1.58	Disagree
Cows udder dried after each cleaning	2.31±1.53	Disagree
Udder is checked after milking	3.97±1.44	Agree

Table 4: Item Statistics (After Milking Hygiene Education)

	Mean±SD
The duration of the training was sufficient	4.31±0.84*
Education content was adequate	4.39±0.72*
Topics were clear and understandable	4.51±0.62*
The training material was sufficient	4.40±0.76*
Explanation for purpose was made	4.55±0.69*
Subjects presented with appropriate methods	4.58±0.62*
Active participation enabled	4.49±0.67*
Educators had enough communication with the participants	4.70±0.51*
Education given with samples	4.60±0.59*
Education contributed professional development positively	4.46±0.70*
Education gave new knowledge	4.41±0.75*
Education gave practical information	4.38±0.75*
Gained information will be useful	4.49±0.73*
Theoretical knowledge applied	4.68±0.54*
Examples used in training were useful	4.55±0.58*

*All parameters strongly agree.

The distribution of SCC values obtained from districts after hygiene education were shown on Table 9. When the distribution according to the provinces was examined, it was found that the SCC values of 79 (50.3%) of 157 farm milk samples decreased.

DISCUSSION

Due to its complex biochemical structure and high-water capacity, raw milk is an excellent medium for microorganisms. Some of these microorganisms, mainly total viable mesophilic microorganisms, are indicators in determining the hygienic properties, both in the protection of milk quality and in the process from raw milk production to consumption (Üzüm 2006).

The acceptable numbers of SCC in the raw milk have been determined by the relevant authorities in EU, and Health and Hygiene Directive (92/46/EEC) requires raw milk SCC should not exceed 400000cell/mL (Europa 1992). According to the Turkish Food Codex (2009) on Raw Milk and HeatTreated Milks, raw cow milk should contain less than 100000cfu/mL TVC and less than 400000cell/mL SCC.

Mastitis is one of the most important problems of herd management. Determination of mastitis status of herd is carried out via somatic cell count taken from the tank milk. If tank milk SCC is low, but there are a lot of clinical cases, it is concluded that the problem is environmental, whereas if SCC is high but the number of clinical cases is low, it is concluded that the problem is contagious (Mingala et al. 2020).

The high level of SCC in mastitic milk cause changes in milk enzymes and results in the breakdown of proteins and fats. One of these enzymes, plasmin, increases the breakdown of casein due to the increase in SCC which reduces casein level in the milk. This disruption begins with SCC rising above 100000cells/ml. Depending on the changes in the milk composition in, the quality of the cheese cloth reduces during the production of cheese. Increased activity of proteolytic enzymes in high somatic cell reduces the amount of product and causes impaired aroma (Kirk 2005; Elbayoumy et al. 2020).

In this study, it was determined that the mean TVC and SCC values obtained before the milking hygiene training were above the norm values. As a result of the milking hygiene training given to the farm workers, the laboratory analyses showed that the average TVC and SCC values decreased. 143 farms were found above 100000cfu/mL, and after the milk hygiene training, the TVC value of 12 farms was lowered to 100000cfu/mL. It is also observed that 14 farms with norm values of <100000cfu/mL exceeded higher than 100000cfu/mL norm values after training. In conclusion, 17 (11%) of tank milk samples were below the norm value of 100000cfu/mL out of 157 farms. 27 farms reduced their SCC values below 400000cells/mL out of 56 farms with a norm value of ≥400000cells/mL after hygiene training, whereas 30 of 101 farms with a value of <400000cells/mL exceeded 400000cells/mL. When overall results were evaluated, hygiene was improved by 11% in TVC value and 1% in SCC value after training.

Table 5: The descriptive statistical data of the mean values

	N	Min	Max	Mean±SE
Pre-Education TVC Value (cfu/mL)	157	11000	6305000	1067528.02±96685.59
Post-Education TVC Value (cfu/mL)	157	14000	11089333	946026.00±104772.47
Pre-Education SCC Value (cell/mL)	157	10000	1826333	401512.84±29828.83
Post-Education SCC Value (cell/mL)	157	10000	2000000	397728.23±30922.27

Table 6: TVC Cross tabulation [n (%)]

		Post-education		
		≥100000cfu/mL	<100000cfu/mL	Total
Pre-education	≥100000cfu/mL	131 (91.6)	12 (8.4)	143 (100.0)
	<100000cfu/mL	9 (64.3)	5 (35.7)	14 (100.0)
Total		140 (89.2)	17 (10.8)	157 (100.0)

Table 7: SCC Cross tabulation [n (%)]

		Post-education		
		≥400000cell/mL	<400000cell/mL	Total
Pre-education	≥400000cell/mL	29 (51.8)	27 (48.2)	56 (100.0)
	<400000cell/mL	30 (29.7)	71 (70.3)	101 (100.0)
Total		59 (37.6)	98 (62.4)	157 (100.0)

Table 8: Number of farms about TVC changing after education according to the districts [n (%)]

District	Increased or not changed	Decreased	Total
Çine	26 (76.5)	8 (23.5)	34 (100.0)
Efeler	16 (64.0)	9 (36.0)	25 (100.0)
Germencik	4 (57.1)	3 (42.9)	7 (100.0)
Karpuzlu	14 (53.8)	12 (46.2)	26 (100.0)
Koçarlı	19 (63.3)	11 (36.7)	30 (100.0)
Kuyucak	6 (42.9)	8 (57.1)	14 (100.0)
Nazilli	6 (37.5)	10 (62.5)	16 (100.0)
Söke	1 (20.0)	4 (80.0)	5 (100.0)
TOTAL	92 (58.6)	65 (41.4)	157 (100.0)

Table 9: Number of farms about SCC changing after education according to the districts [n (%)]

District	Increased or not changed	Decreased	Total
Çine	21 (61.8)	13 (38.2)	34 (100.0)
Efeler	17 (68.0)	8 (32.0)	25 (100.0)
Germencik	2 (28.6)	5 (71.4)	7 (100.0)
Karpuzlu	14 (53.8)	12 (46.2)	26 (100.0)
Koçarlı	11 (36.7)	19 (63.3)	30 (100.0)
Kuyucak	7 (50.0)	7 (50.0)	14 (100.0)
Nazilli	5 (31.3)	11 (68.8)	16 (100.0)
Söke	1 (20.0)	4 (80.0)	5 (100.0)
TOTAL	78 (49.7)	79 (50.3)	157 (100.0)

Values in parenthesis indicate %.

In a previous study, 6.04logcells/mL; 6.60logcells/mL; 6.09logcells/mL; 6.25logcells/mL and 6.83logcells/mL SCC were determined in Elazığ, Samsun, Malatya, Şanlıurfa and Erzurum provinces. All the analyzed samples showed higher value than 100000cells/mL. 11 of the samples (2.5%) were between 100000-500000cells/mL, 139 of them (31.6%) were between 500000-1000000cells/mL and 290 of them (65.9%) had SCC value more than 100000cells/mL (Patır et al. 2010).

Akın et al. (2016) reported that TVC in farm milk was 5.24-5.74logcfu/mL and that TVC in collectors' milk was 6.45-7.01logcfu/mL. Diler and Baran (2014) found that TVC of tank milk were between 2.8 and 6.8logcfu/mL and 36.7% of the samples met the criteria stated in the Turkish Food Codex (2009).

To be able to process high quality milk and dairy products, the hygienic quality of the processing steps (animal health and care, hygiene in stall, udder cleaning, cleaning and disinfection of milking machines, equipment etc.) should be improved. In this manner, milking hygiene trainings for farm workers are of great importance. Naing et al. (2018) stated that in order to improve milk quality, farmers should be trained properly.

In this study a total of 1400 farm workers were trained in milking hygiene by specialist trainers, then educational evaluation surveys were conducted. Milk samples were also collected from the farms where the training was conducted, and TVC and SCC values were determined. As results showed that TVC values decreased in 65 (41.4%) farms and SCC values decreased in 79 (50.3%) farms out of 157 farms. It was concluded that the training was effective on the basis of the districts and envisaged hygiene procedures should be implemented to ensure the sustainability of hygiene in farms. A study conducted by Mu´nera-Bedoya et al. (2017), aimed to find out how human behaviors affect the milk process also concluded that SCC was associated to knowledge of animal handling, training of milkers, and milking site.

Conclusion

Milk quality is an important issue in the commercial area as the farmer who produces high quality milk sells it with higher price and gains more profit. While the factories produce high quality dairy products using high quality milk, the demand of consumer to these products increases. Therefore, increasing milking hygiene by improving the farmers' knowledge and changing their attitudes by training would be crucial for healthy milk and dairy products.

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