

CHAPTER III

MATERIALS AND METHODS

This research was divided into three parts which study in eleven milk collecting centers of six dairy cooperatives in Chiang Mai Province and Lamphun Province;

- 1) Chiang Mai dairy cooperative: five milk collecting centers
 - 1.1) Milk collecting center of member in Sarapee District
 - 1.2) Milk collecting center of member in Sanpahtong District
 - 1.3) Milk collecting center of member in Sankhampang District
 - 1.4) Milk collecting center of member in Sansai District
 - 1.5) Milk collecting center of member in Banthi District
- 2) Pahtung-Huaymo dairy cooperative: one milk collecting center
- 3) Maejo dairy cooperative: one milk collecting center
- 4) Chaiprakarn dairy cooperative: one milk collecting center
- 5) Mae-on dairy cooperative: one milk collecting center
- 6) Lamphun dairy cooperative: two milk collecting centers
 - 6.1) Milk collecting center of member in Banthi District, Maeta District and Muang District
 - 6.2) Milk collecting center of member in Banhong District

Study A: Finding the herd level factors affecting the raw milk quality.

Study design. This study is a case-control study.

Sample size. According to Schlesselman (1982), the formula for the needed sample size is:

$$n = \frac{\left(Z(a) \cdot \sqrt{\left(1 + \frac{1}{c}\right) \cdot p_m \cdot (1 - p_m)} + Z(b) \cdot \sqrt{p_1 \cdot (1 - p_1) + \frac{p_0 \cdot (1 - p_0)}{c}} \right)^2}{(p_1 - p_0)^2}$$

where:

$$p_1 = \frac{p_0 \cdot OR}{1 + p_0 \cdot (OR - 1)} \quad p_m = \frac{p_1 + c \cdot p_0}{1 + c}$$

Z(a) = the value of Student's t at the specified confidence level

Z(b) = the value of Student's t (2-tailed) at the specified power

p₀ = proportion exposed in Control group

p₁ = calculated proportion exposed in Case group

p_m = proportion exposure in the population

1-p_m = proportion non-exposure in the population

c = number of controls per case

OR = estimated Odds Ratio

The total sample size for this study is to be at least 96 farms when;

Z(a) = 1.96 (if confidence level is 95% or a = 0.05)

Z(b) = 0.842 (if confidence level is 80% or b = 0.20)

p₀ = 0.10 (10%)

p₁ = 0.33 (33.33%)

p_m = 0.215

1-p_m = 0.785

c = 1

OR = 4.5 (This odd ratio is on the assumption of $p_0 = 0.10$ and $p_1 = 0.33$ for every investigated factors.)

Method. The raw milk samples from 11 milk collecting centers' bulk tanks (MCC) of 6 dairy cooperatives are collected to find the bacterial counts in the form of the SPC every week for 2 months. Before calculation, the SPC values were transformed to the \log_{10} SPC. MCC are divided into 2 groups by the median value of \log_{10} SPC; Group1: the \log_{10} SPC are less than or equal the median value and Group2: the \log_{10} SPC are more than the median value. Two MCCs from each group are randomly sampling to sampling the farm member. The farm members of each MCC are grouped by the average daily milk production (kg / day) in to 4 groups. The farm members of each MCC are randomly 20% sampling of each milk production's group (stratified random sampling). Then, the questionnaires (adapted from Ajariyakhajorn, *et al*, 2000) are taken by interviewing about the farm characteristics (such as the number of years in dairy farming, the average daily milk production and the mastitis history in the farm etc.) and observing about the milking characteristics (such as the barn's environment, the milking cow's cleanliness and the udder preparation etc.) in order to identify the factors.

Statistical Analysis. Both farm characteristic data and milking characteristic data were analyzed. The bivariable association of the hypothesized variable and the likelihood of high \log_{10} SPC was evaluated at $P = 0.1$ in logistic regression multivariable model. All risk factors that were significant in the bivariable analysis were considered in the multivariable logistic regression analysis. The conditional significance of each of the factor with the odds of high \log_{10} SPC was assessed using a

backward elimination procedure at $P = 0.05$. Factors that resulted in a significant change of the partial likelihood ratio with an associated $P = 0.05$ were allowed to stay in the model. To account for the possible changes in the relationship between correlated variables within a model, the changes in the partial likelihood value (Wald's test) between a saturated and a reduced model was monitored. Biologically, plausible two-way interactions between factors in the final model also were assessed. The model fit statistics were assessed using the Akaike Information Criterion (AIC), the Schwarz Criterion (SC), and the negative of twice the log likelihood ($-2 \log L$) for the intercept-only model and the fitted model. The 95% odds ratio (OR) and the attributable proportion for each variable in the final model were calculated. All analyses were performed using SAS statistical software (SAS Institute, 1999).

Study B: Survey of raw milk quality.

Study design. This study is a cross-sectional study.

Sample size. Eleven milk collecting centers from six dairy cooperatives in Chiang Mai Province and Lamphun Province are studied for this study.

Method. The milk sample (200 ml) of each MCC's tank is collected 1-2 times per month for 12 months. It is measured in 4 categories of milk quality; microbiological quality, antibiotic residues, milk composition, and somatic cell count. All milk samples are stored at about 4-7 °C for a maximum of 3 hours in a cooler box before analysis.

Microbiological quality

Milk samples are tested for microbiological quality by direct method and indirect method. The direct method is defined as the standard plate count, the coliform count and the laboratory pasteurization count. The indirect method is defined as the methylene blue reduction test.

Standard plate count

Each milk sample is diluted in sterile phosphate buffer saline (PBS). Dilutions of 10^{-3} , 10^{-4} , 10^{-5} are conducted for this method. One milliliter of each dilution is mixed with the plate count agar (PCA) by poured-plating in duplicate. All SPC-plates are incubated at $32^{\circ} \pm 1^{\circ}\text{C}$ for 48 ± 3 hours. Plates with 25-250 viable colonies are enumerated. The used dilution and the total number of counted colonies are recorded (Haughtby, et al, 1992).

Coliform count

Each milk sample is diluted in sterile phosphate buffer saline (PBS). Dilutions of 10^{-2} , 10^{-3} , 10^{-4} are conducted for this method. One milliliter of each dilution is mixed with the violet red bile agar (VRBA) by poured-plating in duplicate. All CC-plates are incubated at $32^{\circ} \pm 1^{\circ}\text{C}$ for 24 ± 2 hours. Plates with 15-150 dark red colonies which are ≥ 0.5 mm in diameter are selected. The used dilution and the total number of counted colonies are recorded (Christen, et al, 1992).

Laboratory pasteurization count

Each milk sample is mixed thoroughly and aseptically transferred 5 ml of the milk sample to a sterile test tube. These tubes are placed in the water bath at 62.8°C for 30 minute (low-temperature, long-temperature pasteurization: LTLT). The total

bacterial count of the pasteurized sample is detected as same technique as SPC with different selected dilutions: 10^{-1} , 10^{-2} , 10^{-3} (Frank, *et al*, 1992).

Methylene blue reduction test

Ten ml of each milk sample is mixed with 1 ml of the standard methylene blue solution. All sample tubes are placed in the water bath at 37°C. The duration of the changed color of the milk sample were recorded every 30 min (Luck, 1991).

Milk composition

Milk samples are preheated to 40°C in a water bath (for not more than 20 min) before analysis to melt the fat. The milk composition of milk samples are measured by Milko scan 133B to define as the percentages of milk fat (fat B), protein, lactose, total solid and solid not fat (Bradly, *et al*, 1992).

Antibiotic residues

Milk samples are tested for antimicrobial residues using the test kit of the Department of Medical Science, Ministry of Public Health, Thailand. This test kit is based on the principle of the microbial inhibition technique. Milk samples are preheated to 82°C for 2 min to denature some non-specific natural microbial inhibitors in raw milk. The method is followed the product prescription. When a sample was positive, this sample would be retest to confirm the positive result.

Somatic cell count

Milk samples are preheated to 40°C in a water bath (for not more than 20 min) and mixed thoroughly before analysis. Milk samples are measured the somatic cell count using the automated Foss electronic fluorescent dye method (Fossomatic 300) (Hing, *et al*, 1992).

Statistical Analysis. Descriptive procedures for continuous data included calculation of quartiles; categorized data were summarized as contingency tables. The relationship between months and MCC for SPC, CC, LPC, SCC, Fat, Protein, Lactose, TS and SNF was evaluated using Duncan's procedure and one-way ANOVA. The \log_{10} of SPC, CC and LPC values were used in all calculations. Probability values less than 0.05 were considered statistically significant. All analyses were performed using SAS statistical software (SAS Institute, 1999).

Study C: Survey of the Milk Collecting Center characteristic.

Study design. This study is a cross-sectional study.

Sample size. Eleven milk collecting centers from 6 dairy cooperatives in Chiang Mai Province and Lamphun Province are studied for this study.

Method. All of the milk collecting centers (11 MCC) are interviewed by questionnaire and observation. The MCCs were divided by the same criteria in study A in order to compare the data.

Statistical Analysis. Descriptive procedures for continuous data included calculation of quartiles; categorized data were summarized as contingency tables. The differences of the average of all continuous data between group 1 and group 2 were analyzed by Student's T-test. The median of ratio of total capacity of cooling tanks: total milk weight of each of the MCC was calculated for grouping the MCCs. The relationship between the group of SPC and group of this ratio was analyzed by Fisher exact test. All analyses were performed using SAS statistical software (SAS Institute, 1999).