CHAPTER III

MATERIALS AND METHODS

3.1 Study area and period

The study was performed on May 2013 and the study population included dairy cattle farms in Chiang Mai Province. Chiang Mai involves livestock administrative region 5 and called northern part of Thailand. Chiang Mai Province is situated on the Mae Ping River basin and is 300 m above sea level, surrounded by the high mountain ranges of the Thai highlands. It covers an area of approximately 20,107 km². The highest mountain of Thailand, the 2,565 meter high Doi Inthanon, is located in the province. Chiang Mai is subdivided into 25 districts (*Amphoe*). The districts are further subdivided into 204 sub-districts (*Tambon*) and 1915 villages (*Muban*). The cross sectional study was carried out in Mae Wang district in Chiang Mai Province. This area is located between 18° 47 ´43´´ N and 98°59 ´55´´E.

Figure 3: Mae Wang District Map

3.2 Study design

The study was designed as a cross-sectional study to determine the disease status of bovine tuberculosis in dairy cattle from Mae Wang district of Chiang Mai Province. It was conducted on May, 2013. Dairy cattle were tested with comparative intradermal tuberculin test and compared cell-mediated immune responses at bovine injection site and avian injection site. To compare cell-mediated immune responses for both PPDs, skin fold thicknesses were measured before injection and 72 hours after injection.

The interpretation was based on OIE manual for bovine PPD injection (caudal region). According to OIE guideline, the reaction was considered to be negative if the increase in skin-fold thickness was less than 2mm at injection site. The reaction was considered inconclusive if the increase in skin-fold thickness was 2mm-4mm. The positive reaction was considered if there is 4mm and more skin-fold thickness (OIE, 2009).

Farm owners were asked by questionnaires to assess the risk factors for bTB status in study area. The structure of the questionnaires included general information such as educational status of the farmers, farm experience, breed of the dairy cattle, management and farming system, vaccination and de-worming, purchasing of animals, quarantine practice, bTB knowledge, TB experience and biosecurity status of their farms.

In addition, global positioning system (GPS) instrument was used to describe the locations of tuberculin tested dairy farms on the Mae Wang district map.

3.3 Sample size

3.3.1 Population frame

Population frame of this study was based on cattle population in Chiang Mai (2010-2011) from DLD database.

3.3.2 Study Population

Dairy cattle population from Mae Wang district was subjected to conduct tuberculin test. Most of the dairy cattle in Mae Wang district are Holstein Friesian crossed-breed. The farming system of dairy farms in Mae Wang is open house farming system.

Figure 4: Breed of dairy cattle and farming system in Mae Wang

3.3.3 Sample size calculation

The sample size for tuberculin test was calculated using Win Episcope 2.0. It was assumed that expected prevalence P = 4% (DLD official data), 95% confidence intervals.

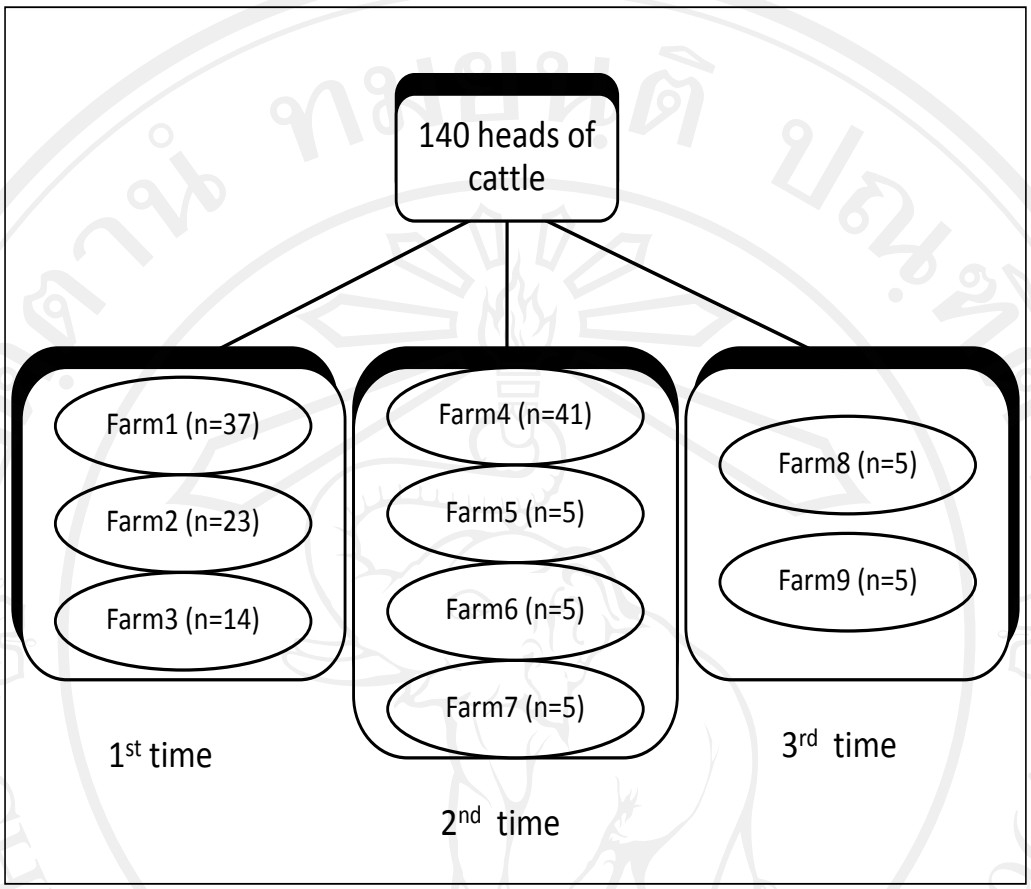

Figure 5: Sample size distribution

3.4 Sample selection

140 cows from nine dairy farms were injected with bovine and avian PPDs. Dairy cattle farms were selected due to geographic distribution and included after agreement by the farmers.

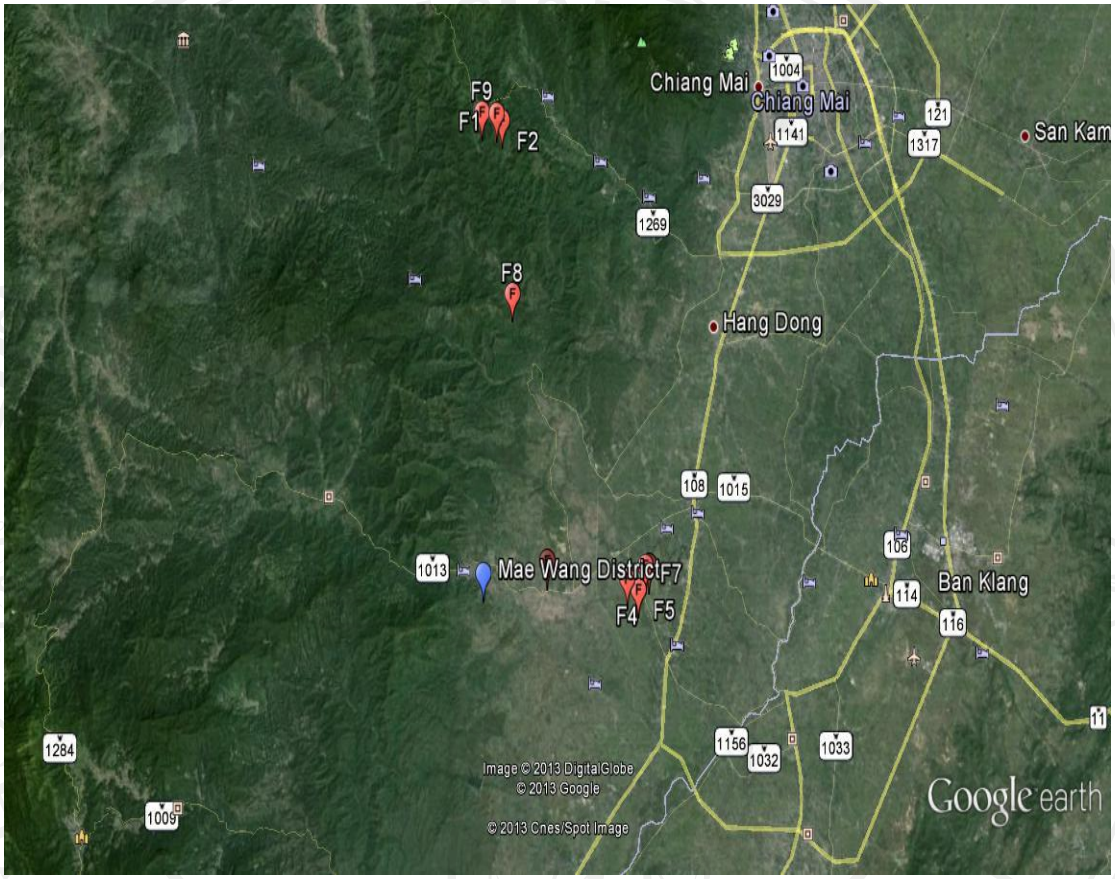

Figure 6: Locations of tuberculin tested dairy cattle farms in Mae Wang District (Chiang Mai Province)

3.5 Tuberculin skin test procedure

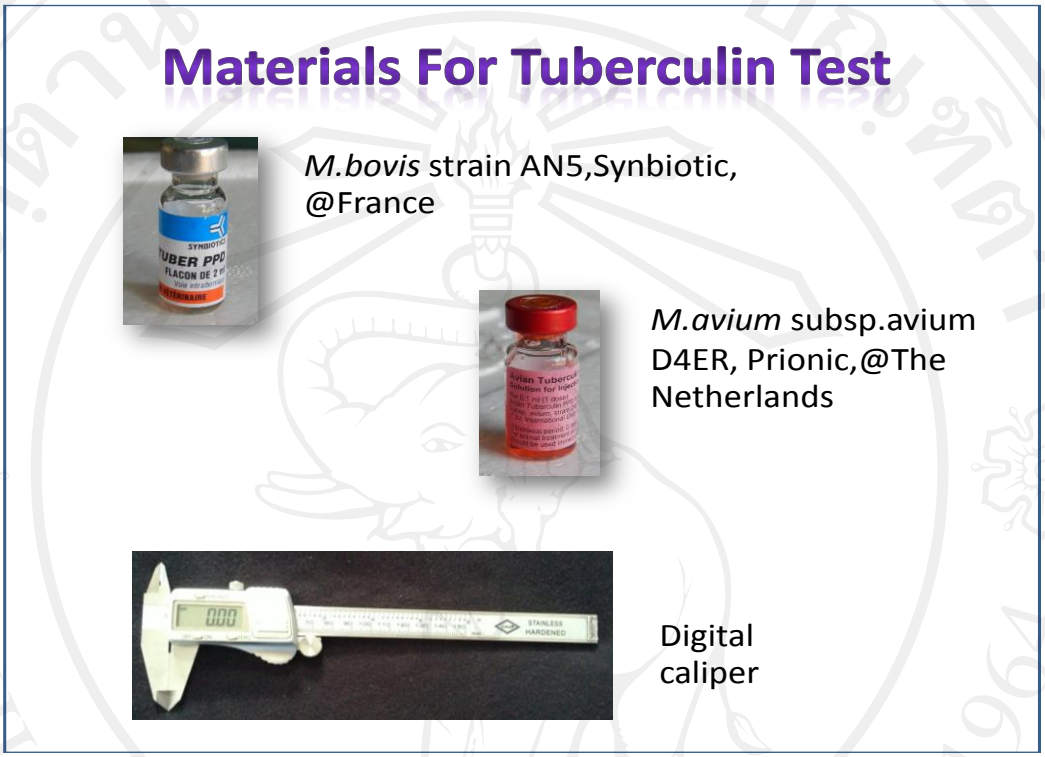

Figure 7: Materials used in tuberculin testing

Dairy cattle farms in Mae Wang District are under the control of Mae Wang Dairy Cooperation Office. Dairy cows were implemented with comparative intradermal tuberculin test including bovine and avian PPDs. In our study, the injection site for bovine PPD was caudal region and avian PPD was injected at cervical region. All of 140 cows were injected with bovine PPD but avian PPD was implemented in 138 cows. To avoid confounding effects, the inclusion criteria for tuberculin tested cows were:

(a) animals > 6 months of age

(b) clinically healthy animals

(c) cow 1 month before and after parturition (Proano-Perez et al., 2009).

1. At caudal region, 0.1ml bovine PPD was injected intradermally. The potency of bovine tuberculin PPD is 20,000 IU/ml (*M.bovis* strain AN5, Synbiotic, @

France) and containing 20 doses per vial. Right site of caudal fold was chosen to inject bovine PPD to be uniformity. The confirmation of corrected injection was formation of pea-like swelling at injection site. Skin thickness of the caudal fold was measured with digital caliper and recorded before injection.

2. At cervical area, 0.1ml of avian PPD from *M.avium subsp.avium* strain D4ER (Prionics, @the Netherlands). The potency of avian PPD is 25,000 IU/ml containing 20 doses per vial. Before avian PPD injection, the cervical area was clipped, clean and shaved about $2cm^2$. Skin thicknesses were measured before the injection and recorded.
3. After 72 hours, researchers visited to tested farms and checked the responses of PPDs at both injection sites and skin swellings were measured again with the digital caliper. Measurements were carried out by the same researcher to avoid manual pressure.
4. Interpretation of positive reactor was followed by OIE guideline depending on the response at bovine injection site. The negative reaction was determined if there was no visible swelling or less than 2mm increase in skin-fold thickness at injection site. The reaction was considered inconclusive if the increase in skin-fold thickness was more than 2mm and less than 4mm. The reaction was considered to be positive if measurement of skin thickness was greater than or equal to 4mm difference.

3.6 Assessment of risk factors for bTB status

To determine the possible risk factors associated with bovine tuberculosis, cattle owners were asked by the questionnaires. In this study, 46 farmers were asked by questionnaires. 9 of 46 farmers or cattle owners answered the questionnaire forms on the same day that their cattle were tested with bovine and avian PPDs. The remaining 37 farmers were invited to Mae Wang Dairy Cooperation Office to answer the questionnaires forms.

Questionnaires were involved on general information of the farm, livestock husbandry, household characteristics such as herd sizes, structure, presence of other

livestock, vaccination/ de-worming of cattle, watering and grazing system, cattle contact with other cattle herds and purchasing of cattle. In addition, questions related to human consumption habits, contact between human and cattle, knowledge of bTB and known TB status in the household were also be asked. Thai version questionnaire forms were used in this study so that the farmers were convenient to answer.

3.7 Analysis method

All of the data were entered Microsoft excel 2007 program and R studio software was used to determine statistical difference between skin-fold thickness measurements. To assess the possible risk factors, questionnaires answers were manipulated using Microsoft excel 2007.