Chapter 3

Materials and methods

This chapter explains the methodology of the study. It consists of the research design, study location, target population, sampling methods and sample size, methods of data collection and data analysis.

3.1 Research design

The main objective of this study was identifying risk or protective factors of FMD in pig and cattle farms in Chiang Mai and Lamphun area. The cross-sectional study was used to describe the farm management, FMD occurrence and the FMD preventive practices. It was cross-sectional study as the data were collected at one point of time (during 2004-2006). The farm management; consist of biosecurity and sanitation procedure, number and type of animals, disease occurrence, farm animal movement, FMD control and prevention strategies included the potential risk factors of FMD outbreak, was identified as the factors. These factors were compared between FMD outbreaks farms and non-FMD outbreak farms. These factor data and history of FMD outbreak during 2003-2005 were simultaneously collected.

In cattle farms, the cross-sectional study was performed to compare between the farm management in FMD outbreaks farms and non-FMD outbreaks farms. In pig farms, because of the number of positive farm was small, the case-control study was undertaken to compare between the farm management in FMD outbreaks farms and non-FMD outbreaks farms. The case was pig farms that has FMD outbreak in

previous year, while the control was all pig farms in the same sub-district (Tambon) of the pig outbreak farm.

3.2 Study location

Selected area was chosen from Chiang Mai and Lamphun provinces. The study area was a boundary surrounded with mountains including twenty-three districts (Amphoe) in Chiang Mai and Lamphun provinces out of total 32 districts (Figure 1 and Table 1).

Chiang Mai and Lamphun provinces were the main animal production area in the Northern Thailand (Department of Livestock Development, 2005). The commercial companies had base offices in Chiang Mai province, for support the contacted farmers in both provinces. The contacted farms were pig and poultry farms. Moreover, in these provinces had more than 6 dairy co-operations and the beef cattle farmers located over the area. This area had being the center of the animal trading. It had the live animal markets and the merchants brought the animal, particularly cattle, to the local market for sell and buy new animal back to their places. The live animal markets opened on Saturday or Sunday.

Thus, this selected area should be a representative of the animal production information, and identified risk factors of FMD outbreak. These information would be supported the government's animal health management and the improvement of FMD prevention in Northern Thailand.

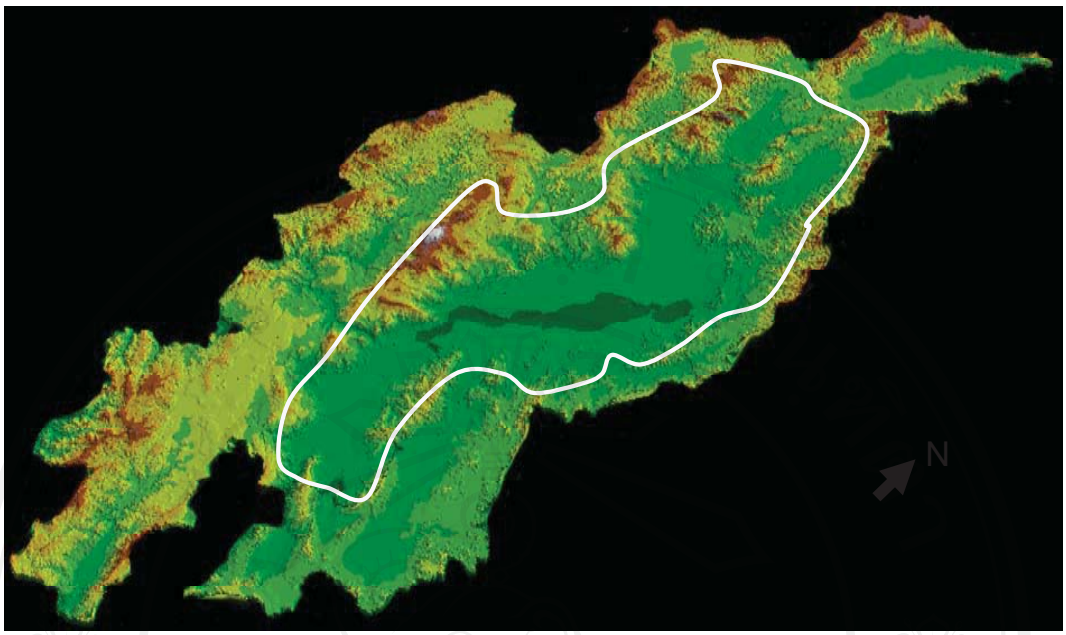

Figure 3.1 Show the geographical characteristic of the study area

Table 3.1 Name of studied Amphoe which locate in Chiang Mai and Lamphun provinces.

No.	Chiang Mai province	Lamphun province
1	Mueang	Mueang
2	Jom Thong	Pa Sang
3	Chiang Doa	Mae Tha
4	Doi Saket	Ban Hong
5	Doi Tao	Ban Thi
6	Proaw	Wiang Nong Long
7	Mae Tang	
8	Mae Rim	
9	Mae Wang	
10	Saraphee	
11	San Kamphang	
12	San Sai	
13	San Patong	
14	Hang Dong	
15	Hod	
16	Mae-On	
17	Doi Lor	

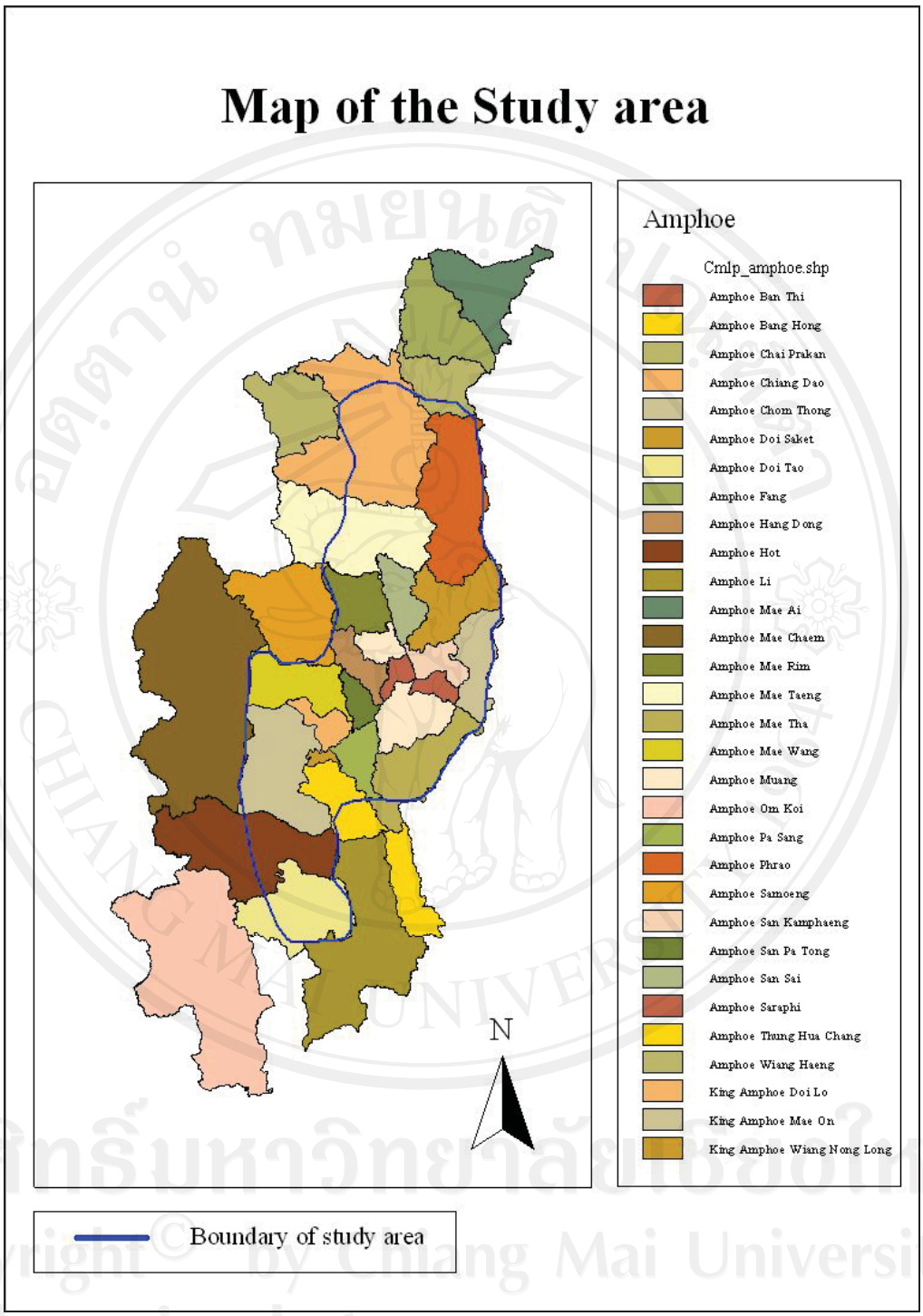

Figure 3.2 Show Map of Amphoe which locate in Chiang Mai and Lamphun provinces.

3.3 Population and sample

The Population of this study was pig farms and cattle (dairy cow, beef cow and buffalo) farms located in Chiang Mai and Lamphun provinces. Chiang Mai province has 17,912 pig farms and 222,457 cattle farms. Lamphun province had 1,869 pig farms and 36,785 cattle farms (Department of Livestock Development, 2005).

The inclusion criterias for the sample recruitment were as follow:

1) Raising pig for sell/cattle for sell or working or milking.
2) Located in Chiang Mai and Lamphun provinces.
3) The farmers willing to participate in this study.

This study included all Amphoe of Chiang Mai and Lamphun provinces in the area which surrounded with mountains. The Tambon, the sub-unit of Amphoe, and the villages, the smallest unit of community in Thailand, which had pig and cattle farms, were included in study area. Then, the random sampling was used to select the sample pig and cattle farms. The total of 899 pig farms and 3,464 cattle farms, which located in Chiang Mai and Lamphun provinces, were studied.

The pig farms were also divided in 4 classes according DLD standard farm practice; farm have less than 50 pigs, 50-500 pigs, 500-5000 pigs and more than 5,000 pigs is called “individual farm”, “small farm”, “medium farm” and “large farm”, the cattle farms were divided in 4 classes according DLD standard farm practice; farm have less than 5 cattle, 5-19 cattle, 20-99 cattle and more than 100 cattle is called “individual farm”, “small farm”, “medium farm” and “large farm”, respectively., respectively.

3.4 Data collection

All data were collected by using the interview form and standardized protocol. The form for pig farmers had a few different from the cattle form. The 6 collectors were trained for the using the interview form and using the protocol as the guideline during collected the data. Before the collection period, all collectors were also tested by collected questionnaires from some samples in the study area. After that the interview form and the protocol were evaluated and adjusted. The data collection was started from June 2006 and was finished in July 2007.

The interview form were designed to collect data concerning general management information of farm, biosecurity and sanitation procedure of farm, number and type of animals, disease occurrence, farm animal movement, FMD control and prevention strategies, history of FMD outbreak during the previous year including management when FMD occurred. Other topics in the interview form include risk factors and preventive factors of FMD of each farm. The interview form was composed of 4 parts:

Part 1: This part is general information of the farm. The questions concerning farm's name, farm's address, farm owner's name, number of animals, number of animal houses, volume of productivities and number of employee.

Part 2: This part is history of FMD outbreak of the farm. The questions concerning number of the outbreak in last year, onset of the outbreak, number of sick animals, number of death animals, number of culling animals and management during FMD occurred.

Part 3: This part is FMD control and prevention strategies of the farm. The questions concerning FMD vaccination program, type of the vaccine, source of the

vaccine, vaccinator, vaccine storage and management when neighboring farm had the outbreak.

Part 4: This part is risk factor of FMD outbreak of the farm. The questions concerning type and pattern of farm, farm management, source of animals, source of feed, source of water, vehicle and personal control and disease control and prevention strategies.

All data were entered into the microcomputer using Microsoft Excel®.

3.5 Geographic Information System (GIS) data collection

Geographic Information System or GIS will be a tool is used in this study to locate the farms, slaughter houses, live animal markets, street, canal, river, and village. One point of cattle or pig farm means one farmer, one point of slaughter house means one owner, one point of live animal market means one market. All GIS data will be collected with Global positioning system (GPS) receiver and be entered into the microcomputer using Arcview® computer program.

3.6 Statistical analysis

The data were entered into the microcomputer using Microsoft Excel®. One question was defined as one variable. The variables were both continuous and category variables. Statistical analyses were undertaken using statistical microcomputer software, SAS®.

The descriptive statistic, frequencies and percentage were used to assess general management information of farm, biosecurity and sanitation procedure of farm, animals, disease occurrence and management when FMD occurred.

Fisher exact test, Mann–Whitney U test and Multivariable logistic regression were used to analyze risk factors and preventive factors of FMD on the history of FMD outbreak.

Fisher exact test was used to determine the associations between two categorical variables. Those were independent and dependent variables. This study, the independent variables were the potential risk factor of FMD, which were categorical variables, and the dependent variable was the occurrence of FMD in the previous year. The purpose of Fisher exact test using in this study was screening factors which had high associated with the dependent variable (the occurrence of FMD). Then, the variables which showed *p*-value less than 0.2 were analyzed with the Multivariable logistic regression (Rajkó & Héberger, 2001).

Mann–Whitney U test was non-parametric statistical test. It was used to compare medians of two non-normal distributions independent variable. This test was similarly to an ordinary parametric two-sample t test on the data after ranking over the combined samples (Quinn & Keough, 2002). To calculate the value of Mann-Whitney U test, I used the following formula:

$$U = n_1 n_2 + \frac{n_2(n_2+1)}{2} - \sum_{i=n_1+1}^{n_2} R_i$$

Where:

U=Mann-Whitney U test

N_1=sample size one

N2= Sample size two

R_i = Rank of the sample size

This study had numerical data likes distance from market, livestock market, slaughter house, feed mills, main road, water source, pasture, nearest farm, number of vehicle or person who came in farm, volume of production and etc. were analyzed by Mann–Whitney U test. Then, the variables which showed *p*-value less than 0.2 were analyzed with the Multivariable logistic regression.

Multivariable logistic regression was used when the dependent variable was nominal and there was more than one independent variable. This test allowed one to predict a discrete outcome, such as group membership, from a set of variables that might be continuous, discrete, dichotomous, or a mix of any of these. Generally, the dependent or response variable was dichotomous, such as presence/absence or success/failure. The independent variables were a categorical, or a mix of continuous and categorical, multivariable logistic regression was preferred(Scheiner & Gurevitch, 2001).

In this study, the multivariable logistic regression was used with the occurrence of FMD in the previous year as the dependent variable. This variable had 2 results that were occurrence of FMD and no occurrence of FMD. The independent variables were the potential risk factors of FMD occurrence, which were screened by Fisher exact test as mention above.

The dependent variable in multivariable logistic regression was usually dichotomous, that was the dependent variable can take the value 1 with a probability of disease occur (P), or the value 0 with probability of no disease occur (1-P) (S. Zhou et al., 2008). This case, value 1 meaned occurrence of FMD and value 0 meaned no occurrence of FMD.

The relationship between the predictor and response variables was not a linear function in logistic regression, instead, the logistic regression function was used, which was the logit transformation of P:

$$P = \frac{e^{(\alpha - \beta_1 x_1 + \beta_2 x_2 + \_ + \beta_k x_k)}}{1 + e^{(\alpha + \beta_1 x_1 + \beta_2 x_2 + \_ + \beta_k x_1)}}$$

Where α = the constant of the equation and, β = the coefficient of the predictor variables.

The goal of logistic regression was to correctly predict the category of outcome for individual cases using the most parsimonious model. To accomplish this goal, a model was created that includes all predictor variables that were useful in predicting the response variable. Several different options were available during model creation.

Backward stepwise regression was used in the study as the preferred method of analyses, where the analysis begins with a full or saturated model and variables were eliminated from the model in an iterative process. The fit of the model was tested after the elimination of each variable to ensure that the model still adequately fits the data. When no more variables could be eliminated from the model, the analysis had been completed (Steyerberg, et.al., 1999).

There were two main uses of logistic regression. The first was the prediction of group membership. Since logistic regression calculated the probability of disease occur over the probability of no disease occur, the results of the analysis were in the form of an odds ratio. In this study, the results of the analysis were the probability of

FMD occurrence after controlling for other associated risks. Logistic regression also provided knowledge of the relationships and strengths among the variables.

The process by which coefficients were tested for significance for inclusion or elimination from the model involves several different techniques. The wald chi-square is fairly popular, but it may yield inaccurate results with small sample sizes. Thus the likelihood ratio method might be better. It used the difference between the probability of obtaining the observed results under the logistic model and the probability of obtaining the observed results in a model with no relationship between the independent and dependent variables (Steyerberg, et al., 1999). This was the recommended test statistic to use when building a model through backward stepwise elimination. A significant level was set at 0.05 for all procedure.