# CHAPTER 3

## RESARCH METHODOLOGY

**Research design**

      This study comprised 2 sub-studies; the first one was a study of geographic information system (GIS) and an epidemiological study on avian influenza introduction into poultry farms. The purposes of GIS study were to create GIS database and further results was served as a tool for control and eradication of avian influenza. In epidemiological study, cross-sectional study was designed to determined risk factors associated with avian influenza infection in northern Thailand. Farms/flocks with clinical signs and a positive highly pathogenic avian influenza H5N1 virus isolation which were reported from Department of Livestock Development (DLD) during January-July 2007 were defined as infected group. Farms and flocks without clinical signs and avian influenza H5N1 positive at study area were defined as control group.

**Research area**

      The study was conducted in 2 areas, Chiang Mai-Lamphun and Nan provinces, based on geographical characteristics. The reasons to use this region were that most outbreaks occurred in northern Thailand and outbreaks result to massive slaughter of poultry. There were approximately 400 commercial chicken farms and there was no data regarding backyard chicken raising census in these provinces (DLD, 2003). The districts in study areas were shown in table 1.

Table 1: Districts and sub-districts in study area

| Chiang Mai | Lumphun | Nan |
|------------|---------|-----|
| Mueang | Mueang | Mueang |
| Jomtong | Pasang | Santisuk |
| Chiang Dao | Maeta | Phu Phiang |
| Doi Saket | Banhong | Maejarim |
| Doi Tao | Ban Thi | Wiang Sa |
| Prao | Veiang Nonglong | Na Noi |
| Maetang | | Na Muean |
| Maerim | | Tawangpa |
| Maewang | | Pua |
| Sarapee | | Chiang Klang |
| Sankampang | | Song Khwae |
| San Sai | | Tung Chang |
| Sanpatong | | Chaloem Phra Kiat |
| Hangdong | | Bo Kluea |
| Hod | | |
| Mae-oon | | |
| Doi Lor | | |

**Population and sample**

In GIS study, the census of poultry and farmer in the study area was colleted. For each village, the number of farmer and poultry, species of animal, the poultry market and slaughterhouse in the village were recorded. The geographic position of

farm/village, poultry market and slaughterhouse were determined by Global Positioning System (GPS).

In epidemiological study, the target population was poultry raiser including backyard chicken and commercial poultry farms in Chiang Mai, Lamphun, and Nan provinces. Backyard chicken raisers in confrrmed-H5N1 villages and commercial poultry farmers which had outbreak reported by DLD were participated in this study. For commercial poultry farms, all of them which located in study area were interviewed to collect epidemiological data using structured questionnaire. At least 10% of backyard raisers in each village were also included.

In the disease surveillance study, concurrent with questionnaire administration, cloacal swab samples from poultry were collected for virus isolation. Two-stage sampling technique was used to perform. The first sampling unit was the poultry flock in the village and the second unit was the animal in the flock which randomly selected from each flock. The sample size of an animal for each village was calculated by Win Episcope program version 2.0. The needed sample size was 45 samples per village using the following values: expected prevalence 25 %, confidence interval 99%, and accepted error 10 %( based on Department of Livestock Development, 2004). Cloacal swab were pooled up to five per tube in approximately 2 ml of phosphate buffer solution added with antibacterial agents. Samples were shipped daily to the Veterinary Diagnostic Laboratory Center, Chiang Mai University for laboratory test within 48 hours.

**Definition**

- Poultry: gallinaceous animal which people raise for specific purposes, for instance, meat and egg, companion.

- Avian influenza: poultry disease caused by highly pathogenic avian influenza type A H5N1.

- Outbreak farm: commercial poultry farm or backyard poultry flock which avian influenza had been occurred in the flock. Outbreak farms in this study were collected from DLD reports.

- Outbreak point: Geographical point of poultry farm or backyard poultry flock which avian influenza had been occurred in commercial farm or backyard raising. The points were reported by DLD.

**Data collection**

In epidemiological study, the toll for collecting data was a structured questionnaire to descript demographic characteristics of poultry raising as a group, backyard or commercial raising, and to assess their farm management, practices during disease outbreak. The questionnaire was composed of 5 parts

**Part 1:** This part consisted of 7 questions to describe demographic characteristics and information regarding farm management. This included poultry raiser's name, address, geographic positioning point, type and number of poultry in farm, type and number of building in their farms, production performance, and worker of farms.

**Part 2:** This part consisted of 3 questions to determine previous disease outbreak of target group, timing and duration, control and prevention practices and sick-animal handling during disease outbreak.

**Part 3:** This part consisted of 8 questions to identify disease control and prevention program including vaccination, vaccination program, person who responsible to vaccinated animal, vaccine storing method, and disease prevention practices during disease outbreak of nearby farms.

**Part4:** This part consisted of 32 questions to identify risk factors associated with disease outbreak of target group.

**Part 5:** This part consisted of 2 questions to identify the road which farmer used for animal movement into and out of study areas.

The training workshop was done for the interviewer before conducting in the field data collection. The objectives of the course were to guideline of questionnaire and to reduce bias that may occur in the study. Five participants of research teams attended the workshop in the first workshop and 10 attended the second. After training, the interviewers started to collect data in August 2004 to June 2005. There were 15,530 of backyard chicken raisers and 451 of commercial chicken farmers including quail farm with avian influenza outbreak participated in this study.

GIS study, the data was divided into 2 part, spatial data and non-spatial data. Spatial data, data which used for created map, were collected from government partner. The data consisted of map of spatial boundaries of provinces, districts, sub-districts, and spatial locations of villages, poultry farms, live birds and chicken meat markets, fighting cock places, main roads and their branches, and main natural water ways. The table 2 showed sources of spatial data

Table 2: Sources of spatial data

| Map | Sources |
| --- | --- |
| Spatial location of poultry farms, villages, markets, fighting cock places | Field surveys |
| Spatial boundaries of target area | Department of Provincial Administration |
| Main roads and their branches networks | Department of Land Development |
| Natural water way | Department of Land Development |

Non- spatial data consisted of poultry and poultry raiser census, farm characteristics, information regarding avian influenza outbreak were collected by survey and received from Department of Livestock Development. The table 3 showed sources of non-spatial data.

Table 3: Sources of non-spatial data

| Type of data | Sources |
| --- | --- |
| Census of poultry | Department of Livestock Development |
| Census of poultry raiser | Department of Livestock Development |
| Farm characteristics | Field surveys |
| Disease outbreaks information | Field surveys |

**Virus isolation**

The interviewers collected the data form chicken risers and after that they collected samples from poultry, cloacal swab or tracheal/nasal swab, to test virus isolation. Cloacal swabs were collected in Phosphate Buffer Solution (PBS) with antibiotics, gentamycin (50mcg2ml). The samples were shipped directly to Veterinary Diagnostic Laboratory Center, faculty of Veterinary Medicine, Chiang Mai University and were tested within 48 hours. The samples were tested by inoculate of embryonated chicken egg following to gold standard method described by Office Des International Epizooties guideline. The diagram below shows the procedures.
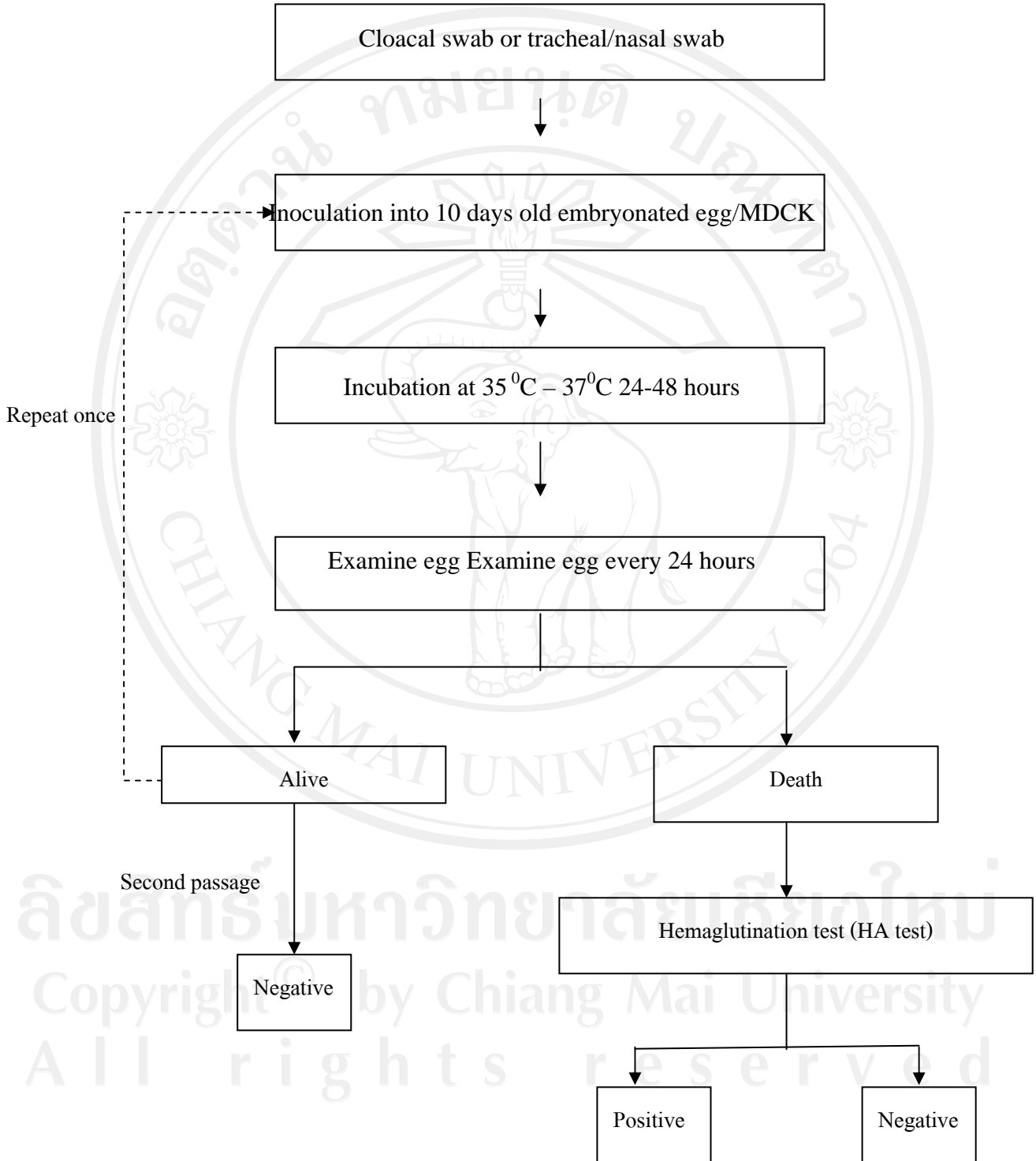
Figure 5. Flow diagram of laboratory procedure for avian influenza virus isolation

**Data analysis**

Data regarding geographic information system were stored and managed in GIS software program (Arc view [R] Version 3.2) which used this program for spatial analysis. Risk area for avian influenza outbreak was analyzed using inverse distance weighted interpolation technique that used risk factors associated disease introduction into target group, to reclass the area, from questionnaire.

Data from questionnaire were analyzed using multivariable logistic regression to determine the risk factors associated with avian influenza introduction into poultry farm/raising areas. A significant level of 0.01 was used to select the variable. The final model was derived using backward stepwise algorithm.