CHAPTER III

MATERIALS AND METHODS

3.1 Place of study

Provinces bordering Mekong Delta, south of Vietnam are Long An, Tien Giang, Ben Tre, Dong Thap, Can Tho-Vinh Long, Tra Vinh, An Giang, Soc Trang, Kien Giang (Fig. 7). Pigs originate from farms where they have close contact with ponds or rivers or watersheds (i.e. biotopes where the intermediate hosts could occur). It was planned that samples are collected at the largest abattoirs in nine provinces bordering Mekong River, south of Vietnam.

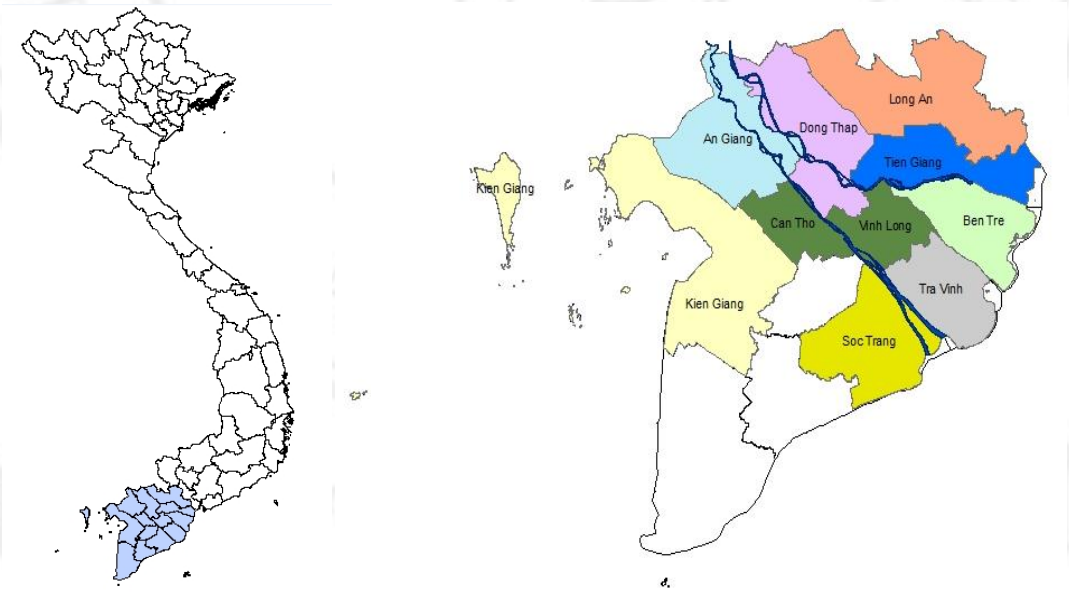

Figure 7 Sampling provinces of Mekong Delta in Southern, Vietnam

3.2 Sample size and sampling method

The study was designed to detect *Alaria alata* mesocercariae in pig carcasses in provinces bordering Mekong River. The sample size was calculated for each province with estimated prevalence 5% (p), 95% C.I., accepted error 5% following the formula:

$$n = (1 - (1 - p)1/d) \times (N - d/2) + 1$$ (Thrusfield, 2005)

The sample size was adjusted with expected sensitivity and specificity of test is 85% and 100%, respectively. Thus, the sample size per province was adjusted to 69. So the total number of tested carcasses was 621.

In general, the farm size is small scale in that the pig keeping was less than 20 pigs/ farm in Mekong Delta (97% pig population) (MARD, 2007). The samples were collected from pigs coming from the small scale and the number of the pigs came up from 90% to 100% at slaughterhouses (RAHO6, 2012, RAHO7, 2012). Slaughtered pigs population of each province is described in Table 3 below.

Table 3 Slaughtered pig population distribution at province level, 2012

No.	Province	No. of slaughtered pig (head) 2012
1	Tien Giang	645,569
2	Ben Tre	396,952
3	Can Tho - Vinh Long	569,442
4	Đong Thap	267,495
5	An Giang	160,516
6	Kien Giang	294,729
7	Soc Trang	264,456
8	Tra Vinh	406,484
9	Long An	283,455

Within each province, the largest slaughterhouses were visited for sampling. If there was no central slaughterhouse, 2 largest slaughterhouses were selected. The total of slaughterhouses (SH) included in this study was 15 and the slaughterhouse size was designed to slaughter from 50 to 500 pigs/ night. In each slaughterhouse, on the days visited, samples were taken from the carcasses coming from the districts belonging to that province. This meant that in total nine provinces were sampled (Table 4), and within provinces, different origins of pigs (communes, districts) were considered.

Table 4 Calculation of Sample size by province

No.	Province	Sampled SH	Sample
1	Tien Giang	1	69
2	Ben Tre	2	69
3	Can Tho - Vinh Long	3	69
4	Đong Thap	1	69
5	An Giang	2	69
6	Kien Giang	2	69
7	Soc Trang	2	69
8	Tra Vinh	1	69
9	Long An	1	69
Total		**15**	**621**

3.2.1 Sample collecting process

Data of the chosen slaughterhouses was collected from sub-Department of Animal Health (sDAH) data, considering basic information such as the number of slaughtered pigs per night, under control of Meat Inspection (MI) teams. When pigs arrived at the slaughterhouse, the traders registered to the MI team at the slaughterhouse about the number pigs sent and be slaughtered on that day, the origin of the animals, etc. Only the collected pigs of small traders with clear origin from backyard farms were included in a sampling list. This was to ensure that the pigs were

raised by traditional farming with low bio-security. Using excel support, the pigs in list were chosen randomly and marked to separate from the others. The pigs were followed through the processing plan and sampled. 50g cheek and 50g peritoneal tissues were collected per carcass immediately after slaughtering. The samples were encoded and stored in an insulation box and transferred to the Center for Testing and Diagnosis Animal Health under Regional of Animal Health No. VI (Raho6), Vietnam. A flowchart of this process is given in Figure 8.

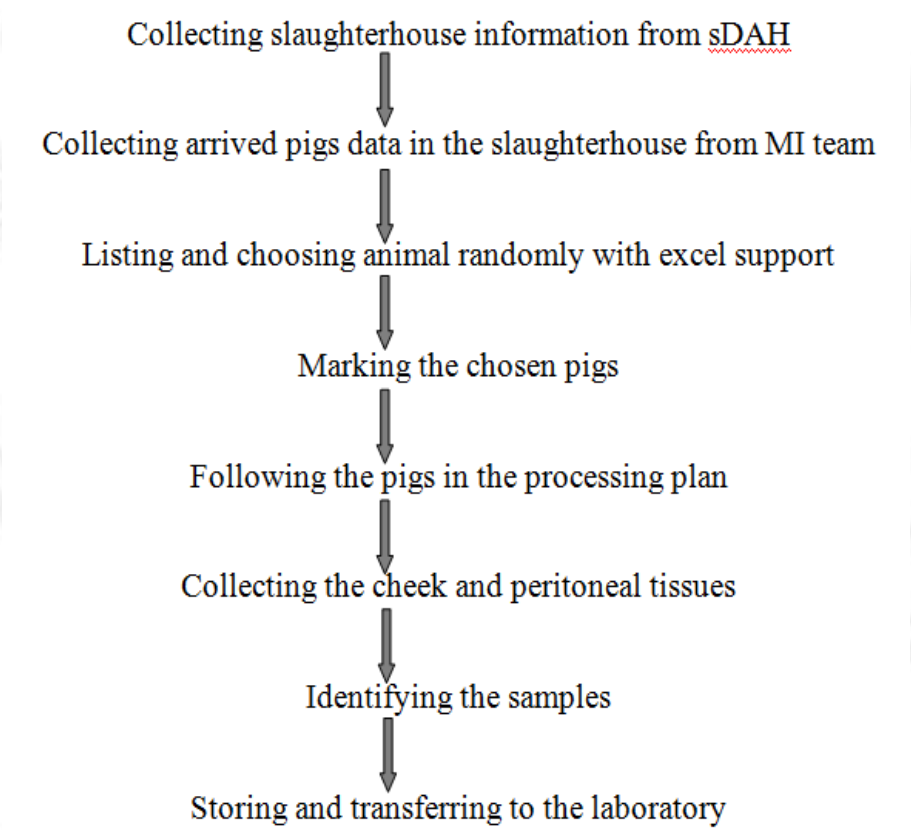

Figure 8 Flow chart of collecting sample process

3.2.2 *Alaria* mesocercaria isolation method

Based on the visualization by stereomicroscope demonstrated that parasites were very motile and the parasite (a) shows a high affinity to liquids and (b) moves actively out of the tissues, a new migration technique (AMT) was developed basing on a larvae migration technique by Riehn *et al*. (2010).

The peritoneal and cheek tissues were mixed, chopped to 5mm size cubes and 30g of these tissues were tested by *Alaria* Migration Technique within 7 days after sampling**.**

3.3 Laboratory analysis

3.3.1 Materials

1.1 Knife or scissors and tweezers

1.2 Cutting board

1.3 Stands, rings, and clamps or multiple funnel stand

1.4 Glass funnel, Ø 10 cm

1.5 Plastic sieves, Ø 9 cm, mesh size 0.8 mm

1.6 Rubber hose, Ø 10 mm, 10 cm long

1.7 Hose clamp, 60 mm

1.8 A microscope

1.9 Petri dishes, Ø 9 cm, marked on their undersides into 10 × 10 mm squares

1.10 Tap water heated to 46 to 48°C cooling down to room temperature

1.11 Balance accurate to 0.1 g or better

3.3.2 Procedure

1. The glass funnels (Ø 10 cm) are supported on the funnel stands.

2. The rubber hose (Ø 10 mm, 10 cm long) is fitted to the funnels stem and closed with a clamp.

3. The sieve (Ø 9 cm, mesh size 0.8 mm) is placed in the funnel.

4. An aliquot of 30 g of the sample material is roughly chopped (0.5 cm edge length).

5. The chopped meat is transferred to the sieve and 150 ml of lukewarm tap water (40-45° C) is filled in the funnel. The sample material has to be totally immersed in the water.

6. The sample is allowed to stand for 30 minutes (not exceed 60 minutes) at room temperature.

7. After 30 min, quickly run off liquid from separation funnel in 2 petri dishes, 20ml/dish.

8. The petri dishes are examined by stereomicroscope at 20x magnification.

Prepare sample

- 30 g mixed from the tissues were cut in 5mm cubes
- Put sample in sieve and place sieve in funnel.

Filtering

- Add warm tap water until all sample is covered with water
- Let stand for at least 30 min.(maximum 1 hr)
- Then quickly run off liquid from separation funnel in 2 petri dishes.

Examinaltion

Examine the sample at 20-40x magnification (stereo microscope).

Figure 9 Flow chart of sample examination