CHAPTER III

METHODOLOGY

1. Conceptural framework

Using the specific antigens which stimulate immunity system to induce the specific antibodies (IgG, IgM). I hope to demonstrate that these antibodies are protective against Ascaris infection.

2. Research question

Do Secretory/Excretory-Somatic antigens of the second and third/fourth larvae forms of Ascaris suum and Ascaris lumbricoides reduce the number of migrating Ascaris larvae from challenge compared to those not immunized?

3. Hypothesis:

3.1. There is no difference in the average number of migrating larvae between 2 groups: experiment and control (Null hypothesis).

3.2. The average number of migrating larvae in experimental group is significantly lower than control (Alternate hypothesis).

3.3. There is no difference in the proportion of mouse free from infection between the experimental and control group (Null hypothesis).

3.4. There is significant difference in the proportion of mice free from infection between the experimental and control groups (Alternate hypothesis).

4. Assumption:

The population in this study were in-bred and cogenic mice (purebred mice, born in a laboratory at animal house). During the time of study, mice were brought up in absolute hygienic conditions. These mice were of the same age and weight. These conditions should create a reasonable expectation that the juice (secretion) obtained should have no specific antibody to Ascariasis at the time they were used for the experiment.

5. Research design:

This is experimental study. In this study, subjects were observed prospectively to see how many migrating larvae will be collected in each mouse in the experimental and control groups after immunization and challenge.

6. Sample specification/population sample:

- Target population: Mice (musmus culus)
- Target sample: Mice were born in laboratory at animal house, in bred and cogenic mice (purebred mice).
- Samples: randomly allocated to the experimental and control groups.
- Inclusion criteria:

  - Mice ≈ 20 g

  - Age ≈ 4 weeks

  - Mice eating well

- Hair was glossy
- Moving quickly
- Mice brought up in absolute hygienic conditions.

- Exclusion criteria:

  Mice having been used in any other experiment.

- Sample size:

  2 N/group is estimeted by formula:

$$2N = 2\ [(Z_\alpha + Z_\beta).\ SD]^2\ /(Mc - mt)^2$$

7. Ethical consideration:

The study did not have any ethical problem.

8. Limitation and obstacle:

Finding ascaris antigens which can induce good protective immunity is very difficult work, it took much time and budget.

9. Out come measurement and data colection/analysis:

- The steps were done by colleagues and I, the outcome measurement were done by other colleagues, they were blinded.
- The results were expressed as the arithimetic mean of the number of larvae recovered by Baermannisation ± standard error of mean.

10. Analysis:

- Data summary: average number of larvae per mouse.

	Experiment	Control
X		
SEM		
95% CI		

- Statistical test: unpaired t-test (one tailed) and wilcoxon's test
- The proportion of mice which have not larvae.

	Larvae (+)	Larvae (-)	
E	A	C	
C	B	D	

- Specification of data handing and collection computer use.